An investigation into the reuse of organic waste produced by the New Zealand Mussel Industry

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Thesis submitted in (partial) fulfilment of the degree of

Master of Applied Science

Auckland University of Technology

June, 2004

“A successful aquaculture system does not have wastes, only by-products, to be used as positive contributors to the surrounding ecosystems.”

- Folke and Kautsky (1992)

Acknowledgements

Firstly I wish to thank and acknowledge all the help and guidance provided by my primary supervisor, Dr Andrea C. Alfaro. I also acknowledge and thank my advisors, Dr Andrew Jeffs (National Institute of Water and Atmospheric Research) and Prof. John Buckeridge (Earth & Oceanic Sciences Research Institute, Auckland University of Technology).

Thanks must also go to the New Zealand Mussel Industry's factories and their employees who provided information, samples and support and without whom this project would not have been possible. Especial thanks to Andrea Blackburn, Lloyd Murphy, Mike Mandeno, Andree Johnston and the New Zealand Mussel Industry Council.

I would like to acknowledge the generosity of Roger Glanville and Bill Eaton of the Materials Laboratory at UNITEC, Auckland, for providing access to their concrete laboratory and equipment; similarly to Timothy Walker, Noel Perinpanayagam and Mark Byrami of the Civil Engineering Laboratory at the University of Auckland for their knowledge, equipment and time. Dr Bryony James of the University of Auckland is thanked for her assistance and for allowing use of the Scanning Electron Microscope.

I am very grateful also to the National Institute of Water and Atmospheric Research and the AUT Faculty of Science and Engineering for all financial support.

Gratitude must also go to my colleagues in the Earth and Oceanic Sciences Research Institute at Auckland University of Technology (especially Dr Steve O'Shea), the EOS Marine Ecology Group, Heather Stonyer and AUT's technical support staff, in particular Chris Whyburd and Yan Wang.

Finally, sincere thanks must go to Marie and Richard Barnaby, Michael Phipps and Andrea Sprosen for their support and patience during this research.

Abstract

Management of organic waste is a major problem for the New Zealand Mussel Industry. Currently most waste is discarded, and this represents a potential loss of both resources and revenue, unless an alternative use for this waste could be developed

Waste types were first identified, then quantified, first seasonally, then annually, to provide an estimate of total industry-wide waste production. Possible uses for this waste were then identified.

Little investigative research has been undertaken on identifying alternative uses for mussel industry organic waste. The uses of organic waste as organic fertilizers, and the economic benefit of adding treated waste products to cement mix to improve its compressive strength and thermal insulation, are two primary objectives of this dissertation. The possibility of using mussel shell in agricultural liming as a substitute is also explored.

The potential value of pre-grade waste as an organic fertilizer was explored by addition of decomposed tissue to tomato seedlings and by monitoring plant development and condition. Growth of treatment and control seedlings was monitored by counting the number of branches, stem heights, leaf numbers and total biomass. Analyses prove *Perna canaliculus* pre-grade organic waste has the potential to be exploited as an expensive, effective organic fertilizer, whereas *Mytilus galloprovincialis* pre-grade organic waste may not. Moreover, there is further potential to develop *P. canaliculus* pre-grade organic waste into an odourless, chemically stable fertilizer product.

The potential value of post-grade waste in cement mixes to improve compressive strength was explored by addition of shell aggregate to cement mix. Analyses indicate that, as an aggregate, mussel shell has little to no structural potential, but does have latent thermal insulating properties.

Recommendations are made to

- Separate *Perna* and *Mytilus* pre-grade waste products.
- Further explore the thermal insulating potential of mussel-shell concrete.

- Further explore techniques for treatment of Perna pre-grade waste as a fertilizer.
- Further explore the use of crushed mussel shell as a potential limestone or sand substitute for agricultural, construction and engineering purposes.
- Explore markets for *Mytilus* potential export, to reduce pre-grade waste production and problems of resettlement.

Table of Contents

List of Figures

List of Tables

List of Acronyms

Universal acronyms

ARC	Auckland Regional Council
AUT	Auckland University of Technology
MDC	Marlborough District Council
NIWA	National Institute of Water and Atmospheric Research
NZMFA	New Zealand Marine Farming Association
NZMI	New Zealand Mussel Industry
NZMIC	New Zealand Mussel Industry Council
NZMIECP	New Zealand Mussel Industry Environmental Code of Practice
NZS	New Zealand Standard
USEPA	United States of America Environmental Protection Agency

Other acronyms

AEA	Air entraining agent
BM	Blue mussel
CA	Control containing a solution of a commercial liquid plant feed diluted in deionised water to recommended specifications
CB	Control comprising 100% deionised water only
GSM	Greenshell™ mussel
HCT1	Trial One concrete mix containing hand crushed mussel shell fragments as an aggregate
ICP - OES	Inductively Coupled Plasma Optical Emission Spectroscopy
IS	Industrial standards
MBT1	Trial One concrete mix containing mixer-broken mussel shell pieces as an aggregate
MBT2	Trial Two concrete mix containing mixer-broken mussel shell pieces as the main aggregate but with additional basalt aggregates included
NPK	Nitrogen: Phosphorus: Potassium, ratio of these elements in fertiliser
AG	Treatment fertiliser containing only decomposed Greenshell™ mussel tissue
BG	Treatment fertiliser containing decomposed Greenshell™ Mussel tissue and calcium oxide derived from shell
DB	Treatment fertiliser containing only decomposed blue mussel tissue
TLPP	Two-linear-parallel-probe

Statement of Originality

‘I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the qualification of any other degree or diploma of a university or other institution of higher learning, except where due acknowledgement is made in the acknowledgements’

(signed)

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(date)

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Intellectual Property and Confidential Material

To the best of my knowledge, this thesis does not contain confidential material. For confidentiality, reference to participating companies/factories was done so by code. Data provided by or pertaining to the participating company/factory (where such data were separated from pooled data in the text) were included only with permission from the said company/factory. No specific references to any factory operating procedures are made where these were found to differ from other factories. Only general, universal procedures are described.

All photographs, tables, charts and diagrams are my own unless stated otherwise in the caption text.

Glossary

The definitions in this glossary include selected basic biological, botanical, engineering and industry-related words, since this thesis transverses three disciplines.

Biological

Bioavailability	The degree and rate at which a substance is absorbed into a living system or is made accessible to organisms
Chitin	A hard polysaccharide that forms part of the exoskeleton of insects, arachnids, and crustaceans
Dinoflagellate	Any of an order (Dinoflagellata) of predominantly marine plankton usually single celled plants
Epibiota / epibiont	Any organisms (esp. benthic) which colonise hard substrate surfaces (either inert or other organisms)
Epifauna	Benthic fauna living on a substrate or on other organisms
Glycogen	A white shapeless polysaccharide ($C_6H_{10}O_5$) that is the most important form in which carbohydrate is stored in animal tissues
Keratin	Various sulphur-containing fibrous proteins that form the chemical basis of horny epidermal tissues such as 'mother of pearl' or hair
Mantle	A heavy fold of tissue between the shell and organ mass – responsible for secretion of shell
Periostracum	Protective keratin-chitin coating on the surface of molluscan shells
Polysaccharide	Any group of carbohydrates comprising long chain simple sugar molecules e.g. glycogen

Botanical

Biomass	The total mass of organisms living in an ecosystem – usually expressed in terms of dry weight
Compost	A mixture consisting of decaying matter used in fertilisation of soil as a stimulus for plant growth
Dry weight	Weight of an organism (plant or animal) after all moisture has been removed
Filtrate	Substance removed from other by washing
Epicotyl	Emergent seedling portion rising from the seed's cotyledonary node
Foliar	Of leaves, both applied to and relating to
Green weight	Weight of fresh plant tissues with all moisture intact (may include roots if desired)
Hypocotyl	Emergent seedling portion from below the seed's cotyledon
Mulch	Protective covering placed on ground to maintain soil moisture, heat, prevent weeds, erosion etc.
Phytotoxicity	Having the potential to inhibit germination or plant growth

Pinna	A leaflet or main division of a pinnate leaf

Engineering

Acicular	Needle-shaped
Aggregate	Any hard inert material such as sand or rock used for mixing with a cementing material to form ceramic products such as concrete
Aliquot	Portion of a solution
Bleeding	Of wet concrete, refers to mix-water which rises to the surface due to sedimentation of solid components in the mix
Calcination	The act of superheating inorganic materials to convert them to simpler compounds or to drive off volatiles e.g. $CaCO_3 \rightarrow CaO$
Ceramic materials	Any of a group of solid products formed using the likes of cement, clay or plaster
Compressive strength	The ability of material to resist a force that intends to crush or buckle
Curing (of concrete)	Pertains to the setting of a wet concrete mix
Interstitial	Relating to a crystalline compound within which small atoms/ions of non-metals inhabit spaces between larger metal atoms/ions in the crystal lattice
Kinetic energy	The energy which is associated with motion
Phonon	Vibrational waves in a lattice
Slab	A thick plate or slice (of concrete paving)
Slump (of wet concrete)	The ability of a wet concrete mix in an open ended cone to sink or fall when the cone is removed. A test for workability
Tamping rod	Steel rod used in the compaction of wet concrete mixes to remove air for testing (usually around 62cm long, 1.6cm diameter with rounded ends)
Tensile	Relating to or involving tension
Thermal conductivity	The ability of a material to conduct heat

Industry

Declumping	Removal of undesirable epibiota from mussel lines and surface of mussel shells
Pre-grade waste	Organic material removed from harvested mussels at the factory, prior to processing. Comprised of epifauna, crabs and macroalgae.

Chapter 1 — General Introduction

1.1 The New Zealand Greenshell™ mussel, *Perna canaliculus*

New Zealand is home to 10 mussel species, but only one of these is cultivated (Walsby, 1993). The New Zealand Mussel Industry (NZMI) is based solely on the cultivation and harvest of *Perna canaliculus* (Gmelin, 1791), the New Zealand Greenshell™ or green-lipped mussel. *P. canaliculus* is endemic to New Zealand and is found from the mid to low intertidal zones of rocky shores, to shallow subtidal areas of the North, South and Stewart Islands (Paine, 1971; Vakily, 1989; Gardner and Thompson, 2001). *P. canaliculus* is easily recognised by its dark green shell with the characteristic bright green 'lip', where the periostracum (outer organic keratin layer) emerges into the inner edge of both valves. The soft tissue is orange in colour for females and ivory/white in males (Walsby, 1993). Mussels have two distinctive life phases, a free-swimming larval stage and a sessile adult stage. The larvae in the water column are approximately 0.3mm (shell length) at 3-5 weeks old and by the time they are ready to settle, they have generally reached a size of 0.25-0.3mm (Jenkins, 1979; Alfaro and Jeffs, 2002, 2003). It is at this 'spat' stage that the juveniles are harvested for cultivation within mussel farms.

1.2 The history of mussel farming in New Zealand

Cultivation of *P. canaliculus* began in the early 1970's following population declines in wild dredge fisheries in the Firth of Thames/Hauraki Gulf and Tasman Bay/Marlborough regions (Hickman, 1989; Hilhorst, 1990; New Zealand Aquaculture Council, 2001; Jeffs, 2002). Today, most mussel culture occurs in the Coromandel area of the Hauraki Gulf (North Island) and in the Marlborough Sounds in the South Island. The majority of the spat (exceeding 70,000 tonnes per year) is sourced from Ninety Mile Beach, Northland (Alfaro *et al*., 2001; Alfaro and Jeffs, 2002).

1.3 The New Zealand Greenshell™ mussel industry

In terms of aquaculture export, New Zealand is most reliant on the culture of *Perna canaliculus* (Jeffs and Holland, 2000; Jeffs, 2002). *P. canaliculus* mussels make up 75% of the value of the entire aquaculture industry and are quick to grow to maturity (Jeffs, 2002). In 2002, the New Zealand Mussel Industry (NZMI) produced 29,000 tonnes of product with a value of $185 million in export sales (New Zealand Mussel Industry Council, 2003). As a comparison, the Hawaiian aquaculture industry, in 2002, experienced record growth for all cultivated species, of which shellfish make up only 32% - gaining US$25.2 million ($NZ 40.2 million) (Sing, 2003).

The NZMI exports to over 60 countries and contributes $50 million to New Zealand's gross domestic product (Jeffs, 2002). With the advent of new aquaculture technology and the expected removal of the current aquaculture moratorium, it has been predicted that by the year 2020 the industry will exceed 140,000 tonnes of annually exported product (Holland and Jeffs, 2000; New Zealand Aquaculture Council, 2001; Chapple, 2003).

P. canaliculus exports were on the rise from 1988 to 1998 before falling between 1998 and 2001 (Fig. 1). This decline was due to the detrimental impact of *Gymnodinium catenatum*, a toxic dinoflagellate microalga responsible for paralytic shellfish poisoning. Toxic algae bloomed previously in 1992/1993 (Judd, 1993), and in May 2000, and in both events caused widespread shellfish contamination (Todd, 2000; Akroyd, 2002). The May 2000 algal bloom was documented as one of this country's most extensive and harmful toxic blooms (Mackenzie and Beauchamp, 2001 c.f. Akroyd 2002). However, the NZMI recovered rapidly from the 2000/2001 bloom and exports are once again increasing.

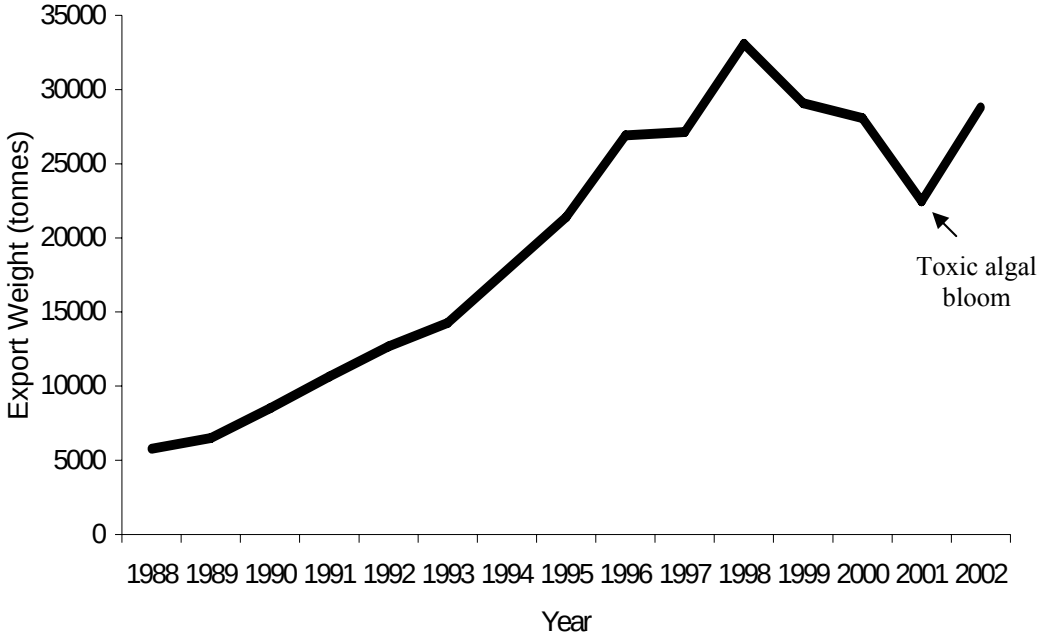

Figure 1: ***Perna canaliculus*** **export summary with data supplied by the New Zealand Mussel Industry Council.**

The Marine Farming Act (1971) regulates the leasing and licensing of the foreshore and seabed for aquaculture purposes (Jenkins, 1979). Contrary to popular belief, marine farming in New Zealand was not "*growing happily*" prior to government intervention, which came in the form of a moratorium, ceasing new marine farm applications (Hodgson, 2003). Marine farming in New Zealand has suffered many hindrances, most stemming from the moratorium placed in November 2001. This legislation prevented the formation of new marine farms, (Chapple, 2003; Louisson, 2003) and enforced farming space restrictions (Thompson, 2002). Other hindrances included broken deadlines on the aquaculture reform bill (Chapple, 2003) and the controversial foreshore and seabed issue (Chapple, 2003b). The aquaculture moratorium was placed in response to the large number of applications received from regional councils and the recognised limitations of current regional coastal plans to manage aquaculture in its entirety (Auckland Regional Council, 2003). This moratorium allowed time for the Government to reform the laws surrounding marine farming and for local councils to improve coastal planning procedures (Hodgson, 2003). In March 2004, the current moratorium on new marine farm applications was set to be lifted. However, due to indecision on the matter, it has been extended to a new deadline of December 2004. When this moratorium is eventually lifted, depending on the new Aquaculture Reform Bill, it may allow for further growth by the establishment of new farms. This growth will be enhanced by the introduction of new marine farm areas, such as the development

of a new zone in Wilson's Bay, 34 km north of Thames, to be introduced by Environment Waikato (Waikato Regional Council). This new area is forecasted to raise the value of Coromandel mussel farming by $40 million, with an increase of 35,000 to 40,000 tonnes of mussels per annum (O'Rourke, 2003). With the potential removal of the moratorium comes the forecasted increase in harvested mussels. This would, in turn, lead to a likely increase in waste production.

The New Zealand Mussel Industry's 38 mussel-product companies have in total 36 privately owned processing plants extending as far north as Auckland and the Coromandel to Bluff in the South Island (V. Robb, pers.comm., 2003) (Fig.2). These factories are responsible for receiving harvested *P. canaliculus* mussels and processing them into various food products. These products include mussels in the half-shell, mussel meat, marinated mussels, crumbed mussels, live whole mussels, smoked mussels and frozen whole mussels. The waste types and quantities produced by each factory are generally directly proportionate to the products they produce. For example, when a factory produces large quantities of mussel meat, double quantities of shell waste are discarded, compared to when they produce mussels in the half-shell. Although there are 39 factories currently in operation in New Zealand (V. Robb, pers.comm., 2003), the amount of waste they produce per annum (and are estimated to produce in future years) is likely to increase (Paul Lupi, pers.comm., 2002). Predicted increases in the growth of the NZMI will thus depend on several factors; amendments and changes in aquaculture legislation, increased consumer demand, the creation of new farms and the carrying capacity of current farms. The latter is currently an important area of research for the National Institute of Water and Atmospheric Research (NIWA), who are devising mathematical models to predict the carrying capacity of marine farms (Ross and Hooker, 2001). These models take into account factors, such as, plankton availability (mussel's primary food source), seasonal changes in climate, temperature and the effect of climate on water column stratification. This in turn affects plankton availability and subsequently mussel growth, condition and yield (Tortell, 1973; Kennedy, 1976; Hickman, 1979; Hickman and Illingworth, 1980; Okumus and Stirling, 1998; Marsden and Weatherhead, 1999; Ross and Hooker, 2001). These factors also affect the feeding, growth and metabolism of mussel larvae prior to settlement and must be taken into account with regard to spatfall forecasting, post-settlement spat mortalities (Jones *et al*., 1996) and larval settlement (Widdows, 1991; Buchanan, 1994; Alfaro *et*

al., 2001; Alfaro and Jeffs, 2002, 2003). These factors must also be considered when predicting whether there will be adequate spatfall to supply a growing industry (Alfaro *et al*., 2001; Alfaro and Jeffs, 2003).

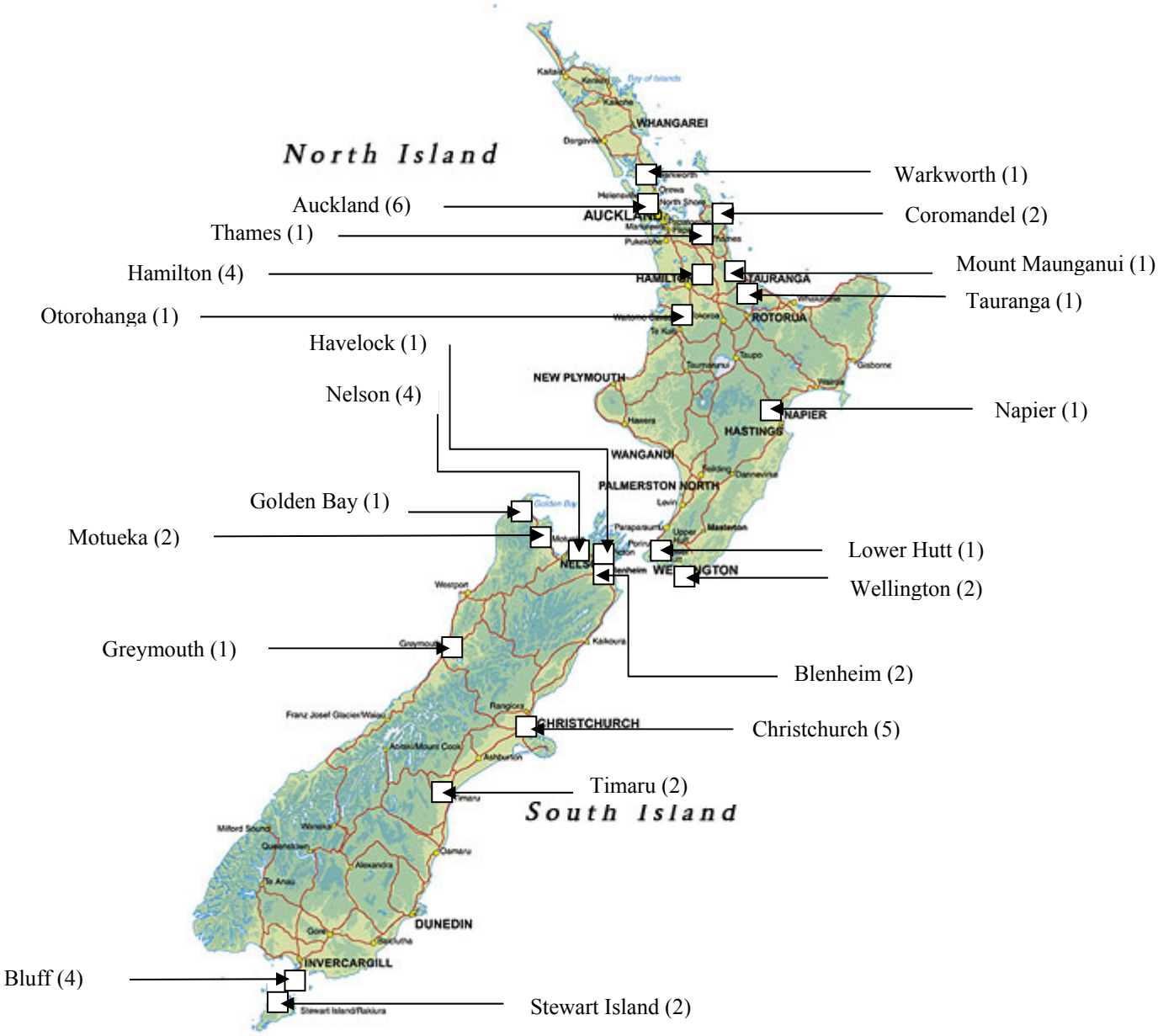

Figure 2: Distribution map of New Zealand Mussel Industry mussel processing factories. Information for map creation sourced from V. Robb (NZ Marine Farming Association).

1.4 Mussel harvesting and processing

In the late 1960's, mussel cultivation techniques were based on the 'Spanish Raft' method that enabled the suspension of 400-600 mussel-encrusted cultivation ropes (Hickman, 1989). This was an efficient technique but was later superseded by the

"long-line" culture method in 1974 (Hickman, 1987; Hickman, 1989) (Fig.3). Longline culture systems were originally developed for the Japanese oyster industry (Imai, 1971). Like the 'Spanish Raft' method, long line cultivation is based on suspended culture techniques but is more advantageous in its simplicity (no large rafts), adaptability to mechanisation, and is easier to construct and transport (Lutz, 1980; Johns and Hickman, 1985; Vakily, 1989).

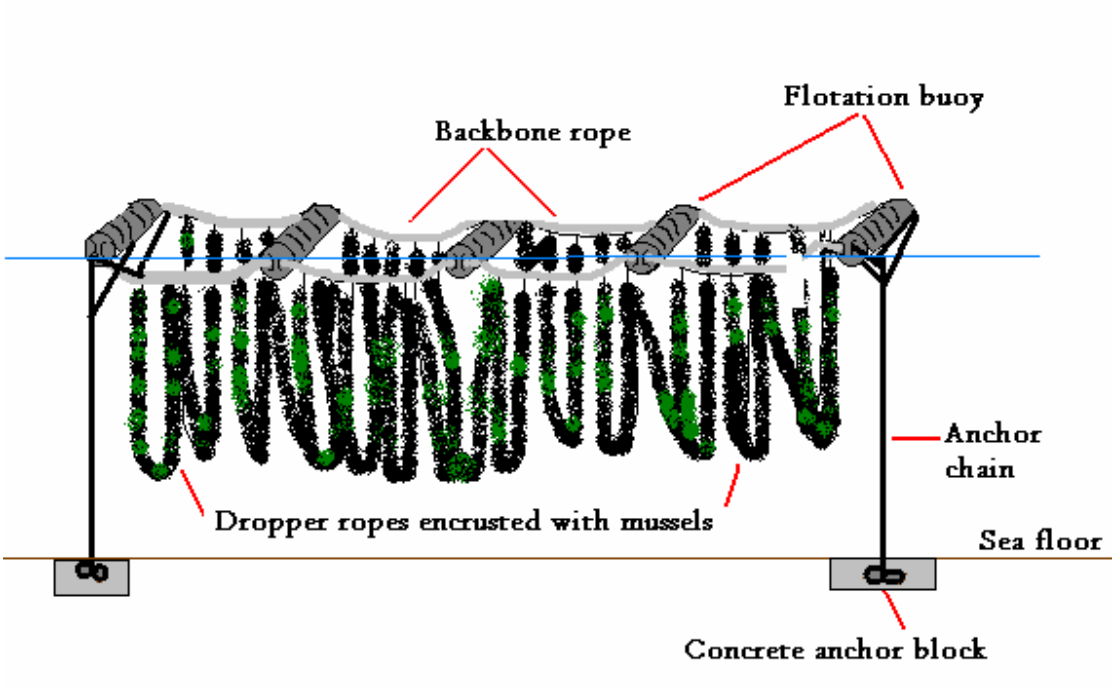

Figure 3: The commonly used "long-line "suspended culture technique utilised in the culture of *P. canaliculus*.. Adapted from Hickman (1987) and Jeffs *et al*. (1999).

1.5 Harvesting techniques

In suspended culture, *P. canaliculus* generally grows from 10 to 75mm in 6 months to 110 to 115mm in one year (Flaws, 1975). Harvesting for market involves the removal of the product from these long-lines when they have reached a marketable size, after around a year of growth (Fig. 4). This process is carried out by hoisting the mussel-encrusted ropes onboard a harvesting vessel, where they are mechanically stripped of the attached mussels. The mussels are then placed in large sacks onboard, shipped to port and transported by truck to the processing factories (C. Barnaby, pers.obs., 2002). Considerable waste, in the form of *Mytilus galloprovincialis* and other epibiota, is removed at this point, mostly by onboard de-clumping (mechanical removal of the mussels form the longlines) and by hand (Fig. 4). This waste is usually discarded directly back into the sea within the mussel farm vicinity.

Figure 4: The MV Pelorus Image, at work harvesting mussels at Port Fitzroy, Marlborough Sounds. December 2002. The dropper ropes are hoisted onboard and are stripped clean of the mussels attached (1). This crewman is removing blue mussels and any noticeable fouling species (2) (Photo: C.Barnaby, 2002).

1.6 Processing techniques

1.6.1 Epibiont removal

After harvesting, epibiotic foulers such as barnacles, macroalgae and sponges must be removed so the shell is 'clean', more attractive to the consumer and easier to process. This is often carried out in a 'de-clumper', which is a rotating device. In this machine, mussels revolve and rub against each other, dislodging fouling organisms, which fall through a grate and into waste bins.

1.6.2 Size grading

Depending on the processes each factory employs, mussels are generally graded into small, medium and large size classes, with the smaller specimens discarded as waste at this point. Broken mussels are removed as waste during the manual quality control check that is carried out after the mechanical size grading.

1.6.3 Pre-cooking

Some of the factories pre-cook the mussels to loosen byssal threads (beards) for ease of removal. Pre-cooking involves immersion in steam-heated water baths at around 85ºC, or treatment with infrared heat. This technique is often employed where hand-removal of byssal threads is carried out.

1.6.4 Byssal threads

Byssi or byssal threads are produced by mussels (and other sessile bivalve species) to facilitate adhesion to substrata. Colloquially referred to as beards, these keratin/collagen-based threads are secreted from the mussel mantle and used to attach to the substratum with strong adhesives (Deming, 1999). The remarkably strong mechanical properties of these threads make them difficult to separate cleanly from the mussel tissue. In the factory, byssal threads are removed either by hand, or mechanically by 'de-bearding' machines (also known in the trade as 'eggbeaters') that tear the threads from the mussel valves, dropping them into a waste container (Fig. 5). This waste is then taken by hand to the main skips and combined with the pre-grade waste (comprising of broken mussels, undersized mussels, crabs and epibionts).

Figure 5: Byssus-waste catching bins at two different mussel-processing factories (Photo: Barnaby, 2002.

1.6.5 Cooking and shell removal

Cooking involves immersion of the mussels into hot water at 90ºC. The mussels are immersed, drained and then the tissue is separated from the shell.

1.6.6 Freezing and packaging

Prior to freezing, mussel meat is cooled in water to 3ºC. The freezing process is carried out in spiral freezer units, which enable thousands of mussels to be simultaneously frozen in an instant. As the mussels are removed from the freezer, they are sprayed with a fine water mist that coats each mussel with a protective thin layer of ice, to help increase shelf life and longevity. The mussels are frozen to –18ºC and are then packed for market. The only exception to this standard process is the method for producing marinated mussels. Mussels intended for marinating are kept separate to the half-shell product and are opened on a shaker. The meat drops from the shell through a grid (for size grading purposes) and is packed into tubs with the pre-made acetic acid-based marinades.

1.7 Summary of waste types

The main waste types produced by New Zealand's mussel processing plants can be summarised as follows (Fig. 6):

- Epibiota (organisms which attach to the mussel long-lines – includes the prolific blue mussel, *Mytilus galloprovincialis*)
- Undersized and broken whole *P. canaliculus* mussels
- Shell waste — both independent of tissue and with tissue fragments attached.
- Water and heat waste
- Packaging waste
- Condiment waste (including oils, marinades and crumbs) – this is minimal.

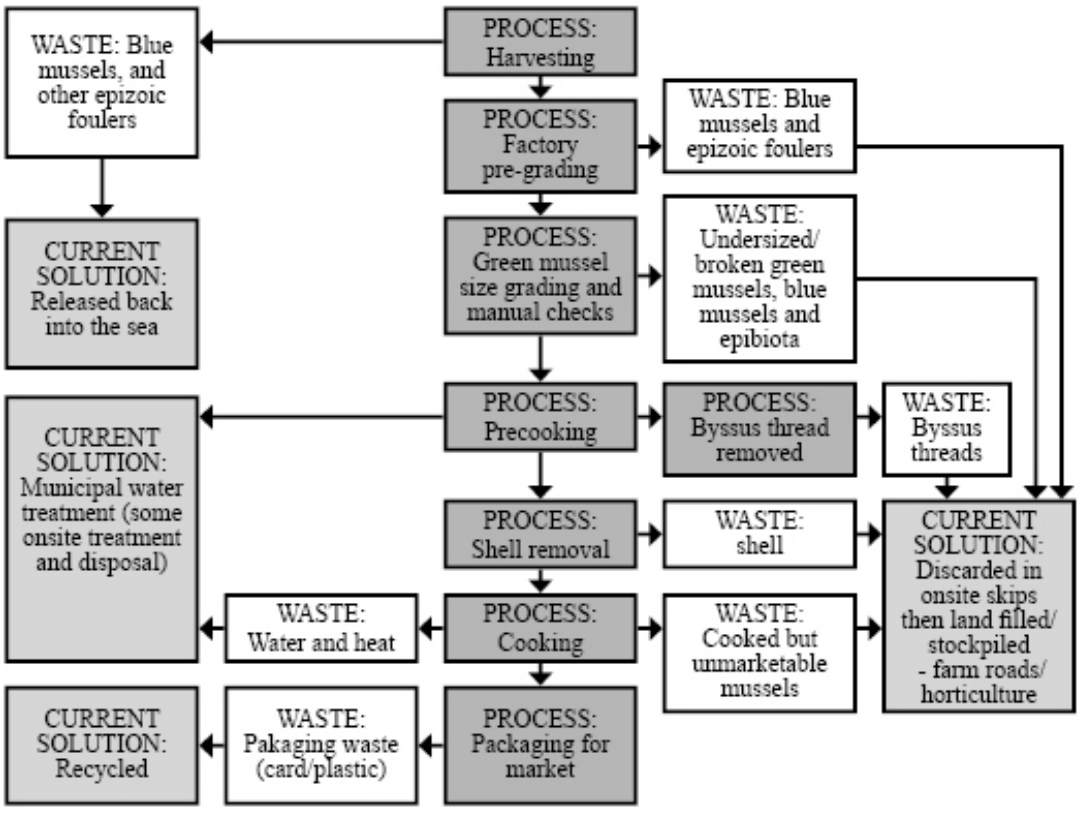

Figure 6: Waste production from harvesting to the marketplace – a generalised model. The white boxes indicate waste types produced by the processes described in the dark grey boxes. The lighter-grey boxes indicate current solutions for existing waste management.

1.8 Waste management in the New Zealand mussel industry

1.8.1 Recognised Waste By-Products

Waste management is a fundamental issue that faces all industries in New Zealand and the world (New Zealand Mussel Industry Environmental Code of Practice, 1997). The New Zealand Mussel Industry (NZMI) generates solid waste products in the form of shell, undersized and rejected mussels, blue mussels, packaging wastes and epibionts that attach to the long-lines adjacent to *P. canaliculus* mussels. According to the New Zealand Mussel Industry Environmental Code of Practice (NZMIECP), effective waste management should scrutinize all waste types produced by this industry and apply the following:

1. *Reduce and avoid waste as it is currently produced*
2. *Consider options for re-use of waste (recycling)*
3. *Treat all waste before disposing*
4. *Utilise 'environmentally-friendly' disposal options for the residual waste*

(NZMIECP, 1999)

In the New Zealand Mussel Industry Environmental Code of Practice (NZMIECP), the NZMI identified principal waste types as packaging waste, mussel shell (as a by-product) and other solid waste including old machinery, office waste and containers. This document did not discuss organic mussel tissue waste (from broken *P. canaliculus* mussels and unwanted blue mussels), epibionts (such as tube worms, barnacles, sponges and macroalgae) or other marine animals such as crabs and scallops that often are harvested when the long-lines are cleaned. The lack of inorganic waste is encouraging for an industry that endeavours to uphold the maxim "*clean, green New Zealand*". However, the NZMI is conscious of the amount of shell waste it does produce and is constantly seeking sustainable, 'eco-friendly' ways in which to recycle

and reduce it (New Zealand Mussel Industry Environmental Code of Practice, 1997).

1.8.2 Current waste disposal techniques

Waste by-products, including undersized mussels, blue mussels, damaged mussels and epibionts are currently discarded within allocated resource consent areas (NZMIECP, 1999). These by-products (known as pre-grade waste) are odorous, attract primary and secondary decomposers and as a result are buried in landfills (A. Blackburn, pers.comm., 2002). Shell waste is currently kept separate and is transported to leased farm paddocks (Fig.7) (M. Mandeno, pers. comm., 2002) and disused logging skid sites (Anon, 2002).

1.8.3 New Zealand initiatives

In New Zealand's Marlborough region, a local vineyard trialled mussel shell incorporated into a mulch to prevent weed growth. In this mulch, shell was found to enhance the vine's ability to withstand 'botrytis bunch' (a wine-grape disease) while increasing grape juice potassium levels (Agnew *et al.*, 2002). However, along with the non-shell mulches used in the trial, the shell containing mulch did not have an effect on yield (Agnew *et al*., 2002). Another New Zealand horticultural initiative was the creation of 'Biosea', a product of Sealord Shellfish Ltd. This product was formulated from fish castings and marketed as a fertiliser for organic farm crops, with much success and positive accolades from farmers (Baumberg, 2002).

From an animal feed viewpoint, a New Zealand company, United Fisheries has developed a mussel-based nutritional supplement for dogs and a mussel meat and shell supplement for horses. The latter utilises waste shell (as a calcium supplement), which is positive for the mussel industry in terms of seeking revenue-generating uses for this by-product (Foundation for Research Science and Technology, 2004). Based on this technology, similar supplements could be formulated with *Mytilis galloprovincialis* (blue mussel) or undersized *P.canaliculus.*

1.8.4 International initiatives

According to Siegel (2000) in the December 2000 edition of *BioCycle,* food additives, health food products, oils, flavourings, soaps, fertilisers, pigments and composts are a few products which can be created from the reuse of seafood waste. The advent of such products decreases waste disposal costs and allows entry into new, lucrative markets (Siegel, 2000).

In Maine, USA, the continual rise of disposal costs has forced local shellfish industries to come up with innovative waste reuse ideas (King, 1996). In 1996, a local Maine farm effectively composted mussel waste, neutralising the odour with nitrogen-rich chicken manure (King, 1996). A composting facility run by the Washington County Commissioners (USA), which manage dead salmon and offal, introduced a mixing area to make the compost more consistent, and to reduce leachate (King, 1996).

Another company in Montville, Maine used sawdust beds to contain leachate from sea urchin residuals, and mixed the incoming waste with existing compost piles to reduce odour (King, 1996). Initiatives such as these show promise in terms of environmental sustainability, while generating income. Other studies have investigated the potential for mussel processing by-products to be developed at a more scientific level, such as in the manufacture of chemical and biological compounds for industrial, medicinal and agricultural uses. Such studies have included the examination of the ability of marine shells to absorb heavy metal pollutants, such as, sulphate and molybdate ions from mining effluents using chitinous shrimp shells (Heu *et al.,* 2003; Moret and Rubio, 2003) and crab shells (Cardenas *et al.,* 2001). Others have taken a more biochemical or biological stance, investigating the addition of shrimp shell waste as a lamb feed to test effects on rumen bacteria and rates of digestion (Cobos, 2002). Others have used crushed shrimp and crab shells as a substrate for isolating chitinolytic micro-organisms with the intention of extracting chitinases — compounds used in the protection of plants against parasites (Wang and Hwang, 2001). Similar microbiological work has been carried out in Spain by researchers seeking an alternative use for their glycogen-rich mussel processing wastes, which are believed responsible for the high levels of eutrophication

along the coast of Spain. The researchers were able to successfully isolate gibberellic acid (used in the promotion of plant growth) and lactic acid (used in food preservation and fermentation) from the waste. (Pastrana *et al*., 1993; Pintado *et al*., 1999).

Figure 7: "Mount Perna" shell waste. Havelock, Marlborough. December 2002. This pile consists of waste shells produced by a local mussel factory as their principal means of disposal. (Photo: A. C. Alfaro, 2002)

Another study investigated the production of antibiotics using mussel/marine processing wastes (MPW) as growing media (Guerra *et al*., 2002). A practical investigation by Murado *et al.* (1993) utilised MPW as a culture for microfungi with the purpose of generating amino acids for animal feeds and supplements for aquaculture species such as Japanese flounder (Kikuchi *et al*., 2002) and salmonids (Gouveia, 1991). Further investigations have examined the use of MPW to be utilised in the cleanup of wastewater. Researchers at the University of Massachusetts in the USA, investigated the use of shell chitin from lobster, crab and shrimp shells, a solid noted for its unique chemical structure and anionic nature and was revealed to have the ability to adsorb various pollutants (Siegel, 2000).

1.9 Scope

The purpose of this thesis was to investigate innovative but cost-effective ways to reuse mussel processing waste. This thesis project is original, since although this waste problem has been recognised since the early 1990's, very little, documented, proactive work has been carried out. There is no published New Zealand data, highlighting paucity in this area of research for the New Zealand Mussel Industry. The aim of this project was to investigate and quantify the potential re-uses for the by-products of mussel factory processing, while seeking possible revenue generation for the industry by the discovery of new, commercially viable products (bio-prospecting). A secondary aim was to become familiar with techniques used in the three different disciplines which this thesis transverses, namely marine biology, terrestrial botany and engineering.

This study included the quantification of organic waste produced by participating factories. The trial of several potential re-use methods were carried out and included fertiliser formulation (from waste tissue) and the use of shell as a substitute concrete aggregate. It also was envisaged that these reuse activities would create a starting point for the formulation of commercially viable products and pave the way for new ideas and future projects. The results of this project will be made available to the New Zealand Mussel Industry Council and participating factories.

Chapter 2—Waste Quantification

2.1 Introduction

2.1.1 Overview

The composition of constituent ratios of New Zealand mussel industry waste has not been described previously in the literature. Accordingly, this is the objective of this chapter. Pre-grade waste, accumulated prior to mussel processing, is removed from both the mussel shells and longlines, comprises epifaunal barnacles, crabs, seaweeds, sea squirts and molluscan taxa. This pre-grade waste is generated at the beginning of the grading process and is kept separate from processed, bulk shell waste (post-grade waste). It must be noted that considerable waste removal is carried out at sea as the longlines are reeled in.

Freshly harvested mussels are delivered to factories with considerable epibiota still attached. Removal of this extraneous material is achieved by a process termed 'de-clumping'— the mechanical agitation of live mussels in rotating cylinders. Mussels are then subjected to automated grading, whereby undersized individuals are discarded, along with other pre-grade waste. The cleaned, graded mussels are further manually screened to remove damaged individuals, and added to pre-grade waste.

Marketable mussels are then processed into half shell mussels (frozen for export), mussel meat (smoked or marinated), live whole mussels and cooked, crumbed mussels.

This process generates the following wastes and potential by-products:

Principal inorganic / non-biological wastes:

- Heat waste (energy)
- Wastewater
- Packaging, equipment and office wastes
- Chemical wastes (cleaning agents – deemed minimal)

Principal organic byproducts:

- Shell – post processing
- Undersized and damaged *P. canaliculus* (and byssal threads)
- Whole *M. galloprovincialis* (blue mussels)
- Pre-grade waste

2.1.2 Pre-grade waste disposal

Pre-grade waste generally is transferred to skips for subsequent burial or stockpiling, often with post-grade waste.

2.1.3 Post-grade waste (shell) disposal

Post-grade waste includes all shell, accumulated after the cooking stages. The shells are deposited onto a conveyer belt and are transferred directly into a truck. Bulk waste shell is accumulated after removal of the cooked tissue. Disposal options vary, and range from landfilling to use as processed roading materials and horticultural applications. Waste destined for roading and horticulture is stockpiled, crushed and mulched.

2.1.4 Objectives

- To determine pre-grade and post-grade waste composition and constituent ratios, and to determine the temporal variation in waste, among factories.

- To estimate cumulative waste production figures across the New Zealand mussel industry

2.2 Methodology

In order to determine the types and amounts of pre-grade and shell waste (post-grade) for the New Zealand Mussel Industry, six mussel processing factories were contacted to obtain access to their waste records. These factories also were asked to provide samples of their waste for further analysis. Three mussel-processing factories provided seasonal waste samples for this project. To maintain confidentiality of sensitive data, the factories were labelled A, B and C. These three factories were based in the South Island of New Zealand. Two additional factories (D, E and F) did not provide seasonal samples but assisted by providing waste figure estimates and related information. These factories were based in the North (D) and South Islands (E, F).

2.2.1 Seasonal differences in pre- grade waste composition

Pre-grade waste samples were obtained in summer, 2002, winter, 2003 and summer, 2004. Approximate seasonal sampling was used to gauge whether there were any significant differences between the types of waste produced (e.g. proportions of *M. galloprovincialis* and *P. canaliculus* to other mollusca, mobile scavengers and epibiota), on a seasonal basis, since productivity and biotic activity can fluctuate with changing water temperatures and associated physical factors.

2.2.2 Pre-grade waste quantification procedure

Each sample was randomly subdivided into three 1 kg sub-samples. Each of these three sub-samples were weighed, before separation into five primary groups – *P. canaliculus*, *M. galloprovincialis*, mobile scavengers (crabs), other molluscan taxa and epibiota - including sea squirts, sponges, seaweeds and barnacles. Each constituent group was weighed and calculated as a percentage of the total 1 kg sub-sample.

2.2.3 Data analysis

Data were tested for significance using a Two-Way ANOVA with Adjusted Sum of Squares, to obtain p-values. Differences among factories' pre-grade waste component types and quantities were established. Checks for significant differences among the amounts of each component, the frequency of component occurrence, effects of different product demands and seasonal differences were also determined by comparisons among the factories. Further pairwise comparisons were obtained by subsequent Tukey Simultaneous Tests. The statistical analysis software used for these data was MINITAB™ Statistical Software v. 13.32.

2.3 Results

2.3.1 Pre-grade waste was found to contain five primary components:

- *Perna canaliculus* (broken and undersized)
- *Mytilus galloprovincialis* (blue mussels)
- Epibiota (including sea squirts, barnacles, macroalgae, sponges and tubeworms)
- Other molluscan taxa (including other mussel species, and juvenile scallops)
- Mobile scavengers (including various crab species)

P. canaliculus shell waste is not included in the pre-grade waste because it is produced at a later stage of mussel processing (after cooking). The amount of bulk shell waste produced was found to be proportional to the type of mussel product processed, that is mussels in the half-shell or mussel meat — the two main products. For example, Factory A harvested approximately 546 lots (sack-loads) within a 4-month period in 2003. Based on factory-worked calculations for this period, 43.98% of the 546 lots were removed as half-shell product, 24.09% was removed as meat and consequently the amount of shell was equivalent to 19.89% shell for the half-shell process and 39.78% shell for the meat process (Fig. 8).

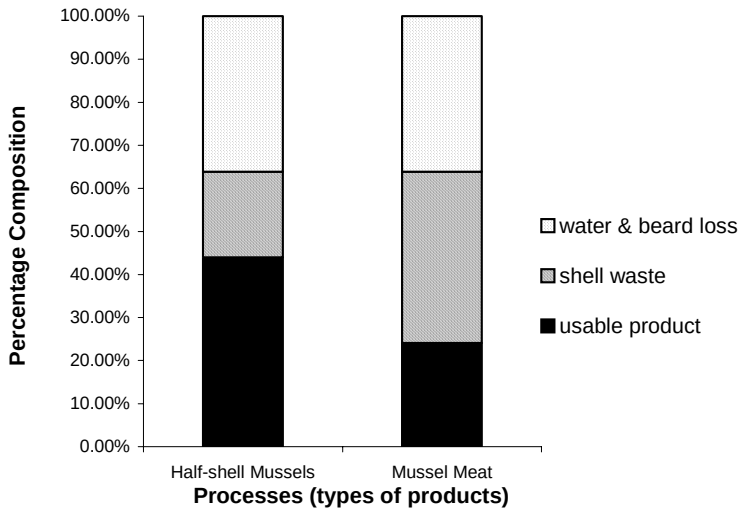

Figure 8: Graph illustrating shell waste generated by factories when mussel meat products were produced.

Table 1: Two-way ANOVA: to determine pre-grade waste constituent differences among seasons (summer, 2002, winter 2003 and summer 2004) and factories (A, B. C).

Perna canaliculus

Source	DF	SS	MS	F	p
Year	2	203308	101654	0.78	0.519
Factory	2	218928	109464	0.84	0.498
Error	4	524269	131067		
Total	8	946505			

Mytilus galloprovincialis

Source	DF	SS	MS	F	p
Year	2	255131	127565	1.10	0.418
Factory	2	74917	37459	0.32	0.742
Error	4	465941	116485		
Total	8	795989			

Epibiota

Source	DF	SS	MS	F	p
Year	2	9093	4546	2.78	0.175
Factory	2	23914	11957	7.32	0.046
Error	4	6533	1633		
Total	8	39540			

Other Molluscan Taxa

Source	DF	SS	MS	F	p
Year	2	972	486	0.67	0.560
Factory	2	952	476	0.66	0.566
Error	4	2889	722		
Total	8	4812			

Mobile Scavengers

Source	DF	SS	MS	F	p
Year	2	606	303	0.96	0.455
Factory	2	304	152	0.48	0.648
Error	4	1258	314		
Total	8	2169			

Table 2: Tukey test results comparing differences between constituent ratio quantities between factories

Tukey: FACTORIES	*Perna canaliculus*	*Mytilus galloprovincialis*	Epibiota	Other Mollusca	Mobile Scavengers
AxB	p=0.5287	p=0.8731	p=0.6397	p=0.6527	p=0.8657
AxC	p=0.9938	p=0.9561	p=0.8726	p=0.5897	p=0.6240
BxC	p=0.5284	p=0.7272	p=0.8980	p=0.9923	p=0.8919

2.3.2. Seasonal pre-grade waste quantification results

2.3.2.1 Summer 2002

Factory B produced the largest quantity of *P. canaliculus* (broken whole mussels and unmarketable tissue) (947±268g per kilogram sample) and Factory A, the least (216g±268g per kilogram sample). The Factory C sample did not yield any other molluscan species in the sample and there were no statistically significant differences in 'other mollusca' quantities between factories (ANOVA; $F_{2,4}$=0.66, p=0.566), nor between years (ANOVA; $F_{2,4}$=0.67, p=0.560) (Table 1).

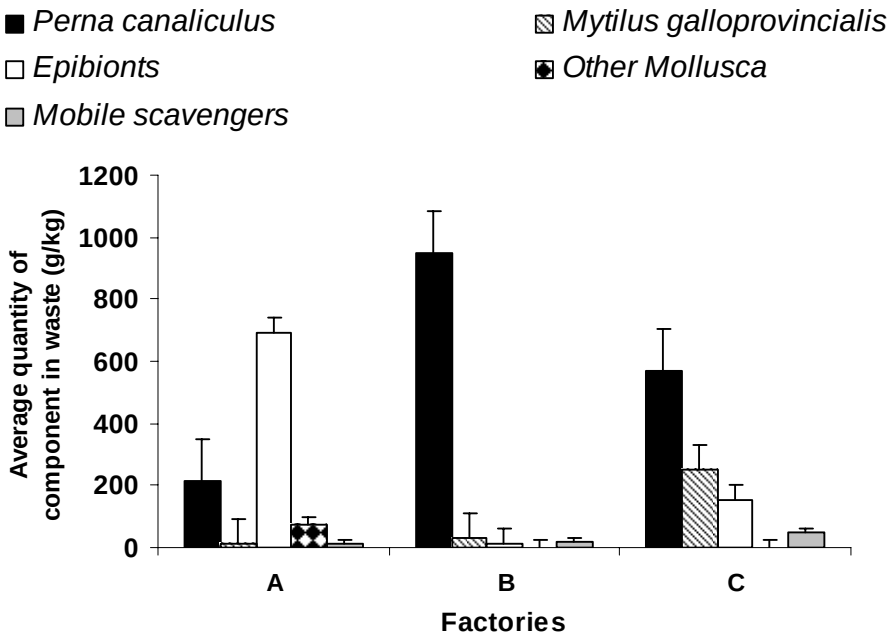

Figure 9: Mean waste quantity of principal components for Factories A, B and C during Summer 2002.

2.3.2.2 Winter 2003

P. canaliculus mussels made up a large proportion of the waste of Factory A (986.g ± 333g per sample) and B's (936 ± 333g) waste in winter, 2003. *P. canaliculus* did feature in Factory C's waste also, but at lower levels. Blue mussels were prolific in Factory C's waste but not significantly (ANOVA; $F_{2,4}$=0.32, p=0.742) in comparison with Factories A and B, which had little to no blue mussels in their waste during this time. The other components were significantly low in

comparison to blue and green mussel wastes (Fig.10). Statistically, however, there were no significant differences between the quantities of each waste component produced between factories during the winter period.

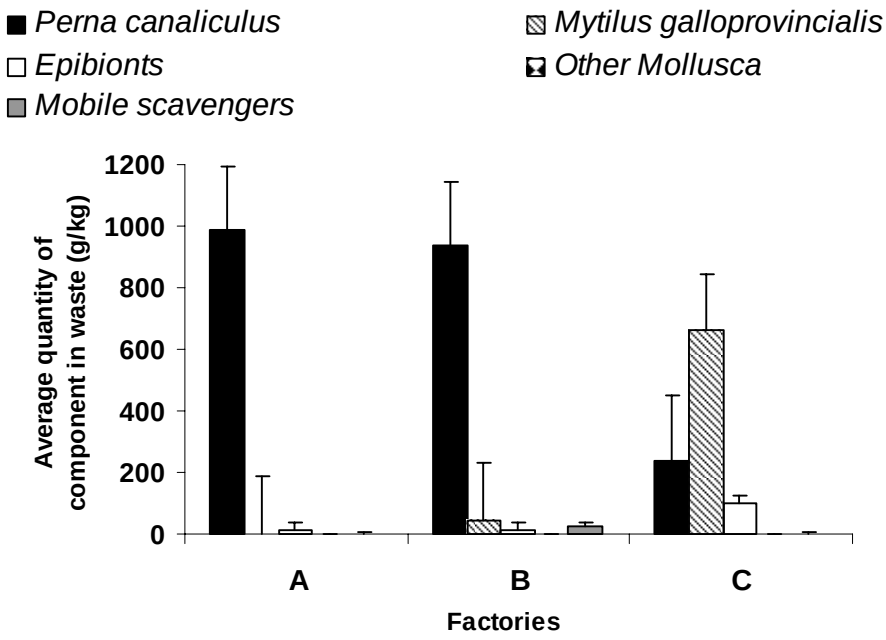

Figure 10: Results of waste quantification (winter, 2003).

2.3.1.2 Summer 2004

In summer 2004, blue mussels made up an apparently large but statistically non-significant proportion of Factory A (882 ± 340g per kilogram sample) and Factory B 402 ± 340g per kilogram sample). Waste green mussels were more prevalent in the pre-grade waste of Factories' B (429 ± 252g) and C (563 ± 252g). Epibiota featured in all factories' wastes but were slightly higher than that of Factories B and C. Again, other molluscan species and mobile scavengers featured the least (Fig. 11).

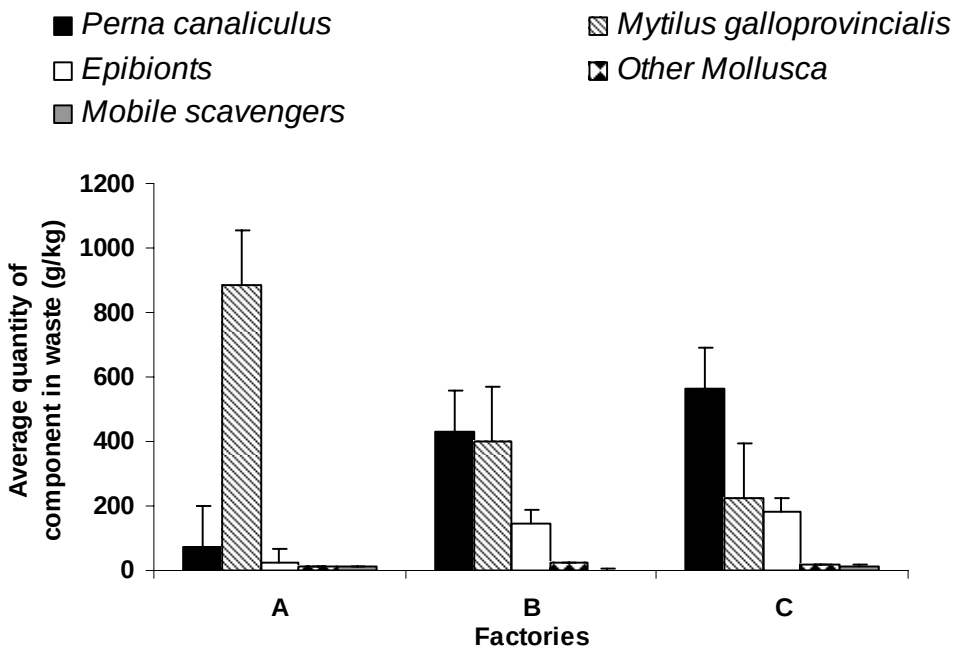

Figure 11: Results of waste quantification (summer, 2004).

2.3.2 Overall trends

Overall, it was found that there was no significant difference in the quantities of *Perna canaliculus*, *Mytilus galloprovincialis,* epibiota, mobile scavengers or other mollusca between the sampling years (Table 1). There were also no differences between the amounts of each of these waste components between the factories, with the exception of epibiota. This group was found to differ significantly in quantity (ANOVA; $F_{2,4}$=7.32, p=0.046) between the factories. A subsequent Tukey Test confirmed the non-significant differences between the factories (all p>0.05) (Table 2).

2.3.3 Pre-grade waste – industry totals

From data provided, it may be estimated that a single factory might produce approximately 45 tonnes of pre-grade waste in one month. If this factory processed for 10 months of the year they would produce 450 metric tonnes of pre-grade waste on an annual basis. To gain an overall picture for the entire industry, if this figure was multiplied by the number of active factories (36) (Fig. 1), it may be estimated that the New Zealand Mussel Industry (NZMI) produces approximately 16,200 tonnes of pre-grade waste per annum.

2.3.4 Bulk dry shell waste (post-grade) – industry totals

An average factory, on a daily basis produces approximately 35m^2 of waste shell per day. An estimated 1m^2 would be about 400kg shell; therefore the daily shell output is equal to 14,000 kg/day. If this factory processed for 200 days per year, they would produce 2,800 tonnes/year of bulk waste shell. Factories do not produce the same quantities of mussel product at precisely the same time, thus it is possible to gauge an estimated figure only. Therefore, from this research it may be estimated that the NZMI produces approximately 100,800 tonnes of shell per year industry-wide. For a more accurate figure, one would need to gain waste production records for every factory in New Zealand. Not all factories keep such records, which was why calculating a precise figure was not entirely possible.

2.4 Discussion and Conclusions

2.4.1 Pre-grade waste

Studies have shown that *Perna canaliculus* is in optimum condition during warmer months and at its lowest during cooler months (Hickman, 1979; Marsden and Weatherhead, 1999). Annual weather variations and phytoplankton levels play an important role in mussel production (Marsden and Weatherhead, 1999) and are likely to be indirectly related to resulting waste outputs. This is because the larger the mussels, the less are discarded as undersized individuals and the less mussels are wasted. This may explain the higher proportion of this species featuring in the pre-grade waste during the winter sampling period (winter, 2003) when individuals may have been smaller overall and many would have been discarded as undersized mussels. Inversely, the reduced amounts of *P. canaliculus* which featured in the two summer seasons examined may therefore be related to the mussels being in optimum condition at this time and therefore fewer undersized individuals would have been discarded. This seasonal influence may also explain why Factory C had a higher proportion of blue mussels in the waste during the winter period (than over the summer months) but does not explain why Factories A and B had little to no blue mussels in their waste at this time.

In actuality, Factories A and B had very few blue mussels in their waste during both summer 2002 and winter 2003 but significant amounts of the blue mussel featured in their wastes in summer of 2004 whereas Factory C had less. The prevalence of the blue mussel, *M. galloprovincialis,* at this later date, may be due to seasonal spawning characteristics. This is because *M. galloprovincialis* is less limited by warmer temperatures than *P. canaliculus* mussels and one would otherwise expect them to be abundant (as mature individuals) in the wastes in all summer months (Kennedy, 1976). However, in this study, statistical analysis showed no differences between the factories in terms of the quantities and presence of each identified component. This is useful, because if future work were to utilise pre-grade from South Island factories, there would probably be few differences in the composition of the waste regardless of where it was sourced. Another reason for observed seasonal differences may be the fact that

different factories had different processes for blue mussel removal. Some factories require the removal of blue mussels at sea where they are discarded back into the sea within the vicinity of the mussel farm. However, others rely more heavily on blue mussel and epibiota removal at the processing factory site.

2.4.2 Other factory wastes

Lincoln Environmental Mussel Industry Environmental Policy (1997) states that the principal factory generated solid wastes are mussel shells and organic material, with minimal chemical wastes. In conjunction with this document, in 1999, the New Zealand Mussel Industry Council produced their Environmental Code of Practice. Section 12 of this document identified three main factory wastes; packaging wastes (cardboard and plastic packaging from incoming and outgoing goods) mussel shell by-product, and other solid wastes that included used containers, office equipment and unused factory equipment. Aside from shell, other organic wastes — including pre-grade waste (found in this research to be a significant component) were not mentioned in this document.

In terms of wastewater, it has been estimated that on a daily basis, one single shellfish-processing factory will produce as much wastewater as 2500 people (Marlborough District Council, 2001). From this research, it is apparent that cooking-waste water (likely to be high in protein from the cooking stages) is either disposed of through municipal channels or treated minimally onsite and discharged (with consents) into neighbouring waterways. Although this project did not examine the waste cooking water, the author was informed that it contains about 3% solids that may have potential to be utilised as flavouring for soups, as a stock or in pet foods. When dehydrated it has a dried fish-like odour and is a light brown powder (C. Barnaby, pers.obs., 2002). There is definite potential for further work concerning the utilisation of this product.

2.4.3 Limitations and recommendations

Larger samples provided on a monthly basis would have been more desirable but this was not logistically feasible and would have incurred additional expense. Problems also arose with the availability of waste from participating companies. This accounts for the fact that there was not a quantifiable sample

from each company for each season. In order to gauge a more accurate idea of spatial differences between different factory locations (and the farms they source from), more thorough factory participation would be required. Although more factories were contacted only a limited few responded favourably and it was found that not all of these factories kept accurate results of waste types and quantities. This accounted for the need to extrapolate and estimate some figures.

It is also recommended that future work encompass quantification and a direct comparison between '*in situ* farm waste' and 'onsite factory waste', in order to gain a broader picture of waste production from all areas of this industry.

As stated in Chapter 1, aquaculture is still growing in New Zealand. With the likelihood of the removal of the current moratorium on new marine farm applications, more waste is likely to be produced in the near future. Thus, further research in waste reuse for sustainable practices is required.

Chapter 3 — Waste Mussel Tissue Recycled as a Fertiliser

3.1 Introduction

3.1.1 Organic fertilisers

Plant fertilisers have been utilised for thousands of years. For example, North American Indians buried fish with seeds to enhance corn crops (Campbell, 1996). Today's sea-derived fertilisers incorporate seaweed, finfish castings, fishmeal and shellfish by-products. These fertilisers contain decomposed organic material. Before such fertilisers can be of use to plants, decomposition must be complete to a level where nutrients have reduced to simple forms that are available for roots to assimilate (Campbell, 1996).

Most agricultural soils are deficient in nitrogen, phosphorus and potassium (N, P, K). These three macronutrients are used as a base for commercial fertilisers. With the increasing popularity of organic horticulture comes a greater demand for natural foliar supplements, fertilisers and soil enhancers. 'Sea derived' fertilisers have been found to provide a wide range of essential nutrients useful for organic plant growth enhancement. Generally, fertiliser sources have been allowed to decompose. This state enables nutrients to be 'unlocked', and facilitates the bio-availability of nutrients for plant absorption.

3.1.2 Previous studies

While it is an excellent practice to completely recycle one form of waste, it is more efficient, both logistically and environmentally, to combine two or more waste streams, to create a usable end product. Combining waste streams is often more economical than dealing with one waste type, and reduces the amount of wastes discarded into the environment. Researchers have examined the combination of aquaculture residuals (from both finfish, crustaceans and bivalve

shellfish) with non-marine wastes such as sawdust (Nicholls *et al*., 2002) and chicken manure (King, 1996). According to Nicholls *et al*. (2002) sawmills in Alaska generate close to half the incoming timber volume as waste sawdust. This sawdust waste is combined with Alaskan fish processing wastes to produce composts, with the sawdust acting primarily as a bulking agent (University of Alaska, 2000; Nicholls *et al*., 2002). The results of related studies found that the sawdust improves pile porosity, aids in the breakdown of the fish components, reduces odours, facilitates moisture absorbency and creates desirable thermal properties for efficient microbial action (Kostov *et al*., 1994; Tiquia *et al*., 1996; Green *et al*., 2004).

Landscapers and domestic gardeners use sawdust-fish waste compost as a soil supplement (Nicholls *et al*., 2002). Similar composting activities have been tested in Maine, USA, where innovative processing approaches have helped local waste disposal facilities reuse the by-products of sea urchin, mussel, scallop, dogfish, herring, groundfish and clam processing (King, 1996). The main initiative was to combine these marine waste streams with chicken manure (from a local fowl farm in Pittston, Maine) by composting. This project was successful, because a useful compost product was created. This compost also had a reduced odour due to the high levels of nitrogen in the chicken manure (King, 1996). Other studies have utilised chitin-based aquaculture waste, which harnesses the anti-microbial properties of chitin, useful for some composts and foliar supplements (Kim *et al*., 1997; Wang and Hwang, 2001; Wang *et al*., 2002). Kim *et al*. (1997) investigated the addition of chitinous crab shell waste (and other waste types) emulsified into several types of combined composts and soil amendments. Considering only the crab shell waste, Kim *et al*. (1997) found that certain amounts of crab shell significantly reduced disease and increased root and shoot yields in bell peppers, in comparison with the control treatments. Wang and Hwang (2001) and Wang *et al*. (2002) also undertook in-depth research into the anti-microbial nature of chitin based waste shells with special reference to plant protection and found similar benefits to Kim *et al*., (1997).

3.1.3 Plant nutrients

Campbell (1996) illustrated the major macronutrients that are essential to plant growth (Table 3). These macronutrients include nitrogen (essential in protein synthesis), phosphorus (essential in energy pathways), potassium (for flowering, stomata operation and water content balancing), calcium (important in cell formation and function) and magnesium (an important component in chlorophyll). Thus, these prior investigations highlight the need for further investigation on the potential for *Perna canaliculus* and *Mytilus galloprovincialis* waste tissue and shell, as fertilisers.

Table 3 Description of the function of five major macronutrients (N, P, K, Ca & Mg) in plants. (adapted from Campbell, 1996).

Macronutrient	Form available to plants	Functions within plants
Nitrogen	NO_3^-, NH_4^+	Forms part of nucleic acids, hormones, proteins and coenzymes
Phosphorus	$H2PO_4^-$ HPO42-	Forms part of nucleic acids, adenosine triphosphate (ATP), phospholipids and coenzymes
Potassium	K^+	Role in protein synthesis, is a solute in water balance and aids in stomatal operation
Calcium	Ca^{2+}	Aids in forming and stabilising cell walls, maintaining membrane permeability and structure, enzyme activation and regulator of some cell responses to stimuli
Magnesium	Mg^{2+}	Component in chlorophyll and activates many enzymes

3.1.4 Principal aim

The aim of this part of the research project was to investigate the potential use of decomposed *Perna canaliculus* and *Mytilus galloprovincialis* tissue as simple organic fertilisers for plants.

3.2 Methods and Materials

3.2.1 Mussel fertiliser formulation

In order to facilitate decomposition of the waste mussels, nine buckets were obtained. Holes were drilled 1cm below the top of the bucket, for aeration. The purpose of the drilled holes was to allow micro-organisms to decompose the tissue rapidly with some degree of air-supply. Microbes active in composting require adequate oxygen to effectively break down organic components and heat is generated during this process (Nordstedt *et al*., 1991). Composts are typically aerated for several months until the thermal decomposition phase has finished (Levy and Taylor, 2003). As time was limited in the project to six months of curing, aeration was provided for the entire duration. These holes were protected from the rain by the fast-sealing bucket lid. Each bucket was filled with 1kg of waste mussel tissue, which was removed from the shells by hand (Figs.12,13). These buckets contained the following components:

- 1 kg of *P. canaliculus* mussel tissue in each of three buckets labelled AG 1-3
- 1 kg of *P. canaliculus* mussel tissue mixed with 1 kg *P. canaliculus* shell in another three buckets labelled BG 1-3
- 1 kg of *M. galloprovincialis* tissue in each of the final three buckets – labelled DB 1-3

The three buckets of each type of 'compost' were used as replicates for each treatment. The filled buckets were then placed in a sheltered paddock. The bucket contents were left undisturbed for three months until decomposition was apparent. Decomposition was noted when liquefaction of the solid tissues (curing) was achieved.

3.2.2. Fertiliser analysis

Due to time constraints, the mussel fertilisers were analysed professionally by R.J. Hill Laboratories Limited (Hamilton, New Zealand). The Dumas

Combustion Method using an Elementar VarioMAX instrument was used to determine total nitrogen concentration. Phosphorus, potassium, calcium and magnesium were analysed by firstly digestion with nitric/hydrochloric acid and the concentrations were determined using Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES). These nutrients were analysed, since they are five of nine macronutrients that are important in plant growth (Table 3, Appendix 2).

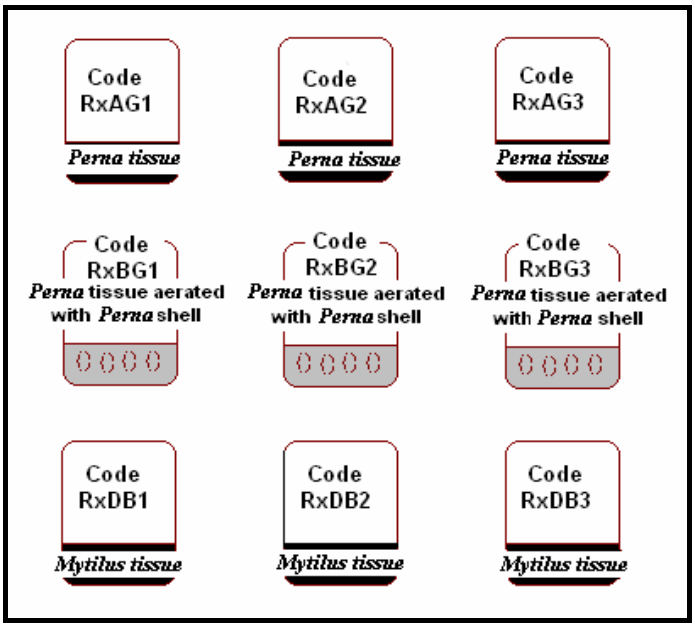

Figure 12: Diagram depicting the curing bins and their corresponding contents. Buckets AG1, AG2 and AG3 contained 1kg of *P. canaliculus* tissue. Buckets BG 1 to 3 contained 1kg of *P. canaliculus* tissue in each, with 1 kg of shell mixed into the tissue in each bucket. Buckets DB 1 to 3 contained 1kg of *M. galloprovincialis* tissue in each bucket.

Figure 13: Bucket containing 1 kg green mussel tissue. Arrow indicates aeration holes that were protected from rain by the sealed lip of the lid.

3.2.3 Preparing the dilutions

Approximately 1 litre of cured fertiliser was removed from each bucket and chilled (<4ºC) to reduce odour emission and to slow further, uncontrolled decay. To prepare the diluted fertilisers, 5, 10 and 20 ml of liquid tissue were pippetted into 2-litre measuring cylinders filled with 2L of deionised water. The cylinder was then inverted twenty times to ensure even mixing. The liquid was then transferred into clean, plastic, labelled bottles. This was carried out for each treatment. The second type of green mussel fertiliser (BG) was supplemented with quicklime or calcium oxide (previously calcined from dried mussel shell). CaO was added to each BG dilution at 1g (for the 5ml: 2L concentration), 2g (for the 10ml: 2L concentration) and 4g (for the 20ml: 2L concentration) respectively. It was decided to incorporate calcium oxide (CaO) from waste mussel shell into the trial fertilisers. CaO is a form of lime that is known to enhance plant growth. It was also viewed as a potential method of reducing shell volumes, while creating a useful, beneficial organic product. The calcium oxide was obtained by 'baking' clean, dry, crushed *P. canaliculus* valves at 1500ºC in a furnace oven. The resulting substance was grey-white shards of shell, which powderised to the touch, after cooling in a desiccator. The material was ground gently with a mortar and pestle.

One of the controls included a selected commercial liquid plant-food concentrate, Yate's '*Lush*'. The dilution instructions (5ml to 2L water) on the packaging were followed for the regular feeding regimes. This control was prepared in the same manner as the trial fertilisers in the laboratory. Thus, 5ml of '*Lush*' was added to 2L of deionised water in a measuring cylinder and inverted 20 times before being transferred to a clean, labelled, plastic bottle. The second control was fresh deionised water, as the only substance added to designated control plants. This control was used to provide baseline growing conditions without fertilisers.

Fresh dilutions for each treatment and control were prepared weekly. Feeding was undertaken once per week. It was decided to wait until the seedling stage (5-7mm) to begin the fertiliser applications, as seeds often germinate regardless of external chemical fertilisation. However, phytotoxic effects can occur and

this was examined by separate testing. Fertiliser applications commenced at the seedling age of two months.

3.2.4. Phytotoxicity germination tests

The phytotoxic potential of each fertiliser (decomposed green mussel tissue (AG), decomposed green mussel tissue with CaO (BG) , decomposed blue mussel tissue (DB), deionised water (CA) and '*Lush'* (CB)) was assessed on seedling germination. This toxicity experiment was important because it provided definitive results regarding the effects of mussel compounds on vegetable plants. The methodology used for this experiment was based on that of Levy & Taylor (2003) who used mink farm wastes, municipal solid waste and sewage sludge. Radish seeds were selected due to fast germinating rates (Levy and Taylor, 2003). Three replicate petrie dishes were obtained for each of the treatments and controls (15 in total). Each petrie dish was lined with one Whatman No.1 glass fibre filter. The purpose of the filters was to imbibe moisture. Five millilitres of each of the liquid fertilisers was pippetted into each dish. Five radish seeds (*Raphanus sativus* L. Cultivar "Scarlett Globe") were placed 1cm apart into each dish with lids. Sixty millilitres of each of the fertilisers and controls (equivalent to 12-34g dry mass) was combined with 120ml of deionised water in beakers and mixed gently with a magnetic stirrer for 1 hour (as per Levy & Taylor, 2003). The wet mixture was then decanted through a Büchner funnel and the filtrate collected. A 5ml sample of each fertiliser or control filtrate was pippetted into the three petrie dishes with five radish seeds. The 15 petrie dishes were placed in darkness at ambient room temperature (~23°C) for 5 days as recommended by Levy & Taylor (2003). After 5 days, the emergence of the epicotyl and hypercotyl were observed and deemed successful germination.

3.2.5 Plant maturity comparisons – fertiliser testing

Tomato seeds (*Lycopersicon esculentum,*L. var. "Moneymaker", Yates) were selected for this experiment. This cultivar is a popular choice with the domestic gardener, to whom this fertiliser product would be marketed, and is a fast growing and successful germinator. Tomato seeds were selected over legumes (peas and beans), which have nitrogen-fixing organs, likely to interfere with obtaining true results for the effects of nitrogen in the fertilisers.

Local topsoil was used in this experiment as the growing medium. This type of medium was selected for its similarity to what is used by domestic gardeners. It was also selected to compare the effect of untreated topsoil on trial plants with soil treated with mussel tissue, regardless of the fact the soil would have an existing nutrient load. The topsoil brand used was *Supa Soil Quality Topsoil,* (a sandy loam) which was purchased locally.

Seedlings were germinated in rearing trays in separate pots. To ensure successful germination rates, 5 seeds per pot were sown at 1cm depths into the damp soil. When germination was observed (by the presence of healthy seedlings, 2-4cm height), all but one seedling in each pot were discarded (thinning).

Lighting was controlled by using a 400-watt high pressure *Sunmaster Super HPS Deluxe* sodium grow lamp. This lamp was set in an *Adjusta Wings* lamp box that directed light below and outwards over the plants. The lamp was set on a controlled timer to deliver a 16:8h light/dark cycle (methods in Levy and Taylor, 2003). This lamp operated off a ballast to control and regulate current. Due to a lack of working space, the plant trays could not be positioned evenly beneath the lamp. Therefore, to ensure each seedling received as close to equal time as possible beneath the light source, the seedling-containing trays position's were rotated anticlockwise every second day beneath the lamp and further sub-rotated (Fig.14).

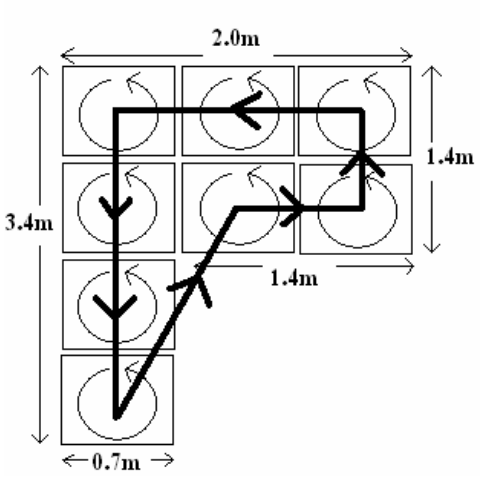

Figure 14: Diagram representing the position of the plant trays and dimensions. The thick, black arrow indicates the direction of tray rotation beneath the grow lamp. Each square represents one tray of plants. The smaller, circular arrows within each tray represent how each tray was sub-rotated, beneath the light source.

3.2.6. Experimental design

The objective of the fertiliser formulation was to test whether blue and green mussel tissues enhance plant growth, and to then determine which dilution enhanced foliar growth the most. Fertiliser BG initially comprised *P. canaliculus* mussel tissue aerated with whole *P. canaliculus* shells in the decomposition buckets. However, for BG, only the liquid tissue was utilised and the valves (included only to aerate the tissue and promote rapid decomposition) were removed prior to dilution with water. However, this fertiliser differed from fertiliser AG (decayed *P. canaliculus* tissue) in that it was combined with calcium oxide that was calcined from dry shells, just prior to application (Table 4).

The experimental design involved the arrangement of 234 seed-rearing pots in 8 trays with 30 pots in each of 7 trays and 24 pots in one tray. Each seed pot was allocated a code for a specific treatment. Each pot was then randomly placed within the 8 trays using a random numbers table generated. The three treatments included a dilution of 5, 10 and 20 ml of tissue / 2L of deionised water (Table 4). The dilution of 5ml corresponded to that recommended by the instructions provided on the commercial fertiliser.

Deionised water was used in this experiment to water the experimental plants. Watering was carried out very second day. Previous research methods used by Levy and Taylor (2003) incorporated local tap water for watering the test plants. However, in this study, deionised water was used to eliminate potential ionic interference from using tap water. Seven millilitres of deionised water were applied to the soil surface of each seedling when they were germinating in seed rearing trays. This quantity was then increased to 14ml when the seedlings grew to 7-10cm in stem height. After transplanting into planter bags (commercial size 3), watering aliquots were increased to 50ml of deionised water. The water quantities and watering regime appeared sufficient for healthy growth with minimal desiccation. This was determined by regular examination of the plants to look for dried leaves and any obvious wilting.

3.2.7 Measurements

Stem height, branch count and leaf count for each plant were measured once per month. Stems were measured to the nearest millimetre from the surface of the soil (at the root base) to the uppermost growing tip (the point where new leaf growth was observed). Leaves (>3mm) in pinna length were counted. When compound leaves were encountered (common in mature tomato leaves), these were counted as one single leaf. Each separate branch was counted regardless of how many leaves it held. The growing tip was included in the count (Fig. 15). Along with these parameters, observations were made regarding plant health and the presence of fruit or flowers.

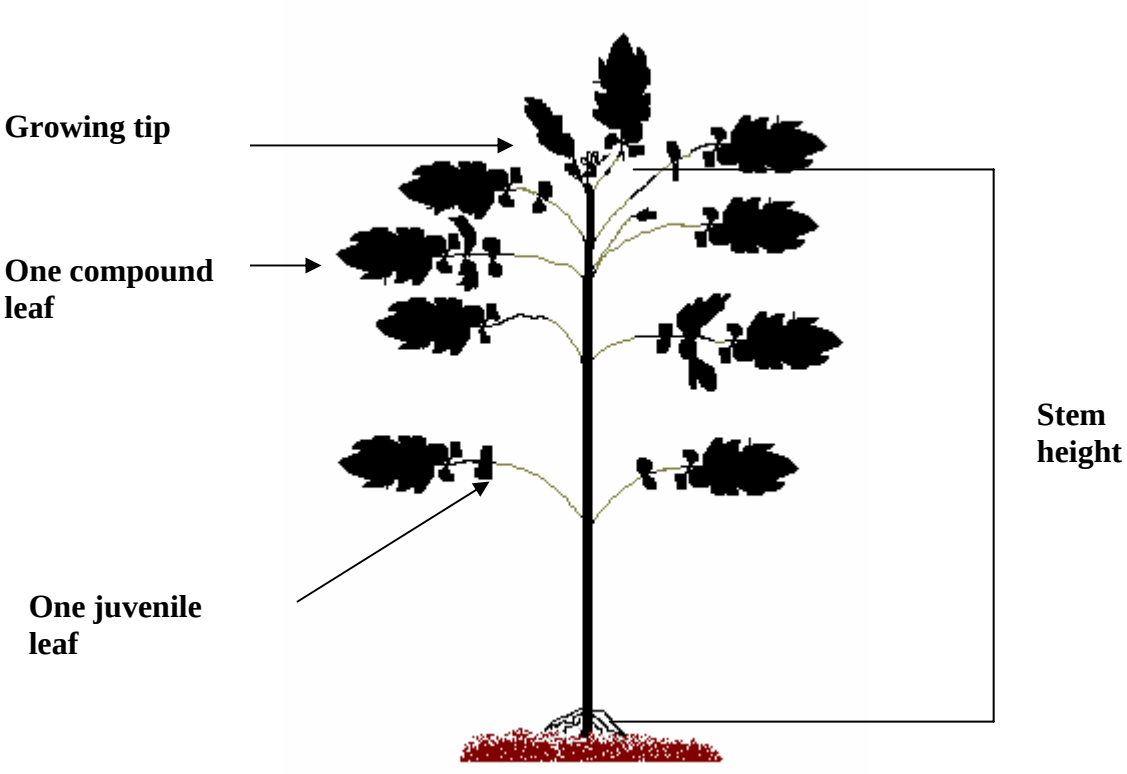

Figure 15: Sketch of a tomato plant defining how stem height, branch count and leaf counts were carried out.

3.2.8 Dry weight biomass

Dry weight was used as an index to total biomass for matured plants (20 weeks old). This method was carried out in accordance with the *Standard Operating Procedure for Plant Biomass Determination #2034 – USA Environmental Protection Agency* (USEPA, 1994). Plants were cut at the base of the stem (at soil level) leaving the roots behind in the planter bag. Root mass differences are only considered when working with multiple species (USEPA, 1994) therefore were not analysed, as only one species was used in this experiment. Plants were

then placed in aluminium weighing dishes (1 plant per dish). Each dish containing one fresh plant was weighed (recorded as green weight) before being placed in an oven at 80ºC for 24 hours. The dried plants were then cooled in a desiccator and reweighed until a stable weight reading was observed. As oven space was limited, plants were refrigerated at 4ºC until oven space was available (no more than 2 days) (USEPA, 1994).

3.2.9 Associated equations and statistical analysis

Determination of biomass and moisture content were obtained with equations in accordance with the US Environmental Protection Agency Standard Operating Procedures 2034:

$$\textbf{Water Content} = \textbf{Fresh Weight} - \textbf{Dry Weight}$$

$$\textbf{Standard Biomass} = \frac{\textbf{Dry Weight (of aboveground tissues)}}{\textbf{Plot Area}}$$

Biomass and moisture differences among treatments were analysed using a General Linear Model ANOVA. A Pearson Correlation was used to relate biomass with moisture content. The statistical analysis software used in all analyses was MINITAB™ Statistical Software v. 13.32.

Nutrient levels in each test fertiliser were analysed for statistical differences by a General Linear Model (GLM) ANOVA, using the date (time) factor as a covariate. This test was followed by a Tukey Test for pairwise comparisons to test differences between rank sum means to confirm the overall value for *p*. Analysis of variance (GLM) was used which uses a regression approach to fit a specified model and was useful as analysis of variance was required to show variations over time in plant growth. The GLM was used in place of repeated measures ANOVA and where a significant p-value was determined using 'time' as a covariate (Table 9). Tukey tests were used again to test for differences among groups. Changes in the soil pH over time were tested for differences using a two-way parametric ANOVA. A one-way ANOVA (unstacked) was used to analyse potential differences between the phytotoxic potentials (responses) of the treatment fertilisers and the controls (fixed treatments)

3.2.10 Soil pH changes

Soil pH was measured on three of six months of the growing period. Three 4g soil samples were randomly selected from each batch of treatments and controls. Each sample was placed in a 100ml beaker with 50ml of deionised water and mixed rapidly using a magnetic stirrer. After 1 hour of mixing, the sample was emptied into a Büchner funnel containing a filter paper (Schleicher and Schuell 595 110mm – equivalent to Whatman No. 1) and the filtrate was collected in a flask. A pH probe was immediately immersed in the filtrate until a stable pH value was achieved. This process was repeated for each sample.

Table 4: Chart describing the treatments and controls used in the plant feeding trials. Fertiliser BG contains an additional admixture of CaO, calcined from *P. canaliculus* shell.

Bucket Contents	Code	Dilution	Plants
1 kg *P. canaliculus* tissue	AG1	5ml tissue : 2L water	AG - 1 to 13
1 kg *P. canaliculus* tissue	AG2	10ml tissue : 2L water	AG - 14 to 26
1 kg *P. canaliculus* tissue	AG3	20ml tissue : 2L water	AG - 26 to 39
1 kg green tissue + 1 kg *P. canaliculus* shell	BG1	5ml tissue : 2L water + 1g CaO (ex shell)	BG - 1 to 13
1 kg green tissue + 1 kg *P. canaliculus* shell	BG2	10ml tissue : 2L water + 2g CaO (ex shell)	BG - 14 to 26
1 kg green tissue + 1 kg *P. canaliculus* shell	BG3	20ml tissue : 2L water + 4g CaO (ex shell)	BG - 27 to 39
1 kg *M. galloprovincialis* tissue	DB1	5ml tissue : 2L water	DB - 1 to13
1 kg *M. galloprovincialis* tissue	DB2	10ml tissue : 2L water	DB - 14 to 26
1 kg *M. galloprovincialis* tissue	DB3	20ml tissue : 2L water	DB - 27to 39
Control	**Code**	**Dilution**	**Plants**
Commercial liquid plant food – Yate's 'Lush'.	CA	5ml liquid : 2L water	CA - 1 to 39
Deionised water	CB	n/a	CB - 1 to 39

3.5 Results

3.5.1 Mussel fertiliser analysis

Out of the mussel fertilisers, decomposed *Perna canaliculus* (AG) was found to yield the highest proportion of nitrogen (9.23g/100g of sample). This value was similar to that of Yate's '*Lush'* (CA) (10g/100g liquid fertiliser). The *P. canaliculus*-CaO blend (BG) contained the least amount of N (1.05g/100g of sample) and decomposed *Mytilus galloprovincialis* (DB) yielded a concentration, similar to that of AG (7.75g/100g of sample). Differences in the averages were significant (ANOVA; $F_{3,6}$=1052.67, p=0.000) (Table 5, Fig.16).

AG yielded the highest mean value for P (1.96g/100g of sample). This was low in comparison with CA (3g/100g of liquid fertiliser). BG yielded the lowest K concentration (0.247g/100g of sample) and the K concentration for DB fell between the other two mussel fertilisers with a value of 1.66g/100g of sample. Differences between the means and the values for '*Lush'* were significant (ANOVA; $F_{3,6}$=132.91, p=0.000) (Table 5, Fig.16).

Of the trial mussel fertilisers, K concentrations were highest in DB (2.2g/100g of sample and lowest in BG (0.7g/100g sample). AG yielded a K concentration of 1.8g/100g sample but all these concentrations were very low in comparison with Lush (6g/100g of liquid fertiliser). Statistically, these differences were significant (ANOVA; $F_{3,6}$=136.52, p=0.000) (Table 5, Fig.16).

No values were available for Mg and Ca concentrations in Yate's '*Lush'*. However, these micronutrients were determined for AG, BG and DB. Calcium concentrations were the highest in BG due to the addition of CaO into the mixture. This addition resulted in a high value of 40.2g Ca/100g of sample. This can be compared to that of AG (5g/100g sample) and BG (0.7g/100g of sample). These means were statistically different (ANOVA; $F_{2,6}$=534.42, p=0.000) (Table 5, Fig.16).

Magnesium levels were higher in DB (0.7g/100g of sample) and similarly, 0.5g/100g of sample, in AG. BG contained the lowest concentration of Mg

(0.1g/100g of sample). These were also found to be statistically different (ANOVA; $F_{2, 6}$=49.56, p=0.000) (Table 5, Fig.16). A Tukey Test was run to test pair-wise comparisons between the mussel fertilisers and the control fertiliser (CA). All differences were statistically significant with three exceptions where differences in N concentration between AG and CA were not significant (p=0.0598) (Table 9). There were also no significant differences in P concentration between AG and DB (p=0.1312) and no differences in K concentration between AG and DB (p=0.3287).

Table 5: General Linear Model ANOVA tables showing significances in differences between macronutrient levels in the three trial fertilisers and Yate's '*Lush*' liquid fertiliser.

Nitrogen

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Fertiliser	3	128.321	128.321	42.774	1052.67	0.000
Error	6	0.244	0.244	0.041		
Total	9	128.565				

Phosphorus

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Fertiliser	3	7.6697	7.6697	2.5566	132.91	0.000
Error	6	0.1154	0.1154	0.0192		
Total	9	7.7851				

Potassium

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Fertiliser	3	20.9879	20.9879	6.9960	136.52	0.000
Error	6	0.3075	0.3075	0.0512		
Total	9	21.2953				

Calcium

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Fertiliser	2	3097.3	3097.3	1548.6	534.42	0.000
Error	6	17.4	17.4	2.9		
Total	8	3114.7				

Magnesium

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Fertiliser	2	0.50953	0.50953	0.25476	49.56	0.000
Error	6	0.03084	0.03084	0.00514		
Total	8	0.54037				

Table 6: Tukey pairwise comparisons for N, P and K levels between the three mussel fertilisers and the commercial liquid fertiliser (CA).

TUKEY:	N	P	K
AGxBG	p=0.0000	p=0.0000	p=0.0043
AGxDB	p=0.0004	p=0.1312	p=0.3287
AGxCA	p=0.0598	p=0.0026	p=0.0000
BGxDB	p=0.0000	p=0.0001	p=0.0010
BGxCA	p=0.0000	p=0.0000	p=0.0000
DBxCA	p=0.0003	p=0.0007	p=0.0000

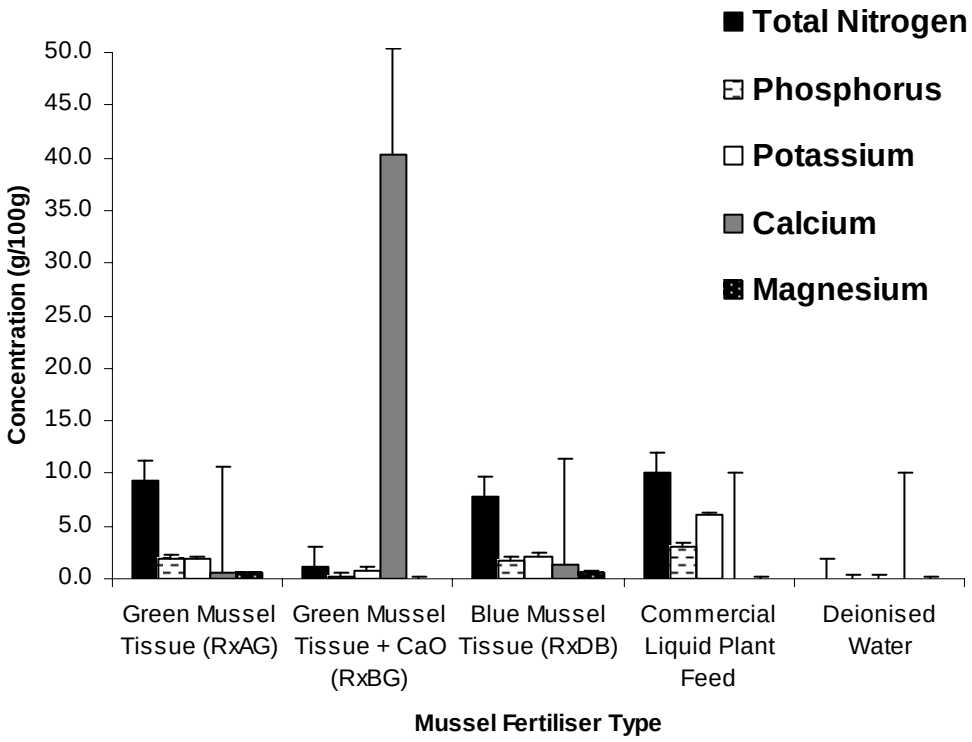

Figure 16: Chemical analysis for the main components of the three trial mussel fertilisers.

3.5.2 Phytotoxicity results

Decomposed *P. canaliculus* tissue (AG) (dilution 5ml: 2L water) resulted in 100% germination. However, the next two dilutions of this fertiliser (10ml: 2L water & 20ml: 2L water) both inhibited 20% of seedling germination. Green mussel fertiliser plus calcium oxide (BG1), at its lowest dilution (5ml sample/2L water), resulted in 60% germination. However, the next dilution of this fertiliser (10ml: 2L) allowed an 80% success rate whereas the highest dilution (BG3;

20ml liquid tissue: 2L water) resulted in 100% germination. Yate's '*Lush*" - commercial liquid plant food (CA) was administered at the same dilution (5ml: 2L water) for all three replicates as per the fertiliser packaging. Theoretically, each test dish should have yielded the same % germination yet one produced 60% germination and the other two replicates, 80% germination. Comparably, the other control, deionised water (CB), in one test dish, (out of the three deionised water supplemented replicates) resulted in 60% germination and the other two dishes, 100% germination.

In terms of germination, an analysis of variance showed no significant differences (p=0.640) between the mussel fertilisers and the two controls (Fig. 17, Table 9).

Table 7: One-way ANOVA for differences in seed germination rates between fertilisers.

Source	DF	SS	MS	F	P
Fert. Type	4	1.733	0.433	0.65	0.640
Error	10	6.667	0.667		
Total	14	8.400			

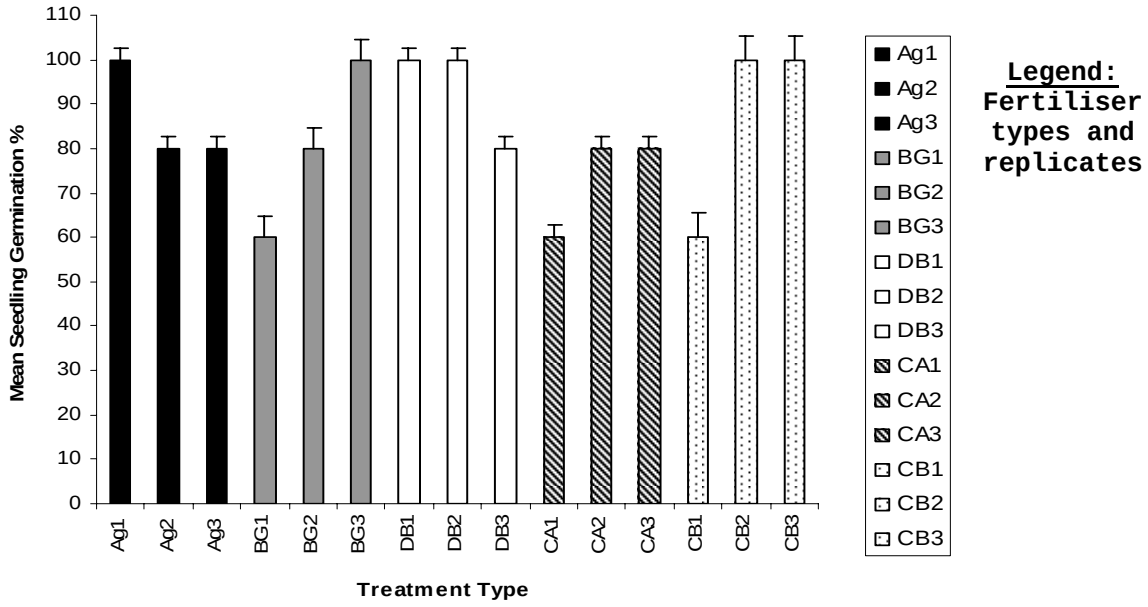

Figure 17: Mean (± SD) percent germination of radish seeds treated with the three mussel fertilisers and the two control treatments.

3.5.3 Plant measurement parameters

3.5.3.1 Leaf count comparisons

The controlled regime resulted in a linear increase in the number of leaves per plant, over time. Differences in leaf counts were statistically significant between the treatments (ANOVA; $F_{4,949}$ =9.29, p=0.000) and also significant over time (ANOVA; $F_{1,949}$=5457.77, p=0.000) (Table 8, Fig. 18).

Varying the concentrations of all mussel fertilisers between 5, 10 and 20 ml per 2 litres of deionised water had no significant difference on the number of leaves counted throughout the study period (ANOVA; $F_{2,947}$=1.02, p= 0.360) (Table 9).

Table 8: ANOVA results for differences between changing leaf counts, stem heights and branch counts (responses), between treatments (model) and over time (covariate).

Leaf counts

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Date	1	1374478	1374001	1374001	5457.77	0.000
Fert.type	4	9353	9353	2338	9.29	0.000
Error	949	238912	238912	252		
Total	954	1622743				

Stem heights

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Date	1	17506456	17507072	17507072	5796.10	0.000
Fert.type	4	6274	6274	1569	0.52	0.722
Error	949	2866448	2866448	3020		
Total	954	20379178				

Branch counts

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Date	1	12282.9	12278.4	12278.4	6932.52	0.000
Fert.type	4	80.0	80.0	20.0	11.29	0.000
Error	949	1680.8	1680.8	1.8		
Total	954	14043.7				

Table 9: ANOVA results for differences between changing leaf counts, stem heights and branch counts (responses), between treatments (model) of varying concentrations (model) and over time (covariate).

Leaf counts

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Date	1	1374478	1373846	1373846	5457.43	0.000
Conc	2	498	516	258	1.02	0.360
Fert. Type	4	9370	9370	2343	9.31	0.000
Error	947	238396	238396	252		
Total	954	1622743				

Stem heights

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Date	1	17506456	17507513	17507513	5799.65	0.000
Conc	2	7793	7719	3860	1.28	0.279
Fert. Type	4	6201	6201	1550	0.51	0.726
Error	947	2858729	2858729	3019		
Total	954	20379178				

Branch counts

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Date	1	12282.9	12277.7	12277.7	6921.65	0.000
Conc	2	0.9	1.0	0.5	0.28	0.754
Fert. Type	4	80.1	80.1	20.0	11.29	0.000
Error	947	1679.8	1679.8	1.8		
Total	954	14043.7				

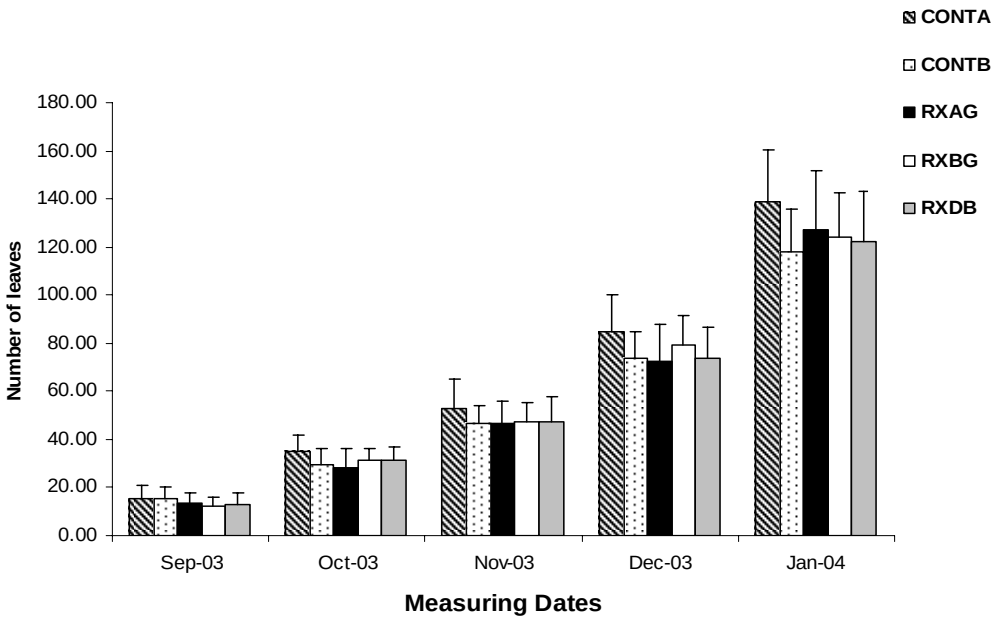

Figure 18: Effects of the trial fertilisers and controls on the number of leaves per plant (average counts for 39 plants per treatment/control).

3.5.3.2 Stem height comparisons

As expected, plant stem heights increased linearly over time. Thus, all differences in stem heights in relation to time were statistically significant (ANOVA; $F_{1,949}$=5796.10, p=0.000). However, apparent differences observed between the different treatments were not significant (ANOVA: $F_{4,949}$=0.52, p=0.722) (Table 8, Fig. 19).

Stem height differences, over time, were not statistically significant between the three concentrations of each fertiliser (5, 10, 20ml/2L water) (ANOVA; $F_{2,947}$=1.28, p=0.279) (Table 9).

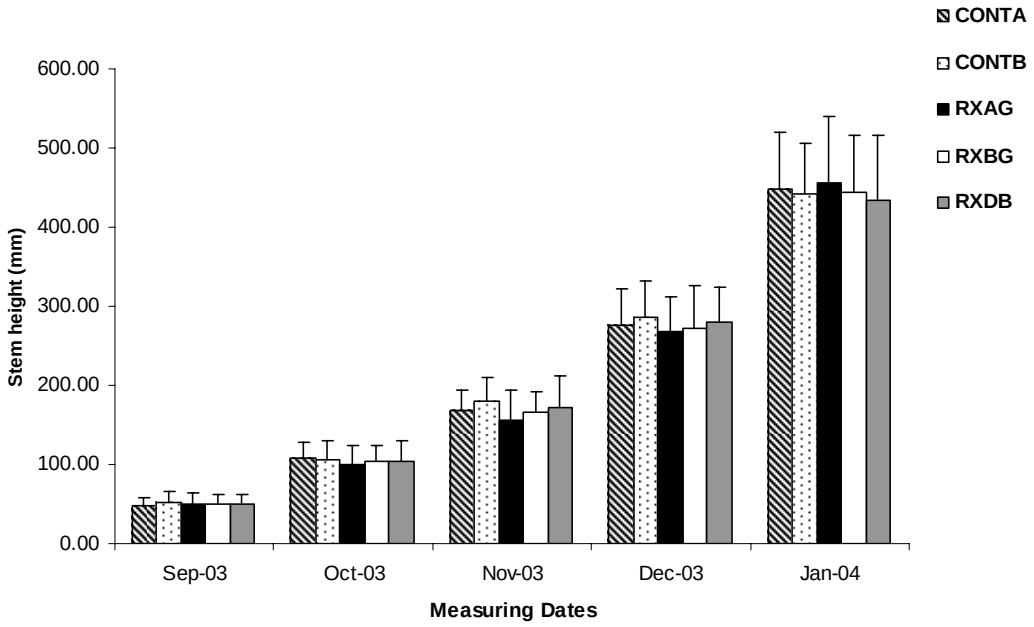

Figure 19: The effect of the trial fertilisers and controls on plant height (averages – each point represents the average height of 39 plants).

3.5.3.3 Branch count comparisons

The general trend of an increase in branches with time was apparent for all treatments over time, with the exception of deionised water-fed plants (CB) which appeared to have a constant total number of branches during two measuring periods, November and December. Despite this one-off fixed value, statistically, differences over time were apparent,

(ANOVA; $F_{1,949}$=6932.52, p=0.000). Differences between the treatments were also significant (ANOVA; $F_{4,949}$=11.29, p=0.000) (Fig.20, Table 8)

No significant differences were apparent in the number of branches counted, when considering the three different concentrations of each fertiliser (5,10,20ml/2L water) fed to the plants for the duration of the study period (ANOVA; $F_{2,947}$=0.28, p=0.754) (Table 9).

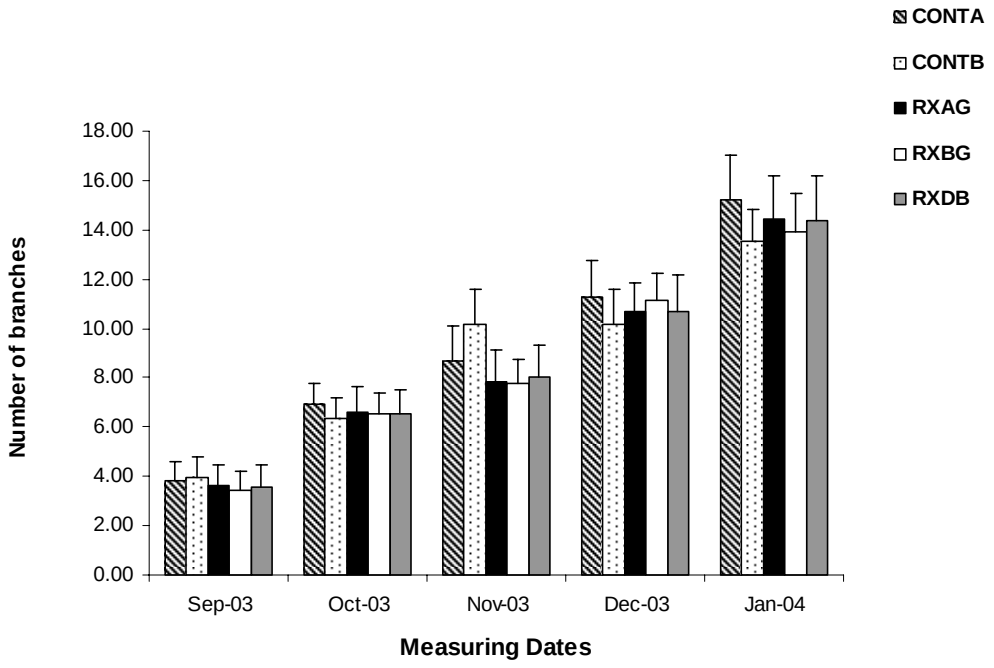

Figure 20: The effect of the trial fertilisers and their controls on the number of branches on each plant (each point is the average number of branches for 39 plants).

3.5.3.6 Final dry-weight biomass

Deionised water-fed plants had the highest overall dry-weight biomass (total =19.13g.m^2) and the highest moisture content of fresh aboveground tissues (547.91g). Commercial liquid fertiliser fed-plants exhibited the lowest overall dry-weight biomass of 14.95 g.m^2 as well as the lowest moisture content of aboveground tissues (385.83g). Plants supplemented with the trial mussel fertilisers had biomass and moisture levels that fell between those of the two controls (Fig. 21, Tables 10,11).

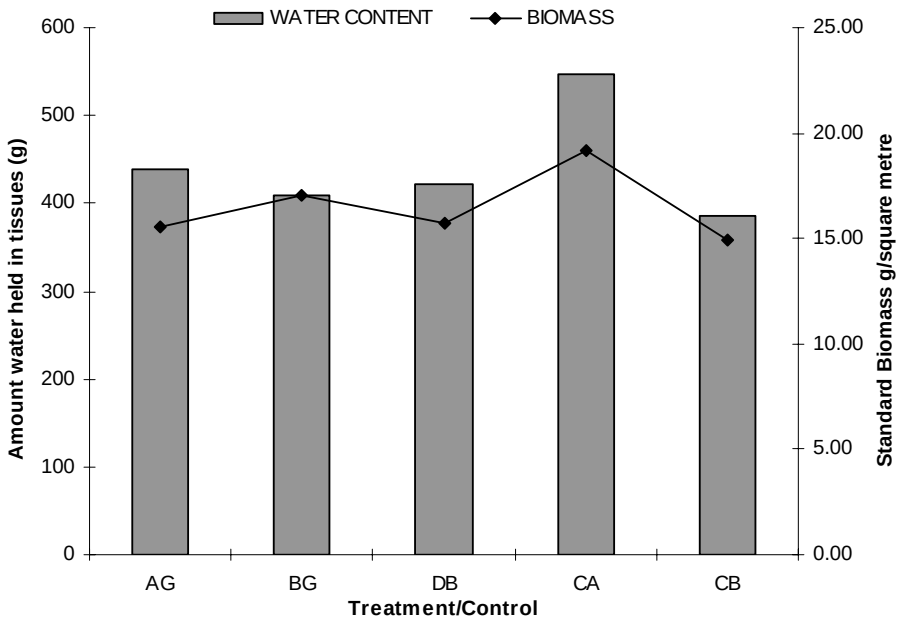

Figure 21: Final biomass and total moisture levels of the trial plants. Highest to lowest ranking in terms of moisture content was CA > AG > DB > BG > CB. In terms of biomass: CA > BG > DB > AG > CB.

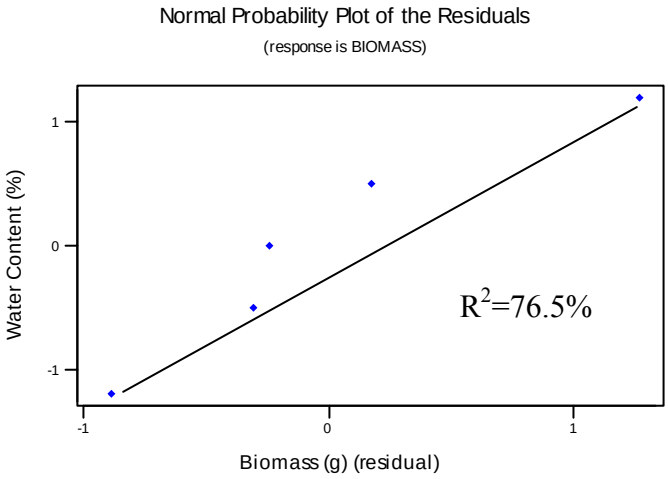

Figure 22: Regression plot showing the relationship between moisture content (predictor) and biomass (response).

Table 10: Regression Analysis: BIOMASS versus WATER.

The regression equation is
BIOMASS = 6.26 + 0.0232 WATER

Predictor	Coef	SE Coef	T	P
Constant	6.257	3.293	1.90	0.154
WATER	0.023172	0.007409	3.13	0.052

S = 0.9320 R-Sq = 76.5% R-Sq(adj) = 68.7%
PRESS = 9.89317 R-Sq(pred) = 10.88%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	8.4951	8.4951	9.78	0.052
Residual Error	3	2.6058	0.8686		
Total	4	11.1009			

Table 11: General Linear Model ANOVA results for: final green-weight, final dry-weight and plant water content, based on resukting effects between type of fertiliser and the concentrations.

Analysis of Variance for FINAl GREEN-WEIGHT

Source	DF	Seq SS	Adj SS	Seq MS	F	P
Fert Type	4	593.54	594.01	148.38	11.68	0.000
Conc.	2	15.64	15.64	7.82	0.62	0.541
Error	176	2235.99	2235.99	12.70		
Total	182	2845.18				

Analysis of Variance for FINAL DRY-WEIGHT

Source	DF	Seq SS	Adj SS	Seq MS	F	P
Fert Type	4	5.5368	5.5287	1.3842	8.60	0.000
Conc.	2	0.1986	0.1986	0.0993	0.62	0.541
Error	176	28.3287	28.3287	0.1610		
Total	182	34.0641				

Analysis of Variance for WATER CONTENT

Source	DF	Seq SS	Adj SS	Seq MS	F	P
Fert Type	4	491.27	491.92	122.82	11.31	0.000
Conc.	2	12.35	12.35	6.17	0.57	0.568
Error	177	1922.73	1922.73	10.86		
Total	183	2426.35				

A Pearson correlation and least-squares regression was used to test the relationship between moisture content and biomass of plant tissue. The R^2 value was 76.5% indicating a seemingly strong relationship between these two variables and showed that generally, the higher the moisture content, the larger the resulting biomass. However, the subsequent

analysis of variance, indicated no significant correlation between moisture and biomass (Regression; p=0.052) (Fig. 22, Table 10,11).

Further analysis of variance indicated significant differences between the final green-weights (weights of the above-ground tissues, prior to desiccating in the oven) and the types of fertilisers and controls (ANOVA; $F_{4,176}$=11.68, p=0.000) but that varying the concentration of the fertilisers had no bearing on the final green-weights (ANOVA; $F_{2,176}$=0.62, p=0.541) (Table 11). A subsequent Tukey Test showed no significant differences between the three fertiliser concentrations (Table 15). Differences in green-weights between the fertilisers and controls were statistically significant between Yate's Lush (CA) and all mussel fertilisers and the deionised water control. All other comparisons for green-weight were not significant (Table 11).

Final plant dry-weight (after removal from the oven) analysis indicated significant differences between plants fed the different fertilisers (ANOVA; $F_{4,176}$=8.60, p=0.000). Similarly for green-weight, there were no significant differences between plants fed the different fertilisers at varying concentrations (ANOVA; $F_{2,176}$=0.62, p=0.541) (Table 11). Tukey Tests showed that there were no significant differences in dry-weights between plants fed the varying fertiliser concentrations (Table 15). Dry-weight differences between plants fed the different treatments and controls resulted in significant differences between CA and deionised water (CB), CA and the *P. canaliculus*-CaO blend (BG) and between CA and decomposed *M. galloprovincialis* tissue (DB). All other comparisons for dry-weight were not significant (Table 11).

Plant water contents were also found to differ significantly between fertiliser types (ANOVA; $F_{4,177}$=11.31, p=0.000) and again, concentration was found to have no effect on any differences between plants (ANOVA; $F_{2,177}$=0.57, p=0.568) (Table 11). Subsequent Tukey tests showed no significant differences in plant water levels between the three concentrations of mussel fertilisers (Table 12). However, statistically significant differences were found between CA and all the

mussel fertilisers as well as CB. A significant difference was also found between AG and CB (p=0.0125) (Table 13).

Table 12: Tukey test results comparing differences in plant dry-weights, green-weights and moisture levels, between plants fed varying fertiliser concentrations (5ml sample/2L water, 10ml sample/2L water and 20ml sample/2L water).

TUKEY: Dry-weight	p-value	TUKEY: Green-weight	p-value	TUKEY: Moisture	p-value
5x10	0.9518	5x10	0.8895	5x10	0.8896
5x20	0.7285	5x20	0.8073	5x20	0.8307
10x20	0.5278	10x20	0.5116	10x20	0.5391

Table 13: Tukey test results comparing differences in plant dry-weights, between plants fed the different fertilisers and controls. Yate's 'Lush' = CA, deionised water only = CB, decomposed *Perna canaliculus* tissue (AG), decomposed *P. canaliculus* tissue + CaO = BG, decomposed *Mytilus galloprovincialis* tissue (DB).

TUKEY: Dry-weight	p-value	TUKEY: Green-weight	p-value	TUKEY: Moisture	p-value
CAxAG	0.0565	CAxAG	0.0266	CAxAG	0.0349
CAxCB	0.0000	CAxCB	0.0000	CAxCB	0.0000
CAxBG	0.0125	CAxBG	0.0000	CAxBG	0.0000
CAxDB	0.0029	CAxDB	0.0006	CAxDB	0.0010
AGxCB	0.0323	AGxCB	0.0156	AGxCB	0.0215
AGxBG	0.9923	AGxBG	0.2036	AGxBG	0.1608
AGxDB	0.8963	AGxDB	0.8344	AGxDB	0.8474
CBxBG	0.0846	CBxBG	0.8360	CBxBG	0.9263
CBxDB	0.2545	CBxDB	0.2093	CBxDB	0.2433
BGxDB	0.9890	BGxDB	0.8014	BGxDB	0.7190

3.5.4 Soil pH changes

A significant difference in soil pH was found between the different fertilisers (ANOVA; $F_{4,37}$=6.57, p=0.000). However, these differences were not significant over time (ANOVA; $F_{2,37}$=2.80, p=0.074) (Fig. 26, Table 17).

Table 14: Statistical summary for soil pH changes.

Source	DF	Seq SS	Adj SS	Adj MS	F	p
Fertiliser	**4**	**2.38572**	**2.47579**	**0.61895**	**6.57**	**0.000**
Month	**2**	**0.52815**	**0.52815**	**0.26408**	**2.80**	**0.074**
Error	**37**	**3.48760**	**3.48760**	**0.09426**		
Total	**43**	**6.40147**				

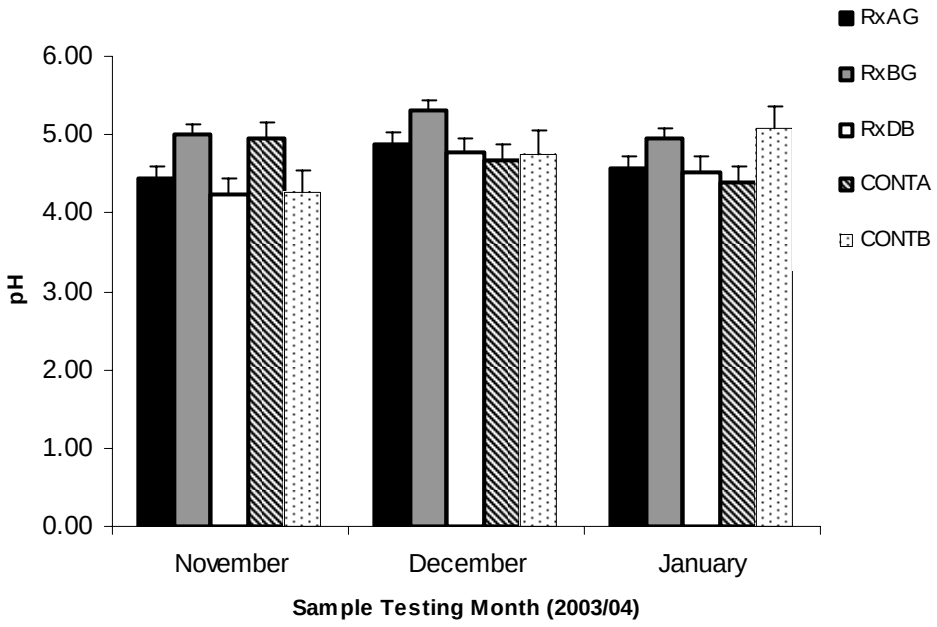

Figure 23: Soil pH changes during the fertiliser trial period.

3.6 Discussion

This chapter explored the potential for *Perna canaliculus* tissue and *Mytilus galloprovincialis* tissue as organic fertilisers. The tissue was accumulated from pre-grade waste from factories, and was allowed to cure (break down) before being administered to tomato plants, as part of a trial feeding regime. The plants were monitored to determine the effects of these fertilisers on growth. Comparisons were made with designated plants supplemented with a proven, commercial all-purpose fertilizer (Yates '*Lush*') with other plants fed deionised water only, as a neutral control. Overall, the *P. canaliculus* (tissue-only) fertiliser was found to be effective, but not as effective as the commercial fertilizer. The *M. galloprovincialis* fertilizer and the *P. canaliculus* – CaO blend-fertiliser showed the least potential in terms of positive effects on plant growth.

3.6.1 Fertiliser analysis

3.6.1.1 Total nitrogen (N)

Nitrogen deficiencies are a common horticultural problem, resulting in limited plant growth and low crop yields (Campbell, 1996). Reduced leaf number and area, as well as, reduced chlorophyll concentration often are observed when plants are deficient in this element (Tei *et al.*, 2002). According to the analysis of the trial fertilisers, *P. canaliculus* tissue fertiliser (AG) contained 9.2% nitrogen (similar to CA, the commercial fertiliser with 10% N); *P. canaliculus* tissue-CaO blend (BG) contained only 1.1% nitrogen and *M. galloprovincialis* fertiliser (DB) contained 7.8% nitrogen. This means that AG contained the closest to optimum levels of N —— if it were to be used as an all-purpose plant feed. This was followed closely by BG. DB would be least likely to be of use as an all purpose plant feed because of its low nitrogen levels in comparison with CA. These differences were significant ($p=0.000$) overall and also between the different fertilisers, with the exception where no differences in N content were apparent between AG and CA. This is promising for

AG as an all purpose fertiliser as its N values are similar to Yate's '*Lush'*, a proven all-purpose commercial fertiliser.

3.6.1.2 Phosphorus (P)

Phosphorus levels in the three mussel fertilisers were lower than that of the commercial liquid plant feed, CA. *P. canaliculus* tissue (AG) contained on average the closest amount of P (2%) to CA (3%) and *M. galloprovincialis* tissue (DB) had the lowest levels of P in comparison (0.2%). Phosphorus deficiency in tomato plants is recognisable as purple discoloration of the leaf veins and undersides and compact growth (Levy & Taylor, 2003). Although the *P. canaliculus*-CaO blend (BG) contained the least amount of P, plants fed this fertiliser did not exhibit any of these symptoms. However, DB plants, did exhibit several cases of unusually short-stemmed compact growth, yet contained 1.7% P. Significant differences between P levels in the mussel fertilisers and '*Lush*' were observed ($p<0.05$) with the exception of AG, where its levels of P were significantly different to DB ($p=0.1312$). Overall, none of the plants exhibited purple discoloration therefore it was assumed that even the low levels of P in the trial fertilisers were adequate for the growth of tomato plants.

Soil treatments containing P are not only beneficial for plant growth but also have been found to immobilise toxic lead irons in soil, reducing the bioavailability of Pb to humans (Tang *et al*., in press). Although Pb contamination is becoming less of a problem in New Zealand over time due to recent removals of the heavy metal from petrol (van Roon, 1999), it is a problem in other countries such as China and the USA (Tang *et al*., in press).

3.6.1.3 Potassium (K)

Fruit development in tomato plants is often hindered by the depletion of leaf potassium, affecting the plant and the fruit quality (Chapagain and Wiesman, 2004). Potassium deficiencies in plants are recognisable by dark blue-green or purple-brown discoloration of leaves and reduced

growth. None of the trial plants (controls included) exhibited signs of deficiencies of P, which was questionable, as CB (deionised water) contained no P. Acute physical signs of K deficiency were not observed but the analytical measures were definitive indicators of lower levels. Out of the mussel fertilisers, DB had the highest proportion of K (2.2%). This was low however in comparison with CA (6% K) and this difference was significant (p=0.000). According to Alcántar *et al*. (1999), when considering the optimum feeding ratios for *Lycopersicon esculentum* Miller, K needs to be the largest component in the NPK ratio followed by N and P respectively, This was not the case for any of the trial or control fertilisers. According to Sobulo and Olorunda (1977), N, P and K can sometimes decrease total sugars and ascorbic acid (vitamin C) values in tomatoes, therefore, the fact that the K proportion in the NPK ratio was low, could result in tomatoes with higher Vitamin C levels, a desirable nutritional quality. Further testing would be required to establish this. These proportions can be compared with results from Ribeiro *et al*. (2000) who analysed the nutrient levels of municipal solid wastes and peat. It was found that municipal solid waste compost contained 0.76% K and peat compost contained 0.07% K. Both these composts were fed to potted geraniums and the proportions of K were sufficient for geranium nutritional requirements. When comparing the municipal solid waste discussed by Ribeiro *et al*. (2000), the mussel fertilisers had a higher proportion of potassium.

3.6.1.4 Magnesium (Mg)

Magnesium is a constituent of chlorophyll. Upon observation of all the test plants (both treatment and control), many appeared to have slightly yellowish leaves (chlorosis). In tomato plants, this is a common indication of magnesium deficiencies (Campbell, 1996). Each trial fertiliser was found to contain very little magnesium (0.5g/100g Mg in fertiliser AG; 0.1g/100g Mg in fertiliser BG and 0.7g/100g Mg in fertiliser DB). Although seemingly similar, these quantities were significantly different, between the fertilisers. These low levels are likely to account for the apparent chlorosis in the trial plants. The plants

fed CA (commercial fertiliser) also exhibited occasional signs of chlorosis. This treatment contained only trace magnesium and chlorosis was also occasionally apparent in those plants fed deionised water (CB).

3.6.1.5 Calcium (Ca)

AG and DB contained low levels of calcium (0.5% and 1.3% respectively), which is acceptable as this metal is required in trace proportions only, in all-purpose fertilisers (Macky, 2000). However, BG was supplemented with calcium oxide derived from mussel shell and as expected, yielded a very high value for Ca (40%). Ca concentrations were significantly different between the fertilisers ($p=0.000$). The high levels of Ca in BG did not appear to adversely affect plant growth or mortality rates. The high level of Ca and the low levels of other nutrients in BG may limit its usage but it may have potential as a crop fertiliser where Ca deficiencies in stock have been recognized (Macky, 2000).

3.6.2 Phytotoxicity

There are many tests that can be run alone or in conjunction with each other to determine the phytotoxic potential of a soil or foliar supplement. These include seed germination, root elongation techniques and germination indices (a factor of relative germination). This study was exploratory and therefore concentrated solely on whether or not the trial mussel fertilisers demonstrated inhibitory properties on radish seedling germination. Overall, none of the trial mussel-based fertilisers or the controls exhibited strong tendencies to inhibit seedling germination. This was apparent from the lack of statistically significant differences between rates of inhibition and germination ($p=0.640$). Fertiliser AG (*P. canaliculus* only) demonstrated 100% germination at its lowest concentration and an 80% germination rate for both other higher concentrations. It is therefore assumed that the higher concentrations of this fertiliser may have slight inhibitory characteristics. This may be due to slightly higher quantities of total nitrogen in this fertiliser than in the other fertilisers. NH_4^+-N has been found to have inhibitory characteristics on the germination of the seeds of some vegetable plants (Tiquia *et al.*, 1996; Hoekstra *et al.*, 2002) and this may account

for the slight reduction in germination for the two higher concentrations of the green mussel fertiliser.

The results from the phytotoxicity analysis indicate opposing inhibitory tendencies for fertiliser BG (*Perna canaliculus* tissue-CaO blend). Germination rates were higher with an increased concentration demonstrating 60% germination for the lowest concentration and 100% for the highest. Statistically however, this difference was not significant and may simply have been due to chance. Calcium is not known to inhibit germination; therefore, the *P. canaliculus*-CaO blend (BG) fertiliser would not exhibit toxicity if supplemented to the soil, adjacent to tomato seeds.

Fertiliser DB (*M. galloprovincialis* tissue) demonstrated 100% germination for both the 5ml and 10ml concentrations, dropping to an 80% germination rate at the highest concentration. However, this difference was not significant and therefore this fertiliser did not demonstrate inhibition.

The commercial liquid plant food (CA) showed slightly higher inhibitory tendencies than the other treatments producing germination rates of 60%, 80% and 80% respectively. Overall, this is still successful in terms of germination yet as the rates of germination are lower than the other treatments an explanation may be that this plant food was designed in terms of N:P:K as a feed supplement for mature plants and as an aid for fruiting and flowering. It may be too nutrient rich for seed germination and thus slightly inhibitory and primarily toxicological (Levy & Taylor, 2003). It's N: P: K rating was 10: 3: 6. The high nitrogen may have been slightly inhibitory in terms of ammonium as previously discussed for fertiliser AG and the higher levels of potassium (increased to enhance flowering) may also have been too nutrient rich. The other control treatment was deionised water (CB). As expected, deionised water generally demonstrated no inhibitory tendencies with the exception of one trial plate in which only 60% of the seeds germinated. This was explained statistically to be due to chance. This result may be considered an outlier because chemically, water should not inhibit germination. Water is imbibed by the seed and is essential because after imbibing, the seed is prompted to release hormones (gibberellins) which in turn prompt the production of digestive enzymes used to release stored foods housed

in the seed's own endosperm (Campbell, 1996). Therefore, without water it is unlikely germination will occur at all. It must be noted that in previous studies, ammonia, copper and zinc have been shown to be the main germination inhibiting agents (Tiquia *et al*., 1996). Copper and zinc were not analysed in the trial fertilisers because they are trace elements and therefore, the slight observed inhibition in each fertiliser may be due to the presence of these metals.

3.6.3 Plant measurement parameters and biomass

Plant leaf area development is directly proportional to the light interception capacity of a plant (Togun and Akanbi, 2003). Although leaf area was not measured, plants with the highest number of leaves would have greater intercepting surface area. During each measuring period, commercial fertiliser-fed (CA) plants yielded the most leaves and branches, followed by those fed *P. canaliculus* tissue (AG) with the second highest of each count. Deionised water-fed (CB) plants yielded the least of both. This was expected due to the greater nutrient content of CA than CB. However, larger leaf surface area has a direct bearing on dry matter accumulation and this may explain why CA plants yielded the largest dry-weight biomass value and CB, the smallest. Differences observed between the leaf counts of the differently treated plants were significant ($p=0.000$).

Taller stem heights were recorded for CB plants during three of the measuring periods whereas plants fed AG had the shortest stems, with the exception of the final measuring date (January) when AG yielded the tallest overall stem heights! However, despite this slight trend, these results were not significant ($P>0.05$). This result does not concur with previous research by Togun and Akanbi (2003) who found that high nitrogen composts resulted in taller stem heights for *L. esculentum* and their control plants (which were not fertilised) yielded the shortest stem heights. Statistically, however, there were no significant differences between the different fertilisers and controls, in terms of stem heights ($p=0.722$), despite the higher N levels in AG and CA. Varying the concentration of the mussel fertilisers also did not significantly affect stem height ($p=0.279$). This suggests that varying the concentration had no direct effect on stem height.

As expected, the number of branches counted for each plant increased significantly with time (p=0.000). One exception was that plants supplemented with deionised water only (CB) (between the two November and December 2003 measuring events) did not change in mean branch numbers, until January 2004, when a large increase was observed (Fig. 19). This may be due to the lack of nutrients in deionised water, which the plants may have required for that stage of maturation, but does not explain the rapid increase in numbers in January 2004. More trials would be required in order to explain this further. Statistically, there were significant differences in branch counts between the treatments but no differences were determined by varying the concentrations of the mussel fertilisers (p=0.754).

Biomass allocation was expressed in terms of dry-weight, as a proportion of green-weight (fresh weight) and allowed for the calculation of plant water (moisture) levels. Green-weight and dry-weight were useful parameters as they provided an overall indication as to which plants grew the largest overall, not only in height but also in terms of the density of leaves, stems, branches and any fruiting bodies.

Plant green-weight differences were found to be significantly different between the different fertilisers and controls (p=0.000). Varying the concentrations of these, however, did not effect green-weight (p=0.541). Dry-weight biomass appeared to be strongly related to water content (R^2=76.5%) but the statistical differences in this regression were slightly insignificant (p=0.052). Varying the types of fertilisers and controls had significant effects on final plant dry-weights (p=0.000) but changing the concentrations did not (p=0.541). Despite having lower levels of NPK in comparison to CA, the *P. canaliculus*-CaO blend (BG)-fed plants yielded a higher total biomass than the *M. galloprovincialis*-fed (DB) plants and *P. canaliculus*-fed (AG) plants. Equally as inexplicable, AG had closer to optimum levels of NPK and yet yielded the lowest total biomass value out of the three mussel fertilisers. The opposite occurred again in terms of moisture content. Although AG plants had the lowest biomass, out of the mussel fertilisers, they had the largest moisture content followed by DB plants and BG respectively. However, these differences were not significant between AG and DB (p=0.8474) and between AG and BG (p=0.1608). In terms of

tomato plants, or any similar vegetable plant, a high biomass or standing crop is desirable over high moisture content therefore, out of the trial mussel fertilisers; BG was the most effective in terms of yielding larger plants. However, why did BG yield the highest biomass out of the mussel fertilisers when it had the lowest NPK values (1.1:0.2:0.7) in comparison with CA (10: 3: 6), which had the highest biomass overall? These results do not concur with Tei *et al*., (2002) who found that the more N that is available to tomato plants, the greater the dry matter accumulation. According to Sandoval-Villa *et al*., (2001), small NH_4^+-N fractions actually improve yield and may enhance vegetative growth and nutrient uptake. This may also account for why the lower levels of total N in BG yielded the highest dry-weight biomass of the three mussel fertilisers. Another explanation may be due to the exceptionally high levels of Ca in BG owing to the addition of CaO in its composition (40%). This concurs with Hamer (2003) who stated that calcium deficiency symptoms of tomato leaves can result in subsequent loss of yield and this may explain why BG (which had very high levels of Ca) had a higher yield in terms of biomass in comparison with the other treatments.

3.6.4 Soil pH changes

Soil pH is a very important factor that must be considered when performing soil treatment plant bioassays (Price, 1990 c.f. Gong *et al*., 2001). Soil pH not only affects cation exchange, but also influences the chemical form in which nutrients assume (Campbell, 1996). pH testing of the treated soils in this bioassay was commenced after two months of treatment, to ensure adequate time for assimilation into the soil. However over the three months of testing, the pH did not significantly change for any treatment, ranging between 4.25 and 5.08 which are considered acidic ($p=0.074$). Nevertheless, tomato plants prefer acidic conditions to alkaline for optimum growth. High pH levels (8+) are required for efficient absorption of magnesium, calcium, sulphur, molybdenum and potassium. Conversely, lower pH levels (6.5 - 5.0) are required for optimum availability of iron, manganese, boron, copper and zinc. Nitrogen and phosphorus are more bio-available at pH levels between 6.0 and 8.0 (Shannon, 1999). Nitrogen can have acidifying effects on soil pH (due to the conversion of ammonium to nitrate with a release of H^+). This may explain why AG, DB and CA contained higher levels of N and overall exhibited a slightly lower soil pH

than BG, which has lower N, levels. P fertilisers do not directly acidify soils but they do increase plant growth. However, plant growth can subsequently acidify the soil due to cationic interactions at root level (Shannon, 1999).

3.6.5 *Perna* versus *Mytilus* as a fertiliser — summary

Despite having similar biochemical composition, the tissue of both mussel species yielded markedly different results in this experiment. *Perna* 'fertiliser' contained significantly higher levels (P=0.000) of nitrogen than Mytilus 'fertiliser' (9.23g/100g sample versus 7.75g/100g sample, respectively). Nitrogen is one of the three principal macronutrients essential for optimum plant growth and maximum yield. Thus the lower levels of this macronutrient in the *Mytilus* 'fertiliser' may explain the significantly lower overall plant tissue yields. The *Perna* fertiliser also contained higher levels of phosphorus but lower levels of potassium than *Mytilus*. Potassium is used predominantly by plants in the formation of fruit and flowers. As the trial plants were not left long enough to allow fruiting and flowering to occur, it should not be concluded that the higher levels of K in the *Mytilus* fertiliser would not have increased yield. This may have been the case had the plants have been left to grow these structures.

3.6.6 Limitations

Methodological limitations were recognized towards the closing stages of the fertiliser experiments. In this experiment, screened, untreated topsoil was used as the growing medium. It was selected for its similarity to common garden soil. However, for more robust, future analyses, clean quartz sand or perlite should be used as a more inert growing media — to further minimise interference from existing micronutrients in the topsoil.

The primary concentration utilised in the liquid fertiliser experiment was 5ml decomposed mussel tissue: 2L water. This, therefore, was the only concentration with a direct control (deionised water, CB). Liquid dilutions (10 ml: 2L and 20ml: 2L) were not controlled for directly. Rather, they were included as comparisons to the controlled 5ml: 2L dilution. Future work should account for these dilutions by directly controlling for them separately.

Adequate working space was a limiting factor. The tray arrangement (Fig.14) was not symmetrical due to a lack of available workspace. However, the experimental design attempted to counteract any 'edge effects' by regular tray rotation.

It must be noted that all fertiliser work was exploratory and would require more time, further experimentation and development into a more stable product.

3.6.7 Recommendations

Each mussel fertiliser could benefit from additions of potassium to bring K levels closer to the commercial all-purpose liquid fertiliser (K), especially if these fertilisers were to be used specifically on tomato crops. It is also recommended that if further research and development were to be carried out, trial plants should be allowed sufficient time to develop fruit and flowers, for a more accurate picture.

The mussel fertilisers were unstable in terms of further decomposition and it is recommended that they be allowed to originally cure for longer than stated in the methodology (reduced in this work due to time restrictions). Odour reduction would also be recommended. It was also assumed that the number of holes punched into the lip of the bucket would allow for sufficient aeration. However, if aeration was insufficient, carbon dioxide may have accumulated in the airspace within the bucket. This was not monitored and future work should account for this possibility. It is therefore recommended that further plant trial work be carried out once the trial fertilisers have been stabilised. This future work should involve nutrient analysis of the plant tissues, which was not possible in this research due to financial and time constraints. Using quartz sand or perlite as a stable, non-reactive medium is also recommended to provide a more robust analysis and to discount effects of growing media on the results. It is also recommended that future studies examine the potential of mussel shell to be incorporated into agricultural paddocks soil horizons to test the potential for the shells to act as aerators and to enhance drainage. The added bonus to aeration would be slow breakdown, with the gradual release of lime into the soil. Thus, one would expect improvements in underlying soil structure over time

3.7 Conclusions

Decomposed *P. canaliculus* mussel tissue showed promise as an all-purpose plant feed in terms of its similarity in NPK proportions to the commercial plant feed (CA). This fertiliser contained similar levels of nitrogen to CA, which is a desirable property. It did however contain lower levels of potassium than CA but this could be amended by the addition of any stable compound containing the K^+ ion, such as potassium permanganate or potassium hydroxide (Chang, 1994). This fertiliser exhibited low phytotoxic potential but had a higher dry-weight biomass than those plants fed deionised water.

Decomposed *P. canaliculus* mussel tissue with supplementary calcium oxide, showed reduced potential in terms of being an all-purpose fertiliser in spite of yielding the highest dry-weight biomass out of all three mussel fertilisers and having low germination inhibitive tendencies. Due to its high levels of Ca, it could be useful as a pasture treatment where calcium deficiency in cows has been identified.

Decomposed *M. galloprovincialis* tissue demonstrated the least potential as an all-purpose fertiliser in terms of the measurement parameters and final dry-weight biomass. It was however more effective than deionised water and did not demonstrate any germination inhibition tendencies. This fertiliser contained higher levels of K than the *Perna* 'fertilisers' and if the plants had been left to mature further and develop fruits and flowers, plant yield may have been higher for those plants supplemented this fertiliser.

In conclusion, *Perna canaliculus* mussel tissue may be useful as an inexpensive, effective organic fertiliser. *Mytilus galloprovincialis* tissue and the *P. canaliculus*-CaO blend were less effective. However, for all three, value would be enhanced if they were stabilised in terms of further decomposition, odour and allowed to cure for as long as possible, to completely release all available nutrients.

Chapter 4 —Potential of mussel shells as a coarse aggregate in concrete

4.1 Introduction

4.1.1 Waste mussel shell and the New Zealand mussel industry

Mussel shells are the main waste by-product generated by the New Zealand Mussel Industry (NZMI) constituting roughly 90% of each factory's waste. This equates to approximately 100,800 metric tonnes of shell per annum. Current reduction and disposal methods include stockpiling, burial in landfills, adding to forest fire-roads in place of stone chips and farm races or in horticulture. In terms of logistics and disposal consents, land filling or burial is rarely a permanent solution, further it is expensive, and has limited life spans with respect to bulk disposal options.

4.1.2 Previous studies and historical overview

Aquaculture has increased significantly in the last twenty years with total harvests accounting for over 25% of all seafood consumed by humans (Siegel, 2000). With this expansion has come an increase in global shell waste. This is a problem which is also becoming increasingly significant for the New Zealand Mussel Industry. Globally, scientists have discovered that natural polymers such as chitin can be extracted from lobster, crab and shrimp shells. Such studies have included an examination of the ability of marine shells to absorb heavy metal pollutants such as sulphate and molybdate ions from mining effluents, using chitinous shrimp shells (Moret and Rubio, 2003), crab shells (Cardenas *et al.*, 2001; Evans *et al.*, 2002) and pollutants from dairying wastewaters (Selmer-Olsen *et al.*, 1996). Some researchers have investigated the reuse of seafood waste in the formation of new edible products. Heu *et al.* (2003) examined the reuse of shrimp heads, shells and tails by determining the nutritional qualities (protein, amino acids and various minerals). Their study

concluded that there is potential for extracting useful proteins and flavourings, for use in existing food products (Heu *et al.*, 2003). A similar nutritional use, researched by Guillou *et al.* (1995) utilised shrimp wastes as colour pigments for assimilation into salmonid diets. Others have taken a more biochemical or biological stance, with one researcher investigating the addition of shrimp shell waste as a lamb feed to test effects on rumen bacteria and rates of digestion (Cobos *et al*., 2002). Others have harnessed the anti-microbial properties of powdered shrimp and crab shells (Wang *et al*., 2002), using the shells as a substrate for isolating chitinolytic micro-organisms. These organisms are used to extract chitinases, which are compounds used in the protection of plants against parasites (Wang and Hwang, 2001). Although encouraging, this reuse technology is not directly applicable to reusing mussel shells due to the relatively low levels of chitin in comparison with crustaceans.

An American company, *The Great Eastern Mussel Farms, Inc*., were faced with a major problem with managing the great quantities of accumulated broken/defective mussels and waste shell. The company solved its waste problem by forming a subsidiary organic compost company called *Coast of Maine*. *Coast of Maine* was able to create two products; the first, a plant bulb soil additive, made from pulverised mussel shells (among other ingredients) with the claim that the shell provides a rich source of calcium and forms a protective barrier, preventing rodents damaging the bulbs. The second product was a soil conditioner that contained 75% mussel waste with other ingredients such as blueberry and herring wastes. The shells in this mix were crushed during composting and were claimed to add sufficient calcium to raise the compost mix pH. Larger shell fragments were also included to aerate and texturise soil (Coast of Maine, website, cited 2003). The aeration concept described by *Coast of Maine,* is concurrent with this dissertation whereby it was found that aeration (by using larger shell pieces) enhanced the breakdown of soft tissue more efficiently. Another international initiative was by a Spanish factory, *Aleco*, a mussel shell treatment plant, in Boiro, Spain. This plant utilises mussel shells to create a bird feed, low technology plastics, cement and sand. The end-product value is estimated to equal approximately €22 / tonne [around NZ$40.00 / tonne] (Anonymous, 2002b).

Past research also documents the incorporation of waste shell into concrete products as coarse aggregates, sand or cement mortar (Falade, 1995; Yoon *et al*., 2003). Falade (1995) described the incorporation of periwinkle shells into concrete as a coarse aggregate, with acceptable compressive strength results. Yoon *et al*. (2003) described the mechanical characteristics of crushed oyster shell. Yoon *et al*. (2003) recognised that along the southern coast of Korea, large quantities of oyster shell was being illegally dumped, adjacent to oyster farm sites. They investigated the potential for this waste shell to be recycled into useful construction materials. In their study, oyster shell was pulverised and combined with sand before being added to cement and water. The quantities of oyster shell were varied as different dosages (20%, 40%, 60%, and 80%) and the compressive strength of each solid sample was determined. Compressive strength was found to decrease with increasing amounts of oyster shell, with an exception where 40% shell yielded an unexpected increase in compressive strength. From these results the authors concluded that crushed oyster shell is a good supplement material for when sand sources are insufficient (Yoon *et al*., 2003). Other studies which have incorporated waste materials into concretes as new aggregates, include coconut shells (Almeida *et al*., 2002), waste oil palm shells (Tay and Show, 1996; Basri, *et al*., 1999), hazelnut shell and black tea waste (Demirbus and Aslan, 1998), steel slag and limestone aggregates (Maslehuddin *et al*., 2003) and wood aggregates (Bouguerra *et al*, 1998; Al Rim *et al*., 1999). Of these studies, unsurprisingly, the black tea waste was found to have the least positive effects on concrete strength!

4.1.3 Properties of mussel shell

4.1.3.1 The mineralogical composition of *Perna canaliculus* shell

As with all bivalves, *P. canaliculus* secretes its shell through the mantle epithelium (Camann, 2003.). The shell's internal structures are comprised predominantly of two polymorphs of calcium carbonate; aragonite (averaging 92%) — forming prismatic or acicular crystals and low-magnesium calcite (averaging 8%) — forming prismatic crystals (Moore, 1973; Checa, 2000; Checa and Navarro, 2001) (Fig. 28). The

presence of the calcite fraction (a central, sub-layer) coincides with the incidence of manganese, itself possibly a by-product of a metabolic reaction, such as respiration (Moore, 1973). However, in some instances this fraction is not present, due to variations in metabolic pathways between species. Consequently, the presence of the calcite portion may be a way for the organism to store the manganese (a poison) safely away from internal organs (Moore, 1973). Strontium has been found to occur as an impurity in aragonite (0.2%), similarly for magnesium (0.16%) in the calcite fraction, as well as the prevalence of the trace elements, Fe, P, Ba and Cu (Moore, 1973). The peripheral, uppermost green-brown layer is the periostracum. This is an organic protein layer, composed primarily of a structural polysaccharide called chitin (Campbell, 1996).

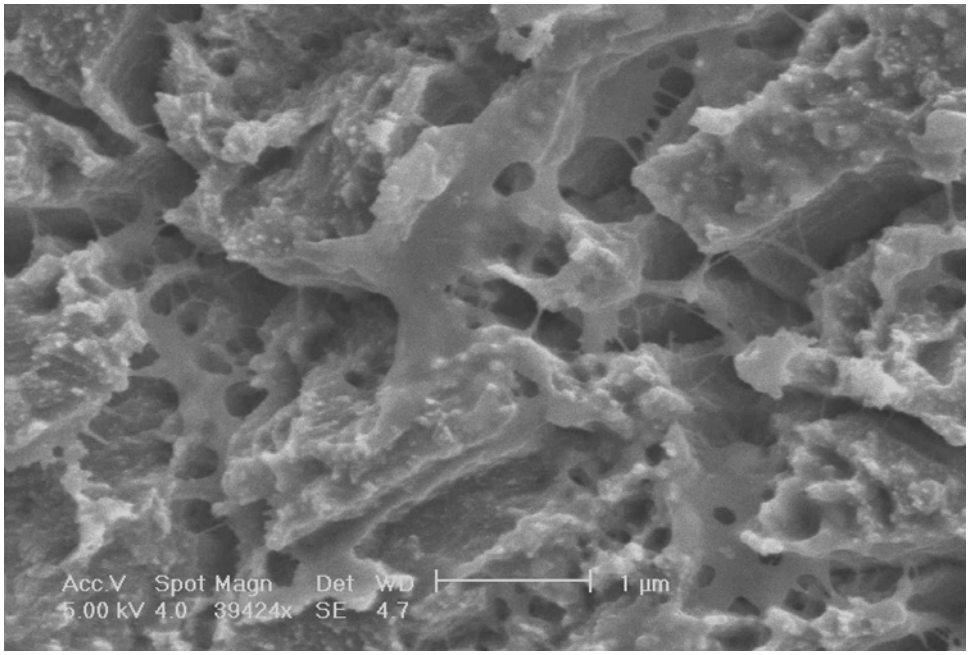

Figure 28: Scanning electron micrograph of a *Perna canaliculus* shell section showing prismatic structure (Photo: B. James, 2002).

4.1.3.2 The basic principles of concrete and its curing

Concrete is a strong, rigid building material consisting of a cured mixture of sand, gravel, water and cement. The most common types of concrete in New Zealand are those formulated with Portland (structural cement) or asphalt (paving materials) cements. Portland cement concrete involves the combination of Portland cement, sand (as a fine aggregate), a coarse aggregate (usually gravel) and water. The aggregates in the mix are used as fillers and strength enhancers. In order to achieve maximum

strength and integrity, it is important to ensure maximum interfacial contact between cement particles and the coarse aggregates, with sand filling the large interstitial spaces. Excess water results in a runny mixture, an increase in porosity or reduced workability and can result in a low overall strength (Callister, 2003).

Curing (concrete hardening to achieve maximum strength) is achieved in water either by immersion, sprinkling, the application of a wet sand layer, misting, by covering in plastic sheeting to keep the moisture in or by the use of a membrane-forming curing substance *(NZ Concrete Construction Standard NZS3109* c.f. Chisholm, 2001). The principle behind sealing moisture around the curing concrete is that water vapour transmission is minimal. 80-90% water retention is the standard relative humidity to allow for maximum curing (Chisholm, 2001).

4.1.3.3 Compressive Strength

Compressive strength is the most important factor when creating structural building materials (Yasar *et al*., 2004). Generally, strength is measured in terms of resistance to stress and strain. A testing load may be applied as tension, compression or shear [torsion] (Callister, 2003).

4.1.3.4 Determination of the bleeding rate of mix-water in wet concrete.

The method of determining the bleeding of concrete measures how much mix water displaces from, and accumulates on the surface of freshly poured and compacted wet cement. Bleeding is caused by natural sedimentation of the solid components prior to initial setting (New Zealand Standard 3112, 1986; Natal Portland Cement, 2003). Bleeding is an undesirable phenomenon that disrupts the water to cement ratio and can have adverse effects on the overall cure, resulting in strength reductions. All concrete bleed tests in this chapter were carried out in accordance with *New Zealand Standard 3112: Part 1: 1986 (Section 8) Determination of Bleeding of Concrete.*

4.1.3.5 Determination of trapped air in wet concrete

Air pot testing is utilised to determine the proportion of air (as a percentage) in a fresh concrete mix and 4%- 4.5% air is standard. Values between 4.5% and 7.0% air will result in a concrete that is 10% weaker that those with lower air contents (T. Walker, pers.comm. 2003).

4.1.3.6 Determination of basic thermal properties

Analysing heat conduction in concrete is imperative when considering energy efficient building designs, thermal loading of structures due to seasonal temperature fluctuations and in ensuring comfort for future inhabitants (Khan, 2002). It is also an important predictor of cracking (Kim *et al*., 2003). Knowledge of the thermal properties of a concrete product is vital in predicting temperature profiles and heat flow (Kahn, 2002). The analysis of thermal properties is another quality-control measure used for pre-cast structures (Principallo *et al*., 2003) and is necessary if such materials are to be utilised as an insulator.

In thermal conduction, heat is transported in solid materials by free electrons and lattice vibration waves called phonons (Callister, 2003). In a conducting solid such as a metal, the abundant free electrons convey an increase in kinetic energy when heat is applied, before rapidly migrating to cooler areas within the solid. Some of this kinetic energy is transferred to the solid's atoms as a consequence of imperfections in the solid's matrix. Non-metallic materials (such as concrete) are thermal insulators because they lack large amounts of these free electrons to conduct heat. Any conductivity that does occur, however small, is carried out instead by the phonons, and these are not as effective in the transport of heat energy as free electron conduction, which occurs readily in a metallic solid (Callister, 2003). Cement and concrete products are porous. In ceramic materials (which include cement, plaster of paris and lime), porosity is thought to have a strong influence on thermal properties, wherein the larger the pore volume, the more reduced the thermal properties. Insulation requires a high level of electrical resistivity (Callister, 2003). Typical room-temperature electrical

conductivity for dry concrete is 10^{-9} Ω-m^{-1} . This value can be compared with polystyrene which has an electrical conductivity value of $<10^{-14}$ Ω-m^{-1} (Callister, 2003). Selected aggregate conductivities include quartz with thermal properties of 4.45 kcal/m hºC, limestone, 2.29-2.78-kcal/m hº C and basalt, 2.47 kcal/m h º C (Kim *et al.*, 2003).

4.1.4 Objectives

This chapter examines the usefulness of concrete containing mussel shells and Portland cement, with structural use intentions. The objective for this section of the project was to test the potential for waste mussel shell to be incorporated into cement as a lightweight coarse aggregate, with the intention of creating concrete for structural or load-bearing uses.

4.2 Methods and materials

4.2.1 Overview

Mussel shell material was incorporated into a wet Portland cement/sand mix as a coarse aggregate in both whole and crushed forms. It was later incorporated with basalt aggregates as an attempt to enhance strength. The resulting concrete products were used to test two potential properties:

- Structural / tensile integrity (compressive strength) including bleeding and percentage air
- Basic thermal properties (thermal insulation potential)

The potential for mussel shell material as a coarse aggregate in concrete was determined by compressive strength tests on cured concrete containing shell. Additional tests included establishing the rate of mix-water bleeding, entrained air content and basic heat-transfer potential (insulation). A systems approach diagram was constructed to illustrate the pathways taken in testing mussel shell concrete. (Fig. 24).

4.2.2 Limitations

Due to time limits, a simple, modified test of relative heat conduction was used on finished, dry concrete. The ideal dry method would have been to utilise a two-linear-parallel-probe (TLPP). The TLPP method, described by Kim *et al*. (2003) involves the drilling of two parallel holes into the sample with the heat source probe inserted into one hole and the temperature sensor into the other. At the time of testing, a TLPP unit was not available and modified procedures had to be undertaken. The modified measures did not provide a value for thermal properties *per se* (Watts / metre Kelvin), as there was no way of measuring current flow (Watts) in terms of time ($W=J.s^{-1}$). Therefore, heat transfer was simply determined in terms of the difference between a base heat (at the source) and the conducted heat at the surface (i.e. the temperature over time of the surface of the slab). This set-up was not carried out in a controlled temperature

environment. External variables, such as changing air currents may have affected the base and top surface temperatures. There was also no way of determining heat dispersal throughout the slab and it was therefore assumed that most of the transferred heat would travel upwards and be recorded by the upper-surface thermocouple. Slabs of polystyrene and an industry-standard-mix slab of similar proportions to the test slabs were used for comparison.

The second trial of concrete (MBT2 — the mix containing basalt aggregates and air entrainment agent in addition to mussel shell) was carried out towards the end of the study and therefore a 28 day test could not be carried out due to time restrictions. However, all other tests including a comparative 7-day compressive strength test were carried out for this mix.

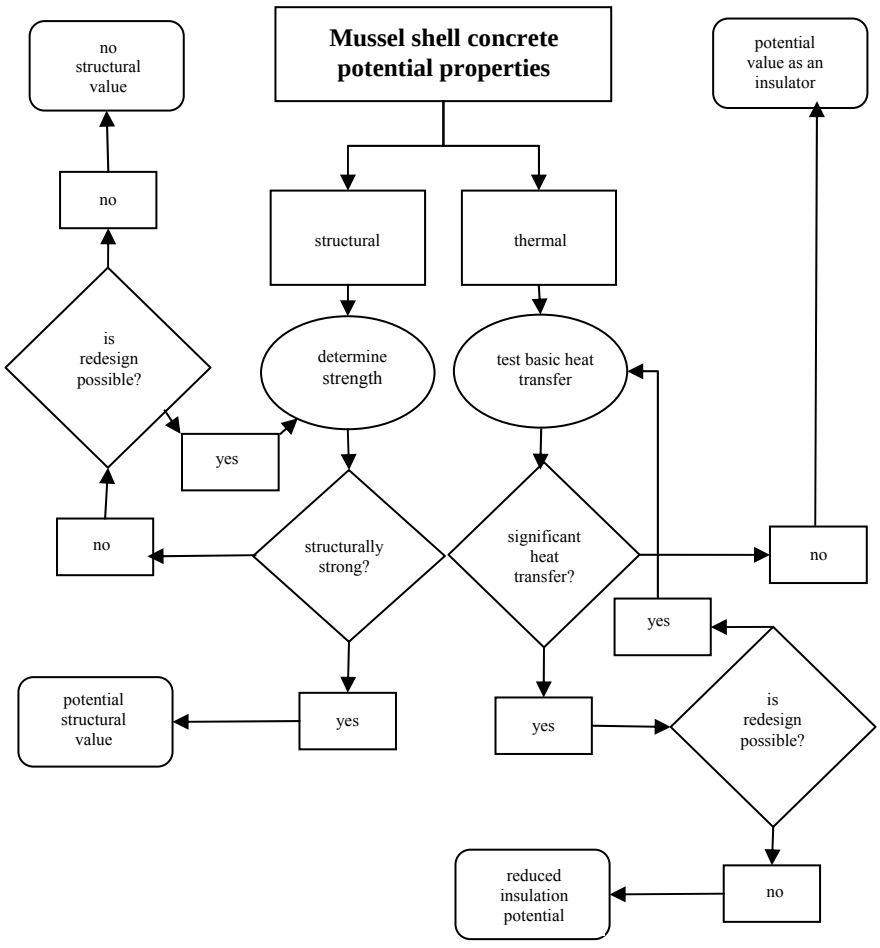

Figure 24: A systems approach to examining potential uses for mussel shell concrete.

4.2.3 Compressive strength

4.2.3.1 Formulation of trial mix 1

Six batches of concrete were produced. Three contained hand-crushed shell as a coarse aggregate (Trial 1, denoted as HCT1) and three contained whole shells (machine-crushed shell broken by the mixer blades, denoted as MBT1) and all six were made to the following formula:

Broken shell (8 kg crushed to approximate 1cm^2 pieces by hand with an iron cylinder) was combined in an industrial mixer with 16 kg of builders-grade sand, 6 kg of Portland cement and 3 litres of tap water. The amount of each component provided adequate quantities of wet mixture to fill at least three cylindrical moulds for testing. Water was added to the dry mix after initial mixing of the dry components. At this stage, a slump test (to gauge potential workability) was carried out. This involved filling a slump cone (Fig. 25) gradually with the test mix no more than 15 minutes after the fresh mix had been created and on a level, rigid, non-absorbent surface. During filling, a tamping rod was used to compact the mix to aid in settling and to remove as much air as possible. At each one-third fill of concrete, the rod was used to strike the mix 25 times — while avoiding hitting the base. When the cone was full, the top was levelled with a trowel and the cone was removed vertically. The empty cone was then set upside down beside the slumped mix and the distance of slump was measured above the highest point of the concrete mix (Fig. 25). All slump values were recorded to the nearest 10mm.

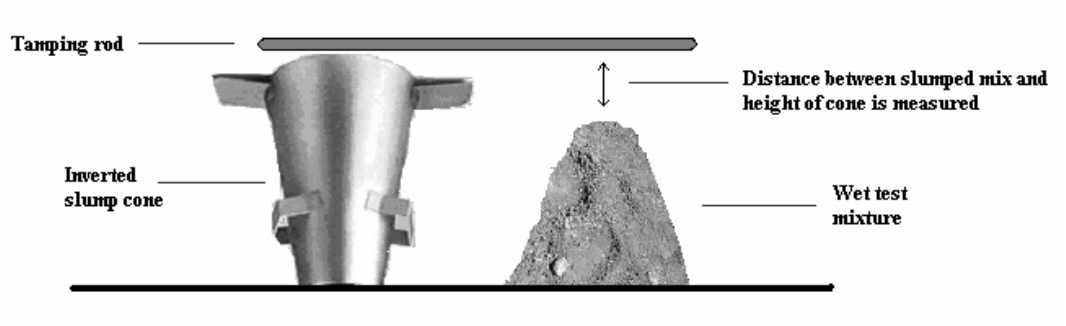

Figure 25: Slump cones used in establishing workability by the slumping of wet mixture.

4.2.3.2 Formulation of Trial Mix 2 (machine-crushed shell — shell broken by the mixer blades — denoted as MBT2)

In response to the results determined for the compressive strength of crushed and whole shell containing concrete (4.3.2.1), it was decided to create an entirely new 10-litre batch to the following specifications:

- Mussel shells 0.5kg
- Sand 10.0kg
- Cement 2.4kg
- 13mm basalt 5kg
- 7mm basalt 7kg
- Water 2.5 litres
- Air entraining agent 65ml

An air-entraining agent (a detergent) was added to trap air in the form of small bubbles. This increases potential workability, finish and can protect the concrete during freeze/thaw cycles

when used outdoors. On this new batch, the following tests were carried out:

- Slump (prior to air testing and immediately after)
- Air pot testing
- Mix-water bleeding
- 7-day mould testing

It must be noted that Trial 2 moulds for 7-day compression testing had the topsides coated with plaster to ensure a smooth, flat surface for even pressure application.

4.2.4 Compressive strength analysis

For compressive strength testing, three cylindrical metal moulds were lubricated with oil (*Formol*) and filled with the test mix; in a similar manner to the slump cone (tamping 25 times at each one-third fill of each mould). Each mould was filled to excess and the surface was struck off (levelled) with a trowel. Each filled mould was left for at least 16 hours (overnight) on a bench top to harden. After 16 hours, the concrete was set enough for the metal moulds to be removed (Fig. 26) and the concrete cylinders were placed in a water bath at room temperature (ponding) according to the *New Zealand Concrete Construction Standard NZS 3109.* After 7 days of curing in water, one test cylinder out of three was removed and allowed to drain for approximately 30 minutes. The cylinder was then placed in a compression stress-strain ram unit with increasing applied load until the first sign of cracking (Fig. 27). The same testing process is carried out of the 14^{th} and 28^{th} days of curing, respectively. In the tests undertaken in this project, the maximum load was measured in megapascals, MPa (SI) (a measure of pressure), where 1MPa = 145 psi or 106 N/m^2) (Callister, 2003).

Figure 26: A test cylinder used in the compressive strength determination.

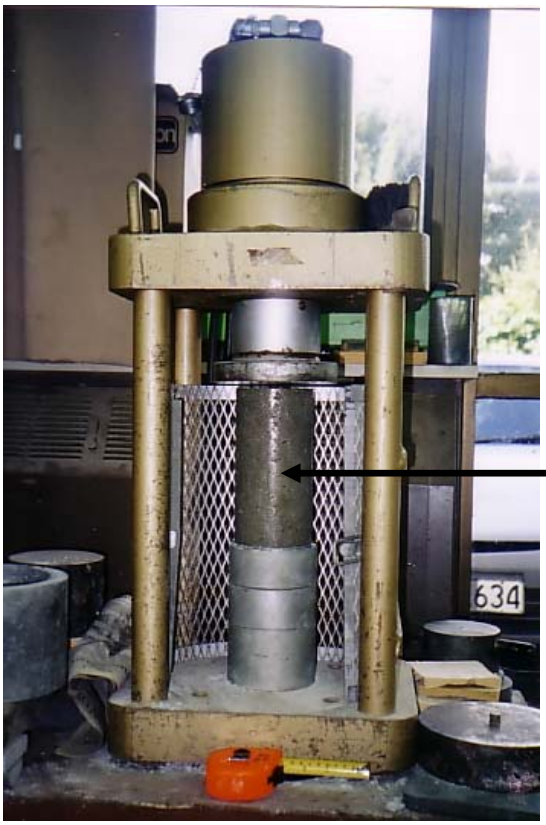

Figure 27: Compressive Strength testing equipment (Photo: C. Barnaby, 2003).

4.2.5 Determination of the bleeding rate of mix-water in wet concrete

A fresh batch of Trial 1 cement mix was made to the same ratio proportions stipulated in 4.2.3.1. This was combined in a small mechanical mixer. A dense cylindrical metal vessel (12 litre capacity) was filled one-third with the wet mix and tamped 25 times with a rounded steel tamping rod, (16mm diameter, 600mm long) to remove air. Another one third was added, tamped and then the final third also tamped. A rubber-headed mallet was used to strike the sides of

the vessel three times to promote compaction. The full vessel was weighed and initial surface bleed water was decanted and discarded. The vessel was placed on a bench and tilted slightly to allow bleed water to pool on one side. At 15-minute intervals the bleed water was pipetted from the surface and placed into a measuring cylinder. The amount of every aliquot at each 15-minute interval was recorded. This process was repeated until bleeding ceased. Bleeding was considered to have ceased when the amount of water drawn from the surface was less than 5ml at the end of an interval. Similarly, this test was carried out for the Trial 2 batch (as per 4.2.3.2 — containing mussel shells, air entraining agent (AEA) and basalt aggregates).

4.2.6 Determination of trapped air in wet concrete

Trapped air quantification was carried out for mix Trial 2 only. A Humboldt brand "*Press-ur-meter* for Measurement of Entrained Air" (Charles R Watts and Co.) was used in this test (complying with NZS 3112 Part 1: 1986) (Fig. 28). The pot was filled in the standard method of one-third at a time with 25 strikes of the tamping rod at each fill. When the pot was filled, a rubber mallet was used to strike the sides of the vessel, two to three times for compaction. The surface was then levelled to ensure a tight seal with the pot lid. The lid was replaced and water was injected through one petcock until all air had escaped through the other. The dial hand was stabilised by pumping to the desired initial pressure line (this unit was calibrated to -2% air). Pressure was applied to the sample to compress the entrained air in the pores. The amount of air present in the sample was read as percentage air. This test was carried out in accordance with the *New Zealand Standard 3112: Part 1: 1986 (Section 9) Determination of the Air content of fresh concrete by the application of pressure.*

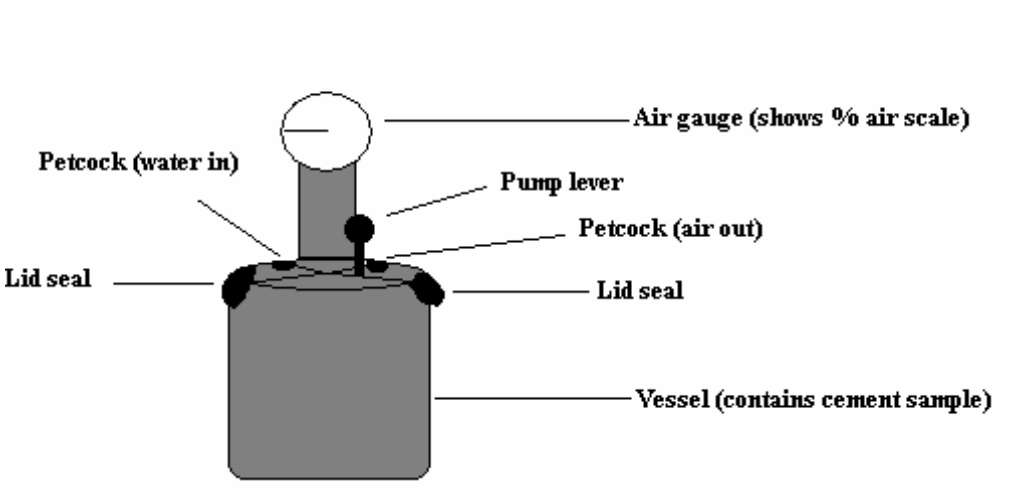

Figure 28: Sketch of an air-entraining meter used to gauge the quantity of entrained air in a fresh concrete mix.

4.2.7 Determination of basic thermal properties

To test the insulation potential of mussel shell concrete (MBT1), a slab (30cm x 30cm x 3cm) of mixer-broken shell concrete was made using residual mix from a compressive strength test batch (Fig. 29). The slab was placed onto a hotplate (set to roughly 100ºC ± 10). A thermocouple wire probe was placed into a pore hole on the underside of the slab and a small square of steel (~3mm thickness) was placed between the thermocouple wire and the hotplate surface to hold the wire in place. A second thermocouple was placed into a pore hole on the topside of the slab and held in place by another square of steel. A limestone brick was placed on top of this square to prevent transferred heat from escaping too rapidly. The hotplate was switched on and allowed to heat to 100ºC (Fig. 30). The ambient (initial) temperature was recorded and from thereon, the temperature was recorded at five-minute intervals until it either levelled off or only changed gradually by one degree, by that point at which it was decided that there would be no further significant changes in temperature. This test was repeated for a polystyrene slab and a concrete stab made to industrial standards (IS), that is, standard methods described in the *New Zealand Standards for Concrete*. These additional slabs were of similar proportions to the test slab and were included for comparison. Each slab was tested twice.

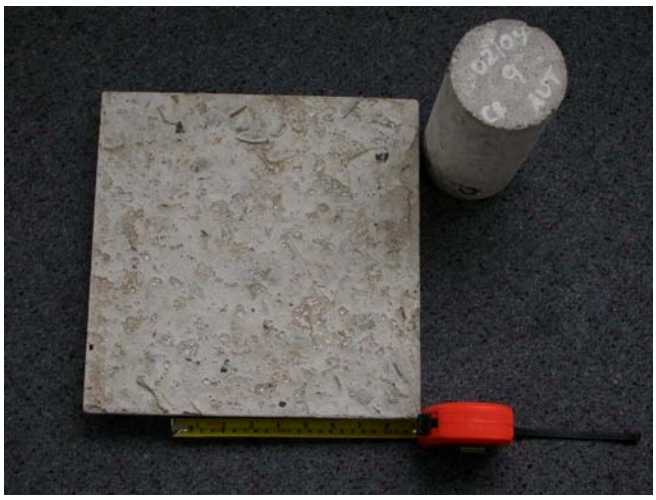

Figure 29: Slab of mussel shell-containing concrete used in the thermal properties determination.

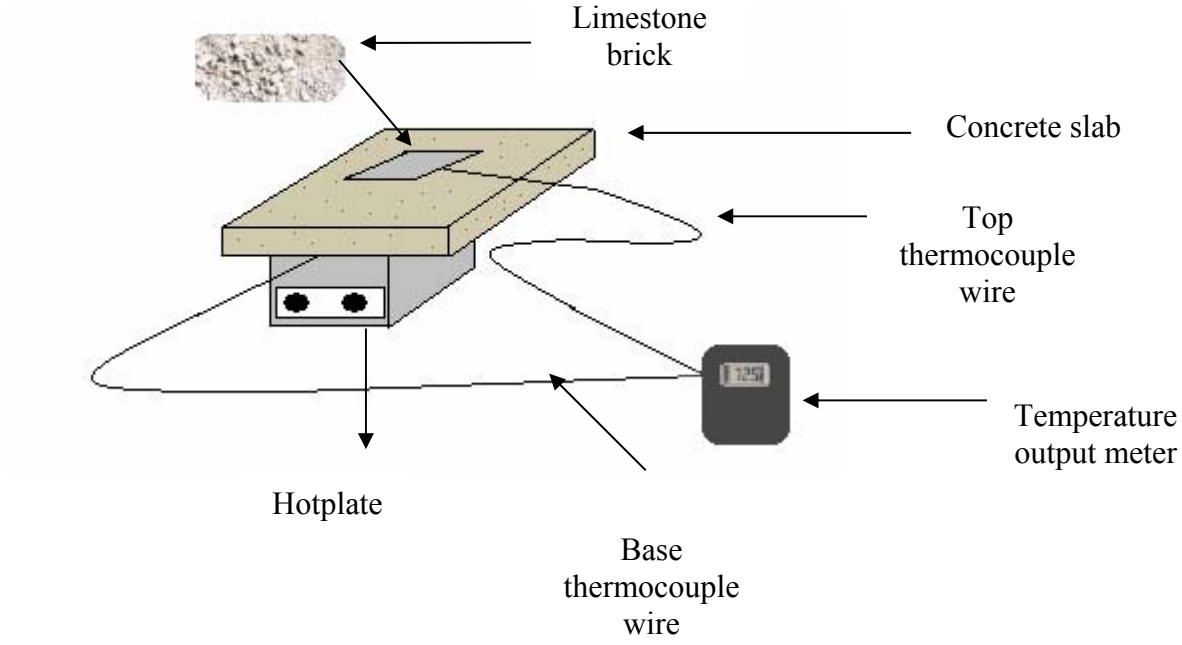

Figure 30: Schematic drawing of the setup used to determine relative heat transfer rates through mussel shell concrete, 'ordinary' concrete (as a control) and polystyrene.

4.2.8 Data analysis

Software packages used for statistical analysis were Minitab™ Statistical Software v.13.32 GraphPad InStat® v.3.06. Concrete slump differences between mix-types was analysed using a one-way ANOVA (Minitab™). '7-14-28 day' compressive strength differences and bleed test data were analysed with

a 2-way ANOVA (Minitab™) and the 7-day only compressive strength tests were analysed for variance using a General Linear Model and Tukey Simultaneous Tests for pairwise comparisons (GraphPad InStat®). Heat transfer differences between composites were analysed using a repeated-measures ANOVA (GraphPad InStat®).

4.3 Results and data analysis

4.3.1 Slump tests

A significant difference was apparent when comparing the slumps of Trial 1 to that of Trial 2, with the Trial 2 slumps more substantial at 100mm (normal, expected range) (ANOVA; $F_{2,7}$=207.64, p=0.0000) (Fig. 31, Table 15).

Table 15: Analysis of Variance for Slump.

Source	DF	SS	MS	F	P
Batch	2	11018.8	5509.4	207.64	0.000
Error	5	132.7	26.5		
Total	7	11151.5			

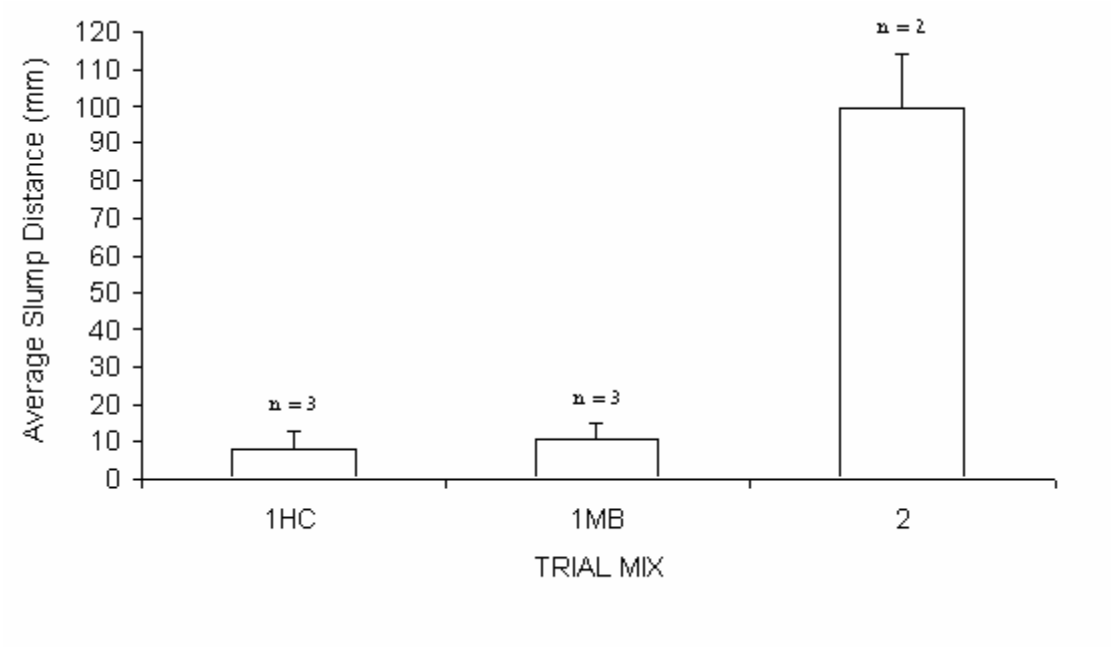

Figure 29: Average slump values for the two trial mixes. Note AEA = air entraining agent. 1HC=Trial 1 (hand crushed); 1MB = Trial 1 (mixer broken).

4.3.2 Compressive Strength of mussel shell concrete

4.3.2.1 Trial 1 Mix

The plotted standard is the theoretical results for 'ordinary' concrete and is included as a comparison. Although exhibiting a slight increase in compressive strength, HCT1 strength increased linearly with respect to time. MBT1 concrete did not exhibit a linear relationship with time. In

comparison with the standard, all values for mussel shells in concrete yielded a significantly low compressive strength. However, statistical analysis showed that there was no significant difference between the compressive strength of HCT1 and MBT1 over the 28-day test period (ANOVA; $F_{2, 12}$=0.63, p= 0.55 (Fig. 32, Table 16).

Table 16: ANOVA: statistical summary for the 28-day compressive strength test.

Source	DF	SS	MS	F	p
Mix type	1	0.97	0.97	0.39	0.544
Day	2	1.26	0.63	0.25	0.781
Interaction	2	3.12	1.56	0.63	0.551
Error	12	29.90	2.49		
Total	17	35.25			

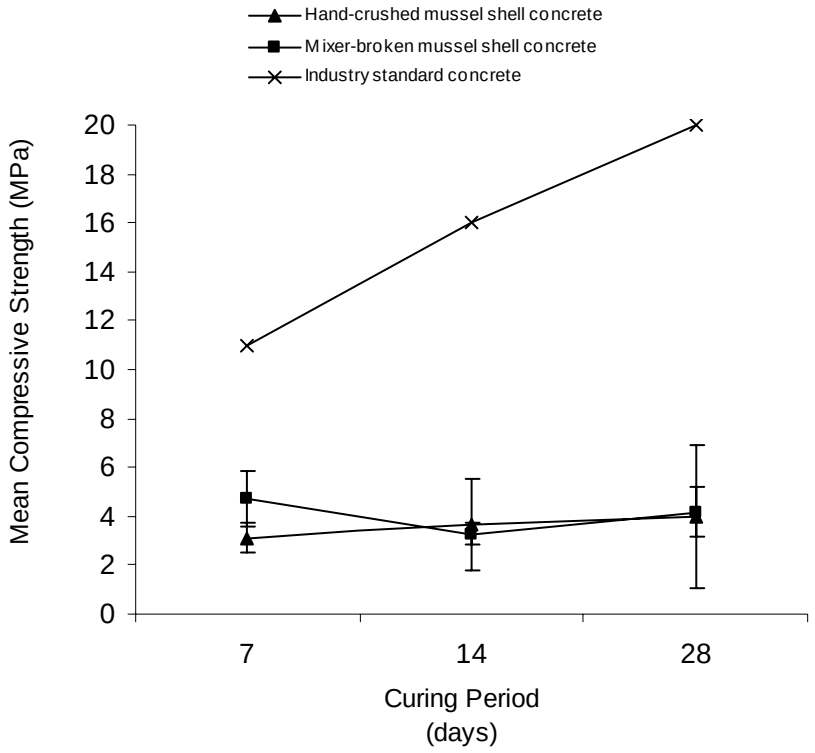

Figure 30: The compressive strength of concrete containing mussel shell as a coarse aggregate at 7, 14 and 28 days.

4.3.2.2 Trial Mix 2

Trial Mix 2 was analysed for 7-day strength only and then compared to Trial Mix 1 and cylinders formulated to industry standard specifications. Although a general linear model ANOVA indicated that the industry standard (IS) samples were significantly stronger than all the trial mussel mixes ANOVA; $F_{1,6}$=144.49, p=0.000) (Fig. 33, Table 17). A subsequent Tukey test indicated that the only significant differences

between 7-day compressive strength-tested mixes, were between the industry standard mix (IS) and each of the three mussel shell mixes (HCT1, MBT1 and MBT2) (p=0.0000, for each comparison) (Table 18).

Table 17: A statistical summary for the 7-day only compressive strength test. Results of a General Linear Model of strength versus mix type.

Source	DF	SeqSS	AdjSS	AdjMS	F	p
Mix type	1	3290.784	290.784	96.92	144.49	0.000
Error	6	4.025	4.025	0.671		
Total	9	294.809				

Table 18: Tukey Test: A statistical summary for the 7-day only compressive strength test. Pairwise test for differences in strength between hand-crushed trial-mix 1 (HCT1), machine-crushed trial-mix 1 (MBT1), machine-crushed trial-mix 2 (MBT2) and the Industry Standard Mix (IS).

TUKEY: Mix Type	p-value
HCT1xIS	p=0.0000
HCT1xMBT1	p=0.1789
HCT1xMBT2	p=0.9270
ISxMBT1	p=0.0000
ISxMBT2	p=0.0000
MBT1xMBT2	p=0.4733

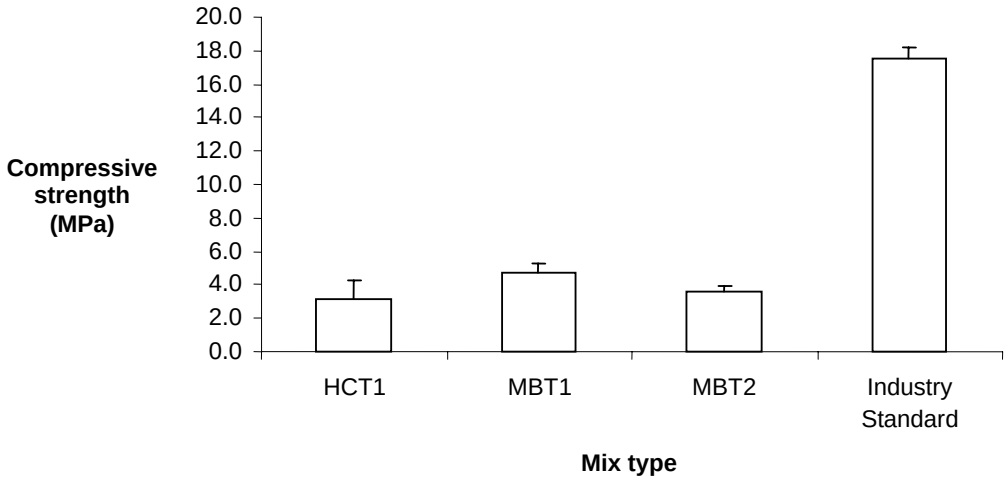

Figure 31: Seven Day only compressive strength test results for a hand-crushed Trial 1 mix (HCT1), a mixer-broken Trial 1 mix (MBT1), a mixer-broken Trial 2 mix (MBT2) and an industry standard mix. Standard deviations: HCT1 = ±1.2; MBT1 = ±0.6, MBT2 = ±0.3; IS = ±0.7.

4.3.3 Determination of the bleeding rate of mix-water in wet concrete

The total quantity of bleeding water was found to be 0.049L (equivalent to approximately 11.55 L/m^3). This can be compared to the industrial standard (IS) mix, which yielded 0.089L (20.99L/m^3). The bleed water displaced completely in one hour which was rapid as the IS concrete with stone aggregates took 3 hours to completely displace all bleed water, which is expected (Fig. 34).

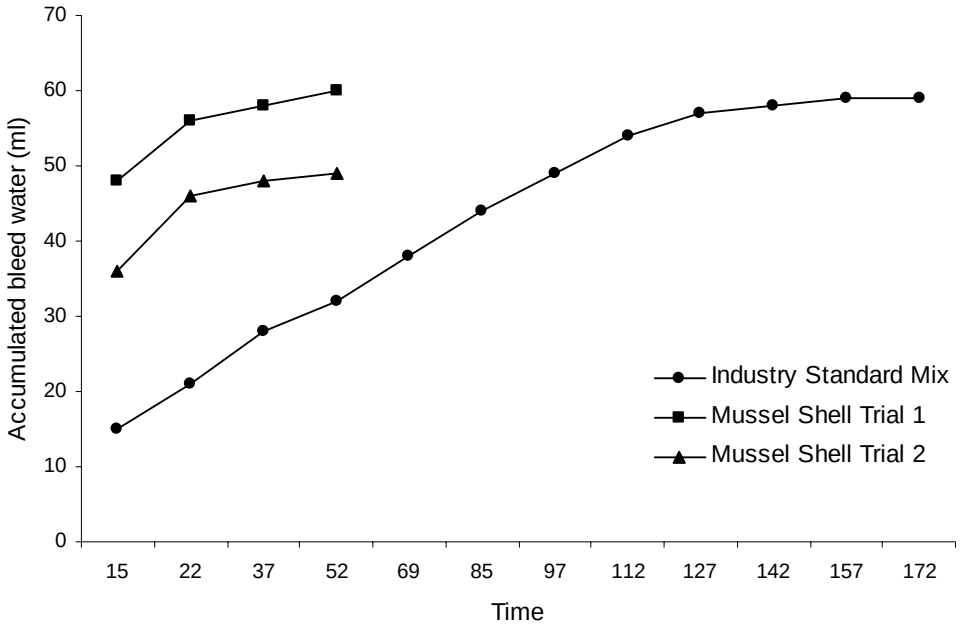

Figure 32: Chart showing bleed water accumulation for both trial mussel shell mixes and an industrial Standard mix.

The total quantity of bleed water for Trial 1 Mix was 9 L/m^3 concrete and Trial 2 Mix bled 7 L/m^3 These total amounts are expected but bled too quickly (between 50 to 60 minutes) in comparison with the industrial standard mix which took almost three hours to excrete all bleed water. A Friedman Test (a non-parametric repeated measures ANOVA) indicated that there was a strong difference with increasing time between the industry standard (IS) mix and the two mussel shell mixes ($p<0.0001$). A subsequent post test (Dunn's multiple comparisons test) indicated strong differences in bleed water outputs with increasing time between the IS mix and mussel shell mix Trial 1 ($p<0.01$) and slightly weaker differences between the IS mix and mussel shell mix Trial 2 ($p<0.05$).

4.3.4 Determination of trapped air in wet concrete

This test was only carried out on batch TRIAL 2 (MBT2) as an additional test for potential strength and workability. Running the air pot test on a fresh batch of this mix yielded a value of 6.5% air. This can be compared to a batch, run using a standard aggregate, ordinary-grade concrete, which yielded an air content of 4.5%. This is the accepted limit for the amount of air contained in a fresh mix, therefore MBT2 contained too much air to be a structurally sound concrete.

4.3.5 Determination of basic thermal properties

A repeated-measures analysis of variance indicated that overall, that there was a very significant difference in the heat transfer over time between the three test slabs ($p=0.0016$). A Tukey-Kramer multiple comparisons test then indicated that between the slabs, significant differences were apparent between T1M (mussel slab) and ISM ($P<0.010$) and between ISM and PolyA ($p<0.010$). There were no significant differences between the mussel slab and the polystyrene slab indicating similar insulation properties ($p>0.050$) (Table 19, Fig.35).

Table 19: Tukey results for thermal conductivity between the test slab (T1M), the industry-standard mix slab (ISM) and the polystyrene slab (PolyA).

TUKEY:Slab Type	p-value
T1M x ISM	P<0.010
PolyA x ISM	P<0.010
T1M x PolyA	P>0.05

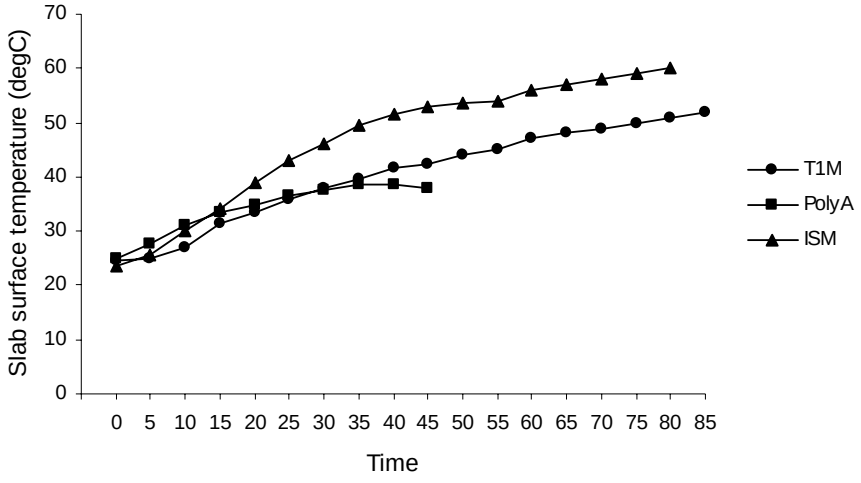

Figure 33: Basic heat transfer test comparisons between composites. T1M=slab created from Trial 1 mix (MBT1); PolyA=a polystyrene slab, an industrially used insulator; ISM = industrial standard mix slab made as per NZS3109.

4.4 Discussion

4.4.1 Structural Testing —Slump and Compressive Strength

There were no significant differences in the concrete strength over time between whole shell and crushed shell aggregates concrete mixes. Moreover, whole shells tended to fragmentise naturally in the industrial mixer and this may account for the lack of significance in the differences between whole shell and hand crushed shell concrete mixes. The results of the compressive strength tests indicated little to no structural integrity in the final concrete product for either crushed or whole shells. This lack of structural strength was likely to be directly related to the brittle nature of mussel shell in comparison to more commonly used stone aggregates. In addition, it was observed that the periostracum (chitinous, organic upper layer) did not completely dry as the concrete cured and this moisture retention may have exacerbated the weakness of the final product. According to Callister (2003), the character of aggregates, more specifically the size distribution, influences the amount of cement-water required. When utilising gravel aggregates, each must be free of silt and clay, as this can decrease adhesion. Mussel shell, however clean, may simply be too smooth for adequate adhesion.

Slump test results were included in this thesis to demonstrate the importance of the water: cement ratio. Inadequate water added into the mixes, resulted in the low slump readings for both Trial 1 mix replicates. The low slump readings are unlikely to be due to the nature of the shell aggregates, as Trial 2 slump had additional non-shell aggregates (basalt) and yet had a 100mm slump, which is expected. In this research, slump was a major factor in describing mix workability and to give an idea if water quantities were sufficient. It is unlikely to have had a direct effect on thermal properties. This may be accounted for by previous research by Uysal *et al* (in press) which states that tests have shown thermal conductivity to fluctuate with slump (due to fluctuations in density), thus no correlation is conclusive.

Placing a piece of litmus paper (for pH testing) on the interface where the damp organic layer (periostracum) contacted the cured concrete, indicated slight

acidity, which would lead to weakness at this point — as cement is naturally alkaline. It can thus be concluded that mussel shell concrete has low structural integrity and is not recommended for use where load-bearing is required. Another reason for the lack of compressive strength may be attributed to the shell pieces being too large for the 2-litre test cylinder, to allow for adequate bonding of the cement particles. According to Lee *et al*. (2002), a cement matrix with a very open pore structure can result in very low or very high strength and durability and this may be referred to as a discontinuous pore structure. The irregularity and shape of whole mussel shells may help to explain the variation in resulting compressive strengths (from high to low to high – Fig. 32) in the whole shell concrete sample.

Mussel shell concrete may have a low compressive strength but that does not mean it is of no use in industry. Pumice concrete has been utilised since 1980 in New Mexico and like mussel shell concrete, has a very low compressive strength ranging from 3 MPa to 16 MPa (mussel shell concrete in this project was found to range from 2MPa to 7.5MPa), and pumice concrete is slowly establishing itself as a useful insulating concrete although more research is required (Lambourne, 2000). In 1998, an Auckland architect, Rick Lambourne, investigated the creation of pumice concrete walls for houses. He found it to be lightweight, have good thermal insulation, excellent soundproofing and fire resistance. The negative aspects to this concrete were the cost of transporting pumice from the source (expensive outside the central North Island), non-uniform raw materials, unpredictable water absorbance, low compressive strength, shrinkage problems and the production of harmful silica dust produced when grinding pumice (Lambourne, 2000). In comparison, mussel shell concrete has a similar compressive strength to pumice concrete, is a stable, inert and lightweight aggregate and does not produce toxic dusts when ground or pulverised.

4.4.2 Determination of the bleeding rate of mix-water in wet concrete

The results indicated that mussel shell concrete (either with shell as the only aggregate (Trial 1 mixes) or mussel shell with basalt aggregates (Trial 2 mix)), had rapid bleed rates in comparison with an adjacently-run industry standard mix, which took almost three hours to release all bleed-water. Bleed quantities

of each trial mix differed and Trial 2 Mix bled slightly faster than Trial Mix 1. Variations in concrete bleeding rates and volumes are attributed to several factors; the cement : water ratio (Tamimi, 1994; Topcu and Elgun, 2004), the quantity and reactivity of the cement (Wainwright and Ait-Aider, 1995) and relative absorptivity of the aggregates and pore spaces (Bjøntegaard *et al*., in press). The rapidity of the bleeding of the trial mixes may relate to the non-absorbent properties of smooth mussel shell. In addition to this, the larger pore spaces, (determined by the high air content and nature of the unbroken sections of shell umbos—the raised lateral structure above the valve hinges), may reduce the impervious nature of the final concrete product. Thus, these spaces may hold larger amounts of unabsorbed mix water (or air), which bleeds from the surface.

4.4.3 Determination of trapped air in wet concrete

This test was carried out on mix TRIAL B (MBT2) only, because according to ASTM C231-03 Standard Test Method for Air Content of Freshly Mixed Concrete by the Pressure Methods, this test is unsuitable for mixes with light-weight aggregates such as the TRIAL 1 mixes. TRIAL B mix MBT2 contained two basaltic aggregates (13mm and 7mm), which were added as fillers. Without the addition of extra aggregates of varying sizes, the mix appeared too sandy. The results of this test yielded an air volume of 6.5%. This was 2.5% greater than the accepted volume of air in wet mixes (4.5%) (T. Walker, pers.comm., 2003). The more air a wet mix contains, the weaker the resulting concrete is likely to be. One possible reason for the high air volume in MBT2 may be due to the large spaces created by larger, unbroken pieces of shell, especially near to the shell umbo, where the structure is harder and would not have broken completely by the action of the mixer. If mussel shells were to be utilised as a filler in future work, it is recommended that they be crushed, with especial attention paid to ensuring the umbo is flattened.

Pore spaces must also be considered when determining the pH of a composite. The larger a pore space, the more water it is likely to hold and pore water alkalinity affects the durability of concrete structures (Li *et al*., in press). This may have been an issue for the mussel shell concrete. Weak interfacial bonding between cured concrete and mussel shells was observed and a litmus test indicated high alkalinity at the interface. The smooth nature of mussel shells

aside, this lack of adhesion may be due to chemical reasons, whereby the corrosion threshold may have increased and could have resulted in the observed cracking (Li *et al*., in press).

4.4.4 Thermal properties

Most of the literature concerning the thermal properties of concrete examines thermal conductivity, which is equivalent to the quantity of heat that passes in over time through an area, when it's opposite faces are subject to a unit temperature gradient. The mussel shell slab was found to transfer less heat over time than the industry-standard (IS) slab, suggesting it may have greater potential as an insulating concrete product. As a control, it was found that there were similarities in the heat transfer rate between the mussel shell slab and polystyrene, a common, industrially used insulation material. According to Kim *et al*. (2003), several factors influence thermal conductivity (and thus heat transfer) of concrete; early age (<2 days old), aggregate volumes, amount of cement, admixture quantities, fine aggregate fractions and moisture.

Water thermal conductivity (TC) is superior to that of air and may be one explanation for the higher rate of heat transfer in the industry-standard slab (ISM) than the mussel shell slab (Thompson, 1968; dos Santos and Cintra, 1999; Khan, 2002; Shin *et al*., 2002; Demirboga, 2003; Demirboga and Gül, 2003b; Kim *et al*., 2003). The IS slab was 3-4 days old and the mussel shell slab was 6 months old. This was not originally considered a significant problem when comparing the two, because according to Kim *et al*. (2003), age only affects conductivity at 2 days or less. However, as the experiment was being carried out on the ISM slab, a very small amount of condensation was observed on the surface steel plate. This was not observed on the older mussel shell specimen suggesting that the ISM slab may have not cured sufficiently for true comparison. This may be one reason for the higher heat conduction of the ISM slab. This is concurrent with work by Khan (2002) who found that from a 50% degree of saturation, the rate of increase of TC is more significant. Pore size strongly effects thermal (and electrical) conductivity, is correlated with water content effects and is likely to have had a significant effect on simple heat transfer (Bouguerra *et al*., 1998). The TC of lightweight concrete changes

significantly with porosity and in addition, the more aggregates there are in the mix, the more macropores will result (Bouguerra *et al*., 1998). Although the mussel shell slab contained less aggregates by weight than the IS slab, the shell pieces were larger than the basalt aggregates in the IS slab. This may account for the reduced heat transfer in the mussel shell slab. This is because, as porosity increases, thermal conduction decreases (dos Santos, 2003). Another factor that has been found to increase conductivity is large quantities of quartz in a mix (Khan, 2002). However, the IS slab which transferred more heat actually contained less sand (which contains quartz) than the mussel shell slab by ratio, therefore sand in this case can be disregarded as having an effect on the heat transfer.

Thermal conductivity (TC) is also influenced by the physical characteristics of the aggregates (Harmathy and Allen, 1971; Kim *et al*., 2003). Aggregate TC and that of the concretes made with them, depends on the aggregate's internal microstructure and its mineralogy (Harmathy and Allen, 1971). The conductivity of highly crystalline aggregates (i.e. those having a well defined microstructure) is high at room temperature and decreases as temperature increases (Harmathy and Allen, 1971). Unstructured aggregates, such as the basalt in the IS slab, exhibit low TC at room temperature. This increased slightly as the temperature rose, which was apparent for the IS slab and the mussel shell slab (Harmathy and Allen, 1971). It must be noted that the TC of calcium is 201 W/m-K whereas the TC of basalt (which varies according to porosity) is considerably lower, which one reference provides as 1.7 W/m-K as an average value (University of Texas, 2003). Therefore in this case, the aggregates are unlikely to be having the main effect on heat conduction. Supplementary work is required to test this further.

Mussel shell concrete is heavy and because it has some latent insulation properties it may have potential to be used as a bulky insulating product for structures such as free-standing chicken coops, (where heavy load-bearing or tensile strength is not required) where it is necessary for heat to be kept inside. It must be noted that if mussel shell concrete were to be used in the creation of insulation walls or light load bearing floors, it may need to be reinforced with steel truss wires or similar reinforcement such as carbon fibre or plastic (J.

Buckeridge, pers.comm., 2003). Steel trusses have been found in past studies to affect the thermal properties of the walls due to competition between reduced heat transfer by the insulation layer and the through-wall conductivity of the metallic truss wires. It has been recommended that sparse low-conductivity truss wires be utilised in conjunction with a dense insulation layer to enhance thermal performance (Jones and Jones, 1999).

4.4.5 A question of sustainability — limestone mining versus mussel shells

Lime (calcium carbonate) has been used for centuries since ancient Egyptian times and is used today in agriculture, roading, construction, water treatment (as a flocculent), mining, steel manufacture and as a bonding agent in cements and mortars (McDonald's Lime website, cited 05/01/04). Lime in its untreated state is calcium carbonate and this is the predominant chemical compound in mussel shell. When calcium carbonate undergoes thermal decomposition (calcination) at 1400-1500°C it is converted to calcium oxide (quicklime) (Fig. 36). Quicklime is converted to calcium hydroxide (slaked lime) by the addition of water (Fig. 4). Mussel shell lime may contain more beneficial micronutrients (previously assimilated by the live organism), essential for optimum plant growth (A. Blackburn, pers.comm., 2002) and therefore further studies should examine the possibilities.

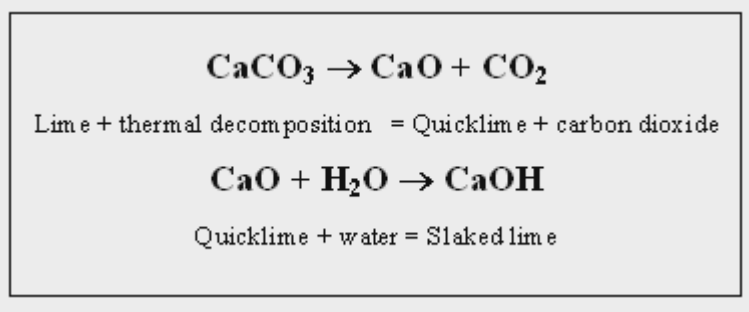

Figure 34: From calcium carbonate to quicklime and slaked lime. Chemical equations.

Limestone quarries are common in New Zealand and yield exceptionally high-grade limestone [purity exceeding 95%] (McDonald's Lime website, cited 05/01/04). Some mining companies, however, resort to limestone blasting. This process is both environmentally detrimental and wasteful as it can destroy the

quarry hill and the limestone in the process. Utilising mussel shell as a source of lime would consequently be an 'environmentally friendly' approach (Fig. 37).

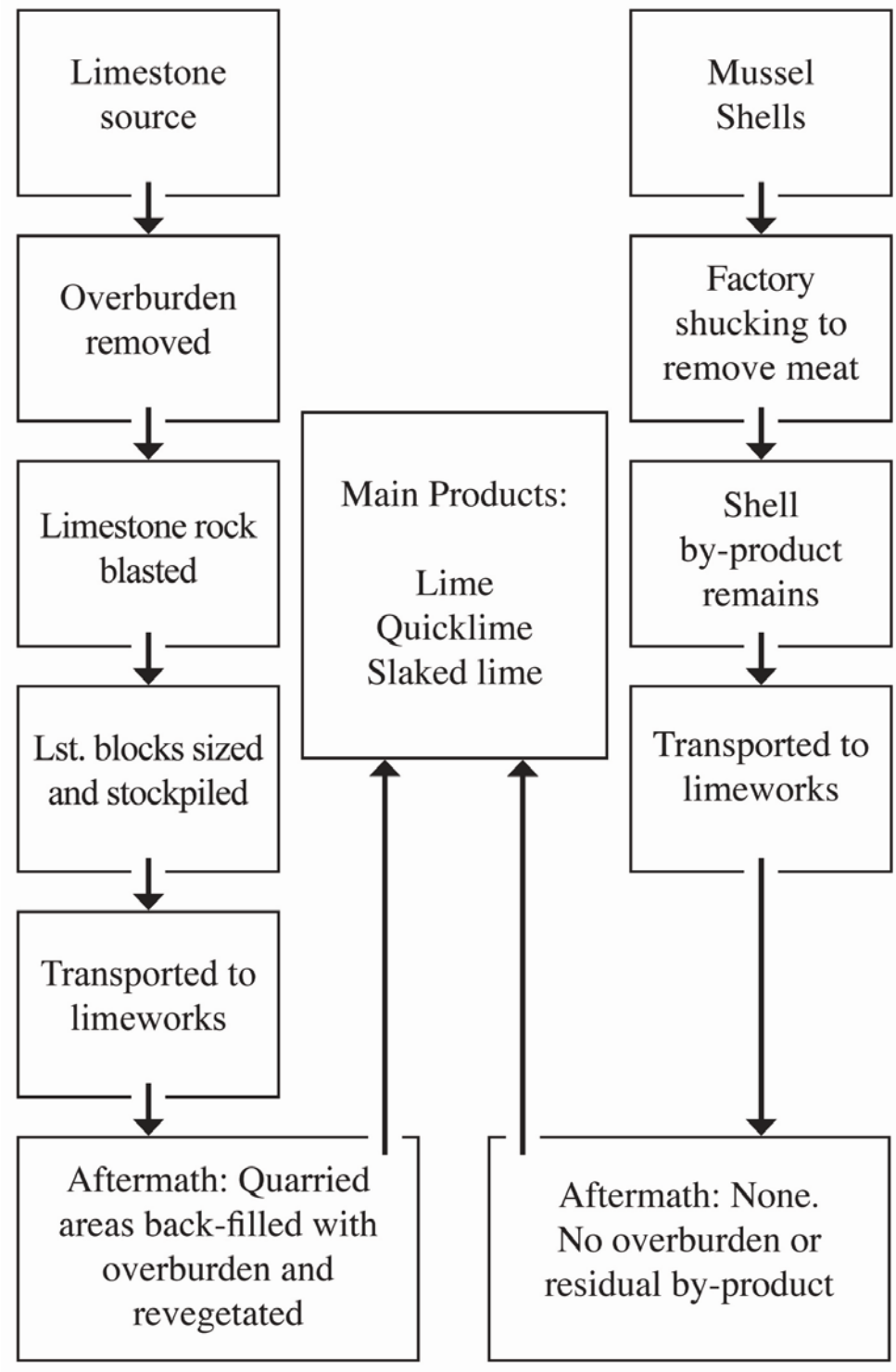

Figure 35: Current conventional lime production versus a theoretical/fictional lime production process using mussel shell as a source. A feasibility comparison.

4.5 Conclusions

Concrete containing mussel shells as a coarse aggregate has very little potential as a structural composite due to a low compressive strength, that is, not suitable for load-bearing. The results however indicate that this composite may have potential insulating properties. Further work, examining specific thermal conductivity over a greater range of temperatures, is required.

Mussel shells can easily be converted to burnt lime products by calcination. This could be useful in cement manufacture. The only environmental concern with calcining mussel shells would be the emission of the greenhouse gas, carbon dioxide. However, this would be the only principal by-product, because unlike conventional lime extraction, no afterburden or damage to the land would result.

Chapter 5 — General Discussion

In addition to bulk shell, the next principal solid waste was found to be pre-grade waste, which *Perna canaliculus* (whole broken and whole undersized) and *Mytilus galloprovincialis* dominated proportionally. The three other main groups identified in this waste (epibionts, other molluscs and mobile scavengers), were found to be insignificant in proportion to the amounts of *P. canaliculus* and *M. galloprovincialis*. Consequently any research into re-use methods should not only target shell waste but also consider whole mussel waste as the quantities estimated suggest that much usable protein is discarded.

The results of the mussel fertiliser trials suggest that *P. canaliculus* tissue may be an effective, inexpensive organic fertiliser when allowed to decompose into simpler compounds, which are more bio-available to plants. It was also found that in comparison, *M. galloprovincialis* showed the least potential as a fertiliser. Overall, it is recommended that future studies focus on *P. canaliculus* fertiliser further, with particular consideration given to stabilising the product from uncontrolled decay and undesirable odour.

The trial incorporating *P. canaliculus* shell into cement mix as a coarse aggregate indicated that the final concrete product did not have any structural potential, and would not conform to New Zealand Standard 3108 (1983). However, additional tests run on heat transfer potential suggested that this product may be useful as an insulator as it demonstrated slower rates of heat transfer than a comparative industrial standard concrete.

In terms of producing other lucrative products, it is recommended that *P. canaliculus* shell (non-calcined) be tested as a fertiliser base or additive. It could be of use for when

traditional limestone stocks decline or to prevent damage to the land by quarrying. *M. galloprovincialis* could be further examined for its potential to be marketed alongside *P. canaliculus* as a meat product, but more specifically for international markets that are already accustomed to this genus of mussel as a culinary delicacy. Due to the uniqueness of *P. canaliculus*, the ubiquitous *M. galloprovincialis* is unlikely to be a competitor on the international markets alongside its green counterpart. Utilising harvested *M. galloprovincialis* would help to solve the problem of resettlement which can occur when they are discarded live, following *in situ* removal from the mussel long lines.

Due to the significant disciplinary boundaries in which this thesis transverses, it is recommended that both an interdisciplinary team and approach is necessary, should further work be undertaken in this area of research.

Appendix 1: Summary of quantification data

Summary results for average component weights from bulk seasonal samples

Season	Year	Factory	*Perna*	*Mytilus*	*Epibionts*	*Other Mollusca*	*Mobile scavengers*
Spring/Summer	*2002*	A	216.18	14.10	691.71	75.81	11.06
Spring/Summer	*2002*	B	947.40	29.25	12.50	1.30	15.90
Spring/Summer	*2002*	C	567.20	250.80	152.08	0.00	50.89
Autumn/Winter	*2003*	A	986.90	0.00	13.10	0.00	0.00
Autumn/Winter	*2003*	B	936.20	43.30	15.20	1.30	28.00
Autumn/Winter	*2003*	C	240.62	661.50	97.87	0.00	0.00
Spring/Summer	*2004*	A	74.09	882.97	23.22	9.44	10.28
Spring/Summer	*2004*	B	429.81	402.42	146.25	21.45	0.07
Spring/Summer	*2004*	C	563.40	224.83	184.14	16.24	13.16

Note: all weights in grams per kilogram of sample

Appendix 2: Results of fertiliser analysis

Analysis was carried out at Hill Laboratories, Hamilton, New Zealand. The following abridged data table was reproduced with permission:

Sample Name	Lab No	Total N (g/100g)	P (g/100g)	K (g/100g)	Ca (g/100g)	Mg (g/100g)
AG1	322866/1	9.500	1.970	1.470	0.442	0.567
AG2	322866/2	9.130	2.170	2.120	0.631	0.528
AG3	322866/3	9.060	1.750	1.880	0.376	0.336
BG1	322866/4	1.020	0.363	0.749	41.700	0.091
BG2	322866/5	0.990	0.182	0.711	42.100	0.084
BG3	322866/6	1.140	0.196	0.741	36.900	0.091
DB1	322866/10	7.640	1.620	1.980	1.330	0.649
DB2	322866/11	7.580	1.730	2.400	0.722	0.670
DB3	322866/12	8.030	1.640	2.130	1.820	0.658

Notes:

1) 1,2,3 refer to replicate samples
2) Nitrogen - Dumas combustion using Elementar VarioMAX instrumentation.
3) P,K,Ca & Mg - nitric/HCl acid digestion and ICP-OES determination
4) Analysis carried out on 06.10.03
5) Initial drying and grinding of samples was done by C. Barnaby at AUT. (samples placed in oven at 80° C for 24 hours, cooled then ground)

Appendix 2A: Summary of fertiliser trial data – mean counts

LEAF COUNT

	Treatmt	Sep-03	SD	Oct-03	SD	Nov-03	SD	Dec-03	SD
n = 39	CONTA	15.23	5.49	34.87	6.98	52.90	12.33	84.82	15.22
n = 39	CONTB	15.21	4.79	29.69	6.48	46.47	7.46	73.55	11.34
n = 39	AG	13.72	4.08	28.49	7.93	46.81	9.17	72.38	15.30
n = 39	BG	12.46	3.50	31.18	4.77	47.21	8.29	79.49	11.94
n = 39	DB	13.08	4.90	31.05	5.56	47.61	9.95	73.67	12.84

		Jan-04	SD	RANK
n = 39	CONTA	138.79	21.85	1
n = 39	CONTB	117.65	18.13	5
n = 39	AG	127.32	24.53	2
n = 39	BG	124.11	18.52	3
n = 39	DB	121.97	21.26	4

STEM HEIGHT

	Treatmt	Sep-03	SD	Oct-03	SD	Nov-03	SD	Dec-03	SD
n = 39	CONTA	47.15	10.59	108.95	18.17	167.28	27.01	276.58	45.03
n = 39	CONTB	52.33	13.17	106.51	24.45	179.20	30.00	285.76	46.55
n = 39	AG	49.83	15.15	99.11	24.37	156.85	36.51	268.14	43.12
n = 39	BG	49.78	12.00	104.46	19.09	165.04	27.89	271.87	54.52
n = 39	DB	49.47	12.17	103.67	27.02	172.12	39.44	279.77	43.83

		Jan-04	SD	RANK
n = 39	CONTA	448.97	70.38	2
n = 39	CONTB	442.49	63.49	4
n = 39	AG	456.92	83.51	1
n = 39	BG	444.24	71.10	3
n = 39	DB	433.57	82.32	5

BRANCH COUNT

	Treatmt	Sep-03	SD	Oct-03	SD	Nov-03	SD	Dec-03	SD
n = 39	CONTA	3.85	0.74	6.92	0.87	8.67	1.44	11.29	1.49
n = 39	CONTB	3.92	0.90	6.36	0.81	10.18	1.39	10.18	1.39
n = 39	AG	3.64	0.81	6.59	1.04	7.86	1.29	10.68	1.20
n = 39	BG	3.46	0.72	6.51	0.86	7.74	0.99	11.13	1.08
n = 39	DB	3.59	0.91	6.56	0.94	8.03	1.32	10.69	1.45

		Jan-04	SD	RANK
n = 39	CONTA	15.24	1.82	1
n = 39	CONTB	13.54	1.30	5
n = 39	AG	14.46	1.76	2
n = 39	BG	13.92	1.59	4
n = 39	DB	14.38	1.82	3

Appendix 2B: Summary of fertiliser trial data – 5mL:2L concentration effects (mean counts)

Parameter	Treatment	Sept	SD	Oct	SD	Nov	SD	Dec	SD	Jan	SD
Leaves	AG	13.15	4.20	27.92	7.35	44.33	6.98	70.17	11.30	128.50	23.61
	BG	13.23	4.19	31.31	5.62	44.69	7.16	81.31	7.16	125.00	23.13
	DB	12.38	5.25	31.00	5.37	49.23	8.61	76.46	10.89	121.33	16.26
	CONTA	15.31	7.63	35.92	8.85	50.46	15.58	81.62	12.97	142.54	23.92
	CONTB	14.23	4.64	27.46	8.30	50.46	9.37	70.54	12.78	109.09	12.78
Stems		Sept	SD	Oct	SD	Nov	SD	Dec	SD	Jan	SD
	AG	43.88	13.92	90.92	27.95	151.08	40.47	271.83	35.21	483.83	51.67
	BG	51.23	12.95	105.96	22.83	169.96	26.74	284.38	26.74	455.31	73.36
	DB	46.96	11.29	99.38	33.19	173.00	56.25	286.31	47.10	473.08	62.55
	CONTA	49.31	11.51	107.46	18.13	159.23	29.39	266.23	62.26	470.54	70.69
	CONTB	53.23	14.29	98.46	31.47	178.85	40.45	306.38	76.84	433.73	76.84
Branches		Sept	SD	Oct	SD	Nov	SD	Dec	SD	Jan	SD
	AG	3.62	0.96	6.25	1.14	7.75	0.87	10.83	1.11	14.11	2.07
	BG	3.38	0.77	6.62	0.51	7.38	1.04	11.00	1.04	14.08	1.32
	DB	3.46	1.05	6.38	0.77	8.46	1.05	11.00	1.35	15.00	2.00
	CONTA	3.77	1.01	6.85	1.14	8.62	2.29	11.15	1.34	15.54	2.11
	CONTB	3.85	1.07	6.46	0.78	7.23	1.01	9.69	1.04	12.91	1.04

Appendix 2C: Summary of fertiliser trial data – all concentrations considered (mean counts)

LEAVES	AG				
Conc	**Sept**	**Oct**	**Nov**	**Dec**	**Jan**
5	13.15	27.92	44.33	70.17	128.50
10	13.85	29.38	44.38	74.23	125.23
20	14.15	28.08	51.92	72.58	128.42
LEAVES	**BG**				
Conc	**Sept**	**Oct**	**Nov**	**Dec**	**Jan**
5	13.23	31.31	44.69	81.31	125.00
10	11.54	31.08	48.85	79.23	123.91
20	12.62	31.15	48.08	77.92	123.38
LEAVES	**DB**				
Conc	**Sept**	**Oct**	**Nov**	**Dec**	**Jan**
5	12.38	31.00	49.23	76.46	121.33
10	12.77	29.54	47.75	74.54	120.23
20	14.08	32.62	45.85	71.18	124.50
STEMS	**AG**				
Conc	**Sept**	**Oct**	**Nov**	**Dec**	**Jan**
5	43.88	90.92	151.08	271.83	483.83
10	56.85	103.73	161.42	260.54	448.62
20	48.77	102.29	157.67	272.67	439.00
STEMS	**BG**				
Conc	**Sept**	**Oct**	**Nov**	**Dec**	**Jan**
5	51.23	105.96	169.96	284.38	455.31
10	49.50	99.50	161.23	255.77	435.73
20	48.62	107.92	163.92	275.46	440.38
STEMS	**DB**				
Conc	**Sept**	**Oct**	**Nov**	**Dec**	**Jan**
5	46.96	99.38	173.00	286.31	473.08
10	50.19	101.65	168.58	262.85	384.23
20	51.27	109.96	174.50	290.15	447.50
BRANCHES	**AG**				
Conc	**Sept**	**Oct**	**Nov**	**Dec**	**Jan**
5	3.62	6.25	7.75	10.83	14.11
10	3.54	6.77	7.31	10.77	14.69
20	3.77	6.75	8.58	10.42	14.25
BRANCHES	**BG**				
Conc	**Sept**	**Oct**	**Nov**	**Dec**	**Jan**
5	3.38	6.62	7.38	11.00	14.08
10	3.62	6.38	8.08	11.31	14.18
20	3.38	6.54	7.77	11.08	13.54
BRANCHES	**DB**				
Conc	**Sept**	**Oct**	**Nov**	**Dec**	**Jan**
5	3.46	6.38	8.46	11.00	15.00
10	3.62	6.54	8.00	10.69	14.31
20	3.69	6.77	7.62	10.38	13.83

Appendix 3: Concrete data summary

Date	Location	Mix	First Slump	7 (CS)	14 (CS)	28 (CS)
30.04.03	UNITEC	HCT1	8	3	3.12	3
16.07.03	UNITEC	HCT1	8	2	3.8	5
06.08.03	UNITEC	HCT1	8	4.3	4	4
30.04.03	UNITEC	MBT1	15	5	4.8	7.5
16.07.03	UNITEC	MBT1	15	4	1.2	2
06.08.03	UNITEC	MBT1	15	5.1	3.8	3
28.01.04	UoA	MBT2	100	3.8	*	*
02.02.04	UoA	ISM	100	16.72	*	*

Date	Mix	Air%	Bleed	Bleed Time	Density kg/m3	Made By
30.04.03	HCT1	*	*	*	*	CB
16.07.03	HCT1	*	*	*	*	CB
06.08.03	HCT1	*	*	*	*	CB
30.04.03	MBT1	*	*	*	*	CB
16.07.03	MBT1	*	*	*	*	CB
06.08.03	MBT1	*	*	*	*	CB
28.01.04	MBT2	6.50	0.049L	0:40	1558	CB
02.02.04	ISM	4.00	0.089L	3:05	2520	TW

Cement (kg)		Sand (kg)	Water (L)	Shell (kg)	19mm basalt (kg)
HCT1	6	16	3	8	*
MBT1	6	16	3	8	*
MBT2	2.4	10	2	0.5	*
ISM	12	21.15	6.7	*	26.5

	13mm basalt	7mm Pap (kg)
HCT1	*	*
MBT1	*	*
MBT2	5	7
ISM	24.75	33.15

Ratios:	Cement:	Sand:	Water
HCT1	3	8	1.5
MBT1	3	8	1.5
MBT2	1.2	5	1
ISM	3	5	2

Notes:	
HCT1	Shell was hand-crushed to ~1cm2 shards
MBT1	Whole shell, mixer-broken~varying sizes
MBT2	Air entraining agent added
IS	Industry Standard Mix NZS 3109
CS	Compressive strength

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