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Studies of the Age, Growth and Shell Increment Patterns in the New Zealand Cockle
(Austrovenus stutchburyi)

Jean F McKinnon

A thesis submitted in partial fulfilment of the requirements for the degree of Master of Science
in
Marine Science
at the University of Otago, Dunedin, New Zealand

April 1996
Abstract

*Austrovenus stutchburyi* were collected (n=1100) from Papanui Inlet on the Otago Peninsula and half were marked using the fluorescent dye, calcein, and half were marked by exposing them to an abrupt decrease in temperature which thermally stressed the animals. A proportion of the cockles (n=660) were returned to cages in Papanui Inlet while the remainder (n=440) were maintained in tanks at the Portobello Marine Laboratory. Once a month, ten marked cockles from each treatment from both Papanui Inlet and the Laboratory were collected and sacrificed by immersion in hot fresh water. The thermal stress method failed to leave a discernible mark in the shell and, therefore, these cockles were used for allometric measurement only. The cockles that had been treated with calcein were used for the growth analysis. These shells were cleaned, measured and internal increments examined using the thin section method. Under an ultraviolet light source, calcein was found to be incorporated as a green line in the shell. Measurements from the calcein line to the shell edge allowed a growth rate to be calculated. Thin sectioning of the shells revealed that there are two types of growth increment: larger macro-increments and smaller micro-increments. The calcein mark allowed the periodicity of these to be examined. The results indicate that the macro-increments found in the shell are annual in nature. Analysis of the periodicity of the micro-increments revealed that during the warm months of summer the periodicity of these increments is tidal. However, this periodicity breaks down over winter. In the summer months there was a relationship between the width of micro-increments and the spring/neap tidal cycle, with wider, more complex, micro-increments being laid down during spring tides and narrow, simple micro-increments being laid down during neap tides. Seasonality of growth was determined by examining the marginal increment on the shells and by comparing the actual growth of samples collected in summer and winter. The results support the hypothesis that *Austrovenus stutchburyi* grows slowly in winter and faster in summer. There is also a slowing of allometric growth in summer that may be related to the physiological stress of gametogenesis and spawning. Cockles maintained in the laboratory showed little or no growth for the duration of this study. This may have been due to inadequate or inappropriate food.
To My Parents, John and Kathryn McKinnon,
for many years of putting up with buckets of weird and
wonderful things coming home from the beach and cluttering
up the laundry sink!

&

To Bev Dickson,
Lab Manager extraordinaire, who nursemaided me through this
thesis and without whose skill and expertise a great deal of
research would not get started, never mind finished!
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I would also like to thank the technical staff in the thin sectioning lab of the Geology Department, University of Otago, for their patience and forbearance while teaching me how to make thin sections.

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At the department of Marine Science I would like to thank Daryl Coup for help with computer problems and Deirdre Kennedy for her support particularly for not running away when she heard “Deirdre I need a disc, an order form.............” ad nauseum!

I would also like to thank Miles Lamare for teaching this mathematical numbskull how to create and plot a Von Bertalanffy growth equation!

Big thanks too, to Craig Currie for spending some VERY cold afternoons on the inlet digging cages and cockles.

I would like to thank my fellow students and ex-students of Marine Science for their support, “helpful” comments and distractions. Thanks Meg, Rach, Tania, Rich, Penny, Maree, Miles, Pete C, Brian, Johnson and everyone else!

Finally, I would like to thank my parents John and Kathryn McKinnon whose support has been immense and who are still finding odd things from the beach round the house!
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Table 3.1. Growth parameters for the Von Bertalanffy equation ..................................... 51
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1.1 Periodic Marks in Skeletal Structures of Marine Animals

Periodic marks in the hard parts of a variety of marine organisms have been used in aging research. Age records are known to be laid down in coral skeletons (Risk and Pearce, 1992; Barnes and Lough, 1993; Grange and Goldberg, 1993; Taylor et al., 1993), fish otoliths (Campana and Neilson, 1985; Maillet and Checkley, 1991; Nishimura, 1993), squid statoliths (Arkhipkin, 1991; Arkhipkin and Bizikov, 1991; Jackson, 1994a; Jackson, 1994b), brachiopod shells (Hiller, 1988), bryozoans (Stebbing, 1971; Pätzold et al., 1987), limpets (Kenny, 1977), barnacles (Bourget and Crisp, 1975; Crisp and Richardson, 1975), the arm ossicles of brittlestars (Law, 1995) and bivalve mollusc shells (Clark, 1980; Ropes et al., 1987; Bourget et al., 1991; Breen et al., 1991; Tan Tui and Prezant, 1993). Periodic marks laid down in the hard parts of some marine animals can provide information about the animals' age and growth pattern and on environmental variables and lifestyle changes experienced by the animal. Study of such marks can provide a great deal of information for environmental science, behavioural ecology, chronobiology and fisheries science.

1.2 A Brief History of Growth Analysis of Bivalve Molluscs

The analysis of age of marine animals using the periodic marks laid down in calcitic structures has been conducted for many years. The most common animals for this type of study have, to date, been commercially important groups of marine animals, e.g., fin fish. Understanding age and growth in marine animals is important as they provide information on the dynamics of populations and on the impact of exploitation on those populations. Thus, age and growth rate information is crucial for the management of a fisheries species (Smith, 1992). While mollusc fisheries can be large, they have not always been perceived as being as important as the more traditional fin fisheries. However, some researchers have, over the years, studied the aging and growth of mollusces. Orton (1926) studied Cardium edule in the River Yealm near Plymouth. He found that
handling the cockles caused them to lay down "disturbance rings" and he used these to measure the amount the cockle had grown over one month. Orton (1926) also observed "rings" in *Mytilus edulis* that were grown at the mouth of the River Yealm. This was a comprehensive study that took several years to complete and provided some of the first data on the growth and production of lines in the shells of bivalve molluscs.

By the late 1950's, more research was being done on the aging and growth of marine bivalves, including the work of Green (1957) who found that specimens of *Scrobicularia plana* lived for 16 -18 years and reached a length of 54mm. Mason (1957) found that growth rings on the shell of *Pecten maximus* were annual. These scallops ceased growing in winter and resumed growth in the spring. Annual growth was found to be the greatest in the first two to three years of life, after which the growth rate decreased. In addition, it was found that the scallops grew more quickly in shallow water than in deeper water.

Bivalve growth has also been studied by geologists. Rhoads and Pannella (1970) found that age and the season of death of *Gemma gemma* could be determined from the shell growth patterns. This study showed that both physiological and environmental effects were recorded in the shell structure.

In recent years, considerable effort has been put into research on molluscan shell structure, growth and aging. Advanced technology and techniques, such as electron microscopy (Clark, 1980b; Isaji, 1993), allow more information to be gleaned from each shell. This information includes the identification of shell material (Carter, 1980), the effect of anaerobic metabolism (Gordon and Carriker, 1978), how the crystals are arranged within the shell microstructure (Fritz et al., 1991; Carriker, 1992; Belda et al., 1993), and how long it takes the animals to lay down the shell (Lutz, 1976; Nolan and Clarke, 1993; Palacios et al., 1994).

In New Zealand most research on age and growth determination has been on fish, both marine and freshwater. However, Cranfield and Michael (1993) have studied the surf clams *Mactra discors, Dosinia anus, Mactra murchisoni, Paphies donacina* and *Spisula aequalatera* to determine their growth rate and age.

### 1.3 Types of Increments Found in Bivalve Mollusc Shells

#### 1.3.1 Terminology

There has been variation in the terminology used to describe the periodic layers found in bivalve shells. Such layers have been called growth lines, rings, bands, zones and increments. The term growth ring has been the commonest descriptor
but it has problems associated with the fact that the phenomenon it describes has a three dimensional structure. Typically, the internal shell of a bivalve is made up of repetitive, concentric ‘light’ and ‘dark’ areas, with one light area plus one dark area forming the so called “annual ring”. Within the “annual rings” there are often finer-scale periodic marks. For clarity in this study, the term “increment” or “macro-increment” will be used when referring to the large “annual rings”, and the term “micro-increment” will be used when referring to the fine-scale periodic marks found in some bivalve shells (section 1.3.2) (Fig 1.1).

Fig. 1.1 Types of increments found in bivalve molluscs. Not to scale

1.3.2 Increment types

There are several different types of increments in bivalve shells (Fig 1.1). In addition to the increments that are assumed to be annual and caused by seasonal fluctuations in variables such as temperature, there are increments that are associated with stress. These stress marks can be physiological, e.g., spawning can be associated with an interruption of somatic growth, and thus produce a check mark (Sheppard, 1985). Alternatively a variety of external physical events can produce a similar stress mark in the shell. Siltation causes an interruption in somatic growth in the Nile River bivalve, Corbicula consobrina, and this is accepted to be the annual check mark in this species (Adam, 1990; el Moghraby and Adam, 1990). “False annual rings” are also laid down by this species. This is due to a decrease in water temperature from November to January. House and Farrow (1968) found that storm events and gales were recorded as irregular disturbance increments in the shell of Cardium edule.

Many bivalves produce very fine increments (micro-increments) between the main increments. The periodicity of these micro-increments varies and is
dependent upon species and conditions. The fine increments may be laid down tidally, daily or sub-daily. In addition, micro-increments that are identified as being tidally influenced may be of two different types, simple or complex (Evans, 1972), depending on the spring/neap tidal cycle. The simple micro-increments have two distinct boundaries. The complex micro-increments also have two distinct boundaries, but have an additional, less distinct, inner boundary that divides the increment into two sections. It was found that the complex micro-increments corresponded with spring tides while the simple micro-increments corresponded with neap tides. Evans (1975) showed a similar micro-increment pattern recording the spring/neap tidal cycle in the shell of the cockle, Clinocardium nuttalli, but not in Penitella penita, a rock boring clam from the same area. This pattern was also be seen in the prismatic layer (Table 1.1) of the shell of Mytilus edulis. This pattern of micro-increments related to the spring/neap tidal cycle and was only seen in Mytilus edulis from the intertidal zone (Richardson et al., 1990). In intertidal Mytilus edulis a wide micro-increment was found to correspond to neap tides, while a narrow micro-increment corresponded to spring tides. The spring/neap tidal patterns found in the shell of Mytilus edulis by Richardson et al., (1990) are the reverse of those found in the shells of Clinocardium nuttalli by Evans (1975) and also the reverse of the findings of this study of the patterns in the shells of Austrovenus stutchburyi. In addition, Richardson et al., (1990) found that this pattern of tidal deposition was not apparent in Mytilus edulis from a sub-tidal environment and in the shells of those individuals raised in the laboratory with no tidal influence, only weak micro-increments with no apparent environmental periodicity being found. This indicates that there may be an endogenous rhythm of shell deposition. These weak patterns would be of little use in the study of short-term variations in shell growth (Richardson et al., 1990). Furthermore, some studies have been unable to explain the pattern of fine micro-increments as functions of day, tides or exposure (Jones, 1981; Tanabe, 1988 and Bourget and Brock, 1990). Validation of the periodicity of growth increments, both the macro-increment and the micro-increment, is, therefore, of paramount importance if meaningful interpretation of growth and age are to be obtained.

1.4 Factors Affecting Bivalve Growth

Bivalve growth can be influenced by a variety of external factors. These have been found to include temperature, tidal emersion, latitude, physico-chemical factors and storms.
1.4.1 Temperature

Green (1973) investigated the growth of an arctic intertidal population of *Macoma balthica* and found that the summer air temperature had a strong positive influence on growth. Richardson et al., (1980c) found that the growth of *Cerastoderma edule* was affected by low sea temperatures. In a study which compared *Cerastoderma edule* from cool temperate and from sub-arctic areas, it was found that the more severe cold of the sub-arctic site caused a complete cessation of growth in the winter. In comparison, *Cerastoderma edule* from a cool temperate area continued to grow in winter but at a reduced rate.

1.4.2 Tidal exposure

The influence of tides (i.e., tidal emersion and immersion) can sometimes be seen in the shell of bivalves as a micro-increment. Richardson et al., (1980b) found that tidal level had a very important influence on the growth rate of *Cerastoderma edule*. They found that the rate of growth was proportional to the fraction of time that the animal was immersed and able to feed.

*Mytilus edulis* also shows tidally induced micro-increments (Richardson et al., 1990), and considerable variation in individual growth rates, both within and between populations. A tidally induced micro-increment pattern was evident in the prismatic shell layer of *Mytilus edulis* and this was related to the spring/neap tidal cycle.

Several studies have shown that many bivalves possess an endogenous periodicity of shell deposition in addition to that which is tidally entrained (e.g. *Cerastoderma edule* Richardson et al., 1980a). *Tapes philippinarum* (Richardson, 1987), *Spisula subtriangulata* (Richardson, 1988) and *Mytilus edulis* (Richardson, 1989) all show an endogenous circatidal periodicity in shell deposition. However, Bourget and Brock (1990) found that the pattern of deposition of micro-increments in *Cerastoderma edule* did not support the hypothesis of an endogenous circatidal rhythm of deposition. They found that the number of micro-increments decreased with increasing age and, furthermore, there were intra-populational differences in deposition and clarity of micro-increments. Thus, Bourget and Brock (1990) suggested that there was a plasticity in deposition periodicity according to the origins and age of experimental specimens.
1.4.3 Latitude

Several researchers have found a latitudinal trend in the growth rate of bivalves. The hard clam, *Mercenaria mercenaria*, has been studied throughout its geographical range in the northern hemisphere (Ansell, 1968). It was found that in the north growth takes place only in the summer, while further south growth is continuous throughout the year. Although temperature clines are important variables, other factors, particularly food availability, are probably important in determining the rate of growth within the limits set by temperature (Ansell, 1968).

Tanabe and Oba (1988) found a north-south cline in the growth rate of northern hemisphere *Phacosoma japonicum* with growth slower in the north. A similar latitudinal cline has been found by Iglesias and Navarro (1990) who studied *Cerastoderma edule* in Spain. It was found that in these bivalves there was an increase in growth rate the further south they were sampled.

1.4.4 Other environmental variables

Appeldoorn (1983) found that latitude, siltiness, and level of sediment hydrocarbons affected growth of bivalves. High siltiness and high concentrations of sediment hydrocarbons reduced growth. However, the positive influence of temperature was the dominant factor and this was associated with latitude.

House and Farrow (1968) worked with the European edible cockle, *Cerastoderma edule*, and found that storms and gales could cause a cessation of growth for the duration of bad weather. Slowing or stopping of growth have also been attributed to physico-chemical factors. Smit et al., (1992) found that the growth of small zebra mussels (*Dreissena polymorpha*) correlated positively with seasonal chlorophyll-a concentrations.

These findings on the external influences on bivalve growth have been corroborated by other workers on a variety of other species including *Clinocardium nuttalli* (Evans, 1972), *Chione [Austrovenus] 1 stutchburyi* (Coutts, 1974), *Geukensia demissa* (Lutz and Castagna, 1979), *Mya arenaria* (MacDonald and Thomas, 1980), *Arctica islandica* (Ropes and Jearld, 1987), *Phacosoma japonicum* (Tanabe, 1980),

---

1 *Chione stutchburyi* was reclassified as *Austrovenus stutchburyi* in 1979 (Jones, 1979). For clarity, I will refer to this animal as *A. stutchburyi* throughout this text regardless of the date of the cited reference.
Modiolus modiolus (Anwar et al., 1990) and Panopea zelandica (Breen et al., 1991).

1.5 The Physiology of Deposition of Bivalve Shell Material

The bivalve shell consists of calcareous and organic elements arranged in a highly organised fashion. In growth studies, bivalve shells are commonly sectioned transversely, from umbo (hinge) to the shell margin along the line of maximum growth. When the shell has been sectioned in this manner, two major kinds of stratification are apparent: shell layers (Table 1.1) and growth increments (Sheppard, 1985). Depending on the species, the shell may be divided into two or three layers. Each of these is formed from distinct structural and, sometimes, mineralogical units (Table 1.1) (Taylor et al., 1969).

Growth increments are "repetitive units of contemporaneous growth detectable across all layers in an accreting tissue" (Sheppard, 1985). Each growth increment is made up of a layer of calcium carbonate and a lamina of an organic material, conchiolin. The conchiolin corresponds to an interruption in calcification and the increment boundary is created by the beginning of calcium carbonate deposition. The growth increments are best observed in the upper (outer) layer of the shell (Pannella and MacClintock, 1968).

Shell growth is controlled by the mantle, through which calcium, calcium bicarbonate and carbon dioxide pass into the extrapallial fluid. Crystals of calcium carbonate are formed in the extrapallial fluid between the mantle and the inner shell surface where these crystals orient and grow on an organic matrix secreted by the mantle (Wilbur, 1976).

Research has indicated that valve movements correlate well with incremental shell growth in bivalves. When the valves are closed, calcification ceases (Thompson, 1975, and Sheppard, 1985). Valve closure seems to be controlled by an endogenous cycle that is influenced by the external environment, such as solar and lunar days, tides, fluctuations in temperature, food and photoperiod (Sheppard, 1985; Beentjes and Williams, 1986; Williams et al., 1993; Palmer, 1995). This is supported by a study of lower littoral zone Austrovenus stutchburyi (Beikirch, 1995). In this study it was found that, in contrast to mid-littoral Austrovenus stutchburyi, individuals from the lower littoral zone show irregular gaping rhythms. Beikirch (1995) postulated that regular exposure to environmental tidal cycles is important for the stability of the endogenous rhythm of this species.
Variations in increment thickness, crystallography, pigment, translucency and topographical features, such as notching or grooving of the shell surface, are all indicative of a change in shell growth in a variety of species (Pannella and MacClintock, 1968; Clark, 1974; Berry and Barker, 1975; Evans, 1975; Hall, 1975; Thompson, 1975; Richardson et al., 1979). A notch or sharp depression on the outer surface of the shell are often associated with the boundary of a macro-increment. The notch may include remnants of the periostracum. It is thought that the notches result from a period of shell growth where the mantle (which secretes the shell) did not extend to the shell edge. This suggests a period during which the shell remained nearly closed, preventing the mantle from extending to its usual position (Clark, 1979).

Information about the deposition of the molluscan shell has been derived predominantly from studies of bivalves. However, work with other classes of molluscs has corroborated these results, eg, the work of Saleuddin (1976) on the formation of the periostracum in the pulmonate snail, Helisoma duryi duryi.

1.6 Shell Structure

The shell microstructure of bivalve molluscs can be divided into eight different structural types, the uncalcified periostracum and seven calcified types. A cross section through a bivalve shell shows two or three layers\(^1\) (Fig. 1.2) in addition to the outer periostracum. Several of the calcified layers can be further divided according to the arrangement and mineralogy of their crystalline components (Table 1.1). Not all microstructural types are found in any particular shell, nor are they necessarily found in the same layer over different species. In addition, there may be more than one type of crystal structure found within a single shell layer.

\[^1\] Austrovenus stutchburyi has three internal shell layers. Therefore, further discussion will draw upon a three-layered example.
1.6.1. Microstructure of venerid bivalves

In the venerid bivalves, the inner shell layer shows intraspecific variation (Shimamoto, 1991). Homogeneous structure coexists with complex crossed lamellar structure (Table 1.1). The position and proportion of each microstructure varies between individuals. In addition, there may also be geographic variation in the frequency and arrangement of the two microstructure types (Shimamoto, 1991) found that species with a predominantly homogeneous inner shell layer were found between 20°N and 45°N latitude, while species with a predominantly complex crossed lamellar inner layer were found south of 35°N latitude.

In the middle shell layer, a homogeneous structure coexists with a crossed lamellar structure, but there is no intraspecific variation. The crossed lamellar structure is secreted during the later stages of shell growth and can be related to breaks in growth. It is possible that the secretion of the middle layer is controlled by genetic factors (Shimamoto, 1991).

The outer layer in venerids is composed of either a crossed lamellar structure or composite prismatic structure (Table 1.1). In some species, for example, *Callista (Exocallista) brevisiphonata*, these two microstructures coexist.

1.6.2 The shell of *Austrovenus*

*Austrovenus stutchburyi* has an outer crossed lamellar layer which becomes homogeneous in the middle layer (Jones, 1979). There is a prismatic pallial myostracum (Table 1.1) and the inner layer has a complex crossed lamellar to homogeneous structure (Jones, 1979).
<table>
<thead>
<tr>
<th>Micro-structure group</th>
<th>Microstructure variety and description</th>
<th>Mineralogy</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periostracum</td>
<td>The periostracum is the outer uncalcified organic structure which covers the outer-most calcified layer of the shell (the prismatic layer in bivalves). It is often worn away. The periostracum is made of various numbers of layers. It is a quinone tanned protein containing 15 - 17 amino acids, lipids and neutral polysaccharides. The acid mucopolysaccharides commonly associated with calcification do not occur in the periostracum.</td>
<td>Uncalcified protein</td>
<td>Grégoire (1972)</td>
</tr>
</tbody>
</table>
| Prismatic             | A. Simple prismatic. Prisms lack an elongate substructure, boundaries are well defined, non interdigitating and show a moderate length/width ratio.  
-1. Regular simple prismatic. Cross sections of prisms are polygonal and uniform.  
-2. Irregular simple prismatic. Cross sections of prisms are non-polygonal to polygonal and are highly variable.  
B. Fibrous prismatic. Similar to simple prismatic, but the prisms have a large length/width ratio.  
C. Spherulitic prismatic. Prisms have a substructure of elongate sub-units that radiate in three dimensions from a central nucleation to the depositional surface.  
D. Composite prismatic.  
-1. Denticular composite prismatic. The three dimensional divergence of the sub-units is an artifact of deposition on a strongly curved and denticulated shell margin eg in Nucula.  
-2. Nondenticular composite prismatic. Divergence of the sub-units is independent of the shape of the depositional surface.  
-3. Compound composite prismatic. Non denticular composite prisms diverge in three dimensions from a central longitudinal axis toward the depositional surface as an artifact of deposition on a strongly curved and denticulated shell margin. | Aragonite & calcite  
Carter (1980)  
Aragonite & calcite  
Aragonite & calcite  
Aragonite & calcite  
Aragonite & calcite  
Aragonite  
Aragonite & calcite  
Aragonite | Grégoire (1972)  
Carter (1980)  
Carter (1980)  
Carter (1980)  
Carter (1980)  
Carter (1980)  
Carter (1980) |
| Spherulitic           | Extremely rare. Densely packed spherical to subspherical aggregations of elongate structural sub-units radiating from a central nucleation site. | Aragonite | Carter (1980) |
### Laminar

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td>Nacreous. Laminae of polygonal to rounded tablets lying parallel to the depositional surface.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-1. Sheet nacreous. Tablets are irregular stairstep or brick wall stacks.</td>
<td>Grégoire (1972)</td>
</tr>
<tr>
<td></td>
<td>-2. Row stack nacreous. “Parallel, elongate tablets show a vertical stacking mode in vertical sections perpendicular to their length, but non-vertical stacking in vertical sections parallel to their length.”</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-4. Myostracum. The portion of the shell that underlies the muscles. This is a modified form of the nacreous structure where the crystals of aragonite assume a prismatic structure.</td>
<td></td>
</tr>
<tr>
<td>B.</td>
<td>Regularly foliated. “Laminae consist of parallel, elongate blades dipping uniformly over large portions of the depositional surface with a single predominant dip direction.”</td>
<td></td>
</tr>
</tbody>
</table>

### Crossed

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td>Crossed lamellar. Numerous parallel elongate sub-units with two predominant dip directions relative to the shell margin.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-1. Crossed foliated. A variety of crossed laminar where the sub-units are blades or laths.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-2. Crossed acicular. Similar to crossed laminar but with only a few elongate sub-units.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-3. Complex crossed lamellar. Aggregations of elongate sub-units with three or more predominant dip directions (irregular complex crossed lamellar), or they are arranged on the surface of stacked cones (cone complex crossed lamellar).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-1. Complex crossed foliated. A variety of complex crossed lamellar where the sub-units are elongated calcitic blades or laths.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-2. Fine complex crossed lamellar. Similar to irregular complex crossed lamellar, but consists of only a few elongated sub-units.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-3. Cross-matted lineated. Two types of elongated sub-units mixed in the same shell layer 1) irregular aggregations of radiating sub-units and 2) radially elongated aggregations of mutually parallel sub-units.</td>
<td></td>
</tr>
</tbody>
</table>

### Table 11 cont. Shell layers found in molluscs (after Carter, 1980)
| Homogeneous | Irregularly shaped but similar sized crystallites with no regular arrangement except for possible growth layers.  
A. Homogeneous *senso stricto*. Homogeneous structure with major structural units less than 5 μm in diameter.  
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated spicules or spikes</td>
<td>Varially shaped calcified structures sparsely distributed within the periostracum or a tissue layer. Periostracal spikes are known only in aragonite.</td>
<td>Aragonite</td>
<td>Carter (1980)</td>
</tr>
<tr>
<td>Isolated crystal morphotypes</td>
<td>Includes all sparsely distributed, irregularly oriented units that do not form a long lasting, major shell layer. Commonly associated with spaces filled with extrapallial fluid away from the mantle epithelium.</td>
<td>Aragonite &amp; calcite</td>
<td>Carter (1980)</td>
</tr>
</tbody>
</table>
1.7 The Applications of Bivalve Growth Research

Geologists have examined the increments found in extant bivalve species and used them to interpret the increments found in fossil species. These are used as a guide to such things as past climate and sea level changes (Berry & Barker, 1975). Rhoads and Pannella (1970) postulated that the differences in calcification and growth found in shallow water versus deep water species may be useful in the determination of paleo-bathymetry.

Anthropologists have used the internal patterns in bivalve shells for a variety of purposes. One of the more common anthropological uses for mollusc shells has been in the determination of seasonal occupation of prehistoric sites. Shellfish was a staple in the diet of many prehistoric hunter-gatherer people and thus middens often contain a wealth of shells. When the internal patterns of the shells from archaeological sites are examined and compared with those of contemporary shells, it is possible to discover in what season the prehistoric animal was killed. Thus, it can be determined during what seasons the site was occupied (Clark, 1979; Coutts, 1970; Quitmeyer et al., 1982 and Sheppard, 1985).

Generally, aging work with bivalves has only been undertaken to investigate problems with established fisheries (Farrow, 1972). Recent work, however, has involved the use of shell and tissue growth and age analysis to determine optimal depths and other parameters for aquaculture. Emerson et al., (1994) investigated the growth and survival of Placopecten magellanicus in relation to depth. Their results indicated that height above the bottom was a significant source of variation in shell and soft tissue growth, with shell growth twice as fast in animals living on the bottom compared with those living 20 cm above the bottom. Emerson et al., (1994) used regressions of scallop growth versus environmental data collected every two weeks and showed that up to 68% of growth variation could be explained by variation in several aspects of water quality, with temperature and seston quality being the most important predictor variables. They also found that predictor variables changed seasonally and that failure to consider seasonal variation may be a primary factor limiting the ability to construct truly predictive models of bivalve growth (Emerson et al., 1994). They showed that to develop predictive models of bivalve growth empirical research is necessary because predictions of bivalve growth and survival based solely on laboratory studies do not have much relevance to natural populations. In addition, the combination of a number of oceanographic variables may have an as yet unknown effect on the growth of bivalves (Emerson et al., 1994).
Shell growth rates can be used as a record of recent environmental change. Records of storms and gales can be found in the shells of marine bivalves (House and Farrow, 1968; Appeldoorn, 1983). Shell calcite can also record anthropogenic input into the marine ecosystem. This is the basis of the 'mussel watch' programme. The shell and tissue can be analysed for various contaminants (eg, heavy metals). By analysing the chemical make-up of the shell increments, a time series of contamination can be constructed. This programme monitors for the effects of pollutants such as petroleum products, synthetic organic compounds and metals which find their way into coastal and estuarine environments (Pitts and Wallace, 1994).

Pitts and Wallace (1994) examined the lead content in the most recent annual growth increment of Mya arenaria. The lead concentration in the shells was compared to the seasonally averaged dissolved lead at the site. It was found that lead in the shell of this bivalve was concentrated by a factor of 10 000 relative to seawater. This makes Mya arenaria a sensitive indicator for ambient lead concentrations (Pitts and Wallace, 1994). Using samples from each increment, an estimate of change in the concentration of pollutants in sea water can be made, thus providing an historical record against which possible anthropogenic input can be compared.

Thus the study of the growth and age of marine bivalves has many applications and is not restricted to ecology and fisheries science. The shell is a useful recording tool, as it is reasonably permanent, and a great deal of information can be extracted from it, even from fossilised shells.

1.8 Methods of Examining Molluscan Growth Increments

There are many methods for examining the growth increments of marine molluscs. These range from very simple to complex, as outlined below.

1.8.1 External increment counts

The simplest method is to examine the increments found on the outside of the shell. In many species these are quite prominent, clear and easy to count. This method has the advantage that it requires little in the way of equipment or training, only a keen eye, a hand lens and, perhaps, a light source or dissecting microscope. An added advantage of this method is that the animals do not need to be sacrificed to gather the data. Thus, long term data on the same individuals can be collected.
This method has been used by some researchers (Mason, 1957; Green, 1957; and Theisen, 1973). There are, however, major problems with this type of age analysis, particularly when one is examining bivalve shells. The environment in which most bivalves live is harsh, and abrasion by the sediments and the effects of shell borers erodes the surface of the shell. This damage makes the increments on the external surfaces of the shell difficult to see and increases the chances of inaccurate counts. Furthermore, it is difficult, if not impossible, to distinguish between different types of increments on the external surface of a shell. Animals that live in a variable environment will be periodically disturbed and this disturbance may well be recorded in the shell as a disturbance increment. Disturbance increments are difficult to separate from the growth increments. Thus, so called “false increments” are often included in age counts, potentially giving an over-estimation of the age of the animal in question.

Another method of examining external shell increments, which avoids the problem of shell damage, has been used by Brousseau (1979). Brousseau used light transmitted through the shell of Mya arenaria to reveal the growth increments. The distance between each successive growth increment was then measured with dividers and an annual growth rate was calculated using these data. There are, however, some difficulties with this technique. It is only of use with thin-shelled bivalves and there can be difficulty in detecting the first and occasionally the second increment. Thus, there may be a merging of the younger age classes and an underestimation of the age of the animal.

The difficulty of using external growth increments requires finding some other method of aging bivalves. The external increments have internal counterparts, which are not subject to the erosive effects of the external environment and these are what most researchers use to study molluscan age and growth.

1.8.2 Acetate peel

The simplest method to examine internal growth increments is to cut the shell and treat it in a way that the increments in the shell are readily visible. One of the most widely used techniques is the acetate peel method. With this technique, an impression of the cross-sectioned surface of the shell is made on a thin sheet of cellulose acetate (commonly called “acetate paper”) which is rolled onto the polished and etched cut surface of a shell (Fig 1.2) (Appendix A).
Many researchers have used the acetate peel method. Results are generally good, with internal increments clearly visible. However, this is not true for all species or, even, for all populations within a species. Acetate peels of *Mytilus edulis*, in particular, do not always produce clear impressions of the increments. Lutz (1976) found increments in the inner nacreous layer of the shell, but these were faint and finding them depended upon the axis through which the shell was sectioned.

The acetate peel method has the advantage that the internal increments can be examined with no distortion due to erosion, although the effects of boring organisms can still be seen. The method is relatively quick and easy and requires little in the way of expensive equipment. Diamond saws and lapidary wheels for the sectioning and grinding stages of this procedure are widely available. The determination of the optimal etching time for the shell is the most time consuming part of the procedure. However, once this has been determined several specimens can be processed at once.

There are several disadvantages to the method: a) it can take some days for the resin to cure enough to be cut; b) it is very easy to damage the shell surface during the polishing process (if the shell is not too badly damaged the shell can be repolished); c) if the etching time is too long or the acid too strong the shell can be irreversibly damaged (it is vital, therefore, that determination of the correct etching time and acid strength not be carried out on experimental animals); d) optimum etching time varies for the different layers in the shell, so that several peels of the same section may be necessary to get clear replicas of all layers (Sheppard, 1984); e) acetate peels are not useful where the shell to be examined has a nacreous or foliated (Table 1.1) outer layer, e.g., *Mytilus edulis* does not often produce clear peels. In the case of shells with this structure, layers are parallel or sub-parallel to the line of growth. This produces very faint impressions on acetate peels (MacDonald and Thomas, 1980).
In conclusion, the acetate peel technique is a relatively easy and inexpensive method for examining the internal growth increments of some bivalves. However, it should be used with discretion when aging shells that are constructed of large crystalline units, as increments may be faint and difficult to see.

1.8.3 Thin sectioning

The second commonly used method of examining the internal structure of bivalve shells is thin sectioning. The thin sectioning of shells is carried out in much the same way as a geologist sections rock. The procedure has to be adapted slightly to allow for the more fragile nature of the shell (Appendix B).

Thin sections are sensitive enough to enable researchers to distinguish between growth increments and disturbance increments. It is also possible to identify finer micro-increments in the shell structure. Berry and Barker (1975) were able to compare the micro-increments found in the extant bivalves, *Chione undatella* and *Protophaca staminea*, with those found in the fossil bivalves, *Astartella concentrica*, *Myalina subquadrate* and *Conocardium* sp. Thin sections of all shells showed very clear micro-increments. However, the periodicity of the micro-increments differed in the extinct bivalves to that observed in the extant bivalves. The authors suggested that this may have been due to changes in the Earth’s rotation rate and associated tidal activity in the past.

MacDonald and Thomas (1980) showed that while the macro-increments from thin sections could be counted directly from the microscope, it was much easier to count them from a photograph. They concluded that thin sectioning was a reliable tool for the examination of growth increments in bivalve shells, especially when obvious growth increments do not appear on the shell surface.

The advantages of thin sectioning are that the shell microstructure is clearly seen. The section can be examined under a light microscope and a number of variables such as pigmentation, micro-structure, and translucency can all be examined at one time. As the thin section is an actual piece of the shell and not just an impression of the shell, as is an acetate peel, the shell can be marked with a dye, for example calcein. The mark left by the dye can then be used to validate the periodicity of the increments. Storage of thin sections is also easier than the storage of acetate peels. They have no relief to protect and do not curl up as peels have a tendency to do (Clark, 1980).

The major disadvantage is that the method is very time consuming and the shell sections often fracture or chip. This can render a section useless if the shell flakes
off along growth surfaces. It requires practice and patience to be able to carry out this procedure with any success (Pillage, pers comm.).

1.8.4 Other methods

The acetate peel and thin section methods discussed in Sections 1.7.2 and 1.7.3 are the most commonly used methods of examining bivalve growth increments. These two methods are used in the present study. There are, however, many other methods which have been developed to investigate bivalve growth and these are summarised in Table 1.2.
Table 1.2. Methods of examining bivalve growth increments

<table>
<thead>
<tr>
<th>METHOD</th>
<th>STRENGTHS</th>
<th>WEAKNESSES</th>
<th>EFFORT REQUIRED</th>
<th>SPECIES &amp; REFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate Peel</td>
<td>Simple, inexpensive, easily repeated, little equipment required</td>
<td>Brittle shells need to be coated in resin, easy to damage the shell during the polishing and etching processes. Optimum etching time is variable. Some shells do not produce clear peels</td>
<td>Relatively little effort is required. Hand polishing can be time consuming but this depends on the species under examination</td>
<td><em>Mytilus edulis</em> Lutz, 1976</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Mya arenaria</em> MacDonald &amp; Thomas, 1980</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sheppard, 1984</td>
</tr>
<tr>
<td>Thin Section</td>
<td>Simple, inexpensive, shell microstructure, pigmentation &amp; translucency can be easily seen with a standard light microscope. Easily stored</td>
<td>Brittle shells need to be coated in resin, easy to damage the shell during sectioning and polishing</td>
<td>Time consuming</td>
<td>Berry &amp; Barker, 1975</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Mya arenaria</em> MacDonald &amp; Thomas, 1980</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Clark, 1980</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sheppard, 1984</td>
</tr>
<tr>
<td>Auto-fluorescence</td>
<td>Simple, inexpensive</td>
<td>Not all bivalve shells auto-fluoresce</td>
<td>Minimal. Little preparation of specimens required</td>
<td>Unionacean bivalves Tevesz and Carter, 1980</td>
</tr>
<tr>
<td>Scanning EM</td>
<td>Three dimensional, enhanced contrast between growth layers</td>
<td>Expensive, technologically difficult. Requires ultra thin specimens.</td>
<td>Time consuming, requires a lot of preparation of specimens to create an accurate 3D picture</td>
<td><em>Weakley, 1972</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lutz, 1976</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Mercenaria mercenaria</em> Gordon and Carriker, 1978</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sheppard, 1984</td>
</tr>
<tr>
<td>Stereoscan EM</td>
<td>Allows investigation of thicker specimens. Stereo photos clarify shell structures</td>
<td>Expensive, technologically difficult</td>
<td>Time consuming</td>
<td><em>Weakley, 1972</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Cerastoderma edule &amp; C. glacum</em> Whyte, 1975</td>
</tr>
</tbody>
</table>
While there are many methods for examining the increments in calcareous structures, the data collected from such work are of limited use unless the periodicity of the increments can be determined. It is dangerous to assume that the increments must be annual in nature. Past research has shown that increments can be influenced by factors such as tidal emersion, disturbance, food abundance, and temperature (Section 1.4.4). Thus, shell increments can have varying periodicity, with some factors that affect the periodicity of increments occurring only occasionally (e.g., storms etc), while others may be more predictable (e.g., winter decrease in temperature etc).

One method of validating increments is with a mark-recapture experiment. Marking of bivalve shells can be achieved in various ways. Coutts (1974) marked the shell of live Austrovenus stutchburyi by cutting the edge of the upper valve with a saw. The animals were returned to the sample site and collected again after one year. The saw cut was apparent in acetate peels as a notch. Approximately 160 micro-increments were visible between the notch and the shell edge. This number of micro-increments did not appear to relate to any known environmental factor that would normally be associated with shell growth, such as the number of tides. This indicates that more work needs to be done on the
periodicity of the micro-increments of this species. An alternative hypothesis is that the saw cut damaged the shell in such a way that normal growth patterns were interrupted. This is possible if the saw damaged the growing edge (Rowley, pers comm).

Cold shock or thermal stress can be used to mark the shells of living bivalves (Richardson et al., 1979). This method involves keeping the live animals in moist air at a low temperature for several days. Marking in this fashion is best carried out in summer. When the bivalves are thermally stressed, growth stops. The sudden cessation of growth creates a deep cleft in the shell which can be seen in a thin section or acetate peel. This cleft is sometimes associated with the development of a spine in the immediate post-shock region. There is a reduction in growth rate immediately following the thermal stress treatment, which gradually accelerates until it returns to normal. The change in growth rate can be seen in the width of the increments. The increments immediately following the application of thermal stress are thin, but get wider as the animal continues to grow (Richardson et al., 1979).

Another method of marking bivalves is to use a dye which can be incorporated into the shell by the animal. Commonly this dye is fluorescent. Richardson et al., (1979) attempted to mark bivalve shells using tetracycline hydrochloride and alizarin red. It was found that various concentrations of tetracycline hydrochloride were not successfully incorporated. It was found that alizarin red was incorporated into the bivalve shell but it interfered with the normal deposition of the shell and thus confounded the age and growth rate estimates.

There has been some success in marking calcareous structures with dye in other groups of marine animals. For example, Rowley (1990) marked the sea urchin Stronglyocentrotus purpuratus using tetracycline hydrochloride and was able to determine growth rates for this species. Also, the loliginid squids, Loliotus noctiluca and Loligo chinensis, were marked using tetracycline hydrochloride (Jackson, 1990). The antibiotic was used to determine the periodicity of growth increments in the statoliths of these squid species, which could, in turn, be used to determine age and growth rates. However, Pirker and Schiel (1993) used tetracycline in various forms to mark the New Zealand abalone or paua (Haliotis iris) and found that care was needed to determine the best combination of marking method (injection or immersion) and concentration for particular size classes of individuals.

Beckman et al., (1990) investigated the use of calcein as a fluorescent marker in fish otoliths. They found that calcein was very successful in wild fish but there
was some variability in incorporation of calcein into the otoliths of laboratory reared fish. More work is required to determine the reason for this, but a metabolic difference between laboratory reared and wild fish was postulated by the authors. It is also possible that the artificial seawater the laboratory fish were kept in may have interfered with the uptake and deposition of calcein (Beckman et al., 1990).

1.9 Current Study

1.9.1 Description and comparison of the family Veneridae with two similar families

The species used for this study was the common New Zealand "cockle", *Austrovenus stutchburyi*, which belongs to the family Veneridae or Venus clams. Thus, the common name for this species, cockle, is a misnomer. The true cockles, family Cardiidae, have only two representatives in New Zealand and these are deep water species.

New Zealand also has representatives of the family Carditidae or cardita clams (Powell, 1979). These are very similar in general appearance to the venerid clams, but there are some morphological differences. Table 1.3 (a-c) summerises the characteristics of each family. The shell features used in Table 1.3 (a-c) are illustrated Fig 1.4.

The venerid clam *Austrovenus stutchburyi* is an important member of the New Zealand mudflat community. It is often the principle bivalve found on mudflats and in estuaries (Jones, 1983). *Austrovenus stutchburyi* are usually found buried at a depth of 2-4 cm in sandy substrata. However, they can sometimes be found deeper or on the surface. The bivalves finely sculptured shell helps maintain its position within the substratum (Jones, 1983). The clams do not tolerate low salinities as their feeding mechanisms are inhibited by salinities less than 18‰, and the animals die if the salinity drops below 4‰. This results in a general decrease in cockle densities with increasing distance inland from the estuary or inlet mouth, particularly if there is significant freshwater input (Jones, 1983). These cockles form a major part of the diet of several species, including the Short Tailed Sting Ray (*Dasyatis brevicaudatus*) (Ayling and Cox, 1987), the Mud Flat Whelk (*Cominella glandiformis*), the Sand Flounder (*Rhomboseola plebeia*), and the South Island Pied Oyster Catcher (*Haematopus ostralegus finschi*) (Jones, 1983).
Fig 1.4. Sketch of a generalised bivalve shell showing the internal shell structures. Not to Scale.
<table>
<thead>
<tr>
<th>Family</th>
<th>Common name</th>
<th>General</th>
<th>Size</th>
<th>Shape</th>
<th>Hinge Morphology</th>
<th>Pallial line</th>
<th>General Morphology</th>
<th>World wide examples (genus)</th>
<th>N. Z. examples (genus)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veneridae</td>
<td>Venus clams</td>
<td>Large</td>
<td>Medium-large (up to 140mm)</td>
<td>Variable oval-heart-shaped or round</td>
<td>Well developed hinge</td>
<td>Distinct with a moderate pallial sinus at the posterior end</td>
<td>Beaks point forward</td>
<td>Venus, Chione, Mercenaria, Tapes &amp; Dosinia</td>
<td>Austrovenus Dosinia &amp; Bassina</td>
<td>Powell, 1979</td>
</tr>
<tr>
<td></td>
<td></td>
<td>family (&gt; 400 spp)</td>
<td>Variation in shell texture and sculpture</td>
<td>Can be considerably inflated</td>
<td>3 well developed cardinal teeth in each valve</td>
<td></td>
<td>Small heart shaped depression anterior to the beaks is the lunule</td>
<td></td>
<td></td>
<td>Wye, 1991</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Often thick, solid</td>
<td>Lateral teeth poorly represented</td>
<td></td>
<td>Longer posterior depression is the escutcheon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Equivalue</td>
<td>Small anterior lateral tooth</td>
<td></td>
<td>Adductor muscles are equal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Can have strong radial ribs</td>
<td>Posterior lateral tooth</td>
<td></td>
<td>Well developed foot</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Posterior lateral tooth is a long rough ridge</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Family</th>
<th>Common name</th>
<th>General</th>
<th>Size</th>
<th>Shape</th>
<th>Hinge morphology</th>
<th>Pallial Line</th>
<th>General morphology</th>
<th>World wide examples (genus)</th>
<th>N. Z. examples (genus)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carditidae</td>
<td>Cardita clams</td>
<td>Moderate - large family</td>
<td>Moderate (up to 30 mm)</td>
<td>Thick walled</td>
<td>Strongly ribbed</td>
<td>Yellow-brown periostracum</td>
<td>Umbones off centre, often well to the anterior of the shell</td>
<td>Massive cardinals</td>
<td>Lateral either sub-obsolete or absent</td>
<td>Simple with no pallial sinus</td>
</tr>
<tr>
<td>Family</td>
<td>Common name</td>
<td>General Name</td>
<td>Size</td>
<td>Shape</td>
<td>Hinge morphology</td>
<td>Pallial line</td>
<td>General morphology</td>
<td>World wide examples (genus)</td>
<td>N. Z. examples (genus)</td>
<td>References</td>
</tr>
<tr>
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</tr>
<tr>
<td>Cardiidae</td>
<td>True cockles</td>
<td>Large family</td>
<td>Moderate - very large (up to 150mm)</td>
<td>Generally heart-shaped but can be rounded or oval</td>
<td>Well developed cardinal and lateral teeth</td>
<td>Simple pallial line with no pallial sinus</td>
<td>Ligament is external</td>
<td>Cardium &amp; Plagiocardium</td>
<td>Nemocardium &amp; Corculum</td>
<td>Powell, 1979</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chiefly edible</td>
<td>Found in Shallow and deep water</td>
<td>Inflated</td>
<td>Large rounded umbones</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Wye, 1991</td>
</tr>
</tbody>
</table>
1.9.2 Recreational and commercial fisheries for Austrovenus stutchburyi

This species is targeted by recreational fishers. The bag limit is 150 cockles per person per day, with no limit being placed on size. This bag limit is currently under review by the Ministry of Fisheries. In addition, it is the target species of a commercial fishery in Papanui Inlet. It is important to note, however, that research into the fishery biology of this species has been largely confined to biomass estimates for the establishment of a commercial fishery (Stewart et al., 1992). There has been little work on age and growth or on the species' ecology.

The commercial fishery for Austrovenus stutchburyi in Papanui Inlet has resulted in the collection of between 2 and 106 tonnes of shellfish/year in the period from 1983 to 1995 (Pers Comm R. Belton, 1995) (Table 1.4). The commercial collecting is concentrated on the northeast side of the main channel away from the study site (Fig. 2.1). The cockles are hand-collected. Brooms are used to sweep dead shell and some surface sand from the harvest site and a riddler crate (a sluice with precise bar spacing to grade out undersize or dead shell, sand and debris) is used to select the cockles. The cockles are then packed into mesh onion sacks and transported to the landing site in a small vessel.

Harvesting is done to order, thus specific target size varies. However, in the majority of landings, the cockles range in shell length from 35 - 50mm. These are sorted into 5 separate size grades. Smaller cockles are left on the beds. About 1.5% by weight of landings are returned to the beds from the factory as rejects predominantly due to being undersized, damaged or possessing unsightly shells.

<table>
<thead>
<tr>
<th>Year</th>
<th>Landing (tonnes)</th>
<th>Year</th>
<th>Landing (tonnes)</th>
</tr>
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<tr>
<td>1983</td>
<td>2</td>
<td>1990</td>
<td>25</td>
</tr>
<tr>
<td>1984</td>
<td>12(^1)</td>
<td>1991</td>
<td>106</td>
</tr>
<tr>
<td>1985</td>
<td>8(^1)</td>
<td>1992</td>
<td>99</td>
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<tr>
<td>1986</td>
<td>34</td>
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<td>1987</td>
<td>14</td>
<td>1994</td>
<td>42</td>
</tr>
<tr>
<td>1988</td>
<td>8</td>
<td>1995</td>
<td>90 (projected)</td>
</tr>
<tr>
<td>1989</td>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) available records are for calendar year not fishing year

\(^2\) Data on commercial collection of Austrovenus stutchburyi kindly supplied by R. Belton of Southern Clams Ltd Dunedin.
1.9.3 Aims of study and hypotheses tested

Effective management of a fishery is impossible without basic biological data on the target species. This study was designed to complement the biomass estimates carried out by Stewart et al., (1992) and to form the basis for an understanding of the population biology of this species. The primary goals were to i) validate the periodicity of the main increments found in the shell of *Austrovenus stutchburyi* and ii) to validate the periodicity of the micro-increments found within the macro-increments and attempt to relate these to some external factor.

Previous work on this species has suggested that the macro-increments were annual in nature (Coutts, 1974). Coutts (1974) marked the live bivalves by notching the shell edge which may have compromised the results. If the growing edge of the shell was damaged by this technique, growth rate may have been affected. In the current study, the shell was tagged with calcein. This dye is non-toxic and less invasive than other methods of marking calcareous structures. In addition to validation of increment periodicity, seasonality of growth and the relationship of growth to sea surface temperature and photoperiod were investigated and a growth curve was constructed for this species at a site in Otago, New Zealand.

The hypotheses tested were:

a) 1-The macro-increments are annual in nature.

2-There is a seasonal variation in the type of shell, either visually opaque or visually translucent, found on the growing edge of the shell.

b) 1-The number of micro-increments found in the shell is related to the number of times the cockle was exposed to the air, i.e., the number of micro-increments will equal the number of low tides that occurred during the experimental period.

2-The width of the micro-increments is related to the spring/neap tidal cycle, i.e., wider micro-increments will be laid down during spring tides than during neap tides.

c) Shell growth is allometric, i.e., the relationship between shell length and shell width, and shell length and shell height is linear.

d) The amount of new shell growth is positively correlated with sea surface temperature and daylength.
Chapter Two - Methods and Materials

After I had been there about ten or twelve days it came into my thoughts that I should lose my reckoning of time...

Upon the side of this square post I cut every day a notch with my knife, and every seventh notch was as long again as the rest, and every first day of the month as long again as that long one; and thus I kept my calendar, or weekly, monthly, and yearly reckoning of time.

Daniel Defoe,
The life and strange, surprising adventures of Robinson Crusoe

2.1 Study Site

The study site was Papanui Inlet on the eastern (seaward) side of the Otago Peninsula (45° 51’S: 170° 41’ E). The Inlet is sheltered on three sides by hills. Papanui Inlet is tidal and is connected to the sea by a narrow channel on the southern side of the Inlet (Fig. 2.1). The total area of the Inlet is approximately 3.5km². The present condition of the Inlet is very similar to that reported by Rayns (1985) with average depth of less than 1.5 m and a substratum of well sorted fine to very fine sand.

Papanui Inlet is representative of the type of habitat in which Austrovenus stutchburyi is commonly found (Jones, 1983). The Inlet is used for recreational fishing, shellfish gathering, commercial shell fishing and water fowl shooting.

Vandalism has been a significant problem in intertidal studies at Waitati Inlet, which has a great deal of human activity (T. Cameron, pers. comm). While there is easy public access to the clam beds on Papanui Inlet, it is not common to find members of the public at this site. It was hoped that this would reduce the amount of vandalism of experiments.

2.2 Pilot Study

2.2.1 Clam displacement

There is considerable water and sediment movement in Papanui Inlet and there was concern that experimental animals would be lost. It was, therefore, decided to carry out some preliminary work to determine whether or not cages were necessary to prevent loss of experimental animals.

120 clams were collected, spray painted with non-toxic car paint (B. H. T. Quick Dry™), and returned to the Inlet. The clams were reburied in the sand and a painted rock was used to mark the site. After one week the site was checked and 60 of the marked individuals were found. The site was checked again after a
further week. No marked animals were found within a three meter radius of the marked rock at this time. The experiment was repeated and after one week the site was checked and 74 of the marked individuals were found. The site was checked again after a further week and 10 marked individuals were found within a three meter radius of the painted rock. On the basis of these observations it was decided that it would be necessary to place experimental clams in cages to minimise loss.

Fig. 2.1. Site Location, Papanui Inlet. A, B & C are commercial shellfish collection sites.
2.2.2 Cage construction

Six circular cages, measuring 0.5m² in area were constructed. The frame of each cage was made of two hoops of number 8 gauge fencing wire. Plastic garden mesh (20mm) was used to form the sides of the cage. This was lined with 2mm plankton mesh to retain all size classes of clams (Fig. 2.2).

![Diagram of clam cage](image)

Fig. 2.2. Diagram of clam cage

2.2.3 Placement of cages

The cages were placed near the main channel of the estuary (Fig. 2.1.). Previous work has shown that the cages can be affected according to their height on the shore. Cages at a low shore height tend to be damaged by water movement. As the cages are placed higher up the shore the amount of damage decreases. There is little or no damage done to cages at the high shore level (pers. obs.) To confirm this observation, the cages were placed at a low shore level (0.0m = chart datum), a mid shore level (0.5m above chart datum) and high shore level (1m above chart datum). The cages were buried in the sand with only one centimetre of the garden mesh showing above the substratum.

These cages were left for one week and then checked. The low shore level cages had been damaged and the substratum in the cage had been washed out by the tide; the mid shore level cages had not been damaged, but one third of the substratum in the cage had been washed out; the high shore level cages had not been affected visibly. This experiment was repeated, with identical results.
Following the second test, the cages were not reburied, but were left for another week. Again, the low shore level cages showed the most damage; the mid shore level cages were slightly damaged and the high shore level cages were not damaged.

Dobinson et al., (1989) found that shore height affected the growth of clams, with the fastest growth occurring at low and mid shore heights. It was decided, therefore, to place the cages for this current work at the mid shore height which would maximise growth while minimising the effect of the tidal current on the cages and their contents.

2.3 Collection and Marking of Clams

Eleven hundred clams were collected from Papanui Inlet in May 1993. The cockles were selected to be within the size range 20 - 35 mm. This range was chosen for ease of handling. The animals were collected from a high shore level as this is where smaller animals are commonly found (Dobinson et al., 1989).

It was necessary to mark the shell in order to verify the periodicity of the growth lines found within the shells of *Austrovenus stutchburyi*. Two procedures were selected:

i) Thermal Stress. 550 clams were maintained in moist air in a refrigerator at 4°C for 3 days (Richardson et al., 1979). Once they were removed, 330 of them were tagged with blue non-toxic car spray paint (B. H. T. Quick Dry™) and distributed evenly among all 6 cages in the field (Fig 2.3). 220 clams were placed in tanks in the laboratory; these were not painted. In January, 1994, an additional 75 clams were marked and distributed among the 6 cages in the field.

ii) Calcein dye. The remaining 550 clams were stained with a fluorescent calcein dye (500mg/L seawater) (Beckman et al., 1990; Pirker and Schiel, 1993). The clams were left in the dye solution for 24h. Once removed from the dye, 330 clams were tagged with red non-toxic car spray paint (B. H. T. Quick Dry™), and returned to the field site and distributed evenly among the cages. The remaining 220 unpainted clams were placed in tanks at the laboratory; these were kept separate from the thermally stressed animals. In January, 1994, an additional 75 clams were marked and distributed among the 6 cages in the field.

2.4 Field Experiment

It has been shown by Dobinson et al., (1989) that the growth of *Austrovenus stutchburyi* is not density dependent at a maximum density of 387 clams per
0.25 m². At the current study site, five 0.25 m² quadrats were examined to determine clam densities. The clam densities were found to be 192 (SD=6) clams per 0.25 m².

The cages were buried in the sediment, and the substratum inside the cage was sieved and the number of clams found was counted. To maintain the natural density, 110 of the clams sieved from the centre of each cage were removed and replaced with marked clams. The remainder of the natural population was reburied in the cages along with the marked clams. There were 660 marked clams in total in the field (Fig. 2.3).

**Fig. 2.3.** Experimental set-up A) Field, B) Laboratory
In January 1994 an additional 150 animals were collected marked and returned to the field. This was done to provide an estimate of growth during a warmer season.

The cages were checked every fourteen days and were cleared of any algal growth. Minor repairs to the cages were also carried out at this time, if needed.

2.5 Laboratory Experiment

In order to compare the growth of *Austrovenus stutchburyi* in the field and in the laboratory, four tanks were set up at the Portobello Marine Laboratory (Fig. 2.3). The tanks were constructed of black plastic and were 35 cm wide, 45 cm long and 20 cm deep. Seawater was piped into the top of the tank and was drained from a pipe on the side. The seawater was constantly flowing and the tanks were kept at ambient sea temperatures and light/dark cycle. 110 clams were placed in each of the four tanks, giving a total of 440 animals (Fig. 2.3). Fewer cockles were held in the laboratory as it was expected that mortality would be less than for those clams in the field.

Clams kept in the tanks were fed on a culture of *Dunaliella primolecta*, a single celled alga. Algae were cultured in 22μm filtered, microwave sterilised seawater (Kellar et al., 1988). The sterile seawater was infused with nutrients and algae and left to grow for ten days (final cell density = 2.5 x 10⁹ cells L⁻¹)(Appendix C). To feed the clams, the seawater to each tank was turned off for a minimum of two hours. The clams were fed at a rate of 10ml of culture per clam twice a week.

2.6 Sampling Procedure

Once a month between June 1993 and May 1994 ten animals from each treatment were sampled. Thus the total number of clams collected at each sample time was forty, twenty from the field and twenty from the laboratory. Ten each from the field and the laboratory had received the thermal shock treatment, the remainder had been treated with calcein. From February onwards an additional twenty clams were collected from the field. A different cage and tank was sampled each month. The sampling was timed to coincide with a low spring tide, as the study site was more accessible at this time, due to the lower water in a minor channel which had to be waded in order to reach the site.

The clams were sacrificed by immersing them in hot fresh water, after which the flesh was dissected away from the shells and disposed of. The shells were soaked in a 13% solution of sodium hypochlorite for one hour to remove any remaining organic material.
Shell length, width and height (Fig. 2.4) were measured to the nearest 0.1mm using vernier callipers.

![Fig. 2.4. Cockle shell parameters measured.](image)

### 2.7 Preparation and Examination of Shell Sections

The left valve was used in all sections unless it was damaged during sectioning. If the left valve was damaged, then the right valve was used. As venerids are equivalve (Powell, 1979) the assumption was made for the purposes of this study that both valves grew at the same rate.

The shells of those individuals that had been marked by the thermal shock procedure were sectioned along the axis of maximum growth (Fig. 2.5) and examined by the acetate peel method (Appendix A).

![Fig. 2.5. Line of cross section through shell.](image)

The shells that had been treated with the calcein stain were also sectioned along the axis of maximum growth (Fig. 2.5) and thin sectioned (Appendix B).
The acetate peel sections were examined using an Olympus™ compound microscope for evidence of a mark left by the thermal stress treatment. The thin sections were examined using an Olympus Vanox™ microscope with an ultraviolet light source to locate the fluorescent calcein mark. Once the calcein mark had been located, the distance between the dye line and the outer edge of the shell was measured to the nearest 0.01mm using a calibrated ocular micrometer. This gave a measure of the amount of growth from the start of the experiment and helped establish the periodicity of the macro-increments. The type of macro-increment, either opaque or translucent, present at the shell edge was also recorded.

The micro-increments in 67 (n=37 for four months in winter and n=30 for four months in summer, the difference in n is due to shell breakage) Austrovenus stutchburyi shells were counted using the Olympus Vanox™ microscope and a hand counter. The number of micro-increments was determined by taking the mean of three consecutive counts that varied less than 10% of the mean (Jackson, 1994). This technique was used to ensure precision of the counts since another experienced counter was not available. This technique also eliminated a further source of error (ie. inter-observer error). Finally, the width of the micro-increments in eight shells (n=1 for each month for four months in winter and four months in summer) were measured using a calibrated eyepiece micrometer attached to the Olympus Vanox™ microscope. These measurements could then be analysed as a function of tidal height.
3.1 Thermal Stress

When the shells that had been treated by the thermal shock method were examined under a light microscope there was no evidence of the expected cleft in the shell resulting from a sudden cessation of growth. In addition, there was a high mortality rate amongst individuals treated by this method: 218 (39.63%) of the 550 thermally stressed clams died, either during the treatment, or within 24 hours of it. Due to the lack of a marker these shells were used for allometric measurements but not for growth analysis.

3.2 Calcein Incorporation

A fluorescent yellow line was observed when the shells that had been treated with calcein were examined under ultraviolet light (Fig. 3.1). This fluorescent line could be used as a baseline for measurement of growth rate, i.e., the amount of new growth that occurred from the line to the outer edge of the shell. The fluorescent line also provided a starting point for increment counts, and for the determination of the periodicity of both the macro and micro-increments.

3.3 Edge Band Type

The band type, either translucent or opaque (Fig. 3.2), on the shell edge was expressed as percentage opaque bands per sample as a function of month (Fig. 3.4). No shells with opaque edge bands were observed in the months of June, July and August, 1993. In September and October, all the shells sampled had opaque bands. However, in November the percentage of shells displaying an opaque edge band dropped to 70% and a minimum was reached in January, 1994, where 20% of shells sampled showed an opaque edge band. In February and March the percentage of shells displaying an opaque edge band had increased to 60% and 90%, respectively. In April and May there were no shells with opaque edge bands (Fig. 3.4).
It was not anticipated that one would observe translucent shell growth in summer, when shell growth was expected to be rapid and hence opaque. When the clams that were displaying the translucent region on the growing edge of the shell in November, December and January were examined closely, it was discovered that in each case there had been a region of opaque shell deposited before the translucent shell. This indicated that the clams had been growing rapidly but for some reason during summer (November, 1993, January, 1994 and December, 1994) their somatic growth had slowed down. In clam shells examined later in the summer (February, 1994) and early autumn (March 1994) rapid opaque shell growth had resumed in most cases.

The translucent band deposited during summer was different in appearance to those laid down during winter. The summer translucent band was not as wide as the winter band and was often incomplete, terminating in the middle layer of the shell rather than the outer layer as was the case with the winter lines (Fig 3.3).

The percentage of opaque edge bands per sample was compared with mean daylength and mean sea surface temperature. These observations indicate that opaque edge bands are being laid down when the sea surface temperature and daylength are increasing, i.e. during the spring/summer months. There is however, one period during which rapid opaque growth was expected but did not occur. During December 1993, January and February 1994, some shells displayed a change in band type from opaque to translucent (Fig. 3.4).

Fig 3.4 demonstrates that as sea surface temperature and daylength increase in spring/summer so does the percentage of individuals showing an opaque edge band. During late autumn and winter the sea surface temperature and photoperiod decrease. However, this is not reflected in the percentage of individuals showing an opaque edge band. In February and March, 1994 there was an increase in percentage of individuals showing an opaque edge band rather than the expected decrease. By April and May, 1994 no individual sampled showed opaque edge bands, indicating that rapid growth had ceased.
Fig. 3.1. Ultraviolet photomicrograph of a transverse section through the shell of *Austrovenus stutchburyi* showing the fluorescent calcein line (c) left by immersion of the living animal in a solution of calcein. Scale bar=1mm

Fig. 3.2. Photomicrograph of a transverse section through the shell of *Austrovenus stutchburyi* showing translucent (T) and opaque (O) bands. Scale bar=1mm

Fig. 3.3. Summer (s) and winter (w) translucent lines. n=shell notch associated with the winter translucent line. Scale bar=1mm
3.5. Periodicity of Macro-increments

The macro-increments consist of a period of rapid growth characterised by a wide band of opaque shell, followed by a period of slower growth characterised by a narrow band of translucent shell. The translucent shell laid down as a result of slow growth in the winter is generally associated with a notch at the shell surface and the band can be traced through all shell layers (Fig. 3.5). Translucent bands, such as those seen in summer, which are due to other factors (e.g., spawning or storms, see section 4.2.1) are frequently not complete and do not always have a surface notch; alternatively, there may be a notch with no associated translucent band (Fig. 3.5), this means that identification of the winter translucent band can be made with relative ease.

In all animals sampled in April and May 1994 a new translucent band had been deposited following an opaque band. Thus, the periodicity of the macro-increments found in the shell of *Austrovenus stutchburyi* is annual.
Fig. 3.5 Photomicrograph of a transverse section through the shell of *Austrovenus stutchburyi* showing twelve months growth. The shell was marked and placed in the field on the 9th of May 1993 and was collected on the 9th of May 1994. The translucent band (T1) representing the winter reduction of growth in 1993 is visible, as is the translucent band (T2) with its associated notch (n) representing the winter reduction of growth in 1994. A spawning mark (d1) can be seen as notches on the shell surface with a faint incomplete translucent band. A possible disturbance mark (d2) can be seen just prior to the winter notch. As this photomicrograph was taken using reflected light with no UV, the calcein mark is not visible. However, when the section was examined using UV the calcein mark was found immediately prior to the winter band (T1). Shell growth over twelve months (from T1 to the shell edge) in this individual was 6.00 mm. Scale Bar=1mm. (The slightly fuzzy nature of this micrograph is due to the fact that it is a video still, as there was not a large format still camera available)
3.5. Periodicity of Micro-increments

The micro-increments (Fig. 3.7) in the shells of 37 individuals were counted for the first four months of the main experiment, covering the period June to September, 1993. The number of micro-increments in the shells of 30 individuals was also counted from the start (February, 1994) of the supplementary experiment, to the end (March, 1994) of that experiment. These two data sets represented a winter period and a summer period respectively. In each case the count started at the calcein line deposited by the animal and ended at the shell edge. The counts were compared to the number of low tides that had occurred during the period prior to sampling (Fig. 3.6). As discussed in section 1.3.2, the micro-increments were of two types: wide complex increments and narrow simple increments (Fig. 3.8.). Evans (1975) found that at low tide a strip of organic conchiolin was formed in the shell of Cerastoderma edule. There is also a strip of conchiolin found associated with the micro-increments in the shell of Austrovenus stutchburyi. For the purpose of this study it is assumed that this is also laid down at low tide (Fig 3.8).

![Graph](image-url)

**Fig. 3.6.** Number of micro-increments in the shell of Austrovenus stutchburyi and number of low tides for four months in a) winter 1993 (n=37), b) summer 1994 (n=30). Arrowhead indicates time when cockles were marked and returned to the inlet. The bars represent one standard deviation about the mean.

It was found that micro-increments were being rapidly added between January and April, 1994. In January and February, 1994 the number of micro-increments
added was equivalent to the number of low tides. This correlation was not evident at other times of the year. The rate of addition of micro-increments decreases in March, April and May, 1994. Very few micro-increments were added in June and July, 1993. In August and September, 1993 there was a slight increase in the number of micro-increments being laid down (Fig 3.6).

3.5.1 Relationship of micro-increments to the spring/neap tidal cycle

The width of each micro-increment present from the calcein mark to the shell edge was measured and plotted with the tidal heights for the sample periods (n=1 per sample, total n=8) May-September, 1993 and January-May, 1994 (Fig 3.9 & 3.10). These data show that in winter there were few micro-increments laid down. In sample 1 (May-June, 1993) only 2 micro-increments were laid down over the month. In sample 2 (May-July, 1993) 5 micro-increments were laid down over two months. However, in sample 3 (May-August, 1993) micro-increments were being laid down regularly towards the end of that sample period (64 micro-increments). This pattern is repeated in sample 4 (May-September, 1993).

The pattern of micro-increment addition was quite different in from the summer/autumn (January-May, 1994) samples were examined. In the first sample (January-February, 1994) there was a micro-increment laid down during every tidal cycle, with a wide portion being laid down during high tide and a narrow portion laid down during low tide. In sample 2 (January-March, 1994) micro-increments continue to be laid down as in sample 1 until the end of the sample period when the micro-increments ceased to be laid down. This pattern is repeated in samples 3 (January-April, 1994) and 4 (January-May, 1994).

In all cases the micro-increments were in the opaque portion of the shell and were not present in the translucent portions of the shell. Fig. 3.10 shows that the widest micro-increments were laid down during spring tides as the clams at a shore height of 0.5m are covered for a long time and thus more shell could be laid down. Conversely, the narrowest micro-increments were laid down during neap tides, as the clams were covered for a relatively short period of time there was less shell growth.
Fig. 3.7. Photomicrograph of micro-increments in a transverse section of the shell of *Austrovenus stutchburyi*. Scale bar=0.1mm

Fig. 3.8. Record of spring (s) and neap (n) tides in the micro-increments of *Austrovenus stutchburyi*. Within the spring tide increments can be seen a strong outer boundary (o) and a faint inner boundary (i). The boundaries are a layer of conchiolin (c). Scale bar=0.1mm
Fig 3.9. Tidal cycle and micro-increment width for four winter samples a) May-June 1993, b) May-July 1993, c) May-August 1993, d) May-September 1993. Marked animals were placed in the cages on 9 May 1993. Other dates refer to sample times.
Fig 3.10. Tidal cycle and micro-increment width for the four summer samples a) January-February 1994, b) January-March 1994, c) January-April 1994 d) January-May, 1994. Marked animals were placed in cages on 4 January. Other dates refer to sample times.
3.6 Shell Parameters

The shell parameters, width and height, were regressed against length in both the main experiment and the supplementary experiment (Fig 3.11). This analysis showed that the shells of *Austrovenus stutchburyi* grow in an allometric fashion (Fig. 3.11). In the main experiment, the regression of length against width gave $r^2=0.936$ with a correlation coefficient 0.967. Similarly, length against width in the supplementary experiment gave a value for regression of $r^2=0.935$ and a correlation coefficient of 0.968. The regression of length against height gave $r^2=0.815$ and a correlation coefficient of 0.888 in the main experiment. However, the regression of length against height in the supplementary experiment gave $r^2=0.598$ and a correlation coefficient of 0.773. The differences in the $r^2$ values may be explained by the different sample sizes. In the main experiment $n=120$ and in the supplementary experiment $n=40$.

Fig. 3.11. Allometry. Main experiment ($n=120$): a) Shell length versus shell width; b) shell length versus shell height. Supplementary experiment ($n=40$): c) Shell length versus shell width, d) shell length versus shell height.
3.7 Growth

3.7.1 Growth Over Time

Growth was measured from the calcein mark to the shell edge and the measurements were then averaged for each sample. In this experiment it was not possible to follow individuals through the growing season. Thus, each monthly data point represents mean cumulative growth. The animals grown in the field showed little growth during the months of June to August, 1993 (Fig. 3.12). However, from September, 1993 to February, 1994 the growth rate increased. Growth then slowed during the months of March, April and May, 1994 (Fig. 3.12). Figure 3.13 demonstrates the growth of animals that had been left to grow for one month, six months and ten months after marking.

The animals grown in the laboratory showed little growth over time. The average growth of laboratory grown animals over the course of the experiment was 0.06 mm (Fig. 3.12).

Fig. 3.12. Growth in a) field, b) laboratory grown *Austrovenus stutchburyi*. The bars represent one standard deviation about the mean.
Fig. 3.13. Ultraviolet photomicrographs of a transverse section through the shell of field grown *Austrovenus stutchburyi* marked with calcein. A) May 9th - June 5th, 1993. Growth from calcein mark (c) to shell edge = 0.059 mm; B) May 9th - November 2nd, 1993. New growth from calcein mark to shell edge = 3.25 mm; C) May 9th, 1993 - March 1st, 1994. New growth from calcein mark (c) to shell edge = 4.65 mm. Scale bars=1mm.
3.7.2 Relationship of growth to photoperiod and sea surface temperature

Regression analyses were run on monthly growth data to compare them with sea surface temperature (°C) and daylength (hours of light) (Fig 3.13). The data indicate that as temperature and daylength increase so does monthly growth. The most growth was seen when temperatures were high (16.8°C) and daylength was long (14.6hrs).

![Graph showing regression lines](image)

<table>
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<tr>
<td>20</td>
<td>y = 9.295x + 8.011</td>
<td>0.624</td>
</tr>
<tr>
<td>15</td>
<td>y = 6.121x + 9.595</td>
<td>0.752</td>
</tr>
</tbody>
</table>

Fig. 3.14. Regression lines for monthly growth versus a) sea surface temperature and b) photoperiod.

3.8 Growth Curve

A Brody-Bertalanffy growth equation (Ebert and Russell, 1993) was fitted to length at age data (n=86) (Fig. 3.16). The equation used was

\[
S_t = S_\infty (1 - e^{-kt})
\]

where \( S_t \) = size at time \( t \), \( S_\infty \) = asymptotic size, \( k \) is a constant and \( b \) is a scaling factor. The difference equation

\[
y = a + Bx
\]

was taken from the Walford plot (Walford, 1946; Taylor, 1959) (Fig. 3.15.) where length at time \( t \) was regressed against length at time \( t+1 \). The \( \ln(\text{slope})/t \) of this was \( k \). Asymptotic size \( (S_\infty) \) was derived from the equation

\[
S_\infty = a/(1 - B)
\]

where \( a \) is the y-intercept on the Walford plot and \( B \) is the slope.
The scaling factor, $b$, was determined using the following equation,

$$b = \frac{(S_{\infty} - S_0)}{S_{\infty}}$$

where $S_0$ is size at recruitment. Table 3.1 shows the values for these parameters for *Austrovenus stutchburyi* from Papanui Inlet. These data were analysed using Systat version 5.2.1 (Wilkinson et al., 1989) and graphed using Cricket graph version 1.3.2 (Rafferty and Norling 1986-9) (Fig. 3.16).

Table 3.1 Growth Parameters for the Von Bertalanffy Equation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a$</td>
<td>10.043</td>
<td>This study</td>
</tr>
<tr>
<td>$B$</td>
<td>0.731</td>
<td>This study</td>
</tr>
<tr>
<td>$k$</td>
<td>0.3133</td>
<td>This study</td>
</tr>
<tr>
<td>$S_{\infty}$</td>
<td>37.334</td>
<td>This study</td>
</tr>
<tr>
<td>$S_0$</td>
<td>180μm</td>
<td>Stephenson &amp; Chanley, 1979</td>
</tr>
<tr>
<td>$b$</td>
<td>0.9949</td>
<td>This study</td>
</tr>
</tbody>
</table>

![Fig. 3.15. Walford Plot](image)

$$y = 10.044 + 0.73100x \quad R^2 = 0.916$$
Fig. 3.16. Growth curve for *Austrovenus stutchburyi*. A dot represents an actual size at age measurement (n=86 clams) and an open triangle represents the estimated size at age calculated from the Von Bertalanffy Growth Equation.

These data indicate that *Austrovenus stutchburyi* grow rapidly in their first 4-5 years then growth rate slows between years 5 and 12 after which the model predicts that there will be little increase in shell length per year.
4.1 Thermal Stress and Calcein Marking

The marking of living bivalve shells using a decrease in temperature has been successfully used in a number of studies on a variety of species including, Cerastoderma edule (Richardson, 1979; Richardson et al., 1980a, 1980b), Anadara granosa (Richardson 1987a), Tapes philipinarium (Richardson, 1987b), Spisula subtruncata (Richardson, 1988), Mytilus edulis (Richardson, 1989) and Mercenaria mercenaria (Bock and Miller, 1994). In all of these studies the reduction in temperature produced an easily recognisable cleft in the outer layer of the shell. In the present study, Austrovenus stutchburyi was thermally stressed using the method described by Richardson (1979). However, the expected cleft in the shell was not produced. Richardson (1979) found that marking bivalve shells by the method of thermal stress was best achieved during summer. In this study the animals were stressed in the month of May, which in New Zealand is late autumn. It is possible that the ambient temperature was too low to make thermal stress a successful method of marking Austrovenus stutchburyi. More success may be obtained if this method is attempted in January or February when sea temperatures and shell growth reach a peak.

A chemical marker has been used successfully in the study of growth in abalones (tetracycline, Pirker and Schiel, 1993), sea urchins (tetracycline, Rowley, 1990), squid (tetracycline, Lipinski, 1986, Jackson, 1990), fish (calcein, Wilson et al., 1987; Beckman et al., 1990) and bivalves including, Phacosoma japonicum (alizarin red, Tanabe, 1988) and Placopecten magellanicus (alizarin red, Parsons et al., 1993). In each of these studies the marker has been used in mark-recapture experiments to determine growth parameters in the species studied. Tetracycline and calcein are fluorescent markers which bind to alkaline earth metals in fish tissues and bones during periods of growth (Muncy et al., 1990). The antibiotic tetracycline has been the most often used while calcein has not commonly been used. In the study by Richardson (1979) both alizarin red and tetracycline were used to mark the shells of Cerastoderma edule, but neither proved to be useful. Alizarin red interfered with shell deposition and tetracycline was also unsuccessful although details were not provided. Calcein has not been used in bivalve studies possibly due to the fact that it is more expensive than tetracycline. However the present study has shown that calcein is incorporated into the shell structure of Austrovenus stutchburyi creating a clearly visible yellow-green fluorescent band. The expense of calcein is
offset by the relatively low dosage required to produce a fluorescent mark (Pirker and Schiel, 1993). The present study used calcein at a concentration of 500mg/L seawater which was sufficient to produce a strong mark in all the individuals used in the study. Pirker and Schiel (1993) found that to successfully mark adult *Haliotis iris* by immersion a concentration of tetracycline of 600/800mg/L seawater was required to produce a good fluorescent mark in 78% of animals.

While more expensive than tetracycline, calcein has some advantages. It is less damaging to tissues than tetracycline and is more stable in solution, whereas tetracycline is readily oxidised (Pirker and Schiel, 1993). In fish, post-immersion exposure to light was found to reduce detectable levels of oxytetracyline, which reduced the numbers of otoliths in which a mark could be found (Muncy et al., 1990). In addition, calcein has a brighter fluorescence than tetracycline making it much easier to detect in the marked structure (Wilson et al., 1987).

More work, however, is required in some areas of calcein use. Beckman et al., (1990) found that there was some variability in the fluorescent mark produced in laboratory reared fish indicating that there may be a metabolic difference between wild fish and laboratory reared fish. An alternative hypothesis is that calcein may not be effective when used in conjunction with artificial seawater used in the study. This did not appear to be the case with *Austrovenus stutchburyi* used in this study. All individuals showed a strong fluorescent mark whether kept in the laboratory or in the field. There were some differences in methodology between the two studies which may have affected the deposition of calcein.

The most obvious difference was that Beckman et al., (1990) were studying larval or juvenile fish, while the current study was examining adult molluscs. In addition, Beckman et al., (1990) immersed the fish in a low concentration of calcein (1-200mgL⁻¹) for a short period (2-4hrs), whereas in this study the bivalves were immersed in a higher concentration (500mgL⁻¹) for a longer period of time (24hrs). Finally, Beckman et al., (1990) used low salinity (1-15‰) artificial seawater. The current study used normal salinity (35‰) natural seawater. Any or all of these factors may have had an effect on calcein absorption so that direct comparisons between these two studies should be made with care. It is, however, apparent that calcein may become an important tool in the study of the age and growth of marine bivalves.

**4.2 Edge Band Type**

The edge bands in *Austrovenus stutchburyi* are the result of transparency variations. There are two areas of different transparency: an opaque area and a
translucent area. These two components make up an increment. These two different transparencies are associated with differing growth rates. The opaque area is laid down during periods of fast growth, while the translucent area is laid down during a period of slow growth (Coutts, 1970; Clark, 1979; Grizzle and Lutz, 1988). These periods of slow and fast growth do not necessarily occur at the same time of year for all species. Some molluscs have limiting temperatures at both ends of the scale, i.e., a winter and summer check in growth. However, most bivalve growth studies are made in areas where the lower temperature limit is critical and it is therefore more common to find a winter check (Clark, 1979).

4.2.1 Edge Bands as an indicator of seasonality and reproduction

The change in edge band can be followed throughout the year to give an indication of a seasonal influence on growth. Many bivalve species show a seasonal variation in transparency of shell material. These include: *Mercenaria mercenaria* (Clark, 1979; Quitmeyer et al., 1985; Grizzle and Lutz, 1988; Jones et al., 1990), *Spisula solidissima* (Jones, 1980), *Arctica islandica* (Jones, 1980), and *Mercenaria campechiensis* (Jones et al., 1990; Quitmeyer, 1992). The bands formed are of two types, translucent and opaque. In all studies the translucent portion of the shell was laid down during periods of slow growth, while the opaque portion of the shell was laid down during periods of rapid growth. These types of data have been used in the past by anthropologists to determine the seasonal occupation of prehistoric habitations (Coutts, 1970; Rhoads and Pannella, 1970; Clark, 1979; Nichols and Thompson, 1982; Sheppard, 1985). However, it is important to note that different species of bivalves may display rapid or slow growth in different seasons. For example, *Mercenaria mercenaria* has a period of slow growth in summer (Clark 1979; Quitmeyer et al., 1985; Jones et al., 1990), *Spisula solidissima* has a period of slow growth in late summer/autumn, *Arctica islandica* shows slow growth in early winter (Jones 1980) and the Southern Quahog (*Mercenaria campechiensis*) was found to grow slowest in winter (Jones et al., 1990; Quitmeyer, 1992). The current study has shown that *Austrovenus stutchburyi* shows variation in shell translucency according to season. Examination of the shells showed translucent bands typical of slow in the months April to August, which is Autumn and Winter in New Zealand.

It was also found in this study that some individuals of *Austrovenus stutchburyi* laid down translucent bands from November. At this time of the year temperatures are starting to increase but have not reached a maximum so it seems unlikely that increased temperature is the limiting factor in shell growth at this time.
Gametogenesis has been shown to decrease somatic growth in other species of bivalve. The zebra mussel (*Dreissena polymorpha*) shows a decline in growth which was attributed to reproductive activities (Smit et al., 1992). The venerid clam *Mercenaria mercenaria* also shows a smaller, thinner translucent increment in shell cross sections, in addition to the wider temperature induced translucent increment. This thinner increment was attributed to spawning activity (Cunnliffe and Kennish, 1974). It seems likely then that the summer depression in the growth of *Austrovenus stutchburyi* may be related to gametogenesis. This theory is supported by the study done by Larcombe (1971). In this study the reproductive cycle of clams from Te Rauone Beach on the Otago Peninsula was determined using the gonad index method. The gonad index showed that the gonads of *Austrovenus stutchburyi* are ripening in October/November and are either ripe or spent from November to late February. In addition, Booth (1983) found that the main spawning time for *Austrovenus stutchburyi* is December to January. In the present study, the summer translucent bands were evident in 30% of the sample collected in November. This percentage increased each month until the maximum of 80% was reached in January. These data suggest that the summer translucent band is due to a decrease in somatic growth during the period of reproductive activity.

4.2.2 Sea surface temperature, photoperiod and edge band type

Edge band type was related to both photoperiod and sea surface temperature. The relationship between edge band type and variables such as photoperiod and sea surface temperature is likely to be complicated. It may prove to be impossible to separate the effect of photoperiod from that of sea surface temperature. However, Quitmeyer (1992) found that although all causal factors for the timing of incremental shell formation have not been identified, water temperatures seem to have the greatest influence.Quitmeyer (1992) found that the optimal temperature for maximum shell growth in *Mercenaria mercenaria* is 20°C. Above and below this temperature growth decreases and there is no growth at temperatures below 9°C or above 31°C.

This should be investigated for *Austrovenus stutchburyi*. It is likely that as *Austrovenus stutchburyi* in Otago are found in a cold temperate environment the upper limit for growth may be lower than the 31°C Quitmeyer (1992) found for *Mercenaria mercenaria*. Certainly, *Austrovenus stutchburyi* in this study were growing at temperatures <9°C. Average monthly growth of between 0.1-2mm was recorded for sea surface temperatures of 7.2-8.8°C in the months of July, August and September.
While water temperature appears to be very important in the timing of incremental shell growth, other seasonal variables should not be discounted. Seston composition and water quality have been shown to influence the daily growth in *Mercenaria mercenaria* (Bock and Miller, 1994). It should also be noted that a comparison between this and other similar studies cannot readily be made. Geographic location can influence banding patterns due to differences in environmental variables such as ambient water temperature, particularly winter and summer extremes (Grizzle and Lutz, 1988).

### 4.3 Periodicity of Macro-increments

Periodicity of macro-increments has been determined for a number of species. In most studies the periodicity has been shown to be annual. However, the cause of the cessation or slowing of growth which creates the increment varies. Many species have a winter break in growth due to a decrease in ambient temperature. These species include *Cerastoderma edule* (Farrow, 1972), *Macoma balthica* (Gilbert, 1973), *Mercenaria mercenaria* (Cunliffe and Kennish, 1974; Grizzle and Lutz, 1988), *Mya arenaria* (MacDonald and Thomas, 1980), *Panope generosa* (Shaul and Goodwin, 1982), *Geukensia demissa* (Brousseau, 1984), *Chlamys rosealbus* (Silina and Pozdnyakova, 1985), *Phacosoma japonicum* (Tanabe, 1988), *Tawera mawsoni* (Luckens, 1990), *Panopea zelandica* (Breen et al., 1991) and *Mercenaria campechiensis* (Quitmeyer, 1992). Other species have a break in growth at different times of the year and with a different causal factor. For example spring spawning has been shown to cause an interruption in the shell growth of *Pecten maximus* (Mason, 1957), while *Spisula solidissima* (Jones at al. 1978; Jones, 1980) and *Arctica islandica* (Jones, 1980) have a spawning related interruption in growth in late summer/early autumn and late autumn/early winter, respectively. In the Nile River the annual cessation of growth in *Corbicula consobrina*, (el Moghraby and Adam, 1984), *Caelaturn aegyptica*, *Caelaturn teretiuscula* and *Mutela dubia* (Adam, 1990) is related to an annual siltation event when the river is in flood. This occurs during July-October. The tropical bivalves *Meretrix casta* and *Paphia malabarica* have no evidence of a break in allometric growth (Parulekar, 1984).

As has been discussed in section 4.2.1 *Austrovenus stutchburyi* produces two translucent sections of shell, one in summer and one in winter. The two translucent areas are different in appearance. The translucent section laid down in winter is thicker, more complete and is associated with a depression or notch on the outside of the shell. In comparison, the translucent band laid down in summer is fainter, narrower and is often incomplete. This difference in the
morphology of the edge band allows identification of the main period of growth and thus the periodicity of the macro-increment can be determined. This study has supported Coutts (1974) study which found that the periodicity of the macro-increments of *Austrovenus stutchburyi* are annual, with the annual growth being measured from winter increment to the next winter increment.

### 4.4 Periodicity of Micro-increments

Evans (1975) found that at low tide a strip of organic conchiolin was exposed in the shells of *Clinocardium nuttalli*. This is due to anaerobic conditions within the mantle cavity when the shell is shut. During this period organic acids build up in the pallial fluid causing some calcium carbonate to etch away which uncovers the conchiolin (Palmer, 1995). It is this dark strip that forms the boundaries of the micro-increments.

The micro-increments in the shell of *Austrovenus stutchburyi* have a regular pattern. In other species this has been shown to be due to a variety of environmental factors, including, solar day for *Pecten diegensis*, *P. vogdesi*, *Argopecten irradians*, *A. gibbus*, *A circularis* (Clark, 1975) and *Placopecten magellanicus* (Parsons et al., 1993), tidal cycle for *Cerastoderma edule* (House and Farrow, 1968; Farrow, 1971; Richardson et al., 1979, 1980a, 1980b, 1980c, 1981; Deith, 1985, Lønne and Gray, 1988), *Clinocardium nuttalli* (Evans 1972, 1975, 1988), *Callista chione* (Hall et al., 1974), *Kellia suborbicularis*, *Chione californiensis*, *Chione undatella*, *Protothaca staminea*, *Mercenaria mercenaria* (Berry and Baker, 1975), *Anadara granosa* (Richardson, 1987a), *Tapes phillipinarium* (Richardson, 1987b), *Spisula subtruncata* (Richardson, 1988). Richardson (1990) found that intertidal specimens of *Mytilus edulis* showed a tidal periodicity in the deposition of micro-increments, but subtidal individuals did not. In addition Hall et al., (1974) found that *Tivela stultorum* has a daily periodicity of deposition that may be tidal but their results were inconclusive. In some cases the periodicity of deposition of the micro-increments could not be attributed to external factors. This type of deposition was found in the shells of *Spisula solidissima* (Jones, 1981), *Chlamys hastata hastata*, *Chlamys hastata herica* and *Hinnites multirugosis* (Clark, 1975). Finally, the micro-increments of the rock boring clam *Penitella penita* were examined by Evans and le Messurier (1972) and by Evans (1975). It was found that the effect of environmental factors such as tides and seasonal fluctuations was minor in this species. Width of the growth increments and microstructure was found to be controlled by the amount of space in the burrow, which is in turn dependent upon substrate hardness.
Thus, it can be seen that there are several causes of micro-increment periodicity. However, the most common appears to be the effect of tides and, in particular, tidal emersion. This is especially so for intertidal bivalves such as *Mercenaria mercenaria*. Coutts (1974) suggested that the micro-increments found in the shells of *Austrovenus stutchburyi* may also have a tidal periodicity. However, the results did not appear to support this. Richardson et al., (1980c) examining the shells and micro-increments of *Cerastoderma edule* found that in winter there were fewer micro-increments than tides, but in summer there was fairly good agreement. A difference between winter and summer micro-increments has also been found in *Clinocardium nuttalli* (Evans, 1988) and *Mytilus edulis* (Richardson et al., 1990). This means that it is possible that the micro-increments of *Austrovenus stutchburyi* followed a similar summer/winter pattern. To examine this, the present study compared the number of micro-increments in the shells of *Austrovenus stutchburyi* with the number of low tides over two four month periods, one in winter and one in summer. The results show that the micro-increments are not laid down in winter and a micro-increment is only laid down for each low tide during the peak growing season. These data explain Coutts' (1974) results where fewer micro-increments were observed than were expected for a tidally controlled phenomenon.

Richardson (1980c, 1990) and Evans (1988) have shown that there is a difference in the deposition of micro-increments during winter when compared to summer deposition. However, Richardson(1980c) found that animals from a cool temperate environment had narrower and fewer winter micro-increments and animals from a subarctic environment had a complete cessation of growth and therefore had no winter micro-increments. The New Zealand cockle, *Austrovenus stutchburyi*, examined in this study came from a cool temperate environment and thus fewer winter micro-increments might be expected. However, most individuals showed no winter micro-increments at all. This indicates that temperature alone may not be the controlling factor in the deposition of micro-increments in this species. Other possible influences on growth such as fluctuations in plankton concentration and turbidity should be examined.

4.4.1 Spring/neap tidal cycle

If micro-increment periodicity is entrained by tidal cycles, then it seems reasonable that the effect of different tidal heights should be recorded in the shell calcite. Several workers have found a relationship between the width of shell micro-increments and the spring/neap tidal cycle.
In the shell of *Austrovenus stutchburyi* there is a pattern of wide micro-increments with well defined outer borders and an additional weak inner border, interspersed with narrower micro-increments which have the well defined outer border but lack the inner border. This is consistent with the micro-increment pattern found in *Clinocardium nuttalli* (Evans, 1972, 1975). Evans (1972, 1975) called the wide micro-increments "complex" increments and the narrow micro-increments "simple" increments. The micro-increments in *Austrovenus stutchburyi* will be referred to using the same terms. Spring/neap tidal patterns have been found in other species. However, in some cases there are differences in the morphology of the micro-increments seen in the shells.

Richardson et al., (1979) found a spring/neap pattern in the shells of intertidal *Cerastoderma edule*, but these micro-increments did not have the "complex" spring tide micro-increments that had been observed by Evans in *Clinocardium nuttalli*. In *Cerastoderma edule* all the micro-increments were of the "simple" type. The major difference was that spring tide micro-increments had strong well defined borders, while neap tide micro-increments had fainter borders.

Richardson et al., (1980b) found that there was a small but significant difference in growth rate during the spring/neap lunar cycle. This was found to be due to tidal level. At mid-to-high tide levels and when submerged on a raft, animals grew fastest at springs and slowest during neaps. Near low water the least growth occurred at springs and the most growth at neaps. The authors attributed this to the reduced periods of immersion during spring tides and continuous immersion during neap tides at this level. Increase in water flow was believed to account for greater growth rates during springs at mean-tide levels and on the raft.

Evans (1988) found that at any particular season the amount of time that *Clinocardium nuttalli* is submerged is the factor that most influences the width of the micro-increment. In animals taken from the middle intertidal, the micro-increments laid down during spring tides (when the animals were only exposed once a day) were twice as wide as those laid down during the previous or following neap tides. Further evidence for the relationship between micro-increment width and coverage time is seen in animals from the high intertidal where nearly every low tide is represented by a micro-increment. In spring tide periods the time of coverage during a high-high tide is considerably longer that during the next low-high tide. This is reflected in the width of the corresponding micro-increments. While time of coverage determines the relative width of neighbouring micro-increments, the actual micro-increment width is also determined by a number of other factors. These may include intrinsic factors (age
and spawning activity) or extrinsic ones (season and vertical position in the intertidal).

Richardson (1988) found that in individuals of *Spisula subtruncata* kept in the intertidal zone at low water of spring tides (LWST) the shell micro-increments showed a pattern related to spring and neap tides. During spring tides when the animals were emersed, distinct semi-diurnal micro-increments were laid down in the shell. However, during neap tides the micro-increments were weaker and ill-defined with an approximate circa-tidal periodicity.

Richardson et al., (1990) examined the shells of low intertidal *Mytilus edulis* and found a spring/neap pattern in the shell. During spring tides, when the animals are emersed twice a day a pattern of strongly defined micro-increments is produced. During neap tides, when the animals remain immersed, weakly defined micro-increments are produced.

Evans (1972) postulated that the smaller the tidal fluctuation, the thinner the micro-increment. Thus the narrow micro-increments are related to neap tides. Because the shells of *Austrovenus stutchburyi* used in this study had been marked with calcein and returned to their home inlet this was easy to test. Each micro-increment from the calcein mark to the shell edge was measured. By comparing that data set with the appropriate tidal data it was found that the simple micro-increments in the shell of *Austrovenus stutchburyi* were related to neap tides while the complex micro-increments were related to spring tides, in a similar fashion to those micro-increments found in *Clinocardium nuttalli*. Evans (1972) stated that "*Clinocardium nuttalli* is probably a particularly sensitive recorder of tidal exposure because it lives just below the surface of the sand." The same can be said of *Austrovenus stutchburyi* which is generally found burrowed 2-4 cm deep below the sand (Jones, 1983). Thus it is not surprising that tidal micro-increments should be found in the shell of this bivalve.

4.5 Shell Parameters

Allometric growth has been extensively used to determine bivalve growth. Shell length has been considered to be the most appropriate parameter to measure for the determination of bivalve growth (Chatterji et al., 1984). In the present study, shell length/shell width and shell length/shell height relationships were analysed. The relationship between these parameters proved to be linear and in the main field study, had regression coefficients of 0.936 and 0.815 respectively. The analysis of shell length against width and height shows that these latter parameters have constant growth with regard to shell length. These data indicate
Firstly the growth rate of the clams may simply lag behind changes in sea surface temperature. Secondly recovery from spawning may have more of an effect on somatic growth than sea surface temperature. Finally, growth rate may not be solely controlled by sea surface temperature. For example, some studies have shown that air temperature plays a more important role in decreasing or halting somatic growth in bivalves than does sea surface temperature (eg Green, 1973).

4.6.3 Growth curve

The growth of *Austrovenus stutchburyi* was found to be well explained by the Von Bertalanffy growth equation. This showed that these clams undergo a rapid growth phase in the first few years after settlement which then slows at about year 5.

*Austrovenus stutchburyi* attains most of its total length by the age of 10. The Von Bertalanffy growth equation predicts that the somatic growth of these clams would be very slow from the twelfth year after settlement with very little shell being added per year after this. These data suggest that *Austrovenus stutchburyi* is a relatively long lived species which may reach ages greater than 20 years. Further work is required on larger specimens of this species to prove or disprove this supposition.

The Von Bertalanffy growth curve is commonly used to describe bivalve growth and it appears to be a good model describing growth in some bivalves, particularly the venerid clams as is seen in *Austrovenus stutchburyi* (this study), *Phacosma japonicum* (Tanabe, 1988), *Mercenaria mercenaria* and *Mercenaria campechiensis* (Jones et al., 1990). However, it should be noted that this model does not describe well the growth of all bivalve species. Theisen (1973) found that the Von Bertalanffy equation was invalid for *Mytilus edulis*. Further, it has been shown that growth can vary between populations, so that a new growth curve must be calculated for each population. For example, Brousseau (1984) and Appeldoorn (1983), found that different growth curves were needed for populations at different latitudes. This is not surprising as many researchers have found that growth rate varies with latitude (e.g., Ansell, 1968; Tanabe and Oba, 1988; Iglesias and Navarro, 1990). Differences in growth rate and thus growth curve have also been found for populations with a smaller geographical separation. Jones et al., (1978) found differences in growth rate between two populations of *Spisula solidissima*, one living onshore and one offshore. This difference was attributed to a temperature difference between the two locations.
4.7 Problems, Limitations and Further Study

As with any study involving living animals, there were some problems and limitations with this study.

1. Experimental growth measures

For the field study, growth was measured in a cumulative fashion, that is, an individual's growth was not followed from month to month. Instead, the animals were placed in the field and different animals were collected each month. Thus, the first sample showed one month's growth, the second two month's and so on. This may have introduced inaccuracies in the growth rate calculations. However, this study was primarily designed to validate the periodicity of the growth increments in the shell; growth rate calculations were secondary objectives. It has been shown in other studies that handling of bivalves may create additional increments or disturbance lines (Orton, 1926, Richardson et al., 1990). The animals examined in this study were handled as little as possible to prevent the creation of additional confounding lines. It would, perhaps, be valuable to do a comparative study to investigate this source of error. Growth could be examined using three different treatments. Some individual's growth rate would be measured as in this study, a second group would be measured each month and a third group would be treated so that each month a new individual is marked at the start of the month and its growth measured at the end of the month. This type of study would serve two purposes: firstly it would show whether cumulative growth measures introduce large errors or not; and secondly, it should be possible to determine whether or not handling causes an individual of this species to lay down a disturbance line.

2. First Increment

Many bivalve studies have shown that the first increment and sometimes the second are very difficult to find (Cole, 1956). Often the newly settled bivalve does not slow or cease growth over the first winter. For the purpose of this study, it was assumed that the first increment found was the first created. However, it should be noted that the age estimates given in this study may be underestimates.

3. Size of Clam

Because the primary aim of this study was to validate the periodicity of the growth increment, the animals were chosen for ease of handling and potential clarity of the internal increments. Also, the clams selected were of a restricted size range. In particular, there were no very small animals (<20mm) and no very large
animals (>35mm). It was felt that small clams would be very difficult to section, while in large animals the growth increments found in the shell get very close together in the later years so that deciphering them becomes nearly impossible (Mason, 1957). This may have had an effect on the growth curve derived for this species. To obtain a more comprehensive growth curve, animals over a greater size range should be aged.

4. Latitude

It should be noted that the results from this study should be used with caution when predicting the age and growth of *Austrovenus stutchburyi* from other areas of New Zealand. In similar studies of European, Asian and American bivalves it was found that there was a difference in growth rate and shell deposition according to latitude (Ansell, 1968; Appeldoorn, 1983; Tanabe and Oba, 1988; Iglesias and Navarro, 1990). It seems reasonable then, to predict that *Austrovenus stutchburyi* growing in the North Island of New Zealand may have different growth characteristics from those living in the South Island of New Zealand. Thus, while this study provides information on the age and growth of individuals from Papanui Inlet it should be used only as a guide to the growth of individuals outside this geographical area.

5. Cage Effect

It is possible that the cages themselves had an effect on the growth of the clams kept in them by creating changes in the micro-habitat (R. Belton pers. comm.). However, other studies have found little effect of cages on the growth of bivalves kept in them (Belanger et al, 1990). The clams in the present study were kept in cages because it proved to be too difficult to relocate them after a week or more in the inlet (section 2.2.1). Thus it was decided that despite the possible effects of the cage on growth it would be necessary to keep the clams caged.

6. Feeding

As discussed previously (section 4.6.1), clams kept in the laboratory did not grow well over the duration of this study. One possibility is that the food provided was inadequate or inappropriate (Bock and Miller, 1994). As *Austrovenus stutchburyi* are filter feeders it was assumed that the single celled alga, *Dunaliella primolecta*, would be a suitable food source. However, it is possible that this was not nutritious enough or not fed in large enough quantities to allow somatic growth. It would be desirable to carry out some feeding trials with this species if they are to be kept with any success in the laboratory. In the field, water samples should be
taken to examine what is present in the water column during the year and thus
give an idea what the clams would be eating in the field. Then clams could be
kept in the laboratory and various concentrations of what is naturally available,
and more artificial foods, such as Dunaliella primolecta could be fed to the clams.
In this way an optimal feeding regime could be created for the maintenance and
growth of these clams in the laboratory.

7. Tide

The production of circa-tidal micro-increments in the shell of Austrovenus
stutchburyi indicates that the tidal cycle influences the growth of this species of
clam. The extent to which this occurs is as yet undetermined. Further work is
required on this aspect of clam growth and may, in part, be achieved by the use
of a tide machine in the laboratory (provided the problems with growth in the
laboratory can be overcome). The machine can be set to have tides of varying
periodicity, e.g., four low tides in 24 hours. This would serve two purposes: firstly
it would completely validate the periodicity of the micro-increments in the shell;
and secondly, it would begin to provide an insight into how much influence the
tides have on somatic growth in the species.

8. Temperature

Temperature has been shown in other species to influence somatic growth
(Green, 1973; Richardson et al., 1980c; el Moghraby and Adam, 1984; Parulekar,
1984; Ramón and Richardson 1992; Tan Tui and Prezant, 1992; Dekker and
Beukema, 1993). It is, therefore, not surprising that there is a positive correlation
between temperature and somatic growth in this species. However, as with tidal
influence, the extent of the influence of temperature on shell growth remains
undetermined. To investigate this clams could be maintained in a controlled
temperature room at a variety of temperatures and the effect on the clams
examined.

4.8. Summary

1. Marking. The fluorescent dye calcein was 100% successful in marking the shells
of Austrovenus stutchburyi. The dye left a strong green line in the shell when
examined under ultra-violet light. Subjecting the clams to a cold shock was
unsuccessful as a method of marking the shell. The expected cleft in the shell did
not occur.

2. Shell transparency. The growth increments in the shell of Austrovenus
stutchburyi are composed of two areas of different translucency, an opaque area
and a translucent area. These were found to correspond to different growth rates. Fast growth produces opaque shell, while slow growth produces translucent shell.

3. **Periodicity of Macro-increments.** The macro-increments were found to be annual.

4. **Periodicity of Micro-increments.** These were found to have a circa-tidal periodicity. The micro-increments were found to be produced only in the summer.

5. **Spring/Neap Tidal Cycle.** The spring/neap tidal cycle was recorded in the shell calcite. During summer, when tidal micro-increments were produced, it was found that there were two types of micro-increment: a wide complex increment which corresponded to spring tides; and a narrow simple increment which corresponded to neap tides.

6. **Growth in the Laboratory.** Individuals of *Austrovenus stutchburyi* maintained in the laboratory showed little or no growth for the duration of this study. Several reasons for this were postulated, including inappropriate food and lack of tidal influence.

7. **Growth in the Field.** Individuals of *Austrovenus stutchburyi* maintained in the field displayed a period of rapid growth in the summer followed by a period of slower growth in the winter.

8. **Growth Curve.** Growth was found to be well explained by the Von Bertalanffy growth equation. Growth was found to be rapid during the first 4 - 5 years after settlement then slow between years 5 and 12 after which the model predicts that there will be little increase in shell length per year.
References


(Ophiocoma scolopendrina, Ophiocoma erinaceus and Macrophiothrix longipeda). MSc, University of Otago.


Rafferty, J. and R. Norling, Cricket graph 1.3.2. (Cricket Software, Malvern, 1986-89).


Appendices
**Acetate Peel Preparation**

To prepare an acetate peel the shell is sectioned through the longest growing axis from the umbo to the outer shell edge (Sheppard, 1984). To prevent the shell chipping or becoming otherwise damaged during sectioning, the shell is often placed in a resin. The resin also provides a firm base upon which to carry out the rest of the procedure. The cut edge is ground flat on wet silicon carbide paper of varying grits, starting with a coarse paper (100 grit) and finishing with a fine one (2000 grit). A lapidary wheel or milling machine can be used at this stage. The block should be ground in a circular motion. This minimises the creation of deep scratches. Final polishing is done by hand with a polishing paste to remove any fine scratches. Several different pastes have been used, including carborundum powder, alumina powder, diamond paste and even brass metal polish or toothpaste. The final polishing should continue until all flaws have been removed from the surface of the block and it looks mirrored under reflected light.

In this study it was found that the shells of *Austrovenus stutchburyi* were brittle when sectioned and had to be embedded in resin to protect them. The best resin for this purpose was an electrical cable jointing resin, "Araldite K142™". Once coated, the shell was sectioned using a small, relatively slow, hand held Dremel™ Saw.

The cut edge of the shell was polished according to the method described above. Alumina (0.05 μm) on wet polishing felt (Leco Lecloth™) gave the best final polish to the sectioned edge of the shell.

The shell, once polished, was etched in acid. The optimum etching time must be determined empirically as it varies considerably depending on the acid used, its concentration and the shell layer. In this study the shells of *Austrovenus stutchburyi* were etched for 1 & 3/4 minutes in 10% HCl. The etching was stopped by washing the shell in distilled water. When the shell was thoroughly dry, it was placed with the polished side up. To provide some support and prevent movement, the shell can be placed in modelling clay (Breen, pers comm). This is important to prevent smudging of the acetate paper.

The cut edge of the shell was flooded with acetone and cellulose acetate paper was rolled onto the surface in a manner similar to the hinge technique used to place cover slips on microscope slides. This ensures that fewer bubbles are formed between the paper and the shell surface. If small bubbles formed, then they could be removed by gently smoothing the paper with either a finger or the blunt end of
forceps. Although minor bubbles can be removed in this fashion, it is preferable not to produce any in the first place, as the acetate paper becomes extremely fragile once exposed to acetone (pers. obs.). The thickness of the paper can vary considerably. Rhoads and Pannella (1970) have used paper up to 3 mm thick. However, in general, thinner paper is used.

The acetate covered shell was left to dry for about 20 - 30 minutes. Once dry, the acetate paper was carefully peeled off. It was quite brittle at this stage and rapid, jerky movements were be avoided to prevent damage to the peel. The peel was placed between two microscope slides for examination. This prevented the peel from tearing or curling. The peel could be examined directly under the microscope or used as a photographic negative (Sheppard, 1984).
Thin Section Procedure

One valve of the shell was embedded in resin and sectioned along the longest growing axis. Half of this was then ground flat using a lapidary wheel and coarse silicon carbide paper (120 grit). Once the cut edge is ground completely flat it was polished until there were no scratches on the cut surface. This was glued to a microscope slide.

There are different glues that can be used to secure the shell to the slide. In some instances catalysed resin has been used. This requires a hot plate to heat the specimen and if the resin boils bubbles are formed which can be difficult to remove. It was considerably easier to use a glue such as "araldite TM" which does not require heating. The slide was left to cure for 24 hours at ambient temperatures (Sheppard, 1984).

Once the glue had dried the shell was reduced in thickness, by sawing off the excess from the back. The remainder was about 3 mm thick. The final desired thickness was then achieved by grinding the block down with silicon carbide paper of a medium (600 grit) to fine (2000) grit and then the section was polished with polishing paste, 0.05μm alumina. The best thickness is when the critical aspects of the shell structure are clearly seen. The optimum thickness for shells varies from 25 - 100 microns, and depends on the shell and the amount of detail required (Sheppard, 1984). The shell sections for this study were on average 30μm thick.

Care should be taken during the final polishing of the section. At this stage the section is very fragile and it is easy to over polish and damage growth layers.
Microwave Sterilisation of Seawater and Nutrient Infusion for Algal Culture

Sterilisation can be achieved by using an autoclave. However, this usually takes some considerable time and may cause precipitation of some of the added nutrients. Microwave sterilisation, using a household microwave oven, is faster. In total it takes about 10 minutes per litre of sea water on the high power setting of the microwave. To ensure even heating the seawater was sterilised in four bursts (3 minutes, then 2 minutes, then 3 minutes and finally 2 minutes). Between each burst in the microwave the sea water was agitated by rotating the flask by hand. This ensured even heating of the water. Algae was added to nutrient media f/2 (Guillard, 1975) (Table C 1)

The solution was infused with 10 ml of algae (Dunaliella primolecta) from a stock solution. This was done in a laminar flow hood to prevent contamination of the stock solution and the new culture. The culture was stoppered with a cotton bung that was then sealed with tin foil. The preparation was kept at 15°C under constant light. The culture takes about 10 days to grow to a cell density of 2.5 x10⁹ cells L⁻¹ seawater and new cultures were grown fortnightly.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Concentration (mg/L seawater)</th>
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<tbody>
<tr>
<td>NaNO₃</td>
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</tr>
<tr>
<td>NaSiO₃</td>
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</tr>
<tr>
<td>NaH₂PO₄</td>
<td>5.00</td>
</tr>
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</tr>
<tr>
<td>CoCl₂·6H₂O</td>
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<tr>
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<tr>
<td>Na₂MoO₄·2H₂O</td>
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<tr>
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</tr>
<tr>
<td>B₁₂</td>
<td>0.5 µg</td>
</tr>
</tbody>
</table>

Table C1 Nutrient composition of media f/2 (Guillard, 1975)