THE EFFECT OF TIDAL LEVEL ON ENERGY BALANCE OF THE GREENSHELL MUSSEL *Perna canaliculus*.

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Abstract

This study investigated the energetics of the greenshell mussel, *Perna canaliculus*, one of New Zealand's most commercially important aquaculture species. Aerial and aquatic rates of O_2 uptake, filtration rate, assimilation efficiency and nitrogen excretion were measured for mussels collected from Taylors Mistake at two shore levels (0.0 (low shore) and 1.0 m (high shore) above mean low water). Measurements were made for winter (May to August) and summer (November to January) collected mussels. I also calculated the dry weight condition index for high and low shore level *P.canaliculus* from Taylors Mistake four or five times during the year. CI was consistently higher for mussels found at the low shore level (yearly mean = 80.3 ± 7.6) than high shore mussels (yearly mean = 68.8 ± 5.1). Scope for growth (SFG) of *Perna canaliculus* was calculated for high and low shore mussels collected during winter and summer.

Aerial and aquatic O_2 uptake increased with body weight with weight exponents between 0.57 and 0.91. Aerial and aquatic oxygen uptake was similar for high and low shore mussels. Aerial $\dot{V}O_2$ was maximal at 10°C during winter and 15°C in summer. This energy conservation was interpreted as a mechanism to reduce desiccation.

Clearance rates were calculated for mussels fed on a monoculture of *Isochrysis galbana*. They were higher for upper shore collected *P.canaliculus* than low shore mussels during both winter (high shore = 3.6 l hr^{-1} g^{-1}; low shore

= 2.5 l hr^{-1} g^{-1}) and summer (high = 2.1 l hr^{-1} g^{-1}; low = 0.7 l hr^{-1} g^{-1}). This was interpreted as an adaptation to compensate for reduced feeding time due to aerial exposure of high shore animals.

Assimilation efficiency of *P.canaliculus* fed on a monoculture of *I.galbana* was constant over a broad range of algal concentrations with an average value of 83%. Assimilation efficiencies were similar for high and low shore mussels.

Excretion rates of ammonia-nitrogen (NH_4–N) were higher in winter than summer. Winter rates of excretion were 59.7 and 20.3 μg NH_4–N hr^{-1} g^{-1} for high and low shore mussels respectively. Summer rates were 14.6 for high shore and 13.7 for low shore mussels. Higher excretion rates during winter are thought to be due to utilisation of bodily reserves due to starvation.

Scope for growth was greater for high shore collected mussels than low shore mussels due mainly to increased clearance rates. When SFG was adjusted to take into account reduced feeding time due to aerial exposure, high and low shore animals had a similar SFG during winter. However, high shore animals had increased SFG during summer. In contrast with findings of SFG calculations, natural growth rates of upper shore *P.canaliculus* appear to be slower than their low shore counterparts. This is reflected by a consistently lower CI and a smaller average shell length (high shore = 57.6 ± 20.8 mm; low shore = 80.2 ± 20.8 mm).

Although *P.canaliculus* has the ability to increase its filtration rate with reduced periods of tidal submersion the increased energy intake appears not to compensate for metabolic demand. During summer high shore mussels would

experience high air temperatures when exposed causing increased anaerobic metabolic rates and risk of desiccation.

It is concluded that the upper tidal limits of *Perna canaliculus* may be limited by physical factors such as temperature stress and desiccation before energetic constraints apply.

General Introduction.

The intertidal zone is a particularly harsh marine environment. Animals living in this zone experience regular and irregular extremes in many physical and chemical factors such as temperature, salinity and oxygen supply. The range of some of these conditions during a single tidal cycle can be very large, in some cases as great as normal yearly variations (Fegley et al., 1992). Sessile marine molluscs, such as bivalves, or limpets and gastropods with limited mobility, are particularly sensitive to desiccation following exposure to air as the tide recedes. The length of aerial exposure is dependent upon height on the shore and tidal level. Other factors affecting tidal height include season, stage in the lunar cycle, barometric pressure and the degree of exposure to wave action (Newell, 1979). During aerial exposure at low tide, intertidal marine invertebrates may have to endure sudden temperature shifts, limited or non-availability of food and lack of dissolved oxygen (Vial et al., 1992).

Many marine invertebrates are morphologically ill-equipped for survival in aerial conditions (Shick et al., 1988). When exposed to air mussels experience problems with collapsing gills, absence of food and inability to flush away waste products. Despite these restraints, mussels form an important and often abundant part of the intertidal community on shores around the world (Harris, 1990).

Two of the main reasons that mussels, and other littoral bivalves, are successful inhabitants of the intertidal zone is that firstly they have a thick protective shell in which they can seal themselves off from the outside world, and secondly they resist wave action by firmly attaching themselves to solid rock with either byssus threads or a proteinaceous cement.

To survive in the intertidal zone, many littoral molluscs have developed metabolic, physiological, morphological and behavioural adaptations. These include the ability to extract aerial oxygen, the capability to withstand prolonged hypoxia and anoxia, development of anaerobic metabolic pathways, increased tolerance to desiccation and tissue freeze tolerance (McMahon & Russel-Hunter, 1977; McMahon, 1988; Widdows & Shick, 1985).

Bivalves that have the ability to respire in air, such as the cockle *Cardium edule*, gape their valves and expose the gills directly to air. Although this allows a good supply of oxygen it also substantially increases the risk of desiccation. Some intertidal species, eg *Polymesoda erosa* a high estuarine mangrove bivalve, minimise the risk of desiccation during gaping by exuding mucus around the edge of the valves. The mucus hardens in air leaving only a small inhalant siphon to allow passage of oxygen to the gills (McMahon, 1988).

The ability to withstand hypoxic and anoxic conditions over a period of time is another important adaptation to intertidal living. Some mussels are able to acclimate to hypoxic conditions. Bayne et al. (1976c) reports that *Mytilus edulis* is able to acclimate to partial pressures of oxygen (Po_2) as low as 80 torr and still show the same rate of oxygen uptake as control mussels held at a Po_2 of 160 torr (normal Po_2 at sea level). Mussels can also tolerate short-term

anoxia. Thamdrup, 1935 (in Bayne et al, 1976c) kept *Mytilus edulis* in anoxic water for 7 days with only 20% mortality. Also some mussels have developed anaerobic metabolic pathways that allow them to function under anoxic conditions (Famme et al., 1981).

When *Mytilus edulis* initially close their valves, during aerial exposure or in response to a predator, they use up O_2 stored in the pallial fluids first. Once this remaining O_2 is used they switch to one of two alternative anaerobic pathways (labelled Path I and Path II on Figure 1). In Path I firstly stored glycogen is converted to pyruvate and a small amount of strombine through the process of glycolysis. Next the pyruvate is converted to alanine in a transamination reaction which also sees stored aspartate converted to oxaloacetate. Finally oxaloacetate is broken down, via malate and fumarate, into succinate. For Path I the end-products of anaerobiosis which accumulate in the tissues are succinate, alanine and strombine. Once the pool of aspartate is exhausted, a process which takes 5-10 hours (Harris, 1990), the mussel is forced to switch to Path II. Path II is a much simpler process than Path I. Glycogen is converted to oxaloacetate via phosphoenol-pyruvate and then oxaloacetate is converted to propionate via malate, fumarate and succinate. The only end-product of Path II is propionate or propionic acid. *Mytilus edulis* can tolerate anoxia until either the build up of end-products reaches toxic levels or they exhaust their supplies of glycogen.

Intertidal invertebrates can be exposed to extremes of temperature. Mussels are capable of withstanding high temperatures. For example, the upper lethal limit for *Mytilus edulis* has been found to range from 27°C to 40.8°C,

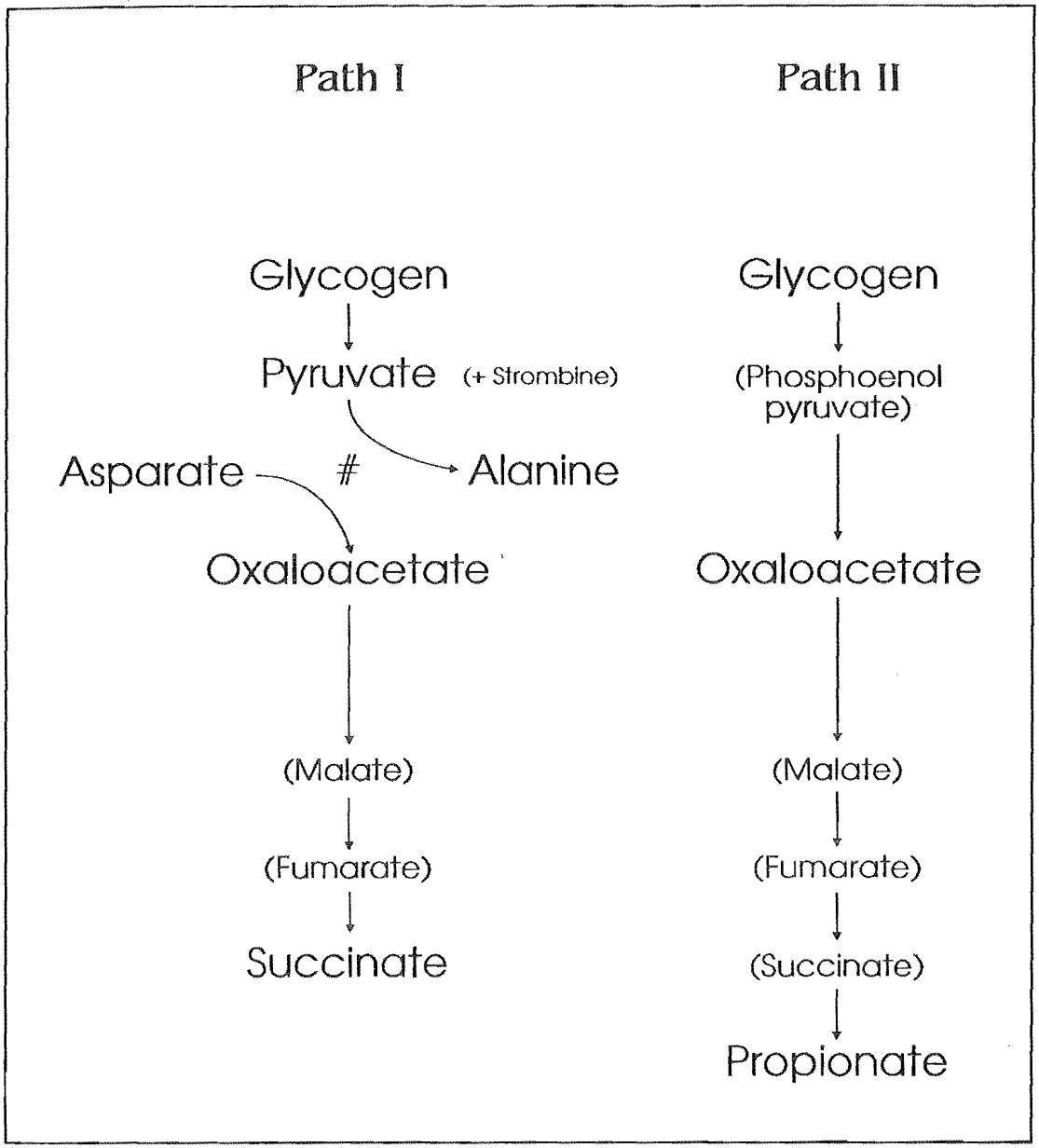

Figure 1 - Anaerobic metabolic pathways found in *Mytilus edulis*.

depending on the acclimation temperature of the animals and the rate of temperature increase (Bayne et al., 1976c). Kennedy (1976) found that *Perna canaliculus* has an upper lethal limit of 33-34°C when acclimated to 20°C. Mussels are also able to withstand temperatures down to −10°C (Bayne et al., 1976c). Survival depends very much on the rate of temperature drop and the availability of alternative external heat sources such as solar energy stored in rock substrates. Some mussel species including *Mytilus edulis* and *Modiolus*

demissus can withstand freezing, being tolerant of a 65% loss of their tissue water as ice (Bayne et al 1976c). Up to 20% of the total tissue water is retained in macro-molecules in the cells and is not osmotically active (Bayne et al. 1976c). Mussels can also withstand a high degree of desiccation. Kennedy (1976) reports that both *Mytilus edulis* and *Perna canaliculus* can tolerate up to 24% body weight loss at 20 to 30°C before significant mortality occurs. Resistance to desiccation and freeze tolerance are related to the ability of mussels to tolerate increased osmoconcentration in the tissues (Bayne et al. 1976c, de Vooys, 1991).

In addition to the adaptations listed above, many mussels show physiological adaptations in their metabolism and energy balance in relation to environmental conditions present at different depths, tidal level or season. These modifications are species specific and may vary within a species depending on geographic location (Widdows et al. 1984).

Perna canaliculus.

The Greenshell Mussel (*Perna canaliculus*) is found along the coasts of the North, South and Stewart Islands. It is commonly distributed from about the mid tide level down as far as 55 metres (Powell, 1979). *Perna canaliculus* is usually found attached to hard rocky substrates, wharf pilings and sometimes on sandy bottoms anchored to gravel and shell fragments (Powell, 1979). *Perna canaliculus* is the basis of New Zealand's largest aquaculture industry (Waite, 1989), in 1986 14900t of farmed mussel were produced, 2500t of this was exported earning $12.2 million (Shiel & Hayden, 1987), in 1987 3904t of mussels

were exported earning $15.4 million (Hayden, 1988). Despite its commercial importance there has been little in the way of scientific research carried out on this species.

In 1972-73 Kennedy examined desiccation and higher temperature tolerance of three common species of intertidal mussels including *Perna canaliculus*. He found that there was a correlation between upper tidal limits of distribution and tolerance to desiccation, with *Mytilus edulis* having a greater resistance to desiccation and distributed higher on the shore than *P.canaliculus* (Kennedy, 1976).

At about the same time R.W. Hickman studied growth in *Perna canaliculus*. He compared growth rates of mussels from sites throughout the length of New Zealand. Hickman found that growth rates were dependent on numerous factors such as light, salinity, temperature, food availability and water currents. He showed that small mussels grow at a faster rate than larger ones; growth rate was greater at higher temperatures (possibly more closely related to higher planktonic productivity); and growth rate was higher at lower latitudes (related to temperature). Hickman also compared intertidal mussels to raft-grown ones and found that intertidal animals grow much slower and have heavier shells than cultured animals (Hickman, 1979).

Hickman and Illingworth (1980) studied cycles of condition[1] of *Perna canaliculus*. They found a clear annual cycle of condition with peak values

[1] Condition is simply a measure of the weight of tissue in a shellfish compared to ıe shell weight. It is commonly used to estimate the reproductive condition of an nimal. The concept of condition will be discussed in detail in chapter 1.

occurring from October to December when the animals spawn. They also found that average condition index declines with increasing latitude.

Kennedy (1984) studied crawling behaviour of three intertidal species of mussel *Perna canaliculus*, *Aulacomya maoriana* and *Mytilus edulis*. He found that *Perna canaliculus* can crawl out from under gravel at a faster rate than *A.maoriana* and *M.edulis*.

Since these papers were completed there have only been a few studies done and these have mainly been on aspects of growth in relation to mussel farming (eg. Hickman et al., 1991; Waite, 1989; Greenway, 1975).

AIMS OF THESIS.

My study had four major aims. The first was to examine variations in condition of *Perna canaliculus* on a seasonal basis and to estimate growth of mussels in their natural habitat, this is covered in chapter 1. The second aim was to estimate aerial and aquatic respiration rates of *P.canaliculus* over a range of temperatures and to relate this to shore level and season. This is examined in chapter 2. My third aim was to examine energy acquisition and efficiency in relation to shore level and season. This is dealt with in chapter 3. The fourth and final aim was to calculate scope for growth of animals from two shore levels during winter and summer. This is covered in chapter 4.

CHAPTER 1

Seasonal variations in condition of *Perna canaliculus*.

INTRODUCTION

Condition is a useful measure of the nutritional and reproductive state of a shellfish. Simply stated it is the relationship between the shell and meat content of an animal. Condition can be measured in several different ways, although there is no established standard method (Lucas & Beninger, 1985). Several workers have suggested possible standards, for example, Crosby and Gale (1990), suggest that,

$$C.I. = \frac{dry\ meat\ weight \times 1000}{internal\ shell\ cavity\ volume}$$

be adopted as the standard scientific method because it is easy to use and gives an indication of the nutritional status of the animal. On the other hand Hickman and Illingworth (1980) suggest,

$$C.I. = \frac{dry\ meat\ weight \times 100}{whole\ weight - shell\ weight}$$

be used for biological studies and,

$$C.I. = \frac{wet\ meat\ weight \times 100}{whole\ (live)\ weight}$$

be used by mussel farms as a fast and reliable method of determining condition. The method adopted should depend on what aspect of condition is to be determined (eg., shell:tissue relationships or potential for tissue growth) and the constraints imposed by availability of time and equipment.

For this study I chose this index,

$$C.I. = \frac{dry\ meat\ weight \times 1000}{dry\ shell\ weight}$$

because this method gives an indication of the proportions of energy division between shell growth and somatic and gametic tissue growth (Crosby & Gale, 1990). It also eliminated the possibility of extra variation being introduced due to uneven draining of tissue which can be a problem when working with wet meats (Hickman & Illingworth 1980). This method of determining condition produces values for *Perna canaliculus* which generally range from 40 to 80 units.

Condition can be affected by many exogenous factors, such as temperature, food supply, season, shore level and pollution as well as endogenous factors like reproductive status and genotype.

There have been very few published studies on condition of *Perna canaliculus*. Hickman and Illingworth (1980) studied changes in condition of *P.canaliculus* from 7 different sites, using 4 indices, over a period of 15 months from December 1973 to February 1975. They compared the various methods of determining condition and discussed factors affecting condition in *P.canaliculus*.

Waite (1989) also looked at cycles of condition in *P.canaliculus* although his study was restricted to sites within the Marlborough Sounds area.

This section of my study had two major aims. The first aim was to examine change in condition over one annual cycle and the second was to compare condition of animals from two different shore levels.

METHODS

Four study sites were chosen on the East coast of the South Island (see Figure 1.1). Each site had to be easily accessible by road, it needed to have extensive beds of *Perna canaliculus* present from mean low water mark (MLW) to the upper extent of their range (about 1.0 metres above MLW).

The sites chosen were:

(a) Taylors Mistake - A north-east facing bay about 30 minutes drive from the campus. This area is a popular recreational spot with easy access to quite extensive mussel beds.

(b) **Tumbledown Bay** - On the south-west side of Banks Peninsula about 80 km from Christchurch by road. This site is very similar to Taylors Mistake except that it is more exposed due to its southerly facing aspect.

(c) **Le Bons Bay** - On the eastern edge of Banks Peninsula about 120 km from Christchurch by road. This site, once again, is similar to Taylors Mistake but the mussel beds are not as extensive.

(d) **Kaikoura** - The Kaikoura site was an exposed rocky shore about 20 km north of the township.

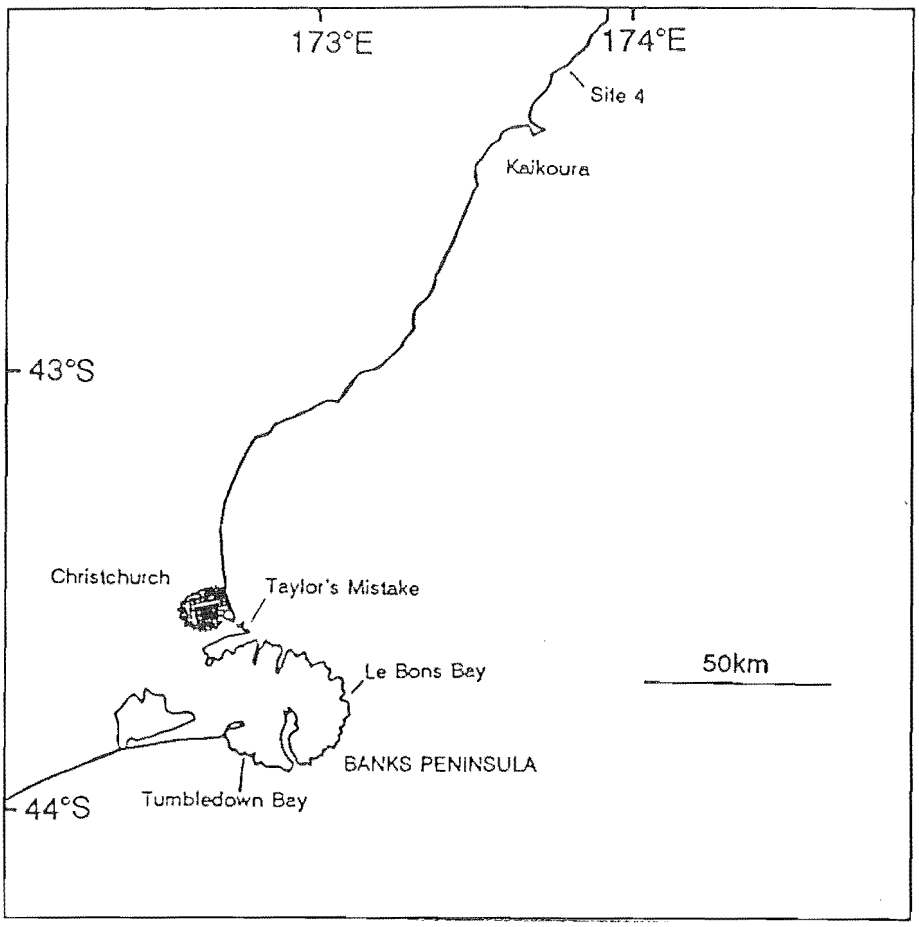

Figure 1.1 - Location of study sites.

Monitoring of condition index was carried out at Taylors Mistake from October 1991 to December 1992. On each collection day 10 to 20 mussels ranging from 20 to 100 mm shell length were collected from two shore levels, 0.0m above MLW and 1.0m above MLW. These shore levels will be referred to as low and high, respectively. The mussels were brought back to the lab in plastic bags and either processed immediately or frozen at −20°C for later use. To determine condition, the mussels were first scraped clean of epibionts, then the byssus threads were trimmed off flush with the edge of the shell. The mussels were then shelled. The meat was placed in clean, dry, preweighed aluminium foil and the shell was put in clean, dry plastic pottles. The foil packets and plastic pottles were placed in a drying oven at 80°C for 24 to 48 hours. The shell and tissue was weighed using an electronic balance.

Any animals found to be parasitised by either the peacrab *Pinnotheres novaezelandiae* or the trematode *Cercaria haswelli* were processed as normal but data produced was not used in later analysis. However, it should be noted that condition indices of parasitised animals were never found to be outside the range of normal unparasitised mussels.

Over a period of 4 days during December 1992, 15 to 20 mussels were collected from high and low shore levels at each of the four sites. Condition indices were calculated to check whether mussels from Taylors Mistake were typical of mussels from Banks Peninsula.

RESULTS

Figure 1.2 shows change in the condition index of *Perna canaliculus* collected from Taylors Mistake from October 1991 through to December 1992. In October 1991 the average condition index was 85, dropped during November then increased during May 1992 before dropping to the yearly low in July 1992. Condition index rose again through spring and by December 1992 was back to peak levels. Throughout the entire study period the average condition of mussels from the high shore level was consistently lower than that of low shore mussels and the pattern of condition index changes was similar for the two tidal levels.

There were no significant differences between average condition indices of mussels collected in Dec 1991 from the four sites. Table 1.1 shows the average Condition Index (C.I.) and standard error of *Perna canaliculus* collected from two shore levels from the 4 sites. Once again low shore level animals had consistently higher average condition than those collected at high shore levels.

Table 1.1 - Average Condition Index of *Perna canaliculus* collected in December 1992.

Site	High Level		Low Level	
	C.I.	Std.Err.	C.I.	Std.Err.
Taylors	74.25	5.37	87.69	4.01
Tumbledown	72.06	2.72	85.66	3.05
Le Bons	72.58	2.26	84.93	2.67
Kaikoura	69.46	1.63	80.05	2.43

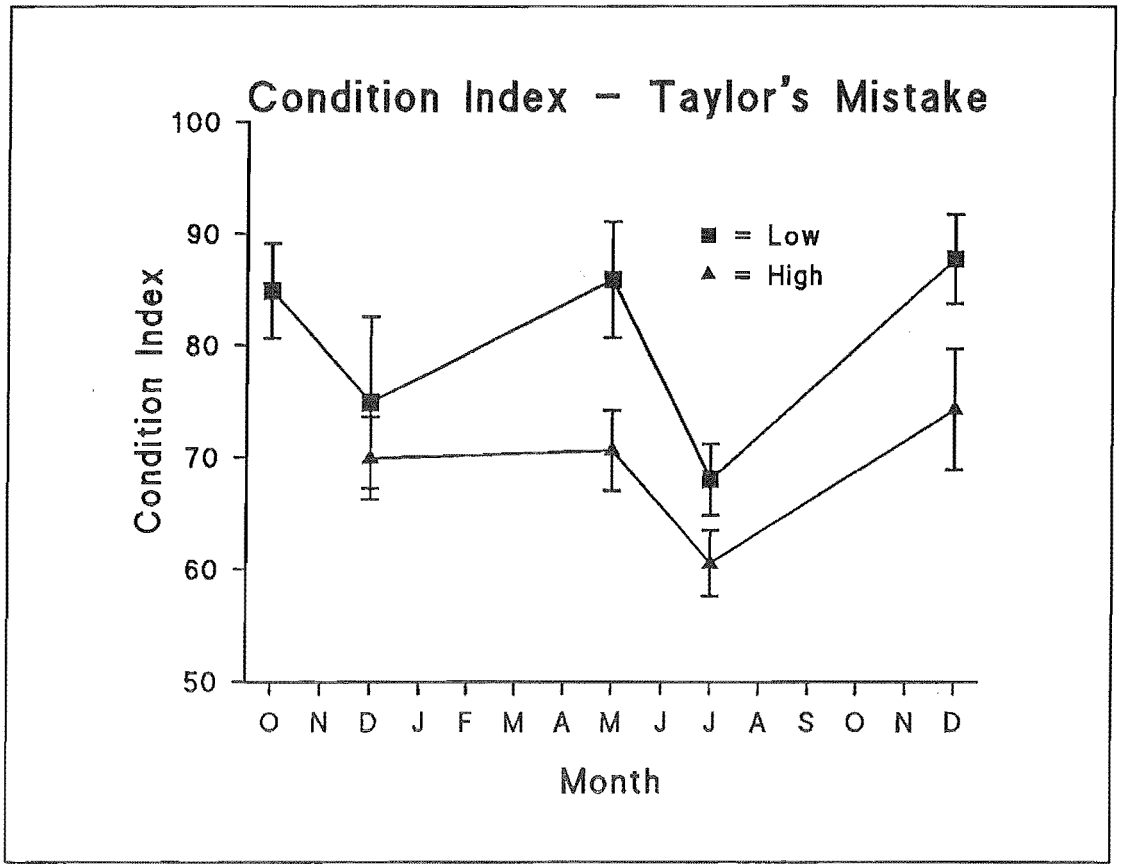

Figure 1.2 - Cycle in condition index of *Perna canaliculus*.

I found no relationship between shell length of *Perna canaliculus* and condition index. Figure 1.3 shows condition index in relation to body size for mussels collected from high shore levels (1.0m above MLW) from Taylors Mistake and Tumbledown Bay and figure 1.4 shows the relationship for animals collected at low shore level (0.0m above MLW).

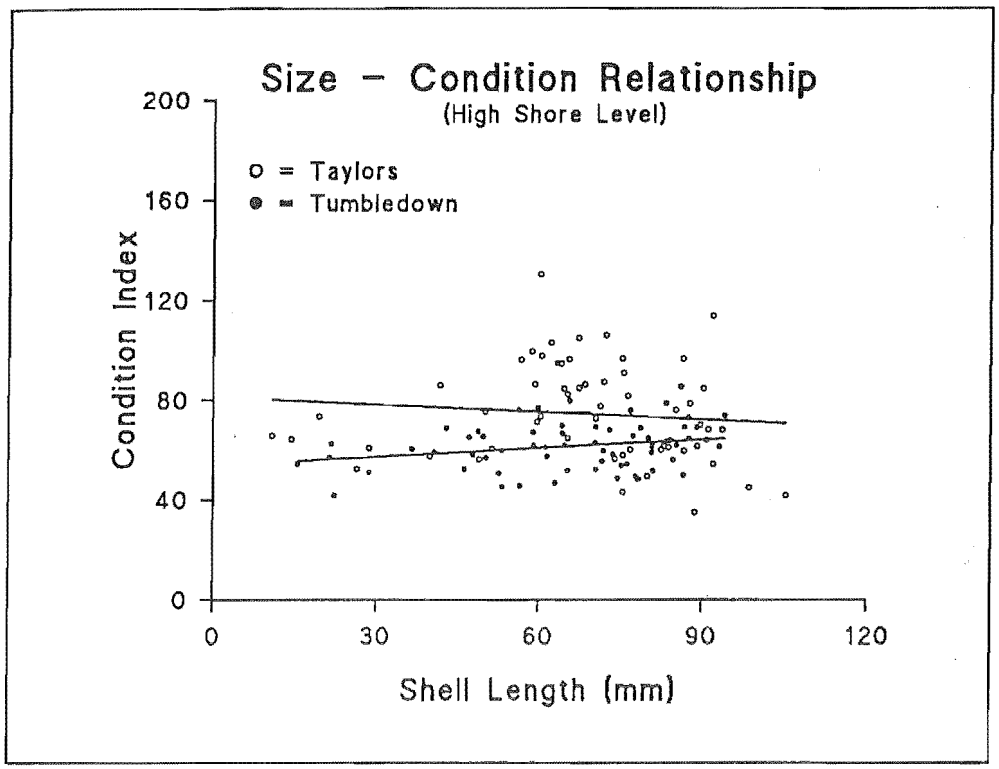

Figure 1.3 - Condition index vs body size for high shore mussels from Taylors Mistake and Tumbledown Bay.

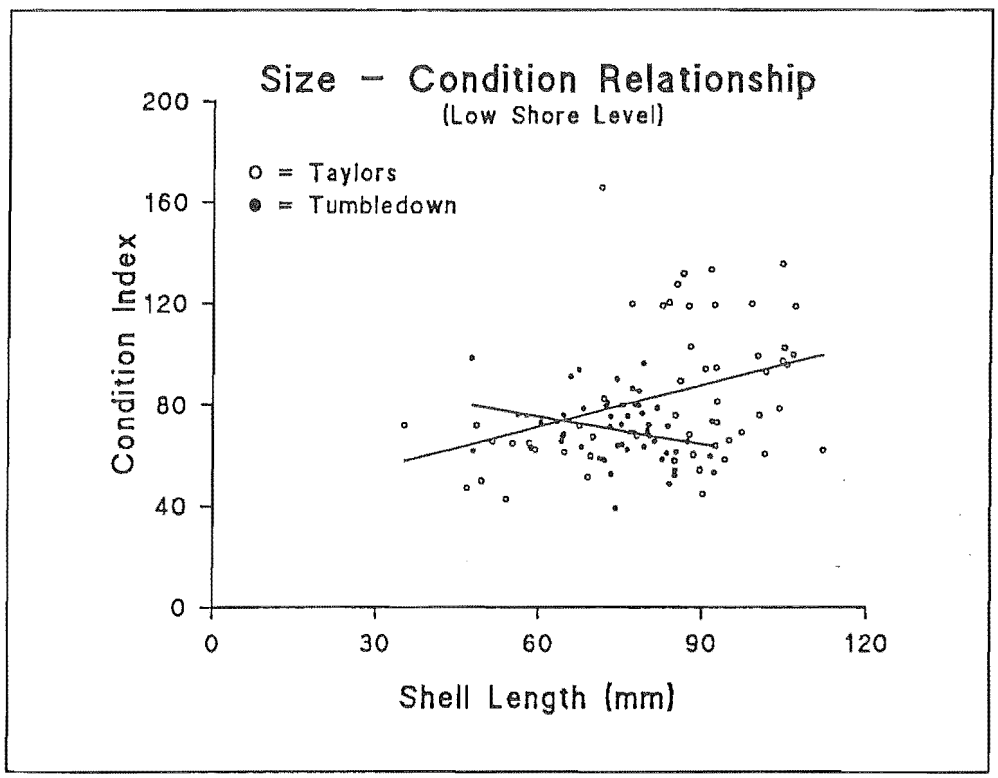

Figure 1.4 - Condition index vs body size for low shore level mussels from Tumbledown Bay and Taylors Mistake.

DISCUSSION

Hickman and Illingworth (1980) describe condition cycle in *Perna canaliculus* from the northern half of New Zealand as beginning with minimum values from June to August then rising until peak levels are reached from October to December. They also noted that at certain sites a second short-lived peak in condition occurs from April to May. My study showed an almost identical pattern in condition index of mussels from Taylors Mistake. However, it is possible that because I did not calculate condition index on a monthly basis I did not find the peak condition levels of *P.canaliculus* from this population.

Perna canaliculus is thought to spawn all year round with major releases during spring/early summer and autumn (Hickman and Illingworth, 1980; Anderlina, 1988; Seed, 1976). The exact stimulus for spawning in mussels is the cause of debate. There are likely to be many factors involved of which temperature seems to be the most important (Seed, 1976). Several workers have found that *Mytilus edulis* can be encouraged to spawn by increasing or decreasing the temperature (eg. Dix & Ferguson, 1984; Parulekar et al., 1982), others have found that mechanical stimulation, such as tugging the byssus threads or pricking the adductor muscle, chemicals and electric shock can also cause release of gametes (Seed, 1976). However, periods during which *Perna canaliculus* spawn coincide with periods of greatest change in sea temperatures (Hickman et al. 1991, Gibbs et al., 1992).

Perna canaliculus collected from similar shore levels at all four sites showed similar condition index. This suggests that mussels from the whole

geographic area were of similar nutritional level. At this time they also appeared to be at a similar stage in their reproductive cycle. *Perna canaliculus* collected from high shore levels had a consistently lower condition index than mussels collected lower on the shore. There are several possible explanations for this finding. Mussels found in the intertidal zone are known to have thicker, heavier shells than those found subtidally (Hickman, 1979; Berry, 1978). This may be a response to more severe intertidal conditions where a thicker shell may provide protection from desiccation or intra- and interspecific competition for space. The thicker shell may also contribute calcium carbonate for buffering against acidic anaerobic end products. The low condition of high-shore animals may also be due in part to reduced soft tissue content resulting from restricted feeding time and the need to regularly use stored glycogen to drive anaerobic metabolism (Small & van Stralen, 1990).

Hickman and Illingworth (1980) have demonstrated in *Perna canaliculus*, that average condition declines as shell size increases. Results from the present study provide little evidence to support this. None of the regression lines fitted to data collected from Taylors Mistake and Tumbledown Bay differed significantly from a horizontal line indicating a constant relationship between size and condition (see figures 1.3 and 1.4). An interesting feature of *Perna canaliculus* from these two sites is the minimum condition value of about 40 units for all shell lengths. Also, mussels greater than 60 mm shell length generally have a greater variability in condition than mussels smaller than this value. Although the reason for this is not evident directly, it is likely that the larger mussels show a greater potential for production of gametes. Small

mussels increase in size mainly by the addition of somatic tissue and therefore may have a more uniform condition index due to the absence of gonad development.

As stated earlier, temperature can affect condition index in mussels. Seed (1976) discusses the relationship between spawning period, temperature and geographical location and suggested that spawning season may be restricted by temperature, especially at extremes of geographical range. He explains that in the Northern Hemisphere, mytilid species found in colder areas have their spawning season restricted by increasing temperature. In more southern, warmer areas species show the reverse trend. Hickman and Illingworth (1980) found that the average condition index of *Perna canaliculus* increases with decline in average annual water temperature. Figure 1.5 shows the relationship between average water temperature and condition index derived from data presented by Hickman and Illingworth (1980).

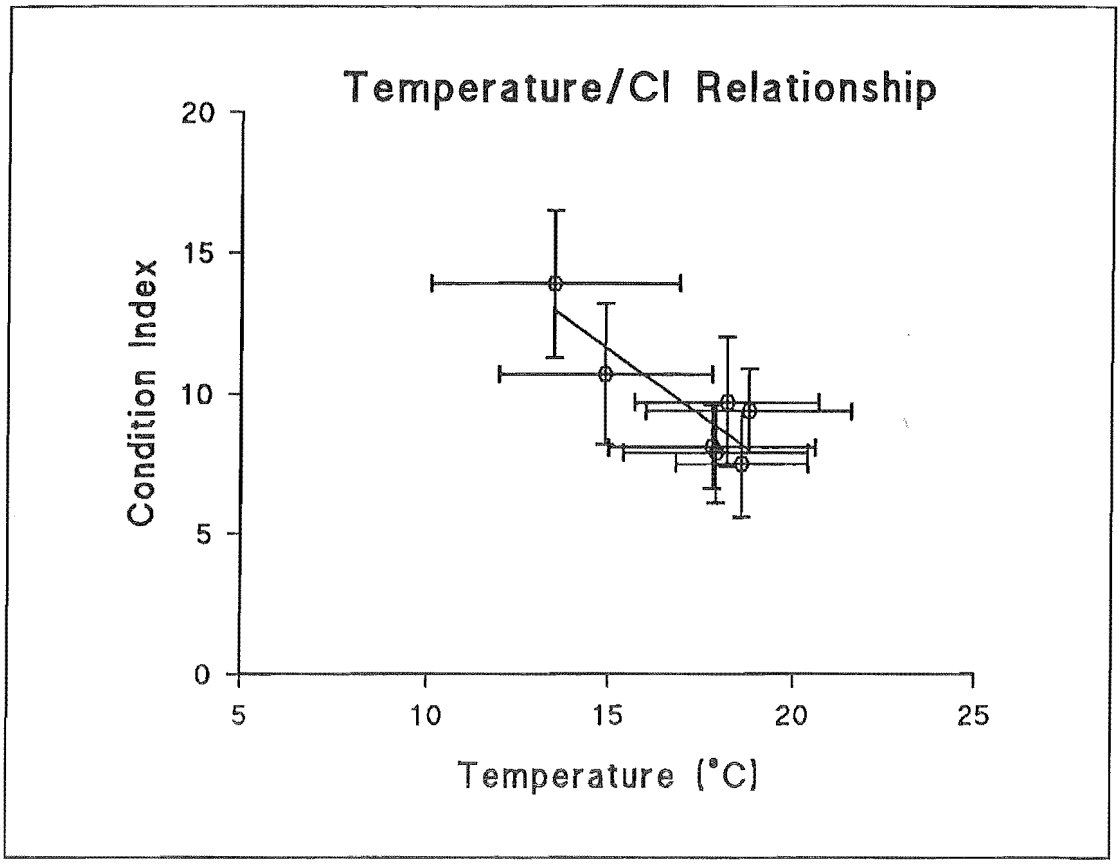

Figure 1.5 - Average yearly condition index vs average temperature derived from Hickman & Illingworth (1980). Note that a wet weight CI was used.

CHAPTER 2

Effects of shore level and temperature on aquatic and aerial respiration.

INTRODUCTION

Respiration rates of mussels are affected by many variables. One of the most commonly studied relationships is the one between respiration rate and body size. This relationship is usually described by an allometric equation:

$$R = aW^b \quad \leftrightarrows \quad \log R = \log a + b \log W$$

Where W = body weight and R = rate of oxygen consumption The parameter *a* indicates the magnitude of the rate of oxygen uptake while *b* is the modifier for body size.

Temperature affects many aspects of invertebrate physiology including feeding, excretion and respiration rates. Q_{10} indicates how metabolic rate is affected by temperature. For most invertebrates Q_{10} is about 2 which means that metabolic rate increases 2 fold for every 10°C rise in temperature It follows then that a Q_{10} of 1 means that metabolic rate has remained constant and a Q_{10} of less than 1 indicates a drop in respiration rate with increased temperature. Q_{10} is calculated as follows:

$$Q_{10} = \left[\frac{V_1}{V_2}\right]^{\frac{10}{t_1 - t_2}}$$

Where V_1 and V_2 are the rates of oxygen uptake at temperatures t_1 and t_2 respectively (Bayne et al., 1976c). In practise Q_{10} itself is a function of temperature and animals often have different Q_{10} values at low and high temperatures.

Aquatic partial pressure of oxygen (Po_2) can vary greatly in the intertidal zone during the course of a tidal cycle. Rock pools are especially variable in their oxygen content, McMahon (1988) found that Po_2 of water in rock pools can vary from 2 to 440 torr and the oxygen content of the water from 3 to 680 μmol l^{-1}. A large number of studies have been carried out examining the effect of declining Po_2 on the respiration rates of a wide range of mussel and other bivalve species. Bayne et al. (1976c) states that mussels are capable of regulating respiration rates under hypoxic conditions until a critical Po_2 is reached. Below this level respiration rate declines. For example, *Mytilus edulis* is capable of regulation of oxygen uptake down to a Po_2 of 70 torr, below this

respiration rate declines (Bayne et al., 1976c). Massabuau et al. (1991) found that the fresh water mussel *Anodonta cygnea* is capable of maintaining oxygen uptake even when Po_2 drops below 10 torr.

Nutritional status also affects respiration rates. When mussels are feeding at their maximum rate they demonstrate the "active rate" of oxygen consumption. During starvation oxygen consumption shows a steady decline until the minimum rate of filtration and digestive activity is reached. This level of oxygen consumption is called the "standard rate", or occasionally "starvation rate". The difference between standard and active rates is called "scope for activity" (Bayne et al., 1976c).

The relationship between respiration rate and temperature, ambient oxygen levels and nutritional status can all be affected by seasonal changes in environmental temperature and reproductive condition.

When intertidal mussels are exposed by the receding tide, filtration of water effectively stops. At this point the animals have two options, either they close their valves tightly and rely on anaerobic processes and thermal tolerance to allow them to survive, or they can gape their valves. This allows some gas exchange and evaporative cooling to take place, but they run the risk of desiccation. Previous studies on intertidal molluscs have found a variety of responses when bivalves are exposed to air. *Mytilus edulis*, for example, closes its valves (Widdows & Shick, 1985) and reduces its metabolic rate (Shick et al., 1988). *Mytilus californianus* also has a reduced metabolic rate when emersed (Bayne et al., 1976c). Other species of bivalve such as the mussel *Perumytilus purpuratus* and the cockle *Cardium edule* gape when exposed to air and respire

aerially until the tide returns or they are forced to close by risk of desiccation or predation (Widdows & Shick, 1985; Vial et al., 1992).

There were 4 major aims for this section of my thesis. First I wanted to estimate aerial and aquatic respiration rates of *Perna canaliculus* and to compare them to other species of bivalve. Secondly I wanted to investigate whether the position of mussels on the shore affected aerial and aquatic respiration rates, especially aerial respiration. The third aim was to investigate the effect of temperature on metabolic rate. In this case metabolic rate was assumed to be a function of respiration rate. Finally I wanted to see if there was any difference in respiration rates between winter and summer animals.

METHODS

During the winter (May to July) of 1992 and the summer (December to February) of 1992/93 groups of mussels were collected from two different shore levels, 0.0 and 1.0 m above mean low water mark (MLW), at Taylors Mistake. The animals were brought back to the department, cleaned of epibionts, had a numbered "bee-spot" glued to their shell and were placed in a recirculating sea-water system (maintained at 15°C) to recover for 24 hours prior to experimentation.

The mussels were randomly assigned to each oxygen uptake experiment. Temperatures of 5, 10 and 15°C were used for experiments on aerial and aquatic oxygen uptake conducted in winter and 10, 15 and 20°C were used during summer. Ten to 20 mussels were used for each experiment.

AQUATIC RESPIRATION.

Aquatic oxygen uptake was measured using closed box respirometry. Individual animals were placed in glass jars (empty volume approximately 250 ml) with pasteurised sea-water. The jars were sealed using silicon rubber bungs. Two syringe needles (18 gauge) were pushed through the bungs and attached to a 1 ml sampling syringe and a 10 ml reserve respectively. Each chamber was suspended in a water bath to ensure that temperature was kept constant. The experimental chambers were left for 15 minutes prior to the beginning of the experiment to ensure that they had equilibrated and to allow the mussels to recover after their brief handling. Five of these experimental chambers were used in each experiment.

Oxygen levels were determined by taking a 1 ml water sample from the experimental chamber and passing it through a Strathkelvin Instruments MC 100 Microcell (volume 70 μl). A Strathkelvin Instruments 1302 oxygen probe and Model 781 Oxygen Meter were used to read O_2 levels. To ensure that the water was adequately mixed prior to sampling, the 1 ml sample syringe was used to mix water in the chamber by repeatedly extracting and then returning approximately 0.5 ml of water over a 30 second period. The 1 ml sample would be taken on the minute. Half the sample would be injected into the microcell as soon as any air bubbles had been cleared from the syringe. The purpose of this first injection was to flush out the previous sample and to start the meter moving towards the correct value. At the beginning of the next minute the second half of the sample was injected and a reading from the meter would be taken 90

seconds later. In this way the meter was given a standard amount of time to stabilise and accurately display the O_2 level of the new sample.

Following this protocol it was possible to make a reading every 3 minutes. Five to 15 animals were used at each temperature for all of the experiments.

Calculations.

Before each experiment the zero value on the oxygen meter was set by injecting a solution of 0.01 M sodium borate ($Na_2B_4O_7$) to which a pinch of sodium sulphate (Na_2SO_4) had been added. It was then calibrated to Po_2 max (calculated at the start of the experiment) by flushing the microcell with distilled water and then opening it to the atmosphere. This meant that the meter accurately displayed the O_2 level of the sample in the microcell in torr.

O_2 consumption in μmol min^{-1} was calculated for each time interval:

$$O_2\ Consumption = \frac{Torr}{time} \times \frac{Vol}{1} \times Capacitance$$

Where Torr was the change in oxygen meter reading between consecutive readings, Time is the number of minutes between readings, Volume is the capacity of the experimental chamber (not including the animal) in litres and Capacitance is the O_2 capacitance of sea-water at the experimental temperature (see Appendix 1).

To convert from moles of O_2 to litres of O_2 at standard temperature and pressure (0°C and 1 atm) the following calculation was made:

$$PV = nRT$$

$$V = \frac{nRT}{P}$$

$$V = \frac{n\ (0.08206 \times 273.15)}{1}$$

$$V = n \times 22.4147$$

PV = nRT is the Ideal Gas Law where P is pressure (in atm), V is volume of gas (in Litres), n is number of moles, R is the gas constant and T is the temperature in Kelvin.

So therefore y moles of O_2 multiplied by 22.4147 = y litres of O_2. The final conversion was from μl min^{-1} to ml hr^{-1}. This was done by the following calculation:

$$y\ \mu l\ min^{-1} \times \frac{60}{1000} = y\ ml\ hr^{-1}$$

AERIAL RESPIRATION.

Aerial respiration rates were measured using a Gilson Differential Respirometer. Individual animals were sealed inside glass experimental chambers with a drop of pasteurised sea water, to ensure high humidity. A small internal vial containing 0.1 ml of 5% potassium hydroxide was used to absorb the carbon dioxide released by the animal. Oxygen levels were measured directly in microlitres by a manometer. Eight experimental chambers and two control vessels were used for each experiment. The 10 chambers were

placed in a water bath and were allowed to equilibrate to the experimental temperature for 20 to 30 minutes prior to commencement of the experiment.

Calculations.

The first step was to convert readings from the respirometer to standard pressure and temperature (STP). Each reading was multiplied by the following conversion factor:

$$Conversion\ Factor = \frac{273.15}{T_{Air} + 273.15} \times \frac{P_B - Sat}{760}$$

Where P_B is Barometric Pressure taken at the beginning of each experiment, T_{air} is air temperature, Sat is the oxygen saturation pressure of water vapour (from tables).

Oxygen consumption in μl/min was then worked out from this equation:

$$Respiration\ Rate = \frac{(R_t - R_{t+1}) \times C}{time}$$

Where R is the manometer reading at time t, C is the conversion factor calculated above and time is the interval between readings in minutes. Finally respiration rates were converted into ml hr^{-1} as in aquatic methods.

ANALYSIS.

All of the calculations were made using a computer spreadsheet package (PlanPerfect 5.1). Paired t-tests were used to compare means using the

computer program Statistix 4.0. Graphs were produced and regression lines fitted using the package FigP.

RESULTS

AQUATIC RESPIRATION.

Aquatic $\dot{V}O_2$ of *Perna canaliculus* was estimated at 5, 10 and 15°C in the winter and 10, 15 and 20°C in the summer.

(a) Winter.

Figure 2.1 shows aquatic $\dot{V}O_2$ for winter collected animals. For both high and low shore animals, oxygen uptake of mussels collected in the winter showed a significant increase with increasing temperature between 5 and 15°C ($p < 0.0029$, $t = 6.49$, d.f. $= 4$ for low shore and $p < 0.0068$, $t = 5.14$, d.f. $= 4$ for high shore animals). The increase between 10 and 15°C was less than that between 5 and 10°C (0.0133 ml/hr/g for 10 to 15°C compared to 0.1252 ml/hr/g fro 5 to 15°C). There were no significant differences between aquatic respiration rates of high and low shore animals at any temperature, nor were there significant differences in oxygen uptake between 5 and 10°C samples and 10 and 15°C samples.

(b) Summer.

Figure 2.2 shows aquatic $\dot{V}O_2$ for summer collected animals. Oxygen uptake of mussels collected during summer showed similar temperature responses to winter collected bivalves. The summer acclimated mussels showed significant increases in rate of aquatic oxygen uptake with increasing temperature between 10 and 20°C ($p < 0.0079$, $t = 6.34$, d.f. $= 3$ for low shore

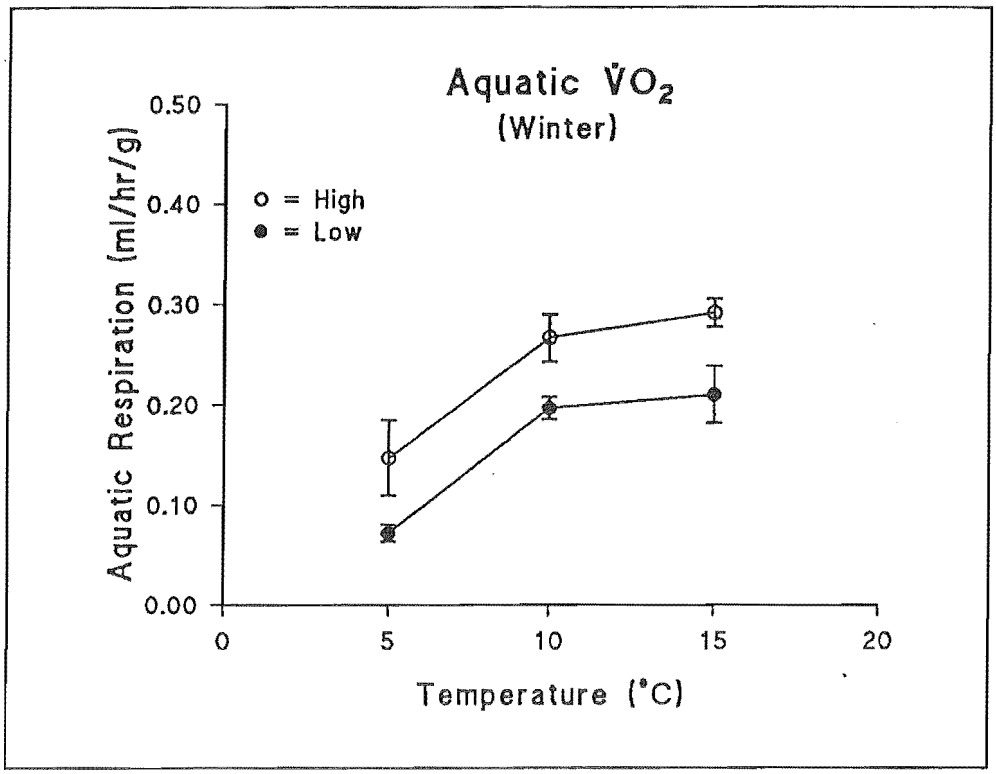

Figure 2.1 - Aquatic respiration rate of *Perna canaliculus* during winter.

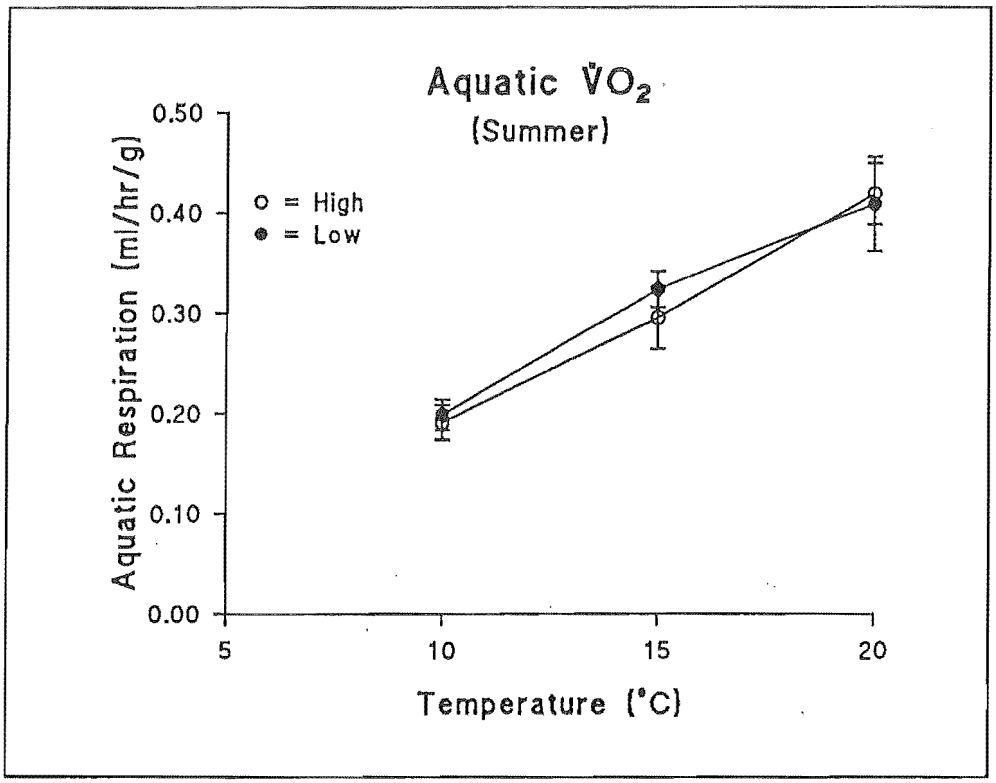

Figure 2.2 - Aquatic respiration rate of *Perna canaliculus* during summer.

animals and $p < 0.0007$, $t = 14.94$, d.f. = 3 for high shore animals). There were no significant differences between aquatic respiration rates of high and low shore animals at any temperature, nor were there significant differences in oxygen uptake between 10 and 15°C samples and 15 and 20°C samples.

(c) Q_{10} values.

Q_{10} values were calculated from average aquatic respiration rates of combined high and low shore, winter and summer animals at 5-10, 10-15 and 15-20°C ranges. The values calculated were 5.39, 1.85 and 1.80 respectively.

AERIAL RESPIRATION.

Aerial $\dot{V}O_2$ of *Perna canaliculus* was estimated at 5, 10 and 15°C in the winter and 10, 15 and 20°C in the summer.

(a) Winter.

Figure 2.3 shows aerial oxygen uptake rates for winter. Aerial respiration rates of *Perna canaliculus* showed an interesting pattern. As expected, $\dot{V}O_2$ increased with increasing temperature up to a peak value at 10°C above which respiration rate declined. There were no significant differences in aerial O_2 uptake between high and low shore animals at any of the exposure temperatures.

(b) Summer.

Figure 2.4 shows aerial respiration rates for summer. Summer collected mussels showed a similar pattern of aerial oxygen uptake to mussels collected in the winter. However, the aerial oxygen uptake of mussels used in summer

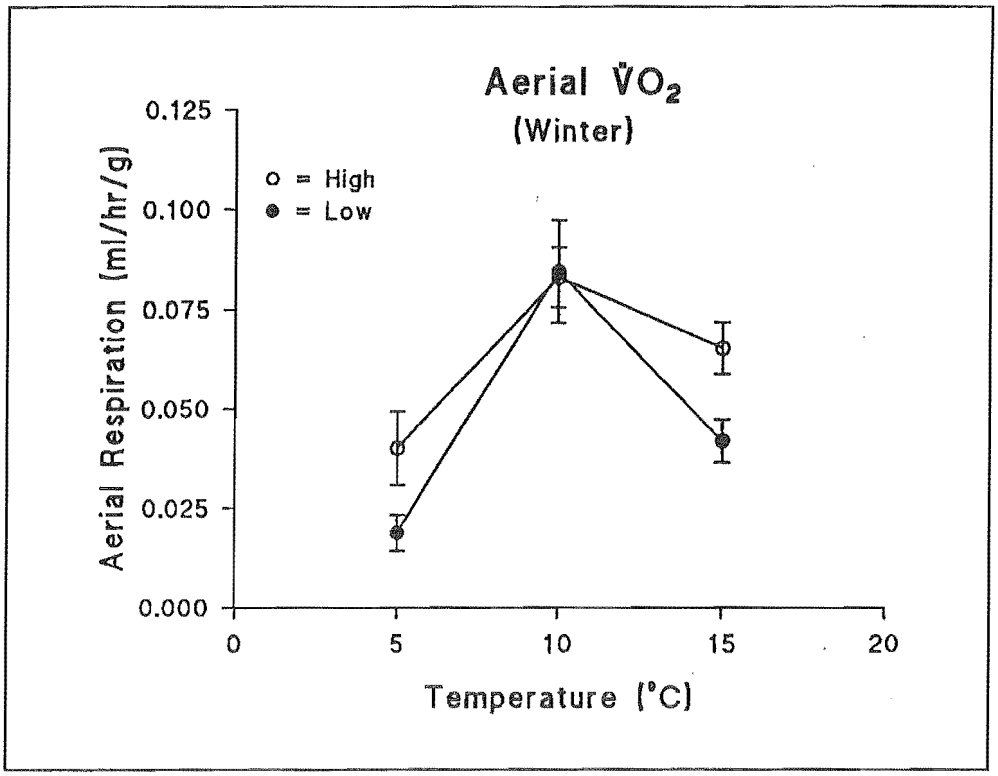

Figure 2.3 - Aerial respiration rates of *Perna canaliculus* during winter.

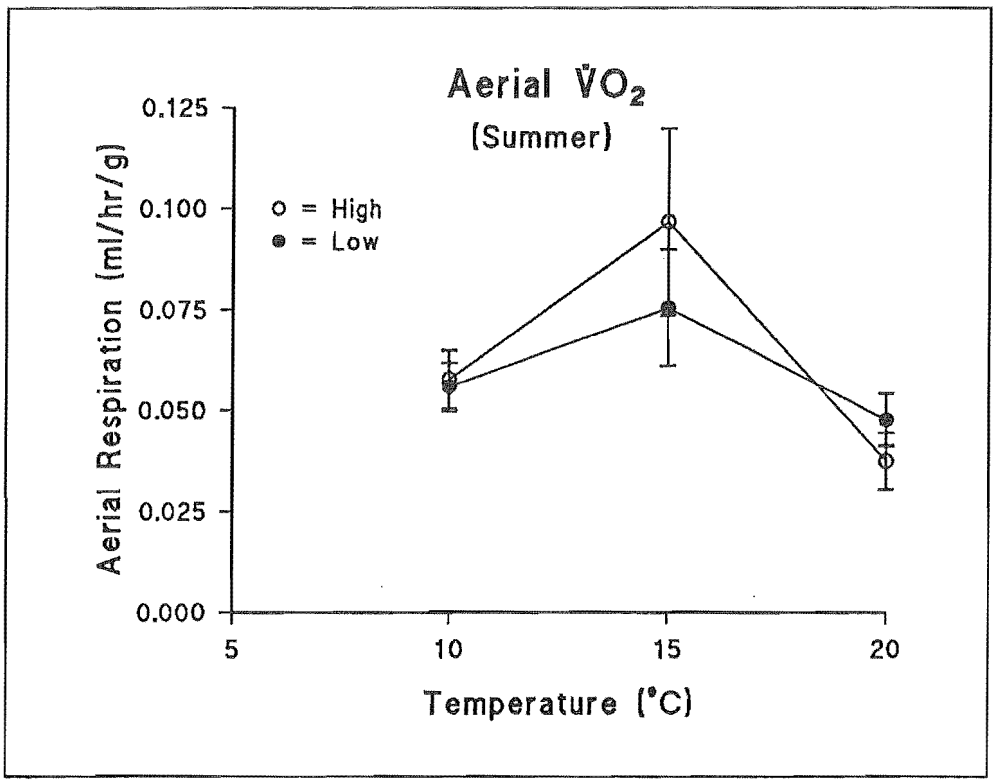

Figure 2.4 - Aerial respiration rates of *Perna canaliculus* during summer.

showed a decline above 15°C. Again there were no significant differences between aerial $\dot{V}O_2$ of high and low shore animals at experimental temperatures.

AERIAL $\dot{V}O_2$: AQUATIC $\dot{V}O_2$ RATIO.

The ratio of aerial $\dot{V}O_2$ to aquatic $\dot{V}O_2$ is a commonly calculated physiological measure for many studies on intertidal animals. This ratio is often used to compare how different species utilise O_2 in relation to their shore level. The aerial $\dot{V}O_2$: aquatic $\dot{V}O_2$ ratio of littoral bivalves is always less than 1, unlike intertidal gastropods which can have ratios of greater than 5:1 (McMahon, 1988a). *Perna canaliculus* had an aerial $\dot{V}O_2$: aquatic $\dot{V}O_2$ ratio at 15°C of 0.21:1 for low shore animals and 0.27:1 for animals collected at high shore levels.

DISCUSSION

Rates of aerial and aquatic oxygen uptake calculated for *Perna canaliculus* were similar to those published for *Mytilus edulis* (Widdows, 1973 - see Figure 2.5), and other species of mussel (see table 2.1). Aerial respiration rates of mussels is almost always lower than aquatic rates (McMahon, 1988a). However, there may be large interspecific variation between the magnitude of aerial respiration rate and the relative uptake compared to aquatic oxygen uptake. This was found in *Perna canaliculus* where the aerial respiration rates represent approximately 25% that of aquatic uptake. The difference between aerial and aquatic respiration rates is usually attributed to reduced energy demands of emersed animals (McMahon, 1988a; Newell, 1979).

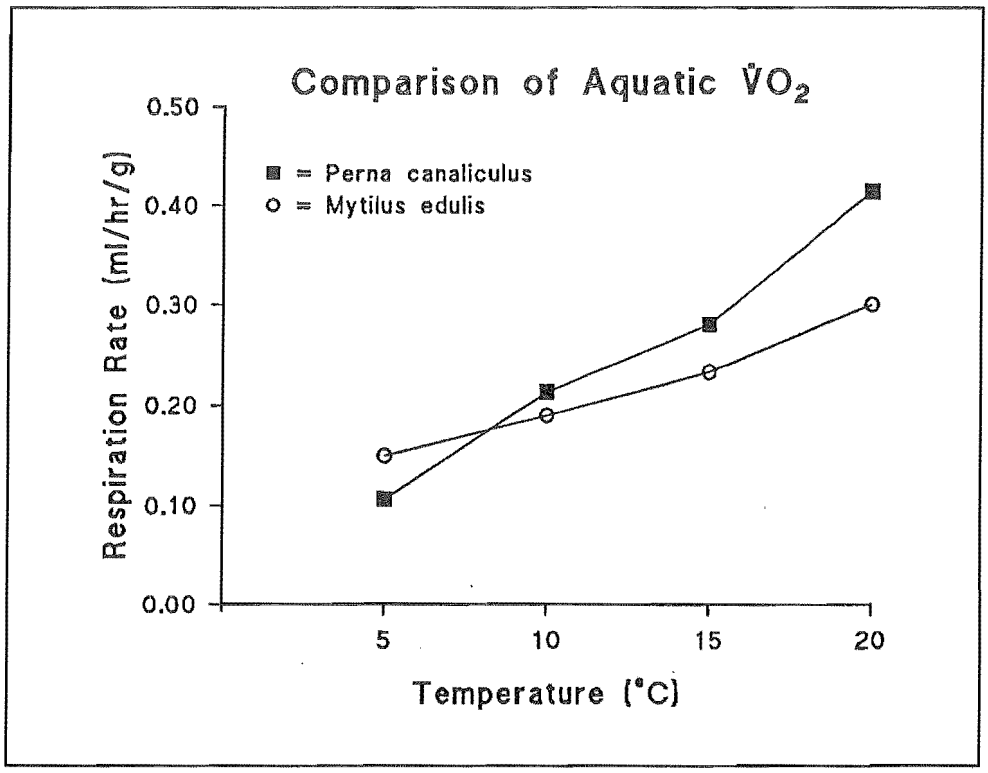

Figure 2.5 - Aquatic respiration rates of *Perna canaliculus* and *Mytilus edulis*.

Table 2.1 - Comparison of aerial and aquatic O_2 uptakes for several species of mussel.

Species		Temp	O_2 Uptake (ml O_2 h^{-1} g^{-1}) Air	Water	Source
Choromytilus meridionalis	I.T.	12°C	0.026	0.17	Griffiths, 1981.*
	S.T.		-	0.23	
Choromytilus meridionalis		12.5°C	-	0.31	Clarke & Griffiths, 1990.*
		21°C	-	0.55	
		30°C	-	0.52	
Modiolus demissus		20°C	0.24	0.38	Bayne et al., 1976c.
Mytilus californianus	Fed	13°C	-	0.54	Bayne et al., 1976a.
	Starved		-	0.23	
Mytilus californianus		13°C	0.20	-	Bayne et al., 1976b.
Mytilus californianus		13°C	0.17	0.23	Bayne et al., 1976c.
Mytilus edulis		?	-	0.36 - 0.39	Vismann, 1990.
Mytilus edulis	I.T.	15°C	0.017	0.20	Widdows & Shick, 1985.
	S.T.		0	0.19	
Mytilus edulis		15°C	-	0.23	Widdows, 1973.
Mytilus galloprovincialis		?	-	0.46	Navarro et al., 1991.
Perna canaliculus		15°C	-	0.26	Marsden & Shumway, 1992.
Perna canaliculus		15°C	0.070	0.28	This Study.
Perna perna		20°C	-	0.34	Bayne et al., 1976c.
Perumytilus purpuratus		10°C	0.84	1.61	Vial et al., 1992.
		20°C	0.31	-	

* These calculations were on the basis of a 50mm shell length animal and NOT dry tissue weight.
I.T. and S.T. indicate intertidal and subtidal respectively.

Body size is a major influence on aquatic oxygen uptake with larger animals having a higher respiration rate. Figures 2.6 to 2.9 show aquatic oxygen uptake in relation to dry tissue weight for each experimental temperature and shore level for winter and summer. All of the regression lines calculated were

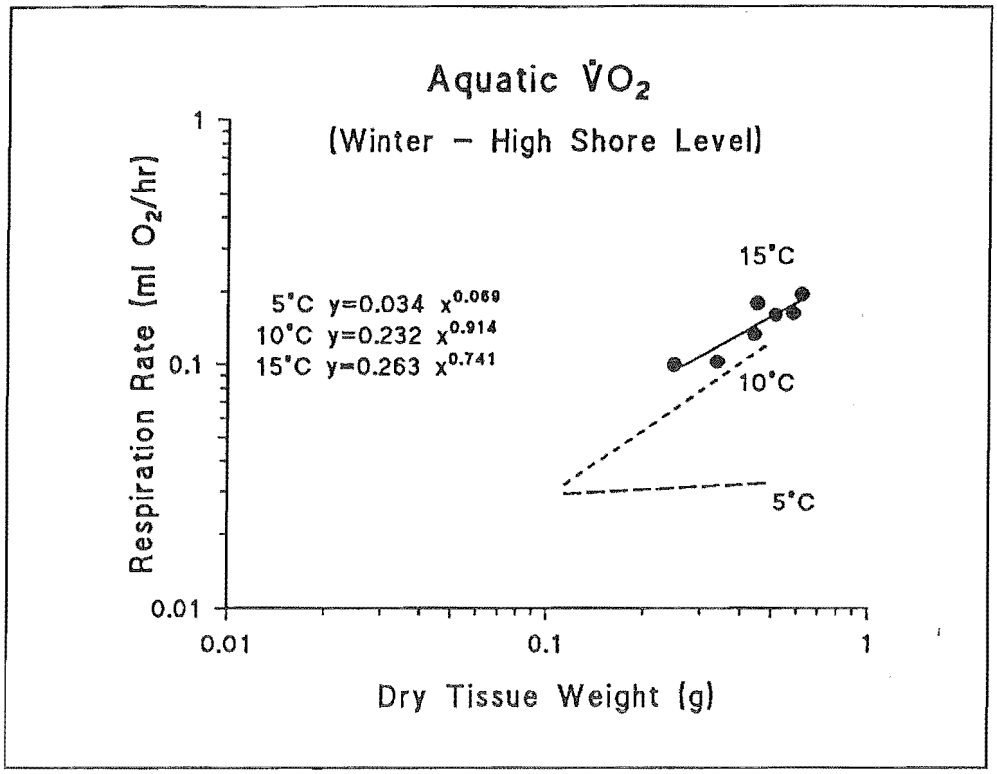

Figure 2.6 - Aquatic respiration rate vs dry tissue weight for winter high shore mussels.

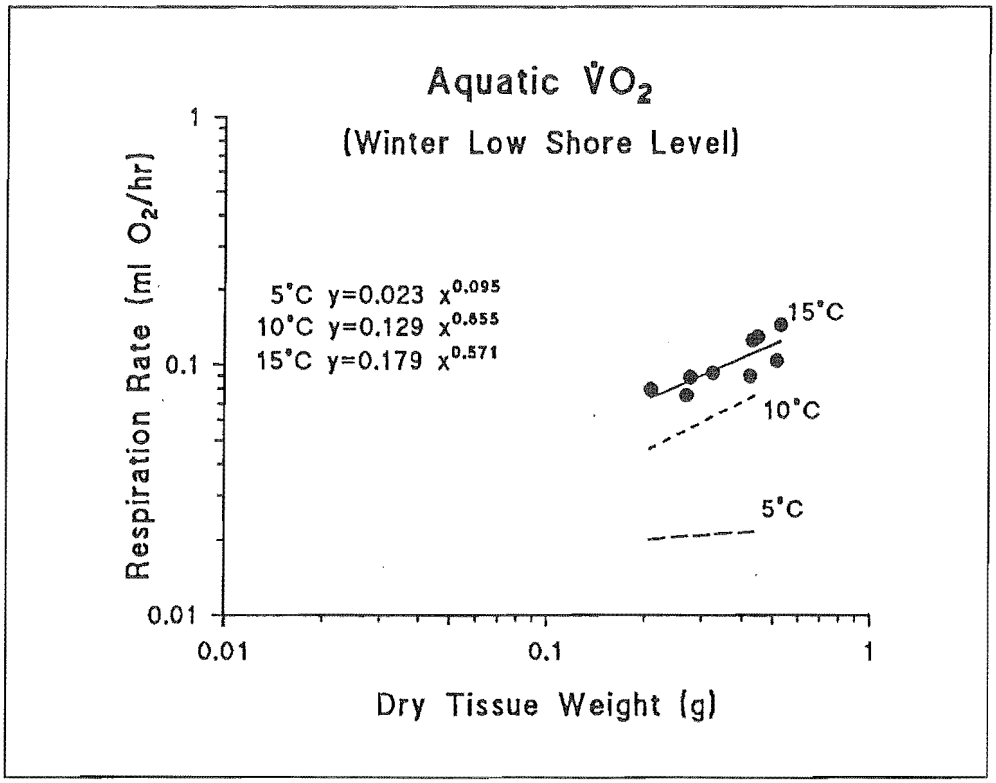

Figure 2.7 - Aquatic respiration rate vs dry tissue weight for winter low mussels.

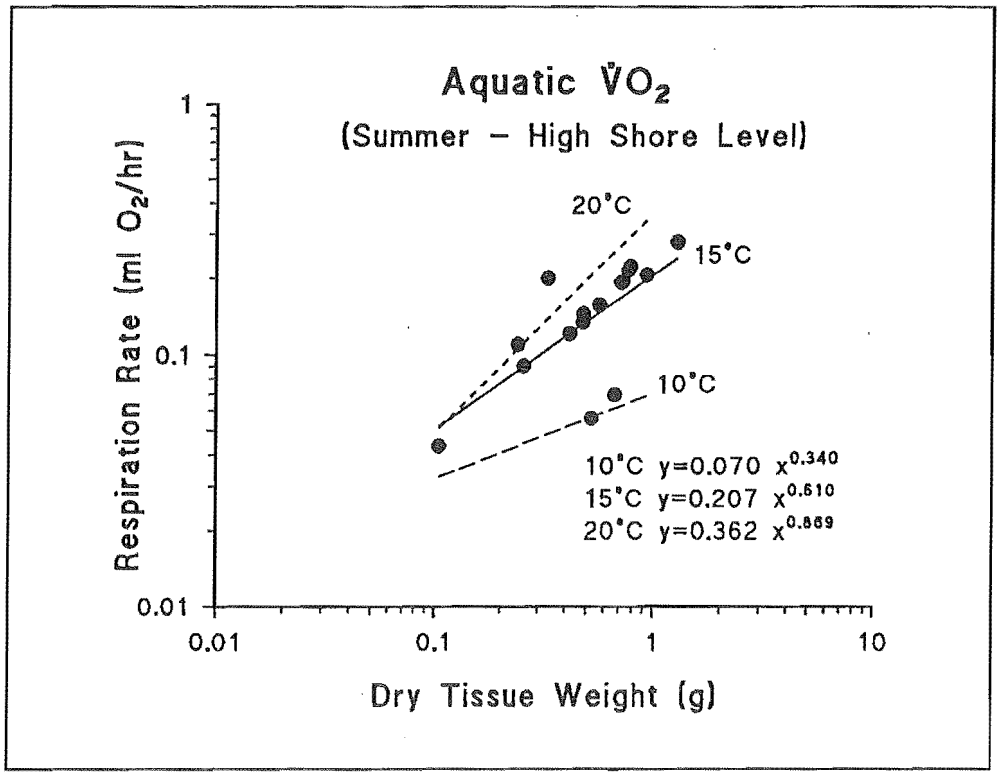

Figure 2.8 - Aquatic respiration rate vs dry tissue weight for summer high mussels.

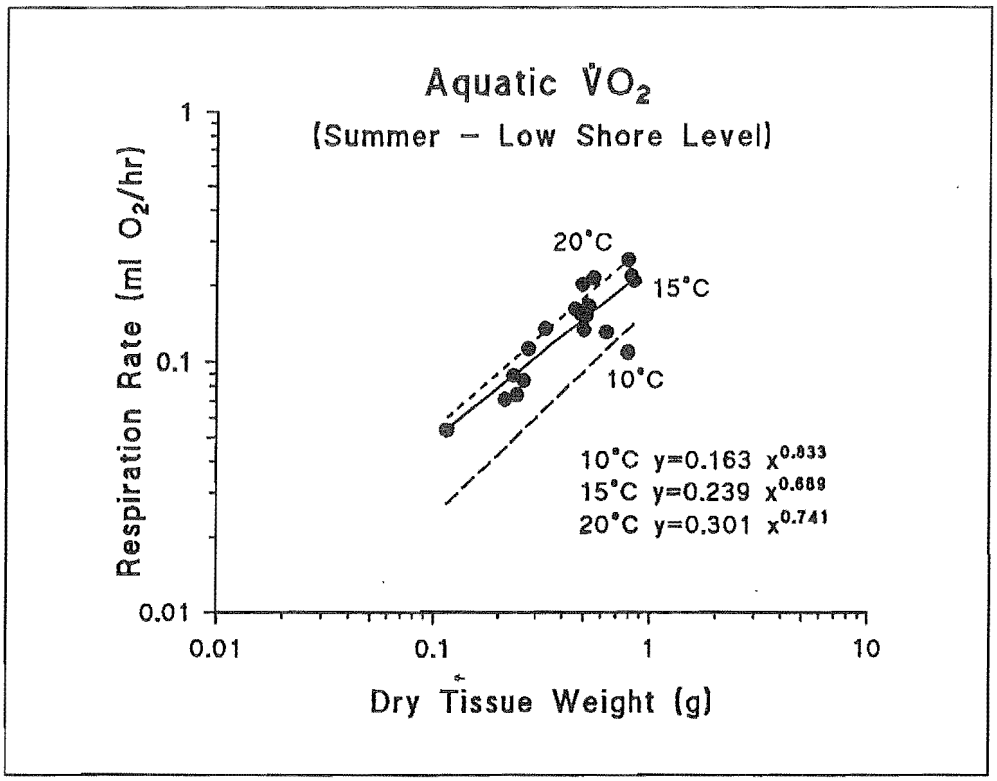

Figure 2.9 - Aquatic respiration rate vs dry tissue weight for summer low mussels.

Table 2.2 - Relationships between oxygen consumption and body weight in *Perna canaliculus*.

Season	Level	Temp	a	b	Sig.
Winter	Low	5	0.023	0.095	n.s.
		10	0.129	0.655	1%
		15	0.179	0.571	1%
	High	5	0.034	0.069	n.s.
		10	0.232	0.914	1%
		15	0.263	0.741	1%
Summer	Low	10	0.163	0.833	5%
		15	0.239	0.689	1%
		20	0.301	0.749	1%
	High	10	0.070	0.340	n.s.
		15	0.207	0.610	1%
		20	0.362	0.869	1%

significant to at least the 5% level with the exception of the experiments carried out at 5°C during winter and at 10°C for high shore summer animals. It appears that at 5°C *Perna canaliculus* from Taylors Mistake reached their minimum level of oxygen uptake which was independent of size. Table 2.2 shows a summary of the allometric parameters *a* and *b* from the regression lines presented on figures 2.6 to 2.9. Both *a* and *b* were within normal ranges reported in Bayne et al. (1976c) (a = 0.164 to 0.698; b = 0.626 to 0.837). By examining parameter *a* it is possible to compare oxygen uptake rates of different groups of animals. As can be seen from Table 2.2, *a* is a function of temperature with higher *a* values (and hence higher respiration rates) being found at higher temperatures. The comparative usefulness of *b* is unclear. It has been suggested that the value of *b* changes in a predictable way and is related to temperature, age or reproductive condition (Bayne et al., 1976c). Q_{10} values for *Perna canaliculus* were also comparable to other mussel species, (see Table 2.3) except for those

derived for the 5 to 10 °C range. This had a much higher than normal value (5.39). The respiration rates for *Perna canaliculus* at 5°C were much lower than expected. I believe that this temperature represents the lower thermal tolerance limit for this species and that the mussels were effectively shutting down their metabolism. This is reflected by the earlier finding that, at 5°C, *P.canaliculus* showed a low, constant rate of oxygen uptake over a wide range of body size.

Table 2.3 - Q_{10} of aquatic respiration rates from several species of mussels.

Species	5-10	10-15	15-20	20-25	25-30	30-35	Source
Mytilus edulis(15°C)	2.25	2.13	2.47	1.10	-	-	Bayne et al., 1976c
Mytilus edulis(15°C)	-	1.55	1.35	1.67	-	-	Widdows, 1973
Mytilus edulis(13°C)	-	-	1.30	1.69	-	-	Bayne et al., 1976c
Modiolus demissus(25°C)	-	-	1.55	1.89	1.86	1.24	Bayne et al., 1976c
Perna perna(25°C)	-	-	1.25	1.17	1.54	2.59	Bayne et al., 1976c
Perna canaliculus(15°C)	5.39*	1.85	1.80	-	-	-	This study.
Mitrella lunata(21.6°C)§	2.89	1.99	2.51	1.28	1.46	0.33	McMahon et al., 1977

Temperatures shown in brackets are acclimation temperatures.
* See text for discussion of this extremely high value.
§ This is a species of intertidal gastropod, not a bivalve. It is here as a comparison.

There were no significant differences between summer and winter aquatic respiration rates at 10 or 15°C. This indicates that *P.canaliculus* may not show much seasonal acclimation of aquatic respiration rates to temperature. If thermal acclimation was occurring then you would expect to find, at a given temperature, either elevated oxygen uptake rates in the winter or depressed rates during summer. *P.canaliculus* may show a shift in lower thermal tolerances, however. This is suggested by the depressed aquatic respiration rates shown by high shore summer animals at 10°C. Aerial respiration rates showed declines at both

the upper and lower temperature used in both winter and summer. McMahon & Russell-Hunter (1977) found a similar response in intertidal gastropods, although for those animals the peak in aerial oxygen uptake was at approximately 25°C. I believe that the decline in aerial oxygen uptake at the higher experimental temperatures was due to valve closure. *Perna canaliculus* most likely reduce periods of valve gaping as a mechanism for desiccation avoidance in the natural environment. Another possible explanation is that the mussels have reached their upper temperature limit. However, Kennedy (1976) has previously found that the upper lethal limit of *Perna canaliculus* was between 24 to 26.9°C. The temperatures used during my experiments were lower than this level and within the normal temperature range found at Taylors Mistake (Kennedy, 1976). The temperature above which there was a decline in oxygen uptake was higher for animals collected during summer than winter indicating that during summer *Perna canaliculus* has a higher thermal tolerance.

It has been suggested that the ratio of aerial to aquatic rates of oxygen consumption would be higher for species that live higher in the littoral zone (McMahon, 1988a). This has been studied in a wide range of intertidal organisms. Bridges (1988) reviewed the respiratory adaptations shown by intertidal fish. He found that species which spend a greater amount of time in air have a higher aerial $\dot{V}O_2$: aquatic $\dot{V}O_2$ ratio. Murdoch & Shumway (1980) studied rates of aerial and aquatic oxygen uptake of six species of chiton in relation to shore level. They found that species found highest on the shore had a higher aerial respiration rate relative to their aquatic rate of oxygen uptake. In contrast Horn (1985) found that patterns on aerial and aquatic respiration of the

chiton *Chiton pelliserpentis* were more closely related to energy conservation than position in the intertidal zone. McMahon (1988a) found that intertidal gastropods show an increase in average aerial:aquatic ratio with increased shore level. He also found some evidence that this also occurs in intertidal bivalves, with species found low on the shore having aerial respiration rates 10 to 20 % of aquatic respiration rates. In contrast, mussels found at the top of the littoral zone having values of 50 to 75% of the aquatic rate. Table 2.4 shows the ratio of aerial to aquatic respiration rates from several intertidal bivalve species. Note that although the ratio for *Perna canaliculus* is higher at the mid-shore level than the low shore, this is due mainly to the slightly different aquatic respiration rates found during winter.

Table 2.4 - Ratio of aerial to aquatic oxygen consumption for various intertidal bivalve molluscs.

Species	Zone	Exp.Temp.	Aerial Vo_2: Aquatic Vo_2	Source
Cerastoderma glaucum	Low	15°C	0.14:1	Boyden 1979
Mytilus edulis	Mid	20-30°C	0.05:1	Widdows et al. 1979
Mytilus galloprovincialis	Mid	25°C	0.14:1	Widdows et al. 1979
Mytilus californianus	High	13°C	0.74:1	Bayne et al. 1976
Modiolus demissus	Mid	20°C	0.63:1	Kuenzler 1961
Geukensia demissa	High	20°C	0.56:1	Widdows et al. 1979
Perumytilus purpuratus	Mid	10°C	0.52:1	Vial et al. 1992
Perna canaliculus	Mid Low	15°C 15°C	0.27:1 0.21:1	This Study

Modified from R.F. McMahon 1988.

To summarise, *Perna canaliculus* from high and low shore levels showed no difference in rate of aerial and aquatic oxygen uptake. There were no differences found between summer and winter rates of aquatic oxygen uptake in relation to temperature. However, mussels collected in summer showed a higher temperature tolerance before curtailment of aerial respiration activity than winter collected individuals. Respiration rates, Q_{10} values and aerial $\dot{V}O_2$: aquatic $\dot{V}O_2$ ratios found for *Perna canaliculus* were all within the normal ranges published for this and other species of marine mussel.

The differences in aerial oxygen uptake recorded here for *Perna canaliculus* most probably reflect adaptations and desiccation rather than a strictly temperature response.

CHAPTER 3

Energy acquisition and efficiency.

INTRODUCTION

FILTRATION RATE.

The lamellibranch gill has two major purposes, as a surface for gas exchange and as a sieve to extract suspended matter from the surrounding water (Bayne et al., 1976c; Jørgensen, 1975). There are three major components to the filter feeding process:

1) *Transport of water past the feeding structure.* This is usually given the term "pumping rate" or sometimes "ventilation rate" although the latter term usually refers to respiration.

2) *Separation of particulate material from the surrounding medium.* This is called "filtration rate".

3) *Transport of captured particles to the mouth.*

In mussels, water is passed across the gills by cilia generated currents (Jones et al., 1992). Pumping rate can be varied by the animal as a means of regulating food intake. Pumping rates also vary between species. Meyhöfer (1985) calculated that *Mytilus californianus*, a mussel found on rocky exposed surfaces, has a pumping rate of 0.085 cm^3 sec^{-1} cm^2 while *Clinocardium nuttallii*, a sediment dwelling bivalve, has a pumping rate of 0.145 cm^3 sec^{-1} cm^2. Mussels also rely to a certain extent on naturally occurring water currents (Cole et al., 1992). Particulate matter is trapped in a layer of mucus on the gills and passes as a string across the palps and into the mouth. Particles which are too large may be rejected at this stage. There is some debate over whether mussels are capable of selectively ingesting food particles. Bayne et al. (1976c) and Winter (1978) both state that mussels are unable to selectively particles, where as Kiørboe et al. (1980) suggests that *Mytilus edulis* is capable of selecting between algal cells and silt. Over the past fifty years many studies have been carried out on the feeding mechanism of mussels. It has been found that individual mussels are capable of varying their filtration rate, and that filtration rate can be altered independently of ventilation rate and vice versa (Bayne et al., 1976c). Vahl (1972, in Bayne et al., 1976c) has found that *Mytilus edulis* is capable of retaining extremely small particles including bacteria and viruses (Charles et al, 1992; Møhlenberg & Riisgård, 1978).

The standard method used to determine feeding activity is to measure clearance rate, which is the amount of water completely cleared of food particles

for a given period of time. If the experimental animals retain 100% of the food that passes across their gills then filtration rate = clearance rate. To assure that this is true many workers use a monoculture of algae known to be retained by the animal and ensure that the concentration of algae is not sufficiently high for the animal to reject excess algae as pseudo-faeces.

One of the most commonly investigated relationships is that between filtration rate and body size. This relationship is usually described using the allometric equation,

$$C = aW^b$$

where C = clearance rate (usually in l h^{-1}) and W = body weight (dry flesh weight). The variable *a* gives an indication of the clearance rate while the exponent *b* serves as a modifier for weight. A value of b greater than 1 would imply that clearance rate increases exponentially with body size, while a value less than 1 indicates that the rate of increase in filtration rate decreases with increased body size. In practice *b* is always less than 1 as gill surface area increases at a lower rate than body weight with increased animal size. Another interesting point about the allometric equation is that at a body weight of 1 gram (or whatever weight unit is being used) clearance rate equals *a*.

Besides body size, clearance rate is also affected by many exogenous factors. Concentration of food particles is known to affect filtration rate. *Mytilus edulis* for example does not filter when food concentrations are very low. However, above a threshold level of food concentration, filtration rate can be remarkably level over a large range (Bayne et al., 1976c). In contrast, Waite

(1989) found no levelling of filtration rate for *Perna canaliculus* fed a monoculture of *Isochrysis galbana* over any food concentration. Navarro and Winter (1982) found that when food is at high concentrations, filtration rate of *Mytilus chilensis* declines. Bayne et al. (1987) found that *Mytilus edulis* is capable of acclimation to low food concentrations by increasing digestion efficiency.

Filtration rate is subject to the standard invertebrate temperature responses so that when mussels encounter a decline in temperature, filtration rate also declines. Conversely when temperature increases so does filtration rate.

The effect of tidal level on filtration rate is poorly understood. Segal et al. (1953, in Bayne et al., 1976c) found that *Mytilus californianus* from the lower intertidal zone have a higher clearance rate than those found at a higher level. In contrast Bob Wear (pers. comm.) has found that *Perna canaliculus* from the intertidal zone have a higher clearance rate than those cultured subtidally on mussel lines. Kreeger, et al. (1990) found no difference in filtration rate of *Geukensia demissa* collected from intertidal and sub-tidal levels. Widdows & Shick (1985) also found that *Mytilus edulis* from the intertidal zone showed no increase in clearance rate or absorption efficiency to compensate for reduced feeding time.

Salinity is also known to affect mussel clearance rates. Navarro (1988) found that a decline in salinity resulted in a drop in filtration rate of *Choromytilus chorus*. Also Theede (1963, in Bayne et al., 1976c) found with *Mytilus edulis* that change in salinity either up or down resulted in a drop in the filtration rate.

ASSIMILATION EFFICIENCY.

Once filtration rate has been established the next step is to measure how efficiently the food is utilised. The term given to this is assimilation efficiency. It is usually calculated as the difference between the ratio of organic to inorganic content of the ingested ration and of the faeces produced. This is calculated with the following equation (from Conover, 1966):

$$AE = \left[\frac{(F - E)}{(1 - E)(F)}\right] \times 100$$

where AE = assimilation efficiency, F = ratio of ash-free dry weight to dry weight of the ingested food, and E = ratio of ash-free dry weight to dry weight of the faeces produced. Many mussels are capable of maintaining a fairly constant assimilation efficiency over a wide range of food concentrations and body size (Navarro & Winter, 1982; Waite, 1989; Hawkins & Bayne, 1991). For *Choromytilus chorus* assimilation efficiency is not affected by decrease in salinity (Navarro 1988).

NITROGEN EXCRETION.

Many marine mussels have been found to excrete large amounts of nitrogen (James et al., 1987). Nitrogen is usually excreted as ammonia-nitrogen (NH_4–N) and to a slightly lesser extent amino–N (Bayne et al., 1976d). Under normal conditions amino–N represents approximately 10% of nitrogen loss. However, when the animals are placed under stress, by starvation or salinity

change for example, amino–N can rise to over 60% of the nitrogen lost (Bayne et al., 1976d).

Excretion rate is usually determined by placing an individual into a closed container with a known volume of water for a set period of time and then analysing the water for nitrogenous wastes.

There were three aims for this section. The first was to determine size related filtration rates for *Perna canaliculus* from two different shore levels during winter and summer. The second aim was to determine rates of excretion for mussels from high and low shore levels during winter and summer. And the final aim was to calculate assimilation efficiency of mussels from Taylors Mistake fed on a monoculture of *Isochrysis galbana.*

METHODS

FILTRATION RATE.

Filtration rate was estimated by determining clearance rate. For this set of experiments 10 to 15 randomly selected mussels of 25 to 85 mm shell length were used. Individual mussels were placed in either 500 or 1000 ml of a monoculture feeding medium of the algae *Isochrysis galbana*. The amount used depended on availability of culture. The feeding medium was kept in suspension by constantly bubbling air through the experimental chambers. Algal concentration was estimated by taking a small sample of culture from the experimental chamber (approx 35 ml) and placing it in a Turner Design Fluorometer. After taking a reading the sample was returned to the experimental chamber to ensure that the volume was not altered. Over the course of the

experiment no more than 1 ml of medium would have been lost which means in the worst case, volume of the experimental medium would not have dropped by more than 0.2%.

Experiments were carried out in a controlled temperature chamber held at 15°C. After being placed in the feeding solution, the mussels were allowed to settle for 5 - 10 mins before the experiment started. Readings were taken every 15 to 20 mins for 90 mins or until algal concentration dropped below detectable levels (<100 cells ml^{-1}).

Calculations:

The first step was to convert fluorometer readings to ln (cells/ml). This was performed by using a regression equation calculated from a calibration curve:

$$\ln(cells/ml) = \frac{\ln(reading) + 6.17404}{0.768324}$$

Next the clearance rate in ml/min was estimated by using the equation from Newell (1979).

$$m = \frac{M}{n}\left[\frac{(\ln C_0 - \ln C_t) - a}{t}\right]$$

$$a = \frac{\ln Conc_0 - \ln Conc_t}{t}$$

Where m = Clearance Rate (vol/time), M = Volume of vessel, n = Number of animals per vessel, C_0 = Initial concentration of algae, C_t = Concentration of algae after time t, t = Time and a = Rate of settlement of particles (from control vessel). The calculations were made using a spread sheet programme. Clearance rate was assumed to be the same as filtration rate.

ASSIMILATION EFFICIENCY.

Assimilation efficiencies were calculated for winter collected mussels. 16 mussels of 20 to 80 mm shell length were selected from each shore level for this set of experiments. All of the experiments were conducted in a controlled temperature chamber at 15°C. Individual mussels were placed in jar with 500 ml of known concentration of algae, determined using fluorometer. Three concentrations of algae were used, they were 1000, 5000 and 10000 cells ml^{-1}. Animals were left in the algal solution until they cleared jars and were then transferred into 500 ml of pasteurised glass-filtered sea water, and left for 24 hours for depuration. The animals were removed from the jars and the water was filtered using pre-ashed and weighed glass filters. The filtrate was gently washed and then dried in an oven at 50 - 60°C for 3 days. The filters were weighed then ashed and re-weighed. Assimilation efficiency was calculated using the methods of Conover (1966).

NITROGEN EXCRETION.

The Phenol-Hypochlorite method of Solórzano (1969) was used to determine ammonia excretion. 16 animals of 25 to 65 mm shell length were

selected from each shore level for this experiment. Individual animals were placed in a glass jar with 200 ml of pasteurised glass filtered sea water with a bubbler for 24 hours. Water samples were then reacted with phenol and hypochlorite using Boehringer urea test kit (Cat. no. 124 788 or 124 770). Absorbance was measured at 640 nm using a Kontron Instruments UVIKON 860 Spectrophotometer.

RESULTS

FILTRATION RATE.

Winter.

Figure 3.1 shows filtration rates for mussels collected in winter from high and low shore levels. *Perna canaliculus* showed size related filtration rates. The regression line fitted to the data for high shore level animals was significant to the 5% level with an r^2 value of 0.38. The line fitted to the low shore data was significant to the 1% level with an r^2 value of 0.79. During winter *Perna canaliculus* from the high shore level showed filtration rates approximately twice that for animals collected from the low level.

Summer.

Figure 3.2 shows clearance rates of *Perna canaliculus* collected from high and low shore levels during summer. Mussels collected in summer showed a clearance rate of about half that recorded for winter collected animals. Once again high shore animals had a higher clearance rate than low shore specimens although animals from the low shore level had a very large variation in filtration

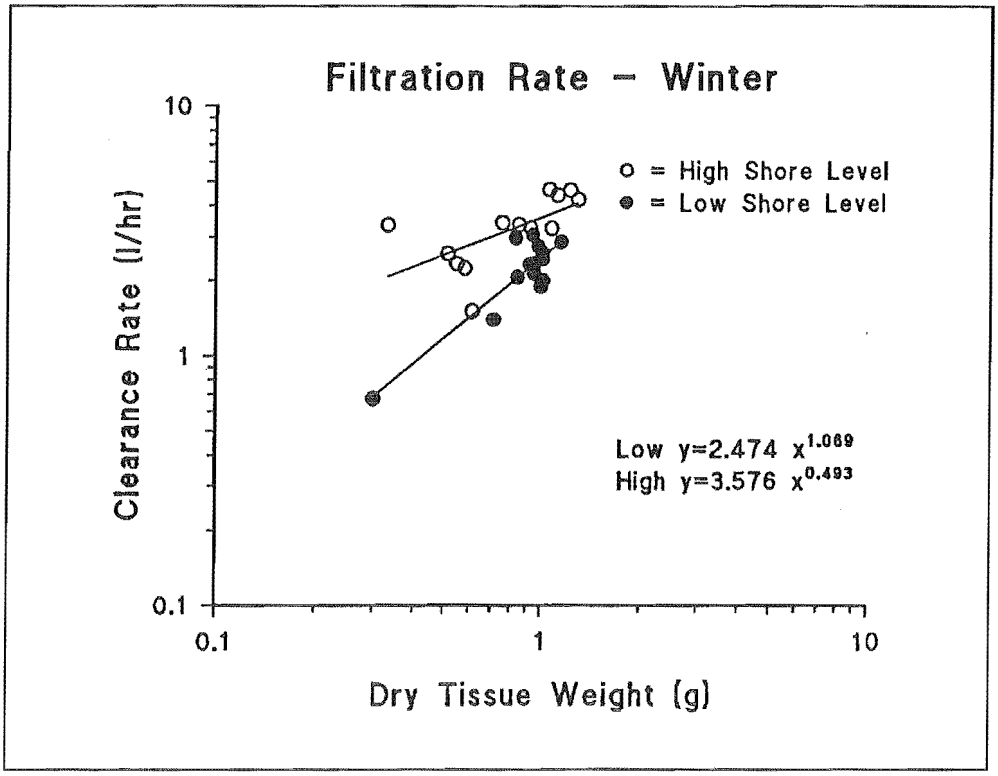

Figure 3.1 - Filtration rate vs dry tissue weight of *Perna canaliculus* during winter.

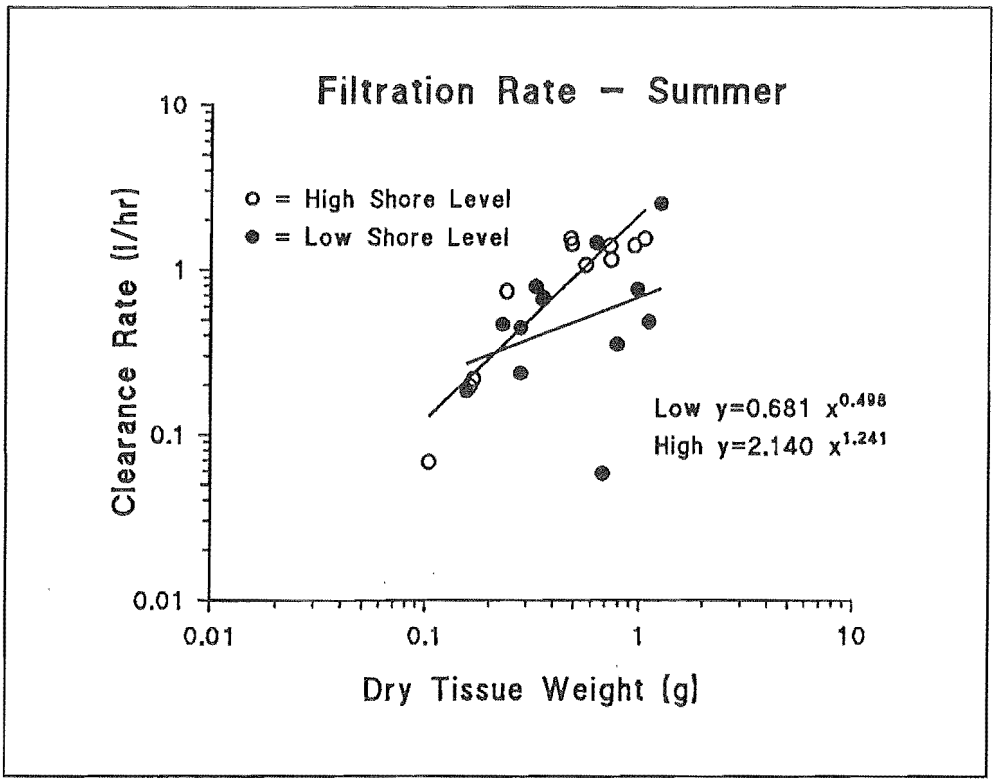

Figure 3.2 - Filtration rate vs dry tissue weight of *Perna canaliculus* during summer.

rate. The regression line fitted to the high shore data was significant to the 1% level with an r^2 value of 0.84. However, the line for the low shore data was not significant with an r^2 value of only 0.12.

ASSIMILATION EFFICIENCY.

Assimilation efficiency of *Perna canaliculus* fed on a monoculture of *Isochrysis galbana* was constant over the range of algal concentrations tested. There was also no significant difference in assimilation efficiency between high and low shore animals (ANOVA, f=0.83, df=23, p = 0.4518). Table 3.1 shows assimilation efficiencies for *Perna canaliculus* at the three tested algal concentrations.

Table 3.1 - Assimilation efficiencies of *Perna canaliculus* fed on 3 concentrations of algae.

Concentration (cells/ml)	1000	5000	10000
Assimilation Efficiency	80.83	84.80	83.24
Std. Err.	1.53	2.22	2.70

NITROGEN EXCRETION

Table 3.2 - Ammonia excretion rate per unit of dry tissue weight (± S.E.)

Ammonia Excretion ($\mu g\ hr^{-1}\ g^{-1}$)	Low	High
Winter	20.3 (± 3.6)	59.7 (± 9.2)
Summer	13.7 (± 2.9)	14.6 (± 5.4)

Table 3.2 shows estimated rates of excretion of NH_4–N by *Perna canaliculus* at 15°C from high and low shore levels during winter and summer. Rates of NH_4-N excretion were higher in winter than in summer. During Winter the rate of excretion was much higher for animals collected from high shore levels compared to those from the low shore (59.7 and 20.3 μg/hr for high and low shore respectively). High shore animals collected during summer also had a slightly higher excretion rate than the low shore animals collected at the same time.

DISCUSSION

There is some controversy regarding the existence of adaptations to reduced feeding time caused by tidal exposure (Bayne et al., 1988). Griffiths and Buffenstein (1981) found no evidence for enhanced filtration rates or assimilation efficiencies in littoral *Choromytilus meridionalis* compared to sublittoral specimens. In contrast, Kreeger et al. (1990) studied *Geukensia demissa* collected from intertidal and subtidal areas and compared the relative utilisation of ^{14}C labelled lignocellulose. He found that intertidal animals showed a significantly higher absorption efficiency than subtidal animals although there was no difference in rates of ingestion. Kreeger suggested that increased absorbtion efficiencies of intertidal animals was caused directly by aerial exposure as the obligatory curtailment of feeding activity allowed food already in the gut to be digested for longer periods of time.

I found that upper shore *Perna canaliculus* collected from Taylors Mistake had a higher clearance rate than low shore animals. It has also been found that

P.canaliculus collected intertidally have a higher clearance rate than those collected subtidally (Bob Wear pers.comm.). This suggests than that *P.canaliculus* from the intertidal zone may show some compensation to reduced food supply by increasing filtration rate. Clearance rates were also highest during the winter. This may have been due to temperature effects as the experiments were carried out at 15°C, which is higher than the winter sea temperature. Waite (1989) found that *P.canaliculus* shows a reduction in clearance rate with an increase in food concentration. Although food concentrations were the same in experiments carried out in winter and summer it may be possible that *P.canaliculus* shows seasonal acclimation to elevated phytoplankton levels found during summer. The range of clearance rates that were calculated for *Perna canaliculus* in this study fall within normal ranges found for other mussel species. Table 3.3 shows clearance rates in l hr^{-1} of several mussel species corrected to 1 g dry flesh weight.

Bayne et al. (1984) found that *Choromytilus meridionalis* and *Perna perna* compensate for poor food quality by increasing gut retention time and gut content. They also found that these two mussel species had a higher clearance rate at a site that had a lower quantity of suspended particulate matter. This finding may be a little misleading as the site with highest quantity also had the lowest food quality. It is possible that reduction of clearance rate may have been a mechanism to increase gut passage time and hence assimilation efficiency. Elwin and Gonor (1979, in Griffiths & Buffenstein, 1981) found that *Mytilus californianus* showed enhanced assimilation efficiency with increased tidal exposure when fed on an algal solution.

Table 3.3 - Clearance rates of various mussel species.

Species	Temperature (°C)	Clearance rate ($l\ h^{-1}\ g^{-1}$)	Source
Aulacomya ater	12.5	1.3 - 1.8	Stuart, 1982.
Choromytilus chorus	12	1.6	Navarro, 1988.
Choromytilus meridionalis	12	3.1 - 5.3	Bayne et al., 1984.
Choromytilus meridionalis	12	2.7 - 5.2	Griffiths & Buffenstein., 1981.
Modiolus demissus	10	3.4	Kuenzler, 1961.*
Modiolus modiolus	12	0.9	Winter, 1969.*
Mytilus californianus	16	1.2	Rao, 1953.*
Mytilus californianus	16	2.1	Rao, 1953; Segal, 1953.*
Mytilus chilliensis	12	0.8 - 1.6	Navarro & Winter, 1982.
Mytilus edulis	12-15	1.3	Willemsen, 1952.*
Mytilus edulis	15	1.7	Theede, 1963.*
Mytilus edulis	15	1.9	Thompson & Bayne, 1974.*
Mytilus edulis	15	2.0 - 4.1	Bayne et al. 1987.
Mytilus edulis	15	1.8 - 2.5	Riisgård & Randløv, 1981.
Mytilus edulis	8-16	2.4	Widdows et al., 1979.§
Mytilus galloprovincialis	?	1.3 - 3.2	Navarro et al., 1991.
Perna canaliculus	15	2.3 - 8.8	Waite, 1989.
Perna canaliculus	15	2.0 - 4.6	Marsden & Shumway, 1992.
Perna canaliculus	15	1.1 - 3.7	This Study.
Perna perna	12	2.8 - 4.4	Bayne et al., 1984.

* These values are derived from Bayne et al. (1976c). § This value was calculated for a 50 mm shell length mussel. Where a range of values is given clearance rate was calculated for a range of experimental conditions such as changing food concentration.

My study showed that *Perna canaliculus* from both shore levels had a similar assimilation efficiency when fed on *Isochrysis galbana* at a range of concentrations (average = 83 %). This is very similar to the findings of Waite (1989) who calculated the assimilation efficiency of *P.canaliculus* (collected from Mahanga Bay, Wellington Harbour), fed on *I.galbana* as being in the range of 82

to 89 %. Waite also found that assimilation efficiency is fairly independent of food concentration (at least over naturally occurring ranges). This has also been found by other workers (Bayne et al., 1989; Navarro & Winter, 1982; Winter, 1978).

James et al. (1987) studied excretion of *Perna canaliculus* collected from mussel lines in the Marlborough Sounds from September 1984 to February 1985. They found that *P.canaliculus* excreted 21.3 μg NH_4–N h^{-1} g^{-1}. Low shore greenshell mussels collected in winter produce a similar amount of NH_4–N (20.3 μg NH_4–N h^{-1} g^{-1}). In contrast with this result, high shore mussels had an excretion rate almost 3 times that of the low shore animals. In addition mussels collected during summer had a lower rate of excretion than winter collected animals. High and low shore, summer collected, mussels had approximately the same excretion rate. Bayne et al. (1976d) report that several studies on *Mytilus edulis* have also recorded a high rates of nitrogen excretion during winter and low ones during summer. It has been suggested that this may be due in part to the animals suffering from starvation during winter. At this time they may be utilising stored glycogen reserves resulting in an increased rate of excretion of nitrogenous wastes. Bayne et al., (1976d) also report a study where mussels collected during summer and winter were taken to the lab and starved. The animals collected in winter showed a large increase in nitrogen excretion while the summer collected animals did not significantly increase nitrogen output. It seems possible then that high shore *Perna canaliculus* produce greater amounts of NH_4–N than their low shore counterparts because they are being regularly subjected to starvation. This is especially true during winter when phytoplankton

levels are at their lowest (Hickman et al., 1991; Barkati & Ahmed, 1990). With the exception of high shore, winter collected mussels, *Perna canaliculus* from this study showed ammonia excretion rates within the recorded ranges of other mussel species (Table 3.4). The unusually high levels in winter collected mussels may suggest that these animals are living at the extremes of their environmental range.

Table 3.4 - Ammonia-nitrogen excretion rates for several species of bivalve.

Species			Excretion Rate (μg NH_4-N $g^{-1}DW$ h^{-1})	Source
Mytilus edulis			6.6 - 22.3	Bayne & Scullard, 1977.*
Mytilus edulis			3.8 - 14.4	Hawkins et al., 1985.
Mytilus californianus			25.2	Bayne et al., 1976a.
Mytilus californianus			23.9	Bayne et al., 1976d.
Geukensia demissa			16.9 - 40.3	Jordan & Valiela, 1982.*
Perna canaliculus			21.3	James et al. 1987.
Perna canaliculus	Winter	Low	20.3	This Study.
		High	59.7	
	Summer	Low	13.7	
		High	14.6	

* These references were from James et al., 1987.

CHAPTER 4

Growth potential in relation to shore level.

INTRODUCTION

This final section of my thesis examines the effect of shore level on the energy balance and potential for growth of *Perna canaliculus*. To date there have been few published studies examining the effects of tidal exposure on growth in *P.canaliculus*. Hickman (1979) studied growth rates of *P.canaliculus* in suspended cultivation and contrasted it with growth of an intertidal population. He found that growth rates of the intertidal population were approximately half that of raft grown mussels. Intertidal mussels grew to an average of 31 mm after 12 months and 48 mm after 18 months. In contrast, cultured mussels grew to 72.5 mm and 108 mm respectively.

Many workers have studied growth in animals indirectly by assessing scope for growth (eg Page & Ricard, 1990). Scope for growth (SFG) is a measure of energy balance of an animal under specified conditions. SFG may

have a positive value indicating that surplus energy is available for somatic and/or gametic growth. SFG may also have a negative value which means that under the calculated conditions the animal will use stored reserves. Scope for growth is calculated according to the standard energy equation (Newell, 1979; Navarro & Winter, 1982),

$$P = C - (F + R + U)$$

where P is the energy available for production, C is the energy input from food consumed, F is energy loss through faeces, R is energetic cost of respiration and U is energy loss through excretion. This equation can be simplified by calculating energy assimilated (A) as energy content of food consumed multiplied by the assimilation efficiency (AE),

$$A = C \times AE$$

this also allows the removal of F from the first equation leaving the formula for scope for growth (SFG) as:

$$SFG = A - (R + U)$$

In order to calculate scope for growth, ingestion rate, assimilation efficiency, respiration rate and rate of excretion must be measured and converted into

energy equivalents (usually J hr^{-1}). Scope for growth is usually given as either J hr^{-1} g^{-1} dry weight or J day^{-1} g^{-1} depending on the needs of the researcher.

Each component of the energy budget is subject to modification due to change in external variables such as temperature and food quality and quantity, or internal factors such as body weight, reproductive condition and season acclimation. Clarke and Griffiths (1990) state that several bivalve species, including *Mytilus edulis*, are capable of adjusting components of their energy budgets in order to maintain a positive scope for growth. Griffiths and Buffenstein (1981) propose that theoretically mussels which live in the littoral zone may be able to enhance energy intake during immersion and suppress metabolic losses during emersion. They also say that in order to survive in the intertidal zone, energy normally put into growth and reproduction may be restricted in order to conserve limited resources.

As mentioned above, food concentration can affect scope for growth. Bayne et al., (1976e) suggest that when food concentrations are very low, scope for growth is very small or even negative, resulting in starvation. This was also found by Clarke and Griffiths (1990), studying the energetics of *Choromytilus meridionalis* trapped in rock pools during low tide. They found that food became depleted very rapidly after isolation and the mussels showed a net loss of energy because they continued to actively filter water low in nutrients. Bayne et al., (1976e) have also shown that, as food concentration increases, the scope for growth also increases to an optimum food concentration. If levels of food continue to increase then the scope for growth declines again as the cost of filtration continues to rise while assimilation efficiency declines. Navarro and

Winter (1982) provide an example of this with *Mytilus chilensis*. This mussel was fed the alga *Dunaliella marina* at three concentrations ranging from 15 × 10^6 to 40 × 10^6 cells l^{-1}. At all three cell concentrations the scope for growth was positive but declined with increasing algal concentration.

Body size is very important in determining scope for growth. At any give food concentration smaller mussels will generally have a higher scope for growth than larger mussels (Bayne et al., 1976e; Chatterji et al., 1984). This is because the increase in respiration costs associated with a larger body size is not matched by an equivalent increase in feeding rate. The net result as a mussel grows larger is that the amount of energy available for further growth declines and so growth rate declines with age (Page & Hubbard, 1987).

Temperature has also been found to affect scope for growth. Widdows (1978, in Newell ,1979) found that gross growth efficiency (which is defined as growth per unit of ingested ration) of *Mytilus edulis* declined as temperature increased from 5 to 25°C. Bayne et al. (1976e) found that scope for growth of *Mytilus edulis* is fairly constant, although always declining, from 5 to 20°C. However, at 25°C it drops significantly as the animals start to reach their upper thermal limit.

There were two aims for this section. The first was to establish the size range of *Perna canaliculus* at high and low shore levels at Taylors Mistake. The purpose of this was to look for field evidence of different growth rates with change in shore level. The second aim was to calculate scope for growth of *P.canaliculus* from high and low shore levels during winter and summer.

METHODS

SIZE FREQUENCY DISTRIBUTION

During December of 1991 two horizontal transects were made at Taylors Mistake at 0.0 and 0.75 m above MLW. Ten random samples were taken at each level with no two samples being closer than 2 m horizontally. For each sample a 25 x 25 cm quadrant was placed on the rock surface at the appropriate level and all the mussels removed within the quadrant. They were placed in plastic bags and returned to the lab where their shell length was measured to the nearest 0.1 mm with vernier callipers. The data were pooled for each shore level and a size frequency graph was produced. The tissues from these animals was used to calculate condition index for December 1991 (see chapter 1).

SCOPE FOR GROWTH

In order to calculate scope for growth of *Perna canaliculus* the following assumptions and constants were derived. Assimilation efficiency of *P.canaliculus* fed on *Isochrysis galbana* was assumed to be 83%, this was derived by taking the average of assimilation efficiencies calculated in chapter 3; *I.galbana* was assumed to have an energy content of 20.1 J mg^{-1} dry weight (Romberger and Epifanio, 1981); *I.galbana* was also assumed to weigh 19.3 pg $cell^{-1}$ (Waite, 1989); the cost of respiration was set at 20.1 J ml^{-1} O_2 hr^{-1} (Waite, 1989) and finally the cost of excretion was assumed to be 10.12 J mg^{-1} NH_4–N (Navarro & Winter, 1982). All other variables were derived from my experimental data (respiration rates were discussed in chapter 2 and energy acquisition and

efficiency in chapter 3). Scope for growth was initially calculated in J hr^{-1} g^{-1} dry tissue weight and then corrected according to the assumption that the high shore level animals are exposed to air for 2½ hours per tidal cycle (5 hours in every 24) to provide scope for growth in J day^{-1} g^{-1}.

RESULTS

SIZE FREQUENCY

Figure 4.1 shows the size frequency distribution of *Perna canaliculus* at the two shore levels examined at Taylors Mistake. Mussels collected from the low shore level averaged 80.2 mm in length with a standard deviation of 20.8 mm while those from the high shore level were on average 57.6 mm long with standard deviation of 20.8 mm.

SCOPE FOR GROWTH

Scope for growth (SFG) was calculated for *Perna canaliculus* at 15°C from high and low shore levels during winter and summer. These calculations assume that mussels feed continuously throughout the day. SFG was found to be higher for mussels collected at high shore level than that for low shore animals. This was due mainly to an increased clearance rate shown by high shore level animals. SFG was also higher for winter collected animals than summer collected animals. Figure 4.2 shows SFG, in J hr^{-1} g^{-1} dry weight, of *P.canaliculus* over a range of algal concentrations. SFG was then adjusted for the reduced feeding time due to aerial exposure (assuming that high shore animals were exposed for 2½ per tidal cycle). Figure 4.3 shows corrected SFG

Table 4.1 - Coefficients of scope for growth regression lines displayed on figures 4.2 and 4.3. (y=a+bx).

Season	Level	a	b
(uncorrected. J hr^{-1} g^{-1})			
Winter	High	-5.89	$11.51x10^{-4}$
	Low	-3.80	$7.97x10^{-4}$
Summer	High	-4.31	$6.89x10^{-4}$
	Low	-4.94	$2.19x10^{-4}$
(corrected for emersion. J day^{-1} g^{-1})			
Winter	High	-111.93	$2.19x10^{-2}$
	Low	-91.29	$1.91x10^{-2}$
Summer	High	-81.86	$1.31x10^{-2}$
	Low	-118.62	$0.53x10^{-2}$

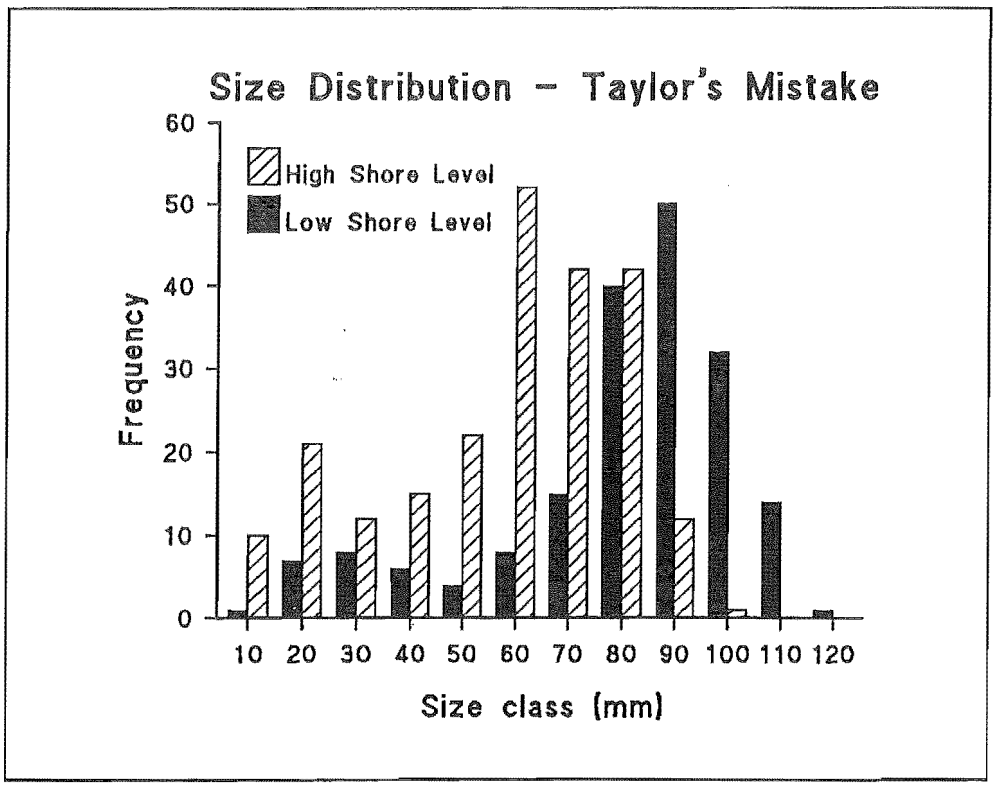

Figure 4.1 - Size frequency of *Perna canaliculus* from Taylors Mistake. (High mean = 80.2 ± 20.8 mm; Low mean = 57.6 ± 20.8 mm).

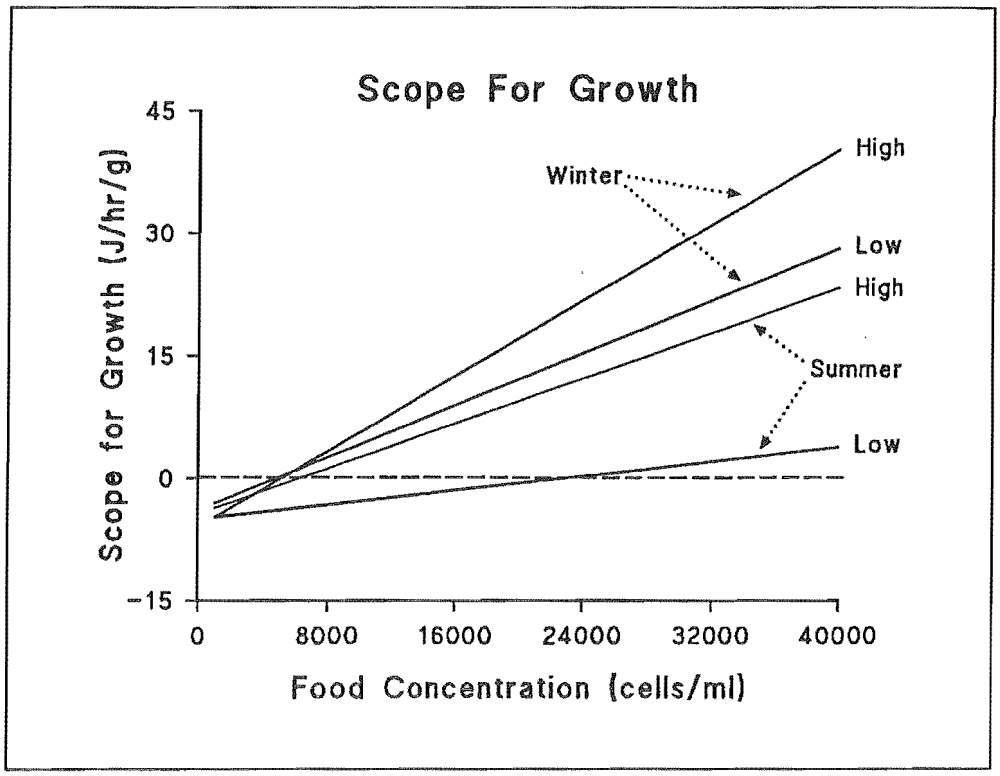

Figure 4.2 - Scope for growth of *Perna canaliculus*.

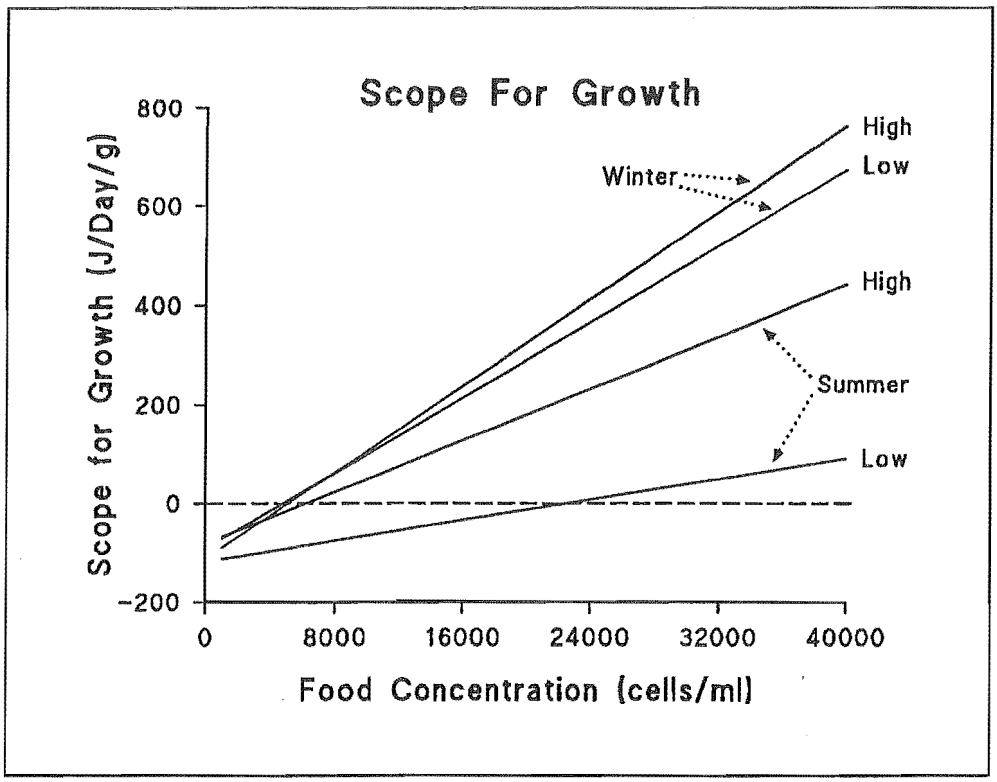

Figure 4.3 - Scope for growth of *Perna canaliculus* adjusted for reduced feeding time (19 hours in every 24).

at a range of algal concentrations in J day^{-1} g^{-1}. Table 4.1 displays the formulas of the lines shown on figures 4.2 and 4.3. These graphs suggest that during the winter the scope for growth of high and low shore mussels is similar. However, in the summer high shore mussels have a greater scope for growth than their low shore counterparts.

DISCUSSION

Scope for growth estimates of *Perna canaliculus* derived from my experimental data were similar to those published for other species of mussel. Navarro and Winter (1982) found that energy available for growth and reproduction of a 1 g dry tissue weight *Mytilus chilensis* fed on a high quality food source (*Dunaliella marina*) ranged from 204.40 to 242.67 J day^{-1} depending on food concentration. These values represented potential increase in dry tissue weight of 0.9 to 1.2% per day. Newell (1979) reports that scope for growth of *Mytilus californianus* fed a ration of 4 to 6% of body weight per day increased from 303.1 to 497.8 J day^{-1} as temperature rose from 13 to 22°C. However, when temperature reached 26°C scope for growth dropped to 62.8 J day^{-1} due to a marked decrease in ingestion rate and a substantial increase in respiration rate. R. Wear (pers.comm.) roughly estimated scope for growth of *Perna canaliculus* as 3.6 J hr^{-1} g^{-1} dry weight for mussels collected subtidally, from mussels lines, and 12.0 J hr^{-1} g^{-1} for intertidal mussels. However, these results did not take into account assimilation efficiency, respiration rate and energy loss through excretion.

Scope for growth calculations were made using data derived from controlled, laboratory based experiments and indicated that *Perna canaliculus* from the high shore level have a higher potential for growth than their low shore counterparts. This suggests that mussels from the high shore are able to compensate for reduced feeding time associated with tidal exposure. However, the scope for growth calculations and the conclusions made were based on parameters which may have been unrealistic (eg, food quality and quantity and mussel size). Also other parameters such as water currents, temperature fluctuations, reproductive condition and parasitism were not taken into account. Any or all of these may have affected the final results.

It is assumed that digestion continues while the animal is exposed to air. If this is true then the mussel is able to continue extracting energy from food ingested during immersion and assimilation efficiency will be higher. Kreeger et al., (1990) found some evidence to support this in the ribbed mussel *Geukensia demissa*. Other workers, for example Griffiths and Buffenstein (1981) studying *Choromytilus meridionalis*, have found no change in assimilation rates with shore level. Assimilation efficiencies of *Perna canaliculus* did not differ between high and low shore levels, although in these experiments the animals remained immersed while digestion was allowed to be completed. It is possible that during aerial exposure digestion activity is halted as an energy saving mechanism.

Scope for growth calculations also assume that the energetic cost of emersion is minimal. While it is true that mussels reduce their energetic costs during aerial exposure by reduction of metabolism (Newell, 1979) and excretion

rate (Bayne, 1976c) the cost of anaerobic metabolism is high in comparison to aerobic metabolism. Also anaerobic metabolism may be associated with several problematic side-effects (Newell, 1979). When exposed to air, mussels have to deal with the problem of build up of CO_2 and other anaerobic end products which may be toxic in themselves. They may also cause a dramatic drop in pH. The effects of aerial exposure may be enlarged due to higher air temperatures during the day (Clarke & Griffiths, 1990). When the mussels are returned to water they have high energy costs associated with repayment of oxygen debt (Bayne, 1976b). These include metabolism of anaerobic end products back into glycogen and replenishment of glycogen which could not be recovered. Upon return to water there is also an increased energy demand due to commencement of filtration, respiration, nitrogen excretion and possibly digestion.

There is also biological evidence to suggest that compensation for reduced feeding time with shore level was not complete in this population of *Perna canaliculus*. Throughout the year, high shore animals had a consistently lower condition index than those found lower on the shore (see figure 1.1 in chapter 1). This suggested that there was less energy available for somatic and gametic tissue growth in high shore mussels. Mussels found at the high shore level have a reduced shell length compared to those found lower down the shore. This suggests that, either resources are being channelled away from shell growth or growth rates are restricted. Suchanek (1978) has also recorded reduced average shell length with increased shore levels in *Mytilus edulis*. Hickman (1980) found that *Perna canaliculus* from the intertidal zone have a

thicker shell than mussels found subtidally. This has also been documented for other mussel species including *Perna perna* (Berry, 1978), *Mytilus edulis* (Rodhouse et al., 1984) and *Mytilus galloprovincialis* (Raubenheimer & Cook, 1990). This suggests that growth in shell thickness may be at the expense of increase in shell length. No information was available on the age structure of this population of mussels which makes determination of natural growth rates impossible. Further work needs to be done in this area to determine whether growth rates are in fact lower in high shore animals.

Despite the limitations of the scope for growth estimates, *Perna canaliculus* does show physiological responses to aerial exposure. Aerial respiration rates of *P.canaliculus* were approximately 25% that of aquatic respiration indicating that at least some of the metabolic needs were met during aerial exposure. *P.canaliculus* also showed a marked decline in aerial respiration rates when temperature increased indicating that desiccation avoidance occurs. *P.canaliculus* from the high shore level also had a higher clearance rate which led to a higher scope for growth of high shore animals in both summer and winter.

Finally, it is important to ask why the increased scope for growth is not reflected in the actual growth rates on the shore. Several features are important here including temperature effects on feeding and metabolism. With low winter sea temperatures it seems likely that scope for growth of *Perna canaliculus* from the two shore levels would be similar. During summer, high air temperatures and increased feeding rates could lead to greater differences in scope for growth. High shore mussels put more investment into shell thickening and are

exposed to greater fluctuations in environmental conditions. Such changes result in interrupted growth patterns. Also, it seems likely that in upper shore animals there is increased metabolic demand due to anaerobic metabolism. These features may explain differences in growth with shore level.

It is concluded that *Perna canaliculus* living in the intertidal zone are not restricted entirely by energetic constraints. Other factors such as desiccation and thermal stress may restrict the range of this animal before energy balance becomes limiting.

Appendix 1 - Tabulated constants used in respiration calculations.

Temperature	O_2 Capacitance	Saturation Pressure of H_2O
5°C	2.0044	6.5
10°C	1.7929	9.2
15°C	1.6218	12.8
20°C	1.4823	17.5
25°C	1.3678	23.8

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REFERENCES

Anderlina, V.C. (1988) Seasonal variations in trace metal concentrations and growth factors in bivalve molluscs from New Zealand and Oman as indicators of marine pollution. Ph.D. Thesis, Victoria University of Wellington, New Zealand.

Barkati, S. & Ahmed, M. (1990) Cyclical changes in weight and biochemical composition of *Mytilus edulis* L. from Lindåspollene, western Norway. *Sarsia* **75** : 217-222.

Bayne, B.L., Bayne, C.L., Carefoot, T.C. & Thompson, R.J. (1976a) The physiological ecology of *Mytilus californianus* Conrad : 1. Metabolism and energy balance. *Oecologia* **22** : 211-228.

Bayne, B.L., Bayne, C.L., Carefoot, T.C. & Thompson, R.J. (1976b) The physiological ecology of *Mytilus californianus* Conrad : 2. Adaptations to low oxygen tension and air exposure. *Oecologia* **22** : 229-250.

Bayne, B.L., Hawkins, A.J.S. & Navarro, E. (1987) Feeding and digestion by the mussel *Mytilus edulis* L. (Bivalvia : Mollusca) in mixtures of silt and algal cells at low concentrations. *J.Exp.Mar.Biol.Ecol.* **111** : 1-22.

Bayne, B.L., Hawkins, A.J.S. & Navarro, E. (1988) Feeding and digestion in suspension-feeding bivalve molluscs: the relevance of physiological compensations. *Amer.Zool.* **28** : 147-159.

Bayne, B.L., Hawkins, A.J.S., Navarro, E. & Inglesias, I.P. (1989) Effects of seston concentration on feeding, digestion and growth in the mussel *Mytilus edulis*. *Mar.Ecol.Prog.Ser.* **55** : 47-54.

Bayne, B.L., Klumpp, D.W., & Clarke, K.R. (1984) Aspects of feeding, including estimates of gut residence time, in three mytilid species (Bivalvia : Mollusca) at two contrasting sites in the Cape Peninsula, South Africa. *Oecologia* **64** : 9-15.

Bayne, B.L., Thompson, R.J. & Widdows, J. (1976c) Physiology I. Feeding, digestion and respiration. In *Marine mussels: their ecology and physiology* (ed. B.L. Bayne). Cambridge University Press, Cambridge, pp 121-206.

Bayne, B.L., Widdows, J. & Thompson, R.J. (1976d) Physiology II. Circulation and excretion. In *Marine mussels: their ecology and physiology* (ed. B.L. Bayne). Cambridge University Press, Cambridge, pp 207-260.

Bayne, B.L., Widdows, J. & Thompson, R.J. (1976e) Physiological integrations. In *Marine mussels: their ecology and physiology* (ed. B.L. Bayne). Cambridge University Press, Cambridge, pp 261-291.

Berry, P.F. (1978) Reproduction, growth and production in the mussel, *Perna perna* (Linnaeus), on the east coast of South Africa. *South African Association for Marine Biological Research, Investigational Report No. 48.* 28 pp.

Bridges, C.R. (1988) Respiratory adaptations in intertidal fish. *Amer.Zool.* **28** : 79-96.

Charles, F., Grémare, A., Amouroux, J.M. & Cahet, G. (1992a) Filtration of the enteric bacteria *Escherichia coli* by two filter feeding bivalves, *Venus verocosa* and *Mytilus galloprovincialis*. I. Experimental study. *Mar.Biol.* **113** : 117-124.

Charles, F., Amouroux, J.M., Grémare, A. & Cahet, G. (1992b) Filtration of the enteric bacteria *Escherichia coli* by two filter feeding bivalves, *Venus verocosa* and *Mytilus galloprovincialis*. II. Modelling. *Mar.Biol.* **113** : 125-131.

Chatterji, A., Ansari, Z.A., Ingole, B.S., & Parulekar, A.H. (1984) Growth of the green mussel, *Perna viridis* L., in a sea water circulating system. *Aquaculture* **40** : 47-55.

Clarke, B.C. & Griffiths, C.L. (1990) Ecological energetics of mussels *Choromytilius meridionalis* under simulated intertidal rock pool conditions. *J.Exp.Mar.Biol.Ecol.* **137** : 63-77.

Cole, B.E., Thompson, J.K. & Cloern, J.E. (1992) Measurement of filtration rates by infaunal bivalves in a recirculation flume. *Mar.Biol.* **113** : 219-225.

Conover, R.J. (1966) Assimilation of organic matter by zooplankton. *Limnol.Oceanogr.* **11** : 338-354.

Crosby, M.P. & Gale, L.D. (1990) a review and evaluation of bivalve condition index methodologies with a suggested standard method. *J.Shellfish.Res.* **9** : 233-237.

de Vooys, C.G.N. (1991) Anaerobic metabolism in sublittoral living *Mytilus galloprovincialis* in the Mediterranean - IV. Role of amino acids in adaptation to low salinities during anaerobiosis and aerobiosis. *Comp.Biochem.Physiol.* **100** : 423-431.

Dix, T.G. & Ferguson, A. (1984) Cycles of reproduction and condition in Tasmanian blue mussels, *Mytilus edulis planulatus*. *Aust.J.Mar.Freshw.Res.* **35** : 307-313.

Fegley, S.R., MacDonald, B.A. & Jacobsen, T.R. (1992) Short-term variation in the benthic quantity and quality of seston available to benthic suspension feeders. *Esturine, Coastal and Shelf Science.* **34** : 393-412.

Famme, P., Knudsen, J. & Hansen, E.S. (1981) The effect of oxygen on the aerobic-anaerobic metabolism of the marine bivalve, *Mytilus edulis* L. *Mar.Biol.Lett.* **2** : 345-351.

Gibbs, M.M., Pickmere, S.E., Woods, P.H., Payne, G.W., James, M.R., Hickman, R.W. & Illingworth, J. (1992) Nutrient and chlorophyll *a* variability at 6 stations associated with mussel farming in Pelorus Sound, 1984-85. *N.Z.J.Mar.Freshw.Res.* **26** : 197-211.

Greenway, J.P.C. (1975) Development of a colony of green mussels, *Perna canaliculus* in Coromandel Harbour, 1971-72. Ministry of Agriculture and Fisheries Fisheries Technical Report No. 141. Wellington, New Zealand. 22 pp.

Griffiths, R.J. (1981a) Population dynamics and growth of the bivalve *Choromytilus meridionalis* (Kr.) at different tidal levels. *Esturine coastal shelf sci.* **12** : 101-118.

Griffiths, R.J. (1981b) Aerial exposure and energy balance in littoral and sublittoral *Choromytilus meridionalis*. *J.Exp.Mar.Biol.Ecol.* **52** : 231-241.

Griffiths, R.J. & Buffenstein, R. (1981) Aerial exposure and energy input in the bivalve *Choromytilus meridionalis*. *J.Exp.Mar.Biol.Ecol.* **52** : 219-229.

Harris, V.A. (1990) Sessile animals of the sea shore. Chapman and Hall publishers. London. 379 pp.

Hawkins, A.J.S. & Bayne, B.L. (1991) Nutrition of marine mussels: Factors influencing the relative utilisations of protein and energy. *Aquaculture* **94** : 117-196.

Hawkins, A.J.S., Salkeld, P.N., Bayne, B.L., Gnaiger, E. & Lowe, D.M. (1985) Feeding and resource allocation in the mussel *Mytilus edulis*: evidence for time-averaged optimisation. *Mar.Ecol.Prog.Ser.* **20** : 273-287.

Hayden, B.J. (1988) Evaluation of species with potential for aquaculture on New Zealand. 48 p. (Draft report held in Fisheries Research Centre library, Wellington).

Hickman, R.W. (1979) Allometry and growth of the green-lipped mussel *Perna canaliculus* in New Zealand. *Mar.Biol.* **51** : 311-327.

Hickman, R.W. & Illingworth, J. (1980) Condition cycles of the green-lipped mussel *Perna canaliculus* in New Zealand. *Mar.Biol.* **60** : 27-38.

Hickman, R.W., Waite, R.P., Illingworth, J., Meredyth-Young, J.L. & Payne, G. (1991) The relationship between farmed mussels, *Perna canaliculus*, and available food in Pelorus-Kenepuru Sound, New Zealand. *Aquaculture.* **99** : 49-68.

Horn, P.L. (1985) Respiration in air and water of the chiton *Chiton pelliserpentis* from high and low zones of a sheltered shore. *N.Z.J.Mar.Freshw.Res.* **19** : 11-19.

James, M.R., Gibbs, M.M & Woods, P. (1987) Excretory products of the green-lipped mussel *Perna canaliculus. Unpublished DSIR Taupo Research Laboratory file report 93.*

Jones, H.D., Richards, O.G. & Southern, T.A. (1992) Gill dimensions, water pumping rate and body size in the mussel *Mytilus edulis* L. *J.Exp.Mar.Biol.Ecol.* **155** : 213-237.

Jørgensen, C.B. (1975) Comparative physiology of suspension feeding. *Ann.Rev.Physiol.* **37** : 57-79.

Kennedy, V.S. (1976) Dessication, higher temperatures and upper intertidal limits of three species of sea mussels (Mollusca : Bivalvia) in New Zealand. *Mar.Biol.* **35** : 127-137.

Kennedy, V.S. (1984) Crawling and clustering behaviour of three species of intertidal mussels in New Zealand. *N.Z.J.Mar.Freshw.Res.* **18** : 417-423.

Kiørboe, T., Møhlenberg, F. & Nøhr, O. (1980) Feeding, particle selection and carbon absorption in *Mytilus edulis* in different mixtures of algae and resuspended bottom material. *Ophelia.* **19**(2) : 193-205.

Kreeger, D.A., Newell, R.I.E., & Langdon, C.J. (1990) Effect of tidal exposure on utilisation of dietary lignocellulose by the ribbed mussel *Geukensia demissa* (Dillwyn) (Mollusca : Bivalvia). *J.Exp.Mar.Biol.Ecol.* **144** : 85-100.

Lucas, A. & Beninger, P.G. (1985) The use of physiological condition indices in marine bivalve aquaculture. *Aquaculture* **44** : 187-200.

Marsden, I.D. & Shumway, S.E. (1992) Effects of the toxic dinoflagellate *Alexandrium tamarense* on the greenshell mussel *Perna canaliculus*. *N.Z.J.Mar.Freshw.Res.* **26** : 371-378.

Massabuau, J.C., Burtin, B. & Wheathley, M. (1991) How is O_2 consumption maintained independent of ambient oxygen in mussel *Anodonta cygnea*? *Respir.Physiol.* **83** : 103-114.

McMahon, B.R. (1988) Physiological responses to oxygen depletion in intertidal animals. *Amer.Zool.* **28** : 39-53.

McMahon, R.F. (1988a) Respiratory response to periodic emergence in intertidal molluscs. *Amer.Zool.* **28** : 97-114.

McMahon, R.F. & Russell-Hunter, W.D. (1977) Temperature relations of aerial and aquatic respiration in six littoral snails in relation to their vertical zonation. *Biol.Bull.* **152** : 182-198.

Meyhöfer, E. (1985) Comparative pumping rates in suspension-feeding bivalves. *Mar.Biol.* **85** : 137-142.

Møhlenberg, F. & Riisgård, H.U. (1978) Efficiency of particle retention in 13 species of suspension feeding bivalves. *Ophelia* **17**(2) : 239-246.

Murdoch, R.C. & Shumway, S.E. (1980) Oxygen consumption in six species of chitons in relation to their position on the shore. *Ophelia* **19**(2) : 127-144.

Navarro, E., Iglesias, J.I.P., Perez Camacho, A., Labarta, U. & Beiras, R. (1991) The physiological energetics of mussels (*Mytilus galloprovincialis* Lmk) from different cultivation rafts in the Ria de Arosa (Galicia, N.W. Spain). *Aquaculture* **94** : 197-212.

Navarro, J.M. (1988) The effects of salinity on the physiological ecology of *Choromytilus chorus* (Molina, 1792) (Bivalvia : Mytilidae) *J.Exp.Mar.Biol.Ecol.* **122** : 19-33.

Navarro, J.M. & Winter, J.E. (1982) Ingestion rate, assimilation efficiency and energy balance in *Mytilus chilensis* in relation to body size and different algal concentrations. *Mar.Biol.* **67** : 255-266.

Newell, R.C. (1979) Biology of intertidal animals. (3rd edition) : Marine Ecological Surveys Ltd. 781 pp.

Page, H.M. & Hubbard, D.M. (1987) Temporal and spatial patterns of growth in mussels *Mytilus edulis* on an offshore platform: relationships to water temperature and food availability. *J.Exp.Mar.Biol.Ecol.* **111** : 159-179.

Page, H.M. & Ricard, Y.O.(1990) Food availability as a limiting factor to mussel *Mytilus edulis* growth in California coastal waters. *U.S.Natl.Mar.Fish.Serv.Fish.Bull.* **88** : 677-686.

Parulekar, A.H., Dalal, S.G., Ansari, Z.A. & Harkantra, S.N. (1982) Environmental physiology of raft-grown mussels in Goa, India. *Aquaculture*. **29** : 83-93.

Powell, A.W.B. (1979) New Zealand Mollusca. pp 371-375 : Collins, Auckland.

Raubanheimer, D. & Cook, P. (1990) Effects of exposure to wave actions on allocation of resources to shell and meat growth by the subtidal mussel, *Mytilus galloprovincialis*. *J.Shellfish.Res.* **9** : 87-93.

Riisgård, H.U. & Randløv, A. (1981) Energy budgets, growth and filtration rates in *Mytilus edulis* at different algal concentrations. *Mar.Biol.* **61** : 227-234.

Rodhouse, P.G., Roden, C.M., Hensey, M.P. & Ryan, T.H. (1984) Resource allocation in *Mytilus edulis* on the shore and in suspended culture. *Mar.Biol.* **84** : 27-34.

Romberger, H.P. & Epifanio, C.E. (1981) Comparative effects of diets consisting of one or two algal species upon assimilation efficiencies and growth of juvenile oysters *Crassostrea virginica* (Gmelin). *Aquaculture* **25** : 77-87.

Seed, R. (1976) Ecology. In *Marine mussels: their ecology and physiology* (ed. B.L. Bayne). Cambridge University Press, Cambridge. pp 13-65.

Schiel, D.R. & Hayden, B.J. (1987) Aquaculture in New Zealand: history, potential, and the role of research. *Report of the Ministry of Agriculture and Fisheries, Fisheries Research Centre, P.O. Box 297, Wellington.*

Shick, J.M., Widdows, J. & Gnaiger, E. (1988) Calorimetric studies of bahaviour, metabolism and energetics of sessile intertidal animals. *Amer.Zool.* **28** : 161-181.

Smaal, A.C. & van Stralen, M.R. (1990) Average annual growth and condition of mussels as a function of food source. *Hydrobiologia* **195** : 179-188.

Solórzano, L. (1969) Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnol.Oceanogr.* **14** : 799-801.

Stuart, V. (1982) Absorbed ration, respiratory costs and resultant scope for growth in the mussel *Aulacomya ater* (Molina) fed on a diet of kelp detritus of different ages. *Mar.Biol.Lett.* **3** : 289-306.

Suchanek, T.H. (1978) The ecology of *Mytilus edulis* L. in exposed rocky intertidal communities. *J.Exp.Mar.Biol.Ecol.* **31** : 105-120.

Vial, M.V., Simpfendörfer, R.W., López, D.A., González, M.L. & Oelckers, K. (1992) Metabolic responses of the intertidal mussel *Perumytilus purpuratus* (Lamarck) in emersion and immersion. *J.Exp.Mar.Biol.Ecol.* **159** : 181-201.

Vismann, B. (1990) Field measurements of filtration and respiration rates in *Mytilus edulis* L. an assessment of methods. *Sarsia.* **75** : 213-216.

Waite, R.P. (1989) The nutritional biology of *Perna canaliculus* with special reference to intensive mariculture systems. Ph.D. Thesis, University of Canterbury, New Zealand.

Widdows, J. (1973) The effects of temperature on the metabolism and activity of *Mytilus edulis. Neth.J.Sea.Res.* **7** : 387-398.

Widdows, J., Donkin, P., Salkeld, P.N., Cleary, J.J., Lowe, D.M., Evans, S.V. & Thomson, P.E. (1984) Relative importance of environmental factors in determining physiological differences between two populations of mussels (*Mytilus edulis*). *Mar.Ecol.Prog.Ser.* **17** : 33-47.

Widdows, J., Fieth, P. & Worrall, C.M. (1979) Relationships between seston, available food and feeding activity in the common mussel *Mytilus edulis. Mar.Biol.* **50** : 195-207.

Widdows, J. & Shick, J.M. (1985) Physiological responses of *Mytilus edulis* and *Cardium edule* to aerial exposure. *Mar.Biol.* **85** : 217-232.

Winter, J.E. (1978) A review on the knowledge of suspension-feeding in lamellibrachiate bivalves, with special reference to artificial aquaculture systems. *Aquaculture* **13** : 1-33.