ENERGETICS OF ACTIVITY IN THE ABALONE, *HALIOTIS KAMTSCHATKANA*

by

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

(Department of Zoology)

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

January 1998

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Date 25 Jan 98

Abstract

The purpose of this thesis was to investigate several aspects of the energetic costs of activity in the Northern abalone, *Haliotis kamtschatkana*.

The percentage of daily consumed energy partitioned to activity was calculated by first measuring summer and winter energy budgets in which abalone were inactive during measurements of respiration and mucus secretion, then by measuring respiration and mucus-secretion for active animals by integrating summer and winter activity budgets with energy equivalents for each activity. Energy expenditure due to metabolic changes during exposure to environmental stressors, measured as increases in blood-glucose levels and oxygen consumption, was also related to the summer energy budget. Abalone locomotion was investigated in several ways. Cost of transport was determined for a range of speeds and sizes by inducing abalone to locomote in respirometers and measuring oxygen consumption at known speeds. Relative contributions of aerobic and anaerobic metabolism were measured by analyzing muscle tissues for anaerobic metabolites after locomotion had occurred and comparing amounts of accumulated metabolites to amount of oxygen consumed during locomotion. Pedal morphology during locomotion was also investigated to determine if possible energy-saving changes occur. This was accomplished by videotaping the pedal soles of locomoting abalone and measuring various foot morphometries.

Activity, in the form of increased respiration and mucus secretion, accounted for a substantial portion of daily consumed energy during both seasons. Exposure to predatory seastars significantly increased blood-glucose titers and oxygen consumption, resulting in a metabolic increase equivalent to 0.3% of daily summer energy consumption. Cost of transport for abalone was less than that of other gastropods, possibly due to its larger relative size. Mucus secretion during locomotion did not vary with speed, but was less than the amount needed for substratum adherence. During locomotion, tauropine and D-lactate levels increased significantly in foot muscle while arginine decreased. Morphological analyses of pedal waves showed that the pedal sole area decreased with increasing speed, and the area of the foot

incorporated into pedal waves increased. Together, these changes translated into a decrease in pedal sole area in contact with the substratum.

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Acknowledgments

Many people at UBC provided support and encouragement. My advisor, Tom Carefoot, provided generous funding and ideas. Barbara Taylor, Mike Harris, Richard Kincaid, Steve Land, and J. P. Danko were always available for advice and mechanical expertise. Josie Osborne, Christine Elliot, Anne-Sophie Miel, and Susan Far helped with different aspects of the lab work. John Gosline and members of his lab provided many helpful comments on the manuscript that became Chapters 2 and 6 of this thesis. This project was funded by a Kit Malkin Memorial Scholarship and a University Graduate Fellowship to D.A. Donovan and a Natural Science and Engineering Research Council (NSERC) grant to T.H. Carefoot.

Much of this work was performed at the Shannon Point Marine Center and I am extremely grateful for the support I received there. In particular, Steve Sulkin, Suzanne Strom, Brian Bingham, Gisele Muller-Parker, Dave Schneider, Gene McKeen, Mimi Brainard, and Sherri Rogers provided encouragement and technical support. Other parts of the study were performed at the Bamfield Marine Station where Andy Spencer and his staff provided logistical support during animal collections.

John Baldwin was very generous with his time and expertise and I appreciate his help with enzyme purification and anaerobic metabolite analysis. Much of that work was performed in Peter Hochachka's laboratory. Some of it was done in the Chemistry Department at Western Washington University and I'm grateful to Gerri Prody for arranging laboratory space for me.

Finally, I'm very grateful for the love and support of my family, particularly that of Todd and Fiona Mary.

General Introduction

Increasing world demand for abalone has caused severe declines in most populations, including those of British Columbia's *Haliotis kamtschatkana* (Emmett and Jamieson, 1989). Efforts have been made to manage this resource including closure of the fishery (1990), farming, and reintroducing abalone into depopulated areas. Studies have shown that transplanting *H. kamtschatkana* from wave-exposed habitats to more wave-sheltered habitats leads to increased growth and ultimately to greater population density (Breen, 1986; Emmett and Jamieson, 1989), suggesting that this may be a feasible means to enhance depleted British Columbia stocks. Successful reintroduction and stock enhancement will depend on identification of suitable habitat, which will in turn depend on complete knowledge of the biology of the abalone. Emmett and Jamieson (1989) point out that *H. kamtschatkana* do not grow to marketable size in high wave-exposure areas, but also note that the cause of the decreased growth is not known. Suggestions of inadequate food supplies or high rates of mortality illustrate the need for information about the energy balance of this species.

While there is a substantial body of literature on abalone, the majority of the studies have focused on reproduction (Ino, 1952; Webber and Giese, 1969; Poore, 1973; Shepherd and Laws, 1974), growth (Leighton and Boolootian, 1963; Poore, 1972; Shepherd and Hearn, 1983; Keesing and Wells, 1989;), diet (Leighton and Boolootian, 1963; Barkai and Griffiths, 1986), population biology (Tegner and Butler, 1985; Tegner *et al.*, 1989), and culturing (Uki, 1981; Morse and Morse, 1984; Morse, 1984; Ebert and Houk, 1984). These research topics have been pursued mainly due to their applicability to abalone mariculture. Less work has been done on *H. kamtschatkana* but, again, in such studies reproduction (Breen and Adkins, 1980), growth (Quayle, 1971; Paul *et al.*, 1977; Paul and Paul, 1981), and diet (Paul *et al.*, 1977) have been emphasized. There has also been substantial interest in aspects of population size and distribution as they pertain to abalone fisheries and the worldwide decline in abalone stocks (see Sloan and Breen, 1988).

One area that is not well understood for abalone and, indeed, for gastropods in general, is energetic cost of activity. Activity is potentially costly to gastropods since, along with the requisite rise in respiratory energy expenditure, mucus must also be secreted. Therefore, although gastropods are notably sedentary and slow-moving, any increase in activity has the potential to influence greatly an animal's energy balance. In fact, there are times when abalone move quite quickly, such as when they are being threatened by seastar predators or when they are actively congregating for reproductive purposes. They may also actively forage for food when drift algae is not abundant.

Abalone exhibit a variety of movements including shell-twisting, righting themselves when overturned, trapping algae with raised foot, climbing kelp, elevating their shell during spawning, and locomoting. These movements are accomplished by contraction of a complicated array of muscle fibers in the massive adductor and foot muscles in concert with a hydrostatic system of hemolymph in the muscles (Trueman and Brown, 1985; Voltzow, 1986). Abalone do not have a single large hemocoelic cavity to act as a hydrostatic skeleton and to provide muscular antagonism. Rather, they rely on a three-dimensional array of muscles which Trueman and Brown (1985) have termed a "muscular antagonistic system." As such, the contraction of different sets of muscle fibers running in different directions allows for a wide range of movements. Specifically, the adductor muscle is responsible for changes in posture such as elevating and retracting the shell, twisting, and clamping onto the substratum, while the foot is mostly used during locomotion (Voltzow, 1986).

Most studies on activity in abalone have focused on locomotion. Abalone must locomote in order to forage, escape predators, find adequate refugia, and reproduce, and they do this by generating waves on their broad foot. Several studies have documented the movements of individual abalone and have shown that abalone vary widely in their motility. For example, in Australia the highly motile *Haliotis midae* migrates distances of up to 1 km annually, while members of a sympatric species, *H. cyclobates*, may spend their entire lives on a single rock (Shepherd, 1973). Observations of *H. discus hannai* and *H. sieboldi* over a 24-h period by Momma and Sato (1969) showed an average locomotory range of 2.5 m per day. In

a study of tagged Northern abalone, *Haliotis kamtschatkana*, Quayle (1971) found that average movement was less than 50 m annually, with most occurring laterally along the shore. The longest distance recorded for a tagged *H. kamtschatkana* was 125 m in a year (Emmett and Jamieson, 1989). However, as tagged abalone are often difficult to recover due to the complexity of their environment and cryptic behavior, these types of studies probably underestimate amount of movement.

Along with the different forms of activity described above, abalone have variable states of "alertness." These are characterized by the extent to which the shell is elevated above the substratum and the tentacles are protruded from the mantle cavity. Shell elevation is obtained through contraction of radial and circumferential muscle fibers (Trueman and Brown, 1985), and thus requires energy to maintain. As well, abalone in an alert state often wave their tentacles, presumably to test the water for food or predators, which also requires energy.

In terms of energy balance, activity is important for the single reason that it requires energy. An organism which is expending energy while escaping predators or foraging for food has less energy with which to grow and reproduce. The overall purpose of this thesis, then, is to quantify the cost of activity in the energy budget of the Northern abalone, *Haliotis kamtschatkana.*

In Chapter 1 I measure baseline energy budgets during winter and summer months. Energy budgets have been developed for two other species of abalone, but neither has incorporated energy expended due to activity which would affect both respiration and mucus secretion components of the budget. The seasonal energy budgets measured in this part of the study will serve two purposes: 1) they will allow comparisons to be made with existing energy budgets for abalone, and 2) they will serve as frameworks to which energy budgets with activity included can be compared.

In Chapters 2 and 3 I determine the amount of energy expended by *H. kamtschatkana* due to activity and relate this to the baseline seasonal energy budgets. Chapter 2 focuses on locomotion and quantifies both respiratory energy expenditure and mucus secretion during different rates of locomotion. Respiratory energy expenditure is measured as cost of transport

(the amount of energy expended per unit mass per unit distance) and is then compared with existing cost of transport measurements for five other gastropod species as well as to cost of transport for organisms with other modes of locomotion. Likewise, I measure the amount of energy lost as mucus secretion during locomotion and compare it to mucus secretion during adherence to the substratum. In Chapter 3 I develop a time-energy budget for *H. kamtschatkana*. I first develop a time budget from 24-h observations of laboratory-held animals, then measure respiratory energy expenditure during different activity states. After incorporating the measurements for locomotion described in Chapter 2, I determine the daily energy expended due to activity by combining the energy used during the different activities with the amount of time spent in each activity. These measurements allow me to assess the impact of activity on the baseline seasonal energy budgets from Chapter 1.

Chapter 4 investigates the accumulation of anaerobic metabolites in both adductor and foot muscles during locomotion. These data are necessary to interpret the measurements of energy expended during locomotion, since anaerobic as well as aerobic energy sources are included. They also allow me to determine the extent to which abalone rely on anaerobic energy sources during locomotion.

Chapter 5 investigates the effects of stress on the metabolism of *H. kamtschatkana*. I measure changes in blood-glucose levels and oxygen consumption during exposure to the seastar, *Pycnopodia helianthoides*, a predator which elicits a strong escape response in *H. kamtschatkana* culminating in rapid locomotion away from the seastar. Stress can markedly increase the metabolic rate of organisms, causing potentially large changes in energy balance.

Finally, in Chapter 6 I examine changes in pedal morphology during locomotion from the standpoint of possible energy-saving strategies. Specifically, I measure changes in the area of pedal waves as well as total pedal area in contact with the substratum in relation to locomotory speed. Observations of locomoting abalone indicate that abalone decrease the amount foot in contact with the substratum which possibly decreases the amount of mucus secreted and the amount of friction generated.

Overall, my thesis research will improve our understanding of the impact that activity has on energy allocation in *Haliotis kamtschatkana* and thus provide us with insight into the habitat requirements of this species. It will also further our knowledge of the energetics of adhesive crawling.

Chapter 1. Summer and winter energy budgets for resting abalone

1.1 Introduction

Essential to understanding the biology of a species is knowledge about the energy usage of individuals, as well as populations, of that species. An understanding of the balance between energy intake and outflow allows for insight into growth patterns, reproductive strategies, population dynamics, and mortality. Insights into these life-history parameters can be gained by constructing an energy budget; that is, a catalog of energy consumption and expenditure within a single individual or a population of individuals. The standard energy budget (Petrusewicz, 1967), modified for gastropods, is defined by the equation:

$$\mathbf{C = F + U + P_g + P_r + R + M}$$

where C is the energy consumed, F is the energy lost in undigested material in the feces, U is the energy of nitrogen compounds excreted, P_g is the energy used for the production of somatic tissue, P_r is the energy used for the formation of reproductive tissue, R is the energy used for metabolism, and M is the energy of mucus production. It is obvious, over the long term, that this equation is balanced; that is, the amount of energy consumed by an organism equals the amount of energy expended.

The value of an energy budget is in its exposure of trade-offs between the different budget parameters and in its elucidation of behavioral strategies that enable an organism to optimize energy intake and output (Townsend and Calow, 1981). If an animal is partitioning energy into one parameter, then that energy is unavailable to the other parameters. For example, animals may seasonally decrease somatic growth (P_g) during gametogenesis in order to expend more energy in the production of gametes (P_r), as is the case with some species of abalone (Keesing and Wells, 1989). This example illustrates one of the more simplistic trade-offs that can occur, but the complexity increases greatly when behavioral factors and population parameters are considered. For example, in an attempt to explain reproductive strategies and population fluctuations of mammals, McNab (1980) correlated basal metabolism with the

amount of energy available as different types of foods in the diets of the different species of mammals. Then, since the intrinsic rate of population increase (r) is directly related to standard metabolism, McNab was able to implicate food type as a constraint to reproductive tactics and population fluctuations. Clearly, the degree of complexity increases as several dimensions of the energy budget are considered simultaneously. Adding variables such as season and age of the organism can further complicate the issue.

Energy budgets have been developed for only a few species of marine gastropods [see Bayne and Newell (1983) and Carefoot (1987)], and abalone are no exception. Only two have been reported for *Haliotis*: Barkai and Griffiths (1988) generated a field budget for *H. midae* and Peck *et al.* (1987) developed a laboratory budget for *H. tuberculata*. Both studies provide a sound base of knowledge about the energy allocations in abalone, but both are deficient in one way or another. Specifically, both cite the importance of activity to the respiratory component of the energy budget, yet neither incorporates activity into measurements of respiration. Activity has been found to cause an increase in gastropod respiratory rate by 100-600% of resting values (Houlihan and Innes, 1982), and thus can potentially cause a severe under-estimation of respiratory energy expenditure if not accounted for. Several studies of gastropod energetics have measured the increase of respiratory rate during activity over resting rates (Newell and Roy, 1973; Calow 1974; Fitch, 1975; Crisp, 1979; Houlihan and Innes, 1982), but few have directly related this increase to the overall energetics of the animal or estimated the cost of activity to the animal.

The purpose of this part of the study was to develop a baseline energy budget for *Haliotis kamtschatkana* (one in which respiratory and mucus components were measured on inactive animals), to which measurements of energy utilization during active states could be compared. Energy budget parameters were measured during both summer and winter in order to gain insight into how activity may affect energy allocation during different seasons.

1.2 Materials and Methods

Collection of animals

Abalone (*N*=20; 13-175 g live mass) were collected in the area of the Bamfield Marine Station, British Columbia and transported to the Shannon Point Marine Center, Washington. This size range is slightly smaller than the natural size range, although animals larger than 200 g live mass are rare (Sloan and Breen, 1988). They were held in a large tank with a constant supply of fresh seawater and fed *ad libitum* on *Nereocystis luetkeana*, a preferred kelp food. Since I was initially interested in potential gender differences in the energy budget parameters, 10 females and 10 males were used for each component of the energy budget (unless otherwise stated). None of these abalone was used in other experiments.

Energy Budgets

Energy budgets were calculated for *H. kamtschatkana* by measuring all components at various times over a 16-month period. The components not directly affected by activity, C, F, U, P_g, and P_r, were measured once during summer (June-Aug 1995) and once during winter (Nov 1995-Jan 1996), except for P_g which was measured monthly (March 1995-July 1996) and P_r which was measured once at the end of the experiment. The two components directly affected by activity, R and M, were also measured once during the summer (June-Aug 1995) and once during winter (Nov 1995-Jan 1996), but here they were estimated for inactive animals by measuring each component using quiescent abalone and extrapolating these values over a 24-h period. These measurements of quiescent R and M will be used as comparisons for R and M values derived from active abalone in Chapters 2 and 3.

Temperature ranged from a monthly mean low of 7°C in Feb 1996 to a mean high of 12°C in July 1996. In both years, mean temperature was below 9°C from Nov-Apr. Laboratory temperature was generally 1-2 °C higher than field temperature.

Consumption (C). Abalone were kept in plastic mesh cages and fed pieces of kelp of known mass each day at 1500h over a 4-day period. Uneaten kelp was removed each following day at 1500h and weighed. Each day, three pieces of kelp were placed into empty cages as controls and change of mass recorded. The mass of the uneaten kelp from each abalone's cage was subtracted from the initial mass of the piece and the result corrected for any difference in mass exhibited by the mean of the controls to determine the wet mass of kelp consumed.

To determine the energy content of food eaten, samples of kelp were weighed fresh, then dried at 60°C to constant mass. Samples of dried kelp were combusted in a Phillipson microbomb calorimeter to determine their energy content. Average daily energy consumption ($J \cdot day^{-1}$) for each abalone was calculated by multiplying the daily wet mass of consumed kelp by the energy content per gram wet mass of the kelp.

Feces Production (F). Abalone (N=5; 20-128 g live mass) were held individually in 1-liter aerated plastic containers filled with 5 µm-filtered seawater at ambient temperature over a 4-day period. Kelp of known mass was fed to each animal on the first day, and uneaten remnants removed and weighed on the following day. The abalone were held in the containers for three more days during which feces were collected daily. Animals were not starved prior to the experiment. The feces were dried at 90°C to constant mass then combusted in a microbomb calorimeter. The mean energy value for the feces was used to calculate F in the energy budget.

Nitrogen excretion (U). Individual abalone were placed in sealed containers and maintained at ambient temperature. Duplicate 1-ml aliquots of the water in the containers were collected after 1-h and analyzed for ammonia following the method of Solorzano (1969). Since nitrogen excretion by *H. kamtschatkana* does not fluctuate daily (Taylor and Carefoot, *in prep*), nitrogen excretion was measured at 0900 h and the values extrapolated to a 24-h period. Energy costs ($J \cdot day^{-1}$) were calculated from µg ammonia excretion by multiplying by 24.83 $J \cdot mg\ NH_3^{-1}$ (Elliot and Davison, 1975).

Somatic growth (P_g) and reproductive growth (P_r). Mass and length of each abalone were recorded monthly for the duration of the experiment (16 months). At the end of the experiment, when the animals were ready to spawn (August 1996), they were weighed a final time and removed from their shells. Each abalone was separated into five components: 1) shell, 2) pedal and adductor muscles with head, and tentacles, 3) visceral mass including stomach, digestive gland, and gills, 4) gonad, and 5) hemolymph, mucus, and mantle water that drained off the abalone during dissection. During dissection the visceral mass was separated from the large pedal muscle and the gonad removed from around the digestive gland by aspirating it into a clean glass vial. Wet mass of shell, muscle, viscera, and gonad were recorded for each animal. The soft tissues from each animal were homogenized individually, dried, and energy content determined by combustion in a microbomb calorimeter. Shell caloric content was estimated from Paine (1971), assuming that abalone shell was 1.1% protein and protein has an energy value of 23.83 $J \cdot mg^{-1}$. Since it proved impossible to collect enough mucus and hemolymph from each animal for analysis, and lacking an estimate for the energy value of hemolymph, this mucus and hemolymph portion was assumed to be similar in energy value to pedal mucus (23.97 $J \cdot mg^{-1}$; Calow, 1974).

Somatic growth was determined by regressing monthly mass of each abalone on time, with the slope of the regression being then a measure of growth ($g \cdot month^{-1}$). This method was used, as opposed to subtracting final mass from initial mass and dividing by time, since there was considerable monthly fluctuation in wet mass. Mass change during winter (Nov-Mar) was compared to mass change during summer (Apr-Oct). Daily gain in live mass was converted to energy gain ($J \cdot day^{-1}$) by partitioning total gain in live mass into gain in mass of individual body parts (shell, muscle, and viscera) estimated from the proportion of whole mass that each of these tissues constituted. Each component was then multiplied by the energy content of the respective tissue.

An attempt was made to spawn the animals during summer (August, 1995) using hydrogen peroxide (Morse *et al.*, 1977). However, since only one animal spawned, energy devoted to reproduction (P_r) was estimated from gonad mass (including gametes) at the end of

the experiment. Reproductive growth ($J \cdot day^{-1}$) was determined for each abalone by multiplying gonad mass by its energy content.

Respiration (R). To determine whether *H. kamtschatkana* exhibit diurnal variations in quiescent respiration, abalone (N=5; 27-52 g live mass) were held individually in round, perspex respirometry chambers for 24 hours. Temperature was maintained at 10°C, and oxygen consumption was monitored continuously with a polarographic oxygen electrode connected to a computerized data acquisition system (Datacan, Sable Systems). The respirometer was opened for 2-min periods to replenish the seawater once oxygen tension fell below 60% of ambient. Oxygen consumption was determined for each hour of the 24-h period.

As a component of the energy budget, quiescent metabolic rates of abalone were measured individually over 1-h periods in respirometers as described above. Energy costs ($J \cdot day^{-1}$) were calculated from oxygen consumption ($\mu l\ O_2 \cdot day^{-1}$) by multiplying by an oxycalorific coefficient (Q_{ox}) of 20.88 $J \cdot ml\ O_2^{-1}$ (Elliot and Davison, 1975). This represents a weighted value for the catabolism of carbohydrate, protein, and fat based on the proportion of each found in *N. luetkeana*. Since quiescent oxygen consumption of *H. kamtschatkana* does not fluctuate daily (see Results), oxygen consumptions were measured between 0900-1200 and the values extrapolated to a 24-h period.

Mucus production (M). The amount of mucus needed for substratum adherence by resting animals was determined by allowing individual abalone to attach to a clean glass plate immersed in a tank supplied with fresh seawater. After an abalone had been stationary for 10 min following adherence, it was removed quickly from the plate. A 10-min period was chosen because Davies (1993) found that stationary limpets stop producing mucus within 10 min after attachment. The plate was then rinsed with distilled water to remove salt residues, and dried at 60°C for 30 min. The dried mucus was carefully scraped from the plate and its carbon content determined (NA-1500 Elemental Analyzer, Carlo Erba Strumentazione). Mass of carbon (μg) was converted to dry mass of mucus (μg) by assuming that gastropod pedal mucus is 24.5%

carbon (Peck *et al.* 1993). Mucus dry mass was converted to energy (J) assuming a conversion of 23.97 $kJ \cdot g^{-1}$ mucus (Calow, 1974). Mucus energy was converted to a daily rate ($J \cdot day^{-1}$) by assuming that quiescent abalone adhere once per day then remain fixed to the substratum. This assumption was made for the purpose of estimating mucus secretion by inactive animals. Actual numbers of adherences per day and resultant mucus secretion for active animals will be measured in Chapter 3.

1.3 Results

Energy Budgets

Weight-specific oxygen consumption did not change significantly during the day (Fig. 1-1; $F_{23,92}=1.23$, $p=0.24$, repeated measures ANOVA).

There were no differences in values for any of the energy budget components between male and female abalone (all $t<2.0$, all $p>0.06$), save for some aspects of reproduction. Gonad energy content (±SE) was higher in females than in males (females 23.4±0.9 $J \cdot mg$ dry gonad $mass^{-1}$, males 20.0±0.5; $t=3.21$, $p=0.01$; Table 1-1). However, there was no gender difference in gonad mass as a percentage of total live mass (±SE) (females 6±1 %, males 7±0.6; $t=0.38$, $p=0.71$) and ultimately no difference in yearly reproductive energy expenditure (±SE) (females 4.2±0.7 $kJ \cdot year^{-1}$, males 2.8±0.3; $t=1.89$, $p=0.09$). Thus, values for both males and females were combined for the regressions of energy budget components on mass.

The percentage of total live abalone mass of the various body tissues after dissection and their energy equivalents are presented in Table 1-1. Muscle and shell comprised the largest percentages of live mass (29 and 28%, respectively) followed by mucus/hemolymph (19%), viscera (12%), and gonad (6%).

Regression equations for each $\log_{10}$ component of the summer and winter energy budgets ($J \cdot day^{-1}$) on $\log_{10}$ mass (g) are presented in Table 1-2 and values for each component, calculated for a representative 50-g abalone, are presented in Table 1-3. None of the slopes of the summer regression equations were significantly different from the winter regressions (all

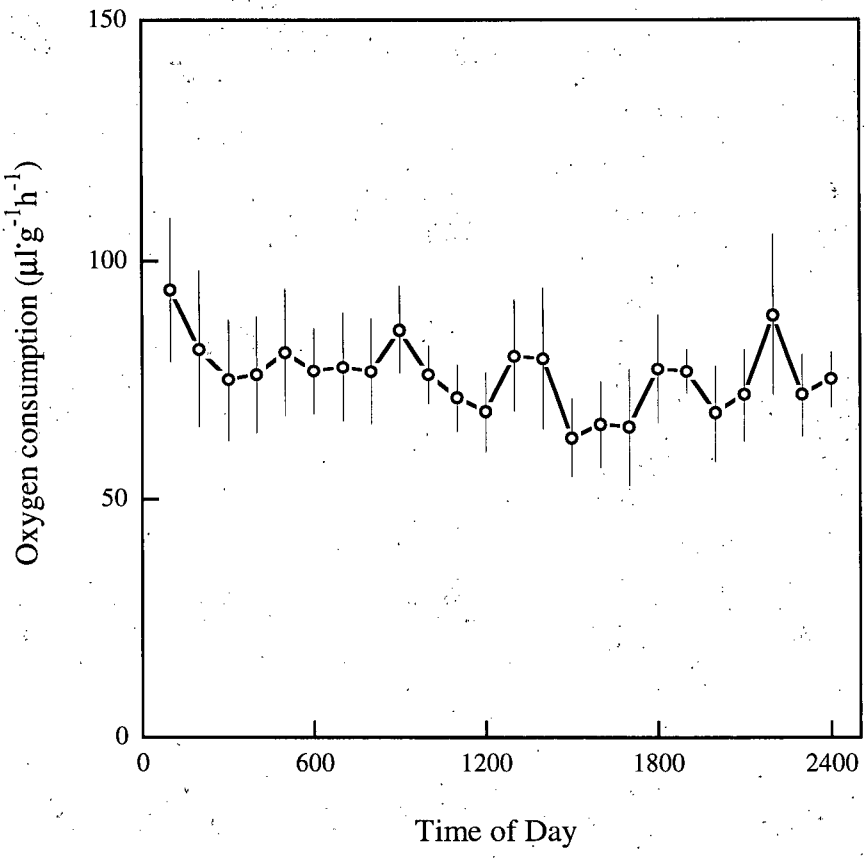

Fig. 1-1. Oxygen consumption of *Haliotis kamtschatkana* (*N*=5) over a 24-h period. Symbols represent mean mass-specific oxygen consumption ± SE.

Table 1-1. Percentage of total live mass of various abalone body tissues after dissection, along with their energy equivalents. Values are expressed as mean ± SE of *N*=20.

Tissue	Percentage of total live mass	Energy equivalent (J·mg dry tissue^{-1})
shell	28 ± 3	0.3*
viscera	12 ± 1	20.2 ± 0.5
muscle	29 ± 3	19.2 ± 0.3
mucus/hemolymph	19 ± 7	24.0**
gonad (females and males)	6 ± 0.5	21.7 ± 0.7
gonad (females)	6 ± 1	23.4 ± 0.9
gonad (males)	7 ± 0.6	20.0 ± 0.5

* Value from Paine (1971).
** Value from Calow (1974) for pedal mucus.

Table 1-2. Regression statistics for the components of summer and winter energy budgets for abalone, *Haliotis kamtschatkana* (n=20). Respiration and mucus components are for resting abalone. Regression statistics are for the equation $\log Y = \log a + b \log m$, where Y is an energy budget component in J·day^{-1} and m is mass in g. p values are derived from Student's t tests.

Energy budget component (Y)	log$a$	b	r^2	t	p
Summer					
consumption (C)	2.18	0.64	0.59	5.15	<0.001
feces (F)	1.45	0.64	0.59	5.15	<0.001
nitrogen (U)	-1.19	0.61	0.21	2.11	0.050
somatic growth (P_g)	1.08	0.56	0.33	2.72	0.016
reproductive growth (P_r)	0.45	0.79	0.60	4.20	0.001
respiration (R; resting)	1.73	0.74	0.77	7.70	<0.001
mucus (M; resting)	-0.17	0.86	0.46	3.67	0.002
Winter					
consumption (C)	2.00	0.55	0.46	3.68	0.002
feces (F)	1.11	0.55	0.46	3.68	0.002
nitrogen (U)	-2.03	0.91	0.27	2.56	0.020
somatic growth (P_g)	-2.39	1.33	0.57	3.99	0.002
respiration (R; resting)	1.44	0.78	0.73	6.97	<0.001
mucus (M; resting)	-0.65	0.85	0.27	2.60	0.018

Table 1-3. Values for each component of summer and winter energy budgets for a representative 50-g *Haliotis kamtschatkana* without activity being included in respiration (R) or mucus secretion (M). Values were calculated using the regression equations from Table 1-2 for resting animals.

	Energy ($J \cdot day^{-1}$)								
						without activity			
	C	F	U	P_g	P_r	R	M	Total	% total of C
Summer	1894	345	< 1	108	62	971	20	1506	
proportion of C (%)		18	< 1	6	3	**51**	**1**		80
Winter	860	111	< 1	1	0	582	6	700	
proportion of C (%)		13	< 1	< 1	0	**68**	**1**		81

t<0.66, all p>0.10), except for the slopes of somatic growth on mass (t=2.02, p<0.05). However, all y-intercepts of the summer regressions were significantly higher than those of the winter regressions (all t>3.44, all p<0.005). Thus, except for somatic growth, the scaled relationship between energy and size remained constant between summer and winter, but summer values were greater than winter values. Consumption and quiescent respiratory rates were 2.2 and 1.7 times greater, respectively, in summer as compared with winter. Respiration was the largest component of both budgets, even without activity. Mucus secretion for these inactive animals accounted for about 1% of total consumed energy during both summer and winter. Somatic growth was negligible during winter. Both energy budgets failed to balance by 19-20%. The missing energy is accounted for by activity of animals, as will be discussed in Chapters 2 and 3.

There was no relationship between shell growth (mm·month^{-1}) and abalone mass (g) (summer and winter regressions of $\log_{10}$ shell growth on $\log_{10}$ mass both t<1.5, both p>0.15). There was, however, a general trend in which abalone showed accelerated growth from April to November, followed by little to no growth until the following March when growth rate increased again. This did not occur with all of the experimental animals: two small abalone exhibited fairly constant increases in shell length throughout the year, and four larger animals did not grow appreciably at any time during the experiment. Mean (±SE) increase in shell length during the summer growth phase was 0.58±0.45 and 0.17±0.26 mm·month^{-1} during the winter.

1.4 Discussion

As expected, values of summer and winter energy budget components differed for *Haliotis kamtschatkana*. For a representative 50-g abalone, winter consumption was only 45% of summer consumption, with nearly all consumed energy going towards maintenance (energy needed by the animal to maintain body tissues without growth; that is, R, M, and U). The proportions of energy lost as feces and nitrogenous waste remained relatively constant between

winter and summer. Winter rates of mucus secretion were 30% of summer rates, but because winter consumption was less than summer consumption the proportion of consumed energy represented by mucus remained constant. Davies (1993) also found seasonal differences in mucus secretion for the limpet *Patella vulgata*, but attributed them to differences in activity. Here activity level was constant during summer and winter measurements (e.g. all abalone were quiescent during mucus collection) so the reduced mucus secretion during winter months must be due to intrinsic physiological or metabolic differences in the abalone.

Two other energy budgets for abalone, one by Peck *et al.* (1987) for the European abalone *Haliotis tuberculata* and one by Barkai and Griffiths (1988) for the South African abalone *H. midae*, are shown in Table 1-4. My summer energy budget can be compared to that of Peck *et al.* (1987) who, based on their data for reproduction, appear to have determined a summer energy budget for their species. *Haliotis kamtschatkana* has a much higher respiration component than does *H. tuberculata*, even before activity has been considered (51% of consumed energy compared to 27%, respectively), while *H. tuberculata* diverts more energy to growth. Barkai and Griffiths (1988) found that *H. midae* loses 63% of consumed energy to feces, a much larger proportion than
measured for either *H. kamtschatkana* or *H. tuberculata* (both 18%). The respiration component for *H. midae* was only 8% of consumed energy, but the authors note it would be higher if they had incorporated activity into their measurements. All three energy budgets indicate little energy lost to nitrogen excretion.

The technique used in this study to collect gonad tissue allowed for analysis of male as well as female reproductive effort. This was not the case in the energy budgets presented by Peck *et al.* (1987) and Barkai and Griffiths (1988) owing to the difficulty of collecting sperm released from live abalone. No difference in reproductive effort was found between males and females in the present study, even though eggs are energetically more costly to produce than sperm (Table 1-1). The animals in this study produced slightly less gonad tissue than did field *H. kamtschatkana*. Carefoot *et al.* (in prep) found that *H. kamtschatkana* collected just prior to spawning in summer have gonad indices (GI: mass of wet gonad divided by mass of live

Table 1-4. Energy budgets for three species of abalone expressed as percentages of C. Proportions of respiration and mucus from the present study were calculated from the summer energy budget. The proportions for *Haliotis tuberculata* were calculated from regression equation in Peck *et al.* (1987) for animals of a size similar to the representative 50-g abalone used for the present study. The proportions for *H. midae* have no mucus entry and P_g and P_r were estimated as a single value.

Species	F	U	P_g	P_r	R	M	Total
Haliotis kamtschatkana[1] (summer)	18	<1	6	3	51	1	80
Haliotis tuberculata[2]	18	1	13	4	27	26	89
Haliotis midae[3]	63	<1	5		8		76

[1] present study, activity not included
[2] Peck *et al.* (1987)
[3] Barkai and Griffiths (1988)

abalone excluding shell) around 10%, with males having a significantly higher GI than females. My mean value of 8% (calculated from Table 1-1 to exclude shell from live abalone mass), with no difference between males and females, was intermediate between their summer and winter GI values. Thus, it is likely that field abalone allocate more energy to reproduction than these laboratory animals did.

Growth of *Haliotis kamtschatkana* in the present study was less than that found in other studies on the same species. Quayle (1971), for example, found a yearly growth increment of 10 mm in animals 70-80 mm long, while my similarly-sized abalone averaged five mm. This could be due to temperature. Maximum shell growth occurs between 13-14°C (Paul and Paul, 1981), temperatures which were rarely reached during the present experiment. Other factors besides temperature are known to affect growth, such as food availability (Leighton and Boolootian, 1963), gametogenesis (Paul *et al*, 1977; Keesing and Wells, 1989), and habitat (Keesing and Wells, 1989).

Although diurnal cycles in oxygen consumption (V_{O2}) were not evident in this study, this could be due to the laboratory conditions. Many species of abalone exhibit nightly increases in V_{O2} due to increased foraging activity [e.g. 42% increase for *H. discus hannai*, Tamura (1939); 20% increase for *H. discus hannai*, Uki and Kikuchi (1975); 20% increase for previously starved *H. diversicolor supertexta*, Jan *et al.* (1981b)]. Since food was abundant in my study, any change in V_{O2} might have been suppressed, as noted by Peck *et al.* (1987), Barkai and Griffiths (1988), and Jan *et al* (1981a) for other *Haliotis* species. Greater night-time activity would potentially raise the respiratory component of the energy budget.

In this chapter, it was determined that a 50-g abalone has a quiescent respiratory energy expenditure of 971 J·day^{-1} during summer and 582 J·day^{-1} during winter. Likewise, mucus secretion during adherence to the substratum was 20 J during summer and 6 J during winter. These values will serve as baselines to which values estimated from time-energy budgets can be compared. Respiratory energy expenditure and mucus secretion during locomotion in abalone will be investigated in Chapter 2, and a time-energy budget will be determined in Chapter 3, allowing these seasonal energy budgets to be modified to include activity.

Chapter 2. Cost of transport and mucus production during locomotion

2.1 Introduction

The energetic costs of locomotion have been studied intensively for running, swimming and flying, but the costs of adhesive crawling are not as well understood. Several studies have shown that crawling snails increase their energy expenditure above resting costs (Newell and Roy, 1973; Calow, 1974), but only two have correlated oxygen consumption with speed to determine cost of transport, the energy needed to transport a unit mass over a unit distance (calculated by dividing mass-specific energy expenditure by speed). Denny (1980) calculated the cost of transport for the terrestrial slug *Ariolimax columbianus*, and Houlihan and Innes (1982) investigated transport costs for four marine gastropod species. Both studies showed adhesive crawling to be energetically more expensive than other types of locomotion. Houlihan and Innes (1982) briefly investigated the effects of animal size on cost of transport for the marine gastropod *Monodonta turbinata*, and they found that total mass-specific cost of transport decreased as size increased.

Mucus is essential for gastropod locomotion. Adhesive crawling involves the secretion of a thin layer of mucus from glands in the foot to form a sheet between the foot and the substratum. Mucus production may be a primary reason that adhesive crawling is so energetically expensive compared with other forms of locomotion (Denny, 1980). As well, the importance of mucus to the gastropod energy budget is well known (Paine, 1971; Calow, 1974; Horn, 1986; Davies *et al*, 1990). Horn (1986) found that mucus production accounted for 70% of consumed energy in the chiton *Chiton pelliserpentis*. Most values are not that high. For example, Calow (1974) estimated mucus production to represent 13-32% of absorbed energy in *P. contortus*, and Carefoot (1967) estimated that mucus accounted for 15% of the energy budget of the opisthobranch *Archidoris pseudoargus*.

Recent studies have focused on intraspecific as well as interspecific variations in mucus production. Davies (1993) showed that the limpet *Patella vulgata* produced varying amounts of

mucus depending on season and habitat, perhaps due to differences in activity level. Peck *et al.* (1993) found that 80% of the daily mucus secretion of the limpet *Nacella concinna* could be attributed to adhesion, with the rest presumably due to locomotory activity. Likewise, Davies *et al.* (1990) found increased mucus secretion in motile compared with stationary limpets. Not only does the amount of mucus secreted change during locomotion, but there is evidence that the nature of the mucus is different. Connor (1986) found that mucus secreted by stationary limpets in the field persisted significantly longer on the substratum than trail mucus.

The purpose of this study is to determine the energetic cost of locomotion of *Haliotis kamtschatkana*. I determine the cost of transport of *H. kamtschatkana* as well as the amount of mucus secreted during locomotion. These values will be used in determining the cost of activity in *H. kamtschatkana*'s energy budget.

2.2 Materials and methods

Animal collections

Northern abalone *Haliotis kamtschatkana* were collected at the Bamfield Marine Station, British Columbia, and transported to the Shannon Point Marine Station, Washington. They were held in large tanks with a continuous supply of fresh seawater at a mean temperature of 10°C during the experiments. The abalone were fed *ad libitum* on kelp, *Nereocystis luetkeana*.

Oxygen consumption and cost of transport

Abalone (N=29; 4-122 g live mass) were placed individually in round, Perspex respirometry chambers (100, 450, 720 and 1850 ml, depending on animal size) at 10°C and were allowed to move freely. A shelf located 1 cm above the bottom of the chamber supported a plastic mesh screen, beneath which rotated a magnetic stir bar. The abalone often circled the respirometer, either on the wall or on the shelf protecting the stir bar. During these revolutions, speeds were measured by recording the time at which distance markers around the side of the respirometer were passed. Oxygen consumption was monitored continuously using a

polarographic oxygen electrode connected to a computerized data-acquisition system (Datacan, Sable Systems, Inc.). Since this system allowed the times between markers to be recorded, oxygen consumption during periods of known speeds could be calculated. Movement usually occurred within the first 15 min of a trial, and at no time did the oxygen partial pressure drop to below 75% of saturation during the trial. Energy expenditure ($J \cdot h^{-1}$) was calculated from oxygen consumption ($\mu l\ O_2 \cdot h^{-1}$) by multiplying by an oxycalorific coefficient (Q_{ox}) of 21.10 $J \cdot ml\ O_2^{-1}$. This represents an accepted value for the catabolism of carbohydrate (Elliot and Davison, 1975) and was chosen because most gastropods rely on glycogen stores for energy during activity (Carefoot, 1987).

Resting metabolic rates were determined for each abalone by measuring oxygen consumption during quiescent periods. If the abalone had been active, resting oxygen consumptions were measured at least 20 min after each activity bout in order to ensure that there was no residual elevation of metabolic rate. Resting oxygen consumption was measured 1-3 times for each abalone, and the resting metabolic rate used to calculate net cost of transport for each animal was the average from the individual animal's trials.

Minimum cost of transport for *H. kamtschatkana* was calculated from the slope of the regression of speed on total oxygen consumption during locomotion (Taylor *et al.* 1970; Full *et al.* 1990). Total cost of transport (total amount of energy needed to transport a unit body mass over a unit distance, including resting metabolism) was calculated for individual abalone for each period of known speed and energy consumption by dividing total mass-specific energy expenditure ($J \cdot kg^{-1}h^{-1}$) by speed ($m \cdot h^{-1}$). Net cost of transport, or the transport cost above resting metabolism, was calculated by subtracting resting mass-specific oxygen consumption from total mass-specific oxygen consumption during locomotion for each abalone, then dividing by the speed at which that abalone had traveled. Net cost of transport represents the energy devoted solely to locomotion, which here includes any postural costs (energy required to hold the body in the posture of locomotion). This method of calculating net cost (as opposed to also subtracting out postural costs) was chosen since total oxygen consumption during locomotion and resting oxygen consumption were both measured directly for each animal,

whereas the postural cost for locomoting abalone was calculated indirectly from the difference between average resting mass-specific oxygen consumption for all the animals and the *y*-intercept of the regression of speed on total oxygen consumption.

Pedal area at rest

Abalone (N=21; 4-150 g live mass) were placed individually in a glass aquarium with a constant supply of fresh sea water and were allowed to adhere to the side of the tank. An animal was left undisturbed until its foot was fully expanded. A 1 cm x 1 cm piece of plastic was placed next to the abalone as a reference, and a photograph was taken. The photographs were analyzed using a Sigma-Scan area-measurement software system (Jandel Scientific, CA, USA) to determine the area of the foot adhering to the glass surface. Wet mass (including shell) and shell length of the abalone were recorded.

Since abalone have body parts with very different densities (i.e. shell, viscera and muscle), the volume of the animal, rather than its wet mass, was used as a measure of abalone size for morphological comparison with foot area. Wet mass was converted to volume by deriving a relationship between mass and volume for *H. kamtschatkana.* Abalone (N=10; 41-191 g live mass) were weighed in air and in sea water. Abalone volume (V) was calculated using the equation:

$$V = \frac{M_a - M_w}{d_w}$$

where M_a is the mass of abalone in air, M_W is the mass of abalone in sea water, and d_W is the density of sea water (assumed to be 1.025 $g \cdot cm^{-3}$).

Mucus secretion

The amount of mucus needed for an abalone to adhere to the substratum was determined by allowing individual abalone (N=16; 17-143 g live mass) to attach to clean glass plates immersed in a tank continuously supplied with fresh sea water. Each abalone was then removed quickly from the plate, using a spatula, after it had been stationary for 10 min

following adherence. This period was chosen because Davies (1993) found that stationary limpets stop producing mucus 10 min after attachment. It is probable that abalone cease to produce mucus much sooner since my observations suggest that they are able to attach to a surface faster than limpets and are difficult to remove from a surface only 30 s after being placed on it. The plate was then rinsed with distilled water to remove salt residues and dried at 60°C for 30 min. The dried mucus was carefully scraped from the plate and its carbon content determined using carbon analysis (NA-1500 Elemental Analyzer, Carlo Erba Strumentazione). Mass of carbon (μg) was converted to dry mass of mucus (μg) by assuming that gastropod pedal mucus is 24.5% carbon (Peck *et al.* 1993).

Mucus secretion during locomotion was determined by inducing similarly sized abalone (N=7; 95-99 mm shell length; mean ± SE resting pedal area 42 ± 4 cm^2) to locomote over glass plates. Three clean 8 cm x 31 cm glass plates were placed in series on glass bowls resting in the tank. An abalone was placed on the first plate and allowed to travel over the middle plate onto the third. The time taken to traverse the middle plate was recorded, the plate was removed, and the mucus collected as described above. This procedure was repeated until each abalone had traversed the middle plate at five different speeds with as wide a range of speed as possible. Locomotory rates ranged from 9.6-105.1 $cm \cdot min^{-1}$ for the seven animals. Trials for an individual abalone were at least 24 h apart.

2.3 Results

Oxygen consumption and cost of transport

Total mass-specific rate of oxygen consumption (V_{O2}) increased linearly with increasing speed, rising by 0.58 μl $O_2 \cdot g^{-1}h^{-1}$ for every 1 $cm \cdot min^{-1}$ increase in speed (Fig. 2-1; N=29; t=2.19; P=0.04). Total V_{O2} was also dependent upon mass (b=-0.15, t=2.59; P=0.02), decreasing with increasing abalone mass. The mean V_{O2} (±SE) of resting abalone (20.7±1.7 μl $O_2 \cdot g^{-1}h^{-1}$) was nearly half the value of the y-intercept of the line relating V_{O2} to speed

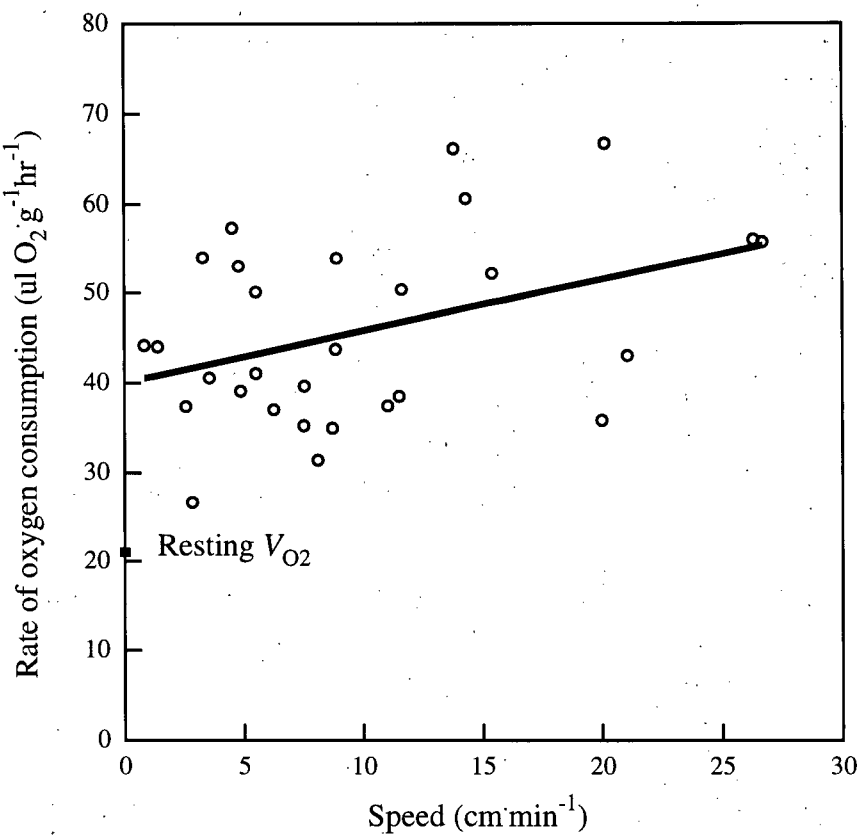

Fig. 2-1. Total rate of oxygen consumption V_{O2} (µl O2 $g^{-1}h^{-1}$) as a function of absolute speed v (cm min^{-1}). The regression line is: V_{O2}=40.1 + 0.58v - 0.15m (r^2=0.35; N=29), where m is body mass (in g). The data presented on the graph have been adjusted for the mass component of the regression equation by calculating the mass component for each abalone and adding it to the measured V_{O2}. The speeds of the individual abalone were then plotted against the adjusted V_{O2} values.

(40.1±4.5 μl $O_2 \cdot g^{-1}$ hr^{-1}) (Fig. 2-1). The difference of 19.4 μl $O_2 \cdot g^{-1}h^{-1}$ is likely to represent a "postural effect" of locomotion for crawling abalone.

The minimum cost of transport, calculated from the slope of V_{O2} *versus* speed (in $m \cdot h^{-1}$), for *H. kamtschatkana* in this study is 20.3 $J \cdot kg^{-1}m^{-1}$ (slope of V_{O2} *versus* speed regression line is 0.96 μl $O_2 \cdot g^{-1}m^{-1}$ when speed is in $m \cdot h^{-1}$). Total cost of transport (total amount of energy needed to transport a unit body mass over a unit distance) and net cost of transport (energy above resting metabolism required for transport, here including postural costs) calculated for individual animals locomoting at a variety of speeds (Fig. 2-2) decreased to minima of 86.0 $J \cdot kg^{-1}m^{-1}$ and 29.7 $J \cdot kg^{-1}m^{-1}$, respectively, at the highest speed measured in the respirometer (maximum speed, 26.8 $cm \cdot min^{-1}$). Because the postural component at high speeds is very small relative to the cost of movement, the total cost of transport should asymptotically approach the net cost of transport, which should in turn approach the minimum cost of transport (Fig. 2-2). Owing to the closed respirometry system, a whole seastar could not be used to stimulate locomotion in the abalone so a tube foot of *Pycnopodia helianthoides* was used. Also, the abalone could not be stimulated continuously with the tube foot so the maximum speed recorded in the respirometer was lower than the maximum speed recorded in the aquarium (113 $cm \cdot min^{-1}$; see Ch. 6).

Both relationships between total and net cost of transport and speed became linear when the variables were $\log_{10}$-transformed. Total and net cost of transport (COT_T and COT_N, respectively, in $J \cdot kg^{-1}$ m^{-1}) decreased both with speed (v in $cm \cdot min^{-1}$) and mass (m, in g), with regression equations $\log_{10}COT_T = 3.35 - 0.90\log_{10}v - 0.21\log_{10} m$ (r^2=0.89, N=29) and $\log_{10}COT_N = 2.29 - 0.69 \log_{10}v - 0.09\log_{10}m$ (r^2=0.48, N=29), respectively. All regressions were significant (all t>2.97; all P<0.006) except the regression of $\log_{10}$ net COT_N *versus* $\log_{10}$ mass (t=0.58; P=0.56).

Pedal area at rest

Log_{10}-transformed resting foot area increased linearly with log volume (Table 2-1). The slope of 0.83 is significantly higher than the predicted slope for isometry (b_o) of 0.67

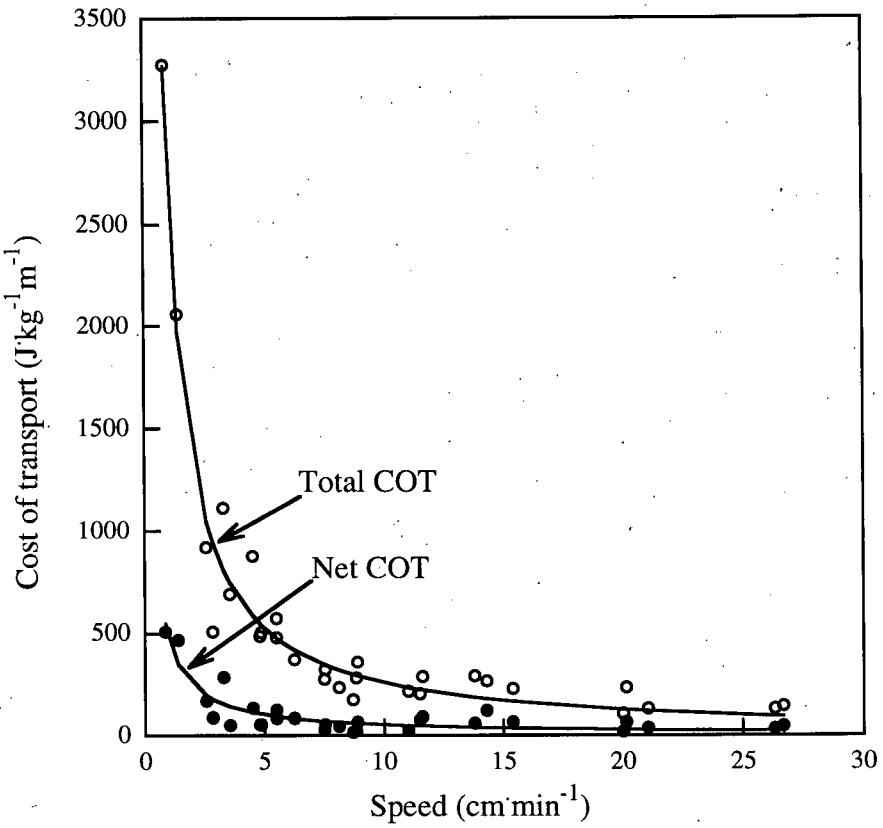

Fig. 2-2. Total and net costs of transport (COT) as a function of absolute speed. Regression equations for $\log_{10}$-transformed data are given in the text. The data presented on the graph have been adjusted for the mass components of the log-log regression equations given in the text by calculating the mass component for each abalone and adding it to the measured cost of transport. The speeds of the individual abalone were then plotted against the adjusted cost of transport for each animal.

Table 2-1. Morphometric relationships between shell length, foot area, abalone volume, and mucus secretion for *Haliotis kamtschatkana*. Regression statistics are for the equation $\log_{10}y = \log_{10}a + b\log_{10}x$.

Relationship (y *versus* x)	$\log_{10}a$	b	r^2	N	Predicted slope for isometry
Resting foot area (cm^2) *versus* abalone volume (cm^3)	-0.11	0.83*	0.96	20	0.67
Resting foot area (cm^2) *versus* shell length (mm)	-3.40	2.51*	0.95	20	2.0
Mucus secretion (µg) *versus* resting foot area (cm^2)	1.11	1.08	0.48	16	1.0

* Indicates that slope differs significantly from the predicted slope for isometry, $P<0.002$.

(t=4.12; P<0.001). Likewise, $\log_{10}$ foot area increased allometrically with $\log_{10}$ shell length (t=3.75; P<0.002). This indicates that the sole of the foot grows disproportionately larger as abalone increase in size.

Mucus secretion

Log_{10}-transformed mucus secretion (μg dry mucus) of stationary animals increased in direct proportion to $\log_{10}$ foot area (cm^2) (Table 2-1). The slope of the regression line was not significantly different from 1.0 (t=0.28; P>0.5), the predicted slope for isometry. Since abalone foot area does not scale isometrically with volume (b=0.83), this means that larger abalone secrete relatively more mucus per unit body volume to adhere to the substratum than do smaller abalone.

None of the regressions of amount of mucus (μg) on speed ($cm \cdot min^{-1}$) was significant (N=7; all P>0.1), so a mean mass of mucus secreted per centimeter traveled was calculated for each animal. Means (±SE) ranged from 2.0±0.4 to 10.0±1.3 μg dry mucus$\cdot cm^{-1}$, with a grand aggregate mean of 5.3±0.7 μg dry mucus$\cdot cm^{-1}$ (N=35). Expressed it terms of abalone foot area, this aggregate mean becomes 0.12±0.02 μg dry mucus$\cdot cm^{-2}$ foot area$\cdot cm^{-1}$ locomoted.

2.4 Discussion

The energetics of locomotion observed in this study were similar to those found for five other gastropod species for which this type of data is available (Denny, 1980; Houlihan and Innes, 1982). Total V_{O2} increased linearly with speed (Fig. 2-1), while cost of transport was high at low speeds and quickly decreased to a minimum as speed increased (Fig. 2-2). Minimum cost of transport is equal to the slope of the regression line of total V_{O2} *versus* speed (Taylor *et al.* 1970; Full *et al.* 1990) and, at high speeds, total cost of transport should reach this value asymptotically as the rate of oxygen consumption increases and resting metabolic costs and postural effect become a smaller proportion of the total metabolic rate. Indeed, total cost of transport was nearly equal to net cost of transport at a speed approximately equal to the

maximum velocity of *H. kamtschatkana* recorded in this study (113 cm·min^{-1}). Thus, 20.3 J·kg^{-1}m^{-1} appears to be the minimum cost of transport for *H. kamtschatkana*. This value is much lower than values for other gastropod species for which similar measurements are available (Fig. 2-3). This may be due to the comparatively large size of abalone, and it would be of interest to measure the cost of transport in larger marine gastropod species to determine whether this trend is consistent. In fact, when net cost of transport is calculated for an abalone of similar size (1.0 g) to the other marine snails in Fig. 2-3, the transport costs of the abalone fall within the range of transport costs for marine snails (147 J·kg^{-1}m^{-1} for abalone compared to 141-485 J·kg^{-1}m^{-1} for the other marine snails; Houlihan and Innes, 1982). For the terrestrial slug (Denny,1980) the high cost of transport of 912 J·kg^{-1}m^{-1} compared with 64 J·kg^{-1}m^{-1} for a similarly sized abalone may be due to differences in the cost of crawling in air *versus* water. Abalone would benefit from the buoyancy provided by water, and mucus requirements may be less in a wet environment. Also, Denny (1980) measured total V_{O2} over a 24 h period and so also recorded oxygen consumption after activity ceased, this taking into account any O_2 debt incurred by anaerobic metabolism. Interestingly, the cost of transport for an abalone (20.3 J·kg^{-1}m^{-1}) is slightly less than the cost of transport of a similarly-sized running vertebrate (26 J·kg^{-1}m^{-1}; Fedak and Seeherman, 1979), but is twice that of a similarly-sized flying bird (10 J·kg^{-1}m^{-1}; Tucker, 1973) and seven times that of a swimming fish (3 J·kg^{-1}m^{-1}; Beamish, 1978). Houlihan and Innes (1982) also found that transport costs for their largest individuals of the marine gastropod *Monodonta turbinata* were below those of similarly-sized running vertebrates (the value represented in Fig. 2-3 is for an average-sized *M. turbinata*).

The linear relationship between energy consumption and speed during adhesive crawling is similar to that found for running homeotherms traveling at their preferred gaits (Hoyt and Taylor, 1981; Taylor *et al.* 1982). In comparison, this relationship is curvilinear for swimming and flying (Brett, 1965; Tucker, 1968). Likewise, total cost of transport decreases with increasing speed in terrestrial locomotion (Peters, 1983; Full *et al.*, 1990). In all forms of locomotion, mass-specific rate of oxygen consumption and transport costs decrease with animal size (Taylor *et al.* 1970; Beamish, 1978; Schmidt-Nielsen, 1984).

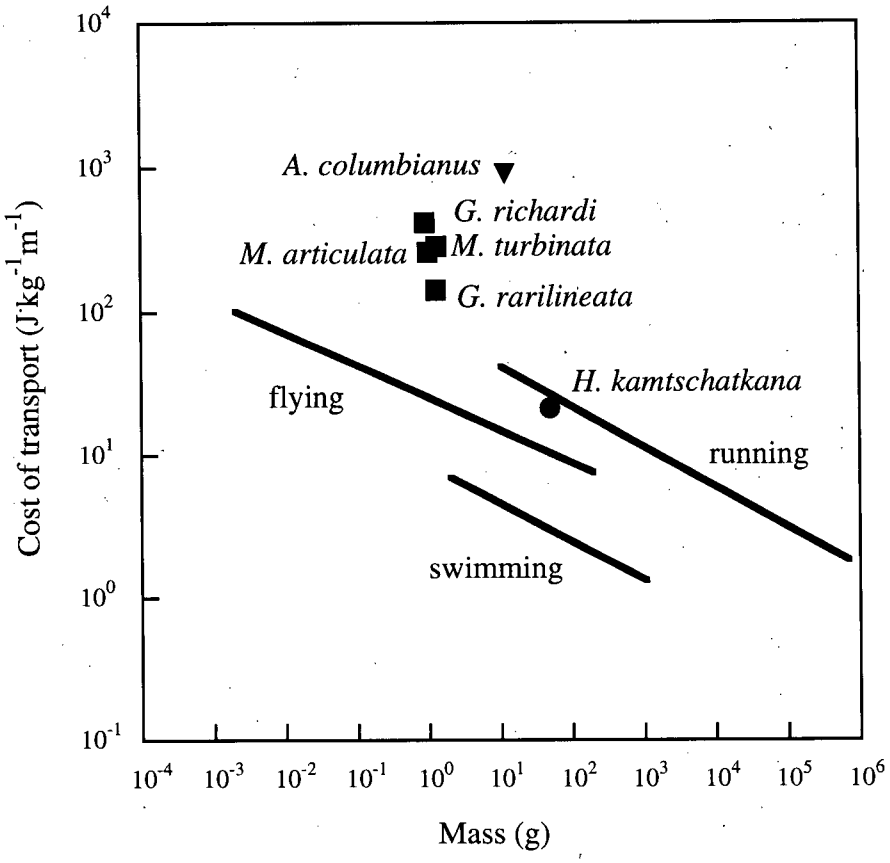

Fig. 2-3. Minimum cost of transport of abalone (*Haliotis kamtschatkana*) compared with that of other marine snails (*Monodonta turbinata, M. articulata, Gibbula richardi, G. rarilineata*; Houlihan and Innes, 1982) and a terrestrial slug (*Ariolimax columbianus*; Denny, 1980). Regression lines for running, flying, and swimming animals were taken from Schmidt-Nielsen (1972).

The y-intercept of the regression of total V_{O2} *versus* speed (40.1 µl $O_2 \cdot g^{-1}h^{-1}$) was 1.9 times larger than the mean resting V_{O2} of the abalone (20.7 µl O_2 $g^{-1}h^{-1}$) (Fig. 2-1). Since the y-intercept represents the rate of oxygen consumption when the animal is not moving, the difference between it and the resting metabolic rate represents the postural effect for locomoting abalone. Schmidt-Nielsen (1972) defines a postural effect as the increase in energy expenditure needed to hold the body in the position of locomotion. Postural costs are probably incurred by abalone due to the lifting of their shells and the subsequent shifting of the visceral mass prior to locomotion, as is the case for other snails (Houlihan and Innes, 1982). Postural effects of similar magnitude have been determined for other gastropods (0.9-1.3 times resting metabolic rate; Houlihan and Innes, 1982), mammals (1.7; Taylor *et al.*, 1970), lizards (1.5; John-Alder *et al.*, 1986) and arthropods (1.5-4.0; Full, 1987; Herreid *et al.*, 1981).

The amount of mucus needed to travel a unit distance was highly variable between animals, and no clear trends were evident with increasing speed. However, the mean amount of mucus secreted during locomotion was low compared with the amount of mucus required to adhere to the substratum. For example, a 95 mm *H. kamtschatkana* secreted 640 µg of dry mucus during adhesion (calculated using the regressions of shell length on resting foot area, and resting foot area on mucus secretion while stationary; Table 2-1). If this abalone were to crawl 10 shell lengths (95 cm) while secreting the same amount of mucus per shell length as used for stationary adherence, then 6400 µg of mucus would be produced. However, since the average amount of mucus secreted per centimeter during locomotion was 5.3 µg dry mucus$\cdot cm^{-1}$, only 504 µg of mucus would actually be produced by this abalone to travel 95 cm. Although these values are only estimates, they corroborate the conclusions of Peck *et al.* (1993) that rates of mucus secretion differed between stationary and locomoting limpets. Davies and Williams (1995) measured values of 21-152 µg dry mucus$\cdot cm^{-1}$ for locomoting limpets *Cellana grata*, which is considerably larger than the 5.3 µg dry mucus$\cdot cm^{-1}$ value determined in the present study for *H. kamtschatkana*, despite the 10-fold larger foot area of the abalone.

The decrease in transport costs with increasing body size in abalone indicates that the proportionately larger pedal soles of larger abalone (Table 2-1) do not increase their cost of

locomotion. The function of a larger foot area may be to increase tenacity when the animal is at rest. Juvenile abalone tend to be more cryptic, occupying crevices that would protect them from heavy surge, while adult abalone are found more often in the open (Sloan and Breen, 1988). Thus, larger abalone may need a disproportionately larger pedal area to allow them to remain affixed to more exposed substrata.

In the present study, cost of transport was calculated solely from aerobic energy expenditure. As such, it may not represent the total amount of energy needed for locomotion. Given the reliance of marine invertebrates on anaerobic metabolism during times of stress (Hochachka, 1980; Gade, 1988) and the open nature of the abalone circulatory system (Bourne and Redmond, 1977*a*,*b*; Jones, 1983), it is likely that abalone make use of anaerobic reserves even at low speeds. In fact, two aspects of the data point to a large anaerobic component during locomotion. First, the steep, negative $\log_{10}$-$\log_{10}$ slopes of both total and net costs of transport *versus* speed (-0.90 and -0.69, respectively) suggest that energy requirements decrease dramatically at high speeds when, in fact, it is possible that a substantial portion of the energy expended at these speeds (the anaerobic component) was not measured. While transport costs may indeed decrease with speed, as they do in other locomotory forms, addition of an anaerobic component would cause this decrease to be less substantial. Second, the minimum cost of transport measured in this study was an order of magnitude lower than the minimum cost of transport calculated by Denny (1980). His measurements of $\dot{V}_{O2}$ included oxygen consumption after activity had ceased, which would have included any O_2 debt incurred by anaerobic metabolism. The extent of the contribution of anaerobiosis to the cost of transport in abalone is the focus of Chapter 4.

Chapter 3. Time-energy budgets and the contribution of activity to the seasonal energy budgets of abalone

3.1 Introduction

While energy budgets, such as the one developed in Chapter 1 for *Haliotis kamtschatkana*, catalog the flow of consumed energy to various energy outputs, energy may be further partitioned within a given energy budget parameter, as is seen in the division of respiratory energy to the variety of activities engaged in by an organism. Beginning with Pearson's (1954) work on hummingbirds, time-energy budgets have been used to investigate activity costs and benefits. A time-energy budget integrates the energetic cost of an activity with the amount of time spent on that activity to quantify respiratory energy expenditure over a period of time. In this respect, time-energy budgets can be used to explain behavioral strategies such as foraging and predator evasion. For example, Huey and Pianka (1981) outline the ramifications of foraging mode (sit-and-wait versus active foraging) of lizards on energy budgeting and on life-history parameters such as daily food consumption, metabolism, clutch size, and morphology. In this case, the energetically inexpensive strategy of sit-and-wait foraging involved a low daily energy consumption which was reflected by a low metabolic rate.

Activity is energetically costly to gastropods due both to increased metabolic rate and to mucus secretion. Many studies have shown a rise of oxygen consumption (V_{O2}) during activity in gastropods (Newell and Roy, 1973; Calow 1974; Fitch, 1975; Crisp, 1979; Houlihan and Innes, 1982). Cost of transport, or the amount of energy needed to transport a unit mass over a unit distance, has also been measured (Chapter 2; Denny, 1980; Houlihan and Innes, 1982). Calow (1974) estimated that 20% of "routine metabolism" of the pulmonate *Planorbis contortus* was devoted to activity. Likewise, several authors have pointed out the importance of mucus as a contribution to molluscan energy expenditure (see Chapter 2)

Metabolic rates of marine organisms depend on a myriad of internal and environmental factors which interact in different ways at different times of the year (Newell, 1973). Seasonal

temperature differences affect both oxygen consumption (see Bayne and Newell, 1983; Carefoot, 1987) and activity (Newell, 1969; Poore, 1972; Newell and Kofoed, 1977). Newell and Pye (1971) showed interaction between activity level and temperature in *Littorina littorea* in that the active rate of respiration was more temperature dependent than the standard rate, suggesting that activity would have a different impact on a gastropod energy budget at different times of the year. Indeed, Widdows and Bayne (1971) found that both filtration rate and oxygen consumption in the mussel *Mytilus edulis* were affected by acclimation to high and low temperatures which, in turn, changed the animal's energy allocations. Evidence for effects of temperature on mucus secretion is more scarce, but Kideys and Hartnoll (1991) found that mucus secretion in the whelk *Buccinum undatum* decreased at low temperatures. Changes in mucus secretion at different temperatures and during different seasons would cause further changes in the impact of activity on an energy budget.

The purpose of this part of the study is to determine the contribution of activity to the seasonal energy budgets of *Haliotis kamtschatkana* developed in Chapter 1, in which the measurements of respiration (R) and mucus secretion (M) were made on quiescent animals. Values for cost of transport and mucus secretion during locomotion from Chapter 2, along with measurements of respiratory energy expenditure during other states of activity, will be integrated with a time budget to determine the daily contribution of activity to the respiration and mucus secretion components of the energy budget. These values will be compared to the values measured in Chapter 1 for inactive animals.

3.2 Materials and Methods

Collection of animals

Abalone were collected at the Bamfield Marine Station, British Columbia and transported to the Shannon Point Marine Center, Washington. They were held in a large tank with a constant supply of fresh seawater and fed *ad libitum* on *Nereocystis luetkeana*.

Time budgets

Activity states of *Haliotis kamtschatkana* were monitored in both laboratory and field, and activity budgets determined from the amount of time spent in different activity states. In total, five states which appeared important to the energetics of *H. kamtschatkana* were identified: 1) quiescent (shell held tightly to the substratum, cephalic and mantle tentacles retracted), 2) alert (shell raised off the substratum, tentacles extended), 3) active (back and forth movements in a small area without moving any appreciable distance), 4) locomoting (moving an appreciable distance in one direction), and 5) feeding. In addition to laboratory and field observations, videotapes were made of locomoting abalone.

Laboratory. Abalone were placed in a large open-air tank exposed to natural light and with a constant supply of fresh seawater. They were observed hourly, and the number of abalone in each of four states (quiescent, alert, locomoting, and feeding) was recorded. This experiment was conducted in summer (June 1994; N=105) and winter (January 1996; N=70). Daily activity budgets were calculated from these summer and winter data.

Field. Activity states (quiescent, alert, and locomoting) of field abalone were recorded during daytime SCUBA dives in Barkley Sound, near the Bamfield Marine Station. Dives were made between 0900-1200 h, in alternate months from April 1994-April 1995. Divers followed 100-m transects and recorded the activity state of all abalone seen (N=52-203 for each outing). These data were used to compare the amount of activity in the laboratory with that in the field.

Videotaped locomotory activity. Abalone (N=15; 70-120 g live mass) were placed three at a time in a glass aquarium (30x50x15 cm) with an adequate flow of fresh seawater (2 $l \cdot min^{-1}$), and were videotaped over a 24-h period. Videotapes were analyzed for 1) rate of locomotion, 2) total distance moved during locomotion, and 3) number of locomotory bouts.

Energy Equivalents for Each Activity

Energy equivalents for quiescent, alert, and active states were determined by measuring oxygen consumption of abalone in each state. Since I was unable to induce abalone to feed in the respirometer, I assumed that energy expended during feeding state was equivalent to energy expended during the active state. Shell posture is similar in both states and both involve muscular activity, thus they appear energetically similar.

Oxygen consumption measurements. Abalone (N=21; 13-144 g live mass) were placed individually in round, Perspex respirometry chambers. Temperature was maintained at 10°C in a water bath, and oxygen consumption was monitored continuously with a polarographic oxygen electrode connected to a computerized data acquisition system (Datacan, Sable Systems). The state of the animal (quiescent, alert, active) was recorded every two minutes for the duration of the trial. Often each animal did not exhibit all states during one trial, so animals were placed in the respirometer multiple times over a period of several days (separated by at least 48-h). Thus, oxygen consumption for each state was measured 2-4 times, permitting an average for each state to be calculated.

Respiratory energy expended during locomotion was estimated from the total cost of transport determined in Chapter 2.

Mucus secretion. Mucus secretion during adherence to the substratum for a 50-g abalone was calculated from the regression of mucus secretion ($J \cdot day^{-1}$) on mass (g) from the inactive energy budgets (Table 1-2). Mucus secretion during locomotion was determined in Chapter 2. Mass of carbon (μg) was converted to dry mass of mucus (μg) by assuming that gastropod pedal mucus is 24.5% carbon (Peck *et al.* 1993). Mucus dry mass was converted to energy ($J \cdot day^{-1}$) assuming a conversion of 23.97 $kJ \cdot g^{-1}$ mucus (Calow, 1974).

3.3 Results

Activity

Laboratory activity. During the summer, definite diurnal trends were seen, with greater locomotion during night time (18%) and increased quiescence during the day (only 1-2% locomoting; Fig. 3-1, top). Peak quiescence occurred during day time, with usually 50% or more abalone being in this state. The abalone fed at a low level throughout the day as kelp was plentiful in the tank.

During the winter abalone were more quiescent and less active than in the summer, and there was less of a diurnal trend (Fig. 3-1, bottom). Peak locomotion was generally 1900-2400h, but the percentage locomoting was no greater than 2-6%. Throughout the day 60% or more of the abalone were quiescent, with an increase in alertness occurring during the period 1700-2300h.

Time spent by an average laboratory-held abalone at each activity during summer and winter is shown in Table 3-1.

Field Activity. Abalone exhibited more activity in the warm summer months (Fig. 3-2). For example, about 20% of all abalone were observed to be locomoting during June, August, and October. Few animals were locomoting during the winter months; instead, they were most often quiescent or alert. No animals were observed feeding.

Videotaped locomotory activity. Locomotion was not continuous, and the average abalone stopped and started again twice for every meter moved. Average rate of locomotion was 1 body length·min^{-1}.

Energy Equivalents for Each Activity

Oxygen consumption measurements: Oxygen consumption increased with activity level over a wide range of abalone mass (Fig. 3-3). The slopes of the $\log_{10}$-$\log_{10}$ transformed regressions

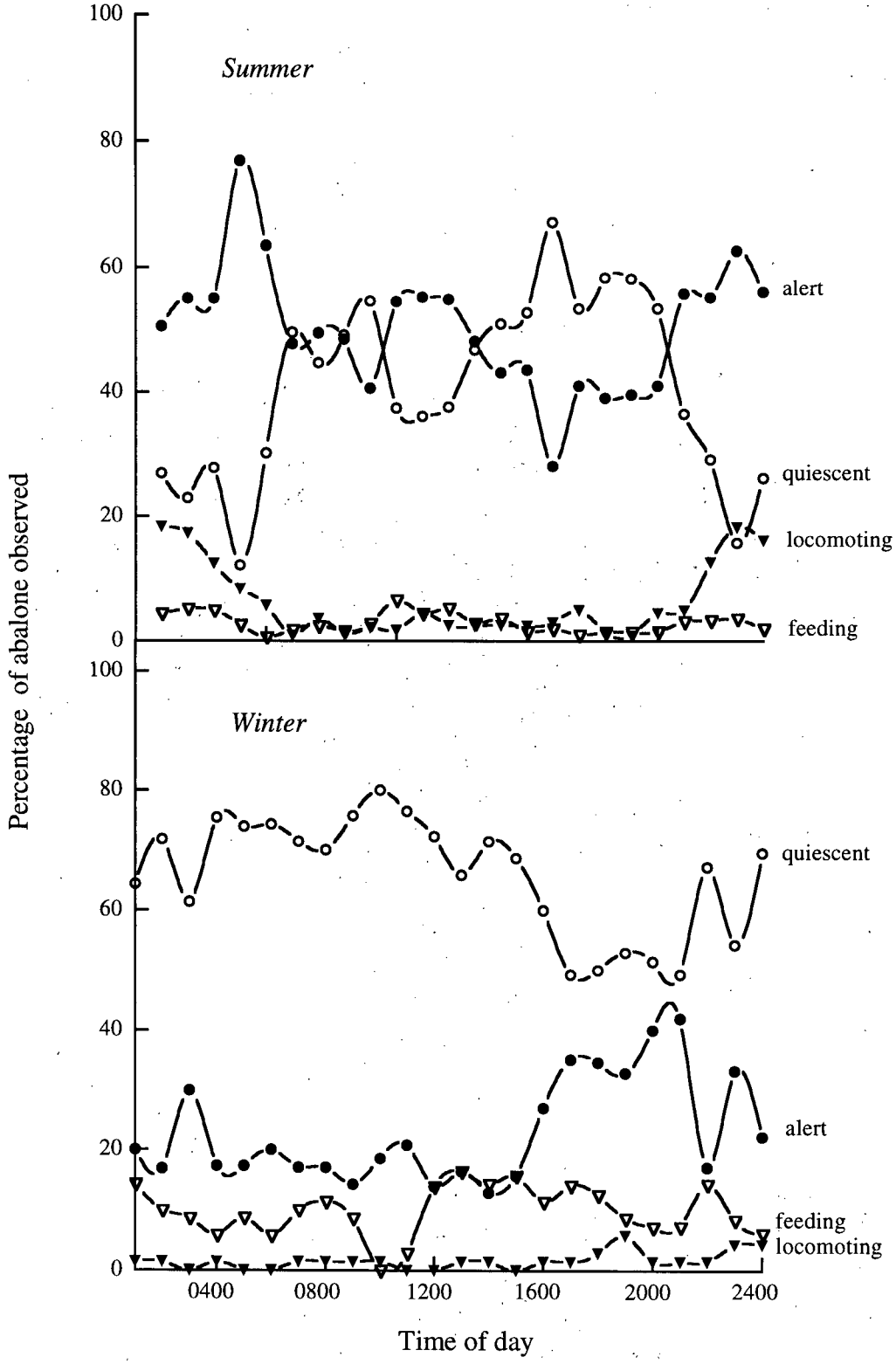

Fig. 3-1. Seasonal activity patterns of *Haliotis kamtschatkana* in the laboratory.

Table 3-1. Average amount of time (h) spent each day by *Haliotis kamtschatkana* in different activity states during summer and winter, in the laboratory.

	Time (h)				
	quiescent	alert	feeding	locomoting	totals
Summer	9.8	12.0	0.7	1.5	24
Winter	15.8	5.5	2.3	0.4	24

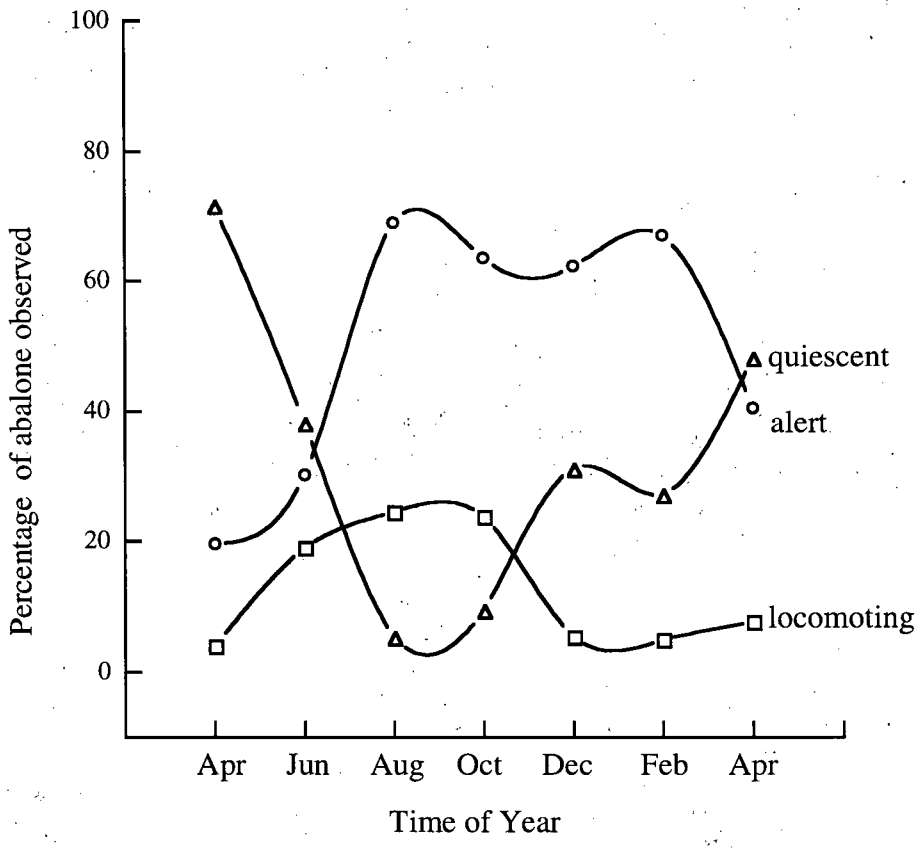

Fig. 3-2. Proportion of *Haliotis kamtschatkana* that were quiescent, alert, and locomoting observed during daytime SCUBA dives in Barkley Sound, British Columbia from April 1994-April 1995. *N*=52-203.

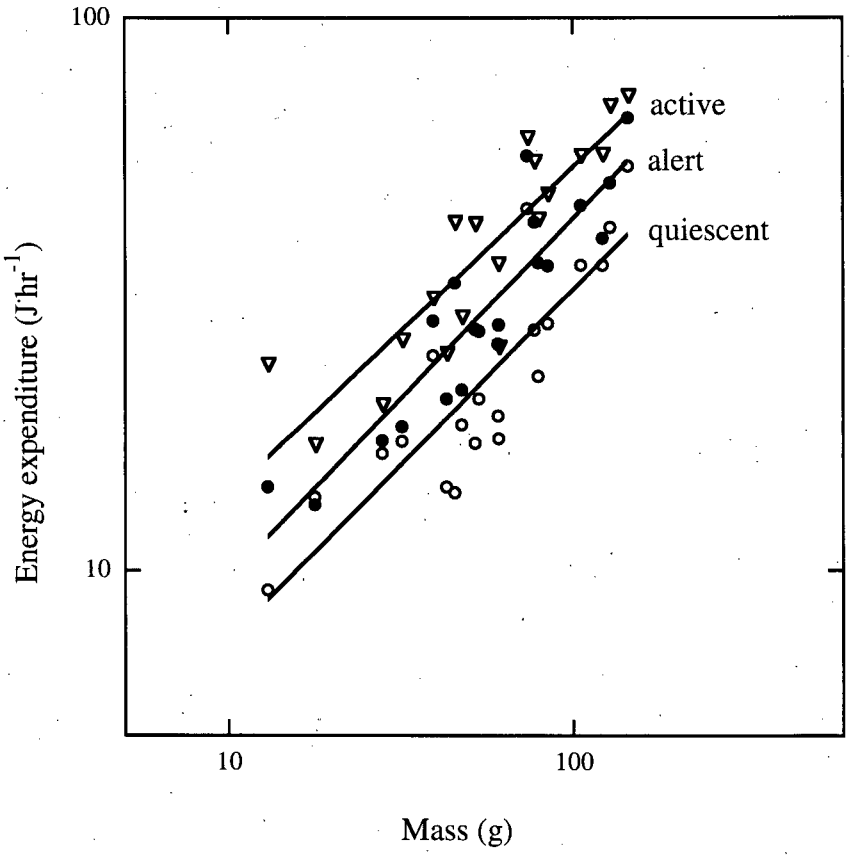

Fig. 3-3. Regression of $\log_{10}$ energy expenditure (E in J·h^{-1}) on $\log_{10}$ mass (m in g) for abalone in three activity states. Regression equations are: quiescent $\log_{10}E = -2.82 + 0.67\log_{10}m$, alert $\log_{10}E = -2.76 + 0.70 \log_{10}m$, and active $\log_{10}E=-2.53 + 0.63 \log_{10}m$. All regressions were significant (all $t>11.26$, all $p<0.001$).

of oxygen consumptions on mass for quiescent, alert, and active abalone were not significantly different ($F_{0.05(2),2,56}$=0.43, p>0.05; ANCOVA) but the Y-intercepts were ($F_{0.05(2),2,56}$=32.7, p<0.001; ANCOVA). For a 50-g abalone, then, oxygen consumption increased 33% from quiescent to alert, and by a further 29% from alert to active. Thus, a 50-g abalone's inactive summer respiratory energy expenditure of 40 J·h^{-1} (calculated from the regression of mass on respiratory energy from the summer energy budget; Table 1-2) would increase to 53 J·h^{-1} while alert, and 68 J·h^{-1} while active. Likewise, the winter quiescent rate of 24 J·h^{-1} would increase to 32 J·h^{-1} while alert and to 41 J·h^{-1} while active.

The total cost of transport of a 50-g *H. kamtschatkana* locomoting at 8 cm·min^{-1} is 151 J·kg^{-1}m^{-1} (calculated from the multiple regression of $\log_{10}$ total cost of transport on $\log_{10}$ mass and $\log_{10}$ speed; Chapter 2). Thus, a 50-g abalone uses 8 J·m^{-1}.

Mucus secretion. The amount of energy lost as mucus secreted while adhering to the substratum for a 50-g abalone was 6 J·day^{-1} during the winter and 20 J·day^{-1} during the summer. Since it was assumed that inactive abalone adhere once to the substratum each day then remain still, this represents a value for energy per adhesion.

Abalone secrete 0.12 ug of dry mucus·cm^{-2} foot area for every cm they locomote (Chapter 2). A 50-g (71 mm) abalone has a foot area of 18 cm^2 (Table 2-1), which yields a mucus secretion rate of 2.2 ug of dry mucus·cm^{-1} locomoted. This converts to 5 J·m^{-1} lost as mucus.

When the time budget is combined with the energetic costs of the different activity states (Table 3-2) a large increase is shown in the respiratory energy component of the budget (R) and in mucus secretion (M) compared with those calculated for inactive abalone (Table 3-3). The new values show that during summer R was 1132 J·day^{-1}, compared with 971 J·day^{-1} from the baseline energy budget for inactive animals, and now represents 60% of total consumed energy rather than 51%, a difference of 9%. Likewise, the estimate of energy lost to mucus secretion for inactive abalone in summer rose from 20 to 315 J·day^{-1}, an increase of 16% of consumed

Table 3-2. Total daily respiratory and mucus energy expenditures for a 50-g abalone. The daily time budget (Table 3-1) was integrated with energetic costs of each activity. Summer and winter quiescent respiratory energy rates ($J \cdot h^{-1}$) were calculated from regressions of energy budget components on mass (Table 1-2). Alert and feeding rates (the latter assumed to be equivalent to alert rates) were calculated from increases over quiescent rates determined by measuring oxygen consumption during different activity states. Distance ($m \cdot day^{-1}$) was estimated from average time spent locomoting (1.5 h in summer, 0.4 h in winter; Table 3-1) and average locomotory rate (8 $cm \cdot min^{-1}$; videotape data). Energetic cost of this movement (cost of transport) was determined in Chapter 2. Number of adherences per day was estimated from average number of locomotory bouts per meter over total distance (videotape data).

Respiration

	time (h)	energy equivalents ($J \cdot h^{-1}$)	distance moved ($m \cdot day^{-1}$)	cost of transport ($J \cdot m^{-1}$)	total energy ($J \cdot day^{-1}$)
Summer					
quiescent	9.8	40			392
alert	12.0	53			636
feeding	0.7	68			48
locomotion			7	8	56
					1132
Winter					
quiescent	15.8	24			379
alert	5.5	32			176
feeding	2.3	41			94
locomotion			2	8	16
					665

Mucus Secretion

	No. adherences per day	energy equivalents ($J \cdot adherence^{-1}$)	distance moved ($m \cdot day^{-1}$)	energy equivalents ($J \cdot m^{-1}$)	total energy ($J \cdot day^{-1}$)
Summer					
adhesion	14	20			280
locomotion			7	5	35
					315
Winter					
adhesion	4	6			24
locomotion			2	5	10
					34

Table 3-3. Values for each component of summer and winter energy budgets for a representative 50-g *Haliotis kamtschatkana.* Respiration (R) and mucus secretion (M) values from Table 1-2 (without activity) have been presented again for comparison with values for R and M calculated from the activity budget (with activity).

Energy ($J \cdot day^{-1}$)

	C	F	U	P_g	P_r	With activity R	With activity M	New total	New % total of C
Summer	1894	345	< 1	108	62	1132	315	1962	
percentage of C (%)		18	< 1	6	3	**60**	**17**		104
Winter	860	111	< 1	1	0	665	34	811	
percentage of C (%)		13	< 1	< 1	0	**77**	**4**		94

	Without activity R	Without activity M	Old total	Old % total of C
From Table 1-3				
Summer	971	20	1506	
percentage of C (%)	**51**	**1**		80
Winter	582	6	700	
percentage of C (%)	**68**	**1**		81

energy. Thus, activity can be shown to account for 25% of the energy consumed during the summer. In the same manner, activity accounts for 12% of energy consumed during the winter.

3.4 Discussion

This study has shown that activity, represented by increased respiration and mucus secretion during locomotion, accounts for 25% of the energy consumed by laboratory-held *Haliotis kamtschatkana* during the summer, and 12% during the winter. In fact, we have probably underestimated the activity component of the energy budget of field *H. kamtschatkana,* since abalone locomote less in the laboratory than they do in the wild. Abalone are known to increase foraging activity when food is scarce (Poore, 1972, Shepherd, 1973; Sloan and Breen, 1988), which did not occur in the laboratory. Likewise, field abalone must locomote to find refugia and escape predation. Shepherd (1986) has shown that motility of *H. laevigata* is related to crevice space, with abalone increasing their locomotion until they find a suitable crevice. Predators of *H. kamtschatkana* include octopus, crabs, fish, and seastars (Sloan and Breen, 1988), and *H. kamtschatkana* exhibits a large increase in locomotory rate in the presence of the seastar *Pycnopodia helianthoides.* Comparison of activity levels in laboratory and field also supports the idea that field abalone are more active than laboratory abalone. During observations of field abalone by SCUBA divers in June, 19% of all abalone observed were locomoting during the daytime. This was not the case during the daytime summer observations in the laboratory (only 2-5% of abalone locomoting during daytime). Winter daytime values were closer to each other, with 5% of abalone locomoting in the field in both December and February, and 0-2% locomoting in the laboratory. Although it was not possible for the divers to discern causes of locomotion in the field, it could have been due to the presence of predators or to searching for food. In fact, large *H. kamtschatkana* appear not to locomote when food is abundant since those living near kelp fields are often observed in home scars (Sloan and Breen, 1988). My laboratory observations indicated that abalone initially

exhibited the foot-raised behavior to capture drift algae when kelp was put into the tank, and they often increased their locomotion and moved towards the kelp.

It is interesting that seasonal differences in activity levels were seen in both field and laboratory. Seasonal variation in activity in field abalone has been explained by storms (Poore, 1972) and reproduction (Ino, 1952). Another possibility could be seasonal shifts in predation intensity. However, none of these explanations is adequate for the laboratory-held animals since predation and storm exposure were absent during both trials, and all of the animals were in close proximity for reproduction (although no animals spawned). It is possible that observed seasonal differences were due to food intake. Although both groups of abalone were fed *ad libitum*, the quality and caloric content of the winter kelp would be greatly decreased, leading to less energy available for movement. However, it is most probable that seasonal differences in activity were due to changing water temperature and the direct dependence of the abalones' metabolic rates on them.

The increase in activity shown in the night-time for laboratory animals agrees with observations on other abalone species (Momma and Sato, 1969; Poore, 1972; Shepherd, 1973). Although field activity was only observed during the daytime in this study, it is likely that the diurnal fluctuations seen in the laboratory are also present in the field since night dives have shown that Northern abalone are more active at night (Sloan and Breen, 1988).

When activity is considered, respiration and mucus secretion comprise two of the largest components of the summer energy budget (60% and 17% of consumed energy, respectively). Respiration was also the largest component of the winter budget, accounting for 77% of consumed energy. For the summer budget, all energy consumed was accounted for (actually, overestimated by 4%) and, for the winter budget, 94% was accounted for. A more meaningful comparison can now be made with the respiration and mucus secretion components of the other two energy budgets for abalone by Peck *et al.*, (1987) and Barkai and Griffiths (1988) considered in Chapter 1 (Table 3-4). Respiration was a much larger component of *H. kamtschatkana*'s budget than it was for either *H. tuberculata* or *H. midae* (60% compared to 27% and 8%, respectively). Mucus was as large a component for *H. tuberculata* as respiration,

Table 3-4. Energy budgets for three species of abalone, with activity factored into the summer budget of *Haliotis kamtschatkana*. Calculations are described in Table 1-3.

Species	F	U	P_g	P_r	R	M	Total
Haliotis kamtschatkana[1] (summer)	18	<1	6	3	60	17	104
Haliotis tuberculata[2]	18	1	13	4	27	26	89
Haliotis midae[3]	63	<1	5		8		76

[1] present study, activity included
[2] Peck *et al.* (1987)
[3] Barkai and Griffiths (1988)

accounting for 26% of consumed energy, while it was less for *H. kamtschatkana* (17%). However, both budgets show that mucus is a substantial component of energy budgets of *Haliotis* spp.

This part of the study has shown that activity has a substantial impact on both summer and winter energy budgets of the abalone *Haliotis kamtschatkana*. In the field, it is likely that more energy would be expended on activity, especially in areas where food is scarce or predation intense, and this has potential ramifications to the dynamics of abalone populations. In the case of predation by organisms eliciting a locomotory escape response, such as the seastar *Pycnopodia helianthoides*, predation would not only affect population dynamics by increasing mortality, but could also cause decreased somatic growth and reproductive effort as energy is diverted from these energy budget parameters to respiratory and mucus components. This would not only cause decreased fecundity due to decreased gamete production, but would also cause a delay in reproductive maturity since abalone start reproducing at a specific size. The result of this would be fewer recruits to local abalone populations.

Chapter 4. Contribution of anaerobic metabolism to locomotory costs in abalone

4.1 Introduction

Chapters 1-3 focused on the contribution of aerobic energy to activity, particularly locomotion, in *Haliotis kamtschatkana*. There was, however, evidence of anaerobic contribution to locomotion. In Chapter 2, the steep slope of $\log_{10}$ cost of transport *versus* $\log_{10}$ speed indicated that energy requirements decreased greatly at high speeds and suggested the possibility of an unmeasured anaerobic component. Likewise, Denny's (1980) measurements of cost of transport for the slug *Ariolimax columbianus*, made over a 24-hour period and thus accounting for oxygen debt, were an order of magnitude higher than those presented here for *H. kamtschatkana*, again suggesting an unmeasured anaerobic component to their locomotion.

Intertidal molluscs have generally well-developed anaerobic capabilities which enable them to cope with fluctuating environmental conditions. They have developed several strategies for increasing anaerobic capabilities, including generating different types of metabolic end-products via novel metabolic pathways and storing fermentable energy (Hochachka, 1980). For example, during functional anoxia (anoxia induced in muscles during exercise due to increased energy demand and lack of adequate blood flow) abalone use a novel anaerobic pathway that reduces glycogen-derived pyruvate to the compound tauropine via the enzyme tauropine dehydrogenase (Sato and Gade, 1986; Gade, 1988; Baldwin *et al.*, 1992). This pathway is similar to other novel pathways found in invertebrates, and these, in turn, are analogous to the lactate/lactate dehydrogenase pathway found in mammals. These novel anaerobic pathways are characterized by the accumulation of "opines" (octopine, strombine, alanopine, and tauropine), and they function to reduce pyruvate in the presence of an amino acid and NADH (Fields, 1983; Gade and Grieshaber, 1986). These pathways ensure a constant supply of ATP and a replenishment of NAD^+, critical for maintaining glycolytic flux, during periods of oxygen deprivation (Gade and Grieshaber, 1986).

Metabolic pathways and their endproducts may vary within an organism depending on the stage of anaerobiosis (early or late after onset; Hochachka and Mustafa, 1972), type of tissue (Gade, 1983, 1988), and type of anoxia (Gade 1983; Meinardus-Hager and Gade, 1986). In this respect, Gade (1988) noted that during functional hypoxia *Haliotis lamellosa* produced mostly tauropine in shell adductor muscle, while only D-lactate was formed in foot muscle. The authors found the same to be true during environmental anoxia (anoxia produced by lack of oxygen in the environment), and similar results have been reported by Baldwin *et al.* (1992) for *Haliotis iris*.

There are other compounds that indicate anaerobiosis besides opines in invertebrates. Phosphagens such as arginine phosphate are often used as a major source of energy by invertebrates in anaerobic conditions (Gade, 1983). In such cases, use of a phosphagen store during anaerobiosis is indicated by a decrease in arginine phosphate levels and an increase in arginine levels. For example, the major part (62%) of the escape response exhibited by the cockle *Cardium tuberculatum* is fueled by the breakdown of arginine phosphate (Meinardus-Hager and Gade, 1986) and, similarly, the major source of energy for swimming in the scallop *Placopecten magellanicus* is supplied by this substrate (de Zwaan *et al.*, 1980; Livingstone *et al.*, 1981). Gade (1988) additionally found that abalone subjected to environmental hypoxia exhibited decreased arginine phosphate levels, while those induced to right themselves until exhaustion did not.

Aerobic versus anaerobic contributions to invertebrate locomotion have been studied in a few organisms. Livingstone *et al.* (1981) found that 96% of the energy used by the phasic muscle of the swimming giant scallop *Placopecten magellanicus* was supplied anaerobically from the breakdown of ATP and arginine phosphate, and from the formation of octopine, while only 6% of the energy was derived aerobically. Conversely, swimming in the squid *Loligo pealeii* appears to be completely aerobic, based on the lack of anaerobic metabolite buildup after burst swimming (Storey and Storey, 1978). Anaerobic contributions to the shell-twisting escape behavior of whelks have also been investigated (Koormann and Grieshaber, 1980; Gade

et al. 1984), but aerobic contributions were not measured. To my knowledge, no study has been done comparing anaerobic and aerobic metabolic output during gastropod crawling.

Abalone, like other gastropods, have an open circulatory system. Blood flows from the heart through an arterial system and into sinuses. It is returned to the heart via a venous system (Crofts, 1929; Bourne and Redmond, 1977a), and flow is unidirectional due to a series of valves in and around the heart (Jones, 1983). Abalone have high peripheral resistance (Bourne and Redmond, 1977b) and low blood pressure (Jones, 1983) which, together, result in a slow circulation time. Since adequate blood flow is essential for continued aerobic respiration, it is possible that this open circulatory system may inadequately supply oxygen to muscle tissues during intense activity in abalone; hence, leading to greater dependence on anaerobic systems.

The purpose of this part of the study is to investigate the extent to which abalone use anaerobic metabolism during locomotion (opine dehydrogenases and arginine phosphate) and then to refine the calculation of cost of transport done in Chapter 2 to include anaerobic energy sources.

4.2 Materials and Methods

Determination of opine dehydrogenase activities in *Haliotis kamtschatkana*

In order to determine which opines may be produced by *Haliotis kamtschatkana* during anaerobiosis, tissues from adductor and foot muscles of *H. kamtschatkana* were first analyzed to determine which opine dehydrogenases were present. The selection of specific enzymes to be studied (shown below) was based on previous studies on opine dehydrogenases in other species of abalone (Gade, 1988; Baldwin *et al.*, 1992).

Approximately 1 g each of adductor and foot muscles were dissected from freshly shucked *Haliotis kamtschatkana* (N=5). The tissues were individually minced with chilled scissors and scalpel, and then immersed in 5 ml of ice-cold 50 mM imidazole-HCl buffer (containing 1mM EDTA and 1 mM dithiothreitol; pH 7.2). The tissues were homogenized with an Ultra-Turrex homogenizer and the homogenates centrifuged for 1 minute at 5000 rpm and

4°C. The supernatants were assayed within 1 hour. The compositions of the reaction mixtures for the various opine dehydrogenases were as follows: *tauropine dehydrogenase*: 80 mM taurine, 2.5 mM pyruvate, 0.15 mM NADH, 50 mM imidazole-HCl buffer, pH 7.0; *lactate dehydrogenase*: 2.5 mM pyruvate, 0.15 mM NADH, 50 mM imidazole-HCl buffer, pH 7.0; *octopine dehydrogenase*: 20 mM arginine, 2.5 mM pyruvate, 0.15 mM NADH, 50 mM imidazole-HCl buffer, pH 7.0; *strombine dehydrogenase*: 200 mM glycine, 2.5 mM pyruvate, 0.15 mM NADH, 50 mM imidazole-HCl buffer, pH 7.0; *alanopine dehydrogenase*: 100 mM alanine, 2.5 mM pyruvate, 0.15 mM NADH, 50 mM imidazole-HCl buffer, pH 7.0; *lysopine dehydrogenase*: 100 mM lysopine, 2.5 mM pyruvate, 0.15 mM NADH, 50 mM imidazole-HCl buffer, pH 7.0. The oxidation of NADH was monitored at 340 nm at 12°C with controls run simultaneously.

Preparation of tauropine dehydrogenase from abalone tissue

Since tauropine dehydrogenase was found to be one of the main opine dehydrogenases in both adductor and foot muscles (see Results) and is unavailable through supply companies, I purified it from adductor tissue of *Haliotis kamtschatkana* following the method of Gade (1986) as modified by Baldwin *et al.* (1992).

Approximately 10-g of adductor muscle was removed from a freshly shucked abalone, quickly diced with scissors, and suspended in 50 mM imidazole-HCl buffer (containing 1mM EDTA and 1 mM dithiothreitol; pH 7.2) at a 1:8 mass/volume ratio. The tissue was homogenized and the homogenate centrifuged for 15 min at 5000 rpm. The supernatant was decanted through filter paper and the pellet resuspended in buffer, homogenized, and centrifuged. The supernatants were pooled and applied to a column (45X2 cm) of DEAE-sephacel (Pharmacia LKB Biotechnology) which had been equilibrated with the homogenization buffer. The enzyme was eluted using a linear gradient of 0-250 mM NaCl in 800 ml buffer. Eluted fractions were tested for lactate dehydrogenase, malate dehydrogenase and tauropine dehydrogenase. Those fractions containing only tauropine dehydrogenase were pooled,

concentrated by membrane filtration (Diaflow), and dialyzed against 80% saturated ammonium sulfate. The purified enzyme was stored at 4°C until use.

A commercial preparation of D-lactate dehydrogenase, the other main opine dehydrogenase found to be present in *Haliotis kamtschatkana* (see Results), was purchased from Sigma, Corp.

Muscle metabolite levels under different experimental conditions

Effects of righting to exhaustion and air exposure on anaerobic metabolites. Abalone were collected from Barkley Sound near the Bamfield Marine Station, Vancouver Island, British Columbia, and transported to the University of British Columbia where they were held in tanks with a recirculating supply of seawater. They were randomly placed into three groups. The first group (N=5) was held undisturbed in the tank and comprised the control. Animals in the second group (N=10) were exercised by placing them on their backs and allowing them to right themselves until exhausted (determined when righting failed to occur within three minutes). To test the effects of environmental hypoxia, animals in the third group (N=12) were kept in moist, cool air for 16 hours. The abalone were removed from their shells immediately following their treatments, and samples of adductor and foot muscles were taken for the muscle metabolite analyses described below. The absolute number of individuals used for analysis of each metabolite was less than the starting totals owing to my initial unfamiliarity with the analyses and consequent wastage of some samples.

Effects of locomotion on levels of anaerobic metabolites. Animals were collected and transported as described above. Tissues from one group of animals (N=5) were sampled when these abalone were quiescent, and these comprised a control group. Another group (N=32) was exercised by inducing each abalone to locomote over a specific distance. This was achieved by touching them with a tube foot of the seastar *Pycnopodia helianthoides*. The time each animal took to locomote 6 m was recorded, thus providing its speed. Abalone were

removed from their shells immediately following their treatment, and samples of adductor and foot muscles were taken for muscle metabolite analyses.

Muscle metabolite assays

Approximately 1-g portions of foot and adductor muscles were removed from a freshly shucked abalone, immediately frozen in liquid nitrogen, weighed, and crushed. Samples were homogenized in ice-cold 6% perchloric acid (1:10 mass/volume) and a 1 ml subsample removed for glycogen analysis. The remaining sample was centrifuged for 15 min at 5000 rpm. The supernatants were neutralized with 5M K_2CO_3 and allowed to stand on ice for 2 h. Supernatants were removed and stored at -80°C until analysis. Similarly, the glycogen subsamples were neutralized and frozen until analysis.

D-lactate and tauropine were assayed in a hydrazine/glycine buffer (1 mM EDTA, pH 9.0). The reduction of NAD^+ was monitored at 340 nm. Commercial LDH was first added to the cuvette and the reaction allowed to run to completion. TDH was then added and the reaction again allowed to run to completion. Glycogen was determined following the method of Bergmeyer (1983). Arginine and arginine phosphate were measured following the procedure of Gade (1985). For arginine, the oxidation of NADH by the enzyme octopine dehydrogenase (Sigma) was monitored at 340nm. Arginine phosphate was determined as the increase in arginine following incubation of the samples in HCl and neutralization with NaCl.

4.3 Results

Opine dehydrogenase activities

Activities of opine dehydrogenase enzymes found in foot and adductor muscles of *Haliotis kamtschatkana* are presented in Table 4-1. Tauropine dehydrogenase was prominent in both adductor and foot with activities (±SE) of 29.0±2.5 IU·g wet tissue^{-1} and 20.1±2.9, respectively. Lactate dehydrogenase was next most abundant and was found in greater

Table 4-1. Opine dehydrogenase activities in adductor and foot muscles of the abalone *Haliotis kamtschatkana* (*N*=5). Values are presented as means ± SE.

	activity (IU·g wet muscle^{-1}min^{-1})	
Enzyme	Adductor	Foot
Tauropine DH	29.0 ± 2.5	20.1 ± 2.9
D-Lactate DH	1.2 ± 0.1	9.1 ± 0.7
Octopine DH	<0.2	<0.2
Strombine DH	<0.2	<0.2
Alanopine DH	<0.2	<0.2
Lysopine DH	<0.2	<0.2

quantities in foot (9.1±0.7) than in adductor (1.2±0.04). Only trace activities of other opine dehydrogenases were found (<0.2 IU·g wet tissue^{-1}).

Muscle metabolite levels under different experimental conditions

Effect of righting to exhaustion and air exposure on anaerobic metabolites. The only significant change for animals induced to right themselves to exhaustion was an increase in adductor tauropine (Table 4-2). Abalone exposed to air for 16-h showed increased levels of tauropine and also D-lactate in both foot and adductor muscles. As well, adductor muscle had significantly more arginine and less arginine phosphate as compared with controls.

Effect of locomotion on anaerobic metabolites. Maximum locomotory rate induced in this experiment was 9.9 body lengths·min^{-1}, a value close to the mean maximum rate of 12.7 body lengths·min^{-1} found in a later part of my study (see Chapter 6). Since none of the regressions of metabolite levels on speed were significant (all $t<1.77$, all $p>0.10$; data not shown here), the following analysis was performed. First, the results for each metabolite were pooled into two groups: those animals traveling slower than 5 body lengths·min^{-1} and those traveling faster than 5 body lengths·min^{-1}. An ANOVA comparing these two groups with the quiescent control animals, followed by Tukey's test, showed that abalone traveling at speeds slower than 5 body lengths·min^{-1} did not show any significant differences in metabolite levels as compared with control animals (Table 4-3). However, abalone moving at speeds faster than 5 body lengths·min^{-1} had increased levels of tauropine, lactate, and arginine in foot muscles.

4.4 Discussion

It is clear from this study that *Haliotis kamtschatkana* uses the same opine dehydrogenase enzymes during anaerobiosis as do other haliotids (Table 4-4), since tauropine dehydrogenase (TDH) and D-lactate dehydrogenase (LDH) were the only opine dehydrogenases found in substantial quantities in adductor and foot muscles. As well, relative

Table 4-2. Concentrations of anaerobic metabolites (μmol·g wet mass^{-1}) in foot and shell adductor muscles of *Haliotis kamtschatkana* induced to right themselves until exhausted and exposed to air for 16-h. Control animals were held undisturbed in a tank with fresh seawater. Values are expressed as means ± SE and the numbers of individuals used for each metabolite analysis are in brackets.

	μmol·g wet mass^{-1} (mean ± SE)		
Anaerobic metabolites	control	Righting to exhaustion	Air-exposed
Foot			
Tauropine	1.1 ± 0.2 (5)[a]	1.4 ± 0.2 (10)[a]	2.7 ± 0.4 (12)[b]
D-Lactate	0.1 ± 0.3 (5)[a]	0.9 ± 0.2 (10)[a]	2.4 ± 0.4 (12)[b]
Glucose	1.4 ± 0.5 (4)	0.9 ± 0.2 (4)	2.4 ± 0.5 (4)
Glycogen†	8.5 ± 1.6 (4)	10.0 ± 1.4 (4)	7.9 ± 0.9 (4)
Arginine	10.3 ± 1.5 (4)	15.6 ± 2.6 (5)	16.4 ± 4.2 (5)
Arginine-P	7.8 ± 3.0 (4)	3.7 ± 0.8 (5)	7.3 ± 2.1 (5)
Adductor			
Tauropine	0.5 ± 0.2 (5)[a]	2.4 ± 0.4 (10)[b]	3.8 ± 0.7 (12)[b]
D-Lactate	0.4 ± 0.6 (5)[a]	0.2 ± 0.04 (9)[a]	2.4 ± 0.4 (12)[b]
Glucose	1.7 ± 0.3 (4)	1.2 ± 0.2 (5)	1.8 ± 0.4 (4)
Glycogen†	8.6 ± 1.3 (4)	8.0 ± 1.7 (5)	6.0 ± 0.5 (4)
Arginine	14.7 ± 3.9 (5)[a]	26.1 ± 1.9 (5)[ab]	39.5 ± 5.0 (5)[b]
Arginine-P	15.5 ± 4.0 (5)[a]	6.6 ± 2.7 (5)[ab]	0.7 ± 5.0 (5)[b]

† glycogen measured as mg·g wet muscle^{-1} (mean ± SE)

[a,b] the presence of different superscripts indicates that values in a group are significantly different (ANOVA; all $F>3.72$, all $p<0.05$); the letters further denote homogenous subgroups (Tukey's test, $p<0.05$)

Table 4-3. Concentrations of anaerobic metabolites (µmol·g wet mass^{-1}) in foot and shell adductor muscles of *Haliotis kamtschatkana* locomoting at various speeds. Values are expressed as means ± SE and the numbers of individuals used for each metabolite analysis are in brackets.

	µmol·g wet mass^{-1} (mean ± SE)		
	Locomotory rate		
Anaerobic metabolites	quiescent	<5 body lengths·min-1	>5 body lengths·min^{-1}
Foot			
Tauropine	0.3 ± 0.1 (5)^{a}	1.1 ± 0.2 (16)^{ab}	1.5 ± 0.3 (16)^{b}
D-Lactate	0.1 ± 0.1 (5)^{a}	0.4 ± 0.2 (16)^{ab}	0.7 ± 0.1 (16)^{b}
Glucose	1.7 ± 0.5 (4)	1.4 ± 0.2 (6)	1.5 ± 0.2 (3)
Glycogen†	8.4 ± 1.6 (4)	6.6 ± 0.8 (6)	5.9 ± 0.7 (3)
Arginine	9.5 ± 1.7 (4)^{a}	12.5 ± 1.2 (11)^{ab}	17.0 ± 1.6 (7)^{b}
Arginine-P	9.2 ± 2.7 (4)	8.4 ± 2.9 (11)	4.9 ± 1.8 (7)
Adductor			
Tauropine	0.7 ± 0.2 (5)	1.1 ± 0.2 (16)	1.4 ± 0.3 (16)
D-Lactate	0.1 ± 0.2 (5)	0.1 ± 0.1 (16)	0.2 ± 0.1 (16)
Glucose	1.6 ± 0.3 (4)	1.3 ± 0.2 (7)	1.7 ± 0.3 (5)
Glycogen†	8.2 ± 1.4 (4)	7.2 ± 1.6 (7)	7.9 ± 1.4 (5)
Arginine	14.9 ± 5.0 (4)	18.0 ± 1.9 (11)	18.6 ± 2.4 (7)
Arginine-P	15.0 ± 4.5 (4)	13.6 ± 3.0 (11)	7.6 ± 2.4 (7)

† glycogen measured as mg·g wet muscle^{-1} (mean ± SE)

^{ab} the presence of different superscripts indicates that values in a group are significantly different (ANOVA; all F>3.72, all p<0.05); the letters further denote homogenous subgroups (Tukey's test, p<0.05)

Table 4-4. Tauropine dehydrogenase (TDH) and D-lactate dehydrogenase (LDH) levels for five species of abalone. Values represent μmol·g muscle^{-1}.

	TDH		LDH		
Species	adductor	foot	adductor	foot	
H. kamtschatkana	29	20	1.2	9.1	present study
H. iris	93	78	4.3	5.6	Baldwin *et al.* (1992)
H. iris	84	64	2.3	2.6	Wells and Baldwin (1995)
H. australis	66	28	2.6	4.3	Wells and Baldwin (1995)
H. lamellosa	44	7	2	11	Gade (1988)
H. lamellosa	40	n.d.	5	n.d.	Sato and Gade (1986)
*H. discus hannai**	12		4		Sato and Gade (1986)

* muscle types not analyzed separately for this species
n.d. = not done

levels between the two muscle types follow the same pattern as other abalone with TDH higher than LDH in both tissues, but with increased LDH found in the foot compared to the adductor. It is interesting that foot LDH is relatively high compared to most of the other species (*H. lamellosa* is the only species with higher levels). Since gastropods which have high levels of anaerobic enzymes are generally those which are often subjected to anaerobic conditions (Morris and Baldwin, 1984), the relatively high levels of foot LDH may indicate that *H. kamtschatkana* is more often subjected to functional hypoxia, and thus possibly more active, than the other species. However, this is purely speculative owing to the lack of information on relative levels of activity of the different species.

The only response of *Haliotis kamtschatkana* to functional hypoxia induced by righting to exhaustion was a 5-fold rise in adductor tauropine (Table 4-2). Thus, it appears that TDH supplies at least some of the energy needed in the adductor for righting, while any energy used by the foot muscle is supplied aerobically. In contrast to this, Gade (1988) and Baldwin *et al.* (1992) found accumulations of both tauropine and lactate in both adductor and foot muscles of *H. lamellosa* and *H. iris*. This indicates a broader response to righting-induced functional hypoxia in these species, with both muscle types deriving energy anaerobically.

When *H. kamtschatkana* was subjected to 16 hours of exposure to moist air, anaerobic energy was supplied by arginine phosphate stores as well as opine dehydrogenases (Table 4-2). In adductor muscle, arginine phosphate levels decreased 22-fold, concomitant with a 2.7-fold rise in arginine levels, while foot levels were unaffected. However, both muscle types exhibited significant increases in tauropine and D-lactate levels. Gade (1988) found similar results in *H. lamellosa* subjected to six hours of anoxia, although in this case arginine phosphate levels in both adductor and foot were affected. Similarly, Baldwin *et al.* (1992) and Wells and Baldwin (1995) found increased levels of tauropine and lactate in both adductor and foot muscles during air exposure in *H. iris* and *H. australis*, respectively.

In this study, functional hypoxia was also investigated by inducing abalone to locomote at a variety of speeds. Changes in metabolite levels indicated that only the foot muscle became anaerobic during locomotion and only at high speeds (Table 4-3). Both tauropine and lactate

levels increased significantly in foot muscle compared with quiescent abalone, but only in animals traveling five body lengths·min^{-1} or faster (there was, however, statistical overlap with the slower group). The evidence for the use of arginine phosphate as an energy source during locomotion was not as clear. Arginine levels showed a significant 1.8-fold increase in foot muscle, but although arginine phosphate levels decreased, this change was not significant, probably due to the large variability in these analyses. Any energy used by the adductor muscle during locomotion appeared to be derived from aerobic sources, as no changes in any of the anaerobic metabolites were found at either high or low speeds.

The significant accumulation of anaerobic endproducts only at high speeds might be explained given the relatively low levels of TDH and LDH in foot muscle of *H. kamtschatkana* compared with adductor muscle. Morris and Baldwin (1984) found that species of gastropods and crustaceans which use more anaerobic energy than other species have higher levels of anaerobic enzymes. Furthermore, tissues which often function anaerobically within an organism will also contain higher levels of these enzymes compared with tissues which primarily function aerobically. Both Baldwin *et al.* (1992) and Gade (1988) suggest that the high levels of TDH in adductor muscle of abalone are a reflection of that muscle's need for quick energy in order to pull the shell close to the substratum in times of danger or stress. Additionally, Gade (1988) suggests that the low levels of TDH and LDH found in foot muscle of *Haliotis lamellosa* is due to the possibly aerobic nature of the slow adhesive gliding for which the foot functions. Given that abalone usually locomote at relatively low speeds (about one body length·min^{-1}; Chapter 3), and given the lower levels of TDH and LDH in *H. kamtschatkana* foot compared with adductor (Table 4-1), it might be expected that foot muscle only resorts to anaerobic metabolism at high speeds.

Given the accumulation of anaerobic metabolites at high speeds, it is now possible to compare amounts of energy derived from anaerobic and aerobic sources during rapid locomotion in abalone. Of all the anaerobic metabolites measured, only foot tauropine, lactate, and arginine changed significantly (Table 4-3). In this study, since abalone locomoted 6 m before tissue samples were taken, these changes in metabolite concentrations represent the

amounts of anaerobic metabolites generated per gram of foot muscle over a 6-m distance. The average abalone traveling faster than 5 body lengths·min^{-1} was 9.2 cm in length and 107 g in live mass. Since total live mass of *H. kamtschatkana* represents 29% muscle (Chapter 1), and my observations indicate that foot comprises 50% of abalone muscle, the average foot mass would have been about 15 g. Taking the values for increases in tauropine and lactate (1.2 µmol·g foot^{-1} and 0.7 µmol·g foot^{-1}, respectively; Table 4-3) and the conversion of 1.5 µmol ATP·µmol tauropine or lactate^{-1}, it can be calculated that 42 µmol of ATP were produced from the reduction of these amounts of taurine and pyruvate. Additionally, although 7.5 µmol·g foot^{-1} of arginine were produced (presumably from the breakdown of arginine phosphate), only 4.3 µmol·g foot^{-1} of arginine phosphate were lost (Table 4-3). Using this smaller value and the equivalence of 1 µmol ATP·µmol arginine phosphate^{-1}, it can be further calculated that 64 µmol of ATP were produced from arginine phosphate. This yields a total of 106 µmol of ATP from anaerobic sources.

The amount of O_2 an average abalone from this study needs to travel 6 m can now be calculated. For an abalone traveling faster than 5 body lengths·min^{-1}, average speed was 6.6 body lengths·min^{-1} (which corresponds to 61 cm·min^{-1}), and average time of locomotion was 10 min. Inserting average speed (cm·min^{-1}) and average mass (g) into the regression of mass specific V_{O2} on speed (Fig. 2-1) yields 59.4 µl O_2·g^{-1}h^{-1}. Thus an 107-g abalone locomoting for 10 min requires 1060 µl O_2 (equivalent to 47 µmol O_2) to travel 6 m. Given the generation of 6 µmol ATP per µmol O_2, this yields a total of 282 µmol of ATP from aerobic sources. Thus, of all energy needed for *H. kamtschatkana* to locomote at high speeds (total cost of transport; 106 µmol of ATP from anaerobic sources and 282 µmol of ATP from aerobic sources, for a total of 388 µmol of ATP), 27% comes from anaerobic and 73% from aerobic sources. If resting metabolism (20.7 µl O_2·g^{-1}h^{-1}) and postural costs (19.4 µl O_2·g^{-1}h^{-1}) are subtracted from total oxygen consumption (assuming these costs are met aerobically), then the 107-g abalone uses 19.3 µl O_2·g^{-1}h^{-1} which converts to 344 µl O_2 or 90 µmol ATP. In this case, anaerobic metabolism would account for 54% of transport costs (minimum cost of transport).

It should be noted that the anaerobic component of high-speed locomotion has possibly been underestimated in this study since the breakdown of ATP stores was not considered. However, Gade (1988) found no significant changes in adductor or foot adenylate levels in abalone induced to right until exhausted. Thus, if ATP stores supply energy during locomotion, the contribution is probably small compared to energy derived from arginine phosphate and opine dehydrogenases.

Addition of this 54% anaerobic component to the minimum cost of transport (COT) determined for *Haliotis kamtschatkana* in Chapter 2 would lead to an increase in COT from 20.3 to 44.1 $J \cdot kg^{-1}m^{-1}$ (20.3, the aerobic component, is 46% of 44.1) This new value places abalone above the regression line of COT on mass for running vertebrates (Fig. 4-1), indicating that transport costs for abalone are more expensive than for a similar-sized running vertebrate. Abalone COT remains within an order of magnitude of Denny's (1980) estimation of COT for the slug *Ariolimax columbianus* (912 $J \cdot kg^{-1}m^{-1}$).

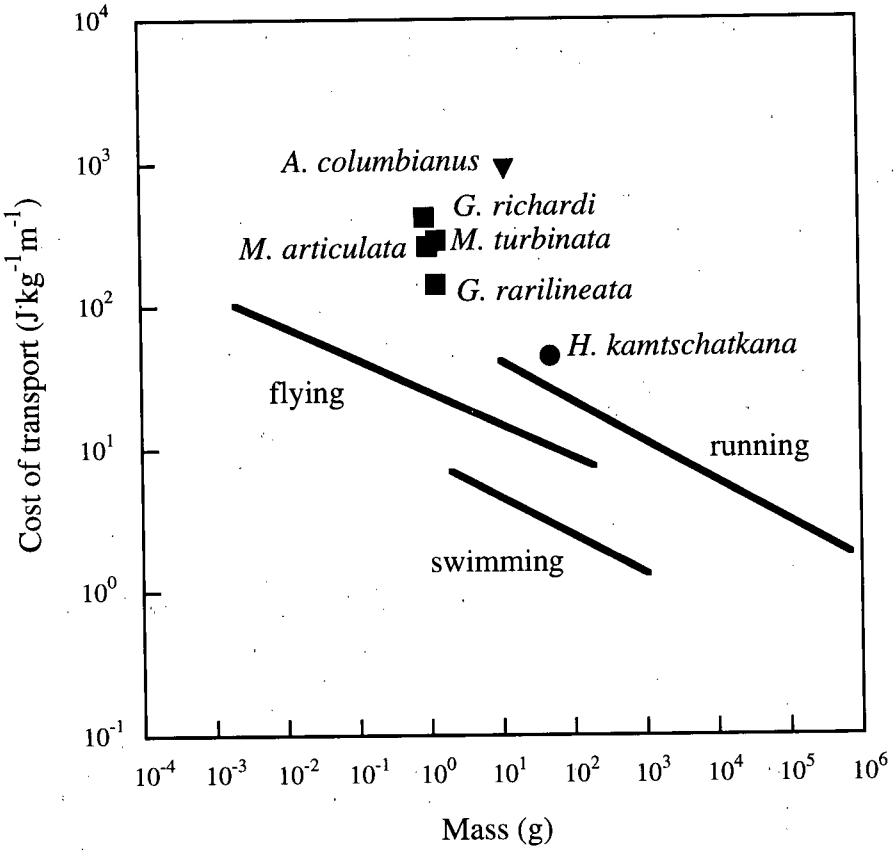

Fig. 4-1. Minimum cost of transport of abalone (*Haliotis kamtschatkana*) compared with that of other marine snails (*Monodonta turbinata, M. articulata, Gibbula richardi, G. rarilineata*; Houlihan and Innes, 1982) and a terrestrial slug (*Ariolimax columbianus*; Denny, 1980). Regression lines for running, flying, and swimming animals were taken from Schmidt-Nielsen (1972). This figure is similar to figure 2-3 except that the value for *H. kamtschatkana* has been revised to include energy from anaerobic metabolism.

Chapter 5. Blood-glucose levels and oxygen consumption of abalone exposed to environmental stressors

5.1 Introduction

In Chapter 3 I calculated that activity accounted for 25% of energy consumed by laboratory-held *Haliotis kamtschatkana* during summer. I concluded that activity probably represented a greater proportion of consumed energy for field abalone since they are more active than laboratory-held animals (Figs. 3-1 and 3-2), and I suggested that the presence of predators could be one reason for these differences in activity. In this chapter, I explore the effects of environmental stress, including that induced by a predator, on the summer energy budget of *H. kamtschatkana.*

A variety of biological indicators are used to determine the effects of environmental stressors on metabolic processes (see Bayne, 1985). As was shown in Chapter 4, the build-up of tauropine and D-lactate reflect a shift to anaerobic metabolism during both functional and environmental hypoxia in *H. kamtschatkana.* Similarly, Wells and Baldwin (1995) used the relative accumulation of these metabolites to evaluate the effects of stress during transport from field to laboratory in two species of abalone. One widely used indicator of stress is change in blood-glucose levels. These have been used to detect environmental stress in crustaceans (Telford, 1968, 1974; Johnson and Uglow, 1985; Santos and Nery, 1987; Spaargaren, and Haefner, 1987; Taylor and Spicer, 1987; Spicer *et al.,* 1990), but have only recently been investigated in gastropods. For example, Ram and Young (1992) found that mild electric shock caused a 50-150% rise in blood glucose levels in *Aplysia californica*, and Carefoot (1994) found that natural stressors such as changes in salinity and temperature, as well as air exposure, caused 1.5-2.3-fold increases above baseline levels in *A. dactylomela.* Although the mechanism leading to increased blood-glucose levels has been well characterized in vertebrates (Pickering, 1981; Tepperman and Tepperman, 1987), it is not as well understood in invertebrates. Whatever the mechanism involved, a rise in blood glucose clearly occurs to

support increased metabolic demands during times of stress in a wide range of invertebrates (Santos and Nery, 1987; Spaargaren and Haefner, 1987; Taylor and Spicer, 1987; Spicer *et al.*, 1990). Thus, one would also expect metabolic rate to increase during stressful periods in abalone. The effects of predator presence on blood-glucose levels in gastropods has not been examined. *Haliotis kamtschatkana* exhibits an energetic escape response when contacted by the seastar *Pycnopodia helianthoides* (see Chapter 2), culminating in rapid locomotion away from the threat. However, even when actual contact does not occur, abalone are more alert and active in the presence of *Pycnopodia* (see Chapter 3). It is possible that merely the presence of *Pycnopodia* can cause changes in blood-glucose levels, and consequently in metabolism, in *H. kamtschatkana.*

Increased oxygen consumption has also been used to determine the impact of stress on invertebrates (Widdows, 1985), but is confounded by the fact that many invertebrates are oxygen conformers. Thus, stressors which change levels of ambient oxygen, such as temperature and salinity, may have multiple effects. However, in the case of predator detection by abalone, an increase in oxygen consumption should indicate a stressful situation.

The purpose of this part of the study is to determine changes in blood-glucose level and oxygen consumption of *Haliotis kamtschatkana* in the presence of a predator in order to investigate the effects of this type of stress on energy allocation. In addition, stress responses to air exposure, temperature, and salinity will be investigated to determine if these stressors could also cause metabolic changes in abalone, and for comparison with other gastropods.

5.2 Materials and Methods

Animal collections

Abalone were collected from Barkley Sound near the Bamfield Marine Station, British Columbia, and held in large outdoor tanks exposed to natural lighting with a constant supply of fresh seawater. They were fed *ad libitum* on *Macrocystis integrifolia*, a preferred kelp food.

Blood-glucose levels

For each experiment, animals were divided into experimental and control groups of equal mean live mass (N=5-8 for each). Hemolymph samples (0.3 ml) were drawn from the pericardial sinuses of individuals using a hypodermic syringe and #21 needle. All samples were immediately transferred to 1 ml containers and frozen for later analysis. Samples were analyzed spectrophotometrically for glucose using the hexokinase method (Sigma Diagnostics Glucose Kit).

In the first experiment, designed to test the effects of exposure to a predator on blood-glucose titers, abalone (63-85 g live mass) were divided into two groups each of N=8. Each group was placed in a tank with a constant supply of fresh seawater at 12°C. At time-zero, a blood sample was taken from each individual. Five predatory seastars *Pycnopodia helianthoides* were then placed into the experimental tank for 30 min. Immediately following removal of the seastars, blood samples were again taken. Subsequent samples were taken hourly for the next three hours.

The second experiment tested the effects of air exposure on blood-glucose titer. Abalone (58-79 g live mass) were divided into two groups each of N=5. A blood sample was taken from each individual. The experimental group was then removed from seawater, placed into a container with moist toweling, and floated in the tank to maintain ambient temperature (12°C). The control group was left in the tank. Blood was drawn from each animal every 1.5 h over a 6-hour period.

To test the effects of temperature on blood-glucose titer, abalone (61-78 g live mass) were divided into two groups of N=5 each and blood samples taken from all individuals. The experimental animals were placed in a 10-liter container of 20°C seawater. After one hour, blood samples were taken from each individual in both control and experimental groups, and the experimental animals were returned to seawater at ambient temperature (12°C). Blood samples were taken every hour for the next three hours from all animals.

In the final experiment, designed to test the effects of salinity stress on blood-glucose level, abalone (60-80 g live mass) were divided into two groups of N=5 each and blood

samples taken from all individuals. The experimental animals were placed in a 10-liter container of seawater at 50% salinity, prepared by diluting a volume of ambient seawater with an equal volume of distilled water at the same temperature (12°C). After one hour, a blood sample was taken from each individual in both control and experimental groups, and the experimental animals were returned to seawater at ambient salinity (32‰). Blood samples were taken every hour for the next four hours from all animals.

Oxygen consumption

Individual abalone (N=9; 60-178 g live mass) were held in flow-through respirometers (0.8 l volume, 40 ml·min^{-1} flow rate) for 60 min to establish baseline rates of oxygen consumption. Oxygen levels were monitored continuously with a Polarographic oxygen electrode connected to a computerized data acquisition system (Datacan, Sable Systems, Inc.). After 60 min, the seawater entering each respirometer was changed from fresh seawater to seawater flowing from a container (10-liter vol) holding five predatory seastars, *Pycnopodia helianthoides* (10-20 cm diameter). Oxygen consumptions of individual abalone were monitored continuously for 60 min following the change of seawater.

Results from repetitive sampling of experimental and control groups were analyzed by repeated-measures analysis of variance (R-MANOVA) coupled with a Student-Newman-Keuls test. In cases where the assumptions for R-MANOVA were not met (ie., such as when distributions were not normal), a Friedman repeated-measures analysis of variance (Friedman R-MANOVA), coupled with a Student-Newman-Keuls test, was used.

5.3 Results

Blood-glucose levels

Exposure to *Pycnopodia* caused a significant change in blood-glucose titers in the experimental abalone over time (X^2=13.5, p=0.009, Friedman R-MANOVA; Fig. 5-1), with

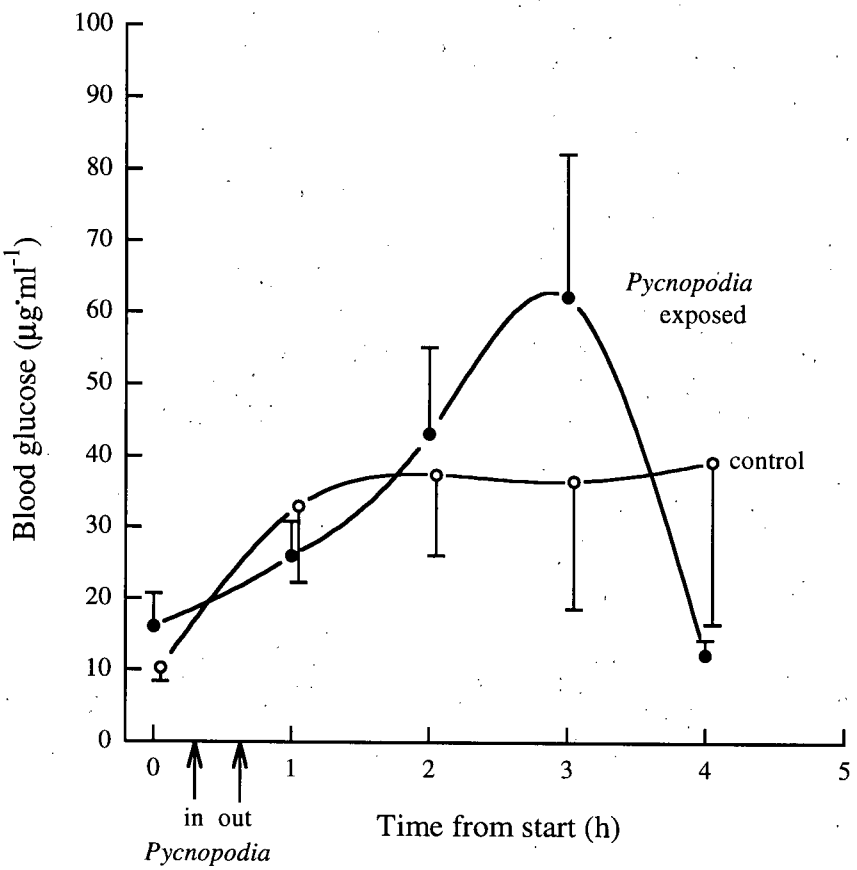

Fig. 5-1. Blood-glucose levels of *Haliotis kamtschatkana* (*N*=8) exposed to the predatory seastar *Pycnopodia helianthoides* along with control animals (*N*=8). Each point represents a mean and vertical bars show standard errors of the mean.

the values at 1-, 2-, and 3-h following the start of the experiment forming a statistically homogenous subgroup (Student-Newman-Keuls, $p<0.05$). A maximum level (±SE) of 62±20 $\mu g \cdot ml^{-1}$ was reached 2.5 h after exposure to the seastars. Blood-glucose levels fell to below control levels by 3.5 h post-exposure. Control levels did not change significantly ($X^2=3.2$, $p=0.53$, Friedman R-MANOVA).

Air exposure also caused a significant change in blood-glucose levels ($F_{4,16}=13.46$, $p<0.001$, R-MANOVA; Fig. 5-2), with values at 3-, 4.5-, and 6-h following the start of the experiment forming a statistically homogenous subgroup which was significantly larger than values at 0- and 1.5-h (Student-Newman-Keuls, $p<0.05$). As in the previous experiment, blood-glucose levels of control animals did not change significantly ($F_{4,16}=2.60$, $p=0.08$, R-MANOVA).

Neither an increase in temperature nor decrease in salinity caused significant changes in blood glucose (both $X^2<4.7$, both $p>0.43$, Friedman R-MANOVA; Figs. 5-3 and 5-4).

Oxygen consumption

To remove the influence of mass on oxygen consumption (V_{O2}), V_{O2}'s for all abalone were converted to that of a "standard" 100-g animal by the equation:

$$V_{O2}\ (x\ g) = (100/\text{experimental mass})^b (V_{O2}\ \text{experimental})$$

where x is 100 g and b is 0.74, the experimentally derived scaled $\log_{10}$-$\log_{10}$ relationship between resting metabolic rate and abalone mass determined previously (Chapter 1).

V_{O2} increased significantly by 30% from a control mean (±SE) of 1.5±0.2 $ml \cdot hr^{-1}$ to 1.9±0.2 $ml \cdot hr^{-1}$, after water previously in contact with seastars was introduced to the respirometer ($t=2.26$, $p=0.05$). Seven animals shifted from a quiescent state to an alert or active state following introduction of the *Pycnopodia* water, and two also exhibited escape locomotory behavior.

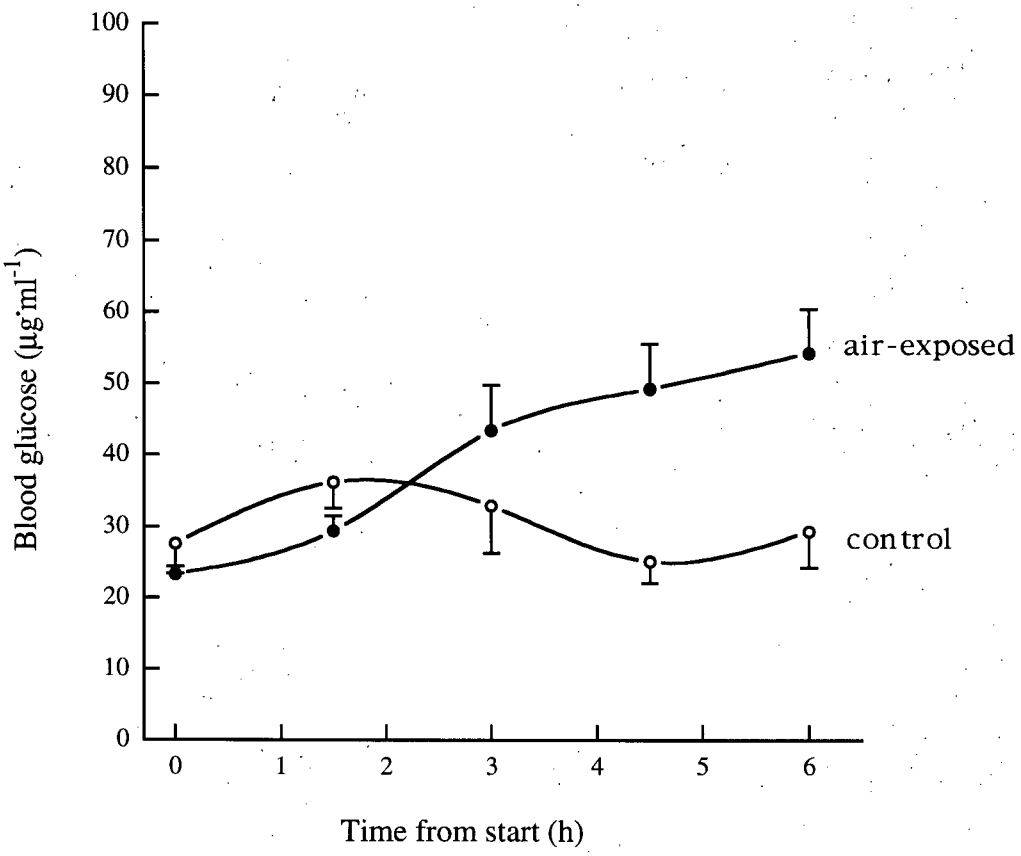

Fig. 5-2. Blood glucose levels of *Haliotis kamtschatkana* (*N*=5) during air exposure along with control animals (*N*=5). Each point represents a mean and vertical bars represent standard errors of the mean.

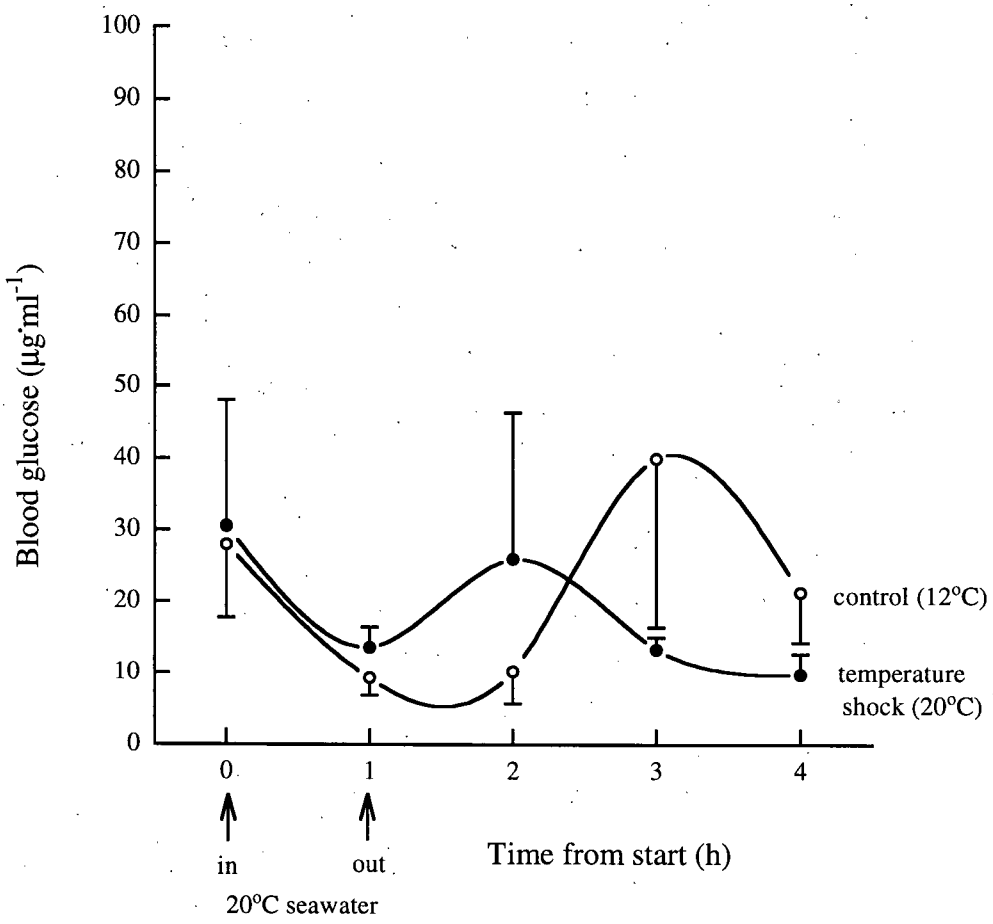

Fig. 5-3. Blood-glucose levels of *Haliotis kamtschatkana* (*N*=5) exposed to 20°C temperature shock along with control animals (*N*=5) at ambient temperature. Each point represents a mean and vertical bars represent standard errors of the mean.

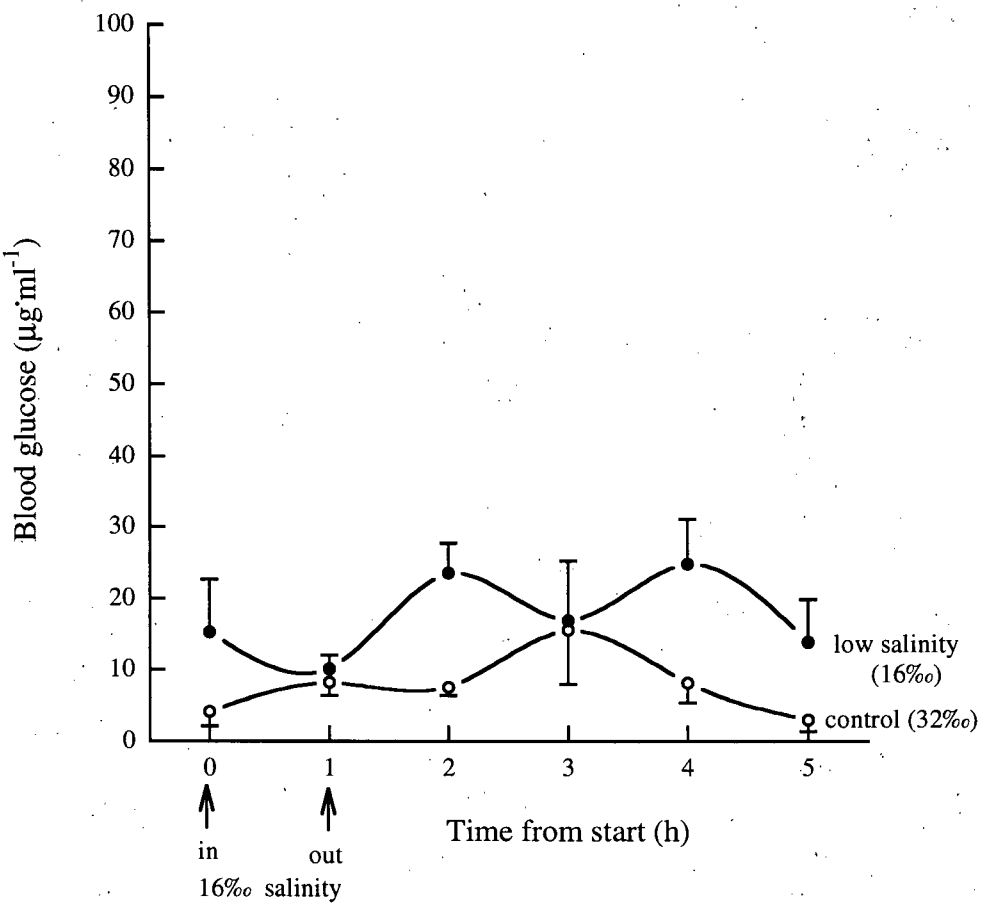

Fig. 5-4. Blood-glucose levels of *Haliotis kamtschatkana* (*N*=5) subjected to salinity shock along with control animals (*N*=5) maintained at ambient salinity. Each point represents a mean and vertical bars represent standard errors of the mean.

5.4 Discussion

This part of the study has shown that the presence of a seastar predator causes increased metabolism in *Haliotis kamtschatkana* in the form of increased oxygen consumption and higher blood-glucose levels. The rise in oxygen consumption exhibited by *H. kamtschatkana* during exposure to the seastar *Pycnopodia helianthoides* can be explained by an increase in activity. The 30% increase in V_{O2} of the test abalone after *Pycnopodia* water was introduced into the respirometer was close to the 33% difference in V_{O2} between abalone in quiescent and alert states found earlier (Chapter 3). Although only two of the test abalone showed actual escape behavior, all were clearly in alert states following detection of the seastar. It is interesting that the predator did not need to be in contact with the abalone to produce these results; rather, compounds presumably exuded into the water were enough to affect the metabolic rate of the abalone. It is well known that gastropods are able to detect minute concentrations of chemicals from food (Kohn, 1961; Carr, 1967; Carefoot, 1982) and that homogenized extracts of predators will elicit escape responses (Bullock, 1953; Kohn, 1961).

Blood-glucose levels were apparently affected by both exposure to *Pycnopodia*. Although blood-glucose levels of both control and experimental rose at the beginning, probably due to the repeated blood sampling, animals exposed to *Pycnopodia* had blood-glucose levels 2x those of control animals 2.5 h after the seastars had been removed from the tank containing the abalone (Fig. 5-1). This increase is possibly explained by mobilization of energy stores in preparation for the escape response. However, Thompson *et al.* (1980) found that the scallop *Placopecten magellanicus* induced to valve snap for 2 min by repeated stimulation with a homogenate of the seastar *Asterias vulgaris* did not exhibit significant changes in blood-glucose levels. Unfortunately, the experimental design (all experimental animals in one tank, all control animals in another tank) leaves doubt about these results. It is possible that some other tank effect caused the changes in blood-glucose levels and it is thus impossible to conclude that seastar presence was the cause of the increase in blood-glucose titers. However, the observed increase in respiration after exposure to *Pycnopodia* is evidence that predator presence does

indeed affect abalone metabolism, thus blood-glucose levels may indeed be elevated by detection of a predator.

Air exposure for 6 h caused a 2-fold increase in blood-glucose titers in *H. kamtschatkana* (Fig. 5-2), which is comparable to the 1.5-fold increase found by Carefoot (1994) in *Aplysia dactylomela* exposed to air for 1.5 h. These values are also similar to those found for crustaceans (2.8-fold increase after 3 h in air for *Nephrops norvegicus*, Spicer *et al.*, 1990; 2-fold increase after 4 h in air for *Carcinus maenas* and 4-fold increase after 4 h in air for *Liocarcinus puber*, Johnson and Uglow, 1985; and 4-fold increase after 2 min in air for crayfish, Telford, 1974). It has been suggested that increased blood-glucose levels in Crustaceans is related to mobilization of energy stores for anaerobic metabolism (Spicer *et al.* 1990) and this may also be the case for *H. kamtschatkana* since they make extensive use of anaerobic metabolism during periods of anoxia (Chapter 4). However, the elevated blood-glucose titers in *H. kamtschatkana* exhibited in this experiment may also be a more general response to a stressful situation since *H. kamtschatkana* rarely encounter air exposure owing to their subtidal habit of life. In this case, mobilization of energy reserves may be in preparation for increased activity to escape the perceived threat. Again, the experimental design casts doubt about the true cause of the changes in blood-glucose levels.

Neither temperature nor salinity shock caused significant changes in blood-glucose titers in *H. kamtschatkana* (Figs. 5-3 and 5-4). In comparison, Carefoot (1994) found elevating effects with both these stressors in *A. dactylomela*, with a 2-fold increase occurring when sea hares were placed in 50% seawater for 2 h and a 1.6-fold increase when they were held in aerated tidepools for 5 h with temperature rises of 6.5°C. Although the evidence showing increased blood-glucose levels during salinity shock in gastropods is minimal, more work has been done in this regard on crustaceans. Interestingly, crustaceans in both hypo- and hyperosmotic conditions exhibited decreased blood-glucose levels (in contrast with the increased levels found by Carefoot, 1994). For example, the brown shrimp *Crangon crangon* exhibited lowest blood-glucose levels at extreme salinities of 5 and 37 ‰ (Spaargaren and Haefner, 1987), and titers in an estuarine crab *Chasmagnathus granulata* dropped to zero at

extreme salinities of 0 and 40 ‰ (Santos and Nery, 1987). Authors of both studies attribute the decreases to increased metabolic demands for osmoregulation. This might also partially explain the lack of change in blood-glucose titers in *Haliotis* and the rise in *Aplysia*. Both are osmoconformers and so would not need energy for osmoregulation. Increased blood-glucose levels in *Aplysia* could be related to other stress-induced responses such as increased alertness.

Thus, both increased oxygen consumption and increased blood-glucose levels during exposure to *Pycnopodia* likely indicate rising metabolic demands for *Haliotis kamtschatkana*. In Chapter 3 I determined that repeated bouts of locomotion resulting from predator evasion could seriously affect the energy budgeting of *H. kamtschatkana*, drawing energy from other allocations and potentially leading to decreased growth and fecundity. It now appears that even the presence of predators (without actual contact and resultant escape locomotion) could cause shifts in energy-budget allocations away from growth as respiratory demands increase. The degree to which this might happen can be estimated from the rise in oxygen consumption following exposure to *Pycnopodia*. Because blood-glucose levels remain elevated, compared with controls, for a 1-hour period following *Pycnopodia* exposure, and assuming that this is accompanied by a concomitant elevation in oxygen consumption, a single exposure to *Pycnopodia* would cost a 100-g abalone 8 J (0.4 ml O_2 multiplied by an oxycalorific coefficient of 20.88 J·ml O_2^{-1}). This represents 0.3% of daily consumed energy during the summer (2880 J for a 100-g abalone, calculated from the $\log_{10}$-$\log_{10}$ regression of consumption on mass, Table 1-1). Although this is small relative to the components of the daily energy budget, repeated exposure to *Pycnopodia* would have the potential to increase substantially the respiratory component of the energy budget of *Haliotis kamtschatkana*. Importantly, this would occur even in the absence of escape locomotion.

Chapter 6. Changes in pedal morphology during locomotion as possible energy-saving strategies for abalone

6.1 Introduction

Abalone display a dramatic escape response when confronted with seastar predators. The response begins with a vigorous twisting of the shell to dislodge the predator's tube feet, followed by rapid locomotion away from the threat. Escape locomotion is visibly different from ordinary locomotion and is characterized by an elevated shell, large-amplitude pedal waves, and a trough running from anterior to posterior such that the middle of the foot is lifted off the substratum. The energetically expensive nature of adhesive crawling (Denny, 1980) suggests that these characteristics of high-speed locomotion, in addition to providing propulsive force, may also have energy-saving benefits to allow abalone to locomote more rapidly than might otherwise be possible.

Most gastropods locomote by generating rhythmic waves on the ventral surface of their broad pedal muscle. The wave pattern differs among species depending on the direction in which the wave travels and the portion of the foot it occupies. Waves that travel from the back of the foot to the front (i.e. in the same direction as the animal is traveling) are direct waves, while waves traveling from front to back are indirect, or retrograde. A wave that reaches from one side of the foot to the other as it travels along the foot is monotaxic, while a wave that occupies only half the foot is ditaxic. In this case, another wave out of phase with the first is found on the other half of the foot. Most marine gastropods are ditaxically retrograde, while the abalone is ditaxically direct. In most marine gastropods, the waves are generated rhythmically (Miller, 1974; Trueman, 1983).

Gastropods with rhythmic pedal waves increase speed by increasing step frequency and/or step length. A step is the distance that a point on the pedal sole travels when a wave passes through it (Miller, 1974). Step frequency can be increased by increasing the velocity of the pedal waves or by increasing the number of waves present on the foot at any given time.

Step size can be increased by changing the length or amplitude of the pedal waves. Abalone increase their locomotory rate by increasing the velocity and, hence, the frequency of their pedal waves (Voltzow, 1986), and there is no evidence in the literature that they change pedal wave length. Miller (1974) describes several gastropod species which dramatically increase pedal wave length in order to increase speed. For example, *Tegula funebralis* increases its wave length from approximately one-third of total foot length during normal locomotion to greater than half of total foot length during escape. Conversely, *Calliostoma ligatum*, a species with waves similar in type and size to *Haliotis* spp., apparently does not change the size of its waves. Miller (1974), however, measured only the length of the waves; there may be other more subtle changes to wave area during rapid locomotion that could be measured.

In fact, characteristics of the escape response described above suggest that the wave morphology of *Haliotis* spp. does change during rapid locomotion. First, abalone differ from most other gastropods in that their pedal waves are lifted off the substratum as opposed to gliding across a mucus layer, and amplitude (the distance that the wave is lifted off the substratum) is known to increase with increasing velocity (Miller, 1974). One might expect that an increase in wave amplitude could increase speed since more propulsive force could be generated as the wave pushes off the substratum, but it may also decrease the energy needed for locomotion since, as the waves are lifted off the substratum, they travel over water instead of mucus. In this case, less force is required because water is less viscous than mucus. Second, at rapid escape velocities, abalone often lift up the middle portion of their foot, and the only part in contact with the substratum is a band along each side. Such a strategy of removing a portion of the foot from being able to produce waves which make contact with the ground and thus generate propulsive force, does not seem likely to contribute to an increase in speed. Rather, it seems designed purely for energy saving since, again, less force would need to be produced when more of the foot is traveling over water. Decreasing pedal surface area would also be energetically beneficial in that less mucus would need to be produced. Adhesive crawling involves the secretion of a thin layer of mucus from glands in the foot to form a sheet between the foot and the substratum. Mucus production may be a primary reason that adhesive crawling

is so energetically expensive compared with other forms of locomotion (Denny, 1980). While these morphological and physiological changes are noticeable at high speeds, it is not clear to what extent they are employed at lower speeds. Subtle changes to the pedal sole may occur such that energy is conserved throughout a range of speeds.

The purpose of this study is to determine if pedal sole morphology changes during locomotion in abalone in such a way that energy could be conserved.

6.2 Materials and methods

Animal collections

Abalone were collected at the Bamfield Marine Station, British Columbia and transported to the Shannon Point Marine Station, Washington. They were held in large tanks with a continuous supply of fresh sea water at a mean temperature of 10°C during the experiments. The abalone were fed *ad libitum* on kelp, *Nereocystis luetkeana*.

Frequency of pedal waves

Wave frequency was compared with speed by counting the waves during periods (usually 20 s duration) in which abalone were moving at a constant speed. Pedal waves were video-taped as abalone (N=8; 39-58 g live mass) locomoted at different speeds across the side of a glass aquarium. A pedal wave is defined as a portion of the foot which is contracting and is visible as a dark region moving across the pedal muscle from posterior to anterior (Fig. 6-1). A stopwatch within the camera recorded elapsed time onto the film so that a period of constant speed could be selected, and the number of waves traveling up the foot during that period were counted using the OPTIMAS digital analysis system (Bioscan, Inc., Washington).

Pedal area and pedal wave variables during locomotion

Abalone (N=8; 13-51 g live mass) were video-taped as described above against a grid of 1 cm x 1 cm markings placed along the side of the aquarium for size and distance reference.

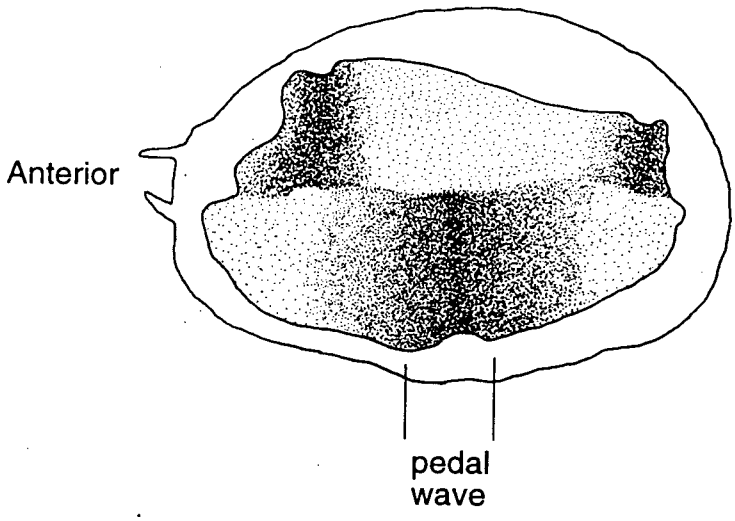

Fig. 6-1. Ventral view of a locomoting abalone as seen through a glass plate. The dark areas represent pedal waves.

Speeds during periods of constant locomotion (five for each animal) were calculated, then a frame from each of these time periods was captured onto computer and measurements made of the abalone's foot. These measurements included total foot area (defined as the area of the foot visible through the glass), pedal wave area (the area of the waves, identified as dark regions on the foot), foot length and foot width. Care was taken to analyze the same wave configuration for each animal. Specifically, the foot was analyzed when one wave on the right side of the foot was at the midpoint of the foot and the two waves on the left side were at the front and back of the foot (see Fig. 6-1).

Maximum speed

Abalone (N=10; 9-135 g live mass) were placed individually in a large tank which had markers every 10 cm along its side. A live predatory seastar *Pycnopodia helianthoides* was held behind the abalone to stimulate rapid escape locomotion. As the abalone locomoted between markers, the seastar was continuously held behind the abalone to simulate a chase. The movement of the abalone was timed between the markers, and maximum velocity was recorded along with the animal's shell length and live mass.

6.3 Results

Pedal morphology

Pedal wave frequency increased linearly with speed for all abalone measured (N=8) (Fig. 6-2). For individual abalone, all frequency (waves min^{-1}) *versus* speed (shell lengths min^{-1}) regression lines were significant (all t>4.91; all p<0.04). The individual regression lines did not differ significantly from each other [$F_{0.05(2),7,20}$=0.44; P>0.5], so the regression lines were pooled and found to have a common slope of 2.42. Thus, to increase speed by 1 shell length per minute, abalone generate 2.4 more waves per minute. However, this increase in frequency is not large enough to account for the increase in speed since, speed more than doubles for a two-fold increase in frequency (Fig. 6-2). Therefore, step length (the distance a

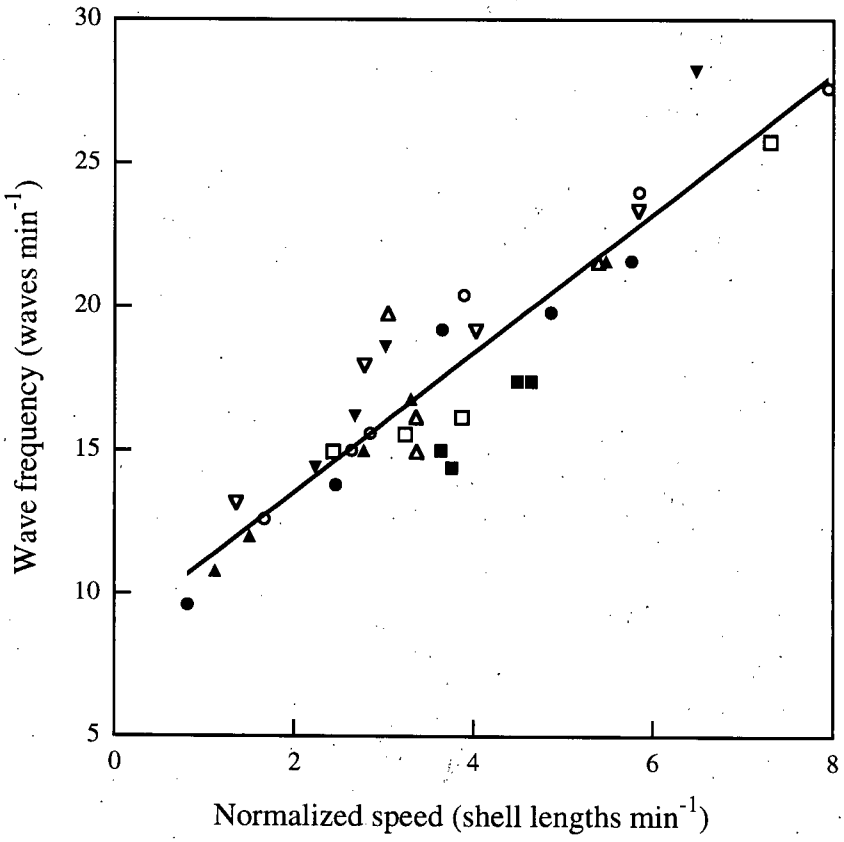

Fig. 6-2. Frequency of pedal waves of locomoting abalone as a function of speed. Statistically significant regression lines were generated for all eight abalone tested. These were not found to be statistically different and they had a common slope *b* of 2.42. Each symbol represents a different abalone (*N*=8).

point on the foot travels during a step) must also increase with increasing speed. Step length was calculated at various speeds using the relationship: $v = f \times L$ where v is speed (in shell lengths·min^{-1}), f is frequency (in waves·min^{-1}) and L is step length (in shell lengths·wave^{-1}) and the regression equation for the pooled data of wave frequency on speed determined above: $f = 8.71 + 2.42\ v$ (Fig. 6-2). As expected, step length increases with increasing speed (Fig. 6-3), but the relationship is not linear because speed and wave frequency are not directly related.

Pedal morphology changed with speed such that less sole was in contact with the substratum at higher speeds. First, total visible sole area decreased with increasing speed (Fig. 6-4). Seven out of eight abalone analyzed decreased the area of the sole of their foot significantly as they increased speed (all $t>2.36$; all $P<0.05$). In these seven abalone, the decrease was linear, and none of the individual regression lines differed significantly from each other [$F_{(2),6,42}=2.22$; $P=0.07$]. Their common regression line had a mean slope (±SE) of -0.021±0.008, indicating that pedal area decreased by 2.1% for each shell length per minute increase in speed. Second, the proportion of the total pedal area occupied by waves increased linearly and significantly with speed in all abalone analyzed (Fig. 6-5; $N=8$; all $t>2.30$; all $P<0.05$). The individual regression lines did not differ significantly from each other [$F_{(1),7,45}=1.17$; $P>0.25$]. They were pooled and found to have a common mean slope (±SE) of 0.018±0.005. Therefore, for each shell length per minute increase in speed, 1.8% more of the foot was incorporated into waves.

No significant trends were found for either foot width or foot length as a function of speed.

Maximum speed

The absolute maximum speed recorded was 113 cm·min^{-1} by a 9.9 cm abalone. This corresponds to a speed of 11.4 shell lengths·min^{-1}. A smaller abalone (5.9 cm) achieved a speed of 14.7 shell lengths·min^{-1}. Mean (± SE) maximal speed of the 10 abalone tested was 12.7 ± 0.7 shell lengths·min^{-1}.

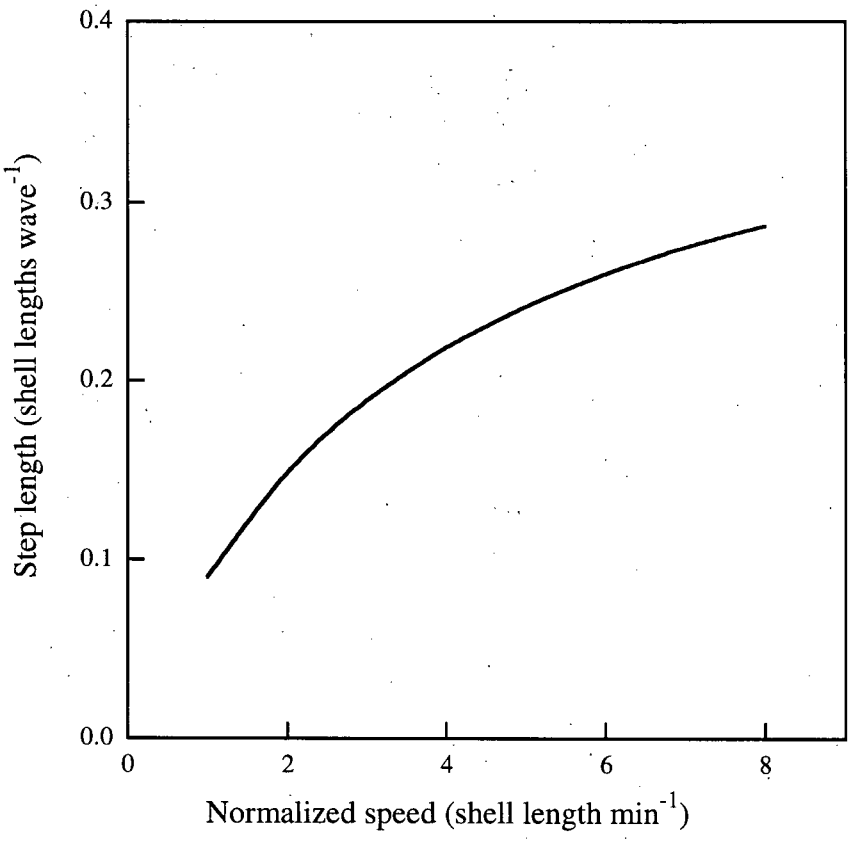

Fig. 6-3. Step length as a function of speed in locomoting abalone. The data were calculated using the equation $v=Lxf$ where v is speed (in shell lengths·min^{-1}), f is frequency (in waves·min^{-1}), and L is step length (in shell lengths·wave^{-1}) and the linear regression equation from Fig. 6-2 of wave frequency on speed for all abalone (f=8.71 + 2.4 v). Points are not shown because of the calculated relationship.

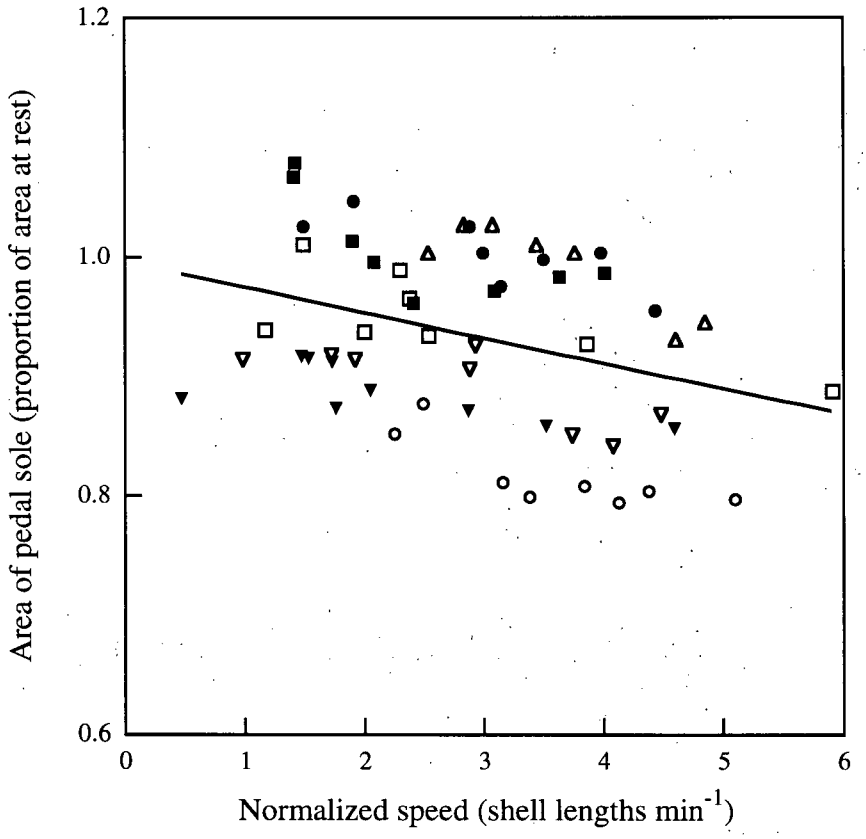

Fig. 6-4. Total area of the pedal sole of locomoting abalone as a function of speed. Statistically significant regression lines were generated for seven out of eight abalone tested. These were not found to be statistically different and they had a common slope *b* of -0.021. The solid line shown here represents a composite of the seven individual regression lines. Each symbol type represents an individual abalone.

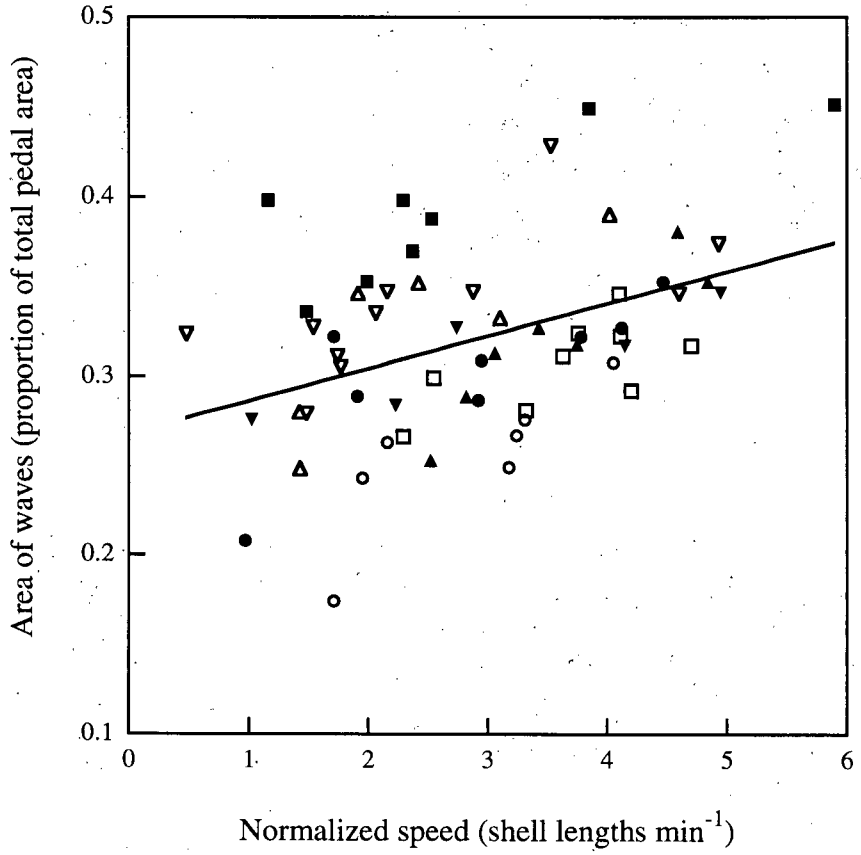

Fig. 6-5. Area of the pedal waves as a function of speed. Statistically significant regression lines were generated for all eight abalone tested. These were not found to be statistically different and they had a common slope *b* of 0.018. The solid line shown represents a composite of the eight individual regression lines and each symbol type represents an individual abalone.

6.4 Discussion

This part of the study demonstrates that the pedal morphology of *Haliotis kamtschatkana* changes with speed such that the amount of the pedal sole in contact with the substratum decreases with increasing speed. This may decrease the amount of energy required for locomotion, potentially allowing abalone to move faster than might otherwise be possible. Indeed, abalone are capable of locomoting more rapidly than are most other gastropod species. The fastest absolute speed recorded in the present study (113 $cm \cdot min^{-1}$ by a 9.9 cm abalone) is 1.5 times faster than the maximum absolute speeds reported for other crawling gastropods by Miller (1974) in a comprehensive survey of locomotion in a large variety of gastropods. The only species having faster rates of locomotion belonged to the specialized leaping Strombidae (223.2 $cm \cdot min^{-1}$ for *Strombus maculatus* and 153.6 $cm \cdot min^{-1}$ for *S. gigas*).

The apparent linear relationship between wave frequency and speed confirms that abalone increase step frequency by increasing the velocity of their pedal waves rather than by increasing the number of waves present on the foot at any moment. This is supported by the observation that no more than three waves were present on the foot at any speed. Likewise, step length increased with increasing speed as a result of a change in pedal morphology. In fact, the pedal morphology of *H. kamtschatkana* changed in two ways as velocity increased: the total visible area of the sole decreased (Fig. 6-4) and, of the pedal area visible, relatively more was incorporated into pedal waves (Fig. 6-5). Although a maximum speed of only 8 shell $lengths \cdot min^{-1}$ was recorded in the video-taped experiments, abalone were able to locomote at nearly 15 shell $lengths \cdot min^{-1}$ when continuously stimulated using a predatory seastar in a large tank, but they could only sustain this speed for a few minutes. If the linear decrease in pedal sole area found in the present study (a 2.1% reduction for each shell length per minute increase in speed) can be extrapolated to this maximum speed, pedal sole area could decrease by as much as 32% during the fastest escape responses relative to that at rest. Furthermore, an additional 27% of the remaining pedal area would be incorporated into pedal waves (i.e. an increment of 1.8% for each shell length per minute increase in speed). To put this into perspective, if an

abalone had a resting visible sole area of 10 cm^2, only 6.9 cm^2 would be visible at 15 shell lengths min^{-1}. Of those 6.9 cm^2, 1.9 cm^2 would be incorporated into waves, leaving only 5.0 cm^2 in contact with the substratum. Thus, the pedal area subjected to friction from the substratum may decrease by as much as 50% at maximal escape velocity relative to the pedal area at rest. It is important to note that such changes will occur throughout a range of speeds. Even at an intermediate speed of 5 shell lengths min^{-1}, pedal adjustment would yield an 18% decrease in resting foot area.

Such changes in pedal morphology and mucus secretion during rapid locomotion presumably lead to a decrease in tenacity. Miller (1974) found that gastropods with larger pedal areas had greater tenacity, that gastropods had maximum tenacity while stationary, and that tenacity decreased as speed increased. Although tenacity was not measured in our study, three observations suggest that our measurements of decreased pedal area associated with increased speed are consistent with a decreased tenacity: (1) abalone moving at high speeds are often unable to cling to the vertical side of an aquarium and fall to the bottom, (2) abalone moving at any speed are easier to remove from the substratum than are stationary animals, and (3) abalone escaping from predators in the field appear to skim the surface of the substratum.

General Conclusion

This thesis has two main components: it describes the importance of activity to the energy budget of the Northern abalone *Haliotis kamtschatkana* and it investigates the energetics of locomotion (both aerobic and anaerobic) of this species. The first part was done over two seasons which allowed a comparison of summer and winter energy budgeting.

For the first part, respiration and mucus secretion were found to be two of the largest components of the summer energy budget, representing 60% and 17% of consumed energy, respectively, and much of the energy devoted to these two components was due to activity. For example, daily mucus secretion increased from 20 $J \cdot day^{-1}$ for inactive abalone to 315 $J \cdot day^{-1}$ for active abalone; and respiration increased from 971 $J \cdot day^{-1}$ to 1132 $J \cdot day^{-1}$. Respiration was also the largest component of the winter energy budget, accounting for 77% of consumed energy. Mucus secretion accounted for only 4% of consumed energy as activity levels decreased during winter. After quantifying these summer and winter energy budgets, I calculated that activity accounts for 25% of total consumed energy during the summer and 12% during the winter. It should be noted that these values probably underestimate the true effect of activity on the energy budgets of field abalone, since field abalone were more active than those in the laboratory possibly due to the presence of predators or the need to forage for food. In fact, detection of the predatory seastar *Pycnopodia helianthoides*, without actual contact by the abalone, caused increases in activity accompanied by a 30% rise in oxygen consumption over resting rates. This rise in oxygen consumption was accompanied by (or followed) a significant elevation of blood-glucose levels. Titers were elevated for 1 hour with a maximum increase of 2x control values occurring 2.5 h after exposure to the seastar. These finding have important implications to the ecology of *H. kamtschatkana* and to the abalone fishery not just in British Columbia but worldwide. Any biological factors in an abalone's habitat which could increase activity could severely alter its energy allocations, causing more energy to be directed towards respiration and mucus secretion, and less towards growth and reproduction. Thus, efforts to reintroduce abalone to local habitats or to bolster local populations should focus on areas

adjacent to large kelp beds which would produce adequate supplies of drift algae and thus minimize the amount of foraging. Likewise, habitats with large numbers of predatory seastars should be avoided, not only to decrease mortality but also to decrease the frequency of predator detection with its consequent increase in metabolic rate and locomotion.

In the second main component of my thesis, locomotory transport costs were quantified for *Haliotis kamtschatkana*. Minimum cost of transport was calculated to be 20.3 $J \cdot kg^{-1}m^{-1}$, lower than cost of transport determined for other gastropods. This is possibly due to the large relative size of abalone since transport costs have been shown to decrease with increasing animal size in other forms of locomotion (running, swimming, and flying). However, too few studies have been done on cost of transport during adhesive crawling in gastropods to draw any definite conclusions on the relationship between transport costs and size. I also initially found that transport costs for *H. kamtschatkana* were lower than transport costs for similarly-sized running vertebrates. However, I was able to somewhat revise this conclusion after factoring in anaerobic metabolism. At speeds great than 5 body lengths$\cdot min^{-1}$ locomoting abalone accumulated significant amounts of tauropine, D-lactate, and arginine in their foot muscles compared with resting animals. This indicates that to fuel rapid locomotion abalone rely on the anaerobic reduction of pyruvate by the enzymes tauropine dehydrogenase and D-lactate dehydrogenase, as well as the breakdown of arginine phosphate. Calculation of ATP equivalents from increased metabolite levels showed that anaerobic metabolism accounted for 54% of the energy expended on locomotion by *H. kamtschatkana* locomoting at high speeds. Addition of this anaerobic component to aerobic energy expenditure caused the cost of transport of *H. kamtschatkana* to be revised to 44.1 $J \cdot kg^{-1}m^{-1}$, which placed it above the regression of cost of transport on size for running vertebrates.

Observations of locomoting abalone indicated that their foot morphology changes during locomotion. In order to determine if these changes could be energy-saving strategies, I investigated pedal sole area and area of the foot incorporated into pedal waves at a range of speeds. As speed increased, abalone decreased the amount of foot in contact with the substratum by decreasing the total area of the pedal sole 2.1% for each shell length$\cdot min^{-1}$

increase in speed. They also increased the area of the foot incorporated into pedal waves by 1.8% for each shell length·min^{-1} increase in speed. Together, these changes translated into a 50% decrease in pedal sole area in contact with the substratum at a maximum escape speed of 15 shell lengths·min^{-1}, relative to the pedal sole area at rest. These changes presumably decrease friction and make locomotion in abalone more cost-effective.

This thesis has improved our understanding of the impact that activity has on energy allocation in *Haliotis kamtschatkana* and has furthered our knowledge of the energetics of adhesive crawling. However, questions still remain about habitat needs of this species. Emmett and Jamieson's (1989) observation that abalone living in exposed environments ("surf" abalone) exhibit reduced growth and often do not reach legal size raises questions about the energetic requirements of living in more turbulent as opposed to more sheltered habitats. This could, in part, be due to the energy costs of living in such an environment. Substratum adherence requires energy in the forms of respiratory energy to maintain muscle tension and mucus energy to remain fixed to the substratum. As hydrodynamic forces tending to pull benthic organisms up and off the substratum increase, it is likely that the amount of energy required to remain attached to the substratum also increases, leaving less energy available for reproduction and growth.

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