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The contribution of anaerobic energy to gastropod crawling and a re-estimation of minimum cost of transport in the abalone, *Haliotis kamtschatkana* (Jonas)

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Abstract

The contribution of anaerobic metabolism to the minimum cost of transport (COT_{min}; energy required to transport a unit mass over a unit distance, excluding maintenance and postural costs) was determined for the abalone *Haliotis kamtschatkana*. Abalone were induced to crawl in water for 6 m at speeds up to 9.9 shell lengths $\cdot$ min^{-1}. Changes in the concentrations of the anaerobic metabolites tauropine, D-lactate, arginine phosphate, and arginine occurred in foot muscle, but only at speeds at or above five shell lengths $\cdot$ min^{-1}. These metabolites did not change significantly in the shell adductor muscle. The amount of anaerobically derived ATP used during crawling was calculated and the values were compared with data for aerobic ATP production. Anaerobic metabolism accounted for 54% of COT_{min}, and the aerobic COT_{min} estimated by Donovan and Carefoot (1997) [Donovan, D.A., Carefoot, T.H., 1997. Locomotion in the abalone *Haliotis kamtschatkana*: pedal morphology and cost of transport. J. Exp. Biol. 200, 1145–1153] was thus adjusted from 20.3 J $\cdot$ kg^{-1} m^{-1} to 44.1 J $\cdot$ kg^{-1} m^{-1}. This new value places abalone above the regression line of $\log_{10}COT_{min}$ on $\log_{10}$mass for running vertebrates, indicating that transport costs for abalone are more expensive than for the average similar-sized runner. The contribution of anaerobic metabolism was also determined for prolonged air exposure and whole animal righting behavior to allow comparisons to be made with other abalone species for which these data, but not data on anaerobic metabolism during crawling, were available in the literature. On this basis, we suggest that in comparison to *H. kamtschatkana*, *Haliotis lamellosa* and *Haliotis iris* may have lower aerobic scopes during crawling, while that of *Haliotis asinina* may be considerably higher.

Keywords: Abalone; Anaerobic glycolysis; Cost of transport; *Haliotis kamtschatkana*; Locomotion; Tauropine dehydrogenase

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1. Introduction

The energetics of activity, particularly crawling locomotion, in *Haliotis kamtschatkana* have been examined (Donovan and Carefoot, 1997; Donovan and Carefoot, in press). Although the focus of these studies was the contribution of aerobic metabolism, we observed evidence of an anaerobic contribution to locomotion. Specifically, while the total cost of transport (COT_{tot}; the total amount of energy required to transport a unit mass over a unit distance, including resting metabolism) decreased with increasing speed as expected, the steep slope of $\log_{10}COT_{tot}$ versus $\log_{10}$speed indicated that aerobic energy expenditure decreased greatly at higher speeds and suggested the possibility of an unmeasured anaerobic component (Donovan and Carefoot, 1997). Although changes in abalone foot morphology occurred as speed increased, such that less foot area was in contact with the substratum, we concluded that these morphological changes could not fully explain the large decrease in aerobic transport costs (Donovan and Carefoot, 1997). Measurements of cost of transport for animals which run, swim, and fly are generally calculated from steady-state rates of oxygen consumption measured when the animals are exercising at predetermined speeds and, with vertebrates, blood lactate levels are often monitored to ensure that only aerobic metabolism is used (Brett, 1965; Tucker, 1968; Taylor et al., 1982). However, comparable steady-state conditions may not be possible, nor biologically relevant, for gastropods. First, it is difficult, if not impossible, to control the speed at which a gastropod crawls (Denny, 1980; Houlihan and Innes, 1982; Innes and Houlihan, 1985; Donovan and Carefoot, 1997). Second, the extent to which anaerobic metabolism contributes to locomotion is unknown and, given the well developed anaerobic capabilities of most gastropods, anaerobic energy sources may be used even at relatively slow rates of crawling. Indeed, many invertebrates use a combination of aerobic and anaerobic metabolism during activity (Full, 1997), thus an accurate estimate of gastropod cost of transport may depend on measurement of both of these energy sources.

Many predominantly sedentary intertidal molluscs display well developed capacities for generating ATP anaerobically when muscle oxygen levels are compromised during short duration bursts of elevated muscle work [see Gäde (1983)]. Strategies for supporting this type of anaerobic metabolism include adequate muscle glycogen reserves to fuel anaerobic glycolysis (Hochachka, 1980), high activities of species-specific pyruvate reductases for the regeneration of cytoplasmic NAD^+ (Fields, 1983; Gäde and Grieshaber, 1986), and high concentrations of the molluscan muscle phosphagen, arginine phosphate (Gäde, 1983). The contribution of anaerobic metabolism to the total cost of locomotion can be determined from the accumulation of pyruvate reductase endproducts and the depletion of arginine phosphate reserves within the muscles used to power movement.

While the amount of ATP generated anaerobically during muscle work has been quantified in a wide range of molluscs (Grieshaber and Gäde, 1976; Koorman and Grieshaber, 1980; Baldwin et al., 1981; Baldwin and England, 1982; Gäde et al., 1984; Meinardus-Hager and Gäde, 1986; Gäde, 1988; Baldwin et al., 1992), studies of the relative contributions of both aerobic and anaerobic metabolism to the total cost of locomotion are limited to a few species. Livingstone et al. (1981) found that 94% of the

energy used by the phasic muscle of the swimming scallop *Placopecten magellanicus* during short burst swimming was supplied anaerobically from the breakdown of ATP and arginine phosphate, and from glycolysis, while only 6% of the energy was derived aerobically. In contrast, ATP generated during slower sustained swimming in the bivalve *Limaria fragilis* is predominantly from aerobic metabolism (72%), with minor contributions from anaerobic glycolysis (2%) and ATP reserves (3%), and a more substantial contribution from arginine phosphate (23%) (Baldwin and Lee, 1979; Baldwin and Morris, 1983). Ratios of anaerobic to aerobic energy production during gastropod crawling have thus far not been investigated.

Abalone, like other gastropods, have an open circulatory system with low blood pressure and slow circulation time (Crofts, 1929; Bourne and Redmond, 1977a,b; Jones, 1983). At rest, relative cardiac output to the pedal musculature is less than to other more oxygen dependent tissues such as kidney, digestive gland, radular muscle, and gonad (Jorgensen et al., 1984) and during sustained exercise blood flow may be shunted away from the pedal musculature, essentially isolating it from the general circulation (Russell and Evans, 1989). As well, it has been argued that the oxygen binding properties of haemocyanin, the respiratory protein found in abalone blood, indicate that the abalone circulatory system is adjusted towards oxygen storage rather than rapid oxygen delivery to working muscles (Wells et al., in press). Thus, it is probable that the circulatory system of abalone is unable to maintain oxygen supplies to the pedal musculature during intense activity, leading to greater dependence on anaerobic metabolism.

The purpose of the present study was to investigate the extent to which abalone use anaerobic metabolism during crawling locomotion and to use these data to adjust the calculation of cost of transport earlier proposed for *H. kamtschatkana* (Donovan and Carefoot, 1997) to include any anaerobic energy component. In addition, accumulation of anaerobic metabolites during air exposure and whole animal righting behavior were determined. This allowed the anaerobic capabilities of *H. kamtschatkana* to be compared with those of other abalone species for which similar data, but not data on anaerobic metabolism during crawling, were available.

2. Materials and methods

2.1. Experimental animals

Abalone (85–100 g) were collected from Barkley Sound near the Bamfield Marine Station, Vancouver Island, British Columbia, Canada. They were transported to the University of British Columbia and held in tanks with a recirculating supply of seawater at 10°C for 3–5 days before experimentation. They were fed ad libitum on the kelp *Nereocystis luetkeana*.

2.2. Determination of pyruvate reductase enzymes in abalone muscles

In order to determine which pyruvate reductase endproducts might be produced by *H. kamtschatkana* during anaerobiosis, tissue samples from shell adductor and foot were

analyzed for a range of pyruvate reductase enzymes. Approximately 1 g each of adductor and foot muscles were dissected from freshly shucked *H. kamtschatkana* ($N = 5$). The sample of shell adductor was taken from the point where the muscle attaches to the shell and the sample of foot was taken from the left side of the ventral surface. The tissues were individually minced on ice with chilled scissors and scalpel, and then homogenized in 5 ml of ice-cold buffer (50 mM imidazole–HCl, 1 mM EDTA, 1 mM dithiothreitol, pH 7.2) with an Ultra-Turrax homogenizer. Homogenates were centrifuged for 1 min at 5000 *g* and 4°C, and the supernatants were placed on ice and assayed within 1 h. Pyruvate reductase activity was assayed by following the reduction of NADH at 340 nm using a spectrophotometer coupled to a refrigerated circulator to maintain cuvette temperature at the aquarium-holding temperature of 10°C. Controls lacking substrates were run to allow for nonspecific activity and all assays were performed in duplicate. The compositions of the reaction mixtures for the various pyruvate reductases were as follows: lactate dehydrogenase, 2.5 mM pyruvate, 0.15 mM NADH, 50 mM imidazole–HCl buffer, pH 7.0; tauropine dehydrogenase, 80 mM taurine, 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.

2.3. Metabolite changes in abalone muscles during crawling

In order to test the effects of crawling in water on levels of anaerobic metabolites, animals were first divided into two groups. Tissues (shell adductor and foot muscles) from the control group ($N = 5$) were sampled when these animals were quiescent. The second group ($N = 32$) was exercised by inducing each abalone to crawl over a specific distance. This was achieved by touching them with an excised tube foot of the seastar *Pycnopodia helianthoides* (Brandt). The time taken for each animal to move 6 m was recorded, thus providing a value for average speed.

The abalone were removed from their shells immediately following their respective treatments, and samples of shell adductor and foot muscles were freeze clamped in liquid nitrogen. The absolute number of individuals used for analysis of each metabolite (described below) was less than the starting totals owing to initial unfamiliarity with the analyses and consequent wastage of some samples.

2.4. Metabolite changes in abalone muscles during air exposure and righting behavior

To test the effects of prolonged air exposure and exercise during righting behavior on levels of anaerobic metabolites in shell adductor and foot muscles, abalone were randomly placed into three groups. The first group ($N = 5$) was left undisturbed in the holding tank and comprised the control. Animals in the second group ($N = 12$) were kept

in moist, cool (10°C) air for 16 h. Animals in the third group ($N = 10$) were exercised by placing them upside down on their shells in water, and allowing them to repeatedly right themselves until exhausted (determined when righting failed to occur within 3 min).

2.5. Metabolite assays

Approximately 1 g portions of foot and adductor muscles freeze clamped in liquid nitrogen were weighed and crushed prior to homogenization in ice-cold 6% perchloric acid (1:10 w/v). Following homogenization, the samples were centrifuged at 4°C for 15 min at 5000 *g*. The supernatants were neutralized with 5 M K_2CO_3 and allowed to stand on ice for 2 h. They were then decanted and stored at −80°C until analysed.

D-Lactate and tauropine were assayed enzymatically using D-lactate dehydrogenase (Sigma Cat. No. L2395) and tauropine dehydrogenase isolated from *H. kamtschatkana*, as described in Baldwin et al. (1992). Arginine and arginine phosphate were measured by the method of Gäde (1985) using scallop octopine dehydrogenase (Sigma Cat. No. O1252).

3. Results

3.1. Pyruvate reductase enzymes

The activities of pyruvate reductase enzymes assayed in foot and adductor muscles of *H. kamtschatkana* are presented in Table 1. Only tauropine and lactate dehydrogenases showed any significant activity, with higher activities of tauropine dehydrogenase than lactate dehydrogenase in both muscles. Adductor muscle had a higher activity of tauropine dehydrogenase and a lower activity of lactate dehydrogenase compared to foot muscle.

Table 1
Pyruvate reductase activities in shell adductor and foot muscles of the abalone *Haliotis kamtschatkana* ($N = 5$)

	Activity (IU·g wet muscle^{-1} min^{-1}; 10°C)	
Enzyme	Adductor	Foot
Tauropine DH[a]	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

Values are presented as means±SE.
[a] DH = dehydrogenase.

3.2. Metabolite changes during crawling

The effects of crawling in water on concentrations of tauropine, D-lactate, arginine phosphate, and arginine in the shell adductor and foot muscles of *H. kamtschatkana* are shown in Table 2.

The maximum crawling rate induced in this experiment was 9.9 shell lengths $\cdot$ min^{-1}, a value approaching the mean maximum rate of 12.7 shell lengths $\cdot$ min^{-1} for *H. kamtschatkana* determined previously (Donovan and Carefoot, 1997). The data on metabolite concentrations were first linearly regressed on speed. Since none of these regressions 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 for each muscle type: those animals crawling slower than five shell lengths $\cdot$ min^{-1} and those crawling at or faster than five shell lengths $\cdot$ min^{-1}. With each muscle, an analysis of variance (ANOVA) was then done comparing these two groups with the quiescent control animals, followed by Tukey's tests. The results (see Table 2 for statistics) showed that abalone crawling at speeds below five shell lengths $\cdot$ min^{-1} did not differ significantly in metabolite levels from the quiescent control animals. However, abalone moving at speeds faster than five shell lengths $\cdot$ min^{-1} had increased levels of tauropine, lactate, and arginine in foot muscles relative to the quiescent controls, while metabolites in the shell adductor muscle showed no significant change. There was, however, statistical overlap between the groups.

Table 2
Concentrations of anaerobic metabolites in foot and shell adductor muscles of *Haliotis kamtschatkana* crawling at various speeds

	Metabolite concentrations (μmol$\cdot$g wet mass^{-1})		
		Crawling rate	
Anaerobic metabolites	Control	<Five shell lengths$\cdot$min^{-1}	>Five shell lengths$\cdot$min^{-1}
Foot			
Tauropine	0.3±0.1 (5)[a]	1.1±0.2 (16)[ab]	1.5±0.3 (16)[b]
D-Lactate	0.02±0.1 (5)[a]	0.4±0.2 (16)[ab]	0.7±0.1 (16)[b]
Arginine	9.5±1.7 (4)[a]	12.5±1.2 (11)[ab]	17.0±1.6 (7)[b]
Arginine-P[c]	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)
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)

Values are expressed as means±SE and the numbers of individuals used for each metabolite analysis are in brackets.

[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, $P < 0.05$).

[c] Arginine-P = arginine phosphate.

Table 3
Concentrations of anaerobic metabolites in foot and shell adductor muscles of *Haliotis kamtschatkana* exposed to air for 16 h and induced to right themselves under water until exhausted; control animals were held undisturbed in a tank with fresh seawater

	Metabolite concentrations (μmol·g wet mass^{-1})		
Anaerobic metabolites	Control	Air exposed	Righting to exhaustion
Foot			
Tauropine	1.1±0.2 (5)[a]	2.7±0.4 (12)[b]	1.4±0.2 (10)[a]
D-Lactate	0.1±0.3 (5)[a]	2.4±0.4 (12)[b]	0.9±0.2 (10)[a]
Arginine	10.3±1.5 (4)	16.4±4.2 (5)	15.6±2.6 (5)
Arginine-P[c]	7.8±3.0 (4)	7.3±2.1 (5)	3.7±0.8 (5)
Adductor			
Tauropine	0.5±0.2 (5)[a]	3.8±0.7 (12)[b]	2.4±0.4 (10)[b]
D-Lactate	0.4±0.6 (5)[a]	2.4±0.4 (12)[b]	0.2±0.04 (9)[a]
Arginine	14.7±3.9 (5)[a]	39.5±5.0 (5)[b]	26.1±1.9 (5)[ab]
Arginine-P	15.5±4.0 (5)[a]	0.7±5.0 (5)[b]	6.6±2.7 (5)[ab]

Values are expressed as means±SE and the numbers of individuals used for each metabolite analysis are in brackets.
[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, $P<0.05$).
[c] Arginine-P = arginine phosphate.

3.3. Metabolite changes during air exposure and righting behavior

Abalone exposed to air for 16 h showed increased levels of tauropine and D-lactate in both foot and adductor muscles (Table 3). As well, adductor muscle had significantly more arginine and less arginine phosphate when compared with controls. The only significant change for animals induced to right themselves to exhaustion was an increase in adductor muscle tauropine (Table 3).

4. Discussion

The presence and relative activities of tauropine and lactate dehydrogenases in shell adductor and foot muscles of *H. kamtschatkana* are consistent with the distribution of pyruvate reductases in other haliotids (Gäde, 1988; Sato et al., 1993; Wells and Baldwin, 1995). This identifies tauropine and D-lactate as the pyruvate reductase endproducts expected to accumulate during muscle hypoxia.

When crawling in water, changes in metabolite levels indicated that only the foot muscle became anaerobic, and then only at higher speeds (Table 2). Both tauropine and D-lactate levels increased significantly in foot muscle compared with quiescent abalone, but only in animals travelling faster than five shell lengths·min^{-1}. There was no clear evidence for the use of arginine phosphate as an energy source during crawling. Arginine levels showed a significant increase in foot muscle but, although arginine

phosphate levels decreased, this change was not significant possibly due to the large variability among individual animals. Any energy used by the adductor muscle during crawling appeared to be derived from aerobic sources, as no changes in any of the anaerobic metabolites were found at either high or low speeds.

Using these data on the accumulation of anaerobic metabolites at higher speeds, it is possible to compare amounts of energy derived from anaerobic and aerobic sources during rapid crawling in *H. kamtschatkana* and to modify the estimation of cost of transport for this species based on an earlier estimate by Donovan and Carefoot (1997) as follows. Of all the anaerobic metabolites measured, only foot tauropine, lactate, and arginine changed significantly during crawling (Table 2). In this study, since abalone crawled 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 travelling faster than five shell lengths $\cdot$ min^{-1} was 9.2 cm in length and 107 g in live mass. Since total live mass of *H. kamtschatkana* represents 29% muscle and since the foot comprises 50% of the total muscle mass (personal observation), the average foot mass would have been about 15 g. Since tauropine and lactate increased by 1.2 μmol $\cdot$ g foot^{-1} and 0.7 μmol $\cdot$ g foot^{-1}, respectively (Table 2) and since 1.5 μmol ATP is generated per μmol tauropine or lactate, we calculate that 42 μmol of ATP were produced from anaerobic glycolysis. Additionally, although 7.5 μmol $\cdot$ g foot^{-1} of arginine were produced (presumably from the breakdown of arginine phosphate), only 4.3 μmol $\cdot$ g foot^{-1} of arginine phosphate were lost (Table 2). Using this smaller value and the equivalence of 1 μmol ATP per μmol arginine phosphate, we can further calculate 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 required for an average abalone (from this study) to crawl 6 m can now be calculated. For an abalone travelling faster than five shell lengths $\cdot$ min^{-1}, average speed was 6.6 shell lengths $\cdot$ min^{-1} (which corresponds to 61 cm $\cdot$ min^{-1}), and average time of locomotion was 10 min. Mass-specific oxygen consumption can be calculated by inserting average speed (v, in cm $\cdot$ min^{-1}) and average mass (m, in g) into a previously derived regression of mass specific V_{O2} (μl $O_2 \cdot g^{-1}\, h^{-1}$) on speed and mass ($V_{O2} = 40.1 + 0.58v - 0.15m$; Donovan and Carefoot, 1997), yielding a value of 59 μl $O_2 \cdot g^{-1}\, h^{-1}$ expended during locomotion. Thus, an abalone weighing 107 g and crawling for 10 min would require about 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 crawl at high speeds (COT_{tot}; 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 $O_2 \cdot g^{-1}\, h^{-1}$; Donovan and Carefoot, 1997) and postural costs (19.4 μl $O_2 \cdot g^{-1}\, h^{-1}$; Donovan and Carefoot, 1997) are subtracted from total oxygen consumption (assuming these costs are met aerobically), then a 107 g abalone uses 90 μmol ATP from aerobic metabolism. In this case, anaerobic metabolism would account for 54% of transport costs (COT_{min}).

Addition of this 54% anaerobic component to the aerobic COT_{min} determined for *H. kamtschatkana* (Donovan and Carefoot, 1997) would lead to an increase in COT_{min} 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 95% confidence limit of the point corresponding to a 50 g running vertebrate on the regression line of $\log_{10}COT_{min}$ on $\log_{10}$mass for running animals (Fig. 1), indicating that transport costs for abalone are more expensive than for the average similar-sized runner. [This 95% confidence interval was calculated from the data published in Taylor et al. (1982). Unfortunately, lack of data prevented us from calculating similar confidence intervals for corresponding points on the regression lines for invertebrates]. Adhesive crawling is generally thought to be more energetically expensive than running, swimming, or flying, possibly due to the production of mucus, but it was only with the addition of the anaerobic component that abalone COT_{min} exceeded that of runners. COT_{min} of the slug *Ariolimax columbianus* (Fig. 1) was determined from measurements of oxygen consumption and average speed over 24 h periods (Denny, 1980) and therefore probably accounts for oxygen debt and anaerobic metabolism. However, COT_{min} measurements for the four tropical marine snails (Houlihan and Innes, 1982) and the temperate marine snail (Innes and Houlihan, 1985)

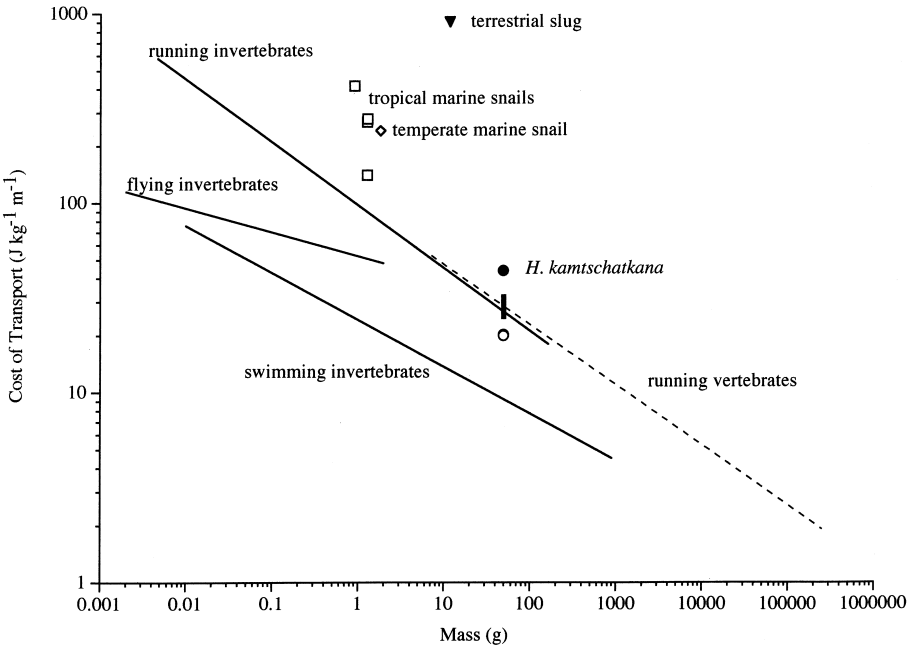

Fig. 1. Minimum cost of transport of abalone (*Haliotis kamtschatkana*) with anaerobic metabolism (closed circle) and without anaerobic metabolism (open circle; Donovan and Carefoot, 1997) compared with that of other marine snails (*Monodonta turbinata, Monodonta articulata, Gibbula richardi*, *Gibbula rarilineata*; Houlihan and Innes, 1982; *Littorina littorea*; Innes and Houlihan, 1985) and a terrestrial slug (*Ariolimax columbianus*; Denny, 1980). Closed symbols denote cost of transport measurements for which anaerobic metabolism was included and open symbols denote cost of transport measurements which do not include anaerobic metabolism. Regression lines for running, swimming, and flying invertebrates are from Full (1997) and the regression line for running vertebrates is from Taylor et al. (1982). The bold vertical line represents the 95% confidence interval of the point corresponding to a 50 g animal on the running vertebrate regression line.

are most likely underestimated since no measurement of anaerobic contribution was made. As well, the regression line for running invertebrates was calculated from measurements of aerobic COT_{min} for a variety of invertebrates (Full, 1997), some of which may rely on anaerobic metabolism during locomotion.

When *H. kamtschatkana* were subjected to 16 h of exposure to moist air, anaerobic energy was supplied from arginine phosphate stores as well as from anaerobic glycolysis (Table 3). In adductor muscle, arginine phosphate levels decreased by 14.8 μmol·g wet mass^{-1}, concomitant with a rise of 24.8 μmol·g wet mass^{-1} in arginine levels, while neither of these metabolites showed significant changes in foot muscle. However, both muscle types exhibited significant increases in tauropine and D-lactate. Baldwin et al. (1992); Wells and Baldwin (1995) found increased levels of tauropine and lactate in both adductor and foot muscles during air exposure in *Haliotis iris* and *Haliotis australis*, respectively. This indicates that these species respond to air exposure in much the same manner as does *H. kamtschatkana*.

The only response of *H. kamtschatkana* to functional hypoxia resulting from righting to exhaustion while in water was a fivefold rise in adductor tauropine levels (Table 3). Thus, it appears that anaerobic glycolysis 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, Gäde (1988) and Baldwin et al. (1992) found accumulations of both tauropine and lactate in both adductor and foot muscles of *Haliotis lamellosa* and *H. iris*. This indicates a broader response to righting-induced functional hypoxia in these species, with both muscle types deriving energy anaerobically, and suggests that these species may be more dependent on anaerobic metabolism during righting behavior than is *H. kamtschatkana*

This greater dependence on anaerobic glycolysis found in *H. lamellosa* and *H. iris* during functional hypoxia induced by righting may indicate that they are also more dependent on anaerobic glycolysis during functional hypoxia induced by crawling. Thus, we might expect these species to have lower aerobic scopes than *H. kamtschatkana* and to switch from aerobic to anaerobic metabolism at speeds lower than five shell lengths·min^{-1}. This is almost certainly not the case for all abalone species however, and an exception may be the tropical abalone, *Haliotis asinina*. This abalone is highly active, often foraging above water on coral outcrops at low tide. During righting experiments, *H. asinina* is able to right itself as many as 70 times at 1 min intervals (Baldwin, unpublished data). In contrast, *H. kamtschatkana* is only able to right itself 25 times at more than 1 min intervals (Donovan, unpublished data). Likewise, while righting causes significant changes in pyruvate reductase endproducts and arginine phosphate in *H. asinina*, these changes are smaller on a wet-mass basis than those found in *H. kamtschatkana* (Baldwin et al., in preparation). This suggests a greater dependence on oxygen for *H. asinina* and possible physiological and/or biochemical adaptations for maintaining aerobic metabolism during locomotion. The extent to which crawling is supported aerobically in this species is presently being investigated.

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