BIOCHEMICAL INDICATORS OF THE TRANSPORTATION STRESS OF THE SOUTH AFRICAN ABALONE (PERLEMOEN), *HALIOTIS MIDAE*, LINN.

S. O. OMOLO

This thesis is submitted in fulfillment of the requirement of the degree of Master of Science (Zoology)

Biochemical indicators of the transportation stress of the South Africa abalone
(Perlemoen), *Haliotis midae,* Linn.
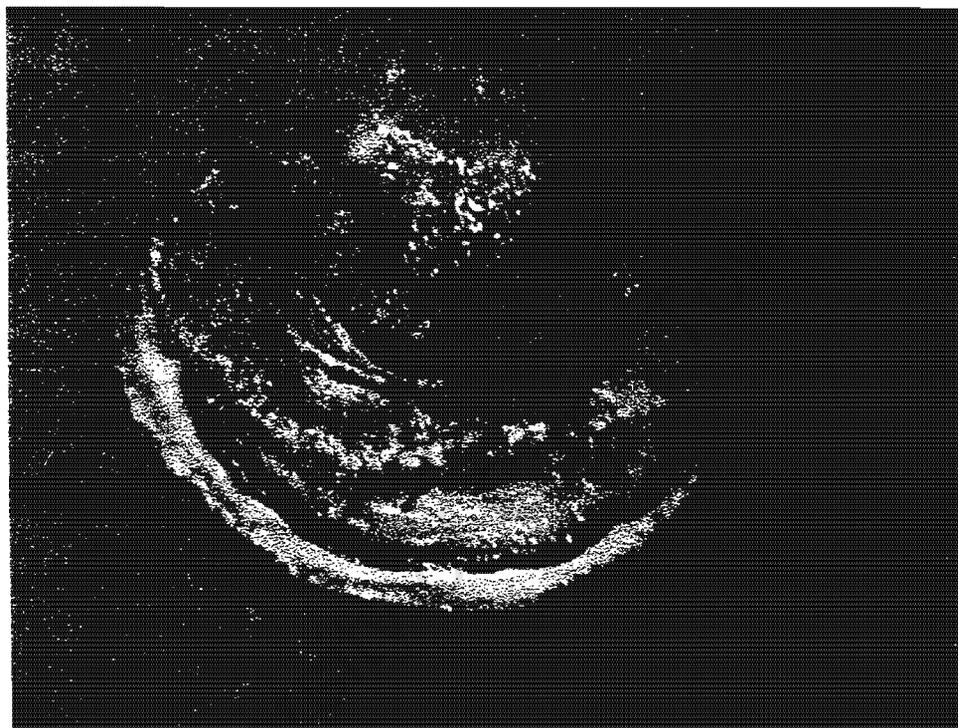

By

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Submitted in fulfillment of

the requirement of the degree of

Master of Science (Zoology).

Department of Zoology, University of Cape Town.

Supervised by Professor Gerd Gäde, Associate Professor Peter Cook and

Emeritus Professor Alec C. Brown.

August, 1999

South African abalone (perlemoen), *Haliotis midae*, Linn.

This thesis is dedicated to my father who never lived to witness, to my mother who lived to witness, to my wife and children with grace and sincerity – Amen

DECLARATION

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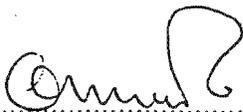
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a) grant the University of Cape Town free licence to reproduce the above thesis in whole or in part, for the purpose of research;

b) declare that

i) This thesis reports the results of original research which I have carried out in the Zoology Department, University of Cape Town, between 1997 and 1999 and it is my own unaided work apart from the normal guidance from my supervisors. Throughout this study I was involved in the determination of metabolites concentrations during simulated transport experiments and interpretation of data, as well as the writing up of the project.

ii) This work has not been submitted for a degree at any other university .

26ᵗ Sept. 1999

Samson Odira Omolo   Date

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ABSTRACT

Biochemical indicators of the transportation stress of the South African abalone (Perlemoen), *Haliotis midae*, Linn.

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Abalone exposed to air incurred greater metabolic and structural stress in muscle than do abalone at rest. Since foot and shell adductor muscle texture and flavor affect economic values of *H. Midae*, the investigation sought evidence of differences attributable to transport of the commercial South African abalone based on environmental and exercise inducement.

Tauropine dehydrogenase from *Haliotis. midae* muscle exhibits its highest activity 53.85 ± 13.56 U g^{-1} wet weight in shell adductor muscle. The activities of octopine dehyedrogenase, strombine dehydrogenase or alanopine dehydrogenase in adductor muscle were very low. Therefore, tauropine dehydrogenase was isolated from adductor muscles for enzymatic determination of tauropine. Tauropine dehydrogenase was purified 8.5-fold with 47% recovery.

During exercise, energy for muscle construction was provided by the breakdown of arginine phosphate and glycogen. Glycogen concentrations significantly ($p < 0.01$) decreased in shell adductor, foot and gill tissues. Exercise caused a significant decline in arginine phosphate and formation of D-lactate in muscle tissues

Glycogen significantly decreased ($p \leq 0.01$) in adductor and foot muscles. Most of the

energy was derived from glycolysis through Embden-Meyerhof pathways resulting in accumulation of tauropine and D-lactate. The energy demand was high and ATP was reduced, but the adenylate energy charge did not show a significant decrease after exercise.

There was significant ($p \leq 0.01$) increase of tauropine and D-lactate in the tissues during prolonged exposure. Adductor muscle exhibits the highest metabolite concentrations.

Exposure to air for 36 h led mainly to the formation of tauropine (6.55 ± 0.39 and 7.76 ± 1.47 μmoles g-1 wet weight at 7°C and 10 °C, respectively) in the adductor muscle, whereas D-lactate production predominated in the foot (5.78 ± 0.17 and 7.58 ± 2.98 μmoles g-1 wet weight at 7 °C and 10 °C respectively).

In addition, glycogen energy derived from the breakdown of arginine phosphate was used during simulated transportation, resulting in increases in arginine concentrations in the tissues. The shell adductor exhibited the highest decrease of arginine phosphate with a concomitant increase of arginine. Energy demand decreased resulting in decrease in energy charge ($p \leq 0.05$). The potential energy from breakdown of arginine phosphate and glycogen substrates expressed as ADP / ATP ratio was highest in gill. Low values of the ADP / ATP ratio are an indication of high available ATP. Since animals were packed inside a humid plastic bag, they used their moisture gill filament surface for aerobic metabolism. But oxygen diffusion was not enough to make any changes in the whole animal tissues.

Heart beat drastically decreased after 24 h exposure to air. Resting animal heart rate was 23 and 26 beats min $^{-1}$. Then after 36 h, heart rate decreased to 2 beat min $^{-1}$.at 7 °C and 10°C.

Oxygen consumption significantly ($p \leq 0.01$) decreased from 3.66 ± 0.51 to 0.62 ± 0.25 ml O2 g^{-1} h^{-1} wet weight after 36 h out of seawater. Animals regained their normal rate of

oxygen consumption immediately on being submerged in seawater after simulated transport stress of 36 h, but values were lower than those of control animals.

There was no mortality experienced during simulated transport of *Haliotis Midae* for 36 h at 7 °C and 10 °C.

ABBREVIATIONS

AlaDH	alanopine dehydrogenase
ADP	adenosine-5'-diphosphate
AMP	adenosine-5'-monophosphate
ATP	adenosine-5'-triphosphate
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
LDH	lactate dehydrogenase
GPT	glutamate pyruvate transaminase
MDH	malate dehydrogenase
NAD^+	nicotine adenine dinucleotide (oxidized)
NADH	nicotine adenine dinucleotide (reduced)
OcDH	octopine dehydrogenase
PAC	perchloric acid
StrDH	strombine dehydrogenase
TaDH	tauropine dehydrogenase
U	enzyme units (μmol product formed per min)

INTRODUCTION

CHAPTER 1.

1. INTRODUCTION.

1. 1. AIMS AND OBJECTIVES

Abalones (*Haliotis* spp.) are in considerable demand as a delicacy (Olley,1971; Olley and Thower, 1977) in many parts of the world but especially in the Far East. This has led to an industry which involves not only harvesting natural populations but also, as such populations have declined, setting up artificial culture of appropriate species (Lo-Chai Chen, 1990; Knauer *et al.*, 1996; Ebert *et al.*, 1984; Uki *et al.*, 1981; Britz, 1996; Gonzalez Aviles and Shepherd, 1996). The industry provides a significant number of employments and brings valuable foreign currency into South Africa. However, one of the major problems faced by the industry is spoilage of the flesh during transport (James and Olley, 1970; Baldwin *et al.*, 1992). The main reasons for such spoilage, particularly in terms of metabolic end products, as well as steps which may be taken to alleviate flesh spoilage, are addressed in this project.

This study was prompted a conspicuous lack of information on biochemical and physiological aspects *Haliotis midae* during transportation. This thesis then examines biochemical and physiological effects of transportation stress in live perlemoen, *Haliotis midae*, muscles under conditions designed to simulate those experienced when live animals are air-freighted to overseas markets.

Aspect of abalone energy metabolism, particularly the accumulation of metabolic end products during air exposure to environmental anoxia and during whole body righting, were investigated. A further aim of the study was to compare *H. midae's* metabolic capabilities with those of other abalone species for which similar data were available An additional objective was to assess some physiological responses (oxygen consumption and heart rate) of *Haliotis midae* to low temperature (7 °C) at which transportation normally takes place, and possibly to suggest improvements to commercial handling and transport procedures.

1. 2. The genus *Haliotis*.

Abalone are herbivorous prosobranch gastropods of the family Haliotidae, Linnaeus 1758 (Hahn, 1989; Fallu, 1991; Shepherd *et al.*, 1992). Between 75 and 100 species have been described world-wide (Shepherd *et al.*, 1992; Jarayabhand and Paphavasit, 1996). All these species are assigned to the genus *Haliotis*. The name is apt and means "sea ear".

1. 3. Morphology of *Haliotis*.

The animals have a flattened, spiral shell with enlarged aperture and rows of small holes along the left side (Day, 1974; Fallu, 1991). The holes are largest towards the anterior and decrease in size towards the posterior, those at the back being almost blocked. The holes are used for both respiration and waste disposal; water enters the gill cavity under the front margin of the shell and exits through the holes. During growth periods, new holes are created and old ones are filled in. The shell hides the large, muscular foot, which is used both for locomotion and for holding firmly onto the substratum. It is protected from predation by a tough outer skin. The margin of the foot has sensory tentacles which detect predators and food. The gut is coiled between the stalk of the foot and the rim of the shell. A thin layer of skin grows out of the stalk and adheres to the surface of the shell, so sealing off the mantle cavity and gut from the external environment.

1. 4. Biology, development and ecology of *Haliotis*.

During spawning (Uki and Kikuchi, 1984) egg and sperm are shed into the mantle cavity, from which they escape into the water. During planktonic development (Newman, 1964, 1966, 1967, 1968, 1969; Fallu, 1991; Shofer and Tjeerdema, 1993; Branch *et al.*, 1994;) the larvae undergo changes from swimming trochophore to veliger, before metamorphosis and settlement as post-larval juvenile. As the shell develops, it grows by adding material onto the anterior right-hand side (Fallu, 1991; Trueman and Brown, 1985) and it rotates on the body as it grows. Shell lengths are

commonly used as an indicator of age. Final body size is related to temperature (Lo-Chai Chen, 1990; Fallu, 1991). The largest species is the Californian abalone *Haliotis rufescens*, reaching a shell length of 290 mm and weighing more than 1.7 kg (Hahn, 1989). *Haliotis midae*, the subject of the present study, attains a shell length of 100 mm.

Abalone are commonly associated with large brown algae, including kelp (Branch *et al.*, 1994), and are exclusively herbivorous, scraping algae off the rocks by means of their radulae. They are active nocturnally. The staple foods of *Haliotis midae* are the kelps *Ecklonia maxima, Gracilaria salicornia* and *Laminaria* (Field *et al.*, 1977; Barkai and Griffiths, 1986; Tarr, 1989; Singhagraiwan *et al.*, 1992; Branch *et al.*, 1994).

Abalone are often named after the colour of the foot; for example brownlip or greenlip (Fallu, 1991; Oakes and Ponte, 1996).

1. 5. The distribution of *Haliotis* species.

Abalone are cosmopolitan gastropods found in temperate and tropical seas, occurring from the intertidal zone down to depths in excess of 400 m (McLean, 1966; Tarr, 1989), although some species, including the South African *H. midae*, are only found in shallow waters. The distribution pattern of the species indicates four distinct faunal regions (Shepherd *et al.*, 1992) - East Africa and the Indian Ocean; Australia - New Zealand; the Japan-China coast; and the Pacific Insular Arc.

Five species of *Haliotis* occur in South Africa (Fig. 1). *H. midae* being found from Cape Columbine on the west coast to about Port Elizabeth in the south-east (Newman, 1965; Kensley, 1973).
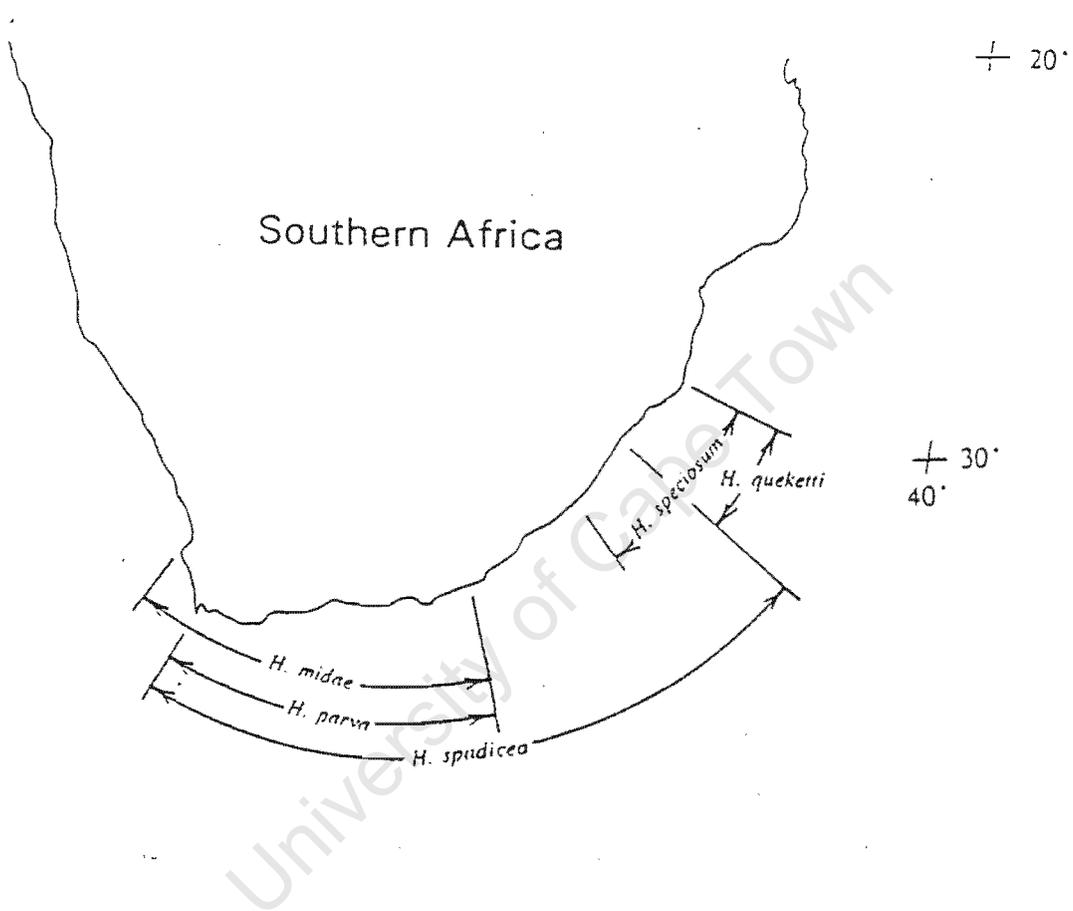
Fig. 1. Distribution of temperate *Haliotis* in South Africa (after Kensley, 1973).

1. 6. Fishery industry.

Among the 100 species of *Haliotis*, only 20 large temperate species support commercial fisheries. Major fisheries production areas are Japan, Australia, New Zealand, USA, Mexico and South Africa (Oakes and Mercer, 1993; Oakes and Ponte,

1996). Abalone products are marketed in different forms live, frozen, canned and dried. Japan and China are the major consumers, taking 80% of the world catch (Redmayne, 1991). About 20 000 metric tons of abalone production with a value of about US$ 300-400 million was estimated in the 1980's (Table 1). The abalone industry faces competition with other major fisheries, forcing the industry into decline (SFRI, 1998; Cook and Grant, 1998).

Table. 1. Estimated supply volume and price structure for major abalone- producing countries. Supply is presented in metric tons (MT) of whole body, including shell. Weight and dollar values were calculated using the conversions given by Oakes and Ponte (1996).

Country	Species	Annual supply (MT)	1993 Price (US$ MT^{-1})
—			
Mexico	*H. fulgens*	2 000	24 000
	H. rufescens		
	H. cracherodii		
South Africa	*H. midae*	600	25 000
Australia	*H. laevigata*	3 600	21 850
	H. rubber		
	H. roei		
Hong Kong	*H. diversicolor diversicolor*	567	22 200
	H. diversicolor supertexta		
Japan	*H. discus hannai*	4 000	66 000
	H. discus		
USA	*H.refescens*	350	25 000

H. carcharoides

H. sorenseni

H. fugens

1. 7. South African abalone fishery.

The abalone fishery in South Africa relies on only one species, *H. midae*, locally known as Perlemoen - a Dutch word for mother of pearl (Tarr, 1989). The fishery is based on subtidal stocks covering a coastal length (Fig.2) of approximately 580 km (Newman, 1964; Tarr, 1992).

In 1953 the Government imposed a fishing size limit of 10.16 cm breadth (12.4 cm shell length) which later was elevated to 11.43 cm (13.8 cm shell length) due to over-fishing. There are four factories for the industry and, according to government legislation, the factories have to market 10% of their products locally. In 1968 each factory was granted a fixed percentage quota and divers were affiliated to specific factories. The quota system was changed to mass quota based on mass delivery to the factories and was expressed as the sum of total abalone catch in each of the fishing zones. The annual Total Allowance Catch (TAC commercial quota) stands at 515 metric tons (SFRI, 1998) which represent a 16% overall reduction since 1995. This reduction in the TAC is an action implemented to compensate for the growth of other sectors of the fishery such as recreation (Sweijd, 1999).
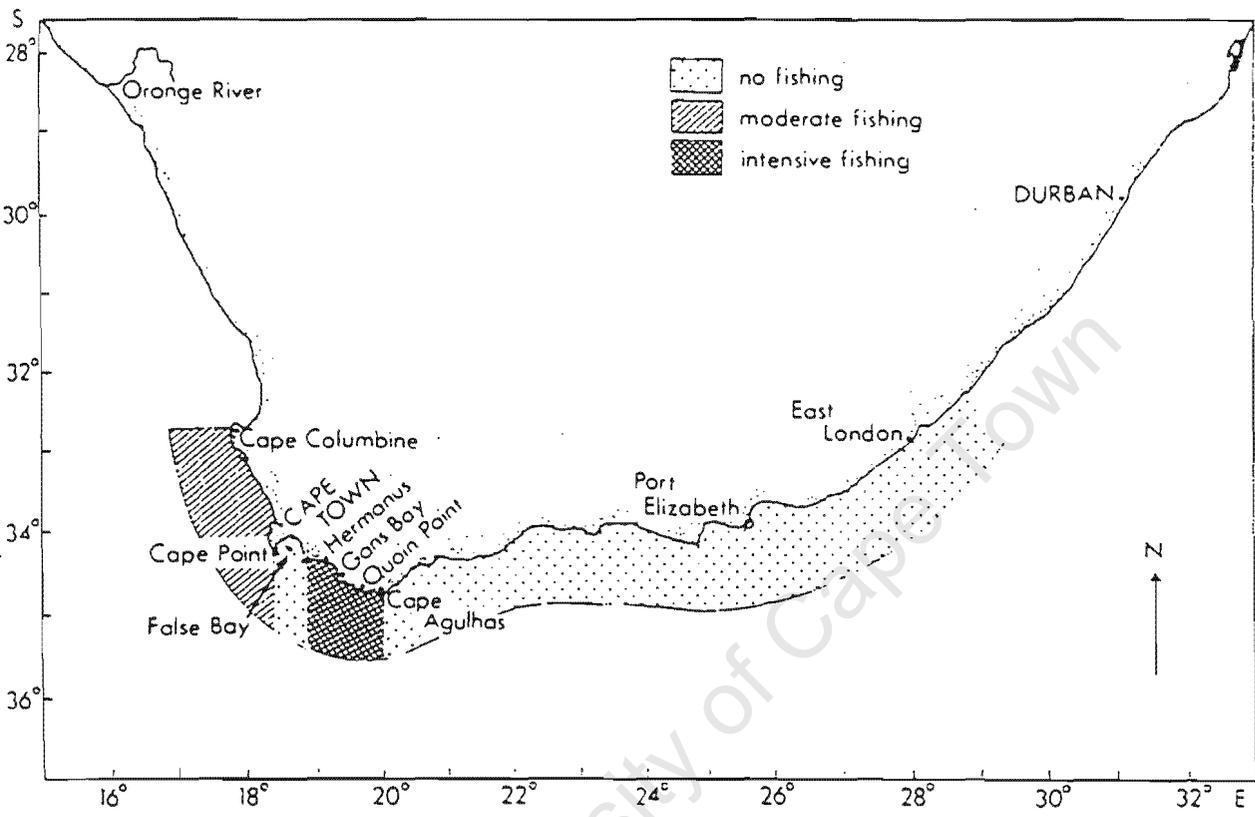
Fig.2. The distribution of *Haliotis midae*, showing the commercially fished areas (after Newman, 1964).

Estimates of catch per unit effort (C.P.U.E.) are illustrated in Figure 3. These show a general declining CPUE over the period between 1960 and 1970 (Tarr, 1997).
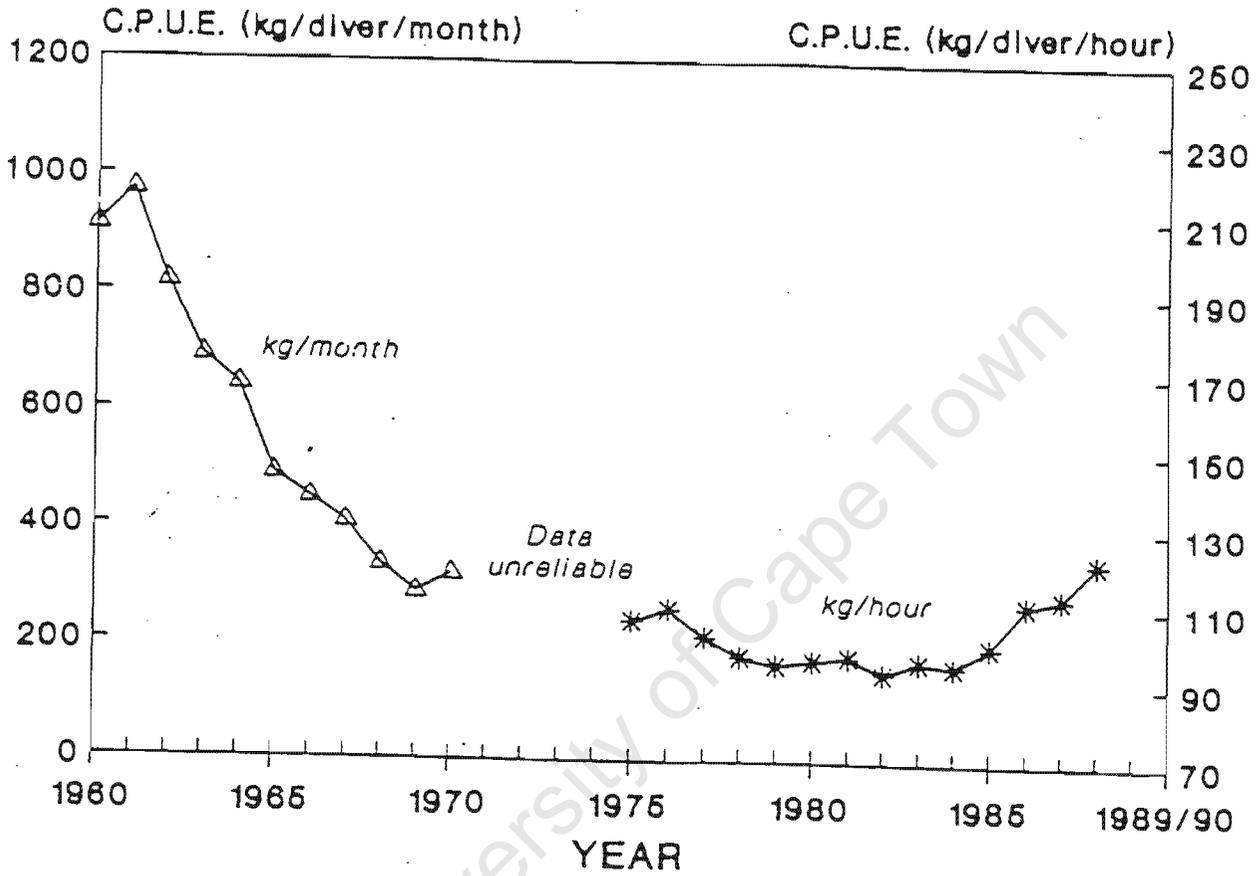
Fig. 3. Catch per unit effort (C.P.U.E.) trends for all areas combined (after Tarr, 1997).

1. 8. Economic importance.

Abalone farming recently attracted the attention of investors as a viable commercial venture resulting in emerging industries. The cocktail abalone fetches high prices in the world market. Areas like California and Japan have succeeded in developing large abalone industries (Oakes and Ponte, 1996), while South Africa is still in the early stage of abalone industry development, but it seems likely that in future farming of South African abalone will intensified with wider market opportunities to the eastern.

For transportation of live abalone, speed of delivery is important; this being done almost exclusively by air. The cost of air freight will always necessitate the use of light packing mass, excluding the possibility of transport in tanks (Dichmont and Przybylak, 1990, 1991; Dela-Cruz and Morris, 1997). Abalone are therefore, transported in cold containers with some humid packing materials.

1. 9. Energy metabolism of molluscs with special reference to Haliotidae.

Many marine invertebrates undergo anaerobic metabolism during periods of oxygen lack, such as exposure in air during low tide. Haliotidae which are often subjected to such conditions during transportation have to develop adaptational mechanisms to enable them to survive. Such adaptation mechanisms are dictated by different enzymes that terminate anaerobic glycolysis in the tissues of many marine invertebrates (Ellington, 1979, 1980; Gäde and Grieshaber, 1986) producing end products such as tauropine, octopine, strombine and alanopine via reductive condensation of pyruvate (Field and Hochachka, 1981; Plaxton and Storey, 1982a,b; Storey, 1983; Nicchitta and Ellington, 1984) with respective amino acids (taurine, arginine, glycine, alanine). During low tide or reduced oxygen tension in mud flats, marine and fresh water invertebrates such as bivalves, gastropods and annelids experience anoxic conditions (de Zwaan, 1977; England and Baldwin, 1983). These

species are able to withstand prolonged environmental anaerobiosis. Their metabolism, during such periods of environmental anoxia, is characterised by co-fermentation of aspartate and glycogen, leading to the formation of end products such as alanine, acetate, succinate and propionate depending on the species. The rate of energy production during anaerobiosis is low, but ATP yield increases (Barringtone, 1979; Ebberink *et al.*, 1979; Livingstone, 1982; Storey, 1985).

The capacity of muscle tissues of *Haliotis* to synthesise ATP at times of excessive locomotory activity by aerobic means is reduced and energy provisions are met during functional anoxia via arginine phosphate and anaerobic glycolysis resulting in the accumulation of lactate and opines as end products as well as the building up of arginine in the tissues (Grieshaber, 1986; Gäde, 1986, 1988; Baldwin *et al.*, 1992). Arginine phosphate is a major source of energy supply during environmental and functional anoxia among *Haliotis* spp.

1. 10. Energy metabolism of *Haliotis* during transport.

Reduced temperature during transportation of live abalone is necessary to minimise metabolic rate and flesh spoilage. It is important that good packaging methods are used during transportation to minimise stress which might lead to accumulation of anaerobic end products (Dichmont and Przybylak, 1990,1991; Morris and Oliver, 1999). Transportation of live seafood at low temperature to minimise mortality has received limited attention by live seafood exporters (Whiteley and Taylor, 1990; Whiteley *et al.*, 1990; Samet *et al.*, 1996).

The metabolic events associated with anaerobic stress during handling and shipping of live abalone is known for *H. iris* (Baldwin *et al.*, 1992; Ryder *et al.*, 1994). Abalone have biochemical mechanisms that enable them to survive when removed from water and these include the synthesis of pyruvate reductase compounds, such as tauropine (Gäde, 1988). Tauropine is suggested to have a biological role in protecting tissues during hypoxia (Baldwin *et al.*, 1992).

Pyruvate reductase end products produced during air exposure, can be used as indicators of anaerobic stress (Bayne *et al.* 1976; de Zwaan and Wijsman, 1976; Baldwin *et al.*, 1992) and these could as well be used to evaluate meat quality (James and Olley, 1970). Shofer and Tjeerdema (1998) suggested that the metabolic state of tissues can be evaluated from the adenylate energy charge, the ratio of concentration of ATP to its degradation products ADP and AMP (Atkinson and Walton, 1967).

1. 11. Oxygen consumption during simulated transport.

The relative abundance of oxygen over carbon dioxide in air compared to water often allows a relative hyperventilation for oxygen uptake and, consequently, carbon dioxide excretion becomes a problem (Dejours, 1981).

Oxygen consumption of *Haliotis kamtschatkana* during locomotion exercise was reported by Donovan and Carefoot (1997), in gastropods (Boyden, 1972; Calow, 1974). They reported a linear relationship of oxygen uptake and crawling both in aquatic and aerial environments. The *Haliotis* foot rhythmic movement is a strategy which saves energy during grazing and escape from predators (Miller, 1974; Trueman, 1983).

Insufficient oxygen consumption during air exposure causes stress that depends on the functioning of blood pigments which are not optimised (Storey and Storey, 1978; Wood and Randall, 1981; Storey, 1983; Morris *et al.*, 1985; McMahon and Burggren, 1988). The acid-base balance of the blood during emersion has been studied in various crustaceans such as lobster *Homarus gammarus* (Taylor and Whiteley, 1989), cray fish *Austropotamobius pallipes* (Taylor and Wheatley, 1980) and crab *Scylla serrata* (Varley and Greenaway, 1992).

1. 12. Heart beat.

The first description of the haliotid cardiovascular system was given by Crofts (1929) for *Haliotis tuberculata*. Later, the vascular anatomy of *H. corrugata* was studied by Bourne and Redmond (1977) and that of *H. cracherodii* by Jorgensen *et al.* (1984). The relationship of *Haliotis ruber* heart rate to water temperature and partial oxygen tension was reported by Russell and Evans (1989). The heart rate decreases when animals are exposed to air. When marine invertebrates are exposed to air during low tide (Trueman, 1967) or subjected to pollutants (Stenton-Dozey and Brown, 1994) heart rate decreases, indicating stress which could result in death of the animal.

The heart beat of *Haliotis midae* was measured during simulated transport to investigate physiological conditions of the stressed animal

MATERIALS AND METHODS

CHAPTER 2.

2.   MATERIALS and METHODS

2. 1.   Experimental animals.

Haliotis midae: Abalone (shell length 90 - 100 mm) were collected by divers from Onrus near Hermanus and from Cape Point. Live specimens were transported to the laboratory and kept in glass aquaria (90 x 32 x 37 cm) in circulating, aerated sea water at an ambient temperature of 15 °C. The specimens were collected by divers using bar knives to lift the abalone from rock substrata. They were carefully removed without injuring the foot. The shell length was measured as the distance between the posterior and anterior extremities of the shell. Size of the animals was chosen to correspond to the market size. Animals were transported on a flat, rigid plastic sheet in a polystyrene box (44 x 38 x 26 cm). The temperature inside the box was kept low by means of an ice block . On arrival they were placed in the aquarium at 15 °C for two weeks before beginning the experiment. They were fed on kelp, which is their dominant food in the natural habitat (Fallu, 1991; Branch *et al.*, 1994). Fresh kelp was provided twice a week. Abalone adhered to the glass walls of the aquarium and were extremely difficult to remove, they were in good condition and no damage to the foot was experienced.

Loligo vulgaris: The enzyme octopine dehydrogenase was needed for one of the biochemical assays described later. The mantle tissue of the squid, *Loligo vulgaris,* was used as a source of this enzyme. Specimens of *Loligo vulgaris* were caught from False Bay by fishermen using line hooks. Six animals, with an average body length of 25.17 ± 1.17 cm, were gutted, skinned and the mantle muscles kept frozen at - 80 °C for future OcDH extraction.

2. 2. Profile of distribution of various dehydrogenases in *Haliotis midae* tissues.

2. 2. 1. Sample preparation.

Abalone (n = 7) were removed individually from the aquarium and tissues removed from the shell immediately by applying thumb pressure to the posterior edge of the adductor muscle where it abuts the shell. The shell adductor, foot and gill were excised for analysis of enzyme activities (Gäde, 1986).

About 1 to 5 g wet weight of tissues were diced with scissors and homogenised (1 : 5 w / v) in ice cold, 50 mM imidazole buffer, pH 7.2, containing 1 mM EDTA and I mM dithiothreitol (DTT), using an Ultra Turrax T- 25 homogeniser. The homogenate was centrifuged at 20 00 x g for 20 minutes at 4°C. The supernatant was kept aside. The pellet was rehomogenised in the same buffer as above and, after centrifugation, the supernatants were pooled.

2. 2. 2. Enzyme assays in crude extract.

Soluble protein was assayed in the clear supernatant according to the method outlined in Gäde (1986). The following enzyme activities were determined in the supernatant: tauropine dehydrogenase (TaDH), lactate dehydrogenase (LDH), octopine dehydrogenase (OcDH), strombine dehydrogenase (StrDH), alanopine dehydrogenase (AlaDH) and malate dehydrogenase (MDH).

All determinations were carried out at 340 nm and 25 °C in 1 ml cuvettes in a Vitatron Universal photometer. The final volume was 1 ml. Determination was performed in duplicate with different concentrations of extract used to ensure linearity. The various enzyme activities were measured under the following conditions:

Tauropine dehydrogenase (EC 1. 5. 1. X) 2.5 mM pyruvate, 0.15 mM NADH, 50 mM imidazole-HCl buffer, pH 7.0. The reaction was started with taurine (80 mM).

Lactate dehydrogenase (EC 1. 1. 1. 28) (pyruvate reductase) 0.15 mM NADH, 50 mM imidazole-HCl buffer, pH 7.0. The reaction was started with pyruvate (2.5 mM).

Octopine dehydrogenase (EC 1. 5. 1. 11) 2.5 mM pyruvate, 0.15 mM NADH, 50 mM imidazole-HCl buffer, pH 7.0. The reaction was started with arginine (5 mM).

Strombine dehydrogenase (EC 1. 5. 1. 22) 2.5 mM pyruvate, 0.15 mM NADH, 50 mM imidazole-HCl buffer, pH 7.0. The reaction was started with glycine (200 mM).

Alanopine dehydrogenase (EC 1. 5. 1. 17) 2.5 mM pyruvate, 0.15 mM NADH, 50 mM imidazole-HCl buffer, pH 7.0. The reaction was started with alanine (100mM).

Malate dehydrogenase (EC 1. 1. 1. 37) 0.15 mM NADH, 50 mM imidazole-HCl buffer, pH 7.0. The reaction was started with oxaloacetate (0.5 mM).

The oxidation of NADH was monitored at 340 nm using a Vitatron Universal photometer equipped with a recorder. One unit of enzyme activity (IU) is taken as the amount of enzyme causing transformation of 1 μmol of substrate / min under standard condition. The absorption coefficient of NADH at 340 nm is taken as 6.3 μmol cm^{-1}.

2. 2. 3. Purification of enzymes from muscles of *Haliotis midae* and *Loligo vulgaris*.

Tauropine and octopine dehydrogenases from *H. midae* and *Loligo vulgaris* tissues were purified for the determination of metabolites from abalone muscles.

Preparation of tissues : All procedures were carried out at 4 °C. Fresh shell adductor muscle of *Haliotis midae* was diced with scissors, suspended in 50 mM imidazole-HCl buffer, pH 7.2 (1 : 5 w / v), containing 1 mM EDTA, 1 mM dithiothreitol and homogenised with an Ultra Turrax T-25 homogeniser. The homogenate was centrifuged for 20 min at 20 000 x *g*. The supernatant was put aside and the pellet rehomogenised in an appropriate volume of the same buffer and centrifuged as before. The combined supernatants formed the crude supernatant, it was brought to 45% saturation with ammonium sulphate under constant stirring and centrifuged as before. The protein pellet was discarded, and the resulting supernatant was then adjusted to 80% saturation with ammonium sulphate, stirred and centrifuged as above. The precipitate was resuspended in 10 ml of homogenisation buffer and was applied in 2.5 ml aliquots to a pre-packed G-25 Sephadex column (1.5 x 8.0 cm) equilibrated in homogenisation buffer. The crude extract was eluted with this buffer to the void volume to remove salt contents.

Tauropine dehydrogenase (TaDH) from *H. midae* tissues: The aliquots from the G-25 Sephadex column were pooled and applied to a pre-packed DEAE-Sephacel column (1.6 x 40 cm) equilibrated with 50 mM imidazole buffer-HCl pH 7.2, containing 1 mM EDTA and 1 mM DTT. After the application of the crude extract, column was washed with 100 ml of equilibration buffer. The unbound substances were washed off the column of the ion-exchanger matrix while proteins such as TDH bind to the matrix under these conditions. Bound enzyme was eluted from the column using a linear gradient of 0 - 180 mM NaCl in 600 ml equilibration buffer. Fractions (10 ml) were assayed for tauropine, lactate and malate dehydrogenases.

Octopine dehydrogenase : About 20 *g* of frozen mantle muscle of squid, *Loligo vulgaris*, was homogenised in (1 : 5 w / v) ice cold 10 mM Tris-HCl buffer, pH 7.5, containing 1 mM EDTA and 1 mM DTT and the procedure repeated. The combine supernatants were passed through a pre-packed
G-25 Sephadex column (1.5 x 8.0 cm) equilibrated with the extraction buffer. The aliquots were pooled and applied to a pre-packed DEAE-Sephacel column (1.6 x 40.0 cm), equilibrated with 10 mM Tris-HCl buffer, pH 5.0, containing 1 mM EDTA and

1 mM DTT. The column was washed with 100 ml of equilibration buffer. Elution

fractions (10 ml) were assayed for OcDH, MDH, LDH.

2. 2. 3. 1. Protein determination.

Protein in the elutant was monitored by measuring the absorbance at 280 nm, using a

LBK Ultrospec spectrophotometer. Protein determination on the pooled enzyme

fractions and of the purified enzymes was performed according to the method of

Lowry *et al.* (1951), with bovine gamma globulin as standard.

2. 3. Incubation of abalone in oxygen-free sea water.

In total 18 abalone were incubated in pairs in an aquarium (21 x 40 17 cm) filled with

sea water (15°C) that had been gassed with pure nitrogen until Po_2 (monitored with an

oxygen electrode, Syland Scientific) reached almost zero mm Hg. The oxygen

electrode was calibrated with air-saturated seawater as determined by Winkler

titration (Strickland and Parsons, 1968). After the animals were inserted, the

aquarium surface was covered with a polystyrene sheet and the water flushed with a

constant, slow stream of nitrogen gas. After 6 h of anoxic incubation, the animals

were removed, and the shell adductor, foot and gill muscles excised, blotted and

frozen in liquid N_2 then stored at -80 °C. Nine abalone were used as control by

placing them in well aerated seawater at 15°C. After 6 h in anormoxia, the animals

were removed and tissues treated as above.

2. 4. Behaviour of *H. midae* during exercise

Nine individual abalone were exercised for 15 to 18 minutes in an aquarium

(25 x 25 x 25 cm) at 15°C with flowing aerated seawater. The animals were placed

upside-down on their shells; their righting movements involved vigorous contractions

of the shell adductor and foot muscles. When the animals had regained their normal

posture, they were immediately inverted again. The procedure was repeated for up to 18 min, when the animal could no longer make any muscular movement and appeared to be too exhausted to right itself. Animals were then killed, the shell adductor, foot and gill muscles excised, frozen in liquid N_2 and stored at - 80 °C.

2. 5. Transport simulation studies on *Haliotis midae.*

In this experiment, the simulated transportation protocol was designed to be as similar as possible to that used in commercial operation.

A clear plastic bag, 74 cm long and 54 cm wide, was packed with foam sponges (2.5 cm thick) which had been soaked in seawater. A perforated polystyrene sheet was placed on top of the sponges with an ice block underneath. Abalone were removed from seawater (7 °C and 10 °C respectively) and packed into the plastic bag resting on the polystyrene sheet and then flushed with pure oxygen. The bag was closed and placed in a polystyrene box (44 x 38 x 26 cm). Polystyrene chips were packed into the box. The lid of the box was then closed with adhesive tape. The boxes were placed in a temperature controlled room at 7 °C and 10 °C respectively and sampled after 6, 12, 24 and 36 hours. The temperature inside the box was monitored with Bailey Bat thermometer throughout the experiment. The adductor, foot and gill tissues were frozen in liquid N_2, then stored at - 80 °C for further analysis.

2. 6. Metabolites in tissues.

Metabolite concentrations were determined in shell adductor, foot and gill of *Haliotis midae* subjected to oxygen free seawater, exercise and simulated transport experiments. Assays were carried out at room temperature, using a Vitatron Universal photometer with a final concentration of 1 ml in the cuvette.

2. 6. 1. Preparation of perchloric acid (PCA) extract.

Perchloric acid extracts (PCA) from frozen tissues were made according to the method of Zebe and Gäde (1993). Frozen tissue samples were ground to powder in a

mortar, cooled by liquid N_2. The powder was then transferred into a pre-weighed Eppendorf tube containing perchloric acid (PCA, 1 M) which was adjusted to a tissue / PCA ratio of 1 : 5. The mixture was sonicated twice for 10 s by means of a Branson sonifier B 30 and centrifuged for 10 min at 20 000 x g (RC5C, Sorvall) at 4 °C. The pellet was resuspended in PCA and the procedure repeated. The combined supernatants were neutralised by adding K_2CO_3 (3 M), The precipitate was centrifuged as above and the final volume of the extract measured. Arginine, arginine phosphate and adenosine nucleotide concentrations from the PCA extract were assayed immediately. About 5 ml of the remaining extract was stored at - 80 for the determination of the other metabolites at later stage.

2. 6. 2. Determination of metabolite concentrations.

Arginine assay: Arginine concentrations were assayed from the PCA extract according to the method described by Gäde and Grieshaber, (1975). The composition of the reaction was 100 mM KH_2PO_4 buffer pH 7.0; 0.2 mM NADH; 2.5mM pyruvate; 27 U/l OcDH.

Arginine phosphate determination: As outlined by Gäde (in Bergmeyer *et al.* 1985), arginine phosphate was hydrolysed in order to obtain arginine. Neutralised PCA (0.1 ml) extract was mixed with 0.1 ml of 1 M HCl and 0.2 ml H_2O , hydrolysed at 100 °C for 90 seconds in a tightly sealed tube. Tubes were immediately chilled in ice and samples neutralised with 0.1 ml NaOH (1 M). The solution was assayed for arginine as above. The difference between the calculated amount of arginine phosphate plus arginine and the previously determined arginine gave the value for arginine phosphate.

Determination of adenosine nucleotide: Concentrations of ATP, ADP and AMP were determined from the PCA extract. ATP concentration was assayed using the method of Trautschold *et al.* (1974) while ADP and AMP were assayed according to the method of Jaworek *et al.* (1974). The energy charge in the muscles was calculated from the data obtained for concentrations of adenosine nucleotides as AEC = [ATP] + 0.5[ADP] / [ATP] + [ADP] + [AMP] (Atkinson and Walton, 1967).

Glycogen determination: Glycogen was precipitated from an aliquot of the PCA extract with 5 ml ethanol, to which 200 μl Na_2SO_4 (saturated) was added. The mixture was kept at low temperature overnight and centrifuged at 10 000 g for 20 min at 4 °C. The precipitate was then re-dissolved in 1 ml distilled water and 25 μl samples were analysed using the anthrone method (Carroll *et al.*, 1955). Absorbance was measured at 585 nm using a Vitatron Universal photometer with glycogen (Sigma Co.) as standard.

D-lactate determination: D-lactate concentrations were determined (Bergmeyer and Graßl, 1984) from PCA extracts, the composition of the reaction mixture being 0.22 mM glutamate buffer pH 9.5; 5 mM NAD^+; 11.6 U / I glutamate pyruvate transminase (GPT); 27.3 U / I. D-lactate concentrations were calculated according to Lambert- Beer's law.

Tauropine determination: Tauropine concentrations from the PCA extracts were determined according to the method described by Gäde (1986).
The composition of the reaction mixture was 0.22 mM glutamate buffer pH 9.5; 5 mM NAD^+; 11 U / I GPT; 27.3 U / I TaDH;. Tauropine concentrations were calculated as above.

2. 7. Oxygen consumption by *Haliotis midae*

Oxygen analyser: Oxygen concentrations were measured using an Ametek S-3A oxygen analyser. A glass tube, filled with soda lime and silica gel, was connected with a rubber tube to the inlet of the sensor of the oxygen analyser. The other end was fitted with a groove to the Indo- Gem hydrodermic syringe.
The flow arrangement as illustrated in Figure 4 draws the gas through the cell and flowmeter with its pump and needle valve. Oxygen partial pressure across the cell generates a DC voltage which feeds to the S-3A readout / control unit for processing and display on a digital panel meter

Sensor   Flowmeter   Pump

Sample → [ ] → [ ] → [ ] →

S-3A Readout / control   Recorder
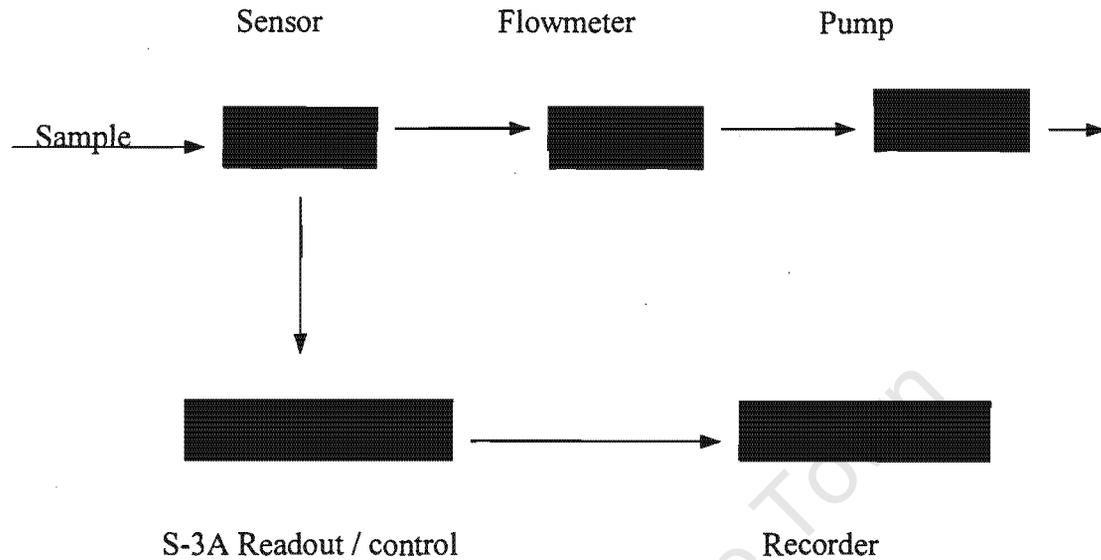
Fig. 4. Flow diagram of Ametek S-3A Oxygen analyser showing the path of oxygen flow through the analyser. Oxygen concentration is displayed on the readout panel.

Oxygen consumption measurements in air: Oxygen consumption by individual *Haliotis midae* was measured in a closed perspex respirometer (1500 ml) held in a temperature controlled room at 7°C and 10°C respectively. A perforated plastic plate at the bottom of the chamber supported the animal. The chamber was provided with a tight lid and a rubber tube connected to a hole into the chamber. Both were air tight. The chamber was then placed in a transport box and closed. Air was sampled from the chamber after 6, 12, 24 and 36 hours using a 50 ml Indo-Gem, hydrodermic syringe. Air samples were passed through a rubber tube connected to a glass tube containing soda lime and silica to absorb carbon dioxide and water respectively before entering the oxygen sensor and analyser. Oxygen consumption was calculated as ml O_2 g^{-1} h^{-1} wet weight.

Oxygen consumption measurements in water: A flow-through perspex respirometer chamber (1500 ml) was fitted with a dissolved-oxygen probe and placed in a constant

temperature room at 7 °C, and used to measure the rate of oxygen consumption. This system consisted of an upper reservoir from which seawater at (7 °C) flowed via the chamber of respirometer into a bottom reservoir from which seawater was pumped back to the top reservoir. The chamber, fitted with a probe, could be instantly shut off from flowing seawater by tourniquet valves on the inlet and outlet hoses, or by closing the exit-taps attached to the top reservoir. The chamber was submerged in a tank of seawater to prevent air-leakage, and a magnetic stirrer below the tank ensured circulation within it. After cleaning off algae and epifaunal organisms, *Haliotis midae* was placed on a perforated disc to acclimatise overnight. Seawater was sieved of micro-organisms using a filter 0.54 μm. The chamber was flushed for 10 minutes with air-saturated seawater before being shut off to monitor oxygen decline. The oxygen probe was calibrated with the oxygen concentrations of air saturated seawater as determined by Winkler titration at 7 °C.

After 20 minutes, when oxygen tension fell to about 80% saturation, the lid was opened and the chamber was flushed with air-saturated seawater before repeating the procedure. About ten readings for each individual were obtained and averaged.

2. 8. Heart beat

The heart beat of *Haliotis midae* was measured by a technique developed by Trueman (1967). A hole of 10 mm diameter was drilled through the shell over the position of the heart without touching the underlying tissues using a Dremel Motor-258-5 drilling machine as described by Segal *et al.* (1953). Fine silver-wire electrodes were inserted through a rubber fitted into this hole so that they just touched the heart muscles. The electrodes were connected in series to an impedance pneumograph (A.C. coupling) and a George Washington oscillograph by a light screened cable (see Fig. 5). Changes in the impedance between the electrodes resulting from pulsatile changes in the volume of the heart or from movements of the animal within the shell were recorded on the oscillograph.

Abalone with implanted electrodes were kept in a 1.5 l tank in well-aerated seawater and allowed to equilibrate overnight at 7 °C before the experiment. The animals were

fed on kelp during this equilibration period as starvation can effect the heart beat (Bayne, 1976).

Individuals were transferred from the equilibration tank into a transport box at 7 °C and left for 30 minutes before recording commenced. Foam sponges (25 cm thick), soaked in seawater, were placed at the bottom of the box. A perforated polystyrene sheet was placed on top of the sponges on which the animals were placed. This was to avoid the animal being in contact with the foam sponges. The temperature in the box was monitored. The box was tightly closed and sealed with adhesive tape.

The heart beat of each animal was monitored for 15 minutes after a period of 6, 12, 24 and 36 h in the transportation box. The experiment was repeated 5 times at each of the exposure times and 5 animals were used per experiment.

For a control experiment, heart-beat was monitored with five implanted animals placed in an aquarium with well aerated sea water.

2. 9. Chemicals.

Enzymes, substrates, co-factors, the adenine nucleotides and reagents used in this study were purchased from Boehringer Mannheim Co. and Sigma GmbH Co., Cape Town. Other chemicals were of analytical grade and obtained from Merck. Tauropine dehydrogenase (TaDH) and octopine dehydrogenase (OcDH) were prepared from shell adductors of *Haliotis midae* and mantle muscles of *Loligo vulgaris*, respectively.

2. 10. Statistical analysis.

The data obtained were statistically analysed using Student's t-test (Zar, 1999). For significance levels refer to legends.

RESULTS

3. RESULTS.

3. 1. Activities of dehydrogenases from crude extracts of various tissues of *Haliotis midae*.

Maximum activities of pyruvate reductases, namely tauropine dehydrogenase, octopine dehydrogenase, strombine dehydrogenase and alanopine dehydrogenase in various tissues of the abalone *Haliotis midae* were measured at rest. Results are depicted in Fig. 6. Tauropine dehydrogenase showed the highest activity in adductor muscles at 53.85 ± 13.56 U g^{-1} wet weight.

Activities of octopine dehydrogenase, strombine dehydrogenase and alanopine dehydrogenase in adductor muscles were all very similar and lowest at 1.96 ± 0.94; 1.93 ± 0.99 and 2.06 ± 1.05 U g^{-1} wet weight, respectively.

Activity of tauropine dehydrogenase in the foot and gill is less than 10% that of the adductor muscle: 4.02 ± 0.77 and 5.78 ± 0.86 U g^{-1} wet weight respectively. Lactate dehydrogenase and malate dehydrogenase are found in all tissues. Lactate dehydrogenase activity is highest in the foot at 12.07 ± 3.31 U g^{-1} wet weight, while malate dehydrogenase showed almost similar activity in shell adductor, foot and gill (Fig. 6).
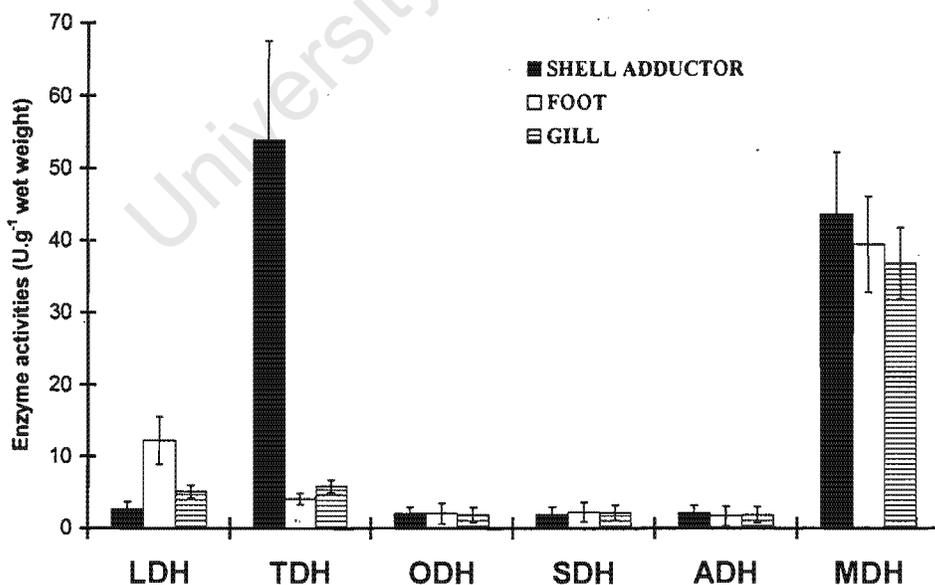
Fig. 6. Enzyme activities for tauropine dehydrogenase (TaDH), octopine dehydrogenase (OcDH), strombine dehydrogenase (StrDH), alanopine dehydrogenase (AlaDH), lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) in tissues of *Haliotis midae*. For each tissue are given means $\pm$ S.D. (n = 7) in units per gram wet weight.

3. 2. Enzyme purification.

3. 2. 1. Purification of Tauropine dehydrogenase (TaDH).

Tauropine dehydrogenase was purified from shell adductor of *Haliotis midae* on a DEAE- Sephacel column (fig. 7). The peak for tauropine dehydrogenase came off the column at 14.40 mM NaCl. The peak for malate dehydrogenase was eluted later at 28.85 mM NaCl. Lactate dehydrogenase which only occurred at low activities in adductor muscle (see 3.1), was not detected in the column fractions or in the initial column wash.

Tauropine dehydrogenase was purified 8.5-fold with 47% recovery from adductor muscle. The final enzyme preparation had a specific activity of 145.51 units mg protein^{-1} (Table 2).

In its purified state TaDH activity was maintained for 4 weeks at 4 °C.

Fig. 7. Elution pattern of tauropine dehydrogenase from extract of *Haliotis midae* adductor muscles on DEAE-Sephacel. The gradient profile (___) is expressed as concentration of sodium chloride added to the equilibrium buffer (50 mM imidazole buffer, pH 7.2). Assay condition for tauropine dehydrogenase activity: 50 mM imidazole / HCl, pH 7.0, 0.15 mM NADH, 2.5mM pyruvate, 80 mM taurine.

The purification procedures of tauropine dehydrogenase through different stages, purity of different fractions, the overall degree of purification and the yields are shown in Table 2.
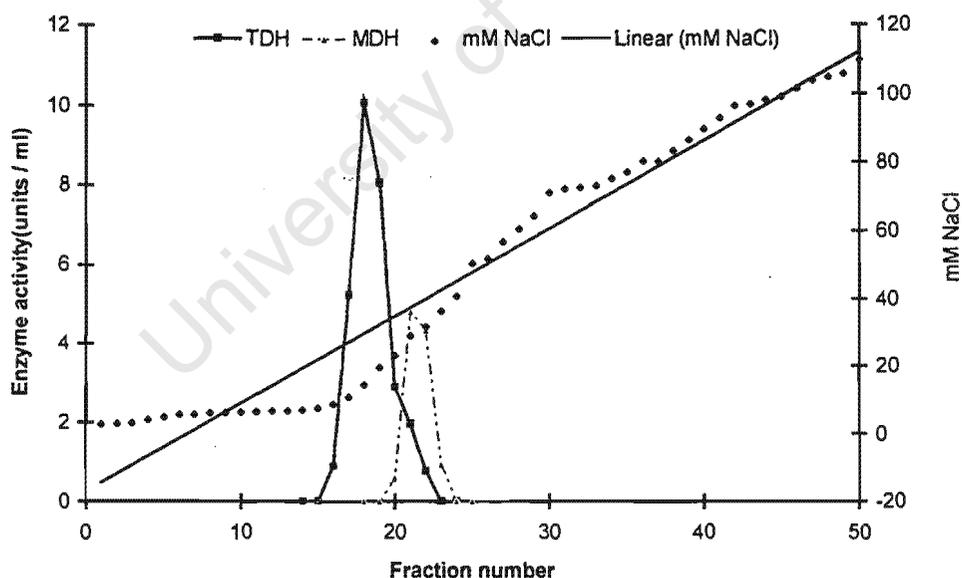
Table. 2. Purification of tauropine dehydrogenase from shell adductor muscle (31 g) of *Haliotis midae*.

Steps	Vol.	Enzyme activity	Total activity	Protein concentration	specific activity	Yield	Purification
	ml	Units/ml	total units	mg/ml	units / mg protein	%	fold
Crude extract	194	12.74	2473.50	0.75	17.05	(100)	1.0
45% (NH₄)₂SO₄	194	11.22	2176.67	1.24	9.03	88	0.53
80% (NH₄)₂SO₄	12.5	92.98	1162.25	0.64	145.51	46.99	8.53

Protein was precipitated with 45% and 80% ammonium sulphate saturation at different steps respectively.

3. 2. 2. Purification of octopine dehydrogenase (OcDH).

Prior to purification of octopine dehydrogenase from extract of *Loligo vulgaris* mantle muscle, appropriate ammonium sulphate concentrations for protein precipitation, and buffer pH were established in pilot experiments.

Appropriate ammonium sulphate concentrations for OcDH precipitation: Crude supernatants of *Loligo vulgaris* mantle muscles were treated with different concentrations of ammonium sulphate. Proteins were precipitated from the supernatant phase at 30% saturation with ammonium sulphate. Further addition of ammonium sulphate increased protein precipitation. Enzyme activities in the pellet also increased with the increase of ammonium sulphate concentrations while enzymes activities in the supernatants decreased. At 60% ammonium sulphate concentrations, most of the enzymes measured were active in the pellet (798.61 units), while enzymes activities in the supernatant phase was low (10.85 units), Table 3. Although enzymes were precipitated between 30% - 60% concentrations

with ammonium sulphate, there was 17.27% protein loss during the processes. Complete separation does not usually occur because there is always some overlap in the solubility of protein in a mixture (Plummer, 1978).

Table 3. Enzyme activities (total units) in supernatant and pellet of *Loligo vulgaris* mantle muscles.
Enzyme activities (Total units).

$(NH_4)_2SO_4$ *	LDH			OcDH			MDH	
%	Supernatant	Pellet		Supernatant	Pellet		Supernatant	Pellet
20	42.63	0		954.83	23.56		65.86	8.77
30	32.21	0.20		796.39	125.56		60.53	12.07
40	22.52	14.67		356.94	440.19		36.80	34.06
50	7.87	25.68		96.52	687.61		12.10	45.30
60	0	38.08		10.85	798.61		3.20	56.77

* Different percentage of ammonium sulphate saturation added to the extract of *Loligo vulgaris* mantle muscles. There was 17.27 % protein activities lose during the procedure.

pH selection for OcDH purification on DEAE-Sephacel matrix: Two ml of DEAE-Sephacel anion exchanger solutions were divided into series of ten test tubes of 15 ml each. Each test tube was equilibrated by washing five times with 5 ml of pre-prepared 10 mM Tris-HCl buffers of different pHs. Two ml of the crude supernatants of the *L. vulgaris* extracts were added into each test tube, well mixed by vortex and left for 15 min for the gel to settle. Supernatants were assayed for OcDH, MDH, LDH activities.

OcDH did not bind on the gel matrix. Most of the proteins were in the supernatant phase at 5 pH exhibiting highest activities of 6.39 ± 0.58 U g^{-1} wet weight. There was a decrease of activities in the supernatants with an increase of pH. At pH 7 activity in the supernatants was 0.03 ± 0.31 U g^{-1} wet weight indicating that protein binds to the DEAE-Sephacel gel matrix. There was no activities for OcDH detected in the supernatant at pH 9. At pH 5 supernatant exhibited no activities for LDH and MDH. Most of MDH binds to the gel matrix (Table 4).

Table 4. Selecting ion exchange pH conditions for isolation of OcDH.

pH	OcDH	LDH	MDH
4.0	-	-	-
4.5	0.12 ± 0.39	-	-
5	3.20 ± 0.15	-	-
5.5	2.01 ± 1.07	-	-
6	0.35 ± 0.09	0.01 ± 0.51	0.13 ± 0.21
6.5	0.20 ± 0.23	0.02 ± 0.71	0.16 ± 0.51
7	0.03 ± 0.31	0.05 ± 0.46	0.19 ± 0.63

Determination of enzyme activities (U / ml) in the supernatants of *L. vulgaris* extracts using 10 mM Tris-HCl buffer with different pHs. There was no activity detected in the supernatant for MDH at pH 5.

3. 2. 1. Purification of OcDH on DEAE-Sephacel column.

Octopine dehydrogenase was purified from *L. vulgaris* mantle muscle on DEAE-Sephacel equilibrated with 10 mM Tris-HCl buffer, pH 5. OcDH did not bind to DEAE-Sephacel matrix under these conditions and was eluted off the column at the 6th and 7th fractions. The enzyme from the peak fractions (Fig. 8) was precipitated again with ammonium sulphate at 80% saturation. The final preparation after ammonium sulphate precipitation was only 4-fold purified and gave a yield of about 11.3 %, but this preparation was free of contaminating dehydrogenases (Table 5).

The enzyme maintained catalytic properties for one month at 4°C. Malate dehydrogenase remained in the column and later eluted at 250 mM NaCl when a salt gradient was introduced.
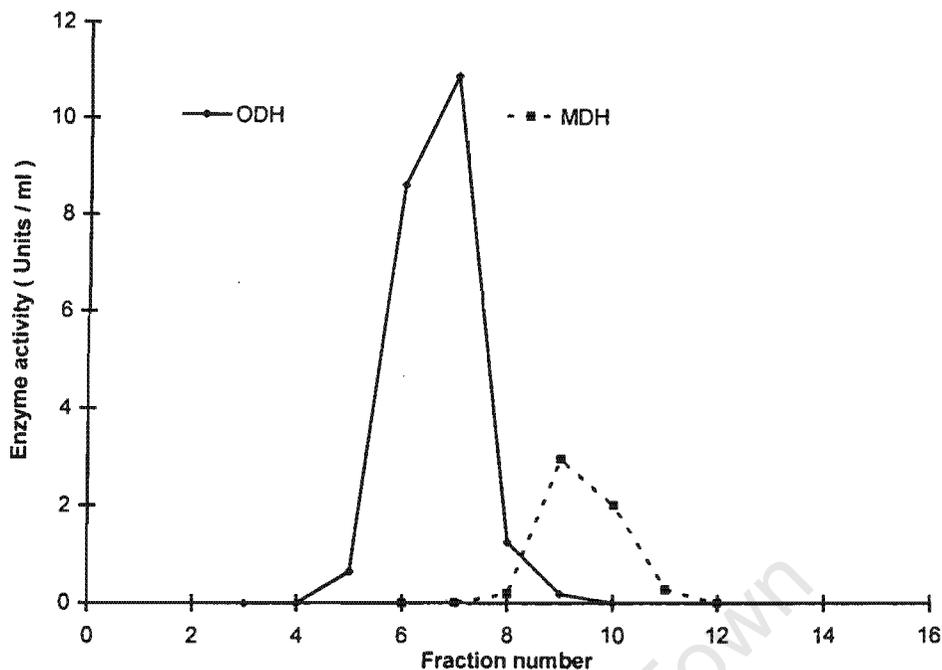
Fig 8. Elution pattern of octopine dehydrogenase from mantle muscle of *Loligo vulgaris* on DEAE-Sephacel, 10 mM Tris / HCl buffer pH 5.0. Assay conditions for octopine dehydrogenase activities : 10 mM Tris / HCl buffer, pH 5.0, 0.15 mM NADH, 2.5 mM pyruvate, 5 mM arginine.

Purification of OcDH through different stages, the efficiency of the different fractions, the overall degree of purification, the yield, the highest specific activity of OcDH (178.7 units mg protein^{-1}) are shown in Table. 5.

Table 5. Purification of the octopine dehydrogenase from *Loligo vulgaris* mantle muscle (50 *g* wet weight).

Step	Vol.	Enzyme activity	Total activity	Protein concentration	Specific activity	Yield	Purification
	ml	Units/ml	units	mg / ml	units / mg protein	%	fold
Crude extract	285	50.81	14 480.85	1.12	45.29	(100)	1.0
30% (NH$_4$)$_2$SO$_4$	285	50.38	14 358.30	2.52	19.97	99.15	0.44
60%(NH$_4$)$_2$SO$_4$	10	163.72	1637.20	0.92	178.73	11.31	3.95

Protein was precipitated with 30% and 60% ammonium sulphate saturation at different steps.

3. 3. Energy metabolism of *Haliotis midae* during environmental and functional anoxia.

3. 3. 1. Behaviour of *Haliotis midae* under conditions of environmental and functional anoxia.

Environmental anoxia: At the early stages of the experiment in anoxic conditions, the animals maintained strong pedal and shell adductor tonus; it was difficult to remove them from the aquarium substratum and they responded actively to poking stimuli. After an incubation time of 4 h, foot muscles were soft with less energetic responses to poking stimuli. There was a significant change of symptoms such as excessive mucus production and the mantle cavity was wide open. The animals appeared shrunk within the shell and the flesh was not firm, it was in an emaciated condition; the foot protruded much further out from the shell margin and was flaccid. It was easy to remove the animal from the substratum. When held out of water, it took the shape of an inverted mushroom. When turned upside-down, there were no muscular or tentacle movements.

Exercise to exhaustion: During initial stages of the exercise, when the animal was turned upside down, there was much movements of the foot and shell adductor to right itself to the normal position. After about 18 minutes of exercise the animal was completely exhausted and was lying upside down on its shell with no sign of muscular movements.

3. 3. 2. Metabolite concentrations during environmental and functional anoxia

Environmental anoxia: The concentrations of metabolites in the shell adductor, foot and gill of the animals incubated in oxygen-free seawater for 6 h are given in Figs. 9 and 10. Tauropine and D-lactate dehydrogenases which were as low as 0.92 ± 0.51; 1.21 ± 0.69 in the shell adductor, 0.89 ± 0.50; 1.85 ± 0.83 in the foot and in

the gill 0.63 ±0.10; 0.65 ± 0.34 μmoles g^{-1} wet weight in controls were the major anaerobic end products After 6 h incubation glycolytic metabolite concentrations (tauropine and D-lactate dehydrogenases) increased to 3.78 ± 1.50; 2.70 ± 0.57 in shell adductor, 1.83 ± 0.35; 3.84 ± 1.42 in the foot and in the gill 2.16 ±1.20; 1.22 ± 0.79 μmoles g^{-1} wet weight .

It was therefore, clearly shown that tauropine (Fig 9), was quantitatively the important end product in adductor muscle and D-lactate in the foot. After 6 h there was also tauropine end products accumulation in the gill tissues.

Fig. 9. Tauropine dehydrogenase concentrations (μmoles g^{-1} wet weight) in shell adductor, foot and gill of *Haliotis midae* incubated for 6 h in oxygen-free sea water. Mean values are given as ±S.D (n = 9). Significance was calculated using Student's t-test. * P ≤ 0.05, ** P ≤ 0.01
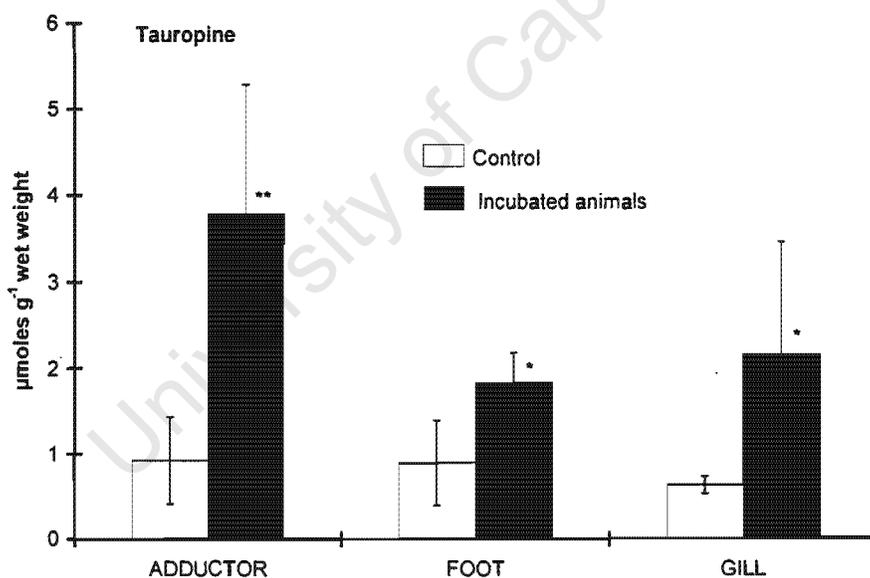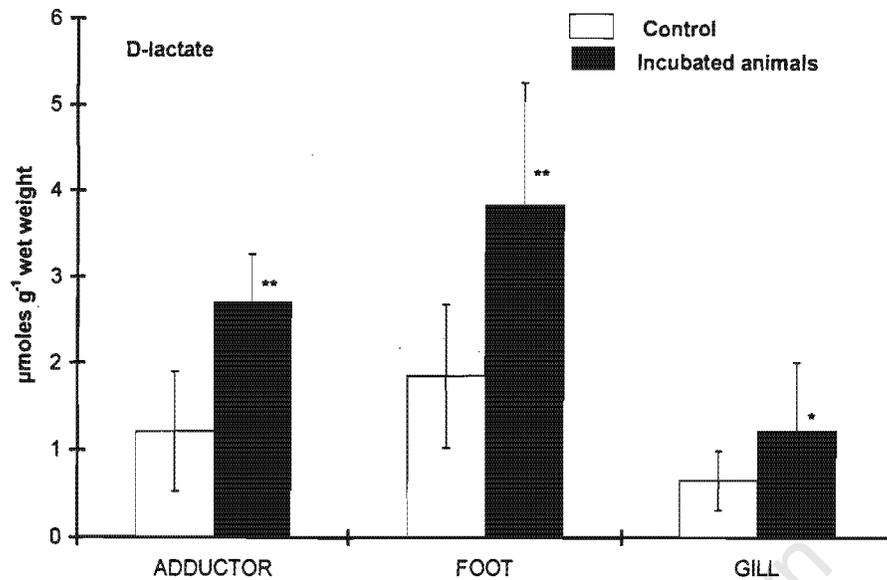
Fig. 10. D-lactate dehydrogenase concentrations (μmoles g^{-1} wet weight) in shell adductor, foot and gill of *Haliotis midae* incubated for 6 h in oxygen-free sea water. Mean values are given as $\pm$S.D. (n = 9). Significance was calculated using Student's t-test. * P $\le$ 0.05, ** P $\le$ 0.01.

Glycogen concentrations decreased drastically (P $\le$ 0.01) in the same tissues (Fig. 11).

During the 6 h anoxia glycogen, which was as high as 62.44 $\pm$ 5.02 in adductor muscle, 57.98 $\pm$ 2.38 in the foot and 20.96 $\pm$ 1.91 μmoles g^{-1} wet weight in the gill muscles in control was the source of fuel for glycolytic metabolism. It decreased to 31.08 $\pm$ 5.42, 23.75 $\pm$ 2.96 and 9.99 $\pm$ 1.61 μmoles g^{-1} wet weight in these tissues respectively. Thus about 50% of the glycogen was consumed in adductor muscle, 41% in the foot and 47% in the gill.
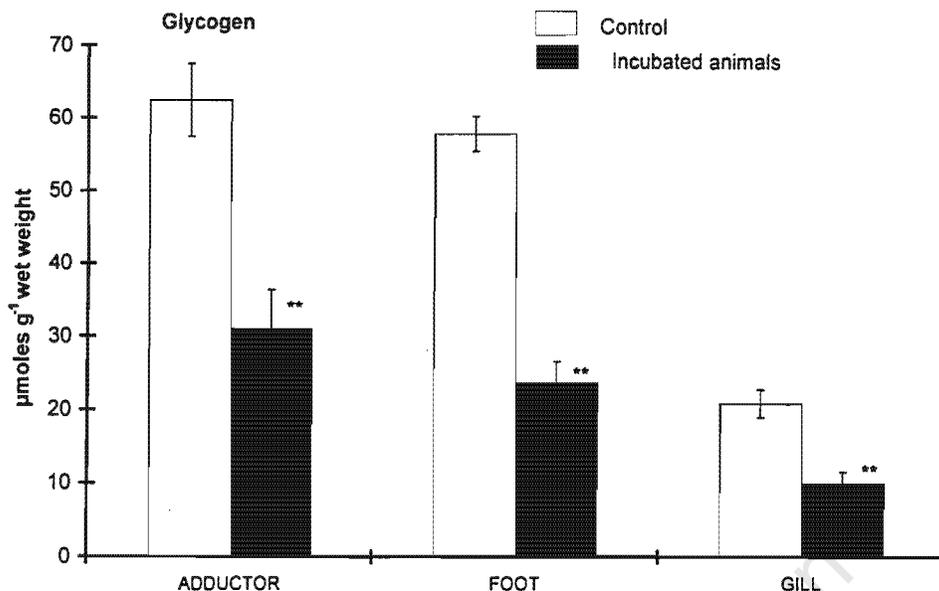
Fig. 11. Glycogen concentrations (μmoles g^{-1} wet weight) in shell adductor, foot and gill of *Haliotis midae* incubated for 6 h in oxygen-free sea water. Mean values are given as ±S.D.   (n = 9). Significance was calculated using Student's t-test. ** P ≤ 0.01.

Utilisation of arginine phosphate on the three tissues was also measured during anoxia. The level of arginine phosphate clearly declined (Fig. 12B) from 24.24 ± 1.38 to 9.87 ± 2.02 μmoles g^{-1} wet weight in adductor muscle, 6.71 ± 0.85 to 3.15 ± 0.38 μmoles g^{-1} wet weight in the and 3.64 ± 0.85 to 2.51 ± 0.67 μmoles g^{-1} wet weight in the gill tissues. At the end of 6 h incubation the level of arginine phosphate was reduced by 59%, 53% and 31% in shell adductor, foot and gill respectively.

The sum of arginine-containing compounds in control animals was about 4-fold higher in shell adductor muscles than in the foot and 6-fold higher than in the gill. Arginine phosphate levels fell drastically (P ≤ 0.01) in the shell adductor resulting in the drastic increase of arginine (Fig. 12A). Arginine phosphate breakdown in shell adductor, foot and gill quantitatively contributed to overall energy production due to the high initial levels of this compound. The sum of arginine and arginine phosphate remained relatively variable.
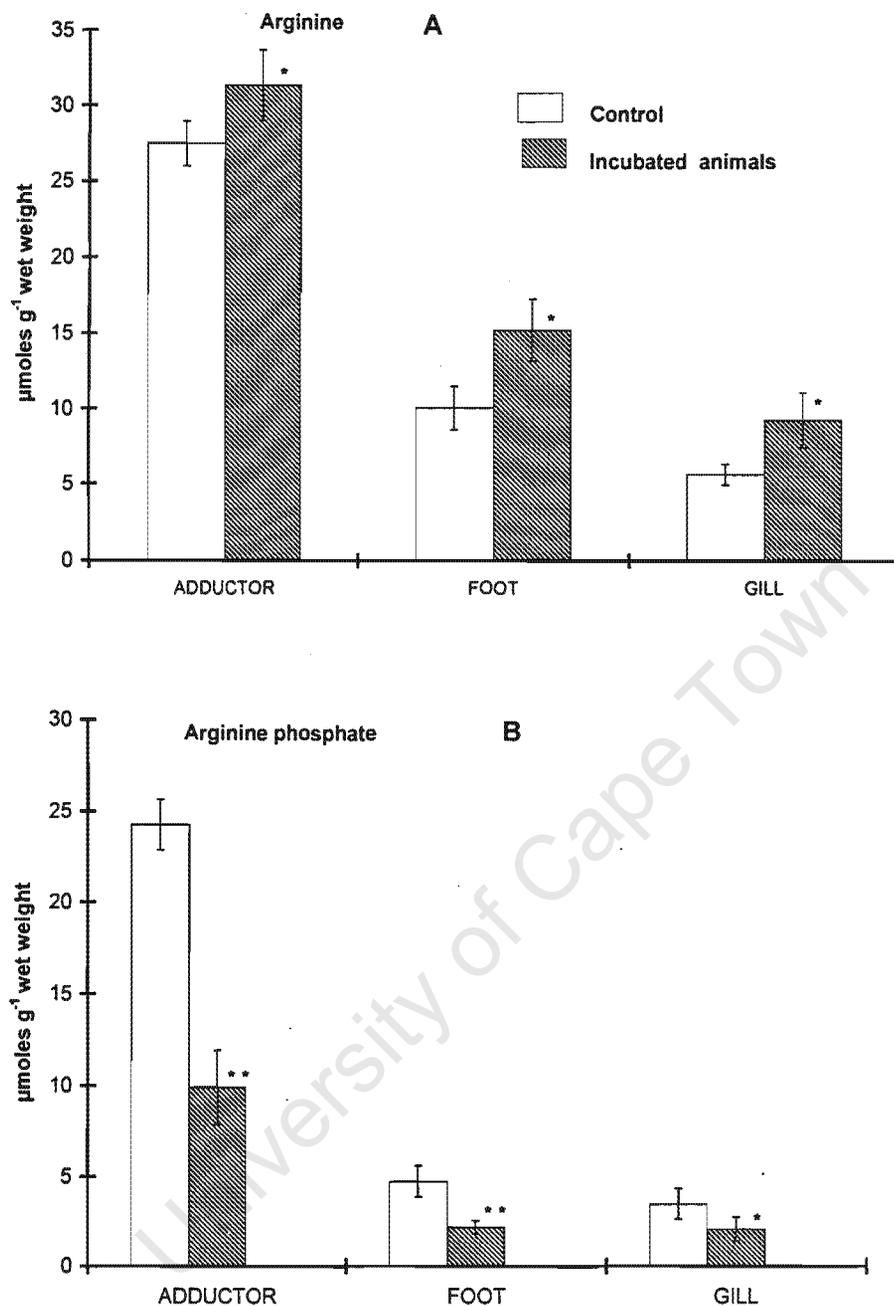
Fig 12. Arginine (**A**) and arginine phosphate (**B**) concentrations (μmoles g^{-1} wet weight) in shell adductor, foot and gill of *Haliotis midae* during 6 h incubation in oxygen-free sea water. Mean values given as $\pm$S.D. (n = 9). Significance was calculated using Student's t-test.* P $\leq$ 0.05, ** P $\leq$ 0.01.

In control animals, the adenylate concentrations of the shell adductor muscle were more than twice those of the foot and gill tissues (Table 6). The concentrations of ADP and AMP increased during anoxia. The increase of ADP and AMP concentrations resulted in a decrease in ATP concentrations in respective tissues. Incubation in oxygen-free sea water had no significant effects on the adenylate

concentrations but the energy charge in the tissues slightly but significantly decreased (P ≤ 0.05). The adenylate energy charge fell from 0.90 ± 0.02 (control) to 0.85 ± 0.03 μmoles g^{-1} wet weight in the shell adductor, 0.83 ± 0.03 to 0.80 ± 0.03 g^{-1} μmoles wet weight in the foot and 0.80 ±0.04 to 0.77 ± 0.04 μmoles g^{-1} wet weight in the gill tissues.

Table. 6.   Concentrations of adenylate (μmoles g^{-1} wet weight) and adenylate energy charge in anoxic *H. midae.*

Control			
	Shell adductor	Foot	Gill
Metabolites			
ATP	4.80 ± 0.96	2.43 ± 0.66	2.58 ± 0.86
ADP	0.85 ± 0.11	0.81 ± 0.19	1.29 ± 0.58
AMP	0.17 ± 0.05	0.12 ± 0.08	0.22 ± 0.16
Σ	5.82 ± 1.07	3.36 ± 0.30	4.09 ± 1.28
Energy charge	0.90 ± 0.02	0.84 ± 0.03	0.79 ± 0.04
Incubated animals			
ATP	3.63 ± 1.16	2.39 ± 0.74	2.29 ± 0.49
ADP	0.96 ± 0.20	1.04 ± 0.24	1.31 ± 0.48
AMP	0.21 ± 0.08	0.22 ± 0.04	0.22 ± 0.08
Σ	4.80 ± 1.33	3.65 ± 0.93	3.82 ± 0.94
Energy charge	0.85 ± 0.03*	0.80 ± 0.03*	0.77 ± 0.04*

Concentrations of adenylate and adenylate energy charge in shell adductor, foot and gill of *Haliotis midae* before and after 6 h incubation in oxygen-free seawater. Mean values given as ± S.D. (n = 9). Significance was calculated using Student's t-test, * P ≤ 0.05.

Functional anoxia: The metabolite concentrations in *Haliotis midae* muscles after 18 minutes exercise are given in Figs. 13 and 14. Tauropine and D-lactate dehydrogenase concentrations increased to 5.41 ± 2.39 and 2.27 ± 1.44 μmoles g^{-1} wet weight in shell adductor; 1.03 ±0.48 and 1.90 ± 0.84 μmoles g^{-1} wet weight in the foot. Tauropine and D-lactate concentrations in the gill tissues also increased to 0.75 ± 0.25 and 1.32 ± 0.49 μmoles g^{-1} wet weight respectively.

Tauropine exhibited a 5-fold increase which was significant (P ≤ 0.01) in shell adductor (Fig. 13). There was slight increase of tauropine concentrations in the foot

muscles and gill tissues, this was not statistically significant. D-lactate also showed significant (P ≤ 0.05) increase in shell adductor, foot and gill tissues (Fig. 14).

Fig. 13. Tauropine concentrations (μmoles g^{-1} wet weight) in shell adductor, foot and gill of *Haliotis midae* exercised for 18 minutes. Mean values are given as ±S.D. (n = 9). Significance was calculated using Student's t-test. ** P ≤ 0.01.
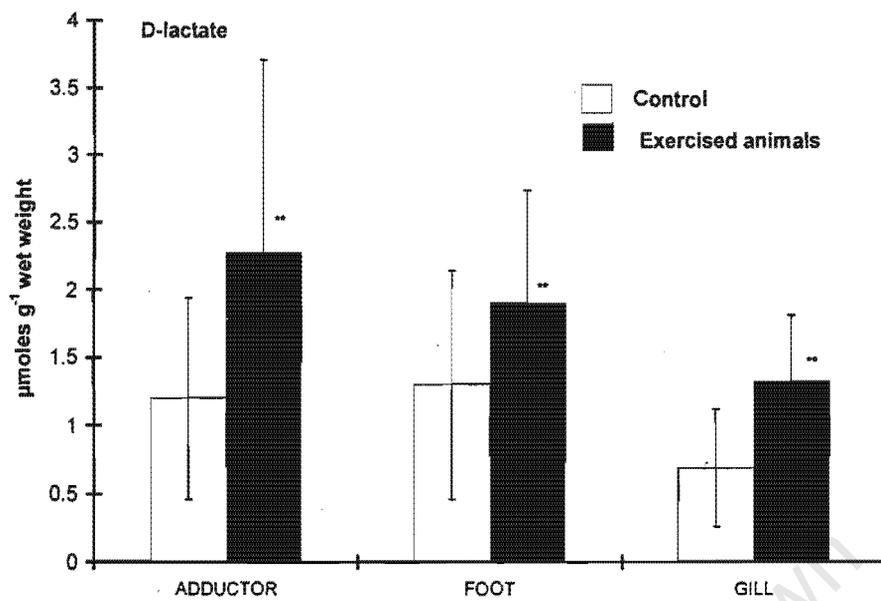
Fig. 14. D-lactate concentrations (μmoles g^{-1} wet weight) in shell adductor, foot and gill of *Haliotis midae* exercised for 18 minutes. Mean values are given as $\pm$ S.D. (n = 9). Significance was calculated using Student's t-test. ** P $\leq$ 0.01.

Glycogen concentrations decreased to half in all three tissues. The glycogen concentrations in shell adductor and foot muscles decrease from 61.34 $\pm$ 5.74 to 37.08 $\pm$ 6.89 and 57.98 $\pm$ 2.38 to 26.36 $\pm$ 6.90 μmoles g^{-1} wet weight respectively, and in the gill tissue it decreased from 20.60 $\pm$ 2.08 to 13.75 $\pm$2.77 μmoles g^{-1} wet weight (Fig. 15).
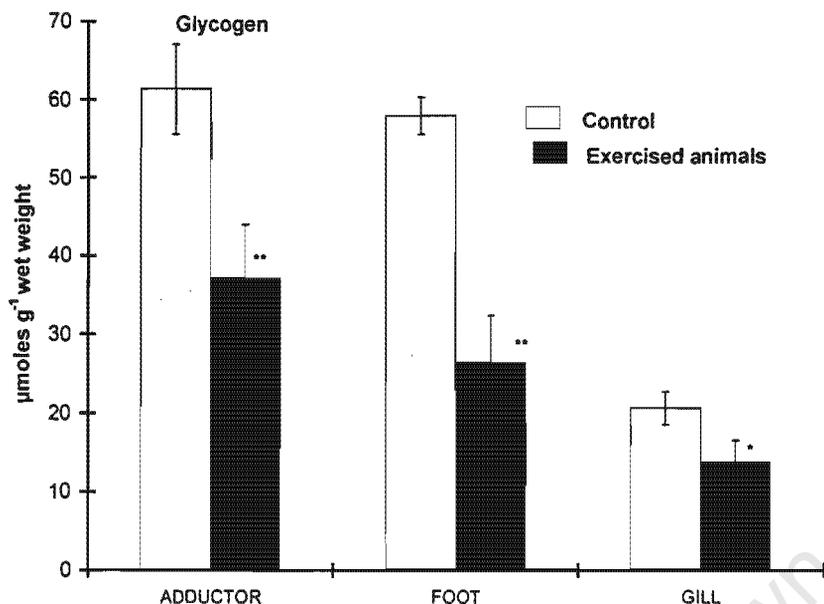
Fig. 15. Glycogen concentrations (μmoles g^{-1} wet weight) in shell adductor, foot and gill of *Haliotis midae* exercised for 18 minutes. Mean values are given as ±S.D. (n = 9). Significance was calculated using Student's t-test. * P $\leq$ 0.05, ** P $\leq$ 0.01.

There was a drastic decrease of arginine phosphate in the shell adductor muscle (P $\leq$ 0.01) after exercise. The decrease in the levels of arginine phosphate was accompanied by a build-up of arginine, as illustrated in Figs. 16A, B. Arginine levels increased in the shell adductor and foot from 27.81 ± 3.90 to 43.12 + 1.33 and 7.85 ± 2.13 to 9.64 + 2.00 μmoles g^{-1} wet weight respectively and in the gill rose from 4.60 ± 0.94 to 5.59 ± 0.58 μmoles g^{-1} wet weight. The arginine phosphate pool in shell adductor significantly diminished from 23.69 ± 4.91 to 8.38 ± 1.67. Arginine phosphate also exhibited decrease in the foot and gill tissues from 6.84 ± 1.81 to 5.32 ± 1.16 and 2.55 ± 0.57 to 1.58 ± 0.58 μmoles g^{-1} wet weight rescpectively The sum of arginine and arginine phosphate was relatively constant.
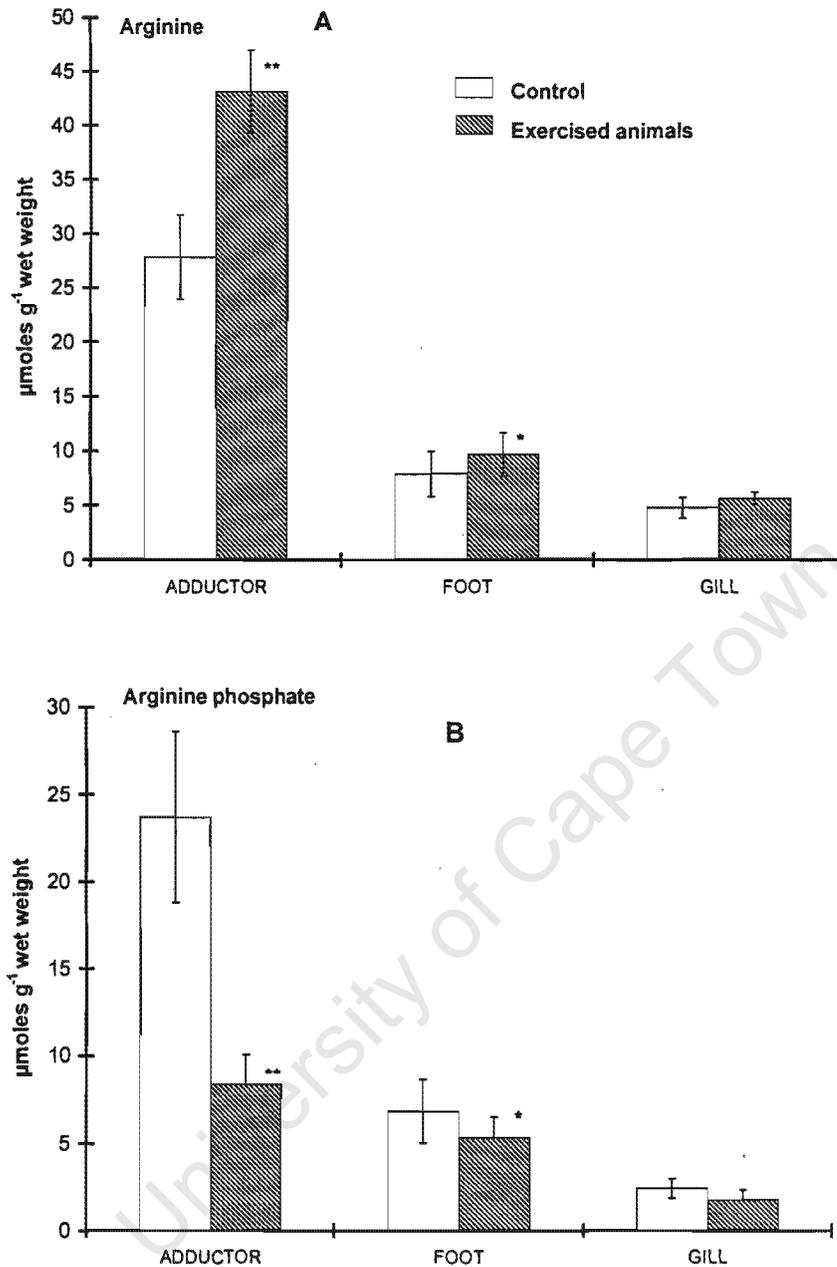
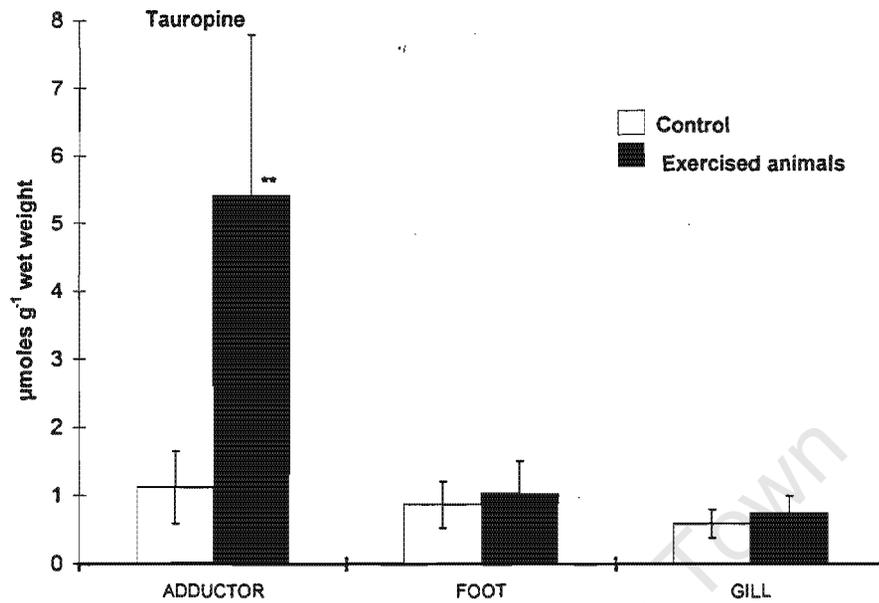Fig. 16. Arginine (**A**) and arginine phosphate (**B**) concentrations (μmoles g^{-1} wet weight) in shell adductor, foot and gill of *Haliotis midae* exercised for 18 minutes. Mean values are given as $\pm$S.D. (n= 9). Significance was calculated using Student's t-test * P $\leq$ 0.05 ** P$\leq$ 0.01.

Exercise had no significant influence on the levels of adenylates and calculated energy charge values (Table 7). There was a slight decrease in the levels of ATP accompanied by a built-up of ADP and AMP in the tissues. The adenylate increases were not significant as compared with resting animals.

Table 7. Concentrations of adenylate (μmoles g^{-1} wet weight) and adenylate energy charge in anaerobic *H. midae*.

Control	Shell adductor	Foot	Gill
Metabolites			
ATP	5.16 ± 1.00	4.24 ± 1.09	3.27 ± 0.90
ADP	0.84 ± 0.08	0.84 ± 0.80	0.93 ± 0.13
AMP	0.20 ± 0.06	0.13 ± 0.05	0.18 ± 0.04
Σ	6.26 ± 0.76	5.21 ± 1.15	4.38 ± 0.90
Energy charge	0.90 ± 0.02	0.89 ± 0.02	0.85 ± 0.04
Exercised animal			
ATP	4.58 ± 0.97	3.98 ± 0.80	3.01 ± 0.16
ADP	0.86 ± 0.14	0.85 ± 0.08	1.08 ± 0.29
AMP	0.21 ± 0.06	0.16 ± 0.04	0.20 ± 0.08
Σ	5.65 ± 0.02	4.99 ± 0.81	4.29 ± 0.68
Energy charge	0.89 ± 0.03 *	0.88 ± 0.02*	0.83 ± 0.03*

Concentrations of adenylate and adenylate energy charge in shell adductor, foot and gill of *Haliotis midae* exercised for 18 minutes. Mean values given as ±S.D. (n = 9). The decrease in energy charge in the tissues was not significant.

3. 4. Energy metabolism during transport of live *Haliotis midae*.

Metabolic stress indicators during transportation of live *Haliotis midae* subjected to

7 °C and 10 °C were investigated and compared (Fig. 17-21). Tauropine and D-lactate concentrations in shell adductor, foot and gill significantly ($P \leq 0.05$; $p \leq 0.01$) increased at 7 °C and 10 °C. The rate of accumulation of pyruvate reductase end products in the three tissues indicated a linear increase as time of transportation increased. Tauropine exhibited a higher rate of accumulation in adductor muscles (0.33 ± 0.12 µmoles g^{-1} h^{-1} wet weight) from 6 to 12 h during transport periods. After 24 h the rate of tauropine accumulation was (0.1 ± 0.01 µmoles g^{-1} h^{-1} wet weight). Rate of tauropine accumulation in the foot and gill also showed a higher rate of increase after 6 h then decreased after 24 h. The rate of accumulation of D-lactate after 6 h of transportation was 0.21 ± 0.01 µmoles g^{-1} h^{-1} wet weight in adductor muscles but, was higher in the foot muscle (0.41 ± 0.02 µmoles g^{-1} h^{-1} wet weight) this level decreases after 24 h. Pyruvate reductase end product in gill tissues remained constant (0.1 ± 0.01 µmoles g^{-1} h^{-1} wet weight) during periods of transportation. There was no significant differences in pyruvate end product accumulation between 7 ° C and 10 °C

At both temperatures, tauropine concentrations in shell adductor greatly exceeded tauropine concentrations in foot muscles (Fig. 17A,B). Lower concentrations of tauropine and D-lactate are indicated in the shell adductor of the control animals, 0.43 ± 0.04 and 0.37 ± 0.07 µmoles g^{-1} wet weight, respectively. In the 36 h period, tauropine concentrations in adductor muscles significantly ($P \leq 0.01$) increased to 6.55 ± 0.39; 7.76 ± 1.42 µmoles g^{-1} wet weight at 7 °C and 10 °C, respectively, although the rate of accumulation was low (0.10 ± 0.01 µmoles g^{-1} h^{-1} wet weight). There was also a significant ($P \leq 0.01$) increase of D-lactate concentrations in the foot to 5.78 ± 0.17; 7.58 ± 2.98 µmoles g^{-1} wet weight respectively at the same temperatures (Fig. 18A,B).

Differences in tauropine and D-lactate concentrations at 7 °C and 10 °C in shell adductor, foot and gill tissues during transportation were not significant.
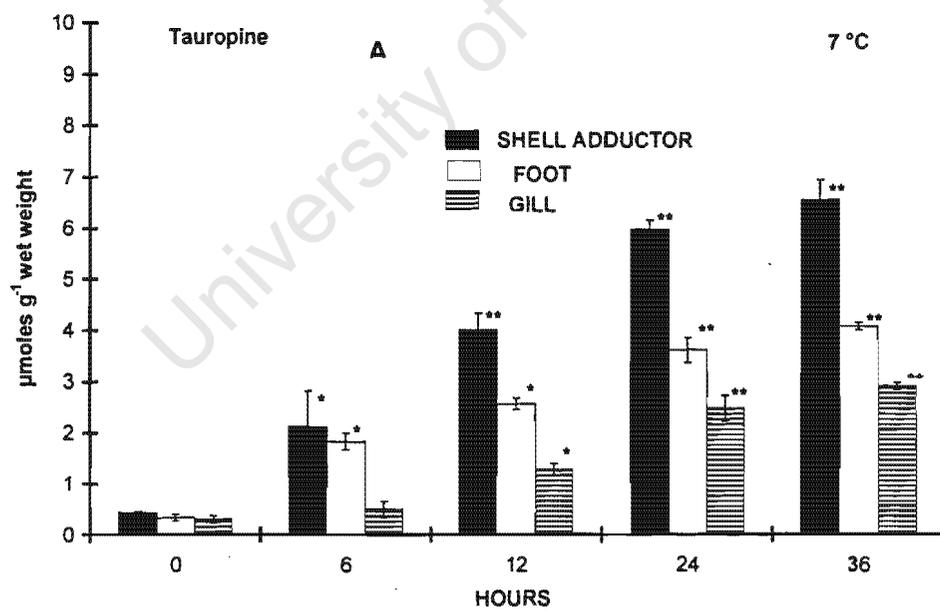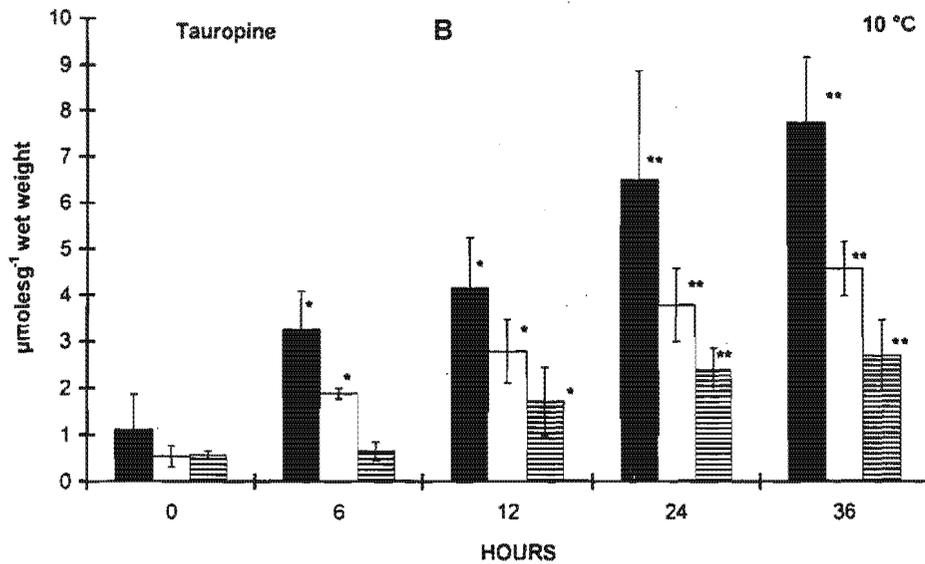
Fig. 17 Tauropine concentrations (μmoles g^{-1} wet weight) in shell adductor, foot and gill muscle of *Haliotis midae* during transport periods 6, 12, 24 and 36 h; 7 °C (**A**) and 10 °C (**B**), respectively. Mean values given as ± S.D. (n = 5 - 6). Significance was calculated using Student's t-test, *P ≤ 0.05, **P ≤ 0.01.
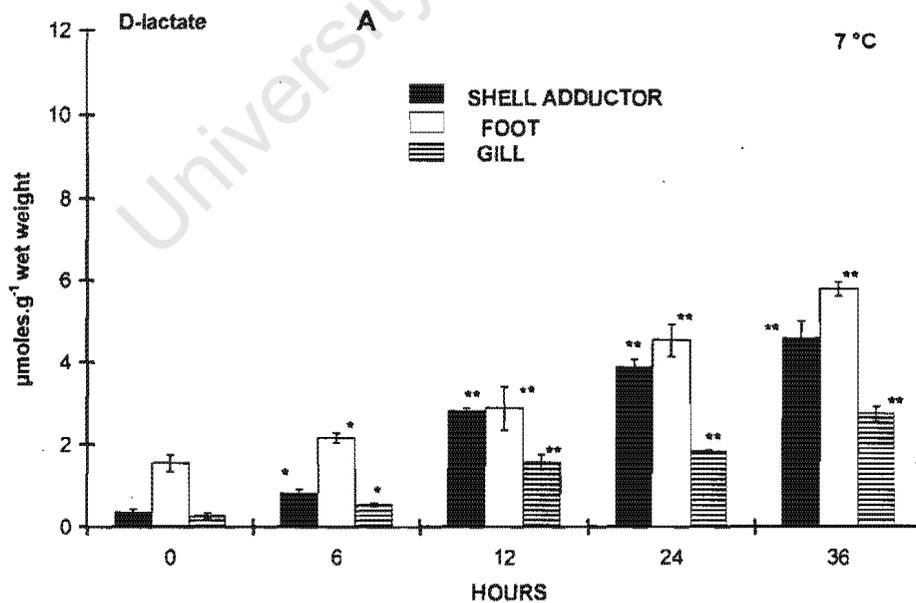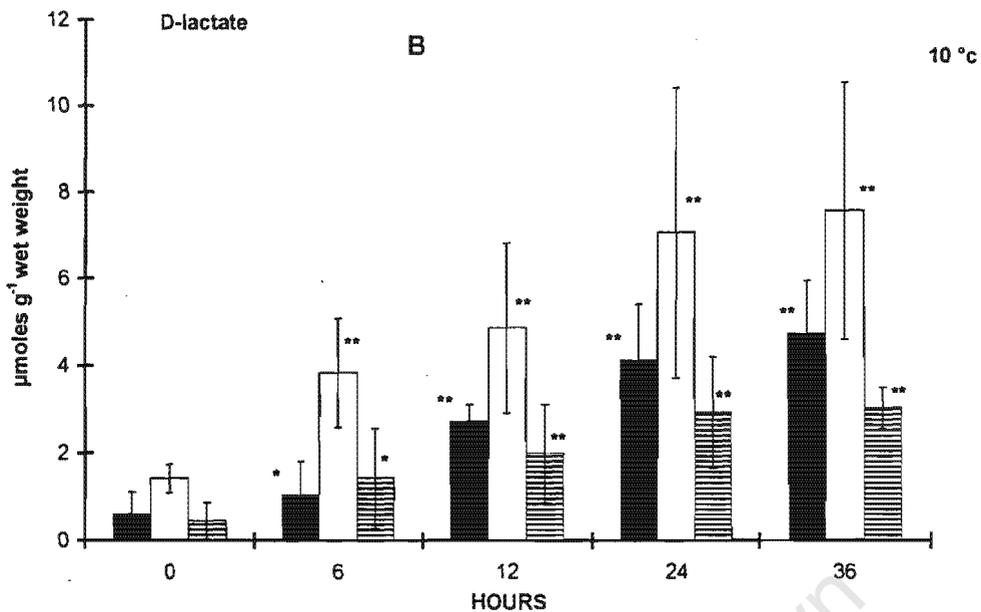
Fig. 18 D-lactate concentrations (μmoles g^{-1} wet weight) in shell adductor, foot and gill muscle of *Haliotis midae* during transport periods 6, 12, 24 and 36 h; 7 °C (**A**) and 10 °C (**B**), respectively. Mean values given as $\pm$ S.D. (n = 5 - 6). Significance was calculated using Student's t-test, *P $\leq$ 0.05, **P $\leq$ 0.01.

As a consequence of anoxic conditions, glycogen concentrations dropped from 59.87 $\pm$ 1.17 to 22.83 $\pm$ 2.13; 62.00 $\pm$ 2.87 to 28.79 $\pm$ 2.44 and 19.54 $\pm$ 1.36 to 5.69 $\pm$ 0.81 μmoles g^{-1} wet weight , in shell adductor, foot and gill, respectively at 7 °C. The control animals indicated high concentrations of glycogen in the shell adductor, foot and gill (Fig. 19).
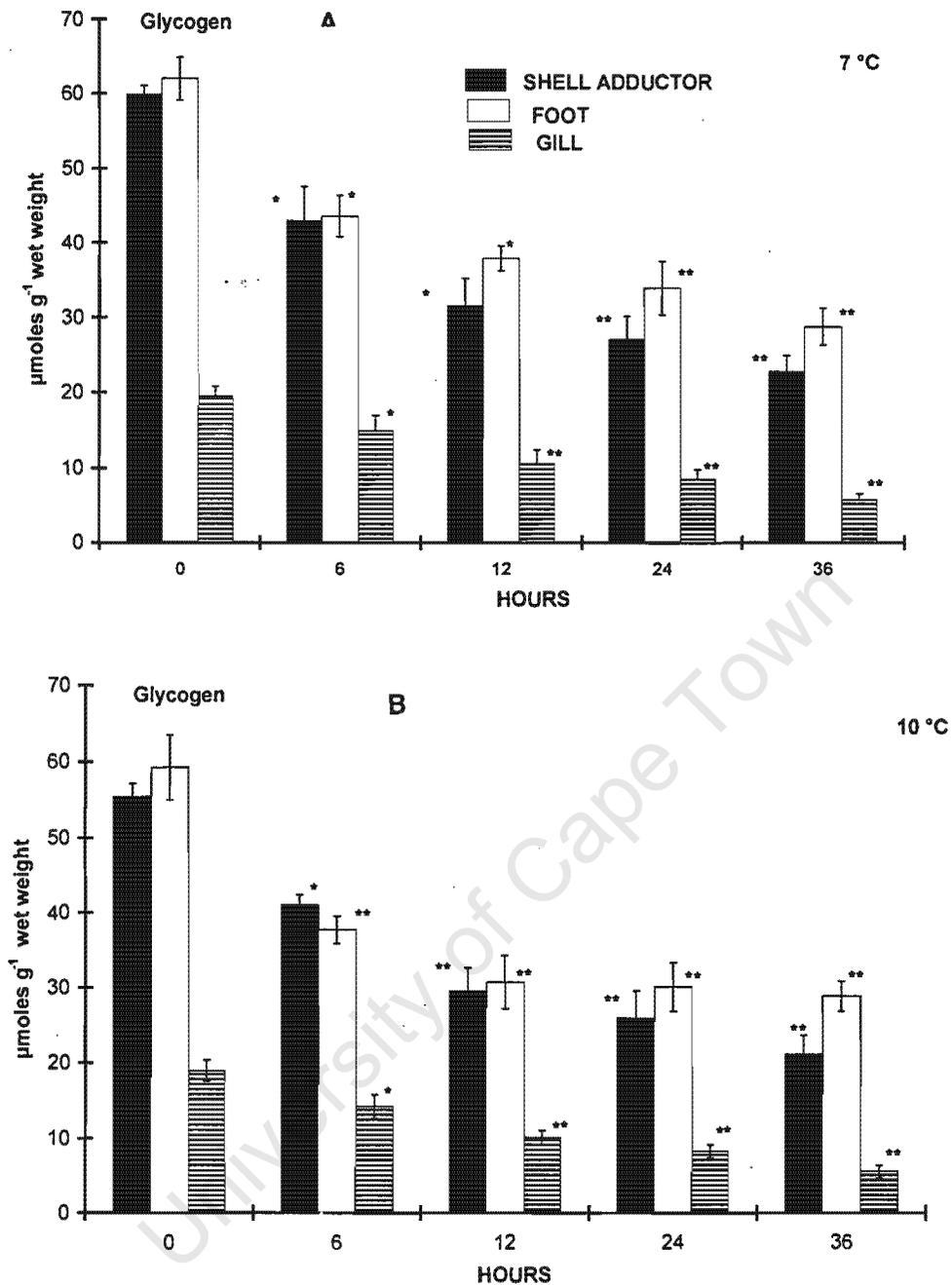
Fig. 19 Glycogen concentrations (μmoles g^{-1} wet weight) in shell adductor, foot and gill muscle of *Haliotis midae* during transport periods 6, 12, 24 and 36 h; 7 °C (A) and 10 °C (B), respectively. Mean values given as ± S.D. (n = 5 - 6). Significance was calculated using Student's t-test, *P $\leq$ 0.05, **P $\leq$ 0.01.

Rate of glycogen breakdown in adductor and foot muscles at the initial stage of transportation were high (3.82 ± 0.21 and 3.06 ± 0.3 μmoles g^{-1} h^{-1} wet weight), then decreased at a rate of 0.35 ± 0.12 and 0.10 ± 0.01 μmoles g^{-1} h^{-1} wet weight respectively after 36 h. Rate of glycogen breakdown in gill tissues at 6 h was 0.75 ± 0.14 μmoles g^{-1} h^{-1} wet weight after 36 h it then decreased at a rate of

0.22 ± 0.02 μmoles g^{-1} h^{-1} wet weight wet weight.

Arginine build up and arginine phosphate breakdown in *Haliotis midae* tissues were measured during transportation at 7 °C and 10 °C. Arginine concentrations significantly (P ≤ 0.05) increased in the tissues (Fig. 20A,B) but differences in arginine concentrations in tissues at 7 °C and 10 °C were not significant during same transportation periods.

There was a decline of arginine phosphate under the experimental conditions. The rate of arginine phosphate breakdown in the adductor muscle was high at the initial stage of transport (0.70 ± 0.15 and 0.81 ± 0.11 μmoles g^{-1} h^{-1} wet weight) at 7 °C and 10 °C respectively and declined at a rate of 0.10 ± 0.01 μmoles g^{-1} h^{-1} wet weight respectively after 36 h. The rates of arginine phosphate breakdown in the foot were 0.58 ± 0.01 and 0.64 ± 0.02 μmoles g^{-1} h^{-1} wet weight, respectively and decreased at a rate of 0.01 ± 0.01 and 0.12 ± 0.01 μmoles g^{-1} h^{-1} wet weight over 36 h at both temperatures. Rate of arginine phosphate breakdown was also indicated by gill tissues at 0.36 ± 0.02 μmoles g^{-1} h^{-1} wet weight after 6 h and decreased at a rate of 0.02 ± 0.01 μmoles g^{-1} h^{-1} wet weight over 36 h.

In resting animals concentrations of arginine phosphate were 19. ± 0.52, 6.42 ± 0.37 and 5.51 ± 0.42 μmoles g^{-1} wet weight in shell adductor, foot and gill, respectively at 7°C whil at 10°C, arginine phosphate concentrations were 20.44 ± 2.07, 7.85 ± 2.07 and 5.81 ± 0.80 μmoles g^{-1} wet weight, in shell adductor, foot and gill, respectively Arginine and arginine phosphate concentrations were higher in shell adductor than foot and gill. Arginine phosphate dropped significantly (P ≤ 0.05; P ≤ 0.01) during the 36 h period of transportation in the same tissues (Fig. 21A,B).

There were no differences observed in the arginine phosphate decrease at 7 °C and 10 °C.
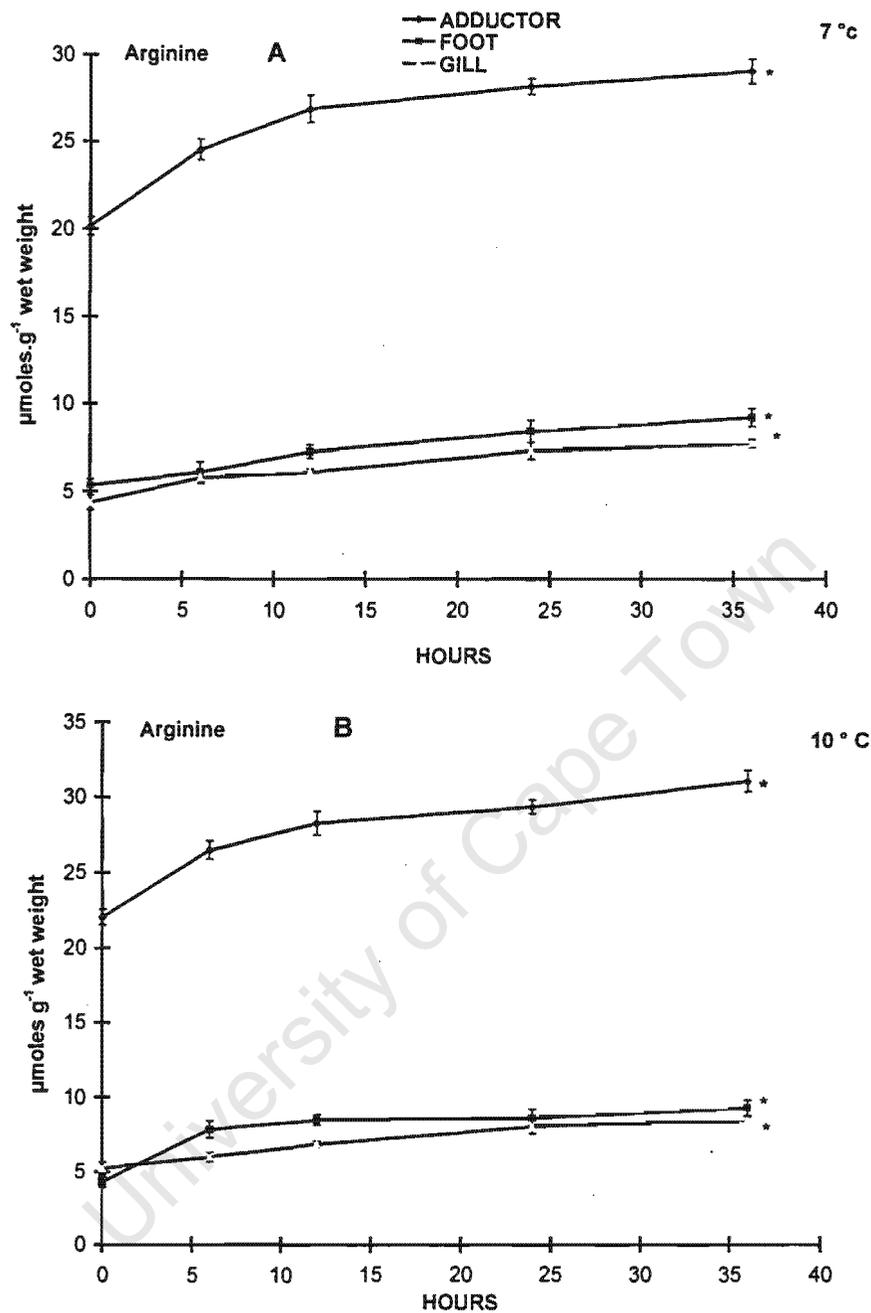
Fig. 20 Arginine concentrations (μmoles g^{-1} wet weight) in shell adductor, foot and gill muscle of *Haliotis midae* during transport period 6, 12, 24 and 36 h; 7 °C (**A**) and 10 °C (**B**), respectively. Mean values given as ± S.D. (n = 5 - 6). Significance was calculated using Student's t-test, *P ≤ 0.05.
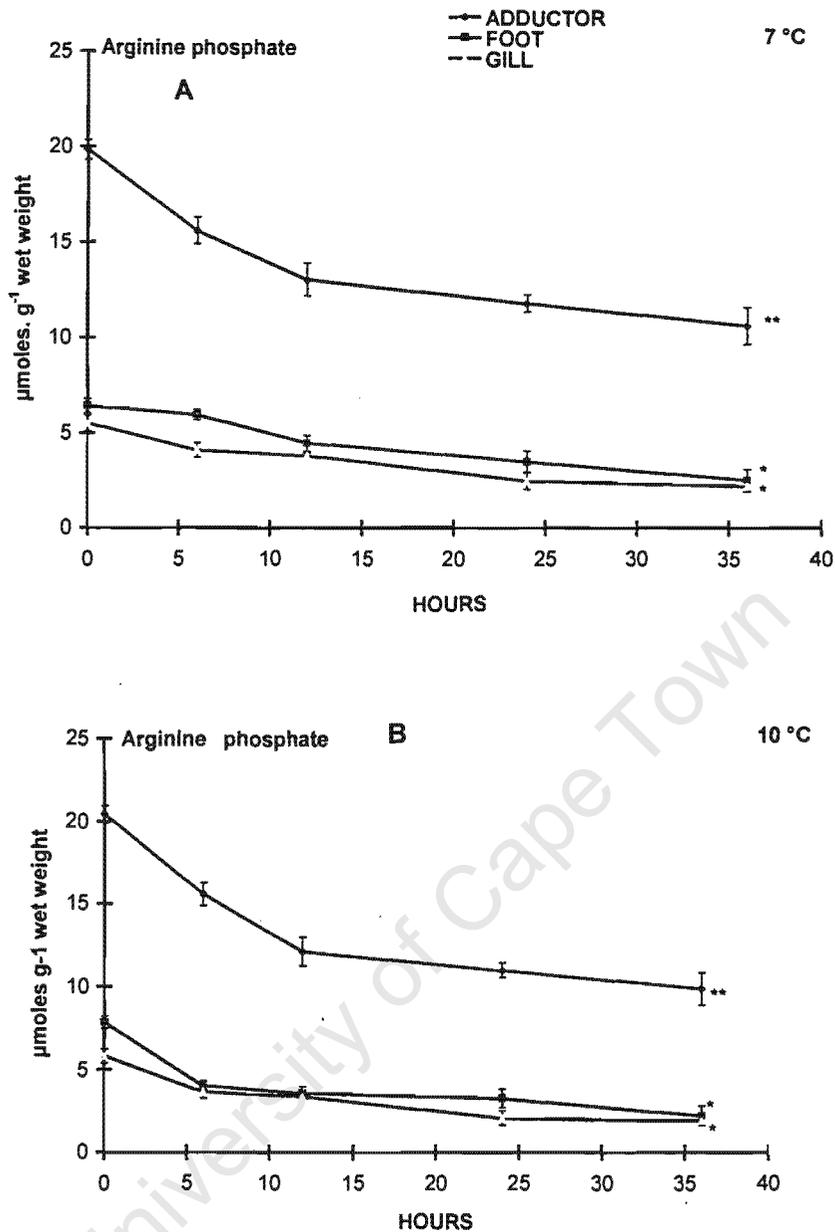
Fig. 21. Arginine phosphate concentrations (μmoles g^{-1} wet weight) in shell adductor, foot and gill muscle of *Haliotis midae* during transport periods for 6, 12, 24 and 36 h; 7 °C (A) and 10 °C (B), respectively. Mean values given as ± S.D. (n = 5 - 6). Significance was calculated using Student's t-test, *P ≤ 0.05, **P $\leq$ 0.01.

Changes in adenylate concentrations and adenylate energy charge in *Haliotis midae* during transport simulation at 7 °C and 10 ° C are shown in Tables 8 and 9. The differences in nucleotide adenylate concentrations in each tissue between 7 °C and 10 °C during the same time of exposure were not significant. Small increases in

ADP and AMP concentrations were observed under the experimental conditions applied. ATP concentrations displayed the greatest change at each temperature over the period of exposure. Concentrations of ADP and AMP showed small increases coinciding with decline in ATP. The total adenylate nucleotide pool was constant up to 36 h but with small changes in the shell adductor and foot.

At 7 °C and 10 °C the observed increases in ADP / ATP ratios in all three tissues were similar (Fig. 22A,B). The gill showed high ADP / ATP ratios. High ADP / ATP ratio is an indication of more ATP generation than when it is low. Since the animals were packed inside a humid plastic bag, they probably use a film of moisture on the gill surface for aerobic metabolism (de Zwaan *et al.*, 1976). Gills are made up of thin cell layers which are sensitive to any change resulting in hypoxic stress. The observed increase in ADP / ATP ratios were higher at 10 °C than at 7 °C.

The rate of decline of adenylate energy charge was high at the initial stage of transportation period in all tissues then slowed down after 24 h period (Fig. 23A,B).

In control animals the adenylate concentrations in the shell adductor muscles were higher than those of the foot and gill by a factor of 1-2. ATP concentrations were higher than ADP and AMP concentrations in shell adductor at exposure temperatures, which may suggest higher metabolic activities in the shell adductor (Lehninger, 1977 :Mahler and Cordes, 1966).
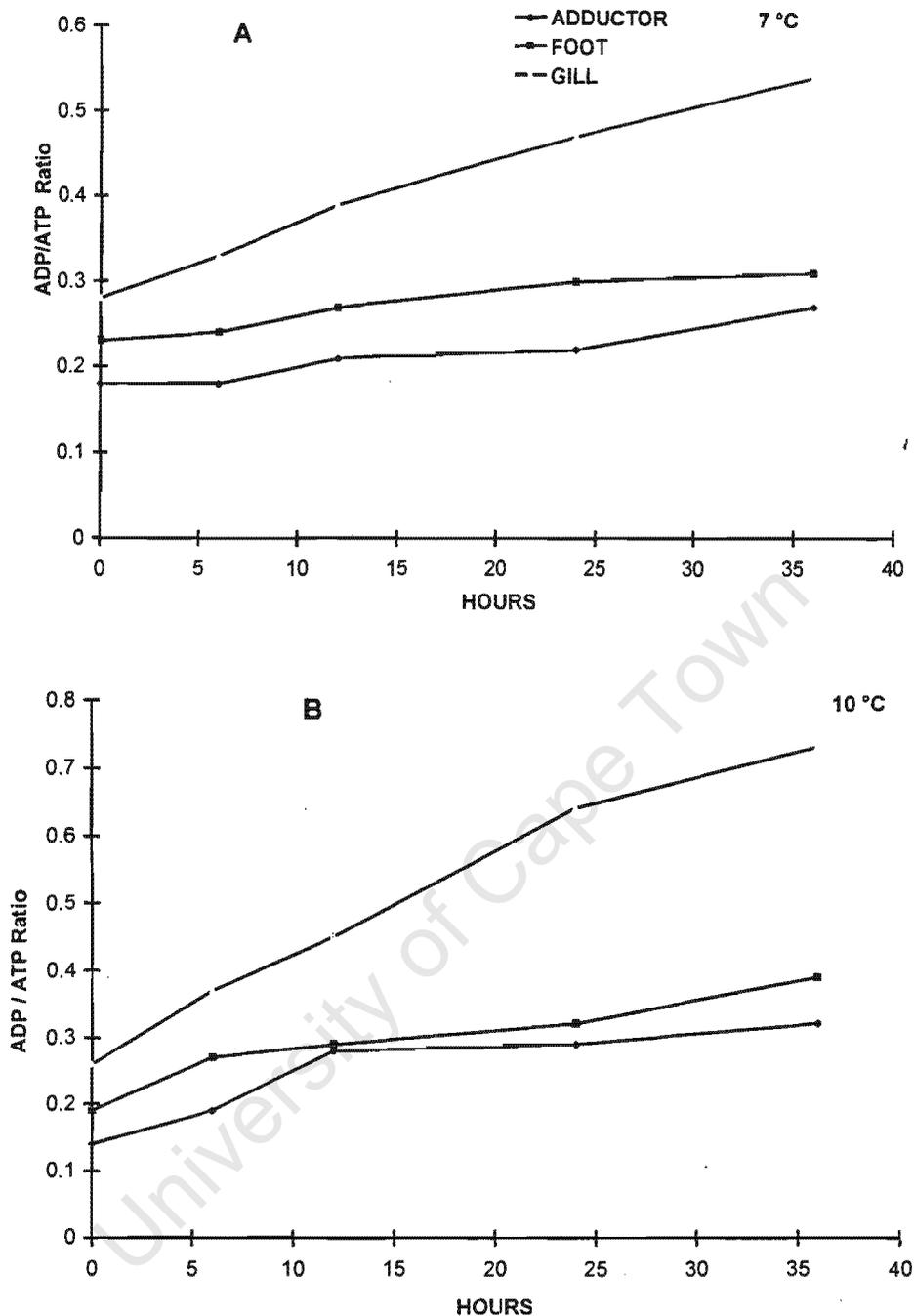
Fig. 22. ADP / ATP ratio of the concentration of ATP and ADP in the shell adductor, foot and gill during transportation of *Haliotis midae* for 6, 12, 24 and 36 h; 7 °C (A) and 10 °C (B). The increase of ADP / ATP ratio as an indication of stress.

Increased levels of ADP and AMP in tissues lead to a lower adenylate charge. ATP concentrations were lower after 36 h of exposure in all tissues. There was significant (p ≤ 0.05) decrease of energy charge in shell adductor, foot and gill tissues at 7 °C and 10 °C. This may suggest that animals exposed in air at these temperatures during transport experience similar stress (Table, 8 and 9). There

were no significant differences in metabolite accumulations in each tissues between the experimental temperatures (7 °C and 10 °C).
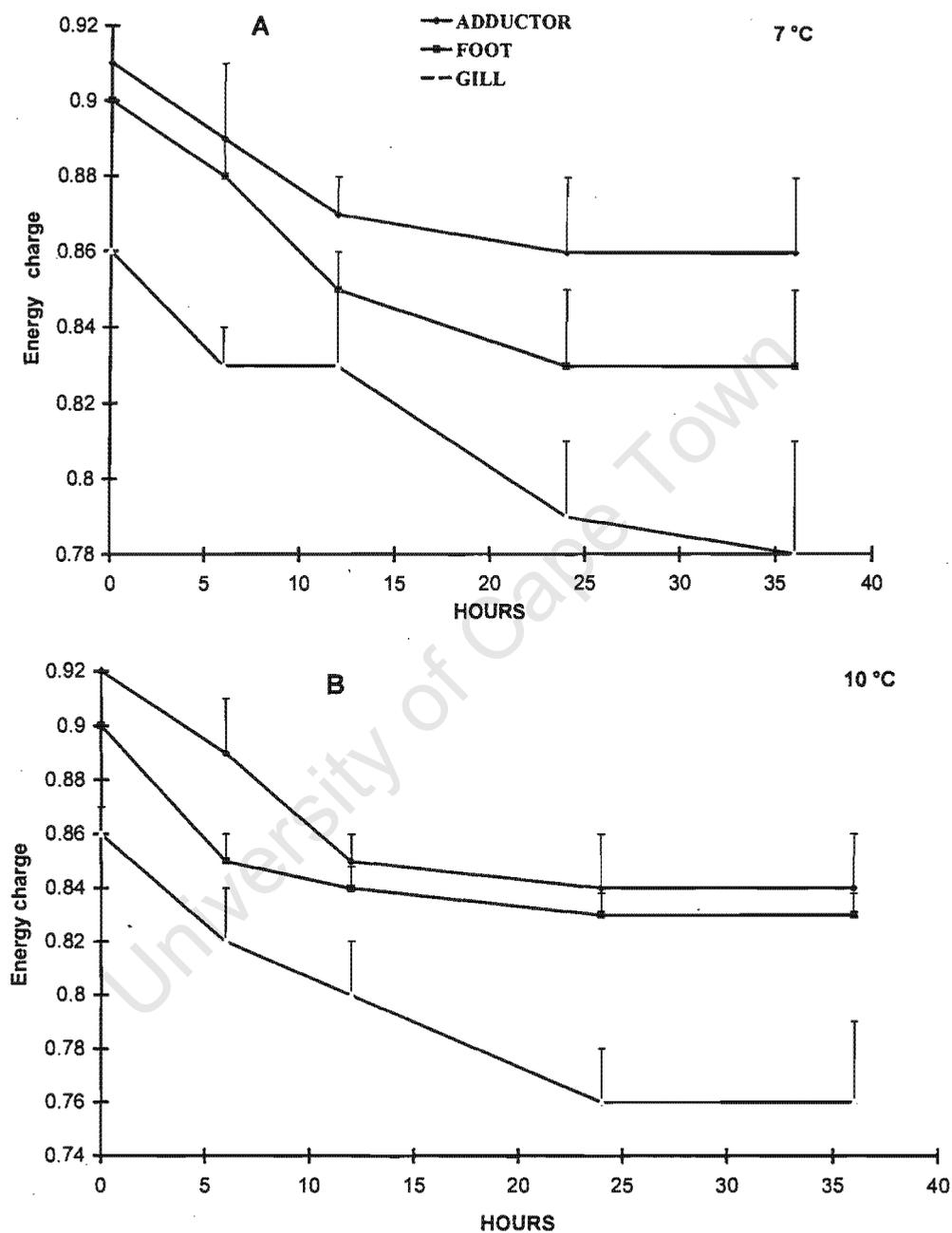
Fig. 23. Energy charge and time of exposure to air of the shell adductor, foot and gill during transportation of *Haliotis midae* for 6, 12, 24 and 36 h.; 7 °C (**A**) and 10 °C (**B**). The decrease in energy charge as an indication of stress.

Table 8. Adenylate concentrations (μmoles g^{-1} wet weight) and adenylate energy charge at 7°C.

Time (h)	Metabolites	Shell adductor	Foot	Gill
control	ATP	4.87 ± 1.22	3.86 ± 1.04	3.24 ± 0.86
	ADP	0.88 ± 0.10	0.89 ± 0.02	0.91 ± 0.05
	AMP	0.10 ± 0.07	0.10 ± 0.05	0.14 ± 0.05
	Σ	5.85 ± 1.31	4.85 ± 1.06	4.29 ± 0.82
	Energy charge	0.91 ± 0.01	0.89 ± 0.05	0.86 ± 0.03
6	ATP	4.85 ± 1.12	3.74 ± 0.72	2.83 ± 0.94
	ADP	0.89 ± 0.10	0.90 ± 0.06	0.93 ± 0.04
	AMP	0.13 ± 0.06	0.12 ± 0.06	0.17 ± 0.02
	Σ	5.87 ± 1.44	4.76 ± 4.72	3.93 ± 0.93
	Energy charge	0.900 ± 0.03	0.88 ± 0.03	0.84 ± 0.04
12	ATP	4.17 ± 1.31	3.42 ± 1.25	2.72 ± 0.86
	ADP	0.89 ± 0.06	0.92 ± 0.07	1.05 ± 0.26
	AMP	0.18 ± 0.08	0.17 ± 0.05	0.19 ± 0.05
	Σ	5.24 ± 1.45	4.51 ± 1.28	3.96 ± 0.05
	Energy charge	0.88 ± 0.03	0.86 ± 0.05	0.82 ± 0.04
24	ATP	4.15 ± 2.02	3.16 ± 1.10	2.66 ± 0.76
	ADP	0.93 ± 0.10	0.95 ± 0.09	1.24 ± 0.40
	AMP	0.23 ± 0.12	0.22 ± 0.06	0.23 ± 0.11
	Σ	5.31 ± 2.10	4.33 ± 1.14	4.13 ± 0.90
	Energy charge	0.87 ± 0.04	0.84 ± 0.04	0.79 ± 0.06
36	ATP	3.42 ± 0.69	3.14 ± 0.44	2.64 ± 0.65
	ADP	0.94 ± 0.08	0.98 ± 0.30	1.43 ± 0.54
	AMP	0.23 ± 0.08	0.24 ± 0.07	0.27 ± 0.08
	Σ	4.59 ± 0.89	4.36 ± 0.54	4.34 ± 1.08
	Energy charge	0.85 ± 0.02*	0.83 ± 0.04*	0.77 ± 0.02*

Adenylate concentrations and adenylate energy charge in shell adductor, foot and gill of *Haliotis midae* during transportation. Mean values given as ± S.D.; (n = 5 - 6). Significance was calculated using Student's t-test, *P ≤ 0.05.

Table 9. Adenylate concentrations (μmoles g^{-1} wet weight) and adenylate energy charge at 10 °C.

	Shell adductor	Foot	Gill
Metabolites			
Time (h)			
ATP	6.36 ± 0.81	4.70 ± 0.74	3.56 ± 0.57
ADP	0.92 ± 0.07	0.90 ± 0.03	0.93 ± 0.05
control AMP	0.12 ± 0.01	0.13 ± 0.07	0.16 ± 0.04
Σ	7.40 ± 0.87	5.73 ± 0.74	4. 65 ± 0.48
Energy charge	0.92 ± 0.01	0.90 ± 0.02	0.86 ± 0.03
ATP	4.95 ± 0.36	3.46 ± 1.20	2.55 ± 0.40
ADP	0.93 ± 0.07	0.94 ± 0.07	0.95 ± 0.03
6 AMP	0.13 ± 0.02	0.18 ± 0.04	0.17 ± 0.01
Σ	6.01 ± 1.12	4.58 ± 1.23	3.67 ± 0.39
Energy charge	0.90 ± 0.03	0.86 ± 0.04	0.82 ± 0.02
ATP	3.29 ± 0.74	3.42 ± 1.85	2.44 ± 0.92
ADP	0.93 ± 0.06	1.00 ± 0.25	1.10 ± 0.33
12 AMP	0.17 ± 0.04	0.21 ± 0.08	0.19 ± 0.02
Σ	4.39 ± 0.73	4.63 ± 0.94	3.73 ± 1.13
Energy charge	0.85 ± 0.03	0.85 ± 0.04	0.80 ± 0.05
ATP	3.19 ± 1.02	3.21 ± 0.72	2.32 ± 0.76
ADP	0.94 ± 0.05	1.02 ± 0.14	1.49 ± 0.48
24 AMP	0.20 ± 0.05	0.23 ± 0.06	0.21 ± 0.05
Σ	4.33 ± 1.04	4.46 ± 0.77	4.02 ± 1.07
Energy charge	0.84 ± 0.04	0.83 ± 0.03	0.76 ± 0.04
ATP	3.16 ± 0.49	2.71 ± 0.79	2.08 ± 1.03
ADP	1.00 ± 0.12	1.06 ± 0.26	1.51 ± 0.53
36 AMP	0.22 ± 0.11	0.26 ± 0.10	0.25 ± 0.09
Σ	4.38 ± 0.47	4.03 ± 1.09	3.84 ± 1.45
Energy charge	0.83 ± 0.02*	0.80 ± 0.02*	0.74 ± 0.05*

Adenylate concentrations and adenylate energy charge in shell adductor, foot and gill of *Haliotis midae* during transportation. Mean values given as ±S.D.; (n = 5 - 6). Significance was calculated using Student's t-test, * $P \leq 0.05$.

3. 5. HEART BEAT.

With the impedance pneumograph it was possible to make continuous recordings of the heart beat for 6, 12, 24 and 36 h under the experimental conditions. The basal heart beat fluctuated between 26 and 21 beats min^{-1} at 7 °C and 10 °C for the control respectively. During the initial stage, animals were wildly moving their bodies which later decreased as time of exposure increased. After 12 h about 75% of the total abalone tested had their feet extended beyond the shell edge, 25% of the animals remained composed in their shell. The mantle cavity was wide open. In this condition the animal was probably depending on the surrounding humidity for oxygen diffusion. At each time of measurement the recording was taken 5 times at 15 min intervals throughout successive measurements. The animals with electrodes inserted remained alive after returning them to aquaria for 3 weeks. The position of the electrodes was checked by dissecting two animals a day after completion of experimentation.

Effect of time of exposure on heart beat: The results of experiments on heart beat of *Haliotis midae* during transport simulation and the response of the heart beat to periods of exposure to air are given in Fig. 24. After 6 h of exposure to air, heart rate dropped to 12 and 15 beats min^{-1} at 7 °C and 10 °C respectively. After 24 h, heart activity became less stable, fluctuating between 2 and 3 beats min^{-1}. Heart beat dropped from 25 to 2 beats min^{-1} as time of exposure reached 36 h. At 24 to 36 h heart beat cessations were pronounced and continued to display erratic rates; the animals were lying with their foot extended beyond the shell margin. They were stressed. At 6 h, regular beat was interrupted by numerous cessations as abalone were exposed to air in the box. At 12 h, heart beat frequency became more wider, adduction increased and followed immediately by cessation of heart beat for about 20 to 25 seconds. At 24 and 36 h, heart beat was recorded at a much lower amplification and gave way to periods of extensive contraction and retardation or suppression (Fig. 24). Periods of suppression often alternated with periods of reduced beat amplitude.

After 24 h beat frequencies were slow and irregular in amplitude, with regular adduction, followed by suppression of heart activity.

When the animal was re-immersed in seawater, the rate and amplitude of the heart beat markedly increased followed by ejection of air bubbles through the holes at regular intervals. Some regular movements were observed, probably as a result of the ejection of pseudofaeces from the mantle cavity and to exert pressure for blood to circulate to the muscle tissues. Such movements were not observed when the animal was exposed in air in the transport box during the day or night.

There was no diurnal variations observed, although abalone are active grazers at night.

The regular pattern of the heart beat in water is probably related to increased oxygen consumption and internal body activities.

The bradycardia shown by *Haliotis midae* appear to be related to lack of oxygen when the heart beat decreased during exposure, the attenuation of the heart beat corresponded to a lower rate of oxygen consumption.

For a control, the measurements were taken from submerged animals in aerated sea water.

Fig. 24. Effect of time of exposure to air on the heart beat of *Haliotis midae* at 7 °C and 10 °C during simulated transport using the impedance technique with AC coupling.

3. 6. OXYGEN CONSUMPTION OF *Haliotis midae.*

Oxygen consumption by individual *Haliotis midae* during simulated transport conditions was investigated and data are presented in Fig. 25. During the experiment animals were not disturbed so that presumably the decline in oxygen consumption from 6 to 36 h was due to exposure to air.

The mean oxygen consumption changed drastically from 3.66 ± 0.51 to 0.62 ± 0.25 ml O_2 g^{-1} h^{-1} wet weight. The oxygen consumption remained low, to give 17% of the control after 36 h. The six animals tested all followed the same pattern, with significant decrease in oxygen consumption ($p \leq 0.01$). There was rise in oxygen consumption after 12 h of exposure. Probably the animals were moving and struggling for the limited oxygen available. During air exposure the gills were clamped together with reduced surface area and therefore there was little oxygen diffusion through the gills.

No differences in oxygen consumption were observed between day and night using the same experimental conditions.

The oxygen consumption of *Haliotis midae* after various periods of transport simulation (at 7 °C) was elevated and reached 2.87 ± 0.21 ml O_2 g^{-1} h^{-1} wet weight within 6 h after transfer to aerated seawater. The rate of oxygen consumption increased with the duration of transportation hypoxic condition to 14% of the resting level (3.66 ± 0.51 ml O_2 g^{-1} h^{-1} wet weight) after 36 h (Fig. 25). Oxygen consumption during recovery remained below that of the control.
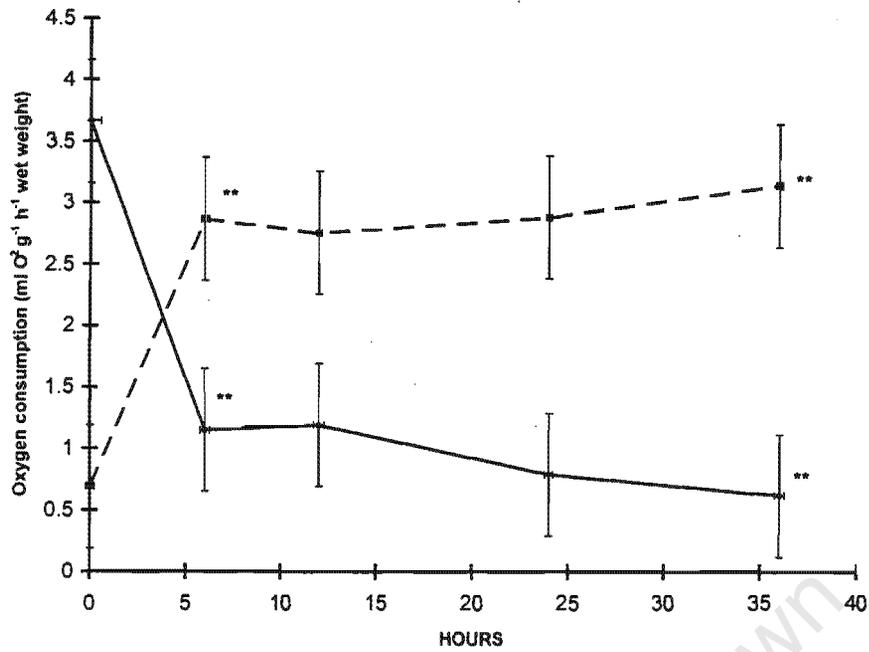
Fig. 25. Oxygen consumption (ml O_2 g^{-1} h^{-1} wet weight) of *Haliotis midae* during (   ) simulated transport an (————) re-submerged in seawater for 6, 12, 24 and 36 h; 7 °C, after simulated transport. Mean values given as ±S.D.(n = 6). Significance was calculated using Student's test, ** P ≤ 0.01.

DISCUSSION

4. Discussion

Invertebrates have the ability to survive under anoxic conditions. During exercise anoxia is confined to muscles which have insufficient oxygen supply or aerobic capacity to provide energy for the increased demand. Therefore *Haliotis midae* suffers from anoxia during movements or escape (Newman, 1966, Tarr 1992) from predators.

The enzymes, tauropine dehydrogenase and lactate dehydrogenase catalyse anaerobic glycolysis reactions during muscular exercise and environmental hypoxia associated with shell movements and locomotion. The activities of these enzymes in *H. midae* adductor and foot muscles showed the same pattern as other abalone species, H. lamellose (Gäde, 1986, 1988), *H.discuss hannai* (Watanabe *et al.*, 1992), *H. iris* (Baldwin *et al.*,) Gäde (1986), Sato and Gäde (1986 and Baldwin et al. (1992) have shown that the enzymes octopine, strombine and alanopine dehydrogenases have no activities in *Haliotis* muscles. Therefore, it could be unusual that they are found in *H. midae* muscles. However, the activities shown in this study (Fig. 6.) for these enzymes could be due to TDH broad specificity to amino acids. It has been previously indicated by Barret and Butterworth (1981), Nicchitta and Ellington (1984) Grieshaber and Kreutzer (1986) that opine dehydrogenase have broadspecificity to amino acids. Octopine dehydrogenase has been shown to exhibit activities with the non-guanidino amino acid lysine (Gäde, 1975). They have also been reported to be distributed in muscular tissues of bivalves *Cardium tuberculatum* (Gäde, 1980), Plaxton and Storey, (1982a,b), *Mytilus edulis* (Ebberink *et al.*; Dando, 1981). Opine dehydrogenase enzymes are important in the maintaince of low cytosolic redox-status (NADH / NAD$^+$ ratio) in invertebrates during anoxic conditions.

4. 1. Metabolic responses to functional anoxia.

Abalone graze extensively and during such activities, oxygen consumption by muscles, exceeds the rate at which oxygen can be delivered by the circulatory system (Donovan

and Carefoot, 1997). Therefore, energy is provided by anaerobic breakdown of glycogen resulting in accumulation of pyruvate reductase end products (tauropine and D-lactate) in adductor and foot muscles. Gäde (1988), Baldwin *et al.* (1992), Wells and Baldwin (1995) have shown that tauropine and D-lactate levels increased during exercise in *H. lamellose, H. iris* and *H. australis* muscles respectively. Tauropine and D-lactate also indicated an increase in *H. midae* muscles.

The result from this study indicate that *H. midae* has a broad response to righting induced functional hypoxia, where adductor and foot muscles derive energy anaerobically. The same has been reported for *H. lamellose* (Gäde, 1986) and *H. iris* (Baldwin *et la.* 1992). *H. midae* and *H. lamellose* dependent on anaerobic glycolysis during functional hypoxia induced by righting as well as *H. asinina*, a tropical abalone which is highly active, often foraging above water on coral outcrops at low tide. *H. asinina* is able to right itself at least 70 times at one minute intervals (Donovan and Carefoot, 1997), while *H. midae* right itself only 25 times at one minute intervals. *H. asinina* can right itself better than *H. midae* probably because it is adapted to a harsher environment than *H. midae*.

Glycolytic flux values (Table 10) after exercise in *H. midae* adductor, foot and gill tissues were 316.67, 66.67 and 17.78 nmoles glycosyl units g^{-1} wet weight min^{-1} respectively which is 1.5 times higher than in *H. lamellose* (206 nmoles glycosyl units g^{-1} wet weight min^{-1}) adductor muscles. Glycolytic flux in adductor muscle of *H. lamellose* was reported by Gäde (1988). *H. midae* shell adductor exhibits higher rate of ATP consumption (33.94 μmoles ATP g^{-1} wet weight min^{-1}) than foot and gill tissues however, ATP consumption rates in *H. midae* foot and gill tissues were similar. Rate at ATP consumption by *H. lamellose* was lower than that shown by *H. midae*; this could be because of the nature of environmental conditions and metabolic mechanisms of these animals.

However, about 1.5μmoles ATP are generated per μmole tauropine or D-lactate (Stryer, 1981) and arginie phosphate decrease, equivalence, 1 μmole ATP per μmole arginine

phosphate. These values can be used to calculate the amount of energy derived from arginine phosphate and also glycolytic flux Table 10 using the method described by Gäde 1988).

The role of arginine phosphate during functional anoxia in *H. midae* muscles was similar to that reported by Gäde (1988) for *H. lamellose*. In the adductor muscle of *H. midae*, arginine phosphate contributed to 70% of the ATP production and glycolysis contributed the remaining 30% of ATP. In the foot arginine phosphate and glycolysis contributed about 65% and 35% for ATP formation, respectively (Table 10)

Table 10. Energy yield, rate of energy consumption and glycolytic flux in shell adductor, foot and gill tissues of *Haliotis midae*.

ATP quivalents
(umoles g-1 wet weight) from

	Glycolysis	Phosphagen of ATP	Rate of consumption (μmoles g^{-1} wet weight min^{-1})	Glycolytic flux (nmoles glycosyl units g^{-1} wet weight min^{-1})
6 h experimental anoxia				
Adductor	4.29	14.37	0.11	15.89
Foot	2.56	2.99	0.05	11.06
Gill	0.86	2.13	0.04	3.17
Exercise				
Adductor	6.44	15.31	33.94	316.67
Foot	0.90	1.52	4.78	66.67
Gill	0.24	0.67	4.91	17.78

Energy yield (μmoles ATP g^{-1} wet weight) rate of ATP consumption μmoles ATP g^{-1} wet weight min^{-1}) and glycolytic flux (nmoles units wet weight min^{-1}) in shell adductor, foot and gill of *H. midae* during environmental (6 h) and functional anoxia (18 min).

4. 2. Metabolite concentrations as a consequence of transport stress in *Haliotis midae* muscles.

In their natural habitat *H. midae* sometimes encounters low oxygen concentrations due to algal blooms and low tides. Their responses to these are similar to their responses during transportation as the animals are exposed to low oxygen concentrations. During exposure to low oxygen concentrations the muscles become anoxic and the animals energy demand in met by anaerobic pathways.

Haliotis midae experiences stress under transportation which causes opine dehydrogenase enzymes activities to enhance production of pyruvate reductase end products. Among gastropods, high pyruvate reductase end products and pH buffering capacity (Morris and Balddwin, 1984: Morris *et al.*, 1985) are associated with the ability to perform active exercise, of anaerobic muscles, during locomotion and predator-prey interactions. These interactions may also be related to the animals habitat and behavioral characteristic such as living in strong wave action (Wells *et al.*, 1998: Donovan and Carefoot, 1997).

Accumulation of tauropine and D-lactate during transportation stress (Fig.17 and 18) may not be conducive to abalone survival or may lead to spoilage of flesh after long periods of exposure to air (James and Olley, 1970: Baldwin *et al.*, 1992: Dybas, 1994). Tauropine has also been suggested to play an important biological (Baldwin, *et al.*, 1992: Wells and Baldwin, 1995) role during anoxia. However, Watanabe *et al.* (1992) showed that starved abalone accumulate less D-lactate, and have improved survival during air exposure.

Mobilisation of glycogen (Gade, 1975: Newsholme and Crabtree, 1986) was adopted by *H. midae* (Table. 11) and may be a strategic mechanism for survival. After 6 h in anoxia, glycogen breakdown was high, resulting in an increase of glycolytic flux reaching the values of 15.89, 11.06 and 3.17 (nmoles glycosyl units g^{-1} wet weight min^{-1}) in adductor, foot and gill tissues respectively (Table 10). These values were lower than values from exercised animals (316.67 and 66.67 (nmoles glycosyl units g^{-1} wet weight min^{-1}) in the same muscles. Gäde (1988) indicated that glycolytic flux in *H. lamellose* adductor muscle was 16.3 (mnmoles glycosyl units g^{-1} wet weight min^{-1}) after 6 h of anoxia. Therefore, *H. midae* responds to air exposure in the same manner as *H. lamellose*.

After 36 h of transport, the animals were exhausted and glycolytic flux was reduced by 91.68% in adductor, 92.32% in foot muscles and 90.45% in the gill tissues. Gill tissues exhibited the lowest values but also showed a decrease with increased transportation time. A decrease in the rate of accumulation of anaerobic and products is a reflection of a decrease in glycolytic flux in the tissues. At the initial stage of transportation, the animals were probably struggling to adapt themselves to the new environment causing the increased glycolytic fluxes. Glycolytic flux was higher at 10°C than at 7°C. Due to increase of glycolytic flux, glycogen levels showed a linear decrease Fig. 19 via the Embden-Meyerhof pathway, leading to an increase of metabolic end products in the tissues. Rates of glycogen depletion were highest at the end of 6 h and thereafter decreased up to 36 h. This is an indication that fermentation of glycogen occurred in *H. midae* tissues, as in other molluscs (Livingtone and de Zwaan, 1983: Gäde, 1983, 1988: Kreutzer *et al.*, 1985). The ability to accumulate lactate during anoxia was also demonstrated in *Nassa mutabilis* (Gäde, 1984) *Cardium edule, C.tuberculatum* (Meninardus-Hager and Gäde, 1981, 1986, 1987). Crisp (1971) and Field (1983) showed that the end product of the equilibrium reaction of lactate dehydrogenase is channeled out of the muscle compartment.

4. 3. Energy production as a consequence of transport stress in *Haliotis midae* muscles.

ATP production during prolonged exposure to air occurred in adductor, foot and gill tissues of *H. midae* by transphosphorylation of phosphagen, and breakdown of glycogen. The adductor muscle contained the highest levels of energy phosphates, ATP and arginine phosphate indicating higher metabolic rate (Gäde, 1988: Baldwin, *et al.*, 1992) than foot and gill tissues. A basic assumption is that the bulk of energy demand during transport is met by the utilization of arginine phosphate and glycolysis for production of ATP. These compounds were in the tissues which was probably enough to meet the energy demand during prolonged hypoxia. Most molluscs use phosphagen and arginine phosphate, as well as glycogen for ATP formation during environmental anoxia and reduce their metabolic rate considerably.

The differences in ATP formation from arginine phosphate and glycolysis between 7°C and 10°C were not significant. This is probably due to the small temperature range taken for this investigation. At higher temperatures metabolic activities increase.

During transport, glycolysis contributed to about 72% of the ATP formation in the adductor muscle, but ATP consumption rate decreased at the end of 36 h while normoxic animals indicated 58% for ATP formation. This seen clearly from the glycogen depletion during transport (Fig. 19). It is also interesting that arginine phosphate contributed a higher percentage for ATP formation in gill tissues. This could be because gills are an important respiratory organ for gas exchange.

ATP production equivalents from glycolysis and phosphagen at the end of the 6 h incubation period were higher in adductor and foot muscles (4.29, 14.37 and 2.56, 2.99 μmoles g^{-1} wet weight) (Table 10) respectively and lower in gills at the end of 36 h. Arginine phosphate contribution to ATP formation during transport was 76% in the adductor muscle. The levels of arginine phosphate were reduced by 25%. This could indicate that arginine phosphate is used as an energy source during exposure to air.

Similar results have been found for other species of molluscs (Meinardus and Gäde, 1981: Livingstone *et al.,* 1981: Gäde, 1981: Gäde, 1980, 1988). Rate of ATP consumption in adductor and foot muscles during transport were 0.11 μmoles ATP g^{-1} wet weight min^{-1} and showed higher values at 10°C than at 7°C due to higher metabolic activities.

The rate of arginine phosphate utilization in the gill tissues was found to be lower than the rate in the adductor and foot muscles and stable during the period of air exposure. This could be a strategic method for *H. midae* to adapt to environmental conditions.

During prolonged hypoxia, anaerobic glycolysis provided the bulk of the energy, and phosphagen the remainder (Table 11). Meinardus and Gäde (1981): Gäde and Ellington (1983): Gäde (1987) indicated that energy utilization by molluscs was higher at the initial stage of environmental anoxia because the animals had to adapt to the environment and the rate of energy consumption decreased with increased air exposure. After 36 h, energy consumption decreased by 85% in the adductor muscle of *H. midae* while in the foot and gill it decreased by 93% and 90%, respectively at 7°C ; over the same period of exposure as indicated previously.

Stenton-Dozey (1989) and Hatake, *et al.* (1995) reported that bivalve molluscs become exhausted after long periods of exposure to air due to low oxygen concentrations and this, accompanied by accumulation of metabolic end products may lead to their death.

Abalone used in this investigation did not die after 36 h, which could probably be attribute to the efficient adaptive mechanism adopted by *H. midae* to survive transport hypoxic conditions.

A comparison of *H. midae* energy output in adductor muscle during transport to that during exercise (arginine phosphate ATP equivalent during exercise was 15.31 μmoles g^{-1} wet weight) showed that *H. midae* adductor muscle exhibited 4-fold lower energy

output during transportation when compared to exercise. Rate of ATP consumption during exercise was 33-fold higher in adductor and foot muscles compared to that during transportation. This is because, during transportation, *H. midae* use less energy than when exercised or when exposed to oxygen-free seawater conditions. Therefore *H. midae* are able to survive for 36 h during transport provided that suitable conditions are met.

Table11. Energy yield, rate of energy consumption and glycogen flux in shell adductor and foot muscles of *Haliotis midae*.

	Temperature		Hours		
		0-6	6-12	12-24	24-36
ATP equivalents					
(umoles g-1 wet weight).					
Glycolysis* Adductor	7°C	5.83	5.01	3.18	1.91
	10°C	5.64	3.89	3.84	2.79
Foot	7°C	4.07	3.15	2.58	2.19
	10°C	5.67	4.80	2.90	1.95
Gill	7°C	2.70	2.18	2.03	0.68
	10°C	2.46	2.40	1.58	0.60
Phosphagen* Adductor	7°C	4.22	2.57	1.25	1.17
	10°C	4.87	3.47	1.12	1.10
Foot	7°C	1.48	0.99	0.98	0.47
	10°C	3.82	1.05	0.45	0.31
Gill	7°C	1.41	1.35	0.27	0.29
	10°C	2.17	0.26	1.29	0.22
Rate of ATP					
Consumption Adductor	7°C	0.03	0.02	0.01	<0.01
	10°C	0.03	0.02	0.01	0.01
Foot	7°C	0.02	0.01	0.01	<0.01
	10°C	0.03	0.02	0.01	<0.01
Gill	7°C	0.01	0.01	<0.01	<0.01
	10°C	0.01	0.01	<0.01	<0.01

Glycolytic Adductor Flux***	7°C	9.22	3.00	**2.44**	1.92
	10°C	11.06	3.92	**2.50**	1.67
Foot	7°C	4.61	4.00	**3.47**	1.42
	10°C	13.50	6.11	**5.72**	3.39
Gill	7°C	5.33	2.56	**1.44**	0.28
	10°C	5.61	3.17	**2.58**	0.72

Energy yield (μmole ATP g^{-1} wet weight)*, rate of ATP consumption (μmoles ATP g^{-1} wet weight min^{-1})** and glycolytic flux (nmoles glycosyl units g^{-1} wet weight min^{-1})*** in shell adductor, foot and gill of *Haliotis midae* during transport simulation exposure periods of 6, 12, 24 and 36 h respectively; 7°C and 10°C. **(in bold).**

4. 4. Heart rates during transport simulation.

Haliotis midae is a subtidal species and rarely experiences hypoxic conditions in its natural habitat. Slow heart beat, accompanied by inadequate oxygen consumption, are likely to increase pyruvate reductase end products during environmental stress. Therefore, it is important to investigate the effect of increased air exposure on heart rate and survival.

An investigation of he heart rate of *Haliotis midae* during transportation indicated that the abalone heart rate ranged between 23 to 26 beats per min when covered by water (control) and 12 to 15 beats per min in air at 7°C and 10°C, respectively. The rates varied from individual to individual. Heart rate patterns were regular in water (Fig. 24) but were irregular during transportation in air. At 10°C the heart rate was slightly higher at 7°C but the difference were not significant.

Haliotis midae showed no acclimation of heart frequency during the transport experiment at 7°C and 10°C. Previous studies on bivalves showed the relationship between temperature and heart rate to be linear, e.g. in *Isognomon alatus* (Trueman and Lowe, 1971); *Mya arenaria* (Lowe and Trueman, 1972); *Mytilus edulis* and *M. californianus* (Picken, 1965; Widdows, 1973a); *Crassostrea gigas* (Lowe, 1974 and *Perna perna* (Bayne, 1976).

As period of exposure increased, heart rate decreased, the lowest rate being 2 beats min^{-1} recorded after 36 h at both temperatures. Decreased in heart rate has been noted in other molluscs species and in isolated heart preparations. Stenton-Dozey (1989) reported that thermoreceptors in the mantle, play an importation sensory role. Since there is an abundance of sensory cells in the foot and adductor muscles (Stenton-Dozey, 1989), even though most appear adapted to detect mechanical changes in external medium, it is reasonable to assume that at least some of these cells functions as thermoreceptors with a possible neural connection to the heart (Russell and Evans, 1989).

Longer transport duration of *H. midae* caused the heart rate to decrease drastically and adductor and foot muscles become feeble. This is a common stress response among molluscs exposed to air with muscles weak accompanied by a decline in heart rate (Trueman, 1967; Trueman and Akberali, 1981). In *M. edulis* (Bayne, 1971) and *S. plana* (Akberali and Trueman, 1979) valve closure leads to a drastic drop in P_{O2} and an increase in P_{CO2} levels in the water in the mantle cavity. Among bivalves, heart rates increase immediately as water temperature increases (Trueman and Lowe, 1971; Lowe, 1974). This response is probably mediated through thermoreceptors in mantle tissues. Upon exposure to air, a drop in oxygen tension, rather than any mechanical effect of closed valves, is believed to slow down the heart rate (Brand and Roberts, 1973; Bayne, 1971, 1976; Booth and Mangum, 1978; Taylor and Whiteley, 1980), however, heart rate response to changes in temperature and oxygen tension are widely documented in molluscs (Taylor, 1976; de Fur and Mangum, 1979; Hill and Welsh, 1966) including abalone (Fujino *et al.*, 1984). Temperature changes act directly on cardiac muscle affecting heart rate (Russell and Evans, 1989). Heart causes muscles cell membranes to

become permeable to ions, leading to acceleration of myogenic self-excitation. Therefore heart rate increased with increasing temperature.

Crofts (1929) demonstrated that the muscular valve of *Haliotis tuberculata* may prevent outflow of blood from the foot, isolating the inner part of the foot or shell adductor tissues from the main heart circulation during active muscular work. Such a phenomenon was not observed in *H. rubber* or *H. kamtschatkana* (Donovan and Carefoot, 1997). *H. rubber* can maintain its heart rate up to a critical oxygen tension of 90-80 mm Hg, after which it decreases. Similar events were reported for the gastropod *Busycon canaliculatum* (de Fur and Mangum, 1979). In bivalves, heart rates slow only after cessation of oxygen uptake, and presumably a decrease in blood PO2, probably suggesting that the heart beat response is direct controlled by blood oxygen levels without involvement of the central nervous system (Bayne *et al.*, 1976; Trueman, 1967; de Fur and Mangum, 1979). This correlation supports the idea that the performance of the cardiovascular system has a simple and direct influence on oxygen consumption.

Retardation of heart beat during exposure may be related to reduced oxygen supply and eventually may lead to suppression of heart beat after 24 to 36 hours. *H. midae* heart rate returned to normal when reimmersed into sea water. Trueman (1967) showed that the rate in *Cardium edule* decreased when exposed to air and immediately returned to a normoxic condition when re-immersion in seawater. Reduced oxygen consumption and bradycardia appear to be a common feature in intertidal molluscs (Hochachka and Somero, 1984) and may be an adaptation to hypoxia during long periods of exposure.

4. 5. Oxygen consumption of *H. midae* during transport.

Oxygen consumption in *H. midae* was measured during transportation and values obtained were higher than those reported for active *Sulculus supertexta* (Nimura and Yamakawa, 1989) and *Haliotis kamtschatkana* and some other gastropods (Houlihan *et al.*, 1981; Houlihan and Innes, 1982a, 1982b). This could be due to the larger animals (100 shell length) used in this study. Oxygen consumption in *H. midae* was reported by

Barkai and Griffiths (1986, 1987) to be related to body size and food intake. This study did not establish any oxygen consumption differences during day and night, although abalone are night grazers. *Haliotis discu hannai* showed an increase (Uki and Kikuchi, 1975) in metabolic rate at night. Anaerobic metabolism in *H. midae* began immediately upon the onset on anoxia or exercise (Fig.15). Such behaviour has been reported in other studies (Meinardus-Hager and Gäde, 1987) based on information of end products. This is because of the absence of capacity for aerobic production of energy (Innes and Houlihan, 1985) or an insufficient supply of oxygen to inner parts of muscle tissues during air exposure.

Haliotis midae showed a significant decrease in oxygen consumption with increased transit time. As soon as the animals were removed from water, oxygen consumption dropped sharply. During this time animals showed a vigorous twisting movement of the body, with the mantle cavity wide open, probably struggling for available oxygen. Prolonged exposure made gills clamp together resulting in a reduction of surface areas for oxygen diffusion (McMahon *et al.*, 1974) Muscular activity was reduced as an adaptation to cope with the decreased oxygen. A fluid film of the surface of the mantle cavity most likely assisted in oxygen diffusion. Houlihan and Innes (1982a) reported that oxygen consumption in *H. kamtschatkana* was directly related to crawling speed. They further indicated that rates of oxygen consumption in active animals were less temperature dependent than in inactive individuals due to muscular activities.

In some gastropods(e.g. *Monodonta* species) the rate of oxygen consumption is higher in air than in water this was not as found in *H. midae*. This may be due to an increase of mucous production in air and the high energy cost involved (Crisp, 1971; Calow, 1974; Innes and Houlihan, 1985). Factors such as temperature, starvation, food availability and water movement (Widdows, 1973b; Widdows and Johnson, 1988; Stenton-Dozey, 1989; Houlihan and Innes, 1982b) have an influence on rate of oxygen consumption.

When animals were returned to well aerated seawater, oxygen consumption returned to pre-normoxic conditions (Fig.25) and it is reasonable to assume that metabolic changes

(Gäde, 1986; Meinardus-Hager and Gäde, 1987) returned to normal levels. Metabolism is reversed by the replacement of phosphagen pools and glycogen (Meinardus-Hager and Gäde, 1987). A process requiring additional oxygen uptake during recovery to provide energy yield ATP to equalize the yield of anaerobic energy production (Surholt, 1977; Ellington, 1983) or by oxidation of glycolytic end products. *H. midae*, display a sharp increase in the oxygen consumption rate (Fig 25) at the onset of recovery. Additional oxygen uptake, seemed to change with the duration of time spent submerged.

Therefore to ensure abalone survival following transport, the following measures should be taken low temperature, oxygen enrichment in containers and careful handling of abalone to markets.

CONCLUSION

CONLUSIONS.

1.) Abalone are to rough handling before and or after harvesting and this could cause mortality as a consequence of accumulation of metabolic endproducts which could also lower meat quality. During transport abalone undergo temperature fluctuations. Increasing in temperature results in increased metabolic rates and hence metabolic end product are high. Abalone accumulated less endoproducts at 7 °C when compared to 10 °C.

2.) Duration of exposure of animals to air is important for survival of abalone. It is possible to transport animals over 24 h if suitable environmental conditions are met with no live animals. This investigation are met with no loss of live animals. This investigation showed that metabolic concentrations increased with an increase of duration of transport. Most shipments take longer than 24 h reach their destination which resulted in animals exhaustion, with soft muscles and awide open mantle cavity. Transportation period s should be as short as possible to alleviate stress. This transported over shorter distances are also found to accumulate andproducts but to lesser extent. After 36 h of exposure to air, abalone accumulated significant ($p \leq 0.01$) amount of tauropine and D-lactate in shell adductor foot gill tissues.

3.) Insulated packing materials controls temperature fluctuations inside containers. During transportation temperature varies and sharp increases or decreases of temperature are harmful to abalone and might increase mortality. Polystyrene boxes should be used, although these may not give 100% insulation. This inside container should be provided with a smooth surface for abalone to attach themselves to conserve energy. Abalone lose much energy in mucous secretion if not provided with good surface for attachment.

4.) Abalone are sub littoral and when exposed to air are to desiccation. Gills are easily affected and therefore oxygen diffusion is impaired, resulting in death immediately or later. Containers should be kept humid to provide a moist environment during transport of the animals to enhance oxygen diffusion.

Oxygen enrichment inside containers provides the animal with oxygen for respiration which improves survival rate. However, even this will be depleted after 36 h trainsit.

5.) Although laboratory experiments provided clear evidence that *H. midae* can survive in air for 36 h. It is acknowledged that in actual transportation, survival would be far more variable due to changes in air pressure or handling. Metabolic interactions within time of exposure to air may magnify absolute measures obtained in laboratory.

6.) If suitable conditions are provided, *Haliotis midae* seems to tolerate hypoxic conditions but, further research is necessary on survival oer longer periods than 36 h to accommodate transportation time to distant markets. Time spent on transportation and trasit should be as short as possible to minimize stress. Further research is also necessary on the regain its normal metabolic mechanism and animal meat quality.

Ammonia level is critical for invertebrates and is also one of the most important end products in sea animals; and should also be investigated to ascertain its role in stressed abalone.

Therefore, *H. midae* can be transported to market in Far East, Japan, China but the most suitable environmental conditions must be provided.

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