THE NUTRITIONAL REQUIREMENTS OF *HALIOTIS MIDAE* AND DEVELOPMENT OF A PRACTICAL DIET FOR ABALONE AQUACULTURE

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CONTENTS

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ABSTRACT

The available literature on abalone nutrition was synthesised and the prospects for developing a complete pelleted dry feed for *Haliotis midae* evaluated. The similar body compositions, digestive structures, enzyme activity, acceptance of a wide variety of feed ingredients and comparable growth performance on formulated diets of various abalone species suggests that they have similar nutritional requirements. Abalone also appear to be similar to other farmed monogastric animals in that digestion is primarily extracelluar and they possess a large, muscular crop and stomach. Abalone energy metabolism is carbohydrate based. They are capable of digesting high levels of dietary protein but their ability to utilise fat is limited. Abalone have been shown to consume predictable amounts of dry feed which is efficiently converted into body weight. Efficiency indices of utilisation of formulated feeds, such as feed conversion ratio, protein efficiency ratio, net protein utilisation and apparent digestibility have been successfully applied to abalone. It was concluded that the prospects for developing complete diets for *H. midae* using a conventional animal feed science model were good. The ability of *H. midae* to utilise a range of proteins which had potential for inclusion in practical diets was investigated. Five protein rich ingredients, namely, casein, fishmeal, soya oil cake, *Spirulina*, and torula yeast, were fed to *H. midae* in semi-purified diets. Two algal diets, fresh *Plocamium corallorhiza* and dried *Ecklonia maxima* were fed as controls. Abalone fed on fishmeal and *Spirulina* based diets displayed significantly higher growth rates than diets containing soya oil cake, torula yeast, casein and *E. maxima*. Growth rates of abalone fed with *P. corallorhiza* were significantly lower than all other diets. All artificial diets yield lower feed conversion ratios (0.7-1.8) and higher protein efficiency ratios (3.3-6.5) ratios than the seaweed control diets (FCR = 2.8-3.4; PER = 2.2-3.0). The results indicated that fishmeal and *Spirulina* were the most suitable proteins for inclusion in practical diets for *H. midae*. The effect of protein level on growth rate and nutritional indices was evaluated by feeding starch bound, fishmeal based diets containing 27, 32, 37, 42 and 47% protein to juvenile *H. midae*. Weight gain was positively related to the level of dietary protein, increasing by 18% between 27% and 47% protein. Protein efficiency ratio declining from 3.2 to 2.3 with an increasing dietary protein content. Feed consumption rate was approximately 1% of body weight per day for all diets. Post-weaning abalone (ca. 10mm shell length, 0.2g) differed from larger juveniles (ca. 35mm, 8g) in their response to varying proportions of dietary protein and energy. The smaller animals appeared to have a lower protein requirement and poorer ability to utilise lipid than the larger juveniles. Proximate analyses revealed that the levels of protein, lipid and carbohydrate in abalone soft tissue increased with increasing dietary levels of these nutrients. Larger juveniles contained significantly higher levels of protein and carbohydrate, but lower levels of lipid, ash and moisture, than the smaller post-weaning abalone. The assimilation efficiency of [U-^{14}C]-arginine by *H. midae* fed diets enriched with [U-^{14}C]-arginine was only 0.45%. Furthermore, supplementation of diets with graded levels of crystalline arginine did not have any effect on growth rates. It was concluded that the prospects for defining the quantitative amino acid requirements of *H. midae* using crystalline amino acids are not promising. Rates of gastric evacuation and enzyme secretion were monitored in juvenile *H. midae* fed an extruded, fishmeal based dry feed. Gut fullness peaked 6h after feed was offered and the bulk of feed consumed was digested within 24 h. Enzyme secretion appeared to begin with the onset of feeding and continued for at least 6h after peak gut fullness was attained. Protease activity increased significantly following ingestion but amylase activity was maintained at a more or less constant level. A low level of lipase activity was observed suggesting that the ability of *H. midae* to digest fat is limited. The growth rate of *H. midae* fed an extruded, fishmeal based feed increased with increasing temperature between 12°C and 20°C. Between 20°C and 24°C a marked decline in growth rate accompanied by a deterioration in feed conversion and increased mortality was observed. Consumption of a dry pelleted feed was shown to be a function of body size and temperature. Based on these data a model which predicts a daily ration for *H. midae* was developed. The present study showed that *H. midae* efficiently utilises extruded dry feeds containing conventional feed ingredients. Although technical difficulties were encountered in measuring apparent digestibility, it was concluded that the prospects for developing practical diets according to established nutritional principles are promising.

CHAPTER 1.

TOWARDS AN ANIMAL FEED SCIENCE MODEL FOR ABALONE CULTURE

INTRODUCTION

Abalone are among the most highly valued seafoods in the world, selling for US$20-40/ kg live weight (Rudd, 1994). The prime demand is in Asian countries where abalone products form part of traditional cuisine and ceremony. Through the 1980's a strengthening of the Japanese Yen coupled with a decline in the worlds abalone fisheries resulted in prices more than doubling in real terms (Rudd, 1994). These conditions have created a classic opportunity for the development of aquaculture, and there has been an upsurge in effort to establish abalone culture in a number of countries. A variety of culture techniques are employed. However in most countries abalone aquaculture is typically a highly intensive, shore based operation. Although most existing farms currently use natural algal feeds, a regular supply of large volumes of seaweed often presents logistical problems, and it would appear that the development of industrial scale abalone production in most countries will be dependent on the availability of nutritionally complete pelleted feeds (Hahn, 1989a; Anon, 1991a; Fallu, 1991; Britz *et al.*, 1994).

Abalone are herbivorous marine gastropods all belonging to the genus *Haliotis*. There are about 100 species world wide (Hahn, 1989a), with the larger species occuring in temperate waters, and the smaller species in tropical and arctic regions. Approximately 22 species are of economic importance, with respect to fisheries and aquaculture (Hahn, 1989a). The South

African abalone (*Haliotis midae*), or "perlemoen" as it is locally known, is a large species attaining 20cm shell length (ca. 1.5 kg). It is highly valued in the Asian markets and thus ninety percent of the local catch is exported. The annual commercial harvest of this species is approximately 600t, which makes up approximately 5% of the global yield (Tarr, 1992). *H. midae* was first propagated in captivity in 1984/5 (Genade *et al.*, 1988) and is a promising aquaculture species, the technology for which is being developed in parallel with abalone species in other countries (Table 1.1).

Table 1.1. *Haliotis* species which are farmed commercially or which show aquaculture potential.

Species	Country	Status	Source
H. asinina	Korea, Thailand	Commercial	Yoo, 1989, Singhagaiwan and Doi, 1993
H. australis	New Zealand	R&D	Tong and Moss, 1992
H. corrugata	California	R&D	Ebert, 1992
H. discus	Japan	Commercial	Hahn, 1989e
H. discus hannai	Japan, Chile, Ireland	Commercial	Hahn, 1989e; Anon, 1994a; Mai *et al.*, 1994
H. diversicolor supertexta	China, Japan, Taiwan	Commercial	Chen, 1989; Hahn, 1989f; Nie, 1992
H. fulgens	California, Chile	Commercial	Ebert, 1992, Anon., 1994a
H. gigantea	Japan, Korea	R&D	Hahn, 1989a; Yoo, 1989;
H. iris	New Zealand	Commercial	Tong and Moss, 1992
H. kamtschatkana	Canada	R&D	P. Fankboner, Simon Fraser University, pers. comm.
H. laevigata	Australia	Commercial	Hahn, 1989i
H. midae	South Africa	Commercial	Britz *et al.*, 1994
H. rubra	Australia	Commercial	Hahn, 1989i
H. rufescens	California, Chile	Commercial	Ebert 1992; Godoy *et al.*, 1992
H. tuberculata	France, United Kingdom	Commercial	Hahn, 1989h,i, Mai *et al.*, 1994

Abalone typically inhabit the sub-tidal zone, feeding on diatoms and microalgae as early juveniles, and then switching to a predominantly macroalgal diet as they grow larger. Their sedentary nature and shallow water habitat make them easily accessible to divers and, in the absence of stringent control, vulnerable to overexploitation. All the worlds abalone fisheries have declined over the last two decades and some, such as those in Mexico and California, have collapsed (Guzman del Proo, 1992; Tegner *et al.*, 1992). Fishery statistics indicate that the global yield declined from 20,000 tons in 1975 to 13,600 tons in 1989 (FAO, 1975, 1989). The shortfall in supply has driven prices to exceptionally high levels, creating an incentive to poach and placing the sustainability of the major abalone fisheries under threat. Given the high demand for abalone, it is unlikely that the supply of abalone from fisheries will increase, and thus the only way to satisfy the market demand for abalone is through aquaculture.

From a biological point of view abalone possess attributes which suit them well to aquaculture (Table 1.2).

Table 1.2. Assessment of the biological characteristics of abalone with respect to development of aquaculture technology.

Positive attributes

* **High stress threshold.** Abalone adapt well to captive conditions. Their growth and health are not easily compromised by the man-made environment, handling etc.

* **High density culture possible.** Abalone are not aggressive or territorial, and can be successfully reared at high density.

* **Disease resistant.** Viral diseases are unknown and abalone are very resistant to bacterial infection.

* **Captive breeding cycle closed.** Hatchery technology is now well established and abalone seed can be produced on an industrial scale on a year round basis.

* **Accept formulated diets.** Abalone readily consume nutritionally complete, dry pelleted feeds.

Negative attributes

* **Slow growth rate.** Abalone grow comparatively slowly (ca. 15-40 mm/yr) and hence the production cycle is long, approximately 3-4 years to a market size of 100g. Genetic selection through line breeding is a very lengthy process as the larger species take 2-5 years to reach sexual maturity (Hahn, 1989a,d)

Abalone aquaculture was first developed in Japan as a means of enhancing recruitment in their declining fishery. Following the pioneering work of Murayama (1935, cited in Hahn, 1989f) and Ino (1952) on abalone propagation, research to develop hatchery technology began in earnest in 1959 at the Tokai Regional Research Laboratory (Hahn, 1989f). Today there are 43 facilities producing seed abalone for restocking, which has greatly improved the harvest of abalone in Japan. The technology developed in Japan has been adapted to species in other countries for reseeding programmes, as well as for the grow out of abalone to market size under intensive culture conditions (Tegner and Butler, 1989; Schiel, 1992; Chen, 1989; Nie, 1992). Growout systems employed include raft or barrel culture in open water (Hahn, 1989i), intertidal tanks (Chen, 1989) and shore based tanks using pumped seawater (Hahn, 1989g). The last option holds the most promise for mass rearing abalone as it best facilitates the intensive management, and control, that this scale of aquaculture demands. Intensive shore based systems for abalone growout which are commercially successful have been established in California (Ebert, 1992), China (Nie, 1992), and Taiwan (Chen, 1989), and are being implemented and further developed in a number of other countries (Table 1.1).

Shore based culture of abalone is a highly intensive form of farming which can be likened to feedlot cattle or broiler chicken rearing. In these systems the cultured animals are confined in man made structures and a high degree of control is exerted over their environment. For example, temperature and photoperiod may be regulated, and the water supply filtered and/or sterilised. A high degree of control over all production processes is necessary in order to minimise risk in such highly capital intensive operations, as well as to ensure that a regular supply of product of uniform size, and quality, can be guaranteed to the market. The existing abalone culture industry is very young and currently contributes less than 10% to the global abalone yield (FAO, 1994). However, it is expected to grow substantially over the next decade to meet the shortfall in supply. Existing commercial operations mainly use seaweed as feed, however, if abalone farming is to become a substantial industry, the development of nutritionally complete pelleted feeds is seen by a number of analysts as being fundamental to its growth (Hahn, 1989f; Anon, 1991a; Fallu, 1991; Britz *et al.*, 1994). Virtually all intensive farming industries rearing monogastric animals, be they aquatic or terrestrial, use nutritionally complete pelleted diets due to the advantages they offer over natural feeds. Britz (1993), highlighted the potential advantages using pelleted feeds for farming *H. midae* in South Africa (Table 1.3).

Proponents of kelp and other seaweeds argue that it is cheaper than pelleted feed, however, the apparent saving is offset by the cost benefits achieved from the low feed conversion ratio and shorter production cycle offered by pelleted feeds. Costs notwithstanding, the major advantage of pelleted feeds lies in their reliability and convenience from a farm management point of view. Harvesting and use of natural feeds is dependent on sea conditions, complicating farm management and adding to the financial risk of such ventures. A final factor in favour of pelleted feeds is farm siting. Research on the temperature requirements of *Haliotis midae* indicates that the southern and eastern Cape is thermally more suitable for abalone farming (Hecht, 1994; Present study, Chapter 4) than the cooler waters of the Western Cape (Figure 4.1). Because kelp does not occur in the Eastern Cape, pelleted feeds make farming possible in this region.

Table 1.3. Comparison of management implications and logistics of using kelp (*Ecklonia maxima*) and pelleted diets for farming *H. midae* in South Africa (Adapted from Britz, 1993).

	Kelp	**Pelleted feed**
Supply	Logistics uncertain. No existing industry base for the harvest and handling of wet kelp. Dependent on sea conditions.	Supply guaranteed. Produced within the infrastructure of the animal feed industry.
Quality	Varies seasonally.	Specification guaranteed by supplier.
Storage	Bulky wet product requiring large tanks. Must be used within a few days.	Concentrated dry product. Shelf life of several months at room temperature.
Handling	Cumbersome due to its wet nature - more labour required in comparison to handling a dry feed.	Easily applied due to concentrated, dry form.
Distribution	Limited to Western Cape. Impractical to transport wet kelp long distances, therefore kelp based farms restricted to this region.	Readily transported. Farming possible in thermally optimal areas where no kelp occurs. Can be exported cost effectively.
Abalone growth	Single species algal diets produce lower growth rates than formulated diets or mixed algal diets (Fleming and Day, 1992, Britz *et al.*, 1994)	Improved diet formulations offer the possibility of significantly enhancing growth rates and feed conversion efficiency.
Feed conversion ratio (Dry feed fed/ wet weight gain)	Approximately 10-15 (C. Clayden, Sea Plant Products, unpubl. data)	0.6 - 1.3 (Britz *et al.*, 1994)
Price	Not commercially available. Farmer must arrange to harvest own supply. Cost estimates ca. R400 - R600/ wet ton (R1.60 - R2.40/ kg dry weight)	Approximately R10/kg. Prices are expected to fall to ca. R5/kg as economies of scale are achieved.

VIABILITY OF FORMULATED FEEDS FOR *H. MIDAE*

Animal feed science is a substantial discipline with very powerful tools at its disposal. When the present project was initiated in 1989, we did not know to what extent they could be applied to *H. midae* since very little work of an applied nature had previously been undertaken on gastropods. Apart from studies on the natural diet of abalone (Barkai and Griffiths, 1986; Uki *et al.*, 1986c; Shepherd and Steinberg, 1992), energetics (Barkai and Griffiths, 1988; Peck *et al.*, 1987), alimentary structure and function (Campbell, 1965; McLean, 1970), and digestive enzymes (Albrecht, 1921, 1923; Oshima, 1931; Clark and Jowett, 1978) only a few publications in Japanese existed on the development of formulated diets for *H. discus hannai* (Ogino and Ohta, 1963; Ogino and Kato, 1964; Uki *et al.*, 1985a; Uki *et al.*, 1985b; Uki *et al.*, 1986b, 1986d). Thus the major challenge we faced was to attempt to apply a conventional animal feed science model to *H. midae* in order to develop a commercial pelleted feed. In other words, would nutritional principles developed for other monogastric groups, such as pigs, chickens, fish and prawns, apply to abalone? Specific questions determining the viability of this approach were:

> * Would *H. midae*, a herbivorous gastropod, accept feeds made from conventional feed ingredients, such as plant and animal meals of marine and terrestrial origin?
>
> * Did it possess the ability to digest and assimilate a concentrated dry feed containing levels of protein, carbohydrate and fat much higher than in its natural algal diet?

Although some marine molluscs do not readily accept artificial diets (Carefoot, 1980, Lee *et al.*, 1991), a review of haliotid nutrition, and exploratory trials with *H. midae,* suggested that the prospects for successfully developing a dry pelleted feed for this species were promising.

Haliotis species, like many other herbivorous gastropods, exhibit a combination of preliminary extracelluar digestion and subsequent intracelluar digestion (Purchon, 1977). Intracelluar digestion is the more primitive method and only a few types of food can be assimilated by purely intracellular digesters (Owen, 1966). This is because only small amounts of food can be efficiently sorted and absorbed, and as a consequence feeding has to be an almost continuous process (Purchon 1977). The evolution of extracelluar digestion has led to the isolation of secretory cells into glandular masses and the development of a muscular stomach. This allows the animal to feed on a greater variety of substrates, as larger masses of food can be manipulated. The digestive efficiency is increased due to the churning of the food with digestive enzymes (Purchon 1977). McLean (1970) found that digestion in *H. rufescens* was largely extracelluar, however, intracellular hydrolysis of at least some material in the midgut gland remains a possibility. These adaptations, which *Haliotis* shares with other farmed monogastric animals, such pigs, chickens and fish, have to a large extent facilitated the application of conventional animal feed science principles. Although the natural diet of abalone has a high moisture content and relatively low nutrient levels, studies with formulated dry feeds have shown that abalone can efficiently digest highly concentrated protein and carbohydrate, but that their ability to utilise high levels of fat is limited (Uki and Watanabe, 1992; Britz *et al.*, 1994; Mai *et al.*, 1995).

Concomitant with a growing interest in abalone culture in Japan in the 1960's the first artificial diets for abalone were developed by Ogino and colleagues (Ogino and Ohta, 1963; Ogino and Kato, 1964) who formulated calcium alginate bound diets containing fishmeal, yeast, dextrin, starch, and a vitamin/mineral premix. Abalone fed with artificial diets containing 27 and 32% protein grew faster than a control group fed on the seaweed, *Undaria pinnatifida,* demonstrating the potential of formulated diets for aquaculture (Ogino and Kato, 1964). Subsequently, other Japanese researchers (Uki and Watanabe, 1992) used the basic formulation developed by these authors to investigate the nutritional requirements *Haliotis discus hannai*. These efforts, reviewed in English by Uki and Watanabe (1992), laid a foundation for the development of practical abalone diets which are now used in commercial abalone culture in Japan (Hahn, 1989f). More recently, the growing interest in abalone culture in other countries, and a growing consensus that artificial feeds will greatly facilitate intensive abalone farming, has stimulated efforts to develop artificial diets in Australia (Gorfine and King, 1991; Mozquiera, 1992), Canada (Taylor, 1992), Chile (pers. comm., C. Wuhrmann, Fundacion Chile, 1994), China (Nie, 1992), France (Hahn, 1989h), Ireland (Mai *et al.*, 1994, 1995), Mexico (Viana *et al.*, 1993), New Zealand (Anon. 1994b, 1994c), South Africa (Britz *et al.*, 1994), Thailand (Singhagraiwan and Doi, 1993), and the USA (Norman-Bodreau, 1989).

The successful studies on the nutrition of Japanese abalone species (Uki and Watanabe, 1992) suggested that the South African abalone would probably also accept an artificial diet. This was subsequently confirmed in exploratory trials with our initial diet formulation (Table 1.4) which was based on a Japanese semi-purified diet (Uki *et al.*, 1985a). Trials undertaken to evaluate the acceptability of the artificial diet demonstrated that it elicited a faster feeding response in comparison to one of *H. midae's* natural diets (*Plocamium corallorhiza)* and that when presented with a choice between the two, it always selected consumed the artificial diet (Dixon, 1992). Dixon (*op cit.)* observed that when the artificial feed was presented to the abalone, it elicited the characteristic abalone "feeding position", which consisted of a lifting of the anterior part of the foot with extended antennae accompanied by vigorous rotation of the shell and visceral mass from side to side, suggesting that the feed was detected by means of chemoreception. The faster leaching of nutrients from the artificial feed compared to *P. corallorhiza* probably accounted for the faster feeding response time observed. However, when both diets were left in the rearing container for an extended period, the artificial diet was preferentially consumed (Dixon, 1992). Powdered kelp (*Ecklonia maxima*) was included in initial dietary formulations as an attractant (Table 1.4), but it was subsequently found that this was not necessary because the diet was consumed just as readily when it was omitted (Britz *et al.*, 1994). This exploratory work suggested that the nutritional physiology of *H. midae* was similar to that to *H. discus hannai*, and that *H. midae*, like other abalone species would probably accept a wide range of feed ingredients of diverse terrestrial and aquatic origin (Table 1.5). The ready consumption of these diverse nutrients is probably an artefact of the unselective feeding habit of these herbivores, which opportunistically consume a wide variety of algae (Shepherd and Steinberg, 1992, Barkai and Griffiths, 1986). This characteristic greatly facilitates the development of formulated practical diets.

Table 1.4. Semi-purified dietary formulation used to feed abalone *Haliotis midae* under culture conditions. Modified from Uki *et al.*, 1985a.

Ingredient	Quantity (%)
Casein	32.0
Dextrin	44.0
Kelp powder	5.0
Fish oil	5.0
Agar	9.0
Vitamin mixture	1.5
Mineral mixture	3.5
	100.0

A major hurdle in the development of artificial diets for abalone is the water stability of feed pellets. Abalone are slow feeders which typically clamp a piece of seaweed under their foot which they then consume over a period of several hours. In water conventional pelleted feeds

Table 1.5. Feed ingredients used in formulated diets for abalone.

Ingredient	Abalone Species	Source
PROTEIN INGREDIENTS		
Abalone viscera silage	*H. fulgens*	Viana *et al.*, 1994
Alcoholic active sludge	*H. asinina*	Sagara and Sakai, 1974
Casein	*H. discus hannai, H. Iris,* *H. midae, H. fulgens*	Uki and Watanabe 1992; Britz *et al.*, 1994; Mai *et al.*, 1995; Viana *et al.*, 1993
Chlorella	*H. discus hannai*	Sagara and Sakai, 1974
Corn gluten meal	*H. discus hannai*	Uki *et al.*, 1985b
Egg albumin, whole egg	*H. discus hannai*	Uki *et al.*, 1985b
Fishmeal	*H. discus, H. midae* *H. fulgens*	Uki and Watanabe 1992; Britz, present study; Viana *et al.*, 1993
Fish silage	*H. fulgens*	Viana *et al.*, 1994
Gelatine	*H. midae, H. discus hannai,* *H. Fulgens,*	Uki *et al.*, 1985b; Knauer *et al.*, 1993; Viana *et al.*, 1994
Meat meal	*H. rubra*	Maguire *et al.* 1993
Rye grass protein concentrate	*H. discus hannai*	Uki *et al.*, 1985b
Semolina	*H. rubra*	Fleming, 1994
Soya meal	*H. discus hannai, H. fulgens*	Uki *et al.*, 1985b; Viana *et al.*, 1994;
Spirulina	*H. midae*	Britz *et al.*, 1994
Squid meal	*H. rubra*	Maguire *et al.*, 1993.
Yeast	*Haliotis discus*	Ogino and Kato, 1964
CARBOHYDRATES		
Alginates	*H. discus hannai, H. midae,* *H. fulgens, H. tuberculata* *H. rubra, H. laevigata*	Uki and Watanabe, 1992; Knauer *et al.*, 1993; Viana *et al.*, 1993; Hahn, 1989h Wee *et al.*, 1992
α-Starch	*H. discus hannai*	Uki *et al.*, 1985b
Corn starch	*H. laevigata, H. rubra* *H. asinina, H. fulgens*	Wee *et al.*, 1992; Viana, *et al.* 1993 Singhagraiwan and Doi, 1993; Viana *et al.*, 1994
Cellulose	*H. discus hannai*	Uki *et al.*, 1985a
Dextrin	*H. discus hannai, H. midae*	Uki and Watanabe, 1992, Mai *et al.*, 1995; Britz *et al.*, 1994
Linseed meal	*H. rubra*	Maguire *et al.*, 1993
Oats	*H. rubra*	Maguire *et al.*, 1993
Wheat flour	*H. discus hannai*	Anon, 1991b.
FATS		
Corn oil		
Cuttlefish	*H. discus hannai*	Uki *et al.*, 1986d
Fish oil	*H. discus hannai* *H. rubra, H. midae*	Uki *et al.*, 1986d; Wee *et al.*, 1992; Britz, in press
Soya bean oil	*H. discus hannai* *H. asinina*	Ogino and Ohta, 1963; Singhagraiwan and Doi, 1993
Sunflower oil	*H. midae*	Knauer *et al.*, 1995a

dissolve within minutes and thus an effective means of binding the abalone diet had to be developed before we could begin any feeding experiments. Semi-purified research diets have been successfully bound with hydrocolloids such as sodium alginate (Uki and Watanabe, 1992), agar, or a mixture of agar and gelatine (Knauer *et al.*, 1993; Britz *et al.*, 1994). Using sodium alginate as a binder Uki and Watanabe (1992) reported very low leaching rates (92%, 98% and 100% recovery of feed after 12h in containers without abalone) for diets bound with 20%, 30% and 40% sodium alginate respectively. Knauer *et al.* (1993) were unable to replicate these low leaching rates using various alginates, and the best result obtained by these authors was a 73% recovery after 12h submersion for a diet bound with 20% of a mixture of agar and gelatine (ratio 1:3). More recently, Mai *et al.* (1995) reported 92% and 86% recovery of sodium alginate bound feed submersed for 8h and 24h respectively. While hydrocolloids have proved useful for binding research diets, they do not hold promise as binders in commercial abalone feeds because they are relatively expensive, their semi-moist nature makes them difficult to store, and a large portion of the dietary nutrients can be lost through leaching (Knauer *et al.*, 1993). Consequently, most commercially available abalone feeds are dry pellets which are bound by means of starch. A company in New Zealand has developed a binding process using casein (Anon., 1994c). Unfortunately, no specific details regarding the binding techniques used to manufacture dry pellets have been published, as the companies concerned all retain this information as proprietary knowledge. The 24h solids leaching rate of commercially available dry pelleted abalone feeds, from South Africa, Japan, and New Zealand, was found to range between 4% and 34% (Britz and Clayden, 1996).

In order to overcome the problem of pellet binding and water stability, research was initiated by the Department of Ichthyology and Fisheries Science at Rhodes University (DIFS) to develop a starch bound dry pellet which could be used as a vehicle for our nutritional formulations. Shrimp feed manufacture technology was found to offer the most appropriate pelleting technology because penaeids, like abalone, are slow feeders requiring water stable feeds. Following initial dry feed pelleting trials at the FOODTEK division of the South African Council for Scientific and Industrial Research, a process was developed by food technologists in the private sector which resulted in exceptional water stability. Less than 5% nutrient leaching occured over 24 hours, and this process has proved to be a major breakthrough in the development of a practical diet for *H. midae* (Britz *et al.*, 1994). Once the dry pellet is submerged, it absorbs water and takes on a rubbery texture similar to seaweed. The pellet is manufactured in the form of flat ribbons allowing abalone to easily clamp it underneath its foot and consume it (Fig. 1.1).

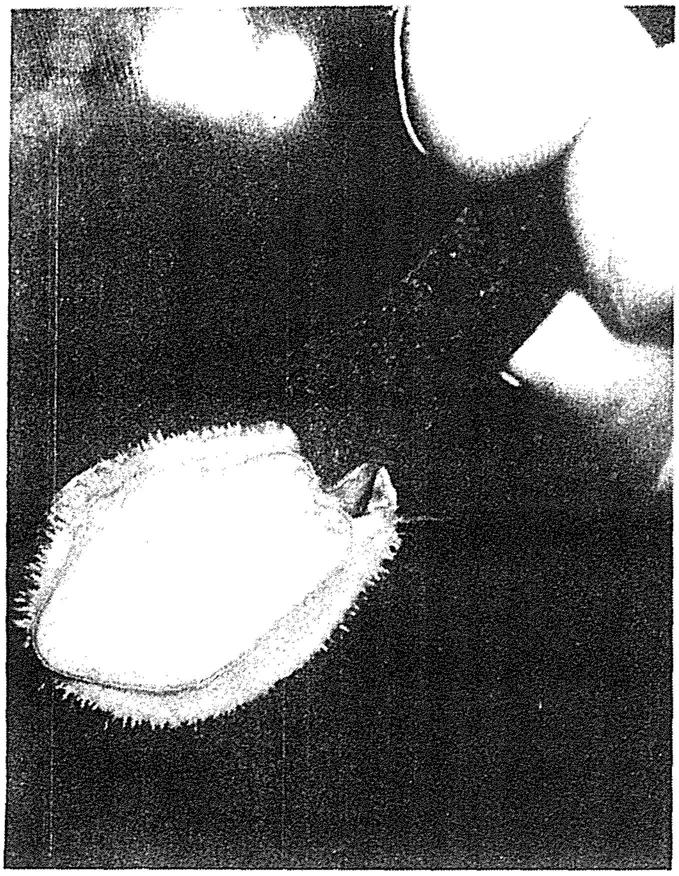

Figure 1.1. Juvenile *Haliotis midae* beginning to feed on a dry feed pellet.

SYNTHESIS OF ABALONE NUTRITIONAL REQUIREMENTS RELEVANT TO THE DEVELOPMENT OF FORMULATED FEEDS

Having established the potential viability of developing a practical pellet for commercial abalone farming, a two pronged approach to the development of nutritionally balanced diet formulations was adopted by the DIFS. Firstly, based on the available nutritional literature and the Departments previous experience in aquaculture nutrition, a "best guess" diet was formulated. Secondly, research into the nutritional requirements of *H. midae* was initiated to refine the "best guess" diet into a scientifically balanced formulation that would be commercially viable. The research presented in this thesis reflects part of this work which has been undertaken in parallel with our other studies on *H. midae* nutrition within the DIFS (Dixon, 1992; Britz *et al.*, 1994; Knauer, 1994; Lyon, 1995; Britz and Clayden, 1996).

In the absence of definitive quantitative studies on the optimal protein, lipid and energetic requirements of abalone, chemical analyses of its body composition and natural diet have provided valuable clues as to the animals nutritional requirements, and have been used as starting points for the development of artificial diet formulations (Knauer, 1994; Mai *et al.*, 1994).

Proximate composition of *Haliotis* and its natural diet

The proximate composition of *H. midae* (Knauer *et al.*, 1994b) mirrors, to a certain extent, its natural diet which contains very low levels of fat, high levels of reserve carbohydrates, and relatively low levels of protein. This is illustrated in Table 1.6, in which the proximate composition of *H. midae* (Knauer *et al.*, 1994b) is compared with kelp (*Ecklonia maxima)*, the dominant algal species in its diet, a range of values typical for algae, and with a fish, the Cape anchovy. Although the level of protein in abalone tissue is considerably higher than seaweed, it is relatively low in comparison to fish (Table 1.6).

Table 1.6. Proximate composition (%) of *H. midae*[a], kelp (*Ecklonia maxima*)[b], a variety of seaweeds consumed by abalone[c], and anchovy (*Engraulis capensis*)[d]

Nutrient	*H. midae* Whole animal	Soft tissue	Kelp *E. maxima*	Seaweed range	*E. capensis*
Protein	31	40	8.1	5 - 25	71
Fat	0.52	0.76	0.5	0.5 - 2.5	11
Carbohydrate	33	48	45.2	40 - 65	0.6
Ash	35	12	25.3	7 - 30	13.4

a. Knauer *et al.*, 1994a.
b. Kelp Products Pty. Ltd., Cape Town, unpublished data
c. Nisizawa *et al.*, 1987.
d. S. A. Sea Products Pty. Ltd., Cape Town, unpublished data

In contrast to most vertebrates which store energy in the form of lipid, a general feature of archeogastropods, including abalone, is energy storage in the form of glycogen (Webber, 1970; Goudsmit, 1972). This is reflected by the high carbohydrate/ low fat composition of *H. midae* tissue (Table 1.6) and indicates that the energy metabolism of abalone, like many other herbivorous gastropods (Emerson, 1967; Livingstone and De Zwaan, 1983), is carbohydate based. Abalone efficiently digest reserve carbohydrate, possessing a wide variety of carbohydrases (Albrecht, 1921; 1923; Oshima, 1931; McLean, 1970; Knauer *et al.*, 1996). Furthermore, enzyme hydrolysis of structural carbohydrates such as cellulose, fucoidan, and carrageenan has been demonstrated in various *Haliotis* species (Bennett *et al.*, 1971; Elyakova *et al.*, 1981; Nakagawa and Nagayama, 1988; Yamaguchi *et al.*, 1989). However, invertebrate herbivores generally hydrolyse structural carbohydrates less well, and the magnitude of their contribution to the digestible energy portion of the diet of abalone has yet to be quantified. Reserve energy is stored as glycogen in muscle tissue and as galactogen in the gonads

(Webber, 1970; Livingstone and De Zwaan, 1983). Glycogen synthesis from ^{14}C-glucose has been demonstrated in *H. rufescens* (Bennett and Nakada, 1968). Concentrations of 2 - 25% glycogen (dry weight basis) have been reported in the digestive gland, foot and mantle of *H. cracherodii* (Webber, 1970). The relatively low feed conversion ratios (dry feed fed/ wet weight gain) obtained in feeding trials with abalone suggest that they efficiently digest and assimilate high levels of carbohydrate in formulated diets (Uki and Watanabe, 1992; Britz *et al.*, 1994). The ability of abalone to utilise high levels of dietary carbohydrate is fortuitous as the inclusion of high levels of starch in pelleted feeds facilitates effective binding and water stability.

While the ash (mineral) content of abalone soft tissue is similar to that of fish (Table 1.6), that of the whole animal is much higher due to the $CaCO_3$ content of the shell. The mineral element profile of *H. midae* tissue (Knauer *et al.*, 1995b) is similar to that of *H. tuberculata* (Bryan *et al.*, 1977) and *H. diversicolor supertexta* (Chen, 1989), suggesting that all abalone have a similar mineral element requirement. Abalone can absorb minerals from seawater (Van As *et al.*, 1975), however, Uki *et al.* (1985a) showed that dietary mineral supplementation improved the growth rate of *Haliotis discus hannai.* As yet, it is uncertain whether the mineral element profile of abalone provides any clue as to its requirements, as has been suggested in fish (Steffens, 1989).

Lipid requirements

Lipids appear not to play a role as a reserve energy source in abalone as their levels are very low in abalone tissue (Table 1.6). They consist mainly of structural lipids associated with cell membrane structure and function. De Koning (1966) found that 70% of the lipid content of *H. midae* consisted of phospholipids and the remaining fraction was rich in sterols. Perhaps not surprisingly, the ability of abalone to utilise dietary lipids appears to be limited. *H. midae* displays low lipase activity, and the growth rate of *H. discus hannai,* fed formulated diets containing lipid ranging from 0 - 20% of the diet, peaked at a level of 5% dietary fat and declined sharply above 10% fat (Uki and Watanabe, 1992). Similarly Mai *et al.* (1995) observed that *H. tuberculata* and *H. discus hannai* grew optimally when diets containing 3.1 - 7.1 % lipid. Abalone are thus able to efficiently utilise higher levels of lipid than they typically encounter in their lipid poor seaweed diet (typically 0.1 - 2.0% of dry weight). Their ability to digest and assimilate dietary lipid appears similar to penaeid shrimps, for which an inclusion rate 6-7.5% is optimal in formulated diets (Akiyama and Dominy, 1989), but considerably lower than most cultured fish and which can efficiently utilise dietary lipid levels ranging from 10 - 20% (Sargent *et al.*, 1989). Uki and Watanabe (1992) observed that it was thus unlikely that substitution of dietary protein with lipid would produce a "protein sparing" effect as has been observed in many fish species (Sargent *et al.*, 1989).

The synthesis of fatty acids from acetate appears to be a general feature of molluscs (Voogt, 1972), however abalone, like other cultured marine species, appear to have a requirement for certain essential long chain highly unsaturated fatty acids (HUFA) which they cannot synthesise themselves (Uki *et al.*, 1986d). Cowey and Tacon (1982) observed that in general, cold water fish and marine species are more exacting in their requirement for essential fatty acids (EFA). Furthermore, they noted that herbivorous fish possess a greater ability to chain elongate and desaturate fatty acids than carnivores, and suggested the same patterns may apply to invertebrates. The fatty acid requirement of fish are, to some extent, related to the feeding habits and position on the food chain (Cowey and Tacon, 1982; Sargent *et* al., 1989), and Cowey and Tacon (1982) further proposed that this principle probably applies to invertebrates. The fatty acid profiles of various abalone species, particularly their high content of 20, 22 and 24 chain length HUFA, do indeed seem to reflect of their diet of marine algae which are rich in these FA (Table 1.7). The generally similar FA profiles of the *Haliotis* species analysed suggests that they probably have similar EFA requirements.

If the FA profiles in Table 1.7 are considered, palmitic acid (16:0), the end product of FA synthesis (Stryer, 1988), makes up a major proportion of the total FA compliment of abalone. In comparison to other marine animals, haliotids have an unusually high content of arachidonic acid (20:4). Red seaweeds (Rhodophyta), which are common in *Haliotid* diets are also rich in arachidonic acid (Sargent *et al.*, 1989). The high levels of arachidonic acid observed in abalone may play a role in their hormonal physiology as prostagladins (of which arachidonic acid is a precursor), are believed to stimulate spawning (Hahn, 1992). *H. midae* is the only species in which a measurable amount of docosahexaenoic acid (22:6) has been observed in specimens from the wild (Table 1.7), however, in *H. discus hannai* fed HUFA in an artficial diet, levels of 22:6 increased to 1.3-2.6% of the fatty acid fraction (Uki *et al.*, 1986d).

The only investigation into the essential fatty acid requirement of abalone has been a quantitative growth trial performed by Uki *et al.* (1986d) in which different combinations of fatty acids of different chain length were fed to *H. discus hannai* in semi-purified diets. These authors found that in a diet containing 5% lipid, with an inclusion of 1% 18:2ω-6 and 18:3ω-3, or 1% ω-3 highly unsaturated fatty acids (ω-3 HUFA, a mixture of 20:5ω-3 and

22:6ω), weight gain and feed conversion efficiency were improved. Supplementation with 18:2ω6 (methyl linoleate) resulted in increased levels of linoleic acid (18:2ω6) and arachidonic acid (20:4ω6) in abalone tissue, suggesting that abalone convert 18:2ω6 to 20:4ω6. Supplementation with 18:3ω-3 (methyl linoleate) resulted in increased levels of linolenic acid (18:3ω-3), eicosapentanoic acid (20:5ω-3) and 22:5ω-3 in abalone tissue. Supplementation with ω-3 HUFA (20:5ω-3 and 22:6ω-3) resulted in elevated abalone tissue levels of 20:5ω-3, 22:6ω-3 and 22:5ω-3. The best growth results were obtained when abalone were fed a diet containing a mixture of linolenic, linoleic and ω-3HUFA (2.5% corn oil and 2.5% cuttle fish oil) which suggests that these are essential fatty acids. Uki *et al.* (1986d) concluded that 1 % of the 5 % level of lipid recommended for inclusion in formulated diets should consist of ω-3 HUFAs.

Protein requirements

Our knowledge of the protein and amino acid requirements of abalone is still fairly rudimentary. Although the seaweeds consumed by abalone generally contain less than 20% protein (Nisizawa *et al.*, 1987), they have been shown capable of digesting high levels of dietary protein (20-50%) in concentrated form (Knauer *et al.*, 1996; Uki *et al.*, 1986b; Taylor, 1992; Viana *et al.*, 1993; Mai *et al.*, 1995). The pioneering Japanese work on artificial diets for abalone first revealed their ability to utilise a wide range of proteins (Table 1.5) and laid a foundation for the development of cost effective practical diets.

Table 1.7. Fatty acid profile of *Haliotis* species. Results are expressed as percentage of total fatty acids (Table from Knauer, 1994).

	Shucked tissue				Viscera	Foot
Fatty acid	*H. discus*[1]	*H. japonica*[1]	*H. midae*[2]	*H. midae*[3]	*H. discus hannai*[4]	*H. iris*[5]
12:0	--	--	--	1.80	0.4	--
14:0	3.9	4.9	4.7	--	14.0	5.1
14:1	--	--	--	--	0.8	0.3
15:0	2.9	3.2	0.7	--	0.7	0.7
15:1	--	--	--	--	0.3	--
16:0	20.9	19.8	33.3	16.25	24.1	22.8
16:1	3.3	4.4	6.3	1.56	5.6	1.8
16:2	--	--	--	--	--	0.2
17:0	--	--	1.7	--	0.5	1.0
17:1	--	trace	--	--	0.5	--
18:0	5.1	3.9	10.3	4.70	2.9	6.7
18:1	16.4	17.1	13.3	7.27	19.3	15.7
18:2	1.2	1.6	1.3[6]	10.86	2.8	0.5
18:3	--	--	0.3	1.11	3.4	1.2
18:4	--	0.9	--	--	3.6	--
19:0	--	--	--	7.22	trace	--
19:1	--	--	--	--	0.3	--
20:0	--	--	1.0	4.02	0.3	--
20:1	4.8[7]	5.9[7]	3.0	2.48	7.4	3.7
20:2	--	--	0.3	0.53	--	--
20:4	10.7[8]	12.3[8]	7.3	3.27	5.1	13.4
20:5	8.8	10.0	7.3	9.55	5.9	8.0
21:5	--	--	--	--	0.7	--
22:0	--	--	--	2.95	--	--
22:1	--	--	3.3[9]	2.47	trace	5.3
22:2	5.5[9]	3.9[9]	--	--	--	--
22:4	2.0[9]	2.6[9]	0.3	3.34	--	3.2
22:5	7.3	8.4	2.0	1.12	1.4	10.4
22:6	--	--	3.3	0.72	trace	--
24:0	--	--	--	5.57	--	--
24:1	--	--	--	4.91[9]	trace	--
24:4	3.5[9]	1.1[9]	--	3.94[9]	--	--

[1] Shimma & Taguchi (1964)

[2] de Koning (1966)

[3] Knauer *et al.*, (1994a)

[4] Hayashi & Yamada (1972)

[5] Bannatyne & Thomas (1969)

[6] 18:2 and 19:0

[7] 20:1 and 18:3

[8] 20:4 and 22:1

[9] Identification uncertain

Proteolytic activity has been observed in the digestive tracts of *H. discus hannai* (Cho *et al.* 1983), *H. gigantea* (Oshima, 1931), *H. midae* (Knauer *et al.*, 1996) and *H. rufescens* (Albrecht, 1921; McLean, 1970), and an ability to hydrolyse high levels of dietary protein thus appears to be a general feature of *Haliotis* species. Knauer *et al.* (1996) found that juvenile *H. midae* reared on a dry 34% protein formulated feed displayed significantly higher levels of protease activity than siblings reared on a natural diatom diet containing 5% protein, illustrating the ability of this species to adapt to a high level of dietary protein formulated diet.

Animals eat to satisfy their energetic needs and do not have an absolute protein requirement (Smith, 1989). Dietary protein is, however, necessary for somatic growth and aquaculture strives to maximise growth and the efficiency of protein conversion into body weight. To achieve this goal a well balanced mixture of essential and non-essential amino acids is required in an optimal ratio with the non protein energy (lipid and carbohydrate) in the diet.

Based on an analysis of the incorporation of labelled (U-^{14}C) glucose into abalone protein, Allen and Kilgore (1975) deduced that *H. rufescens* required the same ten essential amino acids (EAA) as most other groups of animals, namely, arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. No conclusion about the essentiality of tyrosine could be drawn. No quantitative studies on the EAA requirements of any *Haliotis* species have yet been published. It is however generally accepted that the tissue amino acid pattern of an organism approximates the ideal dietary EEA proportions (Cowey and Tacon, 1982; Benitez, 1989) and this assumption has been used in the formulation of diets for *H. tuberculata* (Mai *et al.*, 1994) and *H. midae* (Knauer *et al.*, 1996).

Knauer *et al.* (1996) formulated a practical diet from high quality proteins (fishmeal, casein, gelatine and *Spirulina)* which closely matched the amino acid profile of juvenile *H. midae* (r = 0.90). This diet was fed to early juvenile abalone (3-4 mm SL) resulting in a very low feed conversion ratio (0.44) and a very high protein efficiency ration (PER = 6.64) and percentage of protein deposited (PPD = 32.6). Until quantitative studies are undertaken to establish abalone amino acid requirements their body amino acid profile can be regarded as a good approximation of the requirements of abalone. Crystalline amino acids have been widely used, in purified and semi-purified diets, to determine the EAA requirements of a number of organisms, however, some animals do not readily utilise crystalline amino acids for protein synthesis (Wilson, 1989) and the viability using them to determine the EAA requirements of abalone has yet to be demonstrated. Furthermore, the slow feeding rate and high solubility of crystalline amino acids imposes a technical constraint with respect to the leaching of the supplemental amino acids. Coating of the amino acids with suitable insoluble, yet digestible substances could be considered as a means of overcoming the problem.

A number of studies have investigated the gross protein requirement of abalone by means of growth trials in which graded levels of single proteins such as fishmeal and casein were fed (Ogino and Kato, 1964; Uki *et al.*, 1986b; Taylor, 1992) in formulated diets. Although these studies have attempted to define an "optimal" dietary protein level for abalone, amino acid balance, protein digestibility and protein/ energy ratio, were not taken into account and thus their recommedations with respect to an optimal inclusion level of protein in abalone diets cannot be generally applied. A quantitative definition of an optimal protein level, applicable to practical diet formulation using a range of proteins, requires more knowledge of the amino acid requirements for abalone, the digestibility of the proteins used and an optimal dietary protein/ energy ratio.

Practical feed formulation

While the research on abalone nutrition described above has facilitated the development of practical abalone feeds in Australia (Gorfine and King, 1991; Morrison and Whittington, 1991; Mozquiera, 1992) Japan (Hahn, 1989f), New Zealand (Anon., 1994b, 1994c), and South

Africa (Britz *et al.*, 1994), further research is required before it will be possible to formulate "least costed" diets manufactured from the wide range of available feed ingredients. Existing dietary formulations are fixed formulas whereas the formulation of "least costed" diets is a dynamic process used by the animal feed industry to combine the cheapest feed ingredients which satisfy the nutritional requirements of the animal concerned in an acceptable pellet form. To be able to combine diverse ingredients of varying nutritional content and price, their nutritional value in terms of proximate composition, amino acid profile, and digestibility must be known. Of particular interest is the amount of energy and protein available to the animal in the various feed ingredients.

Indirect methods for determining apparent digestibility have been applied to abalone (Dixon, 1992; Wee *et* al., 1992, Wee *et al.*, in press). Wee *et al.* (in press) evaluated the efficacy of chromic oxide, fibre, ash and acid insoluble (AIA) ash as markers and found chromic oxide and AIA to be the most reliable. These authors fed a fishmeal based diet to *H. rubra* and *H. laevigata* and using Cr_2O_3 as a marker obtained a dry matter digestibility of 46%, protein digestibility of 49% and lipid digestibility of 89%. Dixon (1992) also used Cr_2O_3 as a marker in a casein based diet and obtained a dry matter digestibility of 84% and protein digestibility of 96%. The viability of these techniques pave the way for the routine determination of the digestibility of a wide range of feed ingredients which have potential for inclusion in abalone diets, and ultimately the formulation of "least costed" abalone diets.

Practical feeding

The metabolic rate of abalone, and hence their energetic requirement and rate of feed consumption, is primarily determined by temperature and body size (Hahn, 1989d). If allowed unlimited access to feed, the amount that abalone consume is primarily a function of temperature and body size (Uki, 1981; Uki *et al.*, 1986a; Peck, 1989; Lyon, 1995). Abalone metabolic rate and hence the amount of feed consumed (expressed as a percentage of body weight) decreases with increasing abalone size in a predictable trend (Uki, 1981; Peck, 1989). These trends have been quantified for *H. dicsus hannai* fed an artificial diet and have been used to derive a practical feeding table, in which the recommended ration for abalone is expressed as a function of body weight and temperature (Hahn, 1989f). Because feed constitutes one of the biggest operational costs on a farm, efficient feed application is vital, and such a table is an essential management tool. Overfeeding results in wasted feed and the decaying, uneaten feed can negatively affect water quality, while underfeeding reduces abalone growth rates and extends the length of the production cycle. The energy content of dry, pelleted feeds is much more concentrated than in seaweed and abalone thus consume far less artificial feed. While juvenile abalone typically consume 10-30% of their body weight per day (Hahn, 1989f) when fed on seaweed, they only consume 0.5-5% of body weight per day when fed a dry formulated feed (Hahn 1989f; Britz *et al.*, 1994; Lyon, 1995). This difference, which is due to the difference in the moisture content of seaweed (ca. 80%) and pelleted feed (10%), is also reflected in the feed conversion ratio (FCR), an important economic index of the efficiency of feed utilisation. The feed conversion ratio (feed consumed/ weight gain) of abalone fed seaweed typically ranges from 10-20, while that reported for pelleted feeds ranges from 0.6 - 2.0 (Uki and Watanabe, 1992; Gorfine and King, 1991; Britz *et al.*, 1994), The latter figures are comparable to those for of fish fed dry, pelleted diets (Halver, 1989).

Containers for the growout of abalone have to be designed to ensure ready access to feed. This requires an understanding of the diurnal feeding behaviour and activity of abalone. Abalone are nocturnal feeders and in captive populations 60-80% emerge every evening (Uki, 1981; Knauer *et al.*, 1995a). The different physical characteristics of seaweed and pelleted feeds means that different criteria have to be applied in the design of rearing systems. Seaweeds contain more water and possess a lower density than pelleted feeds. When packed in a tank, seaweed fills up the three dimensional space of the water column ensuring easy access to the feed by the abalone. On existing farms in the USA and Australia (pers. obs.) abalone are reared on vertical surfaces within growout tanks, and water movement, created by means of aeration, moves the seaweed past the abalone which then trap and consume pieces of it. However, ensuring access to artificial feed presents more of a challenge as dry pellets are denser than seaweed, negatively buoyant and a smaller mass of feed is required per unit of abalone mass. Thus abalone cannot effectively gain access to feed while adhering to a vertical surface. While commercial designs for growout systems based on natural feeds are well established in various countries (Hahn, 1989f,g,h), very little experience exists with respect to the design of growout systems for formulated feeds. Existing systems in operation on a small scale in Japan, Australia and South Africa house the abalone within plastic mesh baskets, which contain a horizontal plate as a feeding area. The abalone adhere to surfaces under the plate and the sides of the basket. Although the feed is concentrated in a much smaller area, in comparison to the seaweed based systems, requiring abalone to move to the feed, the designs do provide effective access to feed, as the abalone consume their predicted ration and grow at the expected rates. Overcrowding does not occur on the "feeder plate" as foraging (seemingly random movement within the basket) takes place throughout the hours of darkness, and behavioural observations have demonstrated that less than 5% of abalone actively feed at any instant (Knauer *et al.*, 1995a).

CONCLUSIONS

The similar body compositions, digestive structures, enzyme activity, acceptance of a wide variety of feed ingredients and comparable growth performance on formulated diets of abalone species currently farmed suggests that they have similar nutritional requirements. Abalone appear to be similar to other farmed monogastric animals in that digestion is primarily extracelluar and they possess a large, muscular crop and stomach. Abalone energy metabolism is carbohydrate based. They are capable of digesting high levels of dietary protein but their ability to utilise fat is limited. Various species have been shown to consume predictable amounts of dry feed which is converted into body weight with relative efficiency. Indices of efficiency of utilisation of formulated feeds, such as feed conversion ratio, protein efficiency ratio, net protein utilisation and apparent digestibility have been successfully applied to abalone. It is concluded that the prospects for developing "least costed" abalone diets using a conventional animal feed science model are good.

AIMS AND OBJECTIVES OF THE PRESENT STUDY

The present study forms part of a broader research programme initiated by the DIFS to develop technology for abalone farming based on formulated feeds.

The aim of this study was to investigate the nutritional requirements of *H. midae* with a view to developing a formulated practical feed for abalone farming in South Africa.

The objectives were:

1. To apply existing knowledge on abalone nutrition and feed formulation to *H. midae*.
2. To evaluate the suitability of locally available proteins for inclusion in abalone diets.
3. To investigate the dynamics of digestion in abalone fed a dry formulated feed.
4. To formulate a practical diet and determine the optimal levels of dietary protein and energy using the most promising protein(s) identified.
5. To assess whether *H. midae* was able to assimilate the essential amino acid arginine in crystalline form.
6. To investigate the effect of temperature on the utilisation of formulated feeds by abalone and develop a practical feeding protocol.

CHAPTER 2

AN INVESTIGATION INTO THE ABILITY OF *H. MIDAE* TO UTILISE SELECTED PROTEINS IN ARTIFICIAL DIETS

INTRODUCTION

In intensive aquaculture based on nutritionally complete diets, the key economic parameters of growth rate and diet cost are very sensitive to the quality and quantity of dietary protein. Therefore, in the development of practical diets for a new aquaculture species such as abalone, a great deal of nutritional research initially tends to focus on protein requirements.

As outlined in Chapter 1, our knowledge of the protein requirements of abalone is still fairly rudimentary. The pioneering Japanese work on artificial diets for abalone first revealed the ability of abalone to utilise a wide range of proteins (Table 1.1) and laid a foundation for the development of cost effective practical diets. Uki *et al.* (1985a) evaluated abalone growth using diets containing a number of proteins including casein, soya bean meal, rye grass concentrate, egg albumin, whole egg and fishmeal. They concluded that casein was the most suitable protein for inclusion in artificial diets. However, because of its relatively high cost, casein is unlikely to be widely used as a primary protein source in practical diets in South Africa. The results of Uki *et al.* (1985a,b) furthermore suggested that fishmeal was not a promising protein as growth rates of abalone fed with fishmeal based diets were approximately half those fed on casein based diets. However, in a further study (Uki and

Watanabe, 1986) it was shown that abalone growth improved considerably on diets containing fresh or freeze dried fish. The heterogeneity of fishmeal quality was further highlighted by the results of Viana *et al.* (1993) who observed similar growth rates in *H. fulgens* fed casein and fishmeal diets.

Prior to the execution of the present study, the only published investigations into the effect of dietary protein level on the growth of haliotids were those of Ogino and Kato (1964) and Uki *et al.* (1986b, summarised in English in Uki and Watanabe, 1992) who fed graded levels of casein and fishmeal to *H. discus hannai*. These authors found that growth rate and feed conversion efficiency of abalone on fishmeal based diets were positively related to protein level between 5 and 46.5% dietary protein, whereas protein efficiency ratio was negatively related.

The present investigation consisted of two exploratory trials, undertaken in the initial stages of development of a practical diet for *H. midae*. In the first (Experiment 1), the semi-purified diet developed by Uki *et al.* (1985a) was modified to evaluate the ability *H. midae* to utilise locally available, protein rich feed ingredients. Fishmeal was identified as the most promising primary protein for inclusion in practical diets, and in a second growth trial (Experiment 2), it was used to evaluate the effect of dietary protein level on growth rate and feed utilisation in *H. midae*. Furthermore, in the second experiment starch bound, dry pellets were used for the first time on *H. midae* and their performance evaluated.

MATERIALS AND METHODS

Experimental systems

Both experiments were conducted at our Port Alfred laboratory (33°45'S; 26°00'E) on the Indian Ocean, which utilises sea water pumped from the mouth of the Kowie River estuary. Experimental system parameters are summarised in Table 2.1.

Table 2.1. Experimental system parameters under which the two growth trials were conducted.

Parameter	Experimental regime
Temperature	
Experiment 1	19±2°C
Experiment 2	21.7 ± 1.8°C.
Salinity	35±1 ppt
Water supply	Partial recirculation through a biological filter with 25% replacement per day from Kowie River estuary.
Flow rate	4 exchanges per experimental container/hr
System volume	10 000 l
Experimental containers	Fibreglass, grey epoxy painted, 30cm X 50cm X 26cm (depth), 39 l.
Light regime	12L:12D photoperiod, neon light intensity 200 lux at water surface.
Shelter	Two shelters (150mm long), made from 50mm white PVC piping cut in half longitudinally, were provided for the experimental animals in each container.

Experiment 1: Evaluation of proteins

Experimental diets

Five protein rich sources, namely, casein (91% protein), "low temperature" fishmeal (70% protein; mix of *Engraulis capensis* and *Etrumeus teres whiteheadi*, dried by indirect heat, less than 15h after catch), soya oil cake (51% protein; solvent extracted), *Spirulina* sp. algae (44% protein; harvested from tannery wastewater) and torula yeast (50% protein; sugar cane processing by-product) were selected for evaluation. Two macroalgae, *P. corallorhiza* (20% protein; Rhodophyta) and dried *Ecklonia maxima* (10% protein; Phaeophyta), which are dominant species in the natural diet of *H. midae* (Barkai and Griffiths, 1986), were fed to control groups of abalone. The proteins were included in semi-purified diets (Table 2.2) formulated to contain 30% protein and 5% lipid.

Table 2.2 Ingredient composition (%) and nutrient analysis of the artificial test diets.

	Diet				
Ingredient	Casein	Fishmeal	Soya oil cake	Spirulina sp.	Torula yeast
Casein	31.7	-	-	-	-
Fishmeal	-	44.2	-	-	-
Soya oil cake	-	-	63.4	-	-
Spirulina sp.	-	-	-	42.9	-
Torula yeast	-	-	-	-	57.5
Dextrin	49.3	41.3	19.0	41.2	25.0
Agar	9.0	9.0	9.0	9.0	9.0
Oil[1]	5.0	0.5	3.4	1.9	3.6
Choline chloride	0.5	0.5	0.5	0.5	0.5
Vitamins[2]	1.5	1.5	1.5	1.5	1.5
Mineral [2]	3.0	3.0	3.0	3.0	3.0
	Nutritional analysis (calculated)				
Crude protein	31	29	32	19	29
Total lipid	5.0	5.0	5.0	5.0	5.0

1. Oil was a 50/50 mixture of sunflower seed oil and a fish oil, "Marinol R".
2. Vitamin and mineral composition from Uki *et al.* (1985a)

These inclusion levels were suggested by Uki *et al.* (1985a, 1986b) as being optimal for abalone growth. Subsequent dietary analysis revealed that the protein content of the *Spirulina* diet was only 19% as the batch of *Spirulina* used contained less protein than specified. The total protein content of the ingredients used was determined by the Kjeldahl total nitrogen method. The vitamin and mineral mixtures used were formulated according to Uki *et al.* (1985a).

Following unsuccessful attempts to bind the diets using sodium alginate in the manner of Uki *et al.* (1985a), they were bound with agar using the method described by Knauer *et al.* (1993). The diet mixtures were mixed into liquid agar (60-70°C) and poured into petri dishes where gelling occurred. The diets were made on a weekly basis and stored at 5°C until used. Fresh *P. corallorhiza* was collected every 3-4 days, blotted damp dry, weighed and fed to the abalone. The remaining algae in the experimental containers was reweighed daily after blotting it damp dry. Consumption was calculated on a dry weight basis. Consumption of dried (48h at 70°C) kelp fronds (*E. maxima*), which were rehydrated before feeding, was similarly calculated. Kelp fronds were replaced every third day as they began to disintegrate after this time.

Experimental animals

Juvenile *Haliotis midae* were collected subtidally at Port Alfred, and held in the laboratory for three months prior to the experiment and fed the red alga, *Plocamium corallorhiza*. The abalone were individually labelled by means of plastic "Dymo" tags attached to their shells with quick setting epoxy glue. Abalone were divided into two replicate batches of 15 animals for each test diet. At the beginning of the experiment mean abalone shell length (SL) was

21.2 ± 2.5 mm and weight was 1.76 ± 0.60 g. The growth trial was conducted over 125 days.

Monthly measurements of shell length (SL) and weight of all abalone were made. Before measurement, excess water was drained from the batches of abalone by suspending them in a nylon net bag over a sink for five minutes. SL was measured along the longest axis of the shell to the nearest 0.1 mm using vernier callipers. Weight was recorded to 0.01 g with an electronic balance. Specific growth rate of abalone in each treatment over the experimental period was calculated using the formula:

$$SGR = (Ln(W_f)-Ln(W_i)/t)100$$

where,

SGR = Specific growth rate (% body weight gain.d^{-1})
$Ln(W_f)$ = Natural log of mean final weight
$Ln(W_i)$ = Natural log of mean initial weight
t = Time in days

The experimental abalone were provided with approximately 2-3 times the amount they consumed per day. The amount offered was weighed each day and uneaten food removed, dried and weighed the following day. Daily feed consumption, corrected for leaching, was calculated using the formula:

$$C_g = (F^*L)-R \quad \text{.................... Uki and Watanabe, 1992.}$$

where,

C_g = Consumption (g)
F = Dry weight of feed fed
R = Dry weight of feed remaining after feeding
L = Correction factor for solids leaching

The leaching rate of solids for each diet was determined using a control rearing container without abalone present. The correction factors (L) used were 0.75 for casein, 0.79 for fishmeal, 0.86 for soya oil cake, 0.68 for *Spirulina* sp. and 0.81 for torula yeast.

Daily feed consumption (C_g) was then expressed as a percentage of mean abalone body weight using the formula:

$$C_{\%b.wt} = C_g/W_t*100$$

where,

$C_{\%b.wt}$ = Feed consumption (% of body weight)

C_g = Daily feed consumption

W_t = Mean weight at time t (days)

Mean daily abalone weight was estimated from the rate of weight gain over the experimental period by means of the formula:

$$W_t = W_0*[(SGR/100)+1]^{t-1}$$

where,

SGR = Specific growth rate (% per day)

W_t = Mean weight (g) at time t (days)

W_0 = Mean initial weight

The feed conversion ratio (grams dry feed consumed/grams wet weight gain), protein efficiency ratio (grams weight gain (wet)/ grams protein consumed) and condition factor (weight (g)/length (mm)$^{2.99}$x5575) of abalone were calculated for all treatments. The equation for condition factor was derived from a sample of 3000 length and weight measurements of wild *H. midae* representing the entire size range of the species. The mean condition factor of the sample was set as 1 by means of the constant 5575. The mean condition factor of the experimental animals was 1.05 at the beginning of the trial.

Statistical methods

One way analysis of variance and Scheffe's range test was used to test for differences in abalone length, weight, feed consumption and condition factor between the different dietary treatments. Data for experimental replicates was pooled if no significant difference were found between them. Regression analysis was used to calculate the coefficient of variation for of the EAA profile of each protein in comparison to that of *H. midae* soft tissue.

Experiment 2: Effect of protein level

Experimental diets

Starch bound, extruded diets were formulated with graded amounts of fishmeal to contain 27, 32, 37, 42 and 47% protein (Table 2.3), and fed to juvenile *H. midae* in a 95 day growth trial. Cellulose was increased in the diets as the fishmeal was decreased, and was assumed to be a non-nutritive filler. In the absence of any conclusive studies, the possibility that abalone can utilise cellulose as an energy source cannot be discounted. However, studies on other herbivorous molluscs suggest that structural carbohydrates are poorly digested and do not make a major contribution to their energy metabolism (Galli and Giese, 1959; Livingstone and De Zwaan, 1983).

Growth rates, feed consumption and nutritional indices (FCR, PER, and CF) were calculated as described in Experiment 1. The correction factors (L) used for the leaching rate of solids from the experimental diets were; 27% protein: 0.95, 32% protein: 0.93, 37% protein: 0.90, 42% protein: 0.89, and 47% protein: 0.85. The duration of the experiment was 95 days.

Table 2.3. Percentage composition of semi-purified diets containing graded levels of protein. Dietary designations indicate the level protein inclusion.

	Diet				
Ingredient	**P27**	**P32**	**P37**	**P42**	**P47**
Fishmeal[1]	41.0	48.0	56.0	64.0	71.0
Cellulose[2]	26.7	20.5	13.4	6.2	-
Starch	22.0	22.0	22.0	22.0	22.0
Oil[3]	3.3	2.5	1.6	0.8	-
Choline chloride	1.0	1.0	1.0	1.0	1.0
Vitamins[4]	2.0	2.0	2.0	2.0	2.0
Minerals[4]	3.0	3.0	3.0	3.0	3.0
Cr_2O_3[5]	1.0	1.0	1.0	1.0	1.0
	Nutritional analysis (Calculated)				
Lipid	7.8	7.8	7.8	7.8	7.8
Protein	27.0	32.0	37.0	42.0	47.0

1. "Special A" fishmeal supplied by South African Sea Products (Pty.) Ltd, Cape Town. Derived from a mixed catch of anchovy (Engraulis capensis) and redeye (Etrumeus teres whiteheadi). No formalin was added to the catch, which was processed into fishmeal by indirect heat within 15h of being caught. 2. Sigma brand. 3. 50/50 mix of sunflower oil and "Marinol R", a fish oil produced by Marine Oil Refiners, Cape Town. Vitamins and mineral formulations are listed in Uki *et al.* (1985a). 5. An unsuccessful attempt was made to determine the digestibility of these diets using chromic oxide as an inert marker.

Experimental animals

Hatchery reared juvenile abalone (*H. midae*) spawned at the Sea Fisheries Research Institute, Cape Town, were transferred to our laboratory where they were fed on a starch bound artificial feed for seven months prior to the experiment. The composition of the artificial feed was 30% protein (mixture of fishmeal and *Spirulina)*, and 5% lipid. In the experiment two replicates of 48 abalone (19.9 ± 1.99 mm shell length, 1.46 ± 0.42 g) were used for each dietary treatment. The feeding protocol and measurement of abalone was the same as described in Experiment 1.

At the conclusion of the growth trial, the experimental animals were maintained on their respective diets and faeces collected in an attempt to determine the apparent digestibility of the diets. Each morning at 08h00, the tanks were siphoned clean and the shelters containing the abalone removed and placed in white 10 l plastic basins containing fresh sea water. The water was gently aerated by means of an airstone. After a 2h interval, the abalone were replaced in their tanks, and the remaining faeces siphoned onto preweighed 4.5 cm Whatman filter paper. The filter papers were then oven dried at 60°C for 4h and weighed. The chrome content of the faeces was determined, following digestion of both the filter paper and faeces, using the method of Bolin *et al.* (1952). Apparent dry matter digestibility was calculated using the equation:

$$\text{DMADC} = 100(\%\ Cr_3O_2 \text{ in faeces} - \%\ Cr_3O_2 \text{ in feed})/\ \%\ Cr_3O_2 \text{ in faeces})$$

Statistical methods

One way analysis of variance and Scheffe's range test was used to test for differences in abalone length, weight, feed consumption and condition factor between the different dietary treatments. Data for experimental replicates was pooled if no significant difference were found between them. Linear regression analysis was used to describe specific growth rate (SGR), protein efficiency ratio (PER) and feed conversion ratio (FCR) as functions of dietary protein content.

RESULTS

Experiment 1: Evaluation of proteins

The observed feed consumption rate (dry weight basis) of abalone fed on the artificial diets did not differ significantly and ranged from 0.5% - 0.8% b.wt.day^{-1} (Table 2.4). However, consumption among those fed the algal diets was significantly higher ($P<0.05$) than those fed

Table 2.4. Feed consumption rate, specific growth rate (SGR), length increment, condition factor (CF), feed conversion ratio (FCR), and protein efficiency ratio (PER) of abalone fed on formulated and natural diets. For each parameter, significant differences ($P<0.05$) between the experimental diets are indicated by different alphabetic superscripts. The superscripts for CF are comparable both within and between the initial and final values.

	Diet						
	Casein	*Spirulina* sp.	Soya oil cake	Fishmeal	Torula yeast	*P. corallorhiza*[1]	*E. maxima*[1]
Feed[2] consumption rate (% b.wt.day^{-1})	0.5 ± 0.02^{c}	0.8 ± 0.03^{c}	0.6 ± 0.02^{c}	0.8 ± 0.06^{c}	0.7 ± 0.04^{c}	1.3 ± 0.07^{b}	2.8 ± 0.14^{a}
SGR[2] (% b.wt.day^{-1})	0.6 ± 0.03^{b}	0.8 ± 0.04^{a}	0.5 ± 0.03^{b}	$0.9\pm0.03\text{a}^{b}$	0.6 ± 0.02^{b}	0.4 ± 0.02^{c}	0.6 ± 0.03^{b}
Length increment[2] (µm.day^{-1})	45 ± 3^{b}	$58\pm4^{a,b}$	$41\pm3^{b,c}$	$65\pm4\text{a}^{b}$	$42\pm2^{b,c}$	29 ± 2^{c}	$54\pm3^{a,b}$
Initial CF[2]	1.0 ± 0.02^{a}	1.0 ± 0.02^{a}	1.0 ± 0.02^{a}	1.0 ± 0.03^{a}	1.0 ± 0.01^{a}	1.0 ± 0.02^{a}	1.0 ± 0.02^{a}
Final CF[2]	1.1 ± 0.02^{a}	1.1 ± 0.01^{a}	1.1 ± 0.02^{a}	1.1 ± 0.02^{a}	1.0 ± 0.01^{a}	1.0 ± 0.01^{a}	0.9 ± 0.02^{b}
FCR (g consumed/ g gain)	0.7	0.8	1.0	0.8	1.0	2.8	3.4
PER (g gain/ g protein consumed)	4.7	6.5	3.4	3.9	3.3	2.2	3.0

1. Algal consumption presented on a dry weight basis.
2. Mean ± standard error.

the artificial diets, visibly 1.3% and 2.8% b.wt.day^{-1} for *P. corallorhiza* and *E. maxima* respectively. Abalone fed on the fishmeal and *Spirulina* sp. based diets produced a significantly higher ($P<0.05$) length increment and specific growth rate in comparison to the other formulated diets (Figure 2.1, Table 2.4). Similar growth performance was observed for the casein, soya oil cake, torula yeast and dried kelp diets, however, weight gain of abalone fed *P. corallorhiza* was significantly lower ($P<0.05$) than all other treatments (Table 2.4). Abalone fed on dried kelp increased proportionally more in length than in weight, than those fed the other diets. This was reflected in a significantly lower condition factor (CF) ($p<0.05$) (Table 2.4). The CF of abalone in all other treatments did not change significantly over the experimental period ($p<0.05$) (Table 2.4).

The FCR values of abalone fed the formulated feeds ranged from 0.7 on the casein based diet to 1.0 on the torula yeast based diet. Higher FCR values were obtained for the macroalgae (on a dry weight basis), visibly, 2.8 and 3.4 for *P. corallorhiza* and *E. maxima* respectively (Table 2.4).

Similarly, the conversion of protein (PER) by abalone was less efficient when they were fed on the macroalgae (2.2 and 3.0 for *P. corallorhiza* and *E. maxima* respectively) than on the formulated diets, which ranged from 3.3 for torula yeast to 6.5 for *Spirulina* sp. (Table 2.4).

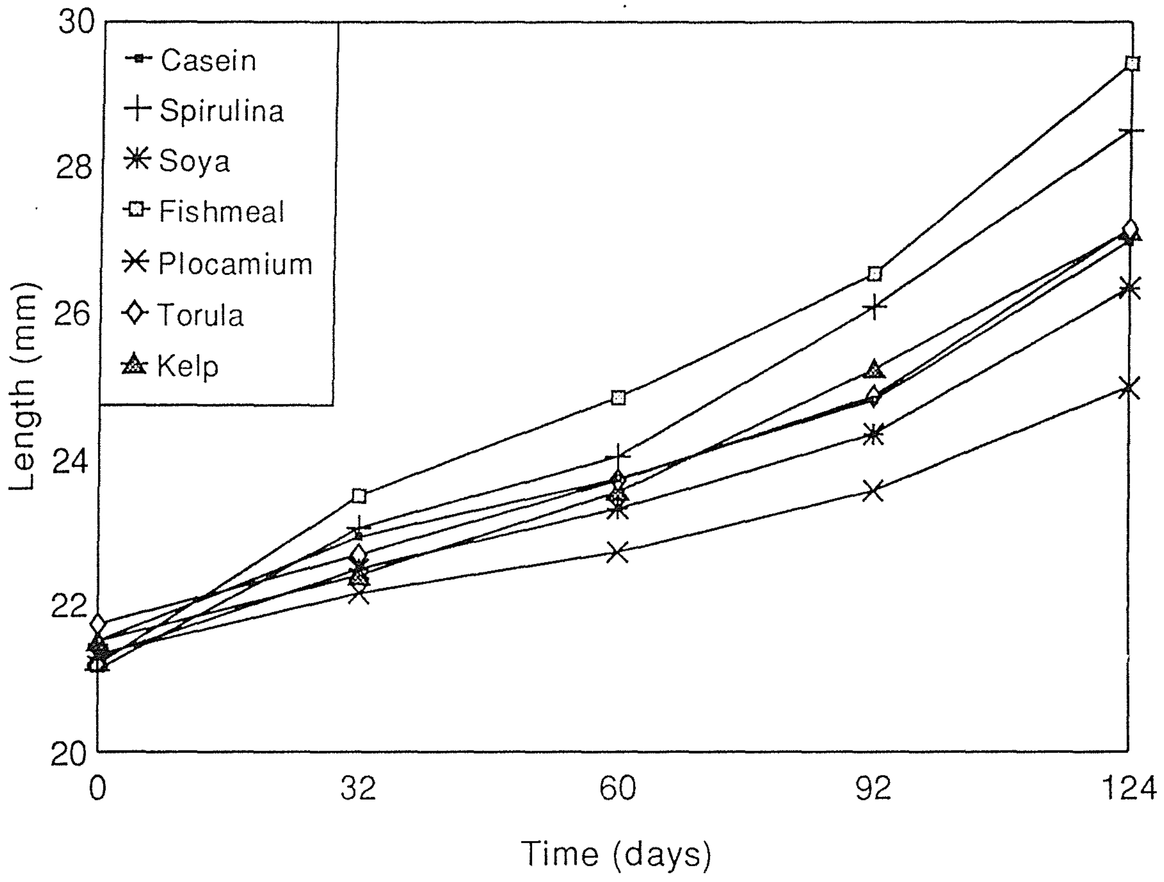

Figure 2.1. Growth rate of H. midae (expressed as shell length) reared on two algal diets and five formulated diets containing different protein sources.

Experiment 2: Protein level

Specific growth rate was positively related to the level of dietary protein ($r^2 = 0.65$, $p < 0.005$), increasing from 0.94 to 1.11 % of body weight/day between 27 and 47% protein (Figure 2.2). Growth rate in terms of length ranged from 2.5 mm/month on the 27% protein diet to 2.9 mm/month on the 47% protein diet (Table 2.5). The final mean weight and length of abalone fed the 47% protein diet was significantly greater than abalone fed the 27% protein diet ($p<0.05$) but did not differ significantly from the other treatments (Table 2.5).

Table 2.5. Means and standard errors for final weight, length, feed consumption rate and condition factor (CF) of abalone fed fishmeal based diets with different levels of protein. CF at the beginning of the experiment was 1.06. Significant differences are indicated by different alphabetic superscripts.

	Dietary Protein (%)				
	27	**32**	**37**	**42**	**47**
Final weight[1] (grams)	3.5 ± 0.11^b	3.6 ± 0.11^{ab}	3.8 ± 0.10^{ab}	3.9 ± 0.13^{ab}	4.1 ± 0.12^a
Final length[1] (mm)	27.8 ± 0.31^b	28.6 ± 0.27^{ab}	28.7 ± 0.24^{ab}	28.5 ± 0.24^{ab}	29.1 ± 0.30^a
Growth rate ($mm.month^{-1}$)	2.5 ± 0.40	2.7 ± 0.05	2.8 ± 0.60	2.7 ± 0.85	2.9 ± 0.55
Feed consumption rate (% of body $weight.day^{-1}$)	1.0 ± 0.03^a	1.0 ± 0.03^a	1.0 ± 0.03^a	1.0 ± 0.02^a	1.0 ± 0.03^a
Condition factor	0.95 ± 0.03^a	0.87 ± 0.01^a	0.92 ± 0.02^a	0.96 ± 0.2^a	0.98 ± 0.03^a

FCR was inversely related to protein level, decreasing from 1.15 at 27% protein to 1.04 at 47% protein ($r^2 = 0.71$, $p < 0.002$). PER was negatively related ($r^2 = 0.76$, $p<0.001$), declining from 3.2 to 2.3 over the range of protein fed (Figure 2.2). Feed consumption rate was approximately 1% of body weight

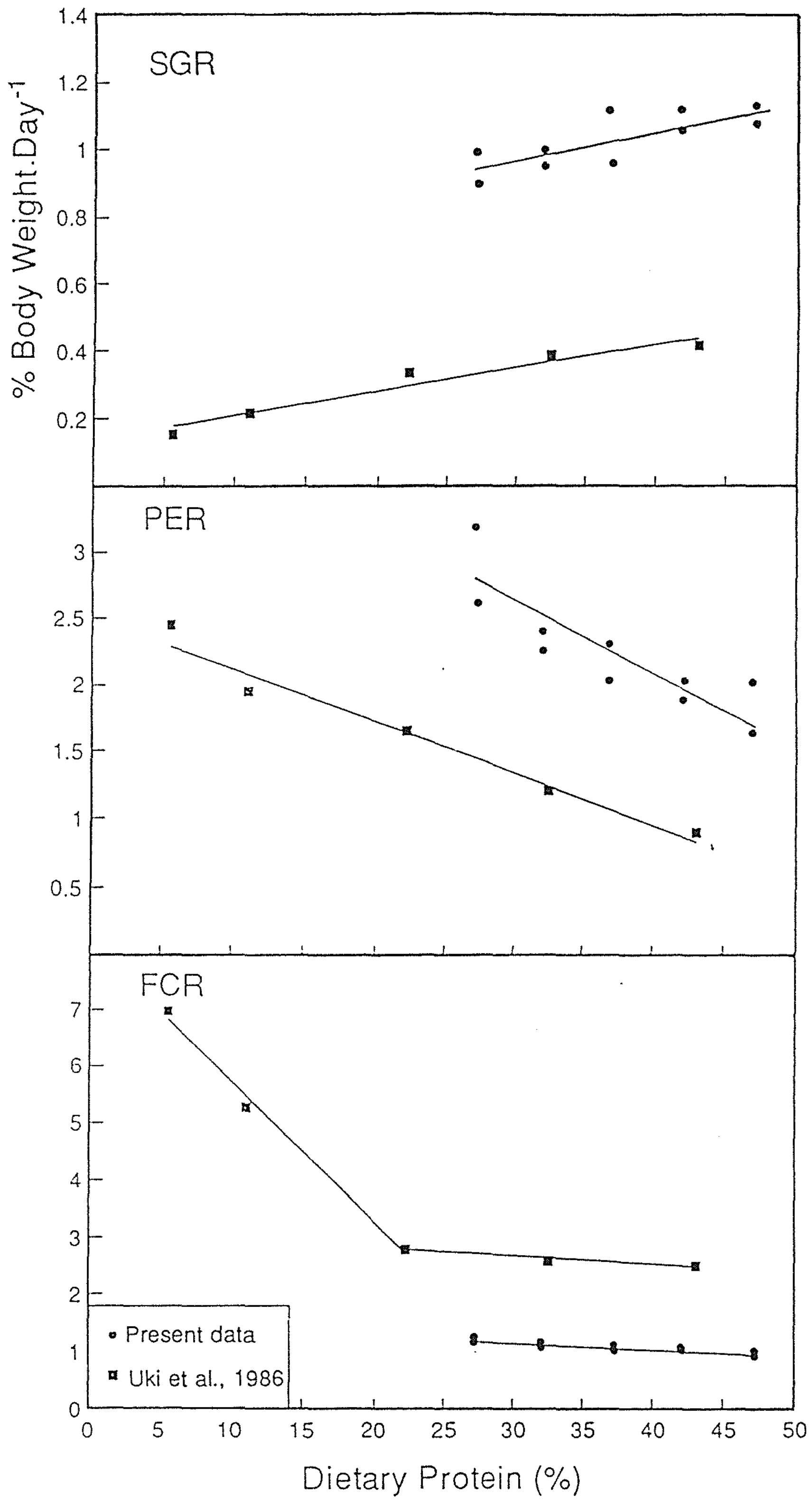

Figure 2.2. Trends for specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) in relation to levels of dietary protein of H. midae (this study) and H. discus hannai (abstracted from Uki *et al.* 1986b) fed fishmeal based diets. The regressions of the present data were statistically significant ($p<0.05$).

per day for all treatments and no significant difference in consumption rate was found ($P<0.01$) between the treatments (Table 2.5). The condition factor of abalone in all groups decreased slightly from the initial value but no significant differences were found between the diets ($p<0.05$) (Table 2.5). The recovery of chrome from the faeces collected was very low and variable and yielded estimates of apparent dry matter digestibility that were clearly erroneous.

DISCUSSION

In general, abalone fed the artificial diets in Experiment 1 performed better than those fed on macroalgal diets, displaying higher rates of weight gain, lower feed conversion ratio's, higher protein efficiency ratio's and condition factors. Faster growth rates on artificial diets in comparison to natural feeds have also been documented in other *Haliotis* species (Hahn, 1989e,f; Morrison and Whittington, 1991; Nie, 1992), illustrating one of the potential benefits of using formulated diets in intensive abalone culture.

Abalone fed on the fishmeal and *Spirulina* based diets performed particularly well and, contrary to expectation, displayed higher growth rates than those fed on the casein based diet. However, abalone fed on the casein diet exhibited the lowest FCR suggesting that the casein diet was more digestible than the other diets. This result contrasts with the finding of Uki and Watanabe (1986) who reported poorer growth and FCR values in *H. discus hannai* fed fishmeal based diets compared to casein based diets (Table 2.6). The source and processing of the fishmeals used in the two studies probably accounts for these differences, although the possibility that *H. midae* and *H. discus hannai* differ in their ability to digest fishmeal cannot be discounted. In the present study low temperature (LT), formalin free fishmeal, made from a pelagic catch was used, whereas Uki and Watanabe (1986) used "white" fishmeal, probably of demersal origin, but provided no processing specifications. However, when raw fish was fed to *H. discus hannai* in the same study, growth was much improved and the FCR and PER values were similar to those produced by the casein based diet. Similarly, Viana *et al.* (1993) also reported that fishmeal and casein produced similar growth rates in *H. fulgens*. These results highlight the possible effect that the processing method may have on protein quality and biological availability of fishmeal. In the present study, casein was only marginally superior to LT-fishmeal in terms of FCR and PER, demonstrating the particularly good quality of this source of fishmeal.

The more efficient feed conversion but lower growth rate of abalone fed on the casein diet in comparison to the fishmeal can be explained by the lower feed consumption rate of the casein diet (Table 2.6). Either casein was not as palatable or attractive as fishmeal, or the lower consumption of the casein diet may be due to an energy/protein imbalance. The good growth performance obtained with fishmeal is encouraging with respect to the development of practical diets because this feed ingredient is readily available and relatively cheap.

The comparatively efficient conversion of feed (FCR = 0.8) and protein (PER = 6.5) by abalone fed on *Spirulina* suggests that this protein source has a high biological value. However, the PER is not directly comparable to the other diets because of the lower protein content of the *Spirulina* diet. Despite the protein content of this diet being only 19% (compared to 30% in the other artificial diets), it produced growth rates just marginally lower than those of the fishmeal based diet. These results indicate that *Spirulina* is a highly digestible protein source.

Table 2.6. Comparison of results obtained by Uki and Watanabe (1986) and the present study for artificial diets containing similar levels of protein (30%) and fat (5%) fed to abalone (H. discus hannai and H. midae respectively). Growth rate (weight gain) is compared by means of a relative growth index (RGI) which expresses abalone growth on a fishmeal diet as a percentage of growth obtained on a casein diet. LT = low temperature.

	RGI %	FCR	PER
1. Uki and Watanabe (1986)			
Casein	100	0.83	4.0
Raw fish (pollock)	78	0.81	4.0
White fishmeal	48	1.58	2.1
2. Present study			
Casein	100	0.7	4.7
LT-Fishmeal	150	0.8	3.9

Abalone fed the diets containing soya oil cake and torula yeast consumed less and exhibited poorer growth rates than those fed on the other artificial diets. However, the comparatively good FCR and PER values produced by these diets suggest that the low feed consumption rate observed might have been due to a lack of palatability, or an energy/protein imbalance.

The poor growth rates and FCR values observed for the seaweeds could be due to a deficiency of essential nutrients, or a low protein to energy ratio since marine algae are, in general, rich in storage carbohydrates but low in protein. Thus the abalone fed the macroalgal diets may have satisfied their energetic requirements primarily from carbohydrate, and ingested insufficient protein to support a high rate of tissue deposition. Although *E. maxima* and *P. corallorhiza* are major constituents of the diet of *H. midae* in the wild, a variety of other algae are consumed which probably provide nutrients which are deficient in the two principal species. Day and Fleming (1992) discuss growth rate depression using single species algal diets and *H. midae* has indeed shown faster growth on a diet of mixed algae than on *E. maxima* alone (C. Clayden, Sea Plant Products, unpublished data). Thus, although a commercially exploitable resource of *E. maxima* exists off the South African Cape coast which could be used as abalone feed, it would appear that supplementation with other algae and/or artificial feed may be necessary in order to obtain growth rates fast enough to justify the intensive shore based farming of *H. midae*.

Abalone appear to require the same ten essential amino acids (EAA) as other groups of animals (Allen and Kilgore, 1975). It is generally accepted that the essential amino acid profile of an organism approximates the ideal balance of dietary amino acids (Boorman, 1980; Cowey and Tacon, 1982; Benitez 1989). Thus, comparison of the amino acid profiles of the proteins used in this study, may help to explain the observed growth and PER results. Comparison of the EAA pattern of *H. midae* soft tissue (Knauer *et al.*, 1994a) with the proteins used in the present study, by means of linear regression analysis revealed that soya oil cake most closely matched the abalone pattern, followed by *Spirulina,* fishmeal and *P. corallorhiza*, torula yeast, casein and *E. maxima* (Table 2.7). However, the proteins with the highest r^2 values did not produce the best SGR and PER values (Table 2.5). For example, although soya oil cake EAA pattern closely matched that of abalone tissue, growth and PER were comparatively poor in abalone fed this feed, suggesting that soya protein is not as digestible as casein, fishmeal or *Spirulina.* It is interesting to note that the arginine content of abalone tissue is relatively high in comparison to the proteins used suggesting that it may have been the most limiting amino acid in these diets, if it is assumed that the body EAA profile of *H. midae* approximates its requirement pattern, and that there is as equal turnover rate of EAA (Table 2.7). A similar conclusion was reached by Mai *et al.*, 1994 who compared the EAA profiles of *H. tuberculata* and *H. discus hannai* with those of selected seaweeds. The poorest PER values were obtained for the seaweeds. In the case of *E. maxima*, this may have been a reflection of its apparently unbalanced amino acid profile which yielded an r-value of only 0.33 when compared to the EAA profile of abalone. Although the amino acid

Table 2.7. Essential amino acid profile of abalone whole soft tissue compared with that of proteins used in the experimental diets. Amino acid values lower than abalone tissue values are indicated in parentheses. The most deficient amino acids in the feed ingredients are underlined and their percentage deficiency in comparison to abalone EAA pattern expressed in the bottom row. Amino acid values are expressed as a percentage of protein.

	Abalone[1]	Torula[2]	Fish	*Spir-ulina*	Soya	Casein	*Ecklonia maxima*	*Plocamium corallorhiza*
Isoleucine	**4.11**	6.20	(3.85)	5.62	4.54	6.82	(2.92)	(3.41)
Leucine	**6.93**	7.50	7.13	8.54	7.30	10.47	(5.76)	(4.45)
Lysine	**6.21**	8.20	7.77	(4.69)	(5.98)	8.50	8.70	(4.79)
Phen. + Tyr.[3]	**7.71**	8.60	(7.04)	7.57	7.68	11.60	11.16	(6.06)
Threonine	**4.99**	5.60	(4.42)	(4.41)	(3.70)	(4.66)	(4.80)	(3.38)
Tryptophan	**0.82**	0.80	1.15[4]	1.30[4]	1.42[4]	1.28[4]	n/a	n/a
Valine	**4.61**	6.20	4.75	6.44	4.50	7.98	4.80	4.20
Arginine	**7.91**	(4.60)	(5.12)	(6.46)	(6.76)	(4.15)	(0.51)	(4.35)
Histidine	**1.82**	2.60	2.88	1.90	2.40	3.09	(1.30)	(1.16)
Meth. + Cys.	**3.44[5]**	2.40	4.63	3.32	2.80	3.71	2.31	8.14
Coeficient of variation of diet protein vs. *H.midae* EAA. r^2 value		0.63	0.73	0.75	0.92	0.52	0.33	0.73
% Deficiency of most limiting EAA		42	35	25	26	47	94	45

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1. The amino acid profile of *H. midae* was taken from Knauer *et al.* (1994a)
2. Amino acid profile of torula yeast supplied by C.G. Smith Pty. Ltd.
3. Tyrosine value (3.81% of protein) for *H. rufescens* from Allen and Kilgore (1975).
4. Tryptophan values from Appendix to Halver (1989).
5. Methionine = 2.09, Cysteic acid = 1.35 (Analysis performed by Dept. Animal Science, University of Natal Pietermaritzburg.

profile of *P. corallorhiza* appeared more balanced (r = 0.73), this diet produced the lowest SGR and PER suggesting that the digestibility and assimilation efficiency of this protein was relatively low.

While the absolute differences in final length and weight of abalone fed fishmeal based diets containing 27 to 47% protein in Experiment 2 were relatively small, there was a significant trend of increasing growth rate with increasing dietary protein level. The experimental period of 95 days was relatively short in comparison to the period required for commercial growout. Given a longer experimental period, the observed differences would probably magnify and be of economic significance.

The slopes of the trends of the nutritional indices (SGR, PER and FCR) in the present results are remarkably similar to those obtained by Uki *et al.* (1986b) who also documented an increase in abalone growth rate, and a decrease in FCR and PER, with an increasing level of fishmeal as dietary protein (Figure 2.2). The major difference between the two studies is that the growth rate and PER values are higher, and FCR lower in the present study, indicating that the LT fishmeal used was more digestible than the "white" fishmeal used by Uki *et al.* (1986b), and/or *H. midae* is better able to utilise fishmeal than *H. discus hannai.*

Despite the poorer efficiency of conversion of protein by abalone with an increasing level in the diet, abalone weight gain (see SGR, Figure 2.2) was 18% higher on the 47% protein diet in comparison to the 27% protein diet, and FCR also improved. The dietary protein level at which abalone growth peaks was not reached in the present study, nor in that of Uki *et al.* (1986b), who fed fishmeal based diets up to 43.1% protein. However, using casein as a protein source, Uki *et al.* (op cit.) determined that growth rate peaked at 46.5% dietary protein. It however appears that abalone growth rate is not very sensitive to the level of dietary protein above a level 25%. The marginally increased growth at higher levels of protein is probably due to an increased availability of limiting amino acids, however, the decreasing PER values indicate that an increasing proportion of the protein was deaminated and used to meet maintenance energy requirements.

Uki and Watanabe (1992) suggested that the optimal protein level for abalone lay between 20 and 30%. This suggestion is based on their use of an index of protein deposition efficiency, i.e. the product of feed conversion efficiency (weight gain/ feed consumed) and net protein utilisation (% net retention of dietary nitrogen). However, this index is not appropriate for commercial diet formulation because the rate of weight gain and cost of ingredients is not taken into account. In the context of aquaculture, rate of weight gain is vitally important, particularly in the case of haliotids, as they are relatively slow growing and the running costs of pumping to land based farms are very high. Therefore, from an aquaculture production point of view, an optimal dietary protein level should be defined in terms of growth rate, or rate of protein deposition, and dietary ingredient cost.

Although the present growth data, and those of Uki *et al.* (1986b), suggest that a dietary protein level higher than 30% may be required to achieve maximum growth rate (G_{max}; Brett, 1979), accurate definition of an optimal protein level requires more information about the energetic requirements of abalone, since consumption in monogastric animals is primarily a function of their requirement for energy (Smith, 1989). To this end, the determination of digestible energy values for all dietary ingredients is required, as well as the establishment of a digestible energy/ protein ratio which promotes G_{max} in abalone.

The attempt at determining the apparent dry matter digestibility of the experimental diets was not successful. It was suspected that the faeces may have been contaminated with pedal mucous and abalone shell fragments.

CONCLUSIONS

In view of the of the better growth rates and efficient conversion of protein observed in abalone fed the fishmeal based diets, it may be concluded that LT-fishmeal is the most promising primary protein source for inclusion in practical diets. *Spirulina* also produced high growth and conversion efficiencies, however, its use depends on *Spirulina* being commercially available and sold for approximately the same price as fishmeal. Casein is not a promising practical feed ingredient in South Africa due its relatively high cost, and the lower growth rates it produced in comparison to fishmeal. However, it is a useful experimental protein for nutritional studies because of its high biological value and wide use as a reference protein. Despite the poor growth rate obtained using soya oil cake and torula yeast as sole dietary protein ingredients, the relatively good FCR and PER values obtained in this study suggest that they could partially replace more expensive proteins, such as fishmeal, in practical diets.

When fishmeal was used as a protein source, abalone growth rate and feed conversion ratio improved between 27 and 47% dietary protein, while PER decreased. Accurate definition of an optimal dietary protein level must, however, await elucidation of an optimal digestible energy/ protein ratio (see Chapter 6).

CHAPTER 3.

DYNAMICS OF DIGESTION AND ENZYME ACTIVITY IN *HALIOTIS MIDAE* FED A DRY FORMULATED DIET.

INTRODUCTION

To optimise economic efficiency, aquaculture strives to maximise feed consumption, feed conversion efficiency and growth rate. To achieve these goals, an understanding of the factors influencing the rate at which the cultured organism digests its feed is vital. Such information can facilitate definition of an optimal ration size and feeding frequency, and may also influence the diet formulation and manufacture processes. Because feed is the single biggest operating cost in intensive aquaculture, extensive work on factors influencing rates of digestion has been performed on economically important fish species (Smith, 1989). However, due to the relative novelty of abalone aquaculture, very little work of this nature has been undertaken on abalone. Nonetheless, the principles of applied nutrition established for fish appear applicable to abalone, as both groups of animals are poikilotherms with analogous digestive structures. Feeding frequency and diet quality have been shown to influence the rate at which abalone digest algae (Foale and Day, 1992; Day and Cook, 1995) but no work has been published on the rates of digestion of artificial diets, despite the importance of such information to the development of cost effective feeds.

The efficient utilisation of artificial diets by abalone can in part be explained by the organisation of the haliotid digestive system which in key respects is similar to that of other farmed monogastric animals. In contrast to more primitive microphageous molluscs, for example *Patella*, which rely on intracellular digestion (Purchon, 1977), haliotids rely primarily on extracelluar digestion (McLean, 1970). This has facilitated feeding on a greater variety of substrates, as larger masses of food, ingested less frequently can be processed. Digestive enzyme efficiency is increased due to the churning of the food with digestive enzymes (Purchon, 1977). Although the moisture and nutrient concentrations of dry pelleted feeds differ markedly from seaweed, the above adaptations appear to facilitate their efficient digestion.

The bulk of algal biomass, excluding water, consists of structural and reserve carbohydrates, and thus abalone consuming seaweed derive most their energy from carbohydrate. Most work on abalone digestive enzymes has therefore focused on the carbohydrases and a wide variety, hydrolysing both structural and reserve carbohydrates, have been characterised (Oshima, 1931; Tanaka *et al.*, 1968; Bennett *et al.*, 1971; Clark and Jowett, 1978; Elyakova *et al.*, 1981; Vonk and Western, 1984; Nakagawa and Nagayama, 1988; Yamaguchi *et al.*, 1989). Very little work has however been undertaken on proteases despite evidence that abalone can efficiently digest and assimilate high levels of dietary protein (Uki and Watanabe, 1992). Oshima (1931) observed weak protease activity in *H. gigantea*, while McLean (1970) described strong proteolytic activity in the crop and midgut gland of *H. rufescens*. Proteolytic activity has also been detected in the digestive tract of *H. discus hannai* (Cho *et al.*, 1983) and in the midgut gland of *H. discus* (Yamaguchi, *et al.*, 1989). Lipase activity has been detected in the digestive juices of the stomach and crop of *H. rufescens* (Albrecht, 1921; McLean, 1970) and in *H. midae* (Knauer *et al.*, 1996).

The work by Knauer *et al.* (1996) showed that protease activity was significantly higher in abalone fed on a formulated feed (35% protein) than on a diatom diet (5% protein), illustrating that *H. midae* is able to adapt to a diet containing much higher levels of protein than naturally encountered. Despite the high level (45-50%) of carbohydrate in the formulated diet used by these authors, significantly higher amylase activity was observed in abalone fed on the diatom diet, perhaps reflecting a greater reliance on carbohydrate as an energy source in low protein diets (Knauer *et al.*, 1996).

The aim of the present study was to quantify the rate at which a dry artificial feed was digested by *H. midae* and to relate this to the dynamics of digestive enzyme activity.

MATERIALS AND METHODS

Experimental animals and system

Fifty hatchery spawned juvenile abalone (46 ± 4 mm shell length (SL), 14 ± 3 g) which had been reared on a dry artificial feed in our Port Alfred laboratory were used in the present study. The animals were held in two tanks in the system described in Chapter 2. The water temperature was maintained at 20°C.

Experimental protocol

The same feed the abalone had been reared on was used in the present study. The extruded dry pellets were starch bound and contained fishmeal as protein. The proximate composition of the pellet was: 34.6% protein, 5.3% fat, 10% moisture, and 44.4% carbohydrate.

The abalone were not fed for three nights prior to the experiment to ensure their guts would be empty. Feed pellets were placed in their rearing container on the fourth evening at 18h00, the beginning of the dark period (0h). A sample of five animals was immediately frozen whole, and further samples of five animals frozen at six hourly intervals over the following 46h. After the abalone had been allowed to feed through the first evening (0-12 h) the holding tanks were siphoned clean to remove the remaining feed.

Processing of gut contents.

Abalone were subsequently defrosted and held on ice while they were prepared for the gut content and digestive enzyme analyses. Following recording of individual length and weight, the shell of each of the abalone was removed, an incision made in the posterior wall of the crop and stomach, and the contents (hereafter referred to as gut contents) scraped into a glass vial. Notes were taken on the colour and texture of the gut contents. The weight of the gut contents collected was recorded and each sample homogenised for 3 min. using an Ika Ultra Turrax T25 homogeniser in three ml of a 0.1 M citric acid - 0.2 M phosphate acid buffer (pH 5.2). This buffer was used as its pH corresponds to the gut of *H. midae* (pers. obs.). A further 2 ml of buffer was used to rinse the residue from the homogeniser into the sample. Samples were then centrifuged at 18,000g for 45m at 4°C, 4 ml of the supernatant pipetted off, diluted to 20ml with buffer and frozen for subsequent enzyme analysis. The solid centrifuged fraction was weighed.

Amylase activity

The amylase assay was based on modified versions of the Bernfield method (Plummer, 1978; Knauer *et al.*, 1996). One ml of a 1% starch solution was added to one ml of sample solution and incubated in a water bath for 10 min. One ml of the colour reagent 3,5-dinitrosalicyclic acid was added. The resulting mixture was heated in boiling water for 5 min, then cooled down to 20° in a waterbath. After 10 min the absorbance was read on a spectrophotometer at 540 nm. Blanks for each sample were prepared by boiling 1ml of sample solution for 10 min and then following the same procedure.

A standard curve was prepared using maltose in the range 0.01 - 0.10 mg maltose/ ml. Amylase activity was defined as mg maltose liberated from starch in 10 min at 20°C. Amylase concentration was defined as amylase activity per g gut content and total amylase activity was expressed as amylase activity of whole gut contents per gram body weight.

Protease activity

The method employed was based on modified versions of the Anson method (Walter, 1984; Knauer *et al.*, 1996). Of each sample, 0.5 ml were added to 2.5 ml of haemoglobin substrate. Blanks for each sample were processed simultaneously using 0.05 M HCl. The mixtures were incubated for 10 min in a waterbath at 20°C and the reactions stopped by addition of 5.0 ml of 0.3 M trichloroacetic acid. Thereafter, 0.5 ml of sample was added to the blanks and 0.5 ml of 0.05 M HCl to the samples. After five minutes at room temperature, each test tube was centrifuged at 3000g for 20 min. Finally, 2.5 ml of the supernatant, 5.0 ml of 0.5 N NaOH and 1.5 ml of Folins reagent were mixed and allowed to stand for 15 min at room temperature. The absorbance of each sample against its blank was read on a spectrophotometer at 578 nm. A standard curve was prepared in the concentration range 0.01- 0.10 mg tyrosine/ml. Protease activity was defined as mg tyrosine liberated from haemoglobin in 10 min at 20°C. Protease concentration was defined as protease activity per g gut content and total protease activity was defined as protease activity of the entire gut contents per gram body weight.

Lipase activity

The activity of lipase was measured using a modification of the method employed by Tietz and Fiereck (1966) and Borlongan (1990). One ml of sample, 1.5 ml of SIGMA lipase substrate and 1.5 ml of the citric acid-phosphate buffer were mixed and incubated for 6h in a waterbath at 20°C. Blanks containing 1 ml of sample were boiled for 10 min and processed simultaneously with the active samples. The reaction was stopped by addition of 3.0 ml of 95% ethanol. The mixture was then transferred to a 50 ml beaker and the test tubes used washed with a further 3.0 ml of 95% ethanol, which was added to the beakers. The activity of lipase was determined via electrometric endpoint determination, using a 0.05 N solution, Each sample and blank were titrated to pH 10.5 and the amount of NaOH needed for the blank was subtracted from the amount required for the sample. Lipase activity was defined as ml of 0.05 NaOH required to titrate a sample to pH 10.5 after 6h at 20°C. Lipase concentration was defined as lipase activity per g gut content and total lipase activity was expressed as lipase activity of the entire gut contents per gram body weight.

Statistical analysis

Gut content mass and enzyme activity for protease, amylase and lipase were analysed as functions of time using one way analysis of variance. Tukey's multiple range test was used to discriminate significant differences with a 95% confidence interval.

RESULTS

Gut content analysis

The change in the mass of the gut contents (solid plus liquid fraction) over a 46h period following feeding is illustrated in Fig. 3.1. The stomach and crop of abalone at the beginning (0h) of the experiment (18h00, day 1) contained a small volume of material with no recognisable food particles. After 6h (24h00, day 1) the gut content of the emergent abalone had increased significantly ($p<0.05$) to a mean of 6% of body weight. Fresh grains of food were clearly identifiable in the crop and stomach contents, which had a light brown colour. Although a further 6h of darkness were available for feeding (24h00, day 1 - 06h00, day 2), the volume of the gut content began to decline after the 6h peak, a trend which continued over the following 18h. After 24h (18h00, day 2) gut content mass had decreased significantly from the peak observed at 6h, and had a darker, more runny appearance with food granules no longer discernable. By 38h the confidence interval for the mean was similar to the prefeeding mean (0h). The mean mass of the solid fraction centrifuged out of the gut contents followed a similar trend, increasing to a peak after 6h, and then decreasing (Figure 3.1). After 24h it was not significantly different to the 0h prefeeding mean ($p<0.05$). Although the holding tank was siphoned clean after 12h, and abalone were not fed on the second evening, the solid fraction of the gut contents increased significantly between 24h (06h00, day 2) and 30h (24h00, day 2) suggesting that the animals may have ingested abalone faecal matter and mucous. The total gut volume did not however increase significantly over the same interval.

Enzyme activity

During the nocturnal feeding period (0 - 12h), total protease activity in the gut content increased significantly from the prefeeding level (0h) and peaked at 12h (06h00) (Fig. 3.2a). A decline in activity was observed over the next 12h and after 24h activity was down to the prefeeding level. Protease concentration in the gut content followed a similar trend although peak concentration occurred slightly later at 18h before decreasing sharply to prefeeding levels at 24h (Fig. 3.2b).

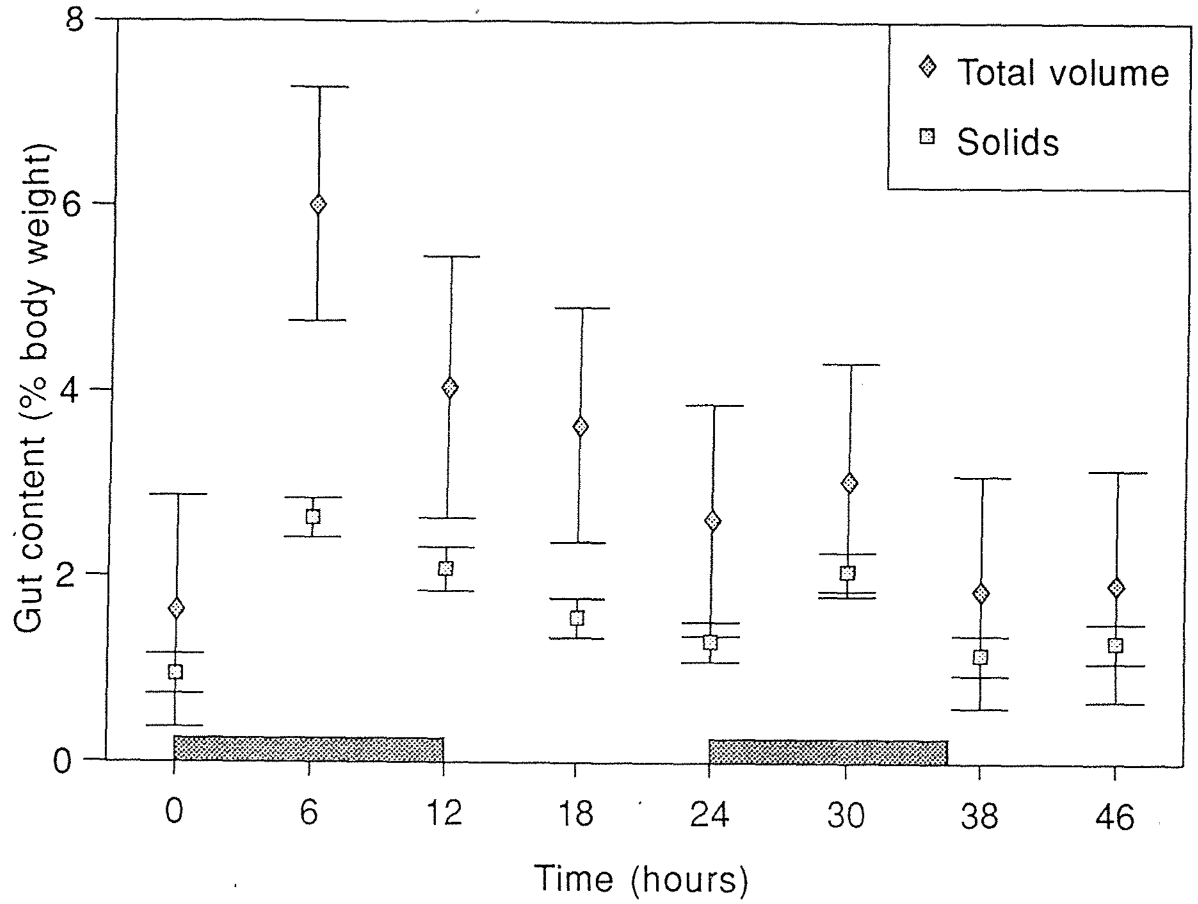


Figure 3.1. Gut content mass of abalone before feeding (0h) and following feeding on an artificial diet. Dark periods are represented by dark bars on the X-axis. The proportion of solids in the gut contents is represented as the percentage mass of the solid fraction centrifuged out of the gut contents. Error bars represent Tukeys 95% confidence intervals. Significant differences between means are indicated by non-overlapping bars.

Total amylase activity and concentration followed a similar pattern, however the increase in activity following ingestion was not as marked as that of protease, and the differences in mean activity were not significant due to the wide confidence intervals (Fig. 3.3a,b). Following peak activity, the decline in amylase activity was more gradual than that of protease, with activity returning to the prefeeding levels 38h - 46h after the commencement of feeding.

Lipase activity was barely detectable over the 46h sampling period, despite the long (6h) incubation period used for the assay. Both lipase concentration and total activity showed no significant trend over the experimental period (Figure 3.4 a,b).

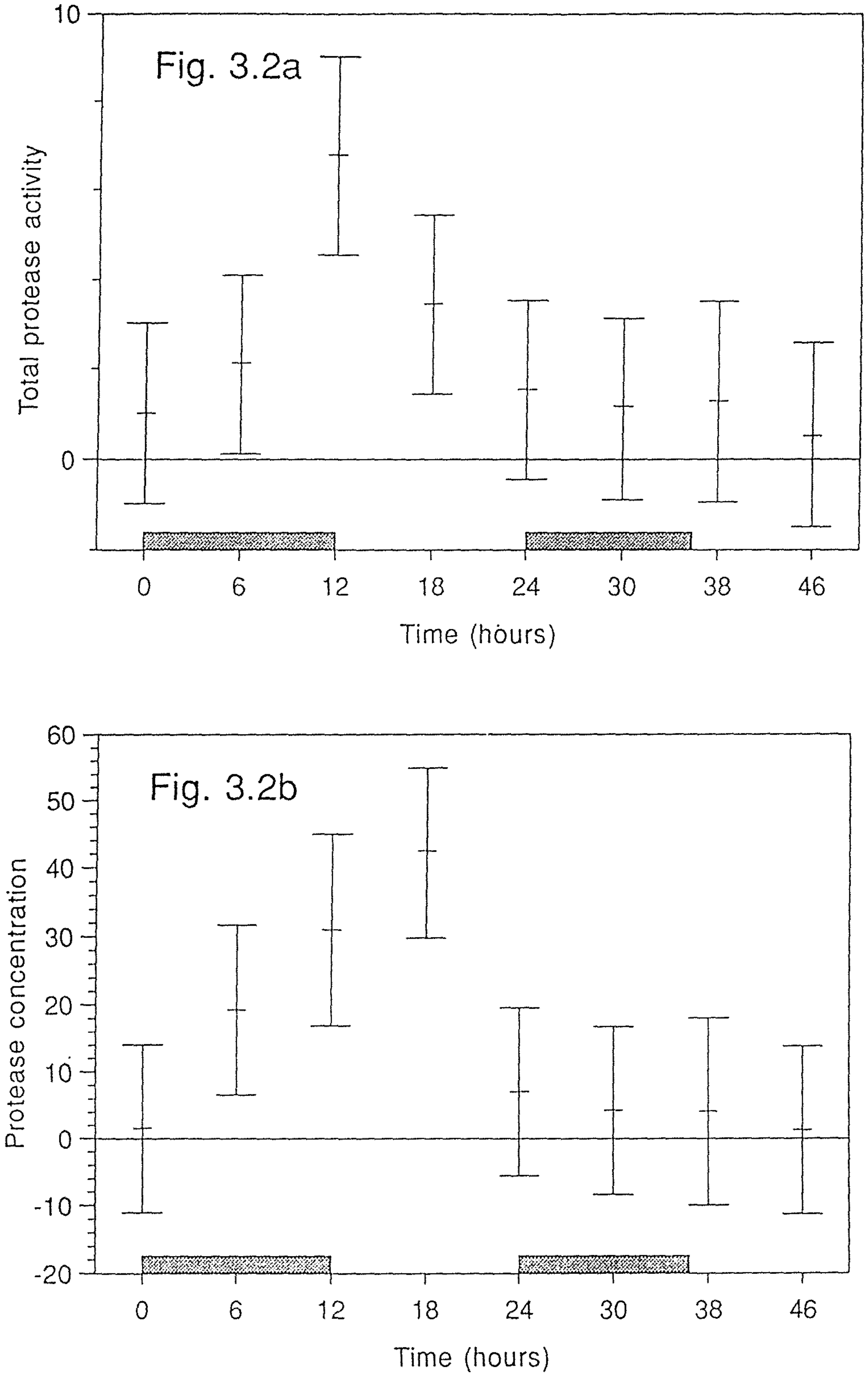


Figure 3.2. Protease activity in the total gut volume (mg tyrosine/ g body weight, Fig. 3.2a) and protease concentration (mg tyrosine/ g gut contents, Fig. 3.2b). Protease activity was defined as mg tyrosine liberated from haemoglobin in 10 min. at 20°C. Error bars represent Tukeys 95% confidence intervals. Significant differences between means are indicated by non-overlapping bars.

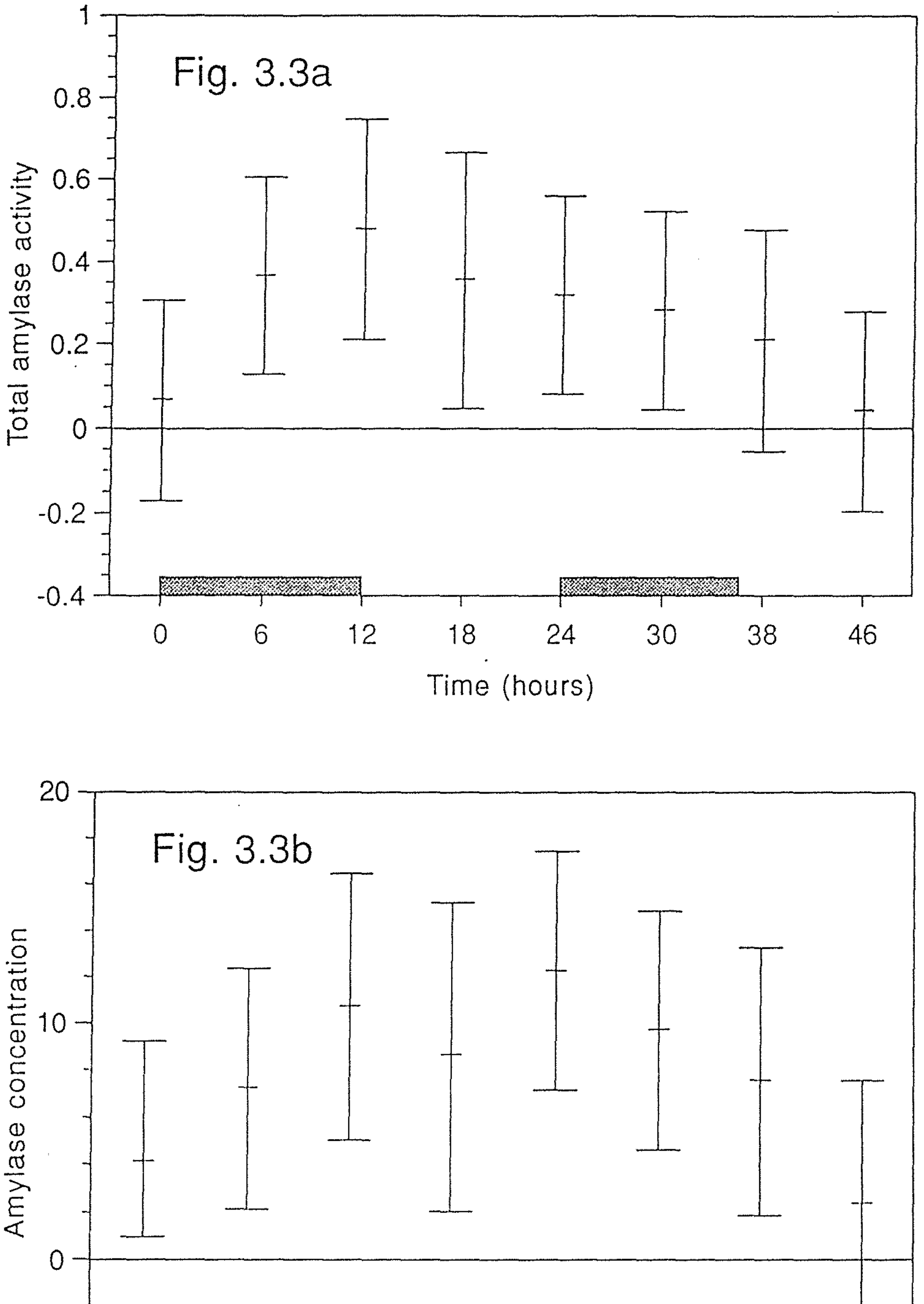
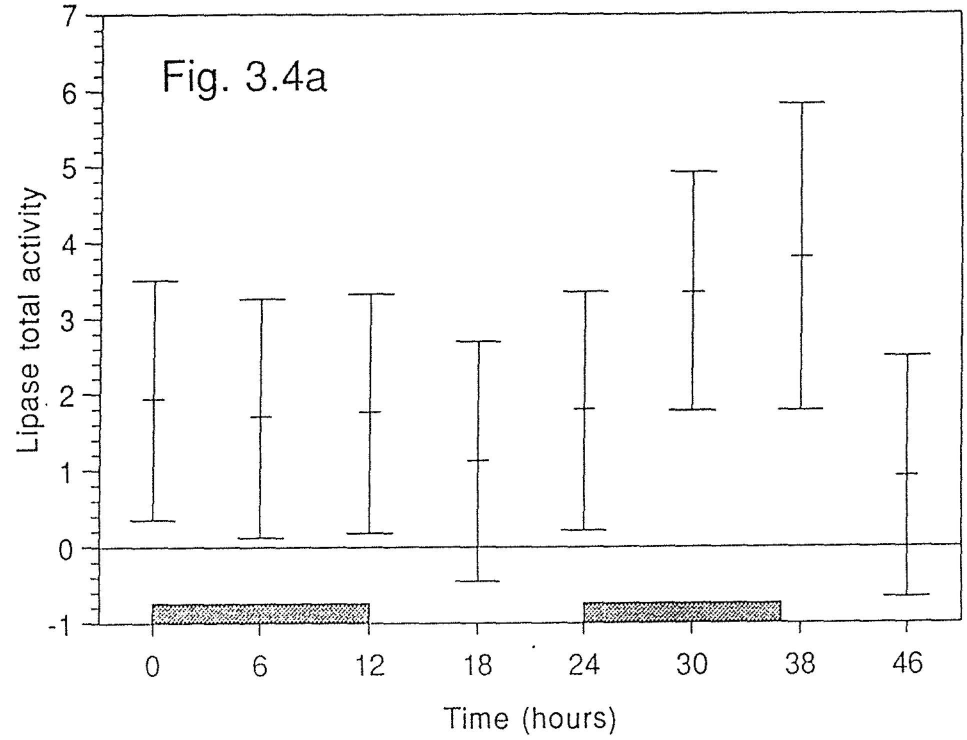


Figure 3.3. Amylase activity in the total gut volume (mg maltose / g body weight, Fig. 3.3a) and amylase concentration (mg maltose/ g gut contents, Fig. 3.3b). Amylase activity was defined as mg maltose liberated from starch in 10 min. at 20°C. Error bars represent Tukeys 95% confidence intervals. Significant differences between means are indicated by non-overlapping bars.



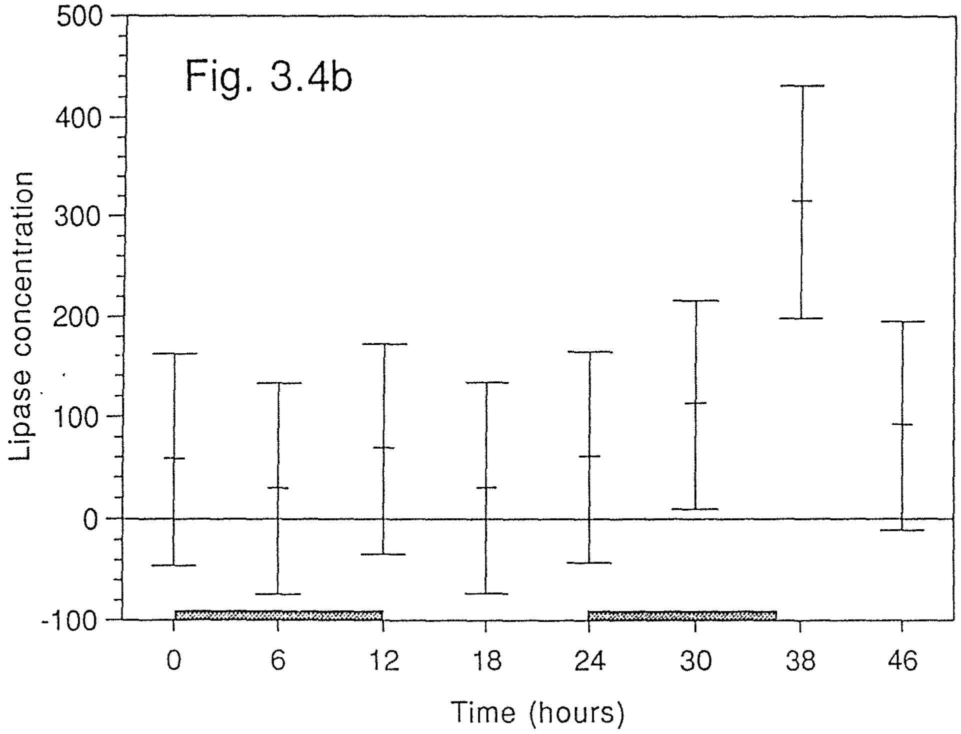


Figure 3.4. Lipase activity expressed as activity in the total gut volume (ml NaOH/ g body weight, Fig. 3.4a) and as lipase concentration (ml NaOh/ g gut contents; Fig 3.4b). Lipase activity was defined as ml NaOH required to titrate a sample to pH 10.5 after 6h exposure of the substrate to abalone gut contents.

DISCUSSION

In the present study, enzyme secretion and digestion in *H. midae* was found to be relatively rapid with the bulk of feed consumed being processed within 24h. This finding is consistent with observations on *Haliotis* feeding behaviour and digestion by other authors.

Studies on feeding behaviour of cultured abalone indicate that most (60-80%), but not all, abalone emerge and feed every night on both natural (Uki, 1981) and artificial feed (Knauer *et al.*, 1996). The present study reflected this trend as observations at the 6h sampling revealed that most abalone were emergent, but that some individuals were stationary inside their shelters. The peak in gut fullness observed at 24h00 is consistent with the findings of Uki (1981), who established that virtually all feed consumed by cultured *H. discus hannai* was ingested between 06h00 and 24h00, and Knauer *et al.* (1995a) who observed that feeding activity in *H. midae* declined after 24h00. The noticeable decline in gut fullness in the second half of the dark period can thus be ascribed to a decline in feeding activity combined with the processes of digestion and absorption.

Rapid digestion is facilitated during ingestion by the radula, which rasps food into small particles thus exposing a large surface area to enzyme activity and promoting the release of soluble nutrients (McLean, 1970). Investigations into the absorption of nutrients at different sites in the digestive tract of *H. rufescens* suggest that absorption begins almost immediately following ingestion (McLean, 1970). For example, in animals fed ^{14}C labelled *Ulva* McLean (1970) detected radioactivity in their blood 15 minutes after feeding and autoradiography revealed absorption on the apical surfaces of the absorptive cells in the midgut gland after 25 minutes. Foale and Day (1992) observed that digestion of preferred algal species in *H. rubra* was rapid, and fragments of a number of preferred algae were not recognisable in the crop and stomach contents after 24h.

Feed quality has however been shown to affect digestion in abalone (Foale and Day, 1992; Day and Cook, 1995) as well as certain fish (Smith, 1989). It has been observed that less preferred algal species are digested more slowly and remain recognisable in the crop for as long as 48h (Foale and Day, 1992; Day and Cook, 1995). From the present results it can thus be concluded that the rate of digestion of the artificial feed was comparable to that of preferred seaweeds.

The effects of feeding frequency and starvation on gastric evacuation time is well documented in fish (Smith, 1989), and some evidence exists which suggests digestion is also more rapid in abalone that are fed more frequently. Foale and Day (1992) observed that the rate of seaweed digestion in *H. rubra* did not differ significantly between animals fed daily and those starved for seven days, however, abalone starved for one month displayed a significantly slower rate of digestion. It can therefore be assumed, with reasonable certainty, that the three day starvation period prior to the present trial probably did not affected the rate of digestion.

The increase in protease and amylase concentrations 6h after feed was offered suggest that secretion of these enzymes is relatively rapid following ingestion. Total protease and amylase activity in the crop and stomach peaked 6 h after peak gut fullness was observed, indicating that enzyme secretion carries on for some time following ingestion. Peak enzyme concentration per gram gut content occurred slightly later at 12h after peak gut fullness, suggesting that the enzymes became more concentrated as digested feed was absorbed from the crop and stomach. The pattern observed is similar to that of fishes, for example, peak protease activity following feeding was observed at 5 h in carp *Cyprinus carpio* (Onishi *et al.*, 1973), 2.5-4 h in African catfish *Clarias gariepinus* (Uys *et al.*, 1987) and 12 h in eel *Anguilla japonica* (Takii *et al.*, 1985).

The increase in protease activity following ingestion was much more marked than amylase activity, with the mean protease activity per gram gut content increasing 20 fold from prefeeding levels. By contrast, mean amylase activity only increased 2.5 fold following feeding and the difference was not statistically significant suggesting that abalone maintain a more or less constant amylase concentration. Interestingly, similar patterns of protease and amylase secretion have been observed in *C. gariepinus* (Uys *et al.*, 1987) and carp (Onishi *et al.*, 1973).

The very low lipase activity observed showed no discernable trends which could be related to the food consumed. This result corroborates the finding of Knauer *et al.* (1996) who also observed low lipase activity in two groups juvenile *H. midae* fed on diatoms and an artificial feed respectively. Low lipase activity is consistent with the low (generally <2%) level of lipid in the natural diet of abalone and the fact that reserve energy in haliotid tissue is stored in the form of carbohydrate (Webber, 1970) and not lipid. The results seen together with the finding that high levels of dietary lipid (>10%) depress growth in abalone (Uki and Watanabe, 1992; Mai *et al.*, 1995; present study, chapter 6) suggest that the ability of abalone to digest lipid is limited.

The present results indicate that enzyme activity cycles of abalone, a drift algal feeder which does not have constant access to feed, are induced solely by feed intake and do not follow a daily rhythm.

CONCLUSION

H. midae processed a meal of artificial feed within 24h at 20°C. The cycle of enzyme activity appeared to be induced by feed consumption. A marked increase in protease activity occured following feeding, however, amylase activity appeared to be maintained at a fairly constant level regardless of gut fullness. *H. midae* appears to possess a low level of lipase activity which is consistent with its carbohydrate based metabolism and the low lipid content of its diet.

CHAPTER 4

EFFECT OF TEMPERATURE ON GROWTH, CONSUMPTION AND NUTRITIONAL INDICES OF *HALIOTIS MIDAE* FED A FORMULATED DIET

INTRODUCTION

Temperature is the most significant environmental controlling factor (*sensu* Fry, 1971) determining the metabolic rate of poikilotherms. An understanding of its effects on the physiology of abalone is of fundamental importance to the management of both fisheries and aquaculture, as environmental temperature directly determines rates of gonadal recrudescence (Uki and Kikuchi, 1984; Hahn, 1989b), larval development (Hahn, 1989c), feed consumption (Uki, 1981; Hahn, 1989e; Peck, 1989), ammonia excretion (Barkai and Griffiths, 1987; Lyon, 1995), oxygen consumption (Uki and Kikuchi, 1975; Barkai and Griffiths, 1987; Lyon, 1995), growth rate (Leighton, 1974; Hahn, 1989e; Peck, 1989) and survival (Hahn, 1989d). From an aquaculture perspective such knowledge facilitates farm siting in thermally optimal areas (Hecht, 1994), the calculation of daily feed rations and the development of other temperature based farm management protocols, and as such, is integral to the development of economically viable technology for the culture of *Haliotis midae*.

H. midae's distribution along the South African coast spans the confluence of the Atlantic and Indian Oceans, ranging from the cold waters of the Benguela upwelling system on the Western Cape coast, to the warmer Eastern Cape coast, which are influenced by the South flowing Agulhas current (Fig. 4.1). Sea temperature records reveal mean monthly temperatures

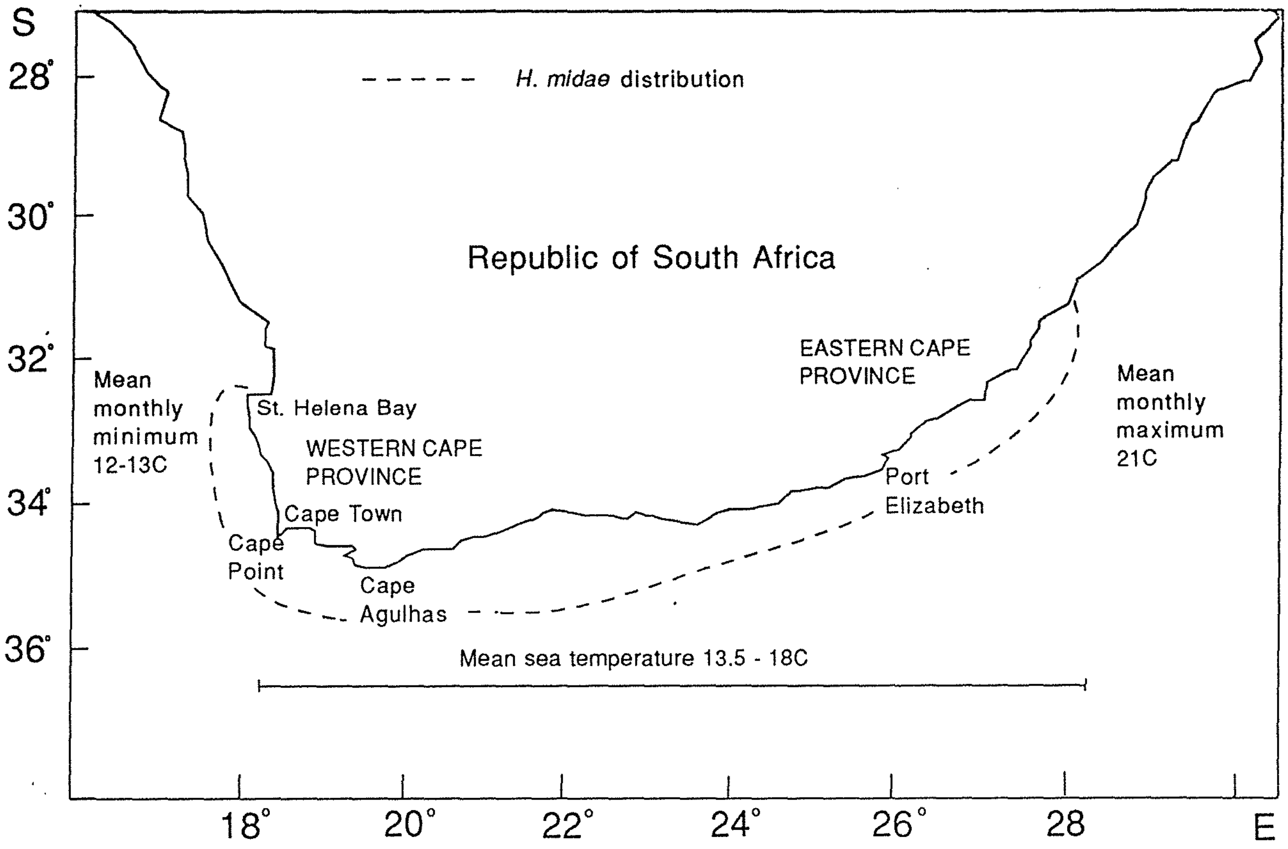


Figure 4.1. Sea surface temperatures across the natural range of *H. midae*.

ranging from a minimum of 12-13°C in the West, to 21-22°C in the east (Greenwood and Taunton-Clark, 1994; Schumann *et al.*, 1995). Newman's (1968, 1969) pioneering work on the biology of *H. midae* first highlighted the influence that temperature exerts on the biology and population structure of natural stocks. An inverse relationship between maximum size and temperature was demonstrated (Newman, 1969), and Eastern Cape populations mature at a smaller size than those in the Western Cape (Newman, 1969; Tarr, 1995; Wood, 1993). In the colder waters west of Cape Point, abalone production decreases (Newman, 1969; Tarr, 1991) and recruitment becomes sporadic (Tarr, 1991). On the basis of growth measurements made at Port Elizabeth (19°E) and Stony Point (26°E), Newman (1969) concluded that abalone growth was faster in the warmer Eastern Cape waters. The validity of this conclusion has however been questioned by Tarr (1995), who gathered more comprehensive age and growth data from a number of populations spanning the distribution of *H. midae,* and found wide variability, but no significant differences in the growth rates of populations in the eastern and western regions of its distribution. The wide variation the growth rate, size at sexual maturity, and maximum size across the natural range of *H. midae*, has profound implications for aquaculture, particularly farm siting, and highlights the need for a better understanding of the effect of temperature on its physiology.

In any form of intensive aquaculture based on the use of complete formulated diets, feed makes up a major portion of the variable costs. Accurate calculation of daily rations is therefore vital to avoid overfeeding, which results in wasted feed and poor water quality, and underfeeding, which results in reduced growth rates. Consumption in most cultured poikilotherms, including abalone, is determined by temperature and body size (Uki, 1981; Hahn, 1989e; Smith, 1989). For example, it has been shown that consumption of artificial feed by *H. discus hannai* ranges from 0.8 - 7.2% of body weight/ day depending on size and temperature (Nihon Nosan Kogyo K.K. Research Centre, cited in Hahn, 1989e). The aim of the present study was to quantify the effect of temperature on feed consumption, growth and nutritional indices in *H. midae* fed a fishmeal based, extruded dry feed, with a view to determining the optimal temperature range for growth and developing a protocol for calculating daily feed requirements.

MATERIALS AND METHODS

Effect of temperature on growth and nutritional indices

Growth rate and feed consumption of early juvenile *H. midae* was determined at temperatures ranging from 12 - 24°C over a three month period.

Young abalone (17.5 ± 1.25 mm, 1.1 ± 0.25g) from a single spawning were obtained from the Sea Plant Products hatchery in Hermanus and stocked at a rate of 30 per tank in self contained recirculating glass aquaria in our Grahamstown laboratory. The water from each tank (70 l) overflowed into a biological filter compartment (36 l) and was pumped back into the tank by means of an airlift. Each tank was siphoned clean once a week and two thirds of the water replaced with fresh, sand filtered, sea water (35ppt) obtained from Port Alfred. The set temperatures for the experimental treatments were 12, 14, 16, 18, 20, 22 and 24°C. The temperature of the laboratory was maintained at 14°C by means of an air conditioner and the water in each tank heated to the predetermined temperature by means of thermostatically controlled aquarium heaters. The temperature in the 12°C treatment was achieved by means of a laboratory chiller unit with a cooling element placed in the filter compartment of the tank. With the exception of the 12°C treatment, two replicates were used for each temperature. Two shelters in the form of longitudinally halved grey PVC piping (80 mm diameter, 20 cm lengths) were provided in each tank.

The abalone were fed extruded dry pellets (Table 4.1) at a rate of approximately 4% of body weight per day, which was well in excess of their requirements, thus ensuring that feed availability did not limit growth. On weekdays uneaten feed was removed from the tanks at 16h00 and replaced with fresh feed. On Fridays, a double ration of feed was placed in the tanks and no feed removed or offered on Saturdays and Sundays. Uneaten feed was oven dried at 60°C for 24h. Loss of solid matter through leaching was determined by placing five feed pellets (ca 1.4g) of feed in the filter compartment of each tank when abalone were fed, removing it after 24h, and drying and weighing the remainder. Consumption, feed conversion ratio, protein efficiency ratio and condition factor were calculated as defined in Chapter 2. Abalone in each tank were measured and weighed monthly in the manner described in Chapter 2.

Table 4.1. Formulation of the diet used to determine the effect of temperature on abalone growth rate and nutritional indices.

Ingredient	Percentage inclusion
Fishmeal	50.5
Starch	47.5
Chromic oxide	1.0
Mineral and vitamin mixture	1.0
	100.0

1. Vitamin A, 2400 IU; Vitamin D3, 400 IU; Vitamin E, 30 mg; Vitamin K3, 14 mg; Vitamin B1, 10 mg; Vitamin B2, 9.0 mg; Vitamin B6, 14.0 mg; Vitamin B12, 0.008 mg; Nicotinic acid, 40 mg; Pantothenic acid 30 mg; Folic acid, 2.4 mg; Biotin, 0.2 mg; Vitamin C, 60 mg; Inositol, 60 mg; Manganese, 10.0 mg; Copper, 0.4 mg; Iron, 4.0 mg; Zinc, 8.0 mg; Selenium, 0.05 mg; Iodine, 0.2 mg; Cobalt, 0.05 mg.

At the conclusion of the three month growth trial, faeces were collected daily from the tanks for a further month in an attempt to estimate the digestibility of the feed at the various experimental temperatures. Faecal collectors were made from rectangular white PVC containers (2 l volume), which contained a 100µm nylon mesh drain (2 cm wide) across the width of one end of the container floor. Every day the two shelters containing the abalone from each tank were placed inside a faecal collector, which was positioned on the bottom of the experimental tank. The collectors were left in the tanks from 09h00 to 16h00 and then gently lifted out the tanks allowing the water to drain through the mesh upon which the faeces were retained. Faeces were scraped off the mesh with a scalpel and oven dried at 60°C in 5ml glass vials. Once faecal collection was completed, the pooled samples from each tank were inspected under a dissecting microscope and any foreign matter (eg. fragments of abalone shell or sand particles) removed. The chrome content of the faecal samples was analysed according to the method of Bolin *et al.* (1952). The dry matter apparent digestibility coefficient (DMADC) for each diet was calculated according to the formula in Chapter 2.

Regressions of length and weight data for each tank over the experimental period were derived. Differences in the rates of length and weight gain were discerned by means of analysis of covariance ($p<0.05$). Replicate length and weight data were pooled if they did not differ significantly and single regressions for each treatment compared in the same way. Feed consumption, leaching rate, and abalone condition factor were compared between temperatures using one way analysis of variance and Tukey's range test ($p<0.05$).

The growth and FCR data were used to derive a model which predicts daily feed consumption as a function of abalone size up to 60mm shell length (SL) using equations modified from Haskell (1959):

$$1.\ C_t = FCR_t \times \%\ \text{weight gain/day}_t$$

$$2.\ \%\ \text{weight gain/day}_t = (2.99 \times dL_t \times 100)/L$$

where,

C_t = Predicted consumption at temperature (t).

FCR_t = Feed conversion ratio observed at temperature (t).

dL_t = Daily shell length increments observed at temperature (t).

L = Shell length for which consumption is predicted

Prediction of weight gain from length increment assumes an allometric relationship which was found to be valid for *H. midae*. Analysis of the length/ weight relationship of 3000 abalone ranging in size from 6 to 150 mm yielded a constant exponent of 2.99. Shell length increment (*d*L) was assumed to be linear up to 60 mm shell length and this is supported by age and growth studies (Newman, 1968; Wood, 1993; Tarr, 1995) on *H. midae*.

Effect of temperature and body size on feed consumption

The experiment was conducted in the partial recirculating system at our Port Alfred laboratory described in Chapter 2. Feed consumption of the extruded dry feed pellet (Table 4.1) was monitored in five size classes of abalone (Table 4.2) at temperatures ranging from 12°C to 24°C at 2°C intervals.

Table 4.2. Size classes of *H. midae* used to determine consumption of the dry formulated feed.

Weight (g)	Length (mm)
0.19 - 0.58	10 - 14.9
0.59 - 1.48	15 - 19.9
1.49 - 4.98	20 - 29.9
4.99 - 12.0	30 - 49.9
12.1 - 94.0	50 - 80

The three smaller size classes of abalone were hatchery reared animals obtained from the Sea Plant Products hatchery in Hermanus, while the larger abalone were collected from the wild at Cape Recife, Port Elizabeth. For each size class three replicates of 25 animals each were stocked per container in the manner described in Chapter 2. Any mortalities during the trial were replaced with individuals of equivalent length and weight from a holding tank in the same system.

Feed consumption was measured at each temperature over consecutive three week intervals per temperature beginning with 12°C. The experimental temperature was increased by 2°C after each run. Following adjustment of the system to the new temperature, a one week acclimation period was allowed followed by measurement of feed consumption for two weeks.

Before each run, all abalone were measured and weighed according to the protocol described in Chapter 2. Abalone which were found to have grown out of their allocated size classes during a run were replaced with smaller individuals in the subsequent run.

The five size classes were fed a fixed amount of feed daily which ranged from 5% to 9% of body weight from the largest to the smallest size class respectively. This was well in excess of their requirements to ensure that consumption was not limited by food availability. Feed was placed in the tanks every evening at 17h00 and removed the following day at 08h00. The remaining feed was placed in an oven and dried to a constant weight at 60 °C. An estimate of the proportion of solid matter lost due to leaching during immersion was obtained by placing feed in a control container with no abalone and following the same procedure as outlined above. Daily feed consumption for each replicate at each temperature was expressed as a percentage of body weight per day, corrected for leaching, according to the formula:

$$C \quad = 100((F)*L)-R)/W*T$$

Where,

C = Consumption (% body weight/day)

F = Dry weight of feed offered (g) over the experimental period

R = Dry weight of remaining feed (g) collected over the experimental period

L = Leaching factor

W = Weight of abalone at the beginning of the experimental period

T = Experimental period (days)

Regression analysis was used to derive equations describing observed feed consumption as a function of abalone size for each temperature. These curves were then plotted with 95%

confidence intervals and the curves for predicted feed consumption (derived using Haskell's equations) superimposed to test whether they fell within the confidence intervals of the observed data.

RESULTS

Effect of temperature on growth and nutritional indices

Observed rates of abalone weight gain increased significantly ($p<0.05$) with temperature to peak at 20°C, and then sharply declined above this temperature. At 24°C the animals barely gained any weight over the three month trial period (Fig. 4.2). The same trends were evident in the length increment and specific growth rate data (Fig. 4.3, Table 4.3). The rate of length increment was well described by means of a linear regression between 14°C and 20°C, however, this rate dropped off sharply above and below this range (Fig. 4.3).

Feed consumption mirrored growth rate, increasing significantly between 12°C and 20°C and then declining to 24°C. Feed conversion and protein efficiency ratios improved between 12°C and 20°C, however, above 20°C both indices reflected a dramatic deterioration. Abalone in the 22°C and 24°C treatments displayed visible symptoms of stress, for example, some individuals did not return to their shelters during daylight hours, became thinner and eventually died. This was reflected by the high observed mortality at these two temperatures (Table 4.3). Mortality was lower at 20°C and below and was primarily ascribed to "walkout" (abalone leaving the water during the dark period). With the exception of the 18°C treatment the condition factor of the experimental animals changed significantly over the experimental period from an initial value of 1.06 ± 0.02 ($P<0.05$). A trend of decreasing condition factor with rising temperature between 12°C and 24°C emerged by the end of the trial, with the abalone at 22°C and 24°C displaying the poorest condition (Table 4.3).

The extruded dry feed pellets used in this study displayed good 24h water stability maintaining a tough, rubbery texture. Loss of solids through leaching increased slightly with rising temperature and ranged from 5.7% at 12°C to 10.7% at 24°C (Table 4.3).

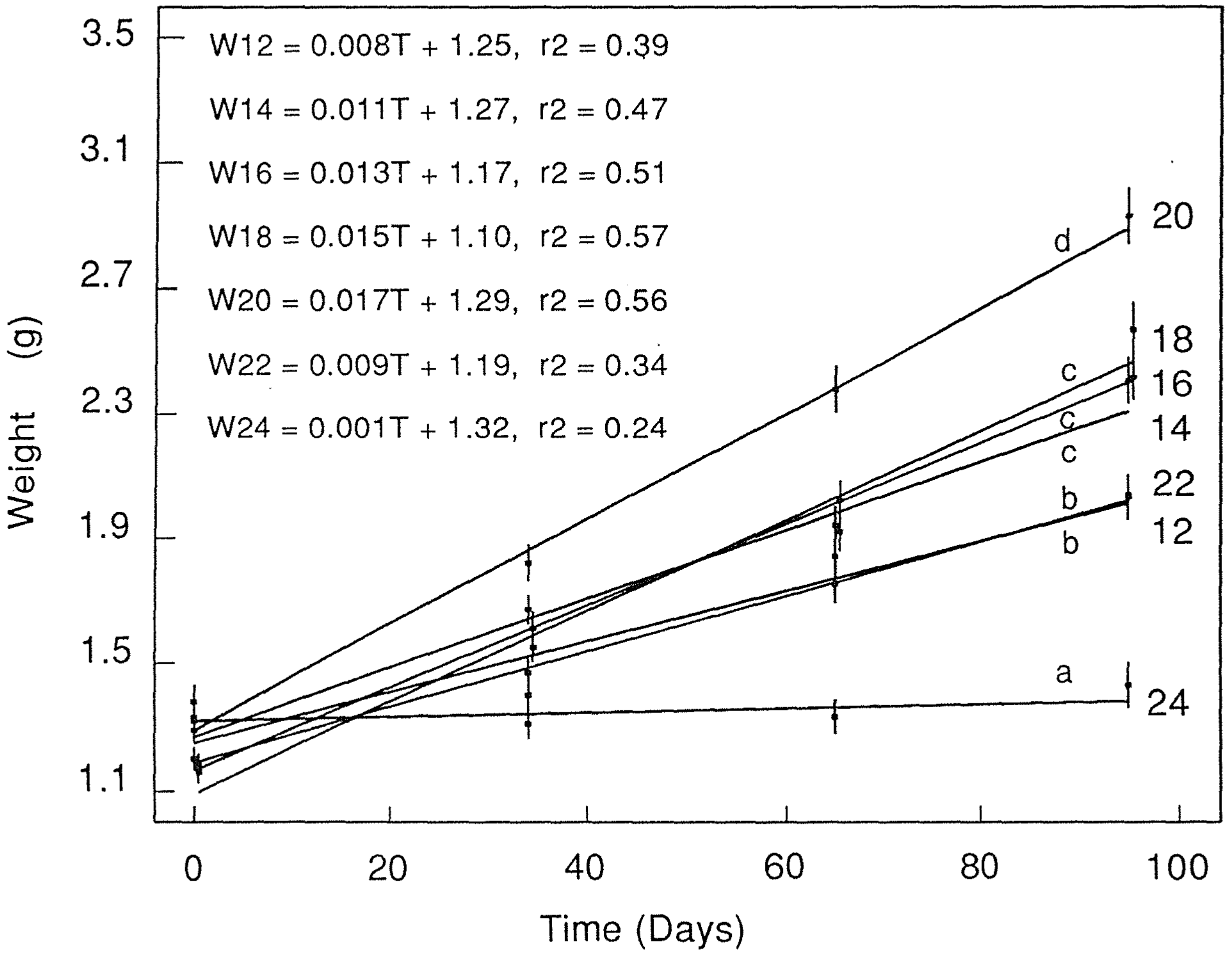


Fig. 4.2. Regressions of raw weight data of juvenile *H. midae* reared between 12°C and 24°C over a three month period. Significant differences ($p<0.05$) in slopes of regressions are indicated by different alphabetic characters. W = weight, T = temperature. Means and standard errors for each temperature are included.

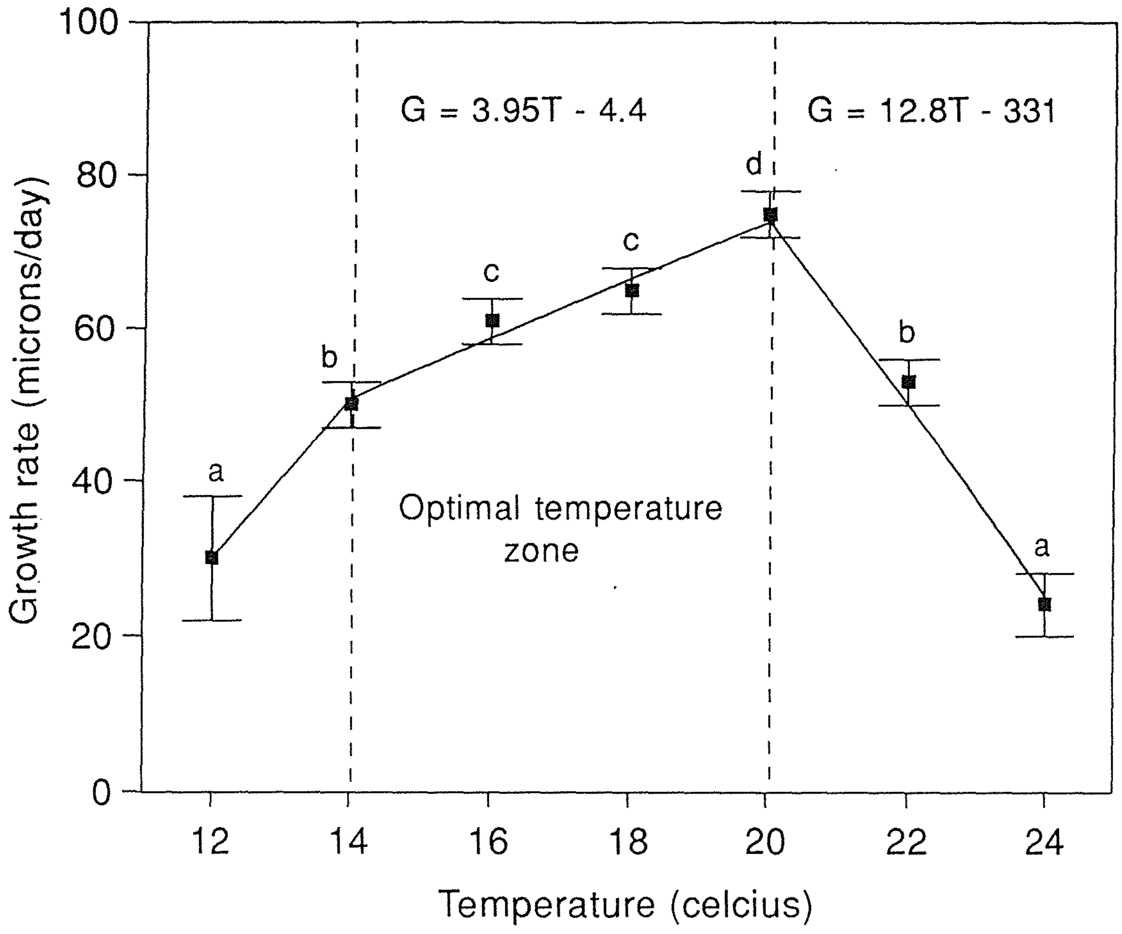


Fig. 4.3. Rate of shell length increment in *H. midae* reared at different temperatures. Significant differences ($p<0.05$) in slopes are indicated by different alphabetic characters. G = growth rate (μ/day), T = temperature.

The attempt to estimate apparent digestibility of the feed using a chromic oxide marker was unsuccessful as the percentage of Cr_2O_3 recovered in the faeces revealed no pattern, and was very variable, producing digestibility estimates ranging from 0 to 50%. Although faeces were examined for foreign matter under a dissecting microscope, it is nonetheless suspected that contamination with other matter, perhaps pedal mucous, caused the generally low and highly variable results.

Table 4.3. Nutritional indices for abalone reared on an extruded dry feed between 12°C and 24°C. SGR = specific growth rate; FCR = feed conversion ratio; PER = protein efficiency ratio. Consumption, condition factor and leaching values are means and standard errors, while other parameters are means only. Different alphabetic superscripts indicate significant differences between temperatures for the parameter concerned. Too few observations (2 replicates) were available to perform statistical analysis on SGR, FCR, PER and mortality.

	Temperature (°C)						
	12	14	16	18	20	22	24
Consumption g/abalone/day	15.7 ± 1.36^{a}	19.1 ± 1.21^{ab}	20.5 ± 1.24^{ab}	21.3 ± 1.26^{ab}	23.5 ± 1.32^{b}	21.7 ± 1.37^{ab}	16.2 ± 1.24^{a}
SGR % body wt.	0.62	0.66	0.76	0.83	0.91	0.55	0.05
FCR	1.37	1.29	1.12	1.15	0.97	1.82	3.02
PER	2.15	2.27	2.63	2.56	3.0	1.62	0.97
Condition factor	1.21 ± 0.02^{a}	1.11 ± 0.02^{b}	0.98 ± 0.03^{c}	1.08 ± 0.02^{b}	0.94 ± 0.01^{c}	0.91 ± 0.01^{d}	0.89 ± 0.03^{d}
Mortality (%)	3.3	4.4	4.4	2.2	1.1	12.2	43.3
24h Leaching (% recovered)	94.3 ± 0.9^{a}	92.9 ± 0.5^{a}	91.3 ± 0.5^{ab}	90.3 ± 0.6^{b}	89.4 ± 0.5^{b}	91.1 ± 0.6^{ab}	89.3 ± 0.6^{b}

Effect of temperature and body size on feed consumption

Observed feed consumption in all size classes increased significantly ($P<0.05$) with temperature to 20°C and then decreased between 20°C and 24°C. Observed consumption decreased significantly ($P<0.01$) at all temperatures in inverse proportion to abalone size (Fig. 4.4). The predicted curves for feed consumption as a function of shell length approximated the observed curves fairly well with most predicted values falling within the confidence intervals of the observed curves. Deviation between expected and observed values was greatest for the smaller abalone size classes (10-20mm SL). This was possibly due to experimental error in the measurement of consumption as the absolute amount of feed consumed in this size class was relatively small (0.07 - 0.41 g per tank/day) in comparison to the larger size classes (cf. 1.97 - 2.97 g per tank/day for the 50-80 mm size class).

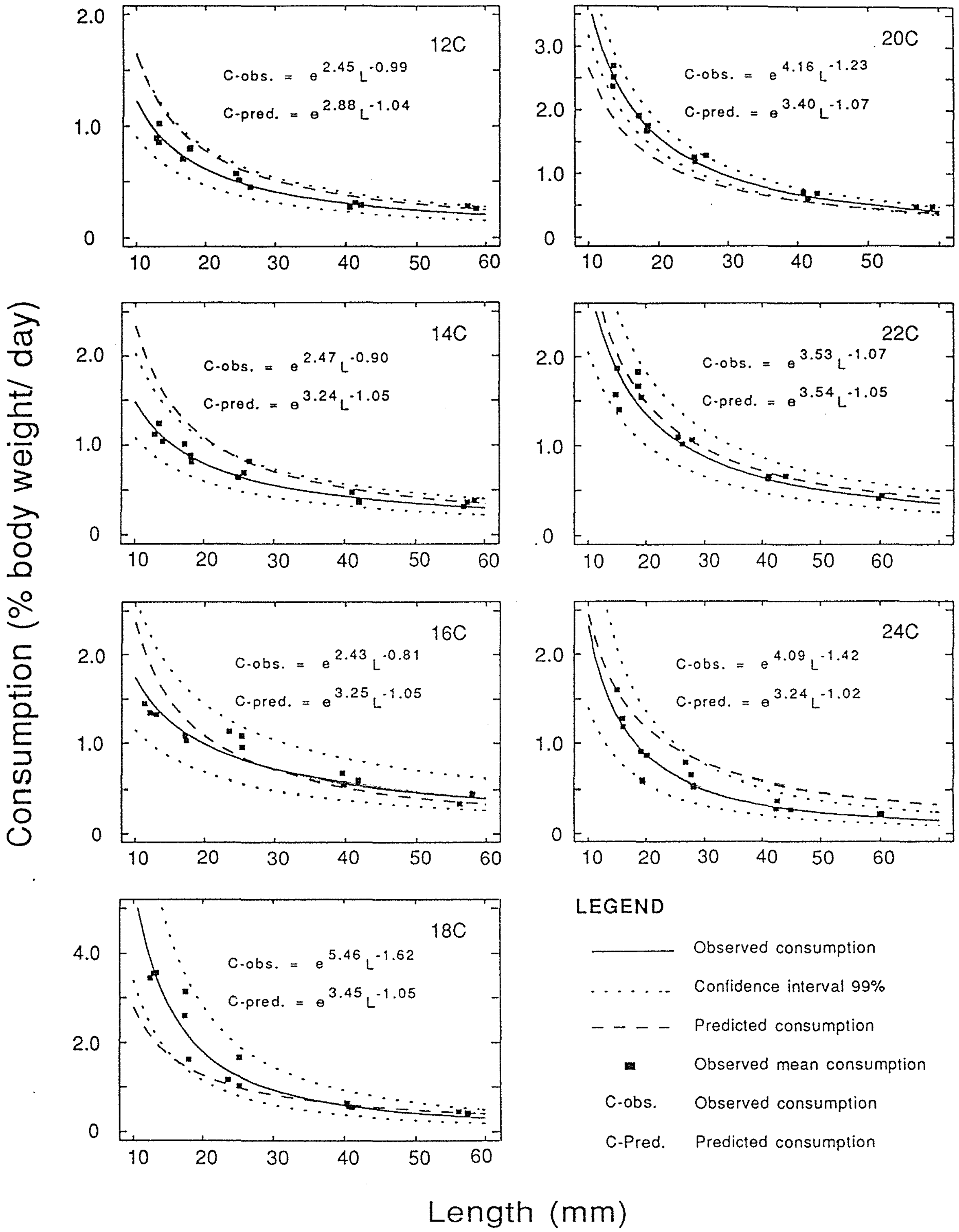


Fig. 4.4. Observed and predicted consumption of a dry formulated feed by *H. midae* at temperatures ranging from 12°C to 24°C. Predicted consumption was estimated using equations modified from Haskell (1959).

DISCUSSION

On the basis of the trends in the present growth rate, consumption, mortality, PER and FCR data it is suggested that a temperature range of 14-20°C is physiologically optimal for *Haliotis midae*. Although mortality was low at 12°C, the observed growth rate at this temperature fell below the linear trend observed between 14°C and 20°C. Above 20°C growth rates declined, feed conversion efficiency deteriorated and mortality increased suggesting a gradual breakdown in physiological processes. This hypothesis is supported by the data of Lyon (1995) who observed that ammonia excretion and oxygen consumption rates in *H. midae*, expressed per kg feed consumed, increased significantly above 20°C. In a practical farming context, it would be advisable to cease feeding when water temperatures exceed 22°C as growth above this temperature is minimal, and feeding may result in stress due to increased ammonia, and reduced oxygen levels.

The suggested optimal temperature range corresponds very well with the mean monthly minimum (13-14°C) and maximum (21°C) sea temperatures experienced across the natural distribution of *H. midae* (Fig. 4.4). Absolute sea temperature minima and maxima recorded within its range are 8°C under upwelling conditions on the west coast, and 25°C during summer on the East Cape coast. While *H. midae* is able to survive acute exposure to such extremes (Hecht, 1994), the present data indicate that they probably would not grow at these temperatures. It is suggested that temperature is the primary determinant of the natural distribution of *H. midae,* as suitable habitat and food appear to exist in adjacent areas outside of its natural distribution (pers. obs), but mean monthly minimum and maximum sea temperatures fall below 13°C and above 22°C to the west and east of its range respectively

(Greenwood and Taunton-Clark, 1994; Schumann *et al.*, 1995). *H. midae's* eastern distribution may also be limited by lethal temperatures, as *H. midae* begins to loose its footing at temperatures above 26.0°C (Hecht, 1994), and temperatures in excess of this occasionally occur to the east of its natural range (Schumann *et al.*, 1995). Furthermore, the sporadic recruitment observed (R. Tarr, Sea Fisheries Research Institute, Cape Town, pers. comm.) at the western extreme of *H. midae's* distribution is likely to be temperature related as abalone larvae have a narrower temperature tolerance than post-metamorphic animals (Leighton, 1974; C. Clayden, Sea Plant Products, pers. comm.), and thus successful settlement in thermally marginal areas is only likely to occur during occasional periods of favourable sea temperature. Leighton (1974) also noted a correspondence between the thermal tolerance range of larvae and the field distribution of *H. corrugata*, *H. fulgens*, and *H. rufescens*.

Tarr (1995) explains the similar growth rates observed in *H. midae* populations in the cooler western, and warmer eastern regions, by citing feed availability rather than temperature as the most significant factor determining growth. However, in an aquaculture context, where feed availability is not limiting, temperature is likely to be the primary factor determining growth rate. The present data show that the optimal temperature range for abalone culture is 18°C - 20°C which corresponds to summer sea temperatures in the Eastern Cape. However, because abalone farming is a shore based activity, and South Africa is subject to a high level of solar radiation, these mean water temperatures could be achieved on abalone farms in the Western Cape if water was retained ashore long enough to be heated by the sun. If the present growth data for 18°C - 20°C are extrapolated, it is predicted that cultured *H. midae* would take 3 - 3.5 years to reach a market size of 80mm or 100g using a formulated feed similar to that used in this study.

Maximum growth rate was observed at 20°C, which is somewhat lower than the 22.5°C predicted by Hecht (1994), who used the regression models developed by Jobling (1983) which estimate optimal temperature for growth from the final temperature preferendum and upper lethal temperature. It thus remains uncertain whether Jobling's (1983) models, which were developed using fish data, are applicable to other groups of cold blooded aquatic animals.

The observed trend of decreasing condition factor (CF) with increasing water temperature is interesting but cannot be entirely explained. Above 20°C the trend can reasonably be explained in terms of the decreasing digestive efficiency and increasing energetic requirement of *H. midae*. However, one can only speculate as to why the CF increased with decreasing temperature below this point. A possible reason may be an increasing partitioning of energy into glycogen reserves as temperature, and hence maintenance energy requirements, declined.

Although 24h solid leaching from the feed pellets used in this study increased significantly with temperature, the feed was highly water stable at all temperatures retaining a tough rubbery texture. This is an encouraging result as it indicates that *H. midae* can be cultured using extruded dry pellets over the entire temperature range experienced within its natural distribution.

The present results indicate that consumption of a dry formulated feed is a predictable function of temperature and body size. The equations derived by Haskell (1959), for predicting feed consumption in rainbow trout on the basis of length increment, provided a good approximation of observed feed consumption in *H. midae*. By combining Haskell's equations (equation's 1 and 2 in methods) with the regression describing shell length increment (Fig. 4.3) in the optimal temperature zone (14°C - 20°C), a simple model for predicting artificial feed consumption was derived:

$$C = 2.99 \times FCR/L \times (3.95T-4.4) \times 100$$

where,

C = Consumption (% body weight per day).
FCR = Feed conversion ratio.
L = Abalone length (microns) for which consumption is predicted.
T = Ambient water temperature (°C).

While the individual FCR data observed at each temperature were used to predict feed consumption for the present data, it remains to be determined whether FCR differs significantly over the optimal temperature range. The average FCR values observed in this range was 1.13 but excludes solids leaching, feed wastage and pellet moisture. If a leaching factor of 10% and pellet moisture content of 10% is included, an FCR value of 1.39 is derived for 14°C - 20°C in the present study. In a practical aquaculture context it will be necessary for each farm to derive an average on site FCR value or function. Obviously, the realised "on farm" FCR will be influenced by the efficiency of feeding practise. The regression derived in this study for predicting abalone length increment as a function of temperature should not be used for abalone larger than 70mm SL, as length increment begins to deviate from a straight line function above this size, and is better described by the Von Bertallanfy growth equation. Although the present data show that *H. midae* consumes a predictable ration as a function of temperature and body size, the animals used in the study were offered feed in excess of their requirements and a further trial should thus be carried out to test whether restriction feeding, based on the equation above, does indeed produce equivalent growth rates. Should the modified Haskell's equation be validated by such an experiment, it will allow the development of highly accurate computer based feeding schedules and greatly facilitate the development of abalone farm management protocols.

CHAPTER 5

AN INVESTIGATION INTO THE ABILITY OF *HALIOTIS MIDAE* TO UTILISE CRYSTALLINE ARGININE

INTRODUCTION

Abalone have been shown to require the same ten essential amino acids (EAA) as most other groups of animals (Allen and Kilgore, 1975), but no studies on their quantitative requirements have yet been undertaken. The amino acid pattern of several farmed organisms has been shown to approximate their dietary requirement pattern (Cowey and Tacon, 1982; Benitez, 1989), and a number of nutritional studies have assumed this to be true for abalone (Mai *et al.*, 1995; Knauer *et al.*, 1996). The quantitative EAA requirements of an organism can be deduced from the EAA pattern of its tissue, if the quantitative requirement for at least one reference amino acid, usually the most limiting, is determined empirically. An animal's quantitative requirement for an EAA is usually determined by adding graded levels of a crystalline amino acid in purified or semi-purified diets deficient in that amino acid (Wilson, 1989). However, a number of animals do not readily utilise crystalline amino acids, for example, it has been found that crystalline amino acids must be neutralised before they can be utilised by common carp (Nose *et al.*, 1974) and channel catfish (Dupree and Halver, 1970), and some crustaceans appear unable to utilise crystalline amino acids (Deshimaru, 1982; Akiyama and Dominy, 1989). The primary aim of the present study was thus to determine whether *H. midae* could assimilate crystalline arginine, and whether its quantitative requirement for arginine could be determined by feeding graded levels of crystalline arginine in a semi-purified diet.

Haliotid amino acid profiles (Florkin and Bricteux-Grégoire, 1970; Allen and Kilgore, 1975; Knauer *et al.*, 1996; Mai *et al.*, 1995) reveal that abalone contain a relatively high level of arginine (ca. 5 - 9% of protein) in comparison to other groups of animals and plants. This is ascribed to the central role that arginine plays in the metabolic economy of molluscs, acting *inter alia* as a precursor to phosphoarginine, the most common phosphogen in molluscs. Most free arginine in molluscs occurs in the form of phosphoarginine (Florkin and Bricteux-Grégoire, 1972; Bishop *et al.*, 1983). Furthermore, arginine is used to sustain glycolysis under hypoxic conditions, for example during periods of vigorous or sustained movement, through the formation of octopine (Florkin and Bricteux-Grégoire, 1972; Bishop *et al.*, 1983). When formulating diets from conventional feed ingredients to match the EAA profile of abalone, arginine is usually the most deficient amino acid and, assuming an equal rate of amino acid turnover in abalone, it has been suggested that it may be the most limiting to growth (Mai *et al.*, 1994). However, this assumption unlikely to be valid for abalone since a proportion of ingested arginine is required to maintain the concentration of non-protein bound tissue arginine for use as phosphoarginine.

Crystalline amino acids are highly soluble and thus losses through leaching are a major impediment to their use in microparticulate diets and in feeds for slow feeding organisms such as shrimp and abalone (Akiyama and Dominy, 1989; Lopez-Alvarado *et al.*, 1994). Attempts to encapsulate crystalline amino acids either as microbound or microencapsulated particles have shown that leaching rates can be substantially reduced. For example, lipid walled capsules have been shown to retain crystalline amino acids long enough for ingestion by marine fish larvae (Lopez-Alvarado *et al.*, 1994). In the present study, oil coating of both the arginine powder and finished feed pellets, was employed to reduce leaching, and measurements of the rates of crystalline arginine leaching were made.

MATERIALS AND METHODS

1. Uptake of [U-^{14}C]-arginine

An extruded, fishmeal based, diet containing 50 µCi of L-[U-^{14}C]-arginine monohydrochloride (Amersham Life Science), and an identical control diet without labelled arginine, were manufactured (Table 5.1). The [U-^{14}C]-arginine was added to the water used in the diet to ensure even dispersion of the chemical in the diet ingredient mixture.

Table 5.1. Formulations of [U-^{14}C]-arginine enriched and control diets used to quantify the efficiency of arginine uptake by abalone.

Ingredient	[U-^{14}C]-Arginine diet (g)	Control diet (g)
Fishmeal	500.0	500.0
Starch	489.0	490.0
L-[U-^{14}C]-arginine monohydrochloride (50µCi)	1.0	-
Mineral and vitamin mixture[1]	10.0	10.0
	1000.0	1000.0

[1] Vitamin A, 2400 IU; Vitamin D3, 400 IU; Vitamin E, 30 mg; Vitamin K3, 14 mg; Vitamin B1, 10 mg; Vitamin B2, 9.0 mg; Vitamin B6, 14.0 mg; Vitamin B12, 0.008 mg; Nicotinic acid, 40 mg; Pantothenic acid 30 mg; Folic acid, 2.4 mg; Biotin, 0.2 mg; Vitamin C, 60 mg; Inositol, 60 mg; Manganese, 10.0 mg; Copper, 0.4 mg; Iron, 4.0 mg; Zinc, 8.0 mg; Selenium, 0.05 mg; Iodine, 0.2 mg; Cobalt, 0.05 mg.

A portion of the [U-^{14}C]-arginine enriched batch was coated with fish oil, and the leaching rate of [U-^{14}C]-arginine from both the oil coated and uncoated pellets was determined. Two grams of pellets were placed in beakers containing 50ml seawater at 20°C, and a series of 1ml water samples were taken over a 24h period. The contents of each beaker was gently stirred before sampling. A volume of 0.5ml from each sample was mixed with 4.5ml scintillation fluid in a scintillation vial and the level of radioactivity in the leachate measured using a Beckman LS2800 scintillation counter. The difference in radioactivity level, measured as disintegrations per minute (DPM), between the leachate of the control and [U-^{14}C]-arginine enriched diet, indicated how much [U-^{14}C]-arginine was lost from the feed pellets. This was expressed as µCi/g feed (1 µCi = 2.22 x 10^6 DPM). In order to determine the total radioactivity in each feed, two grams of pellets were homogenised in 50ml water using an Ika Ultra Turrax T25 homogeniser and analysed as described above.

Fat coated [U-^{14}C]-arginine and control diets were fed to juvenile abalone (11.2 $\pm$ 1.02 mm; 0.24 $\pm$ 0.07g), obtained from the Sea Plant Products hatchery, which had been exclusively reared on an extruded fishmeal based artificial diet. The feeding trial was carried out in a constant environment room in our Grahamstown laboratories. Two fully recirculating glass aquaria were stocked with 30 abalone each for the respective diets. Each system consisted of a 70 l open compartment, into which water overflowed into a 36 l biological filter compartment. Water was returned to the open compartment by means of an airlift pump (Fig. 5.1). The abalone were housed in perforated, transparent plastic jars (2l) with screw top lids placed on the bottom of the open compartment. Water was circulated through each jar by means of an airstone. Water temperature was maintained at 20°C by means of a thermostatically controlled aquarium heater. Two thirds of the water in the system was replaced every week with sand filtered sea water. A 10L:14D photoperiod was maintained with the dark period beginning at 17h00.

Two to three feed pellets (0.30 - 0.45g) were placed in each jar just before 17h00 on weekdays. Uneaten feed was removed and oven dried as described in Chapter 2, in order to calculate consumption. After a two month period the abalone were removed from each tank, measured and weighed whole, and then shucked. The soft tissue weight was recorded and approximately half of the soft tissue of each abalone was placed into a vial and dissolved by addition of concentrated HCl (33%) (total volume = 10x the abalone tissue weight). The level of radioactivity in the samples was then determined in the manner described above.

The difference in the level of radioactivity in the soft tissue of abalone fed the [U-^{14}C]-arginine enriched and control diets was assumed to be due to [U-^{14}C]-arginine uptake. The efficiency of uptake of [U-^{14}C]-arginine was estimated using the formula:

$$^{14}\text{C-Arg. absorption (\%)} = 100((^{14}\text{C-Arg.}_{\text{Abalone}} \text{ x } (W_f - W_i/ W_f))/ (^{14}\text{C-Arg.}_{\text{Feed}} \text{ x FCR})$$

Where,

^{14}C-Arg.$_{\text{Abalone}}$ = [U-^{14}C]-arginine content of abalone tissue (µCi/g wet weight)

^{14}C-Arg.$_{\text{Feed}}$ = [U-^{14}C]-arginine content of feed (µCi/g air dry weight)

W_i = Initial weight of abalone (g)

W_f = Final weight of abalone (g)

FCR = Feed conversion ratio (air dry feed consumed/ wet weight gain)

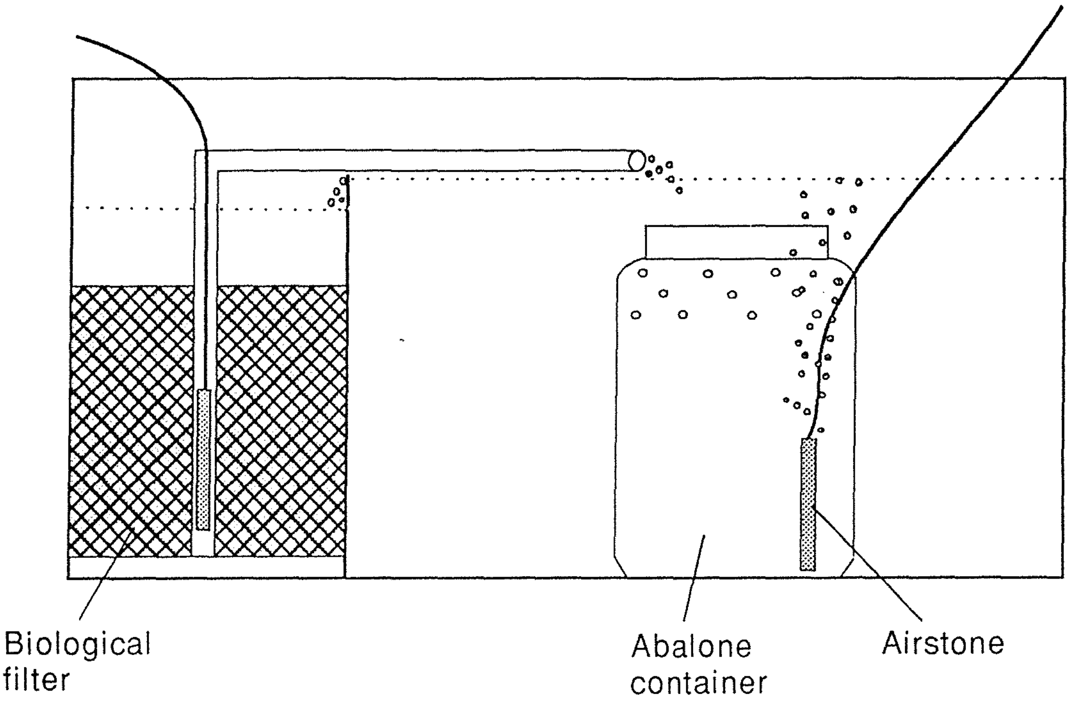


Figure 5.1. Experimental aquaria used to house abalone fed the arginine enriched and control diets.

2. Effect of arginine enrichment on abalone growth rate

In order to evaluate whether arginine supplementation had an effect on the growth rate of *H. midae*, casein based diets were supplemented with graded levels of crystalline L-arginine (Table 5.2). Casein was chosen as a protein source as it contains a good balance of most essential amino acids, but a relatively low level of arginine (ca. 3.5% of protein) in comparison to *H. midae* tissue (ca. 8% of protein). The theoretical arginine content of the experimental diets was 3.5, 8, 12 and 16% of protein. Due to the high solubility of crystalline arginine (15g/100g H_20), the supplemental arginine was suspended in the oil fraction of the test diets, before being mixed with the other ingredients, in order to reduce leaching losses. In addition, the extruded pellets were coated with fish oil to further reduce leaching losses.

Table 5.2. Formulations of crystalline L-arginine supplemented diets fed to juvenile *Haliotis midae*. The diet designation indicates the dietary arginine content.

Ingredient	**Diet**			
	Arg. 3.5	**Arg. 8**	**Arg. 12**	**Arg. 16**
Casein	34.0	34.0	34.0	34.0
Starch	50.0	50.0	50.0	50.0
Fish oil	5.0	5.0	5.0	5.0
L-arginine	-	1.4	2.6	3.8
Vitamin and Mineral mixture[1]	1.0	1.0	1.0	1.0
Cellulose	10.0	8.7	7.5	6.3
	100.0	100.0	100.0	100.0
Analysis (calculated):				
Protein (%)	30.0	30.0	30.0	30.0
Arginine (% of protein)	3.5	8.0	12.0	16.0

1. Vitamin A, 2400 IU; Vitamin D3, 400 IU; Vitamin E, 30 mg; Vitamin K3, 14 mg; Vitamin B1, 10 mg; Vitamin B2, 9.0 mg; Vitamin B6, 14.0 mg; Vitamin B12, 0.008 mg; Nicotinic acid, 40 mg; Pantothenic acid 30 mg; Folic acid, 2.4 mg; Biotin, 0.2 mg; Vitamin C, 60 mg; Inositol, 60 mg; Manganese, 10.0 mg; Copper, 0.4 mg; Iron, 4.0 mg; Zinc, 8.0 mg; Selenium, 0.05 mg; Iodine, 0.2 mg; Cobalt, 0.05 mg.

The arginine content of each diet, before and after 24h submersion in seawater, was quantified in the following manner. Twenty grams of pellets from each diet were placed in shadecloth bags in the a self contained sea water aquarium identical to that used in the growth trial, but with no abalone present. After 24h, the pellets were removed and oven dried at 45°C along with 25g samples of unleached pellets from each diet. The arginine content of the pellets was determined according to the method of Simpson *et al.* (1976) on a Beckman 6300 High Performance Analyser. In addition, to obtain an indication of the rate of arginine leaching, 2g of pellets from each diet were placed in beakers containing 50ml of sea water and the pH change in the seawater monitored hourly over 20h. The contents of the beakers was gently stirred before each pH reading. Arginine is a strongly basic amino acid (isoelectric point =

pH 11.1) and thus causes a pH rise when it leaches into water. The difference between the pH of the leachate of the control (no supplemental arginine) and test diets was thus used as measure of arginine leaching. The leaching rate of each diet was evaluated in triplicate.

The growth trial was run simultaneously with the ^{14}C-arginine trial described above, in the same laboratory. Environmental conditions, the origin of juvenile abalone, and recirculating aquaria used were identical to those described above. The arginine enriched diets were fed to triplicate batches of juvenile abalone, stocked at a rate of 30 per tank. The experiment was run over 117 days and each batch was measured and weighed monthly according to the protocol described in Chapter 2.

The weights and lengths of abalone from each tank were regressed against time and slopes compared by means of analysis of covariance ($P<0.05$). Replicate data were pooled if no significant differences were found, and single regressions for each treatment compared using analysis of covariance.

RESULTS

Uptake of [U-^{14}C]-arginine

The leaching rate of [U-^{14}C]-arginine from the fat coated pellets used in the trial was negligible whereas a high rate of radioactive leaching (ca. 100% after 8h) was detected in the uncoated pellets (Fig. 5.2). It was therefore assumed that the abalone fed the fat coated diet ingested 100% of the [U-^{14}C]-arginine content of the feed consumed.

Abalone fed on both the [U-^{14}C]-arginine and control diets doubled their weight over the experimental period (Table 5.3). The level of radioactivity in the soft tissue of abalone fed on the ^{14}C-arginine was very low with an estimated efficiency of uptake of 0.45% (Table 5.4).

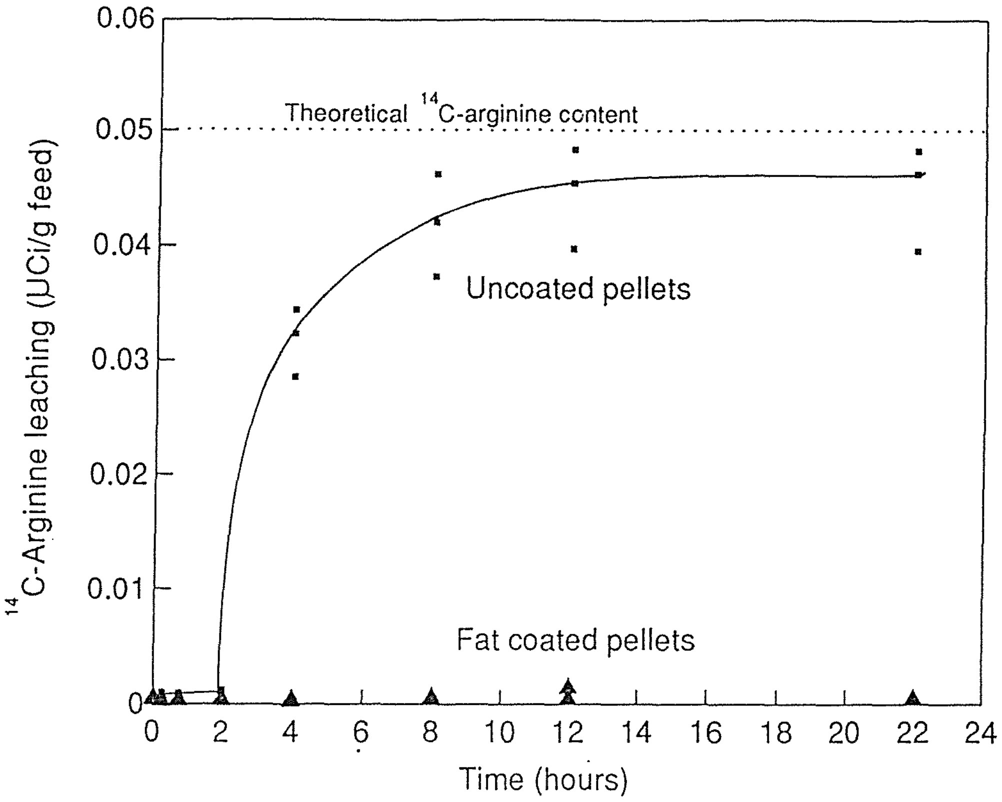


Figure 5.2. Rate of L-^{14}C-arginine leaching from fat coated, and uncoated, abalone feed pellets immersed in 50ml seawater.

Table 5.3. Average weights and lengths, and feed conversion ratio of juvenile *H. midae* fed on a control and ^{14}C-arginine enriched diet. Values indicated as means and standard deviations. Analysis of variance showed the final weights of abalone for both treatments did not differ significantly ($p<0.05$).

Diet	Length (mm)		Weight (g)		FCR (g)
	initial	final	initial	final	
^{14}C-Arginine diet	11.2 ± 1.02	14.6 ± 1.06	0.24 + 0.07	0.53 ± 0.13	1.10
Control diet	11.5 ± 1.03	14.4 ± 1.07	0.23 ± 0.07	0.54 ± 0.12	1.15

Table 5.4. Estimated assimilation efficiency of ingested arginine. Observed values indicated as mean ± standard deviation.

[U-^{14}C]-Arginine Content (µCi/g)			Assimilation efficiency ingested [U-^{14}C]-arginine (%)
Feed pellets Theoretical	Feed pellets Observed	Abalone soft tissue (obs.)	
0.050	0.044 ± 0.003	0.00012 ± 0.00003	0.45 ± 0.12

Effect of arginine enrichment on abalone growth rate

The growth rates of abalone fed on the casein based diets enriched with graded levels of crystalline arginine did not differ significantly from the control diets ($P<0.05$; Table 5.5). Analysis of the levels of dietary arginine revealed that virtually all the supplemented arginine had leached out of the test diets after 24h (Table 5.6). The rate of arginine leaching, indicated by the differences in the pH of the leachate, revealed that crystalline arginine leached rapidly during the first 5h immersion, causing a pH rise proportional to its level in the diet (Fig. 5.3). The levelling of the leaching curve after 5h suggests that almost all the supplemented arginine had leached out of the diets by this time. An estimate of the amount of supplemented arginine ingested by abalone was then made (Fig 5.4), based on the rate of arginine leaching (Fig. 5.3) and the feeding rate of abalone recorded in other studies (Uki, 1981, Knauer *et al.* 1995a). Assuming that abalone consume their feed at a constant rate over an 8h period (Uki, 1981; Knauer *et al.*, 1995a), and that the crystalline arginine leached at a constant rate over 5h, it was estimated that approximately 37% of the supplemented arginine was consumed. This value was then expressed as a percentage of protein consumed (Table 5.6).

Table 5.5. Regressions of length and weight of abalone fed an arginine enriched diets and a control diet over 117 days. Analysis of covariance revealed no significant differences between the slopes of all treatments ($P<0.05$). T = time in days.

Diet	Length (µm)	ln Weight (mg)
Arg. 3.5	$26.0T + 12060$, $r^2 = 49$	$4.50T - 1200$, $r^2 = 33$
Arg. 8	$25.8T + 12080$, $r^2 = 45$	$5.06T - 1230$, $r^2 = 39$
Arg. 12	$25.6T + 11500$, $r^2 = 50$	$5.18T - 1340$, $r^2 = 41$
Arg. 16	$23.3T + 11830$, $r^2 = 45$	$4.32T - 1280$, $r^2 = 34$

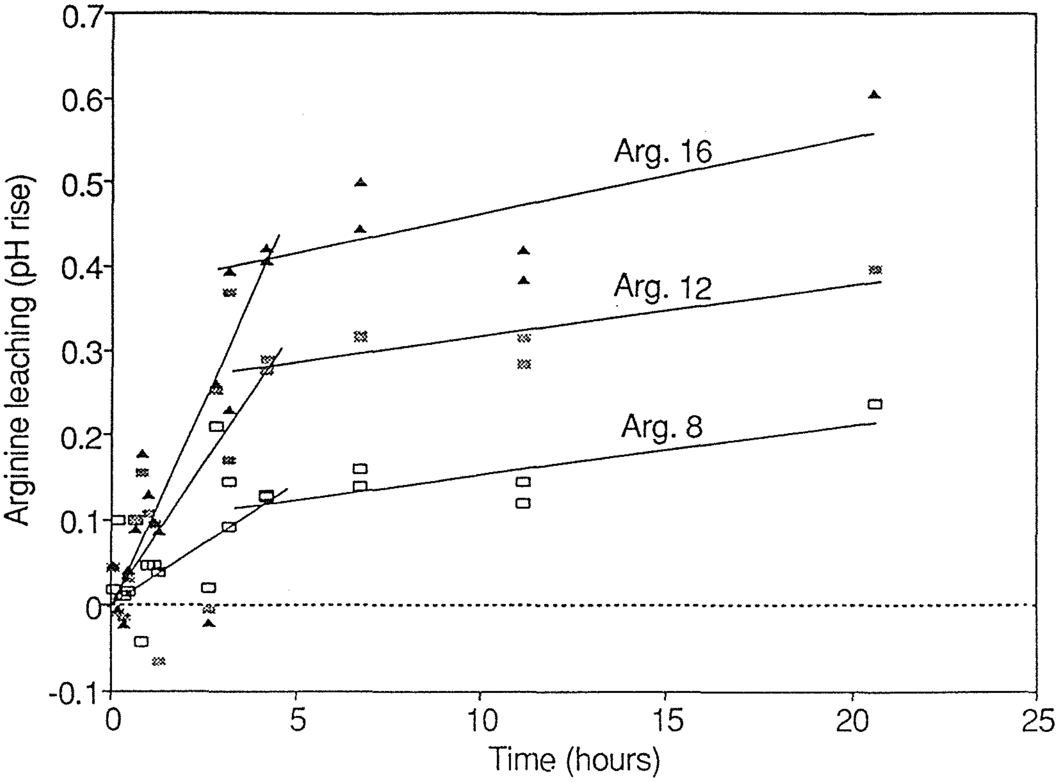


Figure 5.3. Rate of crystalline L-arginine leaching from arginine enriched diets. The rise in pH of water in which the pellets were immersed, relative to that of the control diet, was used as an indicator of the level of crystalline L-arginine. Arg. 8, Arg. 12, and Arg. 16 represent the test diets formulated to contain 8, 12 and 16% of protein as arginine respectively. Curves represent regressions of the plotted points.

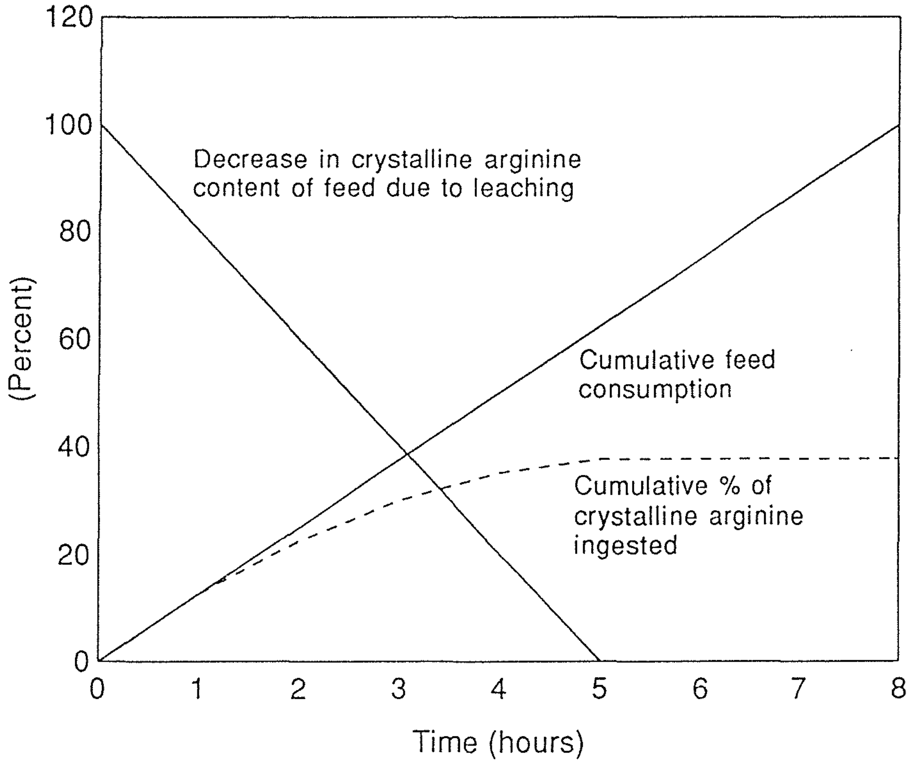


Figure 5.4. Estimate of the percentage of supplemented crystalline arginine ingested by abalone, based on the observed leaching rate of crystalline arginine (Fig. 5.3) and the feeding rate of abalone.

Table 5.6. Leaching loss of supplemented arginine and estimate of the amount of arginine ingested by experimental abalone. All values are expressed as a percentage of protein. The arginine content of the diets after 24h leaching was assumed to represent protein bound arginine. The amount of arginine ingested was calculated by adding the estimated consumption of supplemented arginine (Fig. 5.4) to the amount of protein bound arginine consumed.

	Arginine content of feed (% of protein)			
	Theoretical	Observed	After 24h immersion	Amount ingested
Arg. 3.5	3.5	4.9	4.8	4.8
Arg. 8.0	8.0	9.1	5.3	6.8
Arg. 12	12.0	12.3	5.3	7.9
Arg. 16	16.0	15.6	5.6	9.1

DISCUSSION

Although supplementation with crystalline arginine has been shown to enhance the growth rates of some fish and prawn species (Farmanfarmian and Lauterio, 1979; Wilson, 1989; Lopez-Alvarado and Kanazawa, 1994; Tibaldi *et al.*, 1994), the very low efficiency of uptake (0.45%) of ingested [U-^{14}C]-arginine by *H. midae* soft tissue indicates that abalone cannot utilise crystalline arginine. This is confirmed by the results of the growth trial which demonstrated that supplemental crystalline arginine did not enhance the growth rate of abalone. The low rate of uptake of [U-^{14}C]-arginine is very similar to that recorded for *Penaeus japonicus* by Deshimaru (1982), who obtained a value of 0.6% for this species.

Various reasons have been advanced to explain the poor ability of some organisms to utilise crystalline amino acids, including leaching from the test diets (Wilson, 1989), dietary pH and/or balance of electrolytes (Murai *et al.*, 1983), and differential rates of absorption and excretion of synthetic and protein bound amino acids (Deshimaru, 1982; Murai *et al.*, 1982;

Cowey and Walton, 1988).

The present results appear to discount leaching as a factor accounting for failure of *H. midae* to incorporate crystalline arginine into its tissue. Fat coating effectively minimised leaching of [U-^{14}C]-arginine, however, a substantial amount of arginine leached from the crystalline arginine supplemented pellets which were also fat coated. This apparent inconsistency was probably related to the absolute quatities of arginine used in each experiment. Minute quantities of [U-^{14}C]-arginine were used (0.00362 µg/g feed) whereas the crystalline arginine concentration in the pellets used in growth trial (10.6-48.0 mg/g feed) was 6-7 orders of magnitude higher. Despite the rapid leaching of crystalline arginine from the diets used in the growth trial, the amount of arginine ingested was estimated to range from 4.8% to 9.1% of protein consumed, indicating that effective enrichment was achieved. Notwithstanding these results, the possibility that further leaching losses occurred during the process of ingestion cannot be excluded. The size of feed particle rasped off by the abalone radula is approximately 0.5mm^2 and is immediately swallowed whole without further mastication. Thus while the feeding action of abalone may have resulted in a further loss of crystalline arginine, it is unlikely that all of the remaining of the lipid coated crystalline arginine was lost at this time.

Differential rates of uptake of crystalline and protein bound amino acids, and their subsequent availability at sites of protein synthesis, could account for the low rate of incorporation of crystalline arginine into abalone protein. However, it is interesting to note that despite the relatively large pool of free arginine in abalone tissue (up to double that of protein bound arginine, Florkin and Bricteux-Grégoire 1972; Mai *et al.*, 1994), very little [U-^{14}C]-labelled arginine was taken up.

The pH of the leachate of the diets ranged from pH 5.9 for the control to 6.6 for the diet with the highest level of arginine, and since the crop fluid of *H. midae* has a pH of 5.2 (pers. obs.), free arginine would have been present in its cation form. However, further investigations will be required before any firm conclusions can be reached regarding the relative roles of leaching, pH and electrolyte balance on the ability of *H. midae* to utilise crystalline amino acids. Similarly, a lack of data on the rates of amino acid absorption and excretion in abalone precludes any comment on the rate of crystalline arginine uptake relative to protein bound amino acids.

Although it remains to be empirically determined whether abalone are able to utilise other essential amino acids in crystalline form, the present results suggest that they probably cannot. Futhermore, the high levels of arginine and other free amino acids in abalone tissue raise doubts as to whether the tissue amino acid profiles approximate the animal's requirement pattern. Alternative ways of quantifying abalone amino acid requirements thus have to be considered.

Definition of quantitative EAA requirements through the monitoring of post-prandial levels of free amino acids in the blood of fish has been applied with some success and could be considered in abalone (Wilson, 1989; Mai *et al.*, 1994). The underlying hypothesis is that serum or tissue content of the amino acid should remain low until the requirement for that amino acid is satisfied, and then increase to high levels when excessive amount of the amino acid are fed (Wilson, 1989). Similarly, the rate of tissue amino acid oxidation in plasma can be used to determine an optimal dietary level. The technique assumes that when an amino acid is limiting in the diet, the major portion will be used for protein synthesis, and little will accumulate in the plasma or be oxidised to carbon dioxide, whereas when the quantity of amino acid is supplied in excess, plasma levels will increase and more will be available for oxidation (Wilson, 1989). The only attempt to relate the free amino acid levels in abalone tissue to dietary levels, as well as observed growth rates, has been an exploratory study undertaken by Mai *et al.* (1994) using two species of abalone (*H. discus hannai* and *H. tuberculata*) fed a selection of algae. These authors found that dietary amino acid levels did influence free amino acid levels in abalone tissue. On the basis of the correlation various essential amino acid indices to abalone growth, they proposed that a new index, balance and availability of free EAA in abalone viscera, provided a good estimate of diet protein quality in abalone.

A further alternative would be to vary the EAA levels in abalone diets through appropriate mixtures of whole proteins. For example, Andrews *et al.* (1977) increased the level of dietary arginine fed to channel catfish by substituting gelatin for casein in a semi-purified diet, and observed increased growth rates as a result. For such studies to be meaningful, protein digestibility and ideally, amino acid digestibility, of the proteins used would have to be determined. Furthermore, studies using mixtures of whole proteins are difficult to control due to the possible interactions resulting from the changing proportions of other dietary EAA.

Clearly, quantification of abalone amino acid requirements represents a technical major challenge which will have to be addressed if diet formulation for abalone is to be advanced to a stage where it is possible to manufacture diets from mixtures of practical proteins, which provide essential amino acids in amounts that promote optimal growth.

CHAPTER 6

SIZE SPECIFIC EFFECTS OF DIETARY PROTEIN AND ENERGY LEVEL ON GROWTH AND BODY COMPOSITION OF *HALTIOTIS MIDAE*

INTRODUCTION

A basic tenet of nutritional theory is that animals, with few exceptions, eat to satisfy their energetic requirements (Smith, 1989). While consumption in some herbivorous marine organisms, for example mussels and sea urchins (Bayne and Newell, 1983; Lawrence *et al.*, 1989), is not determined by the energetic content of their food, available evidence indicates that consumption by abalone is indeed related to dietary energy content (Peck *et al.*, 1987; Barkai and Griffiths, 1988). Most pertinently, the amount of artificial dry feed ingested by abalone appears to be governed by their metabolic rate, as consumption in *H. discus hannai* (Hahn, 1989e) and *H. midae* (present study, Chapter 4) has been shown to be a predictable function of body size and temperature.

The economics of aquaculture dictate that the fledgling abalone industry must strive to maximise growth rates, in order to maximise its return on investment. To achieve this the rate of protein deposition must be maximised, which implies that formulated feeds should contain sufficient protein. Moreover, such protein must be readily digestible and provide an adequate quantity of essential amino acids, in the correct proportions. Experience with fishes and other animals fed on complete, formulated diets show that if the level of dietary protein is insufficient, or of low biological value, the animals energetic requirements will be satisfied from non protein energy sources, usually carbohydrates and lipids. This results in growth rate depression and accumulation of fatty tissue (Smith, 1989). Once a dietary protein:energy (P:E) threshold ratio which supports maximum growth has been attained, further increases in the proportion of dietary protein to energy will result in deamination and combustion of protein for maintenance energy requirements. Thus, in order to determine an optimal protein level which supports the maximum growth rate of abalone, dietary energy content must be considered.

Previous studies on abalone protein requirements (Ogino and Kato, 1964; Uki *et al.*, 1986b) have been simple dose response experiments, feeding diets containing graded levels of protein without consideration of the dietary P:E ratio. An obvious constraint has been the paucity of data for abalone on the metabolisable energy value of the protein, carbohydrate and fat content of commonly used dietary ingredients. While exploratory attempts have been made to determine the digestibility and/or digestible energy content of certain ingredients for abalone, such as casein (Dixon, 1992), fishmeal, corn starch and fish oil (Dixon 1992, 1992; Wee *et al.*, 1992, Wee *et al.*, in press) using the indirect method (Cr_2O_3 and acid insoluble ash as markers), insufficient data are available for the information to be of use in feed formulation. Furthermore, the relatively low values obtained for protein digestibility (49%) by Wee and colleagues (op cit.) raise doubts as to the accuracy of these data. Notwithstanding the lack of precise digestible energy data for abalone, sufficient information on the utilisation of various protein, carbohydrate and lipid sources in artificial feeds did exist (Chapter 1) to facilitate the formulation of diets containing a range of P:E ratios.

In the present study, diets containing graded proportions of protein to energy were formulated from practical feed ingredients in quantities that abalone have been shown to be capable of utilising efficiently, and which promote maximum growth rates (Chapter 1). Thus dietary fat was varied between 2% and 10%, protein between 24% and 44%, and carbohydrate between 33% and 58%. It was decided to use fishmeal as the sole protein source in the study because:

> 1) It is efficiently utilised by abalone and forms the bulk of protein in commercial abalone diets manufactured for *H. midae*.
>
> 2) The lack of information on the biological value and amino acid availability of other potential ingredients, would introduce too much uncertainty into any attempt to formulate an "ideal" protein, which matches the amino acid profile of abalone, from a mixture of whole proteins. Furthermore, the present study (Chapter 5) appears to rule out the use of crystalline amino acids in the formulation of an "ideal" protein.

The natural diet of abalone changes markedly over the size range that they are cultured (1 - 80 mm), from a predominantly diatom/ microalgal diet to a macroalgal diet. Very little consideration has however been given to its size specific nutritional requirements (Knauer *et al.*, 1994b) and the implications for artificial diet formulation. The present study was thus used to compare the responses of post-weaning (ca. 10mm; 0.2g) and older juvenile (ca. 36mm; 8g) *H. midae* fed diets containing different proportions of protein and energy. An attempt was made to determine the digestible energy value of the experimental diets using the indirect method (Cr_2O_3 marker).

MATERIALS AND METHODS

Nine fishmeal based, extruded diets were formulated to contain three combinations of protein and carbohydrate, each with three levels of lipid (2%, 6% and 10%), in order to produce a range of protein to energy ratios (Table 6.1). Since the digestible energy values for the ingredients used were not available for abalone, physiological fuel values for fish were used for the purposes of diet formulation to obtain an approximation of the relative proportions of dietary protein and energy. Although it is not entirely acceptable to apply the the digestible energy values obtained for one species to another (Jobling, 1983), this was justified on the basis of the similar feed conversion and protein efficiency ratios that have been observed in fish and abalone fed diets of similar composition (Present study, Chapter 2; Halver, 1989). The values used were 14.65, 18.83, 35.58 kJ/g for carbohydrate (dextrin or cooked starch), protein, and lipid, respectively (Jauncey, 1982; Smith, 1989). The theoretical protein to energy ratio of the experimental diets thus ranged from 18.4 to 41.6 mg protein/ kJ DE (Table 1). However, these values were only intended to be illustrative of the qualitative differences in the P:E ratio between the diets, and during the course of the experiment an attempt was made to determine the actual digestible energy values of the nutrient categories of the experimental diets, using the indirect method (Smith, 1989). To this end an inert marker (Cr_2O_3) was included in the experimental diets. To achieve the lower levels (2 and 4%) of dietary lipid in the respective diets, lipid was extracted from a quantity of fishmeal by boiling it in ethanol at 60-70°C for ten minutes. The fishmeal was then washed over a 100μm nylon mesh using municipal tap water and air dried at 25°C for 3h. The extraction reduced the lipid content of the fishmeal from 11.6% to 2.4%, and the required levels of dietary lipid in the respective formulations were achieved by combining appropriate proportions of fat extracted and unextracted fishmeal.

Table 1. Formulations of extruded dry feeds containing three protein and three energy levels in a three by three factorial design. The diet designations indicate the percentage of protein (P) and lipid (F) in each diet. Diets are arranged in order of increasing P:E ratio form left to right.

Parameter	**Diet**								
	24P/10F	**24P/6F**	**24P/2F**	**34P/10F**	**34P/6F**	**34P/2F**	**44P/10F**	**44P/6F**	**44P/2F**
Fishmeal (11.6% fat)[1]	33.8	33.8	16.0	47.9	47.9	12.5	62.0	45.0	9.0
Fishmeal (2.4% fat)	-	-	16.0	-	-	32.0	-	15.5	48.0
Starch	57.8	57.8	57.8	45.1	45.1	45.1	32.5	32.5	32.5
Fish oil[2]	6.6	2.5	-	5.0	1.0	-	3.5	0.9	-
Vitamin and mineral premix[3]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Chromic oxide	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Cellulose	-	4.0	8.3	-	4.0	8.4	-	4.0	8.4
Analysis (calculated)									
Protein (%)	24.0	24.0	24.0	34.0	34.0	34.0	44.0	44.0	44.0
Lipid (%)	10.0	6.0	2.0	10.0	6.0	2.0	10.0	6.0	2.0
Digestible energy (kJ)	18.4	16.9	15.2	19.2	17.7	15.7	20.0	18.3	16.2
P:E ratio (mg/kJ)	18.4	20.0	21.1	25.0	27.0	28.4	31.0	33.1	35.2

1. LT Fishmeal, Fiskernes Fiskeindustri A.M.B.A., Skagen.
2. Marinol "R", Marine Oil Refiners, Cape Town.
3. Vitamin and mineral premix formulation in Table 4.1.

The diets were fed to two sizes of hatchery reared abalone (10.5 ± 0.15 mm shell length (SL); 0.2 ± 0.01g and 36.3 ± 0.35 mm SL; 7.8 ± 0.25g) in a feeding trial conducted at our Port Alfred laboratory. The two size classes, referred to as "small abalone" and "big abalone",were six and 20 months old respectively. Both originated from the Sea Plant Products hatchery in Hermanus, and had been reared on a fishmeal based artificial feed (34% protein) following weaning from diatoms at 5-6 mm SL. System parameters were as described in Chapter 2. Three replicates for each diet were used which were assigned to the 27 experimental tanks according to a randomised block design. Large abalone were housed in the tanks with shelter as previously described (Chapter 2), and the smaller abalone were housed inside transparent, perforated 2l plastic jars placed within the tanks containing the larger abalone (Fig. 6.1). Circulation of water through each jar was effected by means of an airstone. The larger abalone were reared for 142 days on the experimental diets, while the smaller animals were only reared for the latter 72 days of this period, due to their more rapid rate of proportional change in weight. Abalone were measured and weighed monthly according to the protocol described in Chapter 2. The experimental animals were fed daily at 17h00 from Monday to Thursday at a rate of 2.0g per replicate for the larger size class and 1.0g per replicate for the smaller size class. A triple ration was provided on Fridays and no feeding was performed on

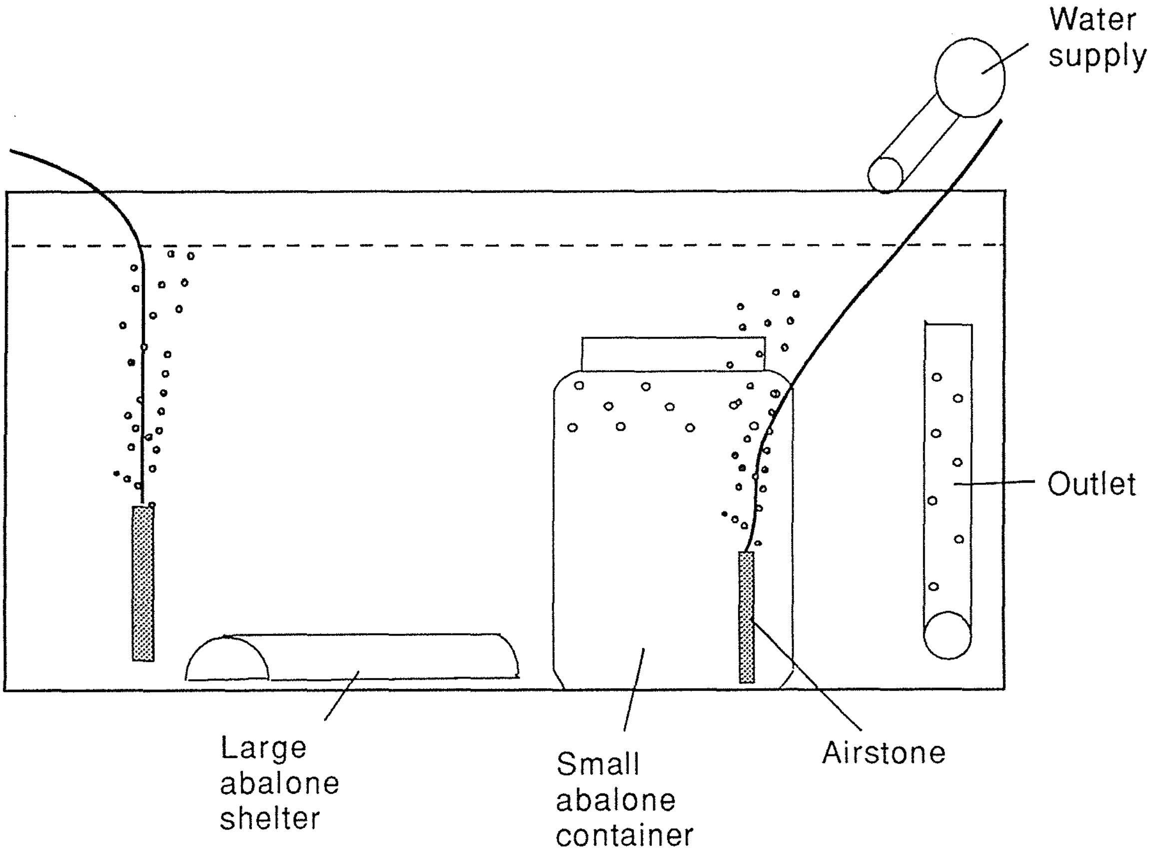


Figure 6.1. Schematic representation of experimental containers in which large (35-40 mm) and small (7 -14 mm) were housed during the growth trial.

weekends. Before placement of feed in the experimental containers, uneaten feed was removed and oven dried at 60°C. In order to quantify the loss of solid matter through leaching for the respective diets, feed was placed in tanks with no abalone present for a 24h period, and then removed and dried as described above. Total feed consumption, adjusted for leaching losses, was calculated for the entire experimental period according to the equation in Chapter 2. Rate of feed consumption for each treatment was then calculated as grams feed consumed per abalone per day, and as a percentage of initial body weight. Feed conversion ratio, protein efficiency ratio, and condition factor were calculated as described previously (Chapter 2). The percentage of protein consumed which was deposited as soft body tissue (PPD) was calculated according to the equation:

$$\text{PPD} = \frac{\text{Final soft body protein content - initial soft body protein}}{\text{Protein consumed}} \times 100$$

Proximate analysis of the soft tissue of both size classes of abalone was performed at the beginning and end of the growth trial. Samples of three large abalone, and all small abalone were taken from each tank and frozen for subsequent analysis. The samples were subsequently defrosted, weighed whole and shucked. Shucked soft tissue was weighed and homogenised using an Ika Ultra Turrax T25 homogeniser. With the exception of lipid, proximate analysis was performed according to standard methods of the AOAC (1984). Thus, moisture content of soft tissue was calculated following drying at 104°C, crude protein content was estimated from total Kjeldahl nitrogen (x 6.25), ash as the residue remaining following combustion of samples at 550°C for 12h, and nitrogen free extract estimated by difference. Lipid content was determined by means of chloroform/ methanol extraction (Folch *et al.,* 1957).

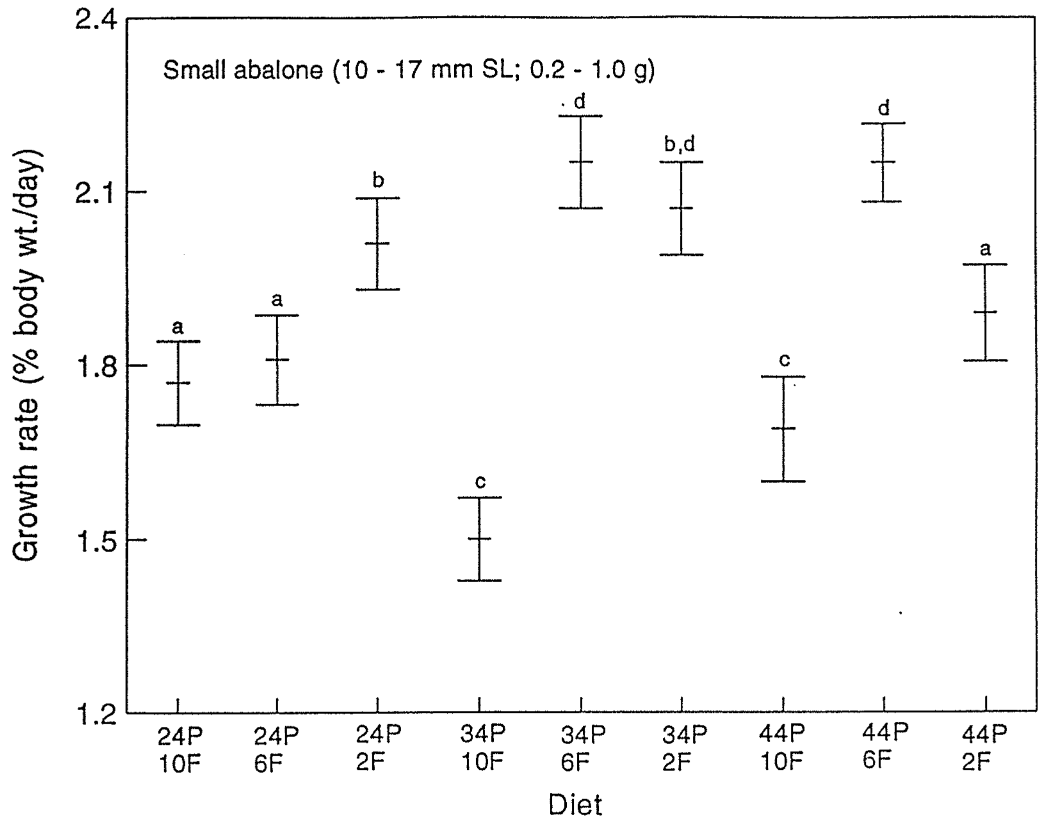
Following the termination of the growth trial, the remaining large abalone were maintained on their respective experimental diets, and faeces collected in order to determine apparent digestibility using Cr_2O_3 as a marker. To avoid contamination of faeces with foreign matter, which was suspected as being responsible for the variable digestibility values obtained in previous attempts (Chapters 2 and 5), only whole faecal "strings" (1-3 cm length) were collected. Previously, all faecal matter observed, which consisted of "strings" and "pellets" (1-10mm length), was collected (Chapter 2 and 4). Faecal strings were gently lifted using a probe and oven dried at 60°C in small glass vials. Faecal collection was performed over a four week period. The chrome content of both feed and faeces was determined according to the method of Bolin *et al.* (1952) and apparent digestibility estimated using the equation in Chapter 2.

Growth rates were determined from the slopes of linear regressions of length and natural log transformed weight data. The slopes were compared for significant differences using analysis of covariance ($P<0.05$). Length and weight data from the experimental replicates were pooled if their growth rates did not differ significantly and single regressions for each treatment compared using analysis of covariance. Feed consumption, FCR, PER, PPD, and condition factor, as defined in Chapter 2, were compared using one way analysis of variance and Tukey's multiple range test ($P<0.05$). The relative influence of dietary protein and fat, and abalone size, on growth rate, FCR, NPU, CF and the proximal composition of abalone soft tissue was analysed by means of multifactor analysis of variance and Tukey's range test.

RESULTS

The rates of weight gain of both small (0.2 - 1.0g) and large (7 - 14g) abalone were significantly affected by both the protein and energy levels in the experimental diets. Moreover, different trends in the growth response of the two size classes were evident (Fig. 6.2). The highest dietary lipid level (10%) appeared to suppress the growth rates of the small abalone, as the three diets containing 10% lipid produced consistently lower growth rates which not did fit a trend which could be related to the P:E ratio of the diets. If the diets containing 2% and 6% lipid only are considered, the growth rates of small abalone increased with an increasing protein/ energy ratio up to a level 34% protein/ 6% fat, then levelled off. The growth rates of those fed the diet with the highest P:E ratio (44P/2F diet) were however significantly lower than the 34P/6F, 34P/2F and 44P/6F treatments (Fig. 6.2). By contrast, the growth rate of the big abalone increased with an increasing proportion of dietary protein to energy up to a level of 44% protein/ 6% fat. As was observed with the small abalone, the growth rates of the larger abalone fed the 44P/2F were lower than those fed the 44P/6F diet. These trends were reflected in the final lengths and weights of the experimental abalone (Table 6.2). The proportional increase in weight of the smaller abalone ranged between 300 and 450%, whereas, that of the larger abalone was only 10-100%.

The leaching rates of the diets, measured as the proportion of solids remaining after 24h immersion, decreased with an increasing inclusion level of starch, and the percentage recovered ranged from 89.5% to 96.4% (Table 6.2). Feed consumption, corrected for leaching losses, and expressed either as mg consumed per individual per day, or as a percentage of initial weight, did not differ significantly between the dietary treatments for both the small and



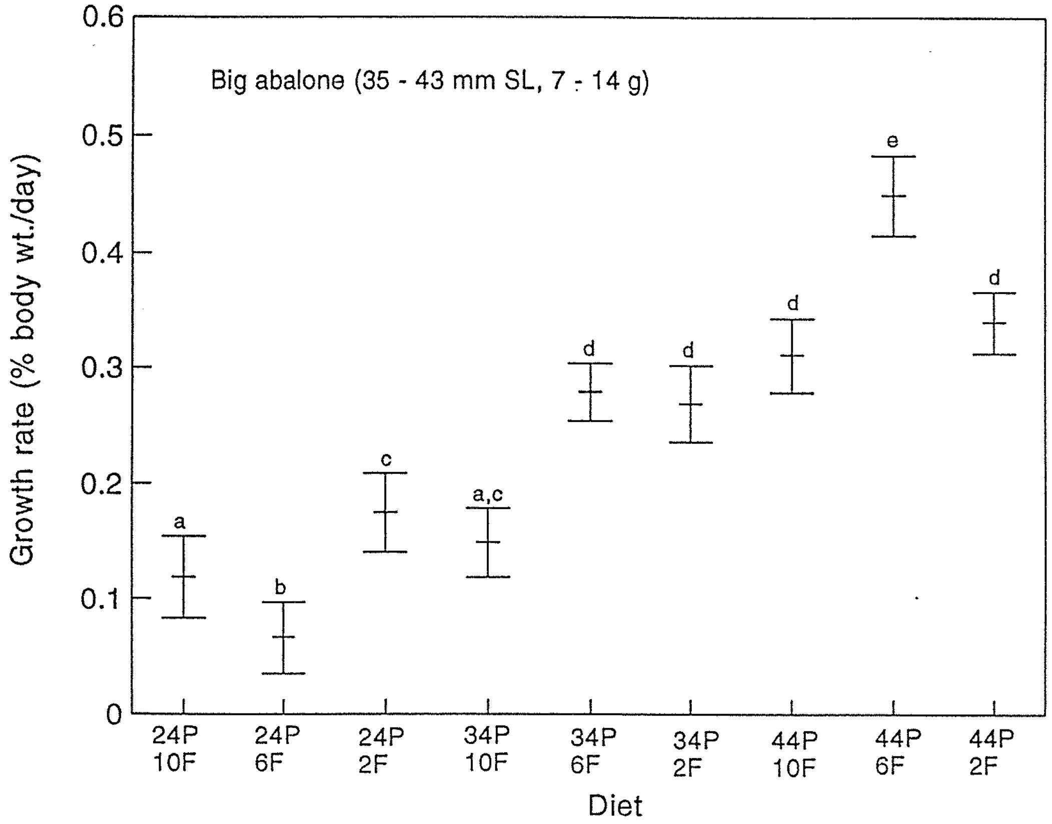


Figure 6.2. Growth rates of two size classes of abalone fed diets containing three protein/ carbohydrate combinations, each with three lipid levels. Diet designations indicate the percentage of protein (P) and lipid (F). Dietary P:E ratio increases from left to right. Growth rates are represented as the mean ± S.E. x 100 of the slopes of natural log transformed weight data. Different alphabetic symbols indicate significant differences ($P<0.05$)

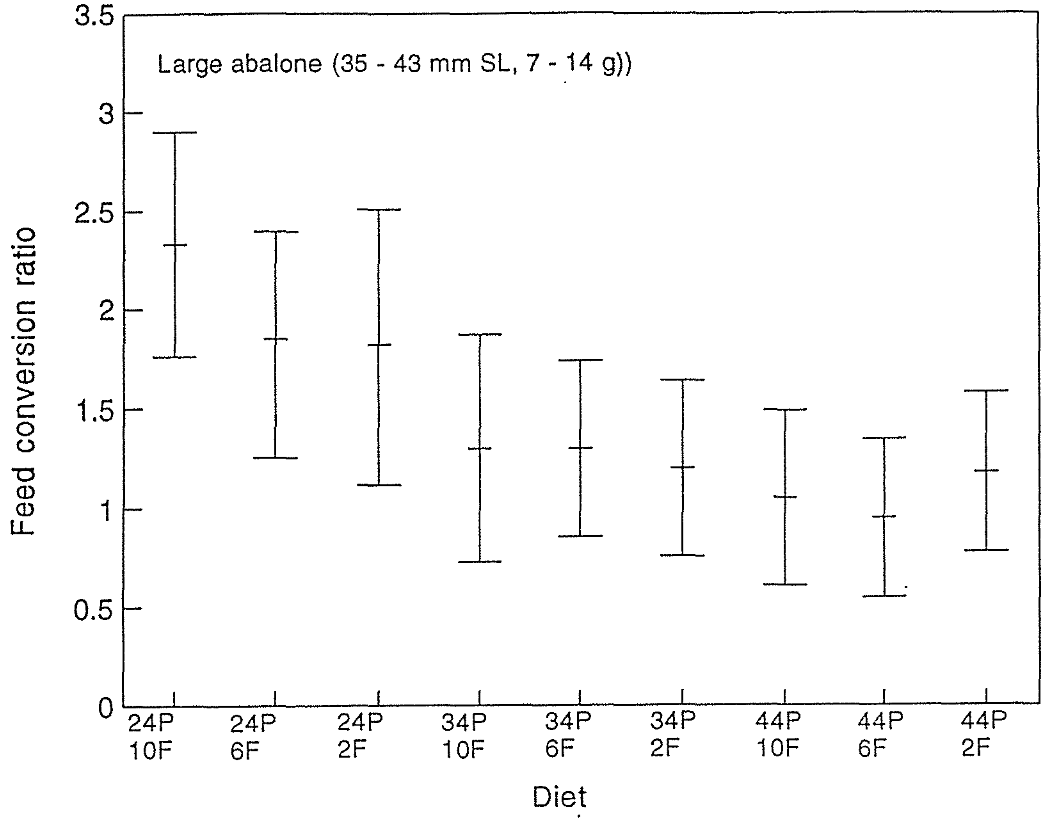
Table 6.2. Nutritional indices for two sizes of abalone reared on extruded dry feeds containing three protein and three lipid levels. The dietary treatments are arranged in order of an increasing P:E ratio from left to right. The diet designations indicate the percentage of protein (P) and lipid (F) in each diet. Values indicated are means with standard errors in parentheses. Different alphabetic superscripts indicate significant differences between treatments for the parameter concerned.

Parameter				**Diet**					
	24P/10F	**24P/6F**	**24P/2F**	**34P/10F**	**34P/6F**	**34P/2F**	**44P/10F**	**44P/6F**	**44P/2F**
Length/ Weight data of Small Abalone									
Initial weight (g)	0.20	0.21	0.20	0.22	0.20	0.20	0.20	0.19	0.19
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
Initial length (mm)	10.6	10.8	10.4	10.8	11.0	10.6	10.6	10.4	10.3
	(0.15)	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)	(0.15)
Final weight (g)	0.69	0.76	0.82	0.63	0.95	0.87	0.67	0.87	0.70
	(0.02)	(0.03)	(0.03)	(0.02)	(0.03)	(0.03)	(0.03)	(0.03)	(0.03)
Final length (mm)	16.4	16.8	17.1	16.2	18.4	17.5	16.6	17.7	16.6
	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)
Length/ weight data of Large Abalone									
Initial weight (g)	7.56	8.21	8.70	7.84	7.37	8.41	7.32	7.40	8.05
	(0.23)	(0.26)	(0.25)	(0.24)	(0.21)	(0.25)	(0.20)	(0.23)	(0.24)
Initial length (mm)	36.3	39.1	36.6	36.2	36.1	36.3	36.2	36.3	36.2
	(0.3)	(0.4)	(0.3)	(0.4)	(0.3)	(0.4)	(0.3)	(0.4)	(0.3)
Final weight (g)	9.14	9.02	11.17	9.88	11.08	12.56	11.93	14.23	12.84
	(0.37)	(0.36)	(0.39)	(0.35)	(0.34)	(0.44)	(0.46)	(0.51)	(0.36)
Final length (mm)	9.1	9.0	11.2	9.9	11.1	12.6	11.9	14.2	12.8
	(0.4)	(0.4)	(0.4)	(0.4)	(0.3)	(0.4)	(0.5)	(0.5)	(0.4)
24h Leaching									
(% recovered)	96.4	95.8	92.8	90.9	93.1	92.9	89.5	89.8	91.4
	(0.8)	(0.9)	(1.0)	(1.0)	(1.0)	(0.9)	(0.9)	(1.0)	(1.0)
Consumption mg/individual/day									
Small abalone	8.7^{a}	7.5^{a}	9.7^{a}	7.5^{a}	9.3^{a}	8.4^{a}	8.3^{a}	9.4^{a}	7.2^{a}
Large abalone	23^{a}	45^{a}	42^{a}	26^{a}	37^{a}	38^{a}	34^{a}	35^{a}	45^{a}
Consumption % init. body wt./d									
Small abalone	4.42^{a}	3.70^{a}	5.10^{a}	3.58^{a}	4.72^{a}	4.40^{a}	4.37^{a}	4.98^{a}	4.25^{a}
Large abalone	0.30^{a}	0.60^{a}	0.49^{a}	0.33^{a}	0.45^{a}	0.47^{a}	0.47^{a}	0.48^{a}	0.61^{a}
Protein efficiency ratio									
Small abalone	2.8^{abc}	4.3^{d}	3.6^{cd}	2.4^{ab}	3.6^{cd}	3.3^{bcd}	1.7^{a}	2.3^{ab}	2.2^{ab}
Large abalone	1.8^{a}	2.4^{a}	3.0^{a}	2.3^{a}	2.6^{a}	2.6^{a}	2.2^{a}	2.5^{a}	2.3^{a}
Condition factor									
Small abalone	0.88^{ab}	0.89^{ab}	0.94^{b}	0.84^{ab}	0.89^{ab}	0.89^{a}	0.87^{a}	0.88^{ab}	0.86^{a}
Large abalone	0.83^{a}	0.83^{a}	0.83^{a}	0.87^{ab}	0.86^{ab}	0.87^{ab}	0.88^{b}	0.91^{b}	0.88^{b}
Mortality (%)									
Small abalone	0.0	0.0	2.2	0.0	0.0	1.1	2.2	2.2	5.6
Large abalone	9.3	8.0	13.1	9.3	5.3	4.0	8.0	13.7	10.6

large abalone. The FCR of the smaller abalone fed diets containing 2% and 6% fat did not differ significantly, however, two-way analysis of variance revealed that diets containing 10% fat produced significantly poorer FCR's (Fig. 6.3; Table 6.3). Protein content did not affect the FCR of small abalone (Table 6.3). The FCR's of the larger abalone decreased with an increasing P:E ratio (Fig. 6.3). Two way analysis of variance revealed that protein level had a significant effect on the large abalone FCR, with those fed 24% protein displaying a significantly lower FCR than those fed diets containing 34% and 44% protein. Dietary lipid level did not significantly affect the FCR of large abalone (Table 6.3).

The percentage of protein deposited by small abalone fed diets containing 10% lipid was significantly lower for diets containing 10% lipid in comparison to those containing 2% and 6% lipid (Fig. 6.4; Table 6.3). The PPD of the latter diets decreased with an increasing level of dietary protein. Two-way analysis of variance showed that the PPD by small abalone was significantly lower for those fed diets containing 44% protein in comparison to those fed diets containing 24% and 34% protein (Table 6.3). By contrast, the PPD of large abalone increased significantly between the 24% and 34% protein treatments, but did not differ significantly between those fed the 34% and 44% protein diets (Fig. 6.4; Table 6.3). Furthermore, PPD was unaffected by the level of dietary fat fed to the larger abalone. The trends in protein efficiency ratio reflected those of PPD (Table 6.2).

The condition factor (CF) of both small and large abalone was influenced by the proportions of protein and energy in the experimental diets. CF of small abalone fed diets containing 24% protein was significantly higher than those fed the higher (34% and 44%) protein diets. Small abalone fed diets containing 2% and 6% lipid displayed a significantly higher CF than those



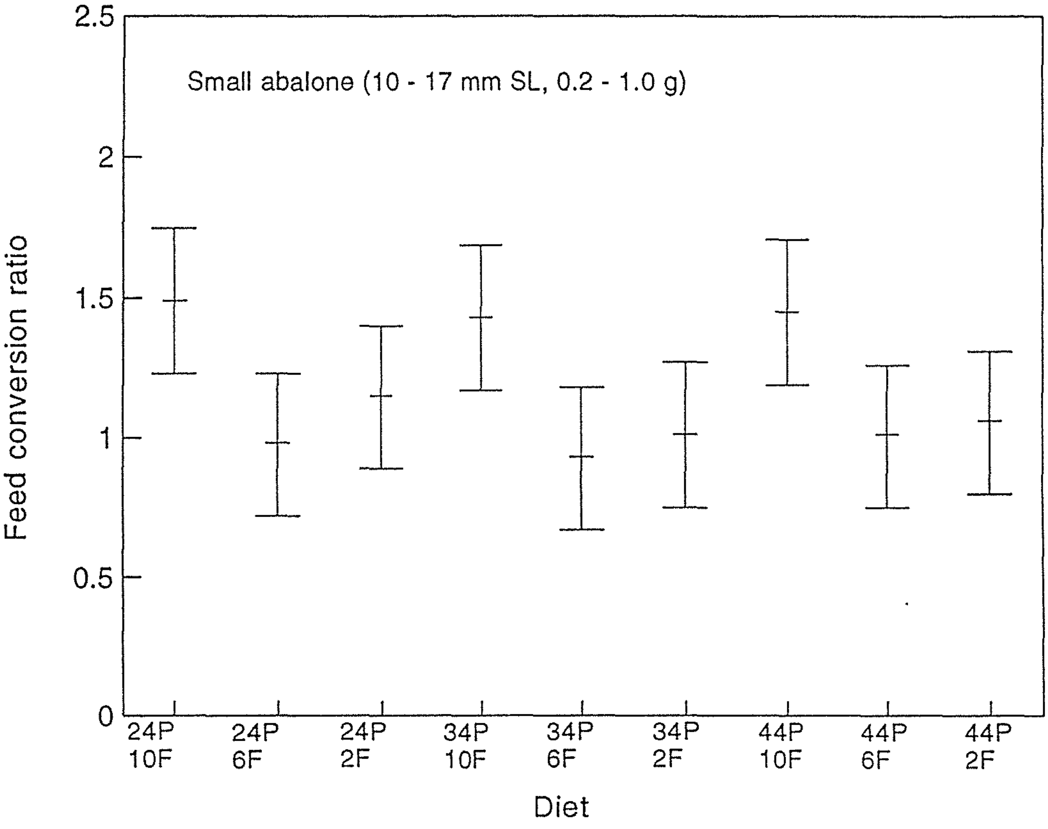


Figure 6.3. Feed conversion ratio's (FCR) of two size classes of abalone fed diets containing three protein/ carbohydrate combinations, each with three lipid levels. Diet designations indicate the percentage of protein (P) and lipid (F). Dietary P:E ratio increases from left to right. FCR is represented as the mean ± Tukey's 95% confidence interval of the three replicates for each dietary treatment. Significant differences are indicated by non-overlapping confidence intervals ($P<0.05$).

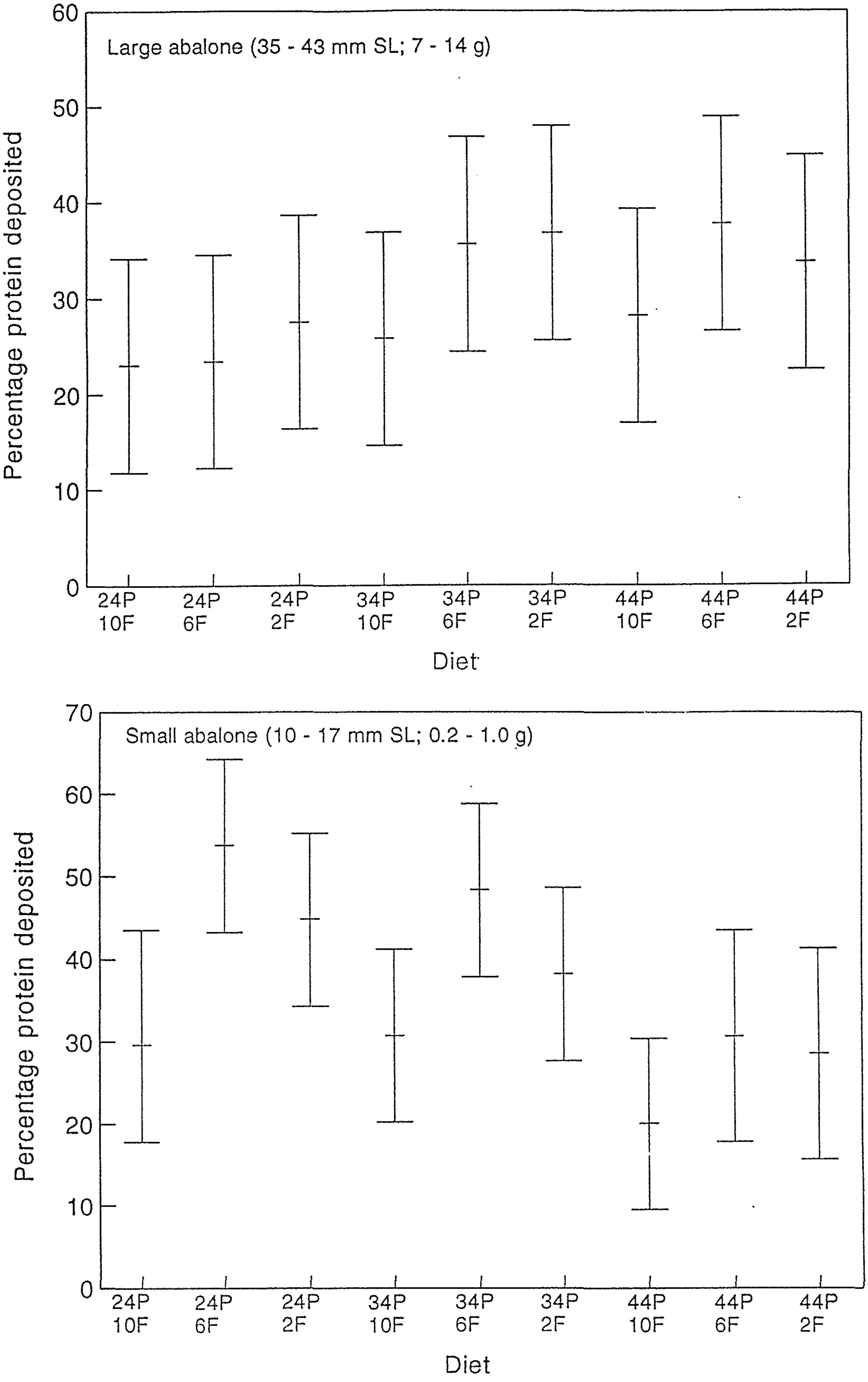


Figure 6.4. Percentage of protein deposited by two size classes of abalone fed diets containing three protein/ carbohydrate combinations. Diet designations indicate the percentage of protein (P) and lipid (F). Dietary P:E ratio increases from left to right. PPD is represented as the mean ± Tukey's 95% confidence interval of the three replicates for each dietary treatment. Non-overlapping confidence intervals indicate significant differences ($P<0.05$).

Table 6.3. Effect of dietary protein/ starch and lipid levels on feed conversion ratio (FCR), percentage of protein deposited (PPD) and condition factor (CF). Means for each effect were calculated using two-way ANOVA. For the respective size classes, significant differences ($p<0.05$) between the means for each effect are indicated by different alphabetic superscripts.

Parameter	Effect	(%)	Small abalone		Large abalone	
			Mean	F	Mean	F
FCR	Protein/ starch	24/58	1.17^{a}	0.48	1.99^{a}	13.3
		34/45	1.20^{a}		1.27^{a}	
		44/33	1.17^{a}		1.07^{b}	
	Lipid	2	1.07^{x}	18.1	1.40^{x}	0.71
		6	0.97^{x}		1.36^{x}	
		10	1.46^{y}		1.56^{x}	
PPD	Protein/ starch	24/58	39.4^{a}	7.8	22.9^{a}	5.6
		34/45	39.1^{a}		33.4^{b}	
		44/33	26.4^{b}		34.0^{b}	
	Lipid	2	37.1^{x}	18.2	31.3^{x}	2.3
		6	44.3^{x}		33.1^{x}	
		10	23.5^{y}		25.8^{x}	
CF	Protein/ starch	24/58	0.90^{a}	4.6	0.83^{a}	14.9
		34/45	0.87^{b}		0.87^{b}	
		44/33	0.87^{b}		0.89^{b}	
	Lipid	2	0.89^{x}	4.0	0.86^{x}	0.2
		6	0.89^{xy}		0.87^{x}	
		10	0.86^{y}		0.86^{x}	

fed the 10% lipid diets (Table 6.2 and 6.3). The CF of large abalone was however unaffected by the level of dietary lipid, but those fed the 24% protein diets yielded a significantly lower CF in comparison the higher (34 and 44%) protein treatments (Table 3).

Mortality of small abalone was low ranging from 0-5%, however, a higher mortality (4-14%) of larger abalone was experienced due to "walkout" (abalone climbing out their tanks during the dark period) during the first month of the experiment (Table 6.2). The problem was solved by placing shadecloth covers over the tanks.

Proximate analyses revealed that the protein, lipid and carbohydrate (nitrogen free extract) content of the soft tissue of both small and large abalone were significantly affected by the experimental diets (Table 6.4). However, ash and moisture content of the respective size classes of abalone did not differ significantly between the experimental treatments (Table 6.5). The protein content of both large and small abalone increased significantly with an increasing dietary protein (and decreasing starch) content, but was unaffected by the level of dietary fat. The protein content of large abalone was significantly higher (ca. 7%) than that of the small abalone (Table 6.5). The lipid content of both size classes increased significantly with the level of dietary lipid, and that of the soft tissue of small abalone was significantly higher than that of the larger abalone (Table 6.5). Nitrogen free extract (NFE) increased with an increasing level of dietary starch (and decreasing protein) content, and small abalone contained a significantly higher percentage than large abalone. NFE was unaffected by the level of dietary lipid. Ash content was not influenced by the dietary levels of protein or fat, but did differ significantly between large and small abalone. Soft tissue moisture content increased significantly with and increasing level of dietary protein (and decreasing starch) content, and large abalone contained a significantly higher level than small abalone.

Results of analysis of the chrome content of faeces collected from the experimental treatments yielded digestibility estimates that were low, and more variable, than expected (Table 6.6) ranging between 8 and 55%. No patterns which could be related to the nutrient content of the diets emerged.

Table 6.4. Proximate analyses of soft tissue for two sizes of abalone reared on extruded dry feeds containing three protein/ carbohydrate combinations, each with three lipid levels. Diets are arranged in order of increasing P:E ratio from left to right. The diet designations indicate the percentage of protein (P) and lipid (F) in each diet. Values indicated are means with standard errors in parentheses. Different alphabetic superscripts indicate significant differences between treatments for the parameter concerned. All values, except moisture, expressed on dry weight basis. Initial values for small and large abalone respectively, were Protein: 51.8% and 57.7%; Lipid: 3.4% and 3.7%; Ash: 19.4% and 17.1%; Nitrogen free extract: 25.4% and 21.5%; and Moisture: 77% and 77.5%.

Category (%)	**Diet**								
	24P/10F	**24P/6F**	**24P/2F**	**34P/10F**	**34P/6F**	**34P/2F**	**44P/10F**	**44P/6F**	**44P/2F**
Protein									
Small abalone	51.7^{a}	51.2^{a}	53.5^{a}	55.4^{ab}	54.1^{a}	55.9^{ab}	56.1^{ab}	57.9^{ab}	61.6^{b}
Large abalone	60.3^{ab}	59.7^{a}	60.2^{ab}	60.4^{ab}	61.8^{ab}	62.0^{ab}	64.1^{ab}	65.7^{ab}	66.5^{b}
Lipid									
Small abalone	5.9^{a}	4.7^{ab}	4.5^{ab}	5.3^{ab}	5.1^{ab}	4.1^{b}	5.9^{a}	5.7^{a}	4.0^{b}
Large abalone	4.1^{ab}	3.6^{a}	3.5^{a}	3.8^{a}	3.85^{ab}	3.4^{a}	4.7^{b}	3.7^{a}	3.8^{a}
Nitrogen free extract									
Small abalone	29.3^{a}	29.9^{a}	28.6^{b}	24.3^{a}	28.7^{a}	25.3^{a}	24.8^{a}	22.9^{a}	20.0^{a}
Large abalone	25.6^{ab}	26.3^{a}	24.9^{abc}	25.8^{ab}	25.0^{abc}	24.7^{abc}	19.4^{c}	19.9^{bc}	20.3^{abc}
Ash									
Small abalone	13.2^{a}	14.2^{a}	13.5^{a}	15.0^{a}	12.2^{a}	14.7^{a}	13.3^{a}	13.5^{a}	14.8^{a}
Large abalone	9.9^{a}	10.4^{a}	11.4^{a}	10.0^{a}	9.4^{a}	9.9^{a}	11.7^{a}	10.7^{a}	9.5^{a}
Moisture									
Small abalone	76.1^{a}	75.6^{a}	76.3^{a}	74.7^{a}	77.8^{a}	76.6^{a}	77.5^{a}	76.4^{a}	77.8^{a}
Large abalone	77.0^{a}	77.8^{a}	77.3^{a}	76.4^{a}	77.5^{a}	78.5^{a}	78.1^{a}	78.3^{a}	80.2^{a}

Table 6.5. Effect of abalone size, dietary protein/ starch and lipid level on proximate composition of abalone fed experimental diets containing three protein and three lipid levels. Means for each effect were calculated using three-way ANOVA. For the respective categories of the proximal analysis, significant differences ($p<0.05$) between the means for each effect are indicated by different alphabetic superscripts.

Category	**Effect**		**Mean**	**F**
Protein	Protein/ starch (%)	24/58	56.1^{a}	29.6
		34/45	58.2^{b}	
		44/33	62.0^{c}	
	Lipid (%)	2	59.9^{x}	3.5
		6	58.4^{x}	
		10	58.0^{x}	
	Abalone size	Small (0.2 - 1.0 g)	55.3^{m}	124
		Large (7 - 14 g)	62.3^{n}	
Lipid	Protein/ starch (%)	24/58	4.3^{a}	3.1
		34/45	4.3^{a}	
		44/33	4.6^{a}	
	Lipid (%)	2	3.9^{x}	26.2
		6	4.5^{y}	
		10	5.0^{z}	
	Abalone size	Small (0.2 - 1.0 g)	5.0^{m}	93.2
		Large (7 - 14 g)	3.8^{n}	
Nitrogen free extract	Protein/ starch (%)	24/58	27.4^{a}	19.9
		34/45	25.6^{a}	
		44/33	21.2^{b}	
	Lipid (%)	2	23.9^{x}	1.2
		6	25.4^{x}	
		10	24.9^{x}	
	Abalone size	Small (0.2 - 1.0 g)	25.9^{m}	8.1
		Large (7 - 14 g)	23.6^{n}	
Ash	Protein/ starch (%)	24/58	12.1^{a}	0.1
		34/45	11.9^{a}	
		44/33	12.2^{a}	
	Lipid (%)	2	12.3^{m}	0.8
		6	11.7^{m}	
		10	12.2^{m}	
	Abalone size	Small (0.2 - 1.0 g)	13.8^{x}	27.5
		Large (7 - 14 g)	10.3^{y}	
Moisture	Protein/ starch (%)	24/58	76.7^{a}	4.1
		34/45	76.9^{ab}	
		44/33	78.0^{bc}	
	Lipid (%)	2	77.8^{x}	2.7
		6	77.2^{x}	
		10	76.6^{x}	
	Abalone size	Small (0.2 - 1.0 g)	76.5^{m}	11.5
		Large (7 - 14 g)	77.9^{n}	

Table 6.6. Results of analysis of chrome content of the experimental diets and of faeces collected from abalone fed the respective diets. An estimate of dry matter digestibility is included. Due to the small weight of faeces collected from each tank, experimental replicates were pooled for chrome analysis. Insufficient faeces were collected from abalone fed the 44P/2F diet for an analysis to be performed.

Diet	Feed weight (mg)	Feed Cr_2O_3 (%)	Faeces weight (mg)	Faeces Cr_2O_3 (%)	Digestibility (%)
24P/10F	28.8	0.89	33.4	1.13	21.5
24P/6F	97.3	0.96	38.4	1.50	35.9
24P/2F	29.9	0.80	26.1	1.13	29.0
34P/10F	98.1	0.92	71.7	2.04	54.8
34P/6F	18.5	0.92	35.3	1.12	17.2
34P/2F	30.5	0.88	28.1	0.96	8.1
44P/10F	29.0	0.80	-	-	-
44P/6F	32.9	0.79	16.6	1.31	39.7
44P/2F	108.0	0.90	21.9	1.08	16.1

DISCUSSION

Although the attempt to estimate the precise digestible energy values of the experimental diets was unsuccessful, the present results yielded trends in growth rate, FCR, PPD, PER, condition factor and body composition which could be related to the proportions of protein and energy in the experimental diets. Furthermore, significant differences in the responses of the smaller and larger abalone emerged indicating that size specific nutritional requirements exist in *H. midae*.

Previous research indicates that the ability of abalone to digest and utilise lipid is limited. For example, low lipase activity has been observed in *H. midae*, and lipid levels between 2.5% and 10% have been shown to produce optimal growth and feed conversion in *H. discus hannai* and *H. tuberculata* (Uki and Watanabe, 1992; Mai *et al.*, 1995). The responses of *H. midae* to the dietary lipid levels used in the present study are broadly consistent with the above observations, however, they provide the first evidence of size specific differences. Amongst the smaller abalone, a high level of dietary lipid (10%) clearly inhibited growth rates, and the correspondingly poor FCR, PPD, and PER values indicate that the utilisation of protein, and perhaps other nutrients, was inhibited as well. Interestingly, consumption did not appear to be reduced by the higher level of energy in these diets, and it is possible that the fat coating around particles of protein and other nutrients inhibited enzymatic digestion, with a resultant poor conversion of feed and protein. Since diets containing 10% lipid appeared to inhibit the effective utilisation of dietary energy by the small abalone, these treatments are excluded from further consideration of the effect of P:E ratio on the growth and nutritional indices of this size class. This inhibitory effect was however not evident among the larger abalone fed the 10% lipid diets, suggesting that larger juveniles can adapt to a higher level of dietary lipid. If the small abalone fed on the diets containing 10% lipid are not considered, dietary lipid level did not significantly affect FCR, PPD, or CF of either size class. The lipid content of these diets did however significantly influence the growth rates of both size classes and it is therefore reasonable to conclude that this was probably due to the calorific content of the lipid.

The lipid content of both small and large abalone soft tissue increased with dietary lipid content. A similar effect has also been documented for *H. discus hannai* and *H. tuberculata*

(Mai *et al.*, 1995). The present results further showed that soft tissue lipid content was not significantly affected by the dietary protein content and hence the dietary P:E ratio. However, the soft tissue of small abalone contained significantly more lipid than large abalone. This further highlights the probability that size specific differences exist in *H. midae* with respect to lipid metabolism. A similar size related difference in the lipid content of wild *H. midae* soft tissue was also documented by Knauer *et al.* (1994b), who recorded levels of 2.4% and 0.76%, in 10 - 20 mm SL and 45 - 55 mm animals respectively. The absolute lipid levels observed in the present study (3.4 - 5.9 % of dry tissue weight) are higher than those recorded in wild *H. midae* (Knauer *et al.*, 1994b), but lower than those observed in *H. discus hannai* (6.3 - 10.2 %) and *H. tuberculata* (5.7 - 9.0) fed artificial diets containing similar levels of lipid to those used in this study (Mai *et al.*, 1995). Fats markedly influence meat flavour, and thus the influence of dietary fat on the taste of cultured abalone is an issue deserving of more attention with respect to consumer acceptance of farmed abalone.

For the larger abalone, growth rates increased with an increasing P:E ratio and peaked in the treatment fed the diet containing 44% protein and 6% fat. This trend is similar to that observed in Chapter 2 where abalone of 20 - 30 mm SL were fed diets containing 27-47% protein. However, the rate of increase in growth rate with an increasing dietary protein level was greater in the present experiment. By contrast, the growth rate of the small post-weaning abalone fed diets containing 2% and 6% lipid increased with P:E ratio up to a level of 34% protein and then levelled off suggesting that these abalone have a lower protein requirement than the larger size class. The pattern of the growth rates of the larger abalone is similar to that observed in *H. discus hannai* of similar size (ca. 32 mm SL), in which growth rate increased with an increasing dietary protein content, up to a level of 43% protein on fishmeal based diets (Uki *et al.*, 1986b). Although this was the highest level of fishmeal protein used by these authors, they also evaluated casein based diets containing up to 55% protein and observed a peak in growth rate at 47% dietary protein.

Size specific differences emerged in the protein related nutritional indices, providing further evidence that the protein requirements of *H. midae* change with size. Visibly, the FCR of small abalone did not change significantly with an increasing P:E ratio, whereas that of the larger abalone improved. A trend of more efficient conversion of feed with an increasing dietary protein content was also reported by Uki *et al.* (1986b) for *H. discus hannai* of similar size (31 - 35 mm SL), and this is the expected response in a fish fed on formulated diets (Steffens, 1989). The declining trends in PER and PPD observed in the smaller size class is also a response typically observed in cultured fishes, and has previously been documented in abalone as well (Uki *et al.*, 1985b; present study, chapter 2). However, the PER and PPD of the larger abalone did not show any clear trend over the range of dietary protein used.

Small abalone fed 34% and 44% protein diets displayed a lower condition factor than those fed 24% protein diets, while the reverse was true for the larger abalone indicating that the higher protein diets promoted the deposition of a greater proportion of soft tissue in this size class. Calculation of condition factor from the results of Uki *et al.* (1986b) for *H. discus hannai* of similar size (31 - 35 mm), revealed a similar trend of increasing CF with increasing protein level in fishmeal based diets.

Although the protein content of the soft tissue in both size classes of abalone increased with an increasing P:E ratio, that of the larger abalone was significantly higher (ca. 7%), lending weight to the hypothesis that the larger abalone have a higher protein requirement. In contrast to the present results, Knauer *et al.* (1994b) recorded similar levels of protein in the soft tissue of post-weaning (10 - 20 mm SL) and larger (45 - 55 mm SL) juvenile *H. midae* collected from the wild. Furthermore, the absolute protein levels recorded (40 - 45 %) were considerably lower than those observed in the present study (ca. 51 - 66%). However, this difference is probably due to the different methods of protein analysis employed. Knauer *et al.* (1994b) used the Folin-Lowry method which, in preliminary work to the present study, was found to yield consistently lower values than the Kjeldahl method. Recalculation of the protein content of a variety of abalone species from different studies (cited in Olley and Thrower, 1977), on a dry weight basis, yielded an average value of 66%, which tends to corroborate the present values.

The substantial increase in soft tissue protein observed in both size classes of abalone, which was related to an increasing dietary protein and decreasing starch level, is interesting. This trend stands in marked contrast to observations on fishes, which tend to maintain a constant level of body protein regardless of the nutritional composition of their diet (Buckley and Groves, 1979). Although dietary lipid may indirectly influence the percentage of dietary protein in fishes, through its effect on the proportion of carcass lipid, dietary protein does not influence carcass protein probably because protein synthesis is genetically determined (Buckley and Groves, 1979). Abalone differ from fish in that energy is not stored as lipid, but as glycogen, and phosphoarginine, the primary phosphogen in molluscs (Bishop *et al.*, 1983). Changes in the levels of these substances could therefore markedly influence the levels of both carcass carbohydrate and protein. For example, the glycogen content of *H. cracherodii* was found to vary between 5 and 25% of the dry weight of abalone muscle tissue (Webber,

1970) and is dependent, *inter alia,* on the nutritional status of the animal (Carefoot *et al.*, 1993; Watanabe *et al.*, 1994). Thus changes in the levels of reserve glycogen could significantly affect the proportions of other categories such as protein. Free arginine levels double that of protein bound arginine have been recorded in abalone (Florkin and Bricteux-Grégoire, 1972), and thus free arginine could equate to as much as 3 - 6% of total protein. In addition to arginine, molluscs possess high levels of free glycine, alanine and taurine, all of which are reduced to opines during anaerobic glycolysis (Gäde and Grieshaber, 1986, Tjeerdema *et al.*, 1991). Levels of free amino acids in abalone are affected by diet composition and starvation (Watanabe *et al.*, 1993; Mai *et al.*, 1994), hypoxic and osmotic stress (Higashi *et al.* 1989; Tjeerdema *et al.*, 1991), and thus fluctuating levels of free amino acids could significantly influence estimates of carcass protein as measured by the total Kjeldahl nitrogen method. The increase in soft tissue protein with an increasing dietary protein, and decreasing dietary starch content, was accompanied by a corresponding decrease in the carbohydrate content of abalone soft tissue. While insufficient data are available to comment on the physiological significance of this result, there nonetheless appears to be a relationship between soft tissue protein and carbohydrate levels, which is related to the proportions of dietary protein and energy.

The apparent increase in protein requirement with size is the converse of what has been demonstrated in commonly cultured fish species (Wilson, 1989). However, in the absence of any other size specific physiological information for abalone any attempt at explanation of this phenomenon is speculative. The more efficient utilisation of protein by small abalone, as reflected in their higher PPD and PER values may in part explain this apparently lower protein requirement. A further consideration in attempting to explain this apparent difference between fish and abalone, is the natural diet of juvenile abalone, which consists predominantly of diatoms and microalgae which are rich in carbohydrates, whereas that of juvenile fish, even omnivorous species such as tilapia and carp, tends to consist predominantly of zooplankton which is richer in protein. It is well documented that juvenile fish rapidly develop a range of protease enzymes (Steffens, 1989), however, the situation in early juvenile abalone is less clear with respect to their ability to digest protein. Knauer *et al.* (1996) showed that early juveniles weaned from diatoms onto high (34%) protein artificial feed developed higher levels of protease activity, and the possibility thus exists that the larger abalone in the present study, which had been reared on an artificial diet of similar composition for a longer period, had developed higher levels of protease activity than the smaller post-weaning abalone.

The PER and PPD values obtained in the present study are comparable to those obtained by Uki *et al.* (1986b) for *H. discus hannai* (Table 6.7). The present values are however closer to those obtained by these authors for casein than for fishmeal, indicating that the "white" fishmeal used in their study was probably of lower biological value than the "low temperature" fishmeal used in the present one. This supposition is corroborated by the similar results obtained feeding casein and fishmeal based diets to *H. midae* (Chapter 2) and *H. fulgens* (Viana *et al.*, 1993). The PPD and PER values in Table 6.7 compare favourably with those of young fishes. For example, studies on the rearing of fingerling rainbow trout with commercial diets typically yield PER values in the range 2.2 - 2.7, and PPD values of 34 - 39 (Steffens, 1989). However, values as high as PER = 3.3 and PPD = 51, have been obtained in fingerling trout fed casein based semi-purified diets (Takeuchi and Watanabe, 1978; Takeuchi *et al.*, 1978). Some caution should however be exercised when considering the absolute PPD for the present study, as they do not include shell protein which constitutes approximately 5% of total protein (Suyama and Sekine, 1965). Furthermore, observed consumption corrected for leaching losses assumes that nutrients were lost in equal proportion from all nutrient categories, whereas in reality it is likely that they were greater from the more soluble components such as minerals. These factors could introduce an error of up to 5 - 10% in the absolute PPD values of the present study. Notwithstanding this possible error, it may be concluded that abalone fed on dry or semi-moist formulated feeds are comparatively efficient converters of protein.

Table 6.7. Comparison of protein efficiency ratio (PER) and percentage of protein deposited (PPD) by abalone and selected fish species.

Species	**Size**	**PER**	**PPD**	**Dietary protein**	**Source**
H. discus hannai	3 - 5 g	1.8 - 3.9	33 - 55	Casein 50 - 20%	Uki *et al.* 1986b
	3 - 5 g	0.9 - 2.0	16 - 19	Fishmeal 63 - 16%	
H. midae	0.2 - 1.0 g	1.7 - 4.3	20 - 54	Fishmeal 44 - 24%	Present results
	7 - 14 g	1.8 - 2.6	23 - 38	Fishmeal 44 - 24%	

The ash content of *H. midae* soft tissue differed significantly between the two size classes, however, the lack of any data on the mineral requirements of abalone precludes any comment on the possible implications of this result with respect to artificial diet formulation.

The indirect method of estimating the apparent digestibility coefficients of the experimental diets failed to yield realistic values, and there was thus no point in measuring the calorific content of the experimental diets and faeces in order to calculate digestible energy values. Thus, while the diets used in the present study undoubtedly represented a range of P:E ratios, the precise level of digestible energy in each diet, as well the relative calorific contributions of the dietary ingredients, remains uncertain. A consideration of the technical problems associated with digestibility studies in abalone and possible approaches to solving them are presented in the final concluding discussion (Chapter 7).

In conclusion, although quantitative P:E ratios were not established for the experimental diets, the present experiment yielded trends in growth rate and nutritional indices that provided a good qualitative indication of the response of *H. midae* to dietary P:E ratio. Size specific differences were observed between larger juvenile and smaller, post-weaning *H. midae* with respect to growth rate, nutritional indices, and body composition, which suggest that the two size classes differ in their requirements for protein and energy. Larger juveniles appear to have a higher protein requirement, and ability to digest higher levels of lipid, than post-weaning abalone. The observed changes in the levels of soft tissue protein and carbohydrate, which were related to the dietary levels of these categories, indicate that abalone differ from fish and other vertebrates in this respect. The present results highlight the scope that exists for further work on the influence of dietary protein and energy on the metabolism and body composition of abalone.

CHAPTER 7

CONCLUDING DISCUSSION

The results of the present study have demonstrated that principles of animal feed science developed for monogastric animals are applicable to *H. midae*, a herbivorous gastropod relying primarily on enzymatic digestion (MacLean, 1970). Moreover, the close correspondence between the results of nutritional work on *H. midae* and other cultured abalone species (Uki and Watanabe, 1992; Viana *et al.*, 1993; Mai *et al.*, 1995) suggests that haliotids have very similar nutritional requirements. This is probably due to the lack of specialisation in abalone with respect to the composition of their natural diet and feeding structures. The acceptance of dry pelleted feeds made from a wide variety of ingredients, by *H. midae* and other species can probably be ascribed to the generalist nature of abalone feeding biology. Consumption of feed by early juvenile *H. midae* conformed to the classic poikilotherm pattern, in that the quantity ingested was related to temperature and body size. This indicates that calorific intake is controlled by the animals metabolic rate. In other words, satiation in *H. midae* is determined by its energetic requirements.

The investigation into the dynamics of digestion of a dry formulated diet revealed that digestion in *H. midae* is analogous to that of a batch reactor (Penry and Jumars, 1987) in that a discrete amount of food is ingested and then processed over a 24h period, whereupon feeding activity again begins. Digestion of the artificial diet was comparatively rapid with enzyme peaks occurring 6-12h after peak gut fullness. The observed rates of digestion were similar to those of preferred algal species, and much faster than those of less digestible algal species which may remain recognisable in the crop for up to 48h (Day and Cook, 1995).

Since the bulk of artificial feed consumed was digested within 24h, feeding of cultured *H. midae* should therefore be a daily process.

The initial work in this project was based on the use of alginate bound diets developed by the Japanese (Uki and Watanabe, 1992) which, although expedient from a research point of view, are not cost effective, or practical, commercially. The development of a starch binding process by a local company during the course of the present project made possible the present investigations into the efficiency of utilisation of dry feeds by *H. midae*, and greatly facilitated the development of a commercial abalone feed in South Africa. Although dry feeds are now commercially manufactured in Japan, New Zealand, Australia and the USA, the present results represent the first published work on the use of dry, starch bound diets in abalone.

Due to the slow feeding mode of abalone, nutrient losses through leaching are a major consideration affecting nutrient availability, FCR (and hence feed cost), and water quality. The low rates of leaching observed in the present experiments indicates that the starch binding technique employed was effective in restricting nutrient losses (up to 96% recovery after 24h leaching). To obtain an accurate measure of consumption, and hence efficiency of feed and protein conversion, leaching losses were corrected for, and very consistent values for these indices were obtained. A wide range of leaching rates (4-34%, Britz and Clayden, 1996) for different commercial abalone feeds have been reported. Therefore if consumption based indices from different studies are to be compared, it is essential that observed consumption be corrected for leaching losses. Work in our laboratories has shown that leaching is very rapid and thus the equation used in the present study (Consumption = Feed fed x leaching correction factor - feed remaining, Uki and Watanabe, 1992), which assumes that all leaching losses occur before abalone begin to feed, provides a realistic approximation of actual losses.

Accurate estimates of consumption are essential in order to calculate the amount of feed required to promote optimal growth rate, with a minimum of wastage. Using a starch bound, fishmeal based formulation, the present study demonstrated that feed consumption in *H. midae* is a predictable function of temperature and body size, as is the case in other cold blooded aquatic organisms cultured on complete diets. The allometric nature of abalone growth made it possible to apply Haskell's feeding equations (Haskell, 1959), originally developed for trout, to *H. midae*. Accurate restriction feeding is essential in abalone, as demand feeding is not possible due to their slow feeding response. The modified Haskell's equations, which can be adapted to observed on-farm performance with respect to growth rate and FCR, provide a simple tool for highly accurate and cost effective feed management. For example, the equations could be incorporated into a computer based farm management model which calculates the required ration for each tank of abalone, based on ambient temperature, size, and biomass of the cultured animals.

Accurate consumption data also make it possible to estimate the quantities of nitrogenous compounds excreted, and regulate water flow rates accordingly. It has been demonstrated that nitrogenous excretion in *H. midae* fed fishmeal based, dry feeds is similar to cultured fish, in that 60-70% of ingested nitrogen is excreted (Lyon, 1995).

The development of industrial scale growout systems based on artificial feeds is now the major challenge facing the developing abalone culture industry in South Africa. The availability of a tightly bound, water stable, feed pellet makes it possible to design growout tank systems around the characteristics of the pellet and the feeding behaviour of the abalone

Abalone are comparatively slow growers taking 2 - 4 years to reach a marketable size of 50 - 100g, and thus production costs are very sensitive to any factor which may enhance growth rate. Since growth rates are dependent upon the quantity and quality of dietary protein, the primary focus of nutritional research on abalone has been its protein requirements (Uki and Watanabe, 1992; Viana *et al.*, 1993). In the present study it was shown that *H. midae* will accept and efficiently utilise a variety of proteins, however, the growth rates observed on single protein rich ingredients were fairly variable. This was ascribed to factors such as palatability, digestibility and amino acid balance. In contrast to the poor growth obtained feeding "white" fishmeal to *H. discus hannai*, the present study revealed that high quality "low temperature" processed fishmeal is an excellent protein source for *H. midae*, promoting higher levels of feed consumption and growth than the other proteins tested. This result highlights the heterogeneity that exists with respect to fishmeal quality, and suggests that for a slow growing, high value species such as haliotids, it makes economic sense to use the best quality fishmeal available. While low temperature fishmeal is clearly an excellent protein to include in abalone diets, scope clearly exists for combining fishmeal with other whole proteins, to produce a balance of essential amino acids that more closely matches that of abalone tissue, and to bring down the cost of the feed ingredients. For example, the present study showed that soya protein is an a good prospect, as its amino acid profile was found to most closely match that of abalone tissue. Furthermore, it is rich in arginine, which is the most deficient essential amino acid if diets are formulated to match the amino acid profile of abalone. Ideally, a database on a range of potential proteins that could be included in abalone diets should be developed, which includes information on their biological value, digestible energy values and amino acid profiles. Once this has been achieved a "least costing" approach to abalone feed formulation will become possible. The difficulties encountered in the present study, with respect to the use of crystalline amino acids and estimation of apparent digestibility, do however present significant technical constraints, which will have to be overcome if abalone amino acid requirements, and the biological availability of dietary proteins, are to be accurately defined and used in the formulation of "least costed" diets.

While the study on the ability of *H. midae* to assimilate crystalline arginine did not conclusively show that abalone cannot utilise crystalline amino acids, it does indicate that the prospects for using them in nutritional studies on abalone amino acid requirements are not promising. The most productive way forward would appear to be to perfect a technique for estimating apparent digestibility. Once this is achieved, and the apparent protein digestibility of various ingredients established, it will be possible to formulate an "ideal" protein from a mixtures of whole proteins, which matches the amino acid profile of abalone. It will be necessary to test whether such a formulation results in better protein utilisation by abalone, as the high free amino acid pool present in molluscs raises questions as to whether the body amino acid composition of abalone does indeed match its requirement pattern. This theory assumes that the turnover rate of all essential amino acids is equal (Cowey and Tacon, 1982), however, the different dynamics of protein bound and free amino acids in molluscs (Florkin and Grégoire-Bricteux, 1972) casts doubt upon the validity of this assumption.

Factors which could have accounted for the low and variable estimates of apparent digestibility in the present study include; an uneven rate of excretion of the Cr_2O_3 marker, absorption of the marker by the abalone, and contamination of faeces with other matter.

Evidence exists that abalone sort food in their crop (MacLean, 1970), and the possibility therefore exists that this may have caused an uneven distribution of marker in the faeces. In digestibility studies on *H. rubra* and *H. laevigata*, Maguire *et al.* (1993) distinguished three types of faeces but found only minor variation in their chemical composition and Cr_2O_3 content. In the present study, abalone maintained on diets containing Cr_2O_3 did however develop a green tinge in their shells indicating that some of the marker was assimilated. Lastly, while efforts were made to avoid contamination of the faeces with uneaten food and other foreign matter, it is possible that reingestion of pedal mucous could have diluted the nutrient concentration in their guts. A comprehensive investigation will however be required to quantify each of these possible effects and devise ways of overcoming them.

The nutritional indices (FCR, PER, and PPD) applied in the present growth trials yielded values in the range reported for fish species (Halver, 1989; Steffens, 1989), and corroborate the view that digestion and assimilation in abalone is analogous to that of other monogastric animals which rely on enzymatic digestion. The nutrition of *H. midae* was however shown to differ from that of fishes in a number of key respects, visibly, its carbohydrate based metabolism and concomitant low capacity for utilising lipid as an energy source. This characteristic is however advantageous with respect to diet manufacture, as supplemental lipid, which tends to inhibit effective pellet binding, can be minimised, and the binding properties of carbohydrates exploited to achieve highly water stable pellets. At the recommended lipid inclusion levels, ca. 2.5 - 7.5% (Uki and Watanabe, 1992; Mai *et al.*, 1995; Chapter 6), dietary requirements can be met by the bound fat in the ingredients, precluding the addition of extra oil.

H. midae was also found to differ substantially from fish in the response of its body composition to dietary levels of protein and energy. The fairly large changes observed in the protein and carbohydrate of *H. midae* soft tissue, do not occur in vertebrates (Buckley and Groves, 1979), and this is clearly an area deserving of more attention. Of particular relevance is the influence of body composition on the organoleptic properties of abalone, as the target market for the product is the Japanese sushi trade, which is very discerning about product quality.

The size specific differences that emerged with respect to protein and energy utilisation are not surprising, as it is well documented that the nutritional requirements of fishes, and other organisms, change with age (Steffens, 1989). The apparently lower protein requirement of young abalone is nonetheless an interesting result, however, a full explanation of the underlying biochemistry must await further investigations.

In conclusion, the prospects for developing diets for abalone according to established nutritional principles look very promising, which bodes well for the development of commercial abalone culture based on artificial feeds. A commercial feed based on the present research is already in production, and evaluation of the feed at research institutions, as well as on abalone farms, in Japan, New Zealand, Mexico, and the U.S.A. has yielded very positive feedback. The availability of a commercially viable dry feed, and feeding protocol, provides a foundation upon which an intensive farming technology for abalone can be built.

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