DEVELOPMENT OF AN ARTIFICIAL WEANING DIET FOR THE SOUTH AFRICAN ABALONE, *Haliotis midae* (HALIOTIDAE: GASTROPODA)

Submitted in Fulfilment of the

Requirements for the Degree of

MASTER OF SCIENCE

of Rhodes University

by

JENS KNAUER

January 1994

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I am indebted to Prof. Thomas Hecht, Prof. John Duncan and Peter Britz for supervision of this project. Their guidance and encouragement was greatly appreciated. A very special thanks goes to Peter Britz for his patience and constant enthusiasm.

Thanks are extended to the staff of the library of the JLB Smith Institute of Ichthyology for their help in supplying literature.

My gratitude to Wayne Barnes of Marine Products (Pty.) Ltd, Craig Clayden, Gustav Groenewald, Donald Spence and Dean Stevens of Sea Plant Products (Pty.) Ltd, as well as Dr. Pierre Hugo and Paul Kabalin of the Old Harbour Marine Biological Laboratory for their part in making the experimental work in Hermanus possible.

Dr. Rob Anderson of the Sea Fisheries Research Institute in Cape Town is thanked for the analysis of the gut contents of juvenile abalone, and Dr. Robin Carter of the C.S.I.R's Institute of Oceanology in Stellenbosch for the supply of juvenile abalone. I am very grateful to Dr. Dean Brady of the Department of Biochemistry and Microbiology, Rhodes University for his help with the mineral element analysis. Thanks are also due to Dr. Horst Kaiser of the Department of Ichthyology and Fisheries Science, Rhodes University for his help, comments and criticisms.

The financial support of Sea Plant Products (Pty.) Ltd., the Foundation for Research Development and Rhodes University is gratefully acknowledged.

Final thanks go to my parents, without whose moral and financial support I could not have pursued my interests in biology.

ABSTRACT

An adequate supply of diatoms during the weaning stage (generally 5 - 10 mm shell length (SL)) is one of the primary constraints to the commercial culture of the South African abalone, *Haliotis midae.* Because of the seriousness of the problem, a project aimed at the development of an artificial weaning diet was initiated.

Initially, the chemical composition (proximate composition, amino acid, fatty acid and mineral element profile) of juvenile ***H. midae*** was analyzed, as a general lack of such information was identified in a review.

Due to the lack of knowledge on the nutritional requirements of *H. midae,* the formulation of the weaning diet was based on the essential amino acid (EAA) pattern of the shucked tissue, and the known nutrient requirements of haliotids. Subsequently, a water stable gel and pellet form of the diet were developed. The best water stability of a gel was obtained with a 1:3 agar/gelatine mixture which retained 70.7 ± 2.7 % of its dry weight after 24 h. Starch based pellets, however, retained 89.0 ± 0.6 % of their dry weight after 24 h.

In a comparative growth trial, pellets produced a significantly better increase in SL and weight than gels after only 15 days. This was probably due to the better water stability of pellets, which resulted in a better nutritional quality than in gels. The feeding behaviour on both forms of the diet did not differ. Activity patterns were exclusively nocturnal and feeding frequency was consistently low. The percentage composition of the pelleted weaning diet, on a dry weight basis, was 5 % casein, 15 % gelatine, 15 % fish meal, 10 % *Spirulina* spp., 2.5 % fish oil, 2.5 % sunflower oil, 21.0 % dextrin, 23.0 % starch, 4.0 % of a mineral and 2.0 % of a vitamin mixture. The correlation coefficient between the EAA pattern of *H. midae* and the dietary EAA pattern was $r^2 = 0.8989$.

Pellets were fed to juveniles in a 30 day growth trial to study the effect of photoperiod (12, 16, 20 and 23 hours of darkness) on growth and general nutritional parameters. A comparative experiment feeding diatoms was conducted under a 12hL: 12hD light regime at the same time. The SL and weight of the juveniles did not increase significantly with an increase in hours of darkness. The growth of juveniles fed on pellets did not differ significantly from those fed on diatoms.

Percentage feed consumption (PFC), percentage feeding rate (PFR), feed conversion ratio (FCR), protein efficiency ratio (PER) and percentage protein deposited (PPD) were determined for the animals fed on pellets. None of the parameters were significantly affected by photoperiod. However, there were trends in that PFC increased with longer periods of darkness, while PPD decreased. The FCRs (0.44 ± 0.04 to 0.60 ± 0.19) and PERs (5.06 ± 1.74 to 6.64 ± 0.77) indicated that juveniles used the feed, and in particular the protein, very efficiently.

Photoperiod did not have an effect on the specific activity of the digestive enzymes amylase, protease and lipase. The specific activity of amylase in the juveniles fed on diatoms was significantly higher than in the pellet fed groups. This was surprising as the main carbohydrate of diatoms is the B-(1—)3) glucan chrysolaminarin, and not starch, a B-(1-34) glucan. Protease specific activity, on the other hand, was significantly higher in the pellet fed groups, indicating an ability to adapt to the high protein content in the artificial diet (35.48 %), compared to diatoms which had a protein content of 5 %. The specific activity of lipase did not differ significantly between groups, probably because of a similar lipid concentration (5 - 10 %) in diatoms and pellets.

Finally, the effect of stocking density, ranging from 1250 to 10,000 juveniles/m^2, on the growth of juveniles was evaluated. A model of hatchery productivity was developed based on this investigation. Hatchery productivity was defined as the number of juveniles per unit space reared

through to the grow-out stage per unit time. The model predicted that maximum productivity would be achieved at a stocking density of 10,000 juveniles/m^2.

The results have shown that *H. midae* can be successfully weaned on an artificial diet, as the growth on the diet was not significantly different to growth obtained on diatoms. Long-term growth trials are needed to confirm these results. The importance of standardized experiments on the nutritional requirements and digestibility of abalone was emphasized. The importance of improved artificial diets, optimal culture conditions, as well as the application of biotechnological techniques to further abalone aquaculture was highlighted.

CHAPTER 1

INTRODUCTION

Abalone are marine snails of the genus *Halioris* and are grouped in the monogeneric family Haliotidae (Cox 1960). As archaeogastropods they are among the oldest and least specialized members of the gastropod class Prosobranchia (Barnes 1980).

Haliotis species are characterized by their single oval shell and their large muscular foot (Hooker & Morse 1985). The shell characters typical of extant haliotids are already evident in 70 million year-old fossils of *Haliotis* species from California (Lindberg 1992). The shell covers the entire dorsal side of the animal's body and is perforated along the side by a line of respiratory pores (Cox 1960). The shape, colour and texture of the shell as well as the number and form of the respiratory pores are often used to identify species (Cox 1960). However, there is a considerable discrepancy in the literature regarding the number of extant *Haliotis* species. Cox (1962) referred to about 130 species and subspecies, Hahn (1989a) and Fallu (1991) to about 100, Hooker & Morse (1985) mentioned 80, Lindberg (1992) 60 - 70, while Tarr (1989) recognized only 50.

Abalone are globally distributed along the rocky shores of all major continents (Lindberg 1992), with the exception of South America and eastern North America (Hahn 1989a). Recently, however, *H. rufescens* has been experimentally introduced into Chile (Godoy *et al.* 1992). The greatest number of species is found in the south and central Pacific and parts of the Indian Ocean (Cox 1962). The most abundant populations are found along the coasts of Australia, Japan and western North America (Cox 1962). Bathymetrically, most species occur between the intertidal and the shallow littoral zone to about 20 m in depth, although the tropical New World species are typically found at depths of 50 - 200 m (Lindberg 1992). Generally, the habitat requirements of abalone include good water circulation to remove wastes and sediment, the correct substrate for settlement and protection from predators, and a good supply of food (Tegner & Butler 1989).

Six species of abalone are found along the shores of South Africa (Muller 1986). The largest of these, *Haliotis midae* Linnaeus 1758, locally known as perlemoen, grows to a size of 230 mm shell length (SL) in 30 years (Newman 1968). It occurs on sublittoral rocky shores between St. Helena Bay (32°45' S, 18°5'E) on the west coast, and Port St. John's (31°58'S, 29°10'E) on the east coast (Newman 1965, Field *et al.* 1977, Muller 1986, Figure 1.1). The greatest abundance of animals is found off the southwestern Cape coast between Cape Hangklip and Quoin Point in depths of less than 10 m, where the animals live in beds of the kelp *Ecklonia maxima* (Tan 1992, Figure 1.1).

The delicately flavoured meat of abalone has attracted man for at least 125,000 years (Voigt 1982). In South Africa many "strandlooper" middens, dating back over 6000 years, consist almost entirely of abalone shells (Avery 1974, Van Noten 1974, Robertshaw 1977, Poggenpoel & Robertshaw 1981, Smith 1981). The first written account of abalone was by Aristotle in the 4th century B.C., who referred to "the other *Patella major"* under the name "Aporrhias". The oldest

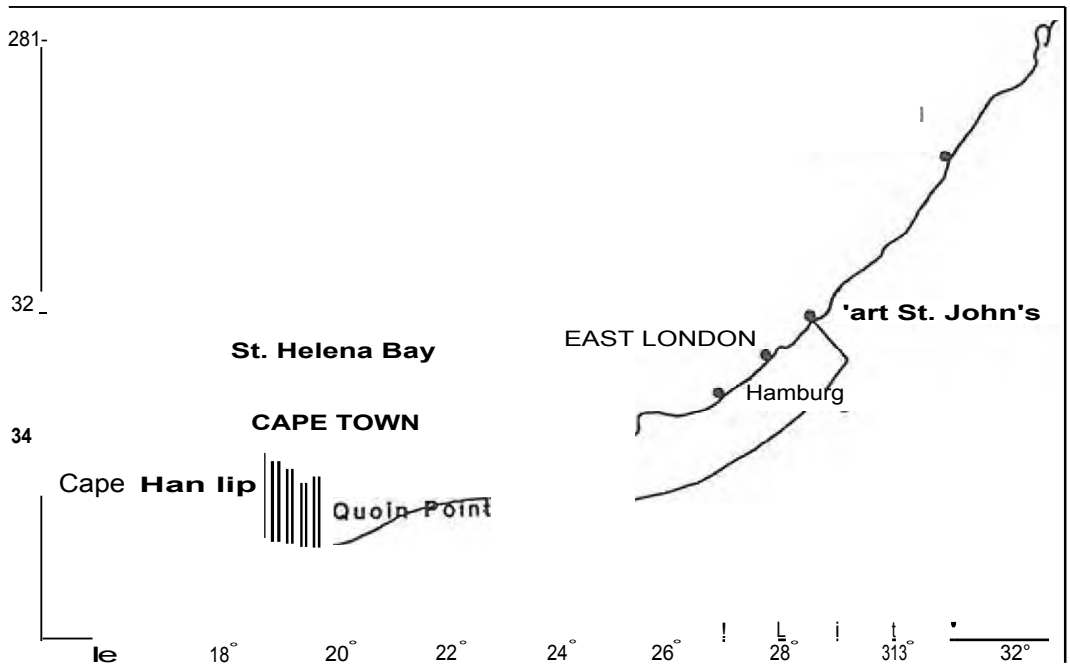

Figure 1.1. Distribution of the South African abalone, *Haliotis midae*. The vertical lines indicate the area of the greatest abundance. The seaward distribution is not drawn to scale.

written record of a fishery for abalone is a report from Japan in the year 425, in which a diver lost his life while diving for an abalone pearl (Cox 1962).

In contrast to prehistoric times, abalone are now considered to be a luxury food (Olley & Thrower 1977). The world-wide demand for abalone is centered in the Far East, especially Japan and China (Tarr 1989). The muscular foot can be purchased canned, dried, fresh or frozen. It is prepared in many different ways, sliced as steaks, cooked with other food or consumed raw (Cox 1960, 011ey & Thrower 1977). The shells are used for decorative purposes, jewellery, and in Asia also in traditional medicines (Cox 1960, Hooker & Morse 1985).

In South Africa, *H. midae is* the only species of commercial importance, due to the small size of the other five South African haliotids (Barkai & Griffiths 1986). In response to a demand for abalone in the Far East, commercial exploitation of ***H.*** *midae* began in 1949 along the south west Cape coast (Tarr 1992). In 1953, 770 tonnes were harvested and 1500 tonnes in 1954. Due to a variable demand abalone catches fluctuated, but peaked at 2800 tonnes in 1965. Since then catches have declined continually due to stock depletion (Tarr 1992). Currently, about 605 tons whole weight/year of *H. midae* are fished commercially (Anonymous 1993). More than 90 % of the catch is exported to the Far East with an estimated value of R16 million (Tarr 1992). A small abalone fishery of 3 tonnes whole weight/year has recently been re-started at Hamburg along the south east coast (Wood 1993, Figure 1.1). Recreational divers are currently restricted to 4 abalone/person/day with a minimum size of 11.43 shell breadth (13.80 cm SL). Furthermore, there is a closed season from August 1 to October 31 each year (Wood 1993). Both of these restrictions also apply to commercial divers.

Commercially important abalone fisheries also exist in Australia (Prince & Shepherd 1992), Canada (Farlinger & Campbell 1992), France (Clavier 1992), Mexico (Guzman del Proo 1992), New Zealand (Schiel 1992), Oman (Johnson *et al.* 1992) and the U.S.A. (Tegner 1989, Farlinger & Campbell 1992, Parker *et al.* 1992, Tegner *et al.* 1992). Similar to the South African situation,

most of these fisheries have also declined over the past two decades (Fallu 1991). Unfortunately, owing to the biology and life history style of abalone, overfishing is inevitable. The animals live in relatively predictable and accessible locations, exhibit slow growth and recruitment is unpredictable (Tegner & Butler 1989). It is not surprising, therefore, that the global harvest from abalone fishery has shrunk from 20,000 tonnes in 1975 (Anonymous 1975) to 13,600 tonnes in 1989 (Anonymous 1989). Simultaneously, there has been an increase in demand, driving retail prices for the meat from US$ 44 - 66/kg in the mid 1980s (Hooker & Morse 1985) to approximately US$ 74/kg in the early 1990s (P.J. Britz 1993, Department of Ichthyology and Fisheries Science, Rhodes University, Grahamstown, South Africa, pers. comm.).

During the 1960s, Japanese fisheries authorities realized that an increase in the demand for abalone could only be met by way of aquaculture (Grant 1981). The basis for abalone aquaculture had already been laid by the research of Murayama (1935 in Hahn 1989b) and Ino (1952). Experimental hatchery work in Japan was started in the late 1950s and soon thereafter animals were successfully grown to a length of 2 cm and sold locally to reseed natural reefs (Tamura 1960 in Shepherd 1976). An intensive research programme to establish culture techniques for abalone was established in the early 1970s (Grant 1981).

The global decline in abalone fishery, the escalating price of the product (Hooker & Morse 1985) and the Japanese research results, triggered an interest in abalone culture all around the world in the late 1970s (Ebert & Houk 1984). Since then, various countries have devoted research efforts to problems such as spawning of adults, rearing and settlement of larvae, and the nutrition of post-settlement animals. Today abalone cultivation is either practiced or being developed in many countries, with the most successful operations being run in Japan, Taiwan and the U.S.A. (Hecht & Britz 1990).

The current status of abalone aquaculture and the culture techniques used in Australia (Hahn 1989c), Chile (Godoy *et al.* 1992), China (Nie 1992), France (Hahn 1989d), Ireland (Hahn

1989c), Japan (Hahn 1989b,e), Korea (Yoo 1989), Mexico (Mazon-Suastegui *et al.* 1992, Salas Garza & Searcy Bernal 1992), New Zealand (Hahn 1989c, Tong & Moss 1992), the U.S.A. (Ebert & Houk 1989, Hahn 1989f, McMullen & Thompson 1989, Ebert 1992), Taiwan (Chen 1989) and Thailand (Singhagraiwan & Doi 1993) have been reviewed.

Currently, there are three options available for abalone farming. Firstly, the release of hatchery-reared juvenile abalone to enhance natural stocks. This was pioneered by the Japanese during the 1960s (Tegner & Butler 1989). Spat is released into suitable natural habitats and left to grow until they are big enough to harvest. Although only minimal investment is required this method of ranching is risky because of the potentially high losses through predation and poaching (Saito 1984, Tegner & Butler 1985, Tong *et al.* 1987). Reported results for seed survival range from 1 - 80 % (Saito 1984, Tegner & Butler 1985). Consequently, this method is only practiced in a few countries such as Japan (Hahn 1989b), China and France (Hooker & Morse 1985).

The second option is open-ocean ranching in containment systems. This allows for high-density cultivation under ideal water quality conditions. Abalone are contained in cages which are suspended from buoys or piers or anchored to the ocean floor. However, the feeding of macroalgae as well as genera] maintenance of the systems are problematic (Hooker & Morse 1985).

Thirdly, abalone can be reared in shore based units in tanks, raceways or ponds using pumped sea water (Hooker & Morse 1985). Globally, this is the most widely used method. Besides the high investment (land, facilities etc.) and operating costs (pumping of sea water, maintenance etc.), the advantages of this system are the relative ease of maintenance and the high degree of control over every step in the production cycle.

In response to the decline *in* the abundance of the natural stocks in South Africa the then Division of Sea Fisheries launched an abalone research programme in 1962 (Newman 1966,

Barkai & Griffiths 1987). Newman (1966) studied the movements, reproduction (1967a), growth (1968) and distribution (1969) of *H. midae* in south west Cape waters. He also summarized the status of abalone research *in* South Africa (Newman 1967b). The history, status and future of the South African abalone fishery was reviewed by Tarr (1989, 1992). Recently, Wood (1993) analyzed the population structure, age, growth, and reproduction of *H. midae* along the east Cape coast. The diet and aspects of the nutritional physiology were studied by Barkai & Griffiths (1986, 1987, 1988), Dixon (1992) and Wood (1993). First attempts to culture *H. midae* were made in the early 1980s when Genade *et al.* (1988) spawned animals and reared the juveniles.

South Africa, in contrast to many developed countries, still has a relatively underpopulated and pollution-free coast line, which is well-suited for the culture of abalone (Hecht & Britz 1992). The main challenge in South Africa, since the technology for the culture of abalone is relatively well established, is to adapt existing techniques to local conditions (Hecht & Britz 1990). The Council for Scientific and Industrial Research (C.S.I.R.) at Stellenbosch, the University of Cape Town and Rhodes University in Grahamstown in combination with the private sector initiated a comprehensive research programme in 1988/89 (Hecht & Britz 1990). The C.S.I.R. and the University of Cape Town have developed techniques for the production of seed abalone, while optimal rearing conditions and the nutrition of abalone have been the focus of research at Rhodes University. Encouraged by the progress in this collaborative research programme, Hecht & Britz (1992) listed *H. midae* as a species on the threshold of commercial production in South Africa.

World-wide, the predominant food used for the culture of abalone are diatoms up to the weaning phase and macroalgae during the grow-out phase (Hooker & Morse 1985, Hahn 1989g). Thus, the nutritional values of different diatom and macroalgae species have been evaluated in many studies (e.g. Paul *et al.* 1977, Uki & Kikuchi 1979, Han *et al.* 1986, Uki *et al.* 1986a).

It is common practice in most countries not to add any nutrients during the larval rearing stage. In the U.S.A. larvae have been reared in sterile water (Fallu 1991), while on the other hand, it

has been traditional practice in Japan to use "green water" for larval rearing. Although it has not yet been quantified, it has been claimed that larvae "do much better if micro-algae are available" (Fallu 1991, p. 71). Generally, however, the nutrition of larval abalone does not present any problems.

One of the major impediments in abalone aquaculture is the provision of food for juveniles from 2 - 3 mm SL onwards (Mozqueira 1992). The supply of diatoms for abalone between 5 - 10 mm SL, i.e. during the weaning stage, has been identified as a major obstacle in abalone nutrition (Ebert & Houk 1984, P.J. Britz 1992, pers. comm.). Diatom growth has to be carefully controlled to maintain the right balance between postlarval growth and food availability, and can even then become a limiting factor (Hahn 1989d). Sudden changes in the diatom film can result in a high mortality rate among juveniles (Hahn 1989d). The low rate of survival of artificially reared abalone from the larval stage to 20 mm SL is partly due to problems relating to the quality and quantity of diatoms (McCormick & Hahn 1983). Adding to this problem, Ebert & Houk (1984) reported that attempts to feed macroalgae to juveniles at the weaning stage resulted in a temporary cessation of growth and an increase in mortality.

The use of macroalgae for the grow-out phase of abalone is also problematic. Mozqueira (1992) summarized three areas of concern. Firstly, it may be ecologically unsound to remove macroalgae from the sea. Secondly, macroalgae are known to host a variety of organisms or their eggs (crabs, seastars, snails) which may be predators or parasites of abalone, or compete for space and food. Thirdly, macroalgae are often only available on a seasonal basis. Hahn (1989g) reported the purchase and storage of kelp to be the single largest cost item of abalone farms in Japan. Collection, processing, storing and feeding of macroalgae is labour intensive, and the processes do not allow for much mechanization. In order to have direct access to macroalgae, the abalone farm should ideally be located close to natural macroalgae beds. This limits the sites for potential farms (Hahn 1989g, Uki & Watanabe 1992). Norman-Boudreau (1988) also stated that the growth of the abalone industry in the U.S.A. has been limited by the availability of natural food, both

in terms of location and quantity. Moreover, Ebert (1992) predicted that due to the patchy distribution of kelp along the coast of the Pacific Northwest of the U.S.A., the growth of abalone aquaculture in that region would be limited.

The most important natural food for the grow-out phase of *H. midae* would be *Ecklonia maxima (Barkai &* Griffiths 1986). Even if harvested on a daily basis, the amounts required would be enormous: to produce 85 tons of medallion sized abalone a year, 9 tons of fresh kelp would have to be supplied daily (Hecht & Britz 1990). Moreover, harvesting of kelp is dependent on sea conditions. The exposed conditions of the South African coast would limit the number of days of harvesting, thereby making a regular supply of macroalgae difficult if not impossible.

The Japanese were the first to recognize the advantages of using artificial diets instead of natural food for the farming of abalone. Artificial diets allow for mechanized production (Hahn 1989g), result in increased survival rates (McCormick & Hahn 1983, Hahn 1989d), and generally produce better growth in weight and length than natural food (Hahn 1989e,g).

Practical diets in gel form have been formulated for *H. discus* (Ogino & Ohta 1963, Ogino & Kato 1964, Sagara & Sakai 1974) and *H. sieboldii* (Sagara & Sakai 1974), while a semi-purified diet for *H. discus hannai* has been developed in the form of a cylindrical paste (Uki *et al.* 1985a). In France, juvenile *H. tuberculata* have been successfully reared on an artificial diet in gel form (Koike *et al.* 1979). A pelleted diet has recently been developed in Mexico for juvenile *H. fulgens* (Viana *et al.* 1993). Practical, extruded diets have also recently been developed in Australia for *H. laevigata* (Morrison & Whittington 1991), *H. rubra* (Gorfine & King 1991), and in South Africa for *H. midae* (Dixon 1992, Britz *et al.* 1994).

To date, artificial diets for the weaning and/or grow-out phase have been used in commercial abalone culture in China (Nie 1992), France (Hahn 1989d), and Japan (McCormick & Hahn 1983, Hahn 1989e,g). The early workers (Ogino & Ohm 1963, Ogino & Kato 1964, Sagara &

Sakai 1974, Koike *et al.* 1979) specifically aimed at providing a weaning diet, but projects to develop an artificial weaning diet have recently again been initiated because of the seriousness of the problem (Chen 1985, Nie *et al.* 1986, Norman-Boudreau 1989a,b, Britz *et al.* 1994).

In South Africa, a pelleted practical diet has already been formulated for the grow-out phase of *H. midae* (Dixon 1992, Britz *et al.* 1994). The major problem for a commercial operation, however, is the adequate provision of food during the weaning stage. Therefore, it was the principal aim of this study to develop an artificial weaning diet suitable for commercial application.

Initially, a review of abalone nutrition was undertaken (Chapter 2) which led to the analysis of the chemical composition of juvenile *H. midae,* presented in Chapter 3. This provided the basis for the formulation of an artificial weaning diet (Chapter 4). The next stage of the project involved the development of a water stable gel and pellet form of the diet (Chapter 5). This was followed by an evaluation of the gel and pellet form in terms of the feeding behaviour and growth of *H. midae* (Chapter 6). To evaluate the diet under culture conditions, the effects of photoperiod on growth and nutritional parameters of juveniles were studied (Chapter 7). Based on an investigation into the effects of stocking density on growth a model of hatchery production was developed, as described in Chapter 8. The study was concluded with a general discussion (Chapter 9).

CHAPTER 2

A REVIEW OF ABALONE NUTRITION

INTRODUCTION

Within the phylum Mollusca the class Gastropoda is by far the largest and most diverse with circa 75,000 existing species and about 15,000 fossil forms dating back to the Cambrium (Barnes 1980). The success of the gastropods appears to be largely attributable to the functional and structural plasticity of their feeding apparatus, particularly the radula and the buccal mass (Owen 1966a, Purchon 1977, Kohn 1983, Hawkins *et al.* 1989). This plasticity has allowed them to develop a multitude of all feeding strategies, ranging from herbivory to carnivory, deposit and suspension feeders to scavengers and parasites (Owen 1966a, Purchon 1977, Barnes 1980, Salvini-Plawen 1988). Herbivory, in contrast to carnivory, is the more primitive feeding mode and is found in archaeogastropod prosobranchs such as *Haliotis* species, some primitive opisthobranchs and pulmonates (Purchon 1977).

The composition of the natural diet of abalone is the only aspect of nutrition that has been extensively studied. In contrast, the nutritional physiology and biochemistry of *Haliotis* species, despite their world-wide economic significance, has not received much attention. Thus, where information on *Haliotis* species is lacking it has been inferred from studies on other herbivorous gastropods. This is possible since the three subclasses, Prosobranchia, Opisthobranchia and Pulmonata, cannot be distinguished on the basis of their food preferences and feeding biology. In particular, prosobranchs and opisthobranchs have radiated with some degree of parallel evolution in their feeding biology (Purchon 1977). Digestive processes discovered in herbivores or carnivores, therefore, generally apply irrespective of the subclass a species belongs to. Where information on physiological processes in gastropods is scarce or absent, studies done on other molluscs have been included.

STRUCTURE AND FUNCTION OF THE ALIMENTARY TRACT

Introduction

Herbivorous gastropods, such as abalone, have a long and complex alimentary tract (Figure 2.1). Detailed information on the anatomy of the alimentary tract of haliotids is scarce. Crofts (1929) published a general anatomical account of *H. tuberculata* with detailed descriptions of the radula, odontophore and associated muscles, and described the organogenesis of the same species (Crofts 1937). The anatomy and function of the alimentary tract of *H. cracherodii* was examined by Campbell (1965), and the digestive processes of ***H.*** *rufescens* were studied by McLean (1970). With the exception of minor details the morphology of the alimentary tract of ***H.*** *rufescens* corresponds to that of *H. cracherodii.* It is reasonable to assume, even in the absence of other studies, that the structure and function of the digestive system is similar in all haliotids. Moreover, Campbell (1965) confirmed the findings on digestive functioning in other archaeogastropods as described by Graham (1949) and Morton (1953).

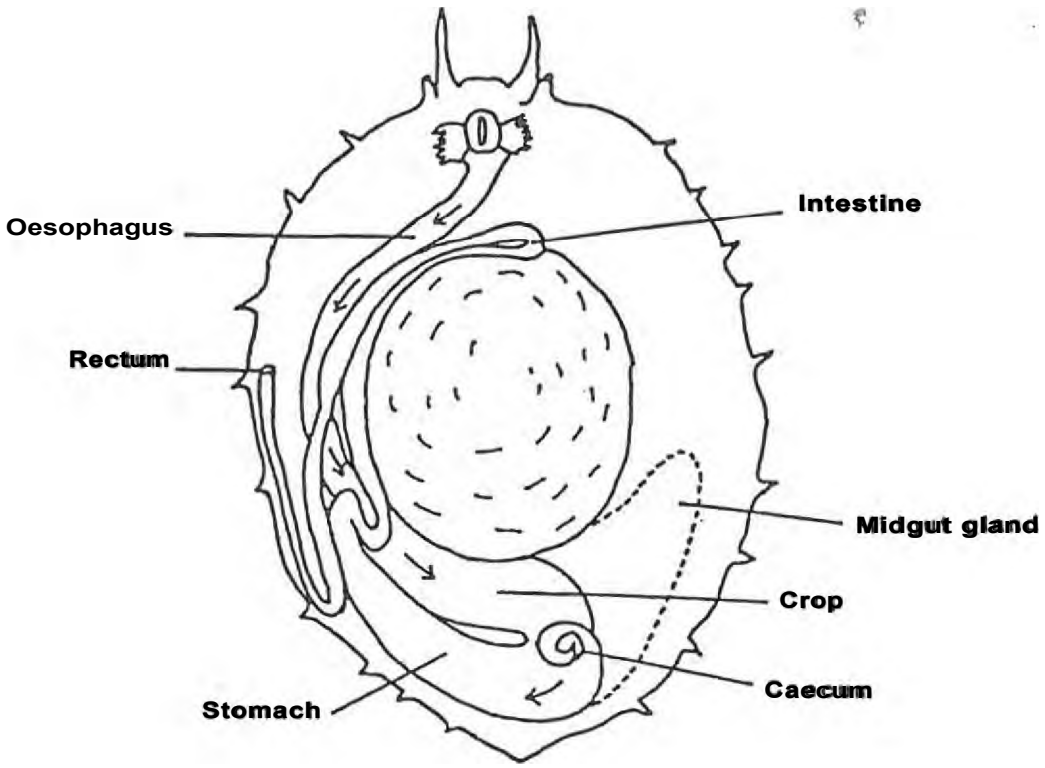

Figure 2.1. Dorsal view of the alimentary tract of abalone (modified from Shepherd 1973).

Buccal Region

The basic feeding apparatus of haliotids comprises a terminal mouth and a buccal cavity containing the projecting odontophore with the radula. The mouth is typically short, muscular and mobile. In *H. cracherodii it* is bordered by lips, which are covered with papillae (Campbell 1965), and probably function in the tasting of potential food items. The radula of haliotids is of the rhipidoglossate type, probably the most primitive gastropod radula (Kohn 1983). Each row of teeth comprises a large central tooth, lined by five lateral and many slender marginal teer h on either side (Kohn 1983, but see Crofts 1929 for more detail). The feeding mechanism of the rhipidoglossate radula was described by Fretter & Graham (1962).

Food particles are rasped off by the radula and transferred to the buccal region. The buccal cavity is lined with a cuticle and contains mucocytes, sub-epithelial glands and mucus-secreting follicles, which mix the food with mucus (Campbell 1965). The lateral wall of the buccal cavity is stiffened by a pair of separate or fused jaws with a denticulate or ribbed surface (Salvini-Plawen 1988). The cavity is continuous with the pharynx, most of which is occupied by the radular apparatus_ A pair of salivary glands opens into the buccal cavity {Campbell 1965).

Oesophagus

The food passes the openings of the salivary glands and is carried down the oesophagus by strong ciliary action (McLean 1970, Salvini-Plawen 1988). The secretions from the salivary glands probably lubricate the radula and also help to transfer the food down the oesophagus (Campbell 1965).

The oesophagus has an anterior, middle and posterior section (Hyman 1967). A dorsal food groove runs down the oesophagus which splits into numerous ridges in the posterior region. In *H. cracherodii* the mid-oesophagus contains paired lateral pouches (or oesophageal pouches) which mix the food/mucus strand with secretions (Campbell 1965). The posterior part of the oesophagus is expanded into a crop, forming the largest lumen in the alimentary tract (Campbell

1965). As the passage of food through the caecum and stomach is slow, food and digestive juices are mixed for extended periods in the crop (Campbell 1965). Thus, it is possible that digestion and absorption already occur in the crop (Owen 1966b, McLean 1970).

Stomach

The stomach (Figure 2.2) is generally divisible into a ventral and dorsal portion. The ventral part includes the openings of the midgut gland, the ciliated sorting area and the intestinal groove. The dorsal part consists of a proximally developed gastric shield and the distally located food/mucus string or protostyle (Salvini-Plawen 1988).

The opening of the oesophagus is separated from the stomach by the stomach fold stretching to the end of the caecum (Fretter & Graham 1976). Ciliated folds from the oesophagus and the stomach, the major typhlosole and the posterior sorting area extend to the tip of the flat, tubular caecum (Campbell 1965). Entering from the oesophagus, the food/mucus strand is directed to the caecum for initial sorting and mixing (Graham 1949). The food material passes to the tip of the caecum and back through the caecal groove. This groove is unciliated, but due to lubrication and pressure from the crop the food particles are moved to the openings of the midgut gland (Campbell 1965).

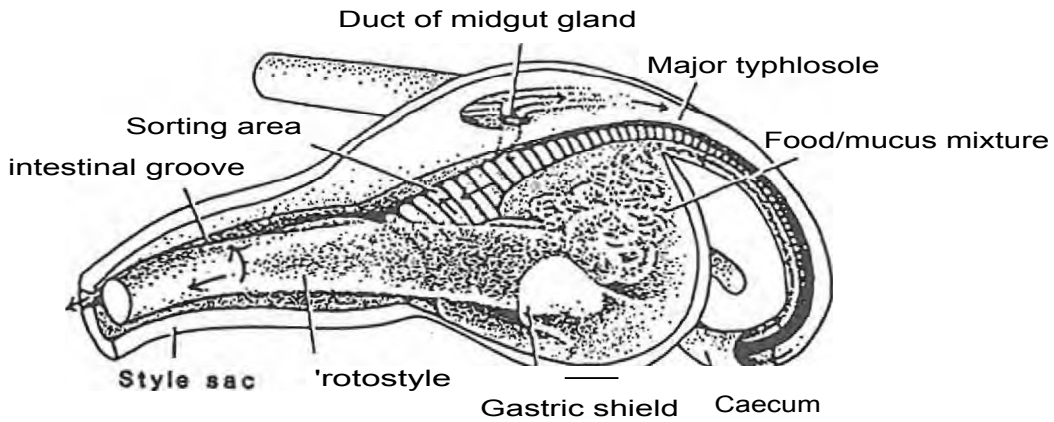

Figure 2.1. The stomach of a generalized prosobranch gastropod (modified from Fretter & Graham 1976).

The stomach contents are a mixture of material from the midgut gland, fine particles sorted out in the caecum, and material from the oesophagus that entered the stomach directly, such as large fragments of kelp or gravel (Campbell 1965). The food material in the stomach is twisted in a rotating strand, the protostyle (Campbell 1965). The protostyle is rotated by the cilia of the style sac (Morton 1953) and rotates against the gastric shield. This is a cuticle like structure, which attains its maximum thickness near the tip of the protostyle (Morton 1953, Salvini-Plawen 1988). By this action the mixing of the protostyle material and the digestive juices is ensured (Fretter & Graham 1976, Salvini-Plawen 1988). At the same time, the gastric shield protects much of the stomach from abrasion (Morton 1953). Rotation of the protostyle allows the removal of faecal material by the posterior sorting area, into the intestinal groove (Graham 1949). The posterior sorting area is comprised of a series of ciliated ridges which cover most of the stomach floor (Campbell 1965). Leighton & Boolootian (1963) found this area to be covered with sand, and speculated that it removes sand grains from the stomach. The intestinal groove is formed by the major and minor typhlosole. Due to the close apposition of the typhlosoles and the ciliary currents on their ridges, the contents of the intestinal groove are isolated from the rest of the stomach and transported directly to the intestine (Graham 1949).

Midgut Gland

The midgut gland, also known as the hepatopancreas or the digestive gland, makes up the bulk of the visceral mass and comprises numerous blind ending tubules. These fuse to form more or less branched ducts, entering the crop and the stomach via one or multiple openings (Campbell 1965, Owen 1966b, Salvini-Plawen 1988). The ducts are lined with a ciliated epithelium (Hyman 1967). The midgut gland is made up of two different cell types. Firstly, digesting/absorbing cells, which in the early stages are provided with cilia. Their function is to absorb, phagocytose and store food. The second cell type contains many mitochondria and has an excretory and/or calciferous function (Owen 1966b, Salvini-Plawen 1988). These cells produce a secretion which is shed into the tubules, the function of which is not known (Graham 1931, Fretter & Graham 1962). A modified form of this cell type has been found to store calcium ions in the tubules of

many pulmonates (Owen 1966b).

Transport of selected particles, together with soluble products from extracellular digestion, to the tubules probably involves muscular contractions of the stomach, rhythmic contractions of the tubules or even a counter-current ciliary system (Owen 1966b, McLean 1970). Generally, in the Gastropoda phagocytosis and subsequent intracellular digestion is known to occur in the midgut gland (Owen 1966b), although McLean (1970) did not find any evidence for this in ***H.*** *rufescens.*

Undigested food particles and waste products coming from the midgut gland enter the intestinal groove (Graham 1949, Fretter & Graham 1976). The content of this channel is referred to as the "liver string" (Fretter & Graham 1976). At the same time, the protostyle is moved towards the intestine forming the "stomach string" (Fretter & Graham 1976).

Intestine

The intestine in herbivorous gastropods is tubular, looped or coiled (Figure 2.1), and generally ciliated. According to Campbell (1965), there are five distinct regions in the intestine of ***H.*** *cracherodii.* Region I and II constitute the intestinal loop. Region I is sparsely ciliated. Region II is thrown into numerous longitudinal ridges and is referred to as the intestinal sorting area (McLean 1970). Cilia on the ridges beat into the grooves, and cilia in the grooves beat away from the stomach. Intermediate sized material is twisted into strands by ciliary action as well as the secretion of mucus. Much of the "stomach string" is composed of the twisted strands formed in this region (Campbell 1965). The intestinal sorting area stops abruptly at region III which extends anteriorly to where the right shell muscle embeds the intestine. Characteristic of this region are the strong ciliary currents on the typhlosoles which are directed into the intestinal groove. Region IV follows the left side of the viscera to below the style sac, shortly before the mantle cavity. In regions III and IV the remaining coarser material is compacted with the previously formed strands. Judging by the length of these regions, which exceeds the rest of the intestine, this is a considerable task. Region V comprises the rectum, which lies mainly in the

mantle cavity. As the faecal rod passes through the intestine it is fragmented by muscular action (McLean 1970). In the rectum, the intestinal contents are compacted and enveloped with mucus. Brough & White (1990), however, concluded that the rectum in the prosobranch *Littorina saxatilis* probably also removed material from the blood and maybe even played a final role in absorption.

The differentiation of the intestine of *H. cracherodii* may be the result of two factors. Firstly, the intestine is shorter relative to total body length than e.g. in limpets. Secondly, the diet comprises a wide size range of ingested material, which in order to be organized into faeces probably requires different functional areas (Campbell 1965).

NATURAL DIET OF ABALONE

The completion of the larval stage, i.e. from fertilization to metamorphosis, takes about 4 - 10 days (Hooker & Morse 1985). The rate of larval development depends on the species and water temperature (Hahn 1989h).

It is commonly held that abalone larvae are lecithotrophic and that no additional food is required during this early ontogenetic phase (Ebert & Houk 1984, Hahn 1989h, Spaulding & Morse 1991). However, larvae which are incapable of capturing particulate food are not necessarily energetically independent of their environment. It has been shown for the larvae of *H. rufescens* that they absorb amino acids (AAs) as well as glucose and maltose from seawater (Jaeckle & Manahan 1989, Manahan *et al.* 1989, Welborn & Manahan 1990, Manahan & Jaeckle 1992). The potential energy gain from these substrates is high. AA uptake, e.g., has been found to account for up to 70 % of the metabolic rate of two day old veliger larvae (Jaeckle & Manahan 1989).

The transition from the larval to the post-larval stage is characterized by settlement, metamorphosis and the deposition of the peristomal shell (Hahn 1989h). In natural environments,

substratum-specific settlement of *Haliotis* larvae occurs on crustose red algae (Corallinaceae, Rhodophyta) (Crofts 1929, Saito 1981, Shepherd & Turner 1985, Prince *et al.* 1987). Consequently, juvenile abalone (1 - 20 mm shell length (SL)) of several species have been found to occur at densities several orders of magnitudes higher on rocks with crustose red algae than similar nearby habitats without (Hahn 1989h). Settlement is induced by a molecule uniquely available on the surface of crustose red algae. The molecule has been shown to mimic the action of the neurotransmitter y-aminobutyric acid (Morse *et al.* 1979, Morse *et al.* 1984, Barlow 1990). However, the presence of diatoms and bacteria (Morse *et al.* 1984) as well as the mucus trails left behind by juvenile and adult abalone (Seki & Kan-no 1981) are also known to induce settlement and metamorphosis.

The composition of the diet of post-larval and juvenile abalone, below the size at which they graze on macroalgae, is poorly understood. It is commonly believed that they graze principally on diatoms and flagellate microalgae (Garland *et al.* 1985, Sakata 1989), presumably because of their high nutritive value (Crofts 1937, Ryther & Goldman 1975, Kohn 1983, Ebert & Houk 1984, Morse 1984, Garland *et al.* 1985). Norman-Boudreau *et al.* (1986) detected diatoms in the digestive tract of post-larval abalone (F_1 of *H. kamtschatkana* x *H. kamtschatkana* or *H. kamtschatkana* x *H. rufescens)* as early as two days after settlement and metamorphosis. After six days, diatoms were always present in the digestive tract, indicating the initiation of feeding. All of the diatoms consumed were of the order Pennales and were less than 10 pm in width. However, Norman-Boudreau *et al.* (1986) pointed out that their method to detect diatoms involved the destruction of soft, organic parts. Thus other possible food items lacking the siliceous frustules of diatoms, such as other microalgae, protozoans or bacteria were possibly destroyed. It is likely that crustose red algae initially compose a major proportion of the diet of post-larvae (Fallu 1991), followed by a rapid switch to diatoms.

The importance of bacteria during the post-larval or any other stage of the life cycle of abalone, and their possible nutritional role are currently not understood_ Garland *et al.* (1985) found

undegraded bacteria in faecal material of juvenile ***H. ruber***[´]. They also detected viable spiral-shaped bacteria at the base of the oral disc. They proposed that these bacteria normally reside in the gut and were disgorged during protrusion of the radula. As yet, there is limited support for a succession amongst the bacterial population in the gut of abalone (Garland *et al.* 1985). Stein (1984) found a population of ***H. cracherodii*** feeding on mats of filamentous sulfur-oxidizing bacteria, associated with subtidal hydrothermal vents off the Californian coast. At least a portion of their nutrition was derived from the digestion of the bacteria, thereby being the only population were the role of bacteria has been identified at least partly.

The post-larval stage ends with the formation of the first respiratory pore (notch stage), after about one to three months (Hooker & Morse 1985). From the notch stage until the onset of sexual maturity, the animals are called juveniles and after that adults (Hahn 1989h).

Aspects of the feeding biology of juvenile abalone less than 5 mm SL have only been studied by Garland *et al.* (1985). Generally, juvenile abalone feed predominantly on diatoms (Hahn 1989g). However, many other food items are also ingested. Tomita & Tazawa (1971), e.g., analyzed the stomach contents of 0 - 2 year old ***H. discus hannai***. In the size range 5 - 11 mm SL they found in decreasing order of abundance (visual estimation) crustose red algae, diatoms, the angiosperm *Phyllospadix iwatensis*, brown algae of the genus *Sphacelaria*, foraminiferans and minor amounts of other algae and even crustaceans. The stomachs of juvenile ***H. midae*** in the size range 11.5 mm - 19.5 mm SL, collected from two sites along the East Cape coast of South Africa, contained the following in decreasing order of abundance (visual estimation): unidentifiable material (often mixed with diatoms, hydrozoans etc.), unicellular algae other than diatoms, diatoms, coralline algae and/or sand, pieces of larger algae and sponge spicules (R. Anderson 1992, Sea Fisheries Research Institute, Cape Town, South Africa, pers. comm.). Juvenile ***H. discus hannai*** and ***H. midae*** therefore appear to be rather indiscriminate feeders.

[´] Synonymous with H. *rubra*

However, whether any of the food items other than diatoms were taken in for their nutritional value remains speculative at this stage.

The feeding behaviour of abalone follows a very characteristic pattern. Genade *et* al. (1988) showed that while post-larval *H. midae* (0.7 mm SL) are already light-sensitive, they are not yet exclusively nocturnal feeders. However, once abalone start feeding mainly on diatoms, the grazing action takes place exclusively during the night (Hooker & Morse 1985, Tutschulte & Connell 1988). When feeding on macroalgae, abalone preferably catch loose pieces of algae drifting in the current, rather than grazing on algae attached to the seabed (Poore 1972a, Shepherd 1973, Tutschulte & Connell 1988). However, these preferences seem to vary from species to species. The animals assume a distinct feeding posture when stimulated by water currents (Shepherd 1973) or chemicals released from nearby food (Hooker & Morse 1985). They extend their chemosensory tentacles and lift the anterior portion of the foot to face the current. Contact with a drifting piece of algae leads to the immediate folding of the foot towards the midline to trap the food item (Hooker & Morse 1985), where it is then secured by the mouth.

Although juveniles up to 5 mm SL show a strong preference for diatoms, they generally switch to graze on macroalgae between 5 and 10 mm SL (Hahn 1989g). This period of transition from one dominant food source to another is termed the weaning phase (Ebert & Houk 1984). It is important to note that the exact timing of weaning is species-specific. Abalone of temperate climates, such as *H. midae,* may continue to feed on diatoms up to 25 mm SL (Fallu 1991). Leighton & Boolootian (1963) provided a possible explanation for the change over from diatoms to macroalgae. They suggested that as the radula grows, the distance between individual teeth becomes too large to rupture diatoms and hence they cannot be digested anymore. However, in a photograph of diatoms isolated from the digestive tract of a one-week old abalone (Norman-Boudreau *et al.* 1986) it was clearly evident that the siliceous frustules of the diatoms were not broken. This indicates that the rupture of the frustules is not a necessity for digestion, which casts a measure of doubt on the suggestion of Leighton & Boolootian (1963). It is also possible that

diatoms can no longer satisfy the energy requirements or specific nutritional needs of juveniles during the weaning stage.

After the weaning stage, abalone feed on macroalgae, whereafter there is no further change in the diet. The composition of the diet and various aspects of the feeding biology of juvenile and/or adult *H. tuberculata* (Stephenson 1924, Crofts 1929, Peck 1989), *H. gigantea* (Ueda & Okada 1939, 'no 1943), *H. kamtschatkana* (Ueda & Okada 1939, Paul *et al.* 1977), *H. rufescens* (Leighton 1961, 1966, Cox 1962, Olsen 1968), *H. discus hannai (Sakai* 1962a,b, Uki *et al.* 1986a), *H. cracherodii* (Leighton & Boolootian 1963, Stein 1984), *H. fulgens* and *H. corrugate* (Leighton 1966, Tutschulte & Connell 1988), *H. australis* and *H. iris* (Poore 1972a), *H. laevigata, H. ruber, H. cyclobates* and *H. scalaris* (Shepherd 1973), *H. roei* (Shepherd 1973, Wells & Kiesing 1989), *H. discus* (Sirenko & Kas'yanov 1976, Harada & Kawasaki 1982), *H. spadicea* (Muller 1984), and *H. sorenseni* (Tutschulte & Connell 1988) have been studied.

The stomach contents of *H. cracherodii* (of unknown size) contained not only macroalgae but also sponge spicules, shell fragments, sand grains, bryozoans, sea urchin spines and hydrozoans (Leighton & Boolootian 1963). Likewise, Shepherd (1973) found sand grains, small gastropods, ascidians, bryozoans, hydroids and detrital matter amongst pieces of macroalgae in the stomach of ***H.*** *laevigata,* ***H.*** *roei* and *H. ruber* of unknown size. It is reasonable to assume that the items other than the macroalgae did not constitute food but were taken in by the grazing action.

The food preferences of abalone differ from place to place, but generally northern hemisphere species feed mainly on brown algae (Fallu 1991). In the south western Cape, brown algae have also been found to make up the bulk of the diet of *H. midae* (Newman 1968). In a later study in the same area, Barkai & Griffiths (1986) identified 18 macroalgae species in the stomach of ***H.*** *midae* (65 - 145 mm SL). The brown algae *Ecklonia maxima* constituted 56 % of the diet by volume, and species of a red algae, *Plocamium* 21 % (Barkai & Griffiths 1986). In eastern Cape waters, however, Wood (1993) found the red algae *Plocarnium corallorhiza* and *Hypnea spicifera*

to be the major dietary components of *H. midae.*

Little is known about the factors affecting the choice of algae in nature. Currently, neither the presence of phagostimulants (Harada 1986, 1987, 1989, 1991, **Sakata** *et al.* 1988, Sakata 1989, Sakata & Ina 1992) or deterrents such as polyphenolics (Steinberg 1984, 1988, Hay & Fenical 1988), nor differences in the morphology or nutritive value of macroalgae (Shepherd & Steinberg 1992) can entirely account for the observed differences in the diets of abalone species. According to Shepherd & Steinberg (1992), feeding preferences may simply reflect differences in the relative abundances of algae in the respective habitats of abalone species.

DIGESTION

Digestive Enzyme Profile

Work on the digestive enzymes of *Haliotis* species has focused almost exclusively on the carbohydrases of the midgut gland (Table 2.1). Several workers have used thc midgut gland of various *Haliotis* species to purify and characterize carbohydrases, such as alginic acid eliminase (Nakada & Sweeney 1967), aryl sulfatase (Orzel 1967, Clark & Jowett 1988, Spaulding & Morse 1991), a-L-fucosidase (Thanassi & Nakada 1967, Tanaka *et al.* 1968, Tanaka & Sarai 1970), 13-glucosidase (Gianfreda *et al.* 1979a), and 13-glucuronidase (Orzel 1967), or groups of carbohydrases, such as **a-(1—A)-glucanases** (Gianfreda *et al.* 1979b), and 13-(1—)4)-glucanases (Gianfreda *et al.* 1979a, Gianfreda *et al.* 1979b).

In Table 2.1 only **the** substrates hydrolyzed by the midgut gland of *Haliotis* species are listed. Some authors stated the enzymes analyzed for, however, it has long been established that enzymes can hydrolyze a number of substrates with identical glycosodic bonds (Bruni *et al.* 1969, Hasegawa & Nordin 1969), and might therefore be not specific for the substrate tested. Most authors also listed the activity and/or specific activity obtained for each enzyme. As they used different methods, pH and temperature regimes to measure activity, the results are not directly

comparable. Therefore, only the capability to hydrolyze a substrate is indicated.

Table 2.1. Carbohydrate substrates hydrolyzed by the midgut gland of *Halions* species.

Substrate	*H.asinina* *H. varia*[1]	*H. discus hannai* *H. sieba*[1]*dii*[2]	*H. japonica*[2]	*H. rufescens*[3]	*IL discus*[4]	
Polysaccharide						
Agar		X				
Alginate		X	X			
Amylopectin	X					
Amylose		X	X			
Carboxymethyl-cellulose	X	X	X		X	
Carboxymethyl-pachyma.n	X					
Canageenan					X	
Cellulose			X	X		
Chitin			X			
Fucoidan					X	
Glycogen				X		
lnulin						
Laminarin	X		X			
Lychenin	X					
B-(1--+4)-Mannan		X	X		X	
Pachyman	X					
Pectic acid		X	X			
Pectin		X	X			
Porphylan					X	
13-(1—>3)-Xylan		X[6]	X[6]		X	
B-(1—>4)-XyL3n						
Yeast glucan	X				X	
Disaccharide						
D-Cellobiose				X		
Lactose				X		
Maltose				X		
a-D(+)-Melebiose				X		
Monosaccharide						
a-L-Fucose		X	X	X		X
B-L-Fucose		X	X			X
a-D-Galactose				X		X
B-D-Galactose		X	X	X		X
N-Acetyl-B-D-glucosamine		X	X	X		X
a-D-Glucose		X	X	X		X
B-D-Glucose		X	X	X		X
B-D-Glucuronic acid		X	X	X		X
a-D-Mannose		X	X	X		X
B-D-Mannose						X
a-D-Xylose						X
B-D-Xylose						X
I3-D-Xylulose				X		

[1] Elyakova *a a*[1]. (1981)
[2] Nakagawa & Nagayama (1988)
[3] Bennett *et* a/. (1971)
[4] Yamaguchi et a/. (1989)
[5] Clark & Jowett (1978)
[6] 341_43) or B-(1-

Among invertebrate herbivores, enzymes hydrolizing reserve carbohydrates (e.g. amylose, starch, laminarin) show the highest activity (Hylleberg-Kristensen 1972, Elyakova *et al.* 1981). Oligosaccharides and structural carbohydrates (e.g. alginic acid, xylan, pectin) are generally hydrolyzed less well, although enzymes acting on cellulose derivatives and chitin are widespread (Hylleberg-Kristensen 1972, Elyakova *et al.* 1981).

The digestive juices of the stomach and the intestine of *H. rufescens* have been shown to contain, among other, the carbohydrases amylase and glycogenase, the protease pepsin and a lipase (Albrecht 1921). The midgut gland of the same species contains amylase, glycogenase, protease and lipase (Albrecht 1923). Oshima (1931) detected the carbohydrases agarase, amylase, cellulase, alginase and maltase as well as a weak protease in the intestine of *H. gigantea.* McLean (1970), working on *H. rufescens,* found strong arnylolytio, proteolytic and lipolytic activity in the crop and midgut gland. The epithelium of the crop and the oesophagus showed a weak positive result for amylase, as well as the intestinal regions III and IV (McLean 1970). Proteolytic activity in the digestive tract of *H. discus hannai* (Cho *et al.* 1983) and in the midgut gland of *H. discus* (Yamaguchi *et al.* 1989) has also been detected. Apart from these studies, work on specific proteases and lipases in *Haliotis* species is lacking.

Diet and Enzymes - are they correlated?

There are contradictory opinions as to whether the set of enzymes present in gastropods match the linkages encountered in the natural diet. Bennett *et al.* (1971) showed that the enzymes of the midgut gland of *H. rufescens* reflected the types of glycosidic bonds present in marine algae. Furthermore, certain enzymes typical of terrestrial organisms were absent. Likewise, Clark & Jowett (1978) found that *H. iris* was capable of dealing with all the linkages commonly found in algal saccharides, provided that small oligosaccharides could be hydrolyzed as well, which they however did not test for. Piavaux (1977) studied the distribution of laminarinases in vertebrates and invertebrates. In the archaeogastropod genera *Patella* and *Turbo,* he found a correlation between strong laminarinase activity and the presence of 13-(1-43)-glucans in the diet

as in diatoms, other protist algae, and brown algae. Carnivorous species generally had weaker activities as these glycosidic bonds were absent in their diet. However, strong laminarinase activity has also been found in the midgut gland of the carnivorous cephalopod *Octopus vulgaris* (Furia *et al.* 1975, Piavaux 1977). According to Piavaux (1977) this seemingly useless enzyme could simply be a remnant of a more primitive digestive arsenal.

Gianfreda *et al.* (1979b), on the other hand, found **that the** presence of a given glucanase activity in a mollusc did not necessarily reflect its feeding habit. This applied specifically **to** B-glucanases, such as cellulase and laminarinase. However, looking at these two enzyme complexes in more detail, Gianfreda *et al.* (1979a) detected that the components of the cellulase complex were present in proportions directly related to feeding habit and species. Endo-l3-(1-44)-glucanase, an enzyme of the cellulase complex hydrolizing B-(1—)4) bonds within the cellulose chain, was present in the midgut gland of *H. tuberculata* but absent in carnivorous gastropods. This supported Piavaux's (1977) view, in that parts of the cellulase complex or one of its basic components were probably an evolutionary relic that survived the evolutionary transition to a carnivore from an ancestral herbivorous stock.

Hylleberg-Kristensen (1972) studied the carbohydrases of 22 marine invertebrates. Only the degradation of laminarin showed some relation to food, but the overall profile of carbohydrases could not be predicted from information on the food of a species. However, the total number of carbohydrases permitted to estimate roughly the natural food of a species. Hylleberg-Kristensen (1972) found the enzyme spectrum of the carnivorous gastropod *Nassarius reticulatus* to be similar to that of the detritus feeding gastropod *Hydrobia ventrosa.* The capability of *N. reticulatus* to hydrolyze alginic acid and alginate, both of which are components of brown algae was extremely puzzling. Again, this could be interpreted as a remnant of an ancient set of enzymes. It is also possible that these enzymes are of use elsewhere in the metabolism of a species (Hylleberg-Kristensen 1972).

Processes and Sites of Digestion

Haliotis species, like many other herbivores, exhibit a combination of preliminary extracellular digestion and subsequent intracellular digestion (Purchon 1977). Intracellular digestion is the more primitive method and only a few types of food can be grazed on by purely intracellular digesters (Owen 1966b). 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 extracellular 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 extracellular. However, intracellular hydrolysis of at least some material in the midgut gland remains a possibility.

It is generally held that enzymes are only produced and secreted in the glandular appendages, i.e. the salivary glands, oesophageal pouches and their derivatives (oesophageal bulb) and the midgut gland (Purchon 1977). In abalone, the midgut gland appears to be the only glandular area producing carbohydrases, proteinases as well as lipases (Owen 1966b).

The Role of Bacteria in Digestion

Studies on marine herbivores of several phyla have shown that gut bacteria aid in the digestion of food (Prim & Lawrence 1975, Fong & Mann 1980, Mann 1982, Dempsey & Kitting 1987, Sweijd 1990). Carefoot (1982) concluded that despite the near absence of such studies on gastropods it was unlikely that they were an exception. He suggested two forms of bacterial participation in the nutrition of gastropods: indirect, through the breakdown of structural carbohydrates (cellulose, alginic acid) for further enzymatic digestion; and direct participation, through the breakdown of storage carbohydrates, such as starch and laminarin. However, Galli & Giese (1959) isolated 18 strains of bacteria from the gut of the archaeogastropod *Tegula funebralis.* Only four of these could hydrolyze algal carbohydrates such as alginic acid, agar and

carrageenan. Moreover, the bacteria were present in small numbers only. Thus the authors concluded that it was unlikely for bacteria to contribute significantly to the breakdown of algae, at least in this species.

Garland *et al.* (1985) studied the role of bacteria in the digestive processes of post-larval and juvenile *H. ruber.* As bacteria in the faeces appeared undegraded, they may perform important metabolic activities in the gut. They also found some indications of a succession of bacterial populations. Apart from this observation the role of bacteria in the digestion of food in any life stage of *Haliotis* species has not been studied.

ABSORPTION

Mechanisms and Sites of Absorption

After metamorphosis, absorption of nutrients takes place mainly in the midgut gland and less extensively in the stomach (Purchon 1977). However, McLean (1970) stated that absorption in *H. rufescens* could already occur in the oesophagus, as tests on the salivary glands and the oesophageal lumen suggested the presence of enzymes. Likewise, it was pointed out by Berrie & Devereaux (1964) and McLean (1970) that absorption by the stomach epithelium is a possibility.

McLean (1970) detected first indications of absorption 25 min after feeding in the absorptive cells of the midgut gland of *H. rufescens.* Absorption occurs through the rnicrovillous distal wall of the absorptive cells (Owen 1966b). Phago- and/or pinocytosis of carbon particles and starch grains has been shown to occur in the midgut gland of *H. cracherodii* (Campbell 1965). However, McLean (1970) did not find any evidence of this in *H. rufescens.* As it is such a general feature of gastropods (Owen 1966b), phagocytosis can however still be assumed to be part of the absorptive mechanisms of abalone.

In the chiton *Cryptochiton stelleri* the absorption of nutrients in the intestine has been shown to occur (Greer & Lawrence 1967, Lawrence & Lawrence 1967). In haliotids, enzymes and algal material are carried into the intestine and intestinal absorption is therefore a possibility (Crofts 1929, McLean 1970).

Absorption of Sugars

In *H. rufescens*, glucose is absorbed by the crop epithelium, the midgut gland and the intestinal sorting area (McLean 1970). The latter may even secrete its own amylase to break down polysaccharides.

D-glucose and D-galactose have been demonstrated to be actively transported across the intestinal mucosa of the snail *Cryptomphalus hortensis* (Barber *et al.* 1975). Competitive inhibition among D-glucose, D-galactose, and 3-methylglucose (Barber *et al.* 1979) as well as inhibition of the transport of all three by phlorizine (Barber *et al.* 1975) suggest that the three sugars use the same transport system. It shows the same or greater affinity for D-glucose than for D-galactose with a much lower affinity for 3-methylglucose (Barber *et al.* 1979). In contrast, D-fructose, L-arabinose, and D-mannitose simply diffuse from the mucosal to the serosal side (Barber *et al.* 1975, Barber *et al.* 1979). Orive *et al.* (1980) detected an energy-independent transport system for glucose in the intestine of the pulmonates *Helix pomatia* and *Anion empiricarum.* No such system has been shown to operate in *Haliotis* species. An active transport mechanism has been proposed for the midgut gland of *H. rufescens* (McLean 1970).

Absorption of Metal Ions

Metal ions such as cadmium, copper, iron, magnesium, manganese and zinc are mineral elements necessary for a variety of metabolic activities, and as such are essential (Stryer 1988). Most of the metal ions are involved in oxygen transport, redox activities and metalloenzymatic reactions (Wurzinger & Hartenstein 1974, Simkiss & Mason 1983).

Detailed studies on the processes of absorption of metal ions in molluscs have only been done on bivalves. Generally, the chemistry of an element dictates its type of uptake mechanism. Iron, zinc and calcium are taken up by the gut and midgut gland of bivalves, whereby the vesicular uptake is preceded by binding of the ion to the mucus (George *et al.* 1976, George & Pirie 1980).

Epithelial uptake of the ions takes place across the gills, the foot, the mantle, and the alimentary tract. Endocytosis of metal ions has been demonstrated in the mantle cells of the clam *Macrocallista maculata* (Bevelander & Nakahara 1966). The vesicle is engulfed by a primary lysosome followed by the formation of a secondary lysosome (Fowler *et al.* 1975). Epithelial endocytosis appears to be important for the uptake of a number of insoluble metals, at least in bivalves.

The digestive cells lining the digestive tubules of gastropods are capable of phagocytosis or pinocytosis of particulate matter. Floculent iron, thorium dioxide are phagocytosed (Walker 1972), chelated iron and other soluble macromolecules are pinocytosed (George *et al.* 1976) by the digestive cells. It is not clear whether there are specific ion pumps for trace metals (Simkins & Mason 1983).

The amount of absorption of metals from food, sediments and sea water depends on the life history and feeding strategy of an animal. In prosobranchs, the dietary import is generally more important than the one from seawater, as shown for the carnivore *Nucella lapillus* and the herbivore *Littorina obtusata* (Young 1975, 1977). The uptake of metal ions is enhanced by the presence of humic and alginic acids (Coombs 1977, George & Coombs 1977), which probably provide a source for ligands.

Amoebocytes

The role of amoebocytes with regard to nutrition is somewhat dubious. They are absent in prosobranchs such as *Patella spp.* (Graham 1931), but present in *H. cracherodii* (Campbell 1965).

McLean (1970) also referred to them, but only Campbell (1965, p. 368) speculated on their nutritional role: "In several sections, starch grains had been carried, presumably by amoebocytes, into the spaces and connective tissue below and between the ridges of the postoesophageal epithelium". However, he adopted Morton's (1953) view that they function in rejecting material. Although it is well established that amoebocytes can ingest particles, it is not known whether they can migrate into the tissues (Owen 1966b). In lamellibranchs it has been found that they ingest fat and diatoms but there is no evidence of their passing into the tissues and blood spaces (George 1952). It is difficult to determine whether amoebocytes in the epithelia of the digestive tract have been phagocytosed in order to utilize the food particles they contain, or whether they are being eliminated from the animal. The latter is supported by observations on the fate of particles injected into the blood stream of oysters (Stauber 1950, Tripp 1960), which indicated that the gut is one of the main pathways for the elimination of amoebocytes containing unwanted material.

FOOD STORAGE AND **METABOLISM**

Storage Carbohydrates: Glycogen

Glycogen and galactogen are the main storage polysaccharides of gastropods. Glycogen occurs in most gastropod tissues and serves as a general source of energy. Galactogen is a specialized energy source in reproduction. It is confined to the albumen gland and eggs of adult pulmonates and the albumen gland region of the pallial oviduct of some prosobranchs (Livingstone & de Zwaan 1983).

Like in other animal tissues, glycogen is made up of D-glucose units, linked via **oc-(1—>4)-glycosidic** linkages. Chains branch off via a-(1—>6)-glycosidic linkages. Chiang (1977) showed for the pulmonate *Biomphalaria glabrata* that the degree of branching (9 %) and the length of the outer branches (40 % of the total glucosyl residues) was similar to those of other animals, including vertebrates. High glycogen stores are found particularly in archaeogastropods (Giese

1966). Concentrations of approximately 5 - 25 % on a dry weight basis have been reported for the midgut gland, foot and mantle of *H. cracherodii* (Webber 1970). Glycogen is stored in two types of connective tissue cells, i.e. granular and vesicular cells (Livingstone & de Zwaan 1983). Granular cells contain cysteine rich glycoproteins, the role of which remains unclear. Vesicular connective tissue cells, on the other hand, are the major storage cells for glycogen and, as such, are important in the nutrition of tissues. In molluscs they are concentrated in the mantle, the midgut gland and the gonad. The cytoplasm and the organelles form a thin rim against the cell membrane, due to the high amount of glycogen stored. The stored glycogen is particulate, and forms either simple spherical particles or large granules composed of the smaller particles. This variability in particle size is reflected in a wide molecular weight spectrum of glycogen (Goudsmit 1972).

Energy metabolism of many gastropods is known to be based on the utilization of carbohydrates (Emerson 1967, Livingstone & de Zwaan 1983). Glycogen synthesis from glucose has been demonstrated by the incorporation of [^{14}C] - glucose (via maltose, maltotriose, and maltotetrose) in the midgut gland, foot and albumen gland of pulmonates such as *Ariolimax colurnbrianis* (Meenakshi & Scheer 1968) and prosobranchs such as *H. rufescens* (Bennett & Nakada 1968). At least in some gastropod species most intermediates and enzymes of glycogen biosynthesis have been detected (Goudsmit 1972).

Phosphorylase, an enzyme involved in the breakdown of glycogen, has been detected in many tissues of *H. rufescens* (Bennett & Nakada 1968). The enzymes of the glycolytic pathway are present in all gastropod tissues (Bennett & Nakada 1968, Goudsmit 1972, Marshall *et al.* 1974, Beis & Newsholme 1975, Zammit & Newsholme 1976, Avelar *et al.* 1978, Zammit *et al.* 1978). This also applies to the Krebs cycle (Goddard & Martin 1966, Coles 1969), although it has not been studied in detail in any gastropod. The pentose phosphate pathway has been demonstrated in *H. rufescens* by measuring glucose-6-phosphate dehydrogenase activity (Bennett & Nakada 1968). Other enzymes of the pentose shunt have also been found, such as 6-phosphogluconate

dehydrogenase and a trans-ketolase-trans aldolase (Bennett & Nakada 1968, Coles 1969).

Glycogen is broken down to glucose. Goddard & Martin (1966) calculated a mean glucose concentration of 1.18 ± .1.08 mM in the blood of gastropods, whereas Livingstone & de Zwaan (1983) calculated 0.74 ± 0.56 rriM. As earlier assays measured total reducing compounds the lower concentration is probably more reliable. The actual range of blood sugar concentrations spans an order of magnitude. There are not only species-specific differences but, as expected, also considerable intraspecific variations (Becker 1972). Trehalose also forms part of the blood sugar. At present its concentration as well as its relationship to carbohydrate storage and mobilization in gastropods is not understood (Livingstone & de Zwaan 1983).

Mucopolysaccharides

Mucopolysaccharides function in lubrication, as mechanical or protective support and components of egg jellies and capsules (Goudsmit 1972). They are heteropolysaccharides with usually two types of alternating monosaccharide units. At least one of these units bears an acidic group, either a carboxyl or sulfuric group. The presence of an acidic sulfur group has been found to be species-specific in gastropods (Goudsmit 1972).

Nucleotide diphosphate sugars are probably the activated precursors for these molecules. Uridine diphosphate (UDP)-acetylgalactosamine, UDP-acetylglucosamine, guanosine diphosphate (GDP)-galactose and GDP-D-mannose have been identified (Goudsmit 1972), but the exact pathways remain rather dubious at this stage. Meenakshi & Scheer (1968) found that [^{14}C] -glucose, [^{14}C] - galactose and [^{14}C] - maltose were incorporated into the galactose, fructose and glucosamine residues of an acid mucopolysaccharide.

The chemical composition of mucopolysaccharides is also species-specific. In association with proteins they are referred to as mucoproteins or mucins. They form highly viscous solutions, thereby decreasing friction during locomotion, providing adhesion, and decreasing desiccation

(Goudsmit 1972).

Mucus production is significant in terms of energy. Peck *et al.* (1987) estimated pedal mucus production of *H. tuberculata* to account for 23 - 29 % of an animals energy budget, depending on the size of the animal. Kideys & Hartnoll (1991) calculated that 27.5 % of the total energy intake of the prosobranch *Buccinum undatum* appeared as pedal and hypobranchial mucus.

Lipids

At present there is little insight as to how molluscs manage to maintain a characteristic composition of lipids, despite the often quite different lipid composition of their diet (Voogt 1983).

In general, temperature influences the lipid composition of membranes, and diet affects the lipid composition of the storage organs (Lewis 1962). Consequently, lipid as well as fatty acid (FA) compositions only reflect particular situations of individual animals. However, McLachlan & Lombard (1980) found a correlation between lipid concentration and temperature in the prosobranch *Turbo sarrnaticus.*

Sterols

They occur either in free form or esterified to FAs (Voogt 1983). Sterols mainly function in regulating the viscosity of cell membranes, an important factor with regard to permeability (Stryer 1988). The sterol composition of a species is very specific when the storage organs, i.e. gonads and midgut gland, are excluded (Idler & Wiseman 1972, Voogt 1972). Gastropods contain mainly cholesterol with only small amounts of C_{28} and C_{29} sterols (Idler & Wiseman 1972).

The sterol composition of archaeogastropods has been extensively studied. While the Trochidae possess the greatest number of components, haliotids have a very simple sterol composition. De Koning (1966a) found sterols to make up 12 % of the total body lipids of *H. midae.* The visceral

sterols of *H. discus hannai* are largely cholesterol and glyceryl ethers (Hayashi & Yamada 1972). The proportions of choline, ethanolamine, inositol, and serine in the phospholipids of the muscle of *H. corrugata* (Simon & Rouser 1969) and *H. midae* (de Koning 1966a,b) are very similar.

There is no data available with regard to the contribution of *de novo* synthesis of sterols or dietary sterols to the sterol pool. Sterol synthesis from mevalonate was shown, e.g. in *H. gurneri* (Teshima & Kanazawa 1974). Little is also known about metabolic processes involving exogenous sterols.

Fatty Acids

The synthesis of FAs from acetate appears to be a general feature of molluscs (Voogt 1972). There are no unique aspects of these pathways in gastropods. FAs form part of sterol esters, acylglycerols and phospholipids. Besides the normal unbranched chain FAs, those with branched chains are also present (Voogt 1983), e.g. 4,8,12 trimethyl-tridecanoic acid. Some of the dienoic FAs have double bonds separated by non-methylene groups (Paradis & Ackrnan 1975).

Proteins

The protein composition of the foot of some *Haliotis* species has received considerable attention (Migita *et* a/. 1959, Kimura & Kubota 1968, Pyeun *et al.* 1973, Song 1973 in 011ey & Thrower 1977). The foot is considered to comprise three distinct anatomical pans, which are made up of different proportions of proteins (011ey & Thrower 1977). The central oval pillar contains mainly paramyosin fibrils (Song 1973 in 011ey & Thrower 1977), while the pedal sole and the epipodium are high in collagen (Kimura & Kubota 1968). Apart from the foot, no other organ has been studied as regards its protein composition.

Amino Acid Transport

Transport systems for AAs in mammals are categorized into various sodium-dependent and -independent systems (Stryer 1988). Gastropod transport systems have not been classified as yet.

Uptake of AAs through the general body surface has not been confirmed for gastropods, but is known to occur in other molluscan classes. The bivalve *Mya arenaria*, e.g., has been shown to remove neutral AAs (alanine, glycine, leucine, methionine, phenylalanine, serine), acidic AAs (aspartic acid), basic AAs (histidine, lysine) and imino acids (proline) from incubation media (Stewart & Bamford 1975, 1976). Stewart (1978) detected a specific transport site for each of the different classes of AAs. However, there seemed to be considerable overlap in the specificity of the sites, especially with regard to the basic and neutral sites.

Metal Ions

Abalone can take up metal ions or mineral elements from both sea water and food in nutritionally sufficient amounts (Fallu 1991). Various blood proteins (Howard & Simkiss 1981) and amoebocytes (Ruddell & Rains 1975) of bivalves have been demonstrated to transport metal ions to very specific storage sites.

Attempts have been made to link the distribution of particular metals to the presence of specific metalloenzymes, e.g. in *H. tuberculata* (George & Coombs 1975). However, comparative studies have shown that other factors are also involved (Simkiss & Mason 1983).

Pore cells have been identified in connective tissue of all three gastropod subclasses. They contain a number of 22 - 24 nm wide pores, which penetrate the cell (Simkiss & Mason 1983). Their function is not understood, but they seem to facilitate the movement of material between the cytoplasm of the pore cell and the extracellular fluid. Pore cells contain metal ions such as copper in intracellular granules. As their endoplasmatic reticulum is very well developed, protein synthesis has been proposed as a function, although the metal ions seem to be associated with the granules rather than the endoplasmatic reticulum (Simkiss & Mason 1983).

CHEMICAL COMPOSITION AND NUTRIENT REQUIREMENTS

Proximate Composition

No total body analysis of any *Haliotis* species has been undertaken to date. The only work of this nature has been on parts of the soft tissue. The foot and the midgut gland of *H. cracherodii* was analyzed by Albrecht (1921, 1923), while Webber (1970) examined the foot, digestive tissue, and the gonad of the same species. The proximal composition of the foot of *H. gigantea nordalis* was determined by Song (1973 in 011ey & Thrower 1977). Sidwell *et al.* (1974) reported results from four literature sources (Intengan *et al.* 1956, Butler 1958, Konosu & Mori 1959, Suyama & Sekine 1965) on the composition of the edible portion of *H. kamtschatkana.* Of the four cited papers, only Butler (1958) positively identified his sample as *H. kamtschatkana.* Suyama & Sekine (1965) analyzed the edible portion of *H. japonicas,* Konosu & Mori (1959) the foot and shell muscle of *H. gigantea,* and Intengan *et al.* (1956) did not identify the species they worked on **at** all. Finally, the composition of the foot of some Japanese species has been analyzed, the results of which were summarized by 011ey & Thrower (1977). However, again there seems to have been some confusion with regard to species identification. The results of the proximate composition of individual tissues have been expressed either as a percentage of wet or dry weight, which does not allow for comparison. However, the proximate composition of abalone is known to vary with size (011ey & Thrower 1977) and gonadal activity. Webber (1970), e.g., showed that prior to spawning, carbohydrate levels in the foot were depleted probably due to gonadal recrudescence.

Based on feeding experiments, Uki *et al.* (1985a, 1986b) determined the quantitative requirements of the major nutrients for *H. discus hannai.* When using easily digestible proteins with high levels of essential AAs (EAAs), 20 -30 % of dietary protein was found to be optimal for the growth of juveniles. The optimum level of lipid was 5 %, but no clear relationship between carbohydrate content and growth has been found.

Table 2.2. Amino acid concentration of *Haliotis* species. Results are expressed as percentage of total protein. Where possible, SD has been added.

	Foot			Shucked tissue
Amino acid	*H. gigantea*[t]	*H. japonica*[2]	*H. tuberculata*[3]	*H. rufescens*[4]
ala	9.6	6.58	10.3	5.45 + 0.14
arg	4.9	9.48	8.9	7.26 ± 1.06
asp	10.2	10.²0	6.6	11.²0 ± 0.72
cys	not known	1.18	not known	0.69 t 0.07
glu	13.3	15.²0	11.5	13.50f 0.52
gly	13.8	7.81	2.7	4.96t 1.02
his	1.3	1.48	1.0	1.97 ± 0.26
ile	3.8	4.18	4.1	3.78 ± 0,49
leu	7.4	8.10	6.4	7.84 ± 0.53
lys	4.9	6.43	5.8	5.99 ± 0.45
met	2.1	2.54	not known	2.60 ± 0.25
phe	².8	3.73	².1	4.07 ± 0.86
pro	5.7	5.21	6.0	2.68 ± 0.31
ser	6.6	5.30	not known	4.²9 ± 025
thr	5.0	5.06	4.4	4.63 ± 0.25
trp	not known	0.88	not known	0.38 ± 0.02
tyr	2.8	3,52	1.7	3.81 ± 0.65
val	4.5	4.36	4.3	4.72 ± 0.42

U.S. Department of Health, Education and Welfare 1972 in 011ey & Thrower (1977)
[2] Suyama & Sekine (1965)
[3] Florkin & Bricteux-Gr6goire (1972)
[4] Allen & Kilgore (1975)

Amino Acids

AA profiles of the muscle have been obtained for *H. japonica* (Suyama & Sekine 1965), *H. tuberculata* (Florkin & Bricteux-Gregoire 1972), and *H. gigantea* (US Department of Health, Education and Welfare 1972 in 011ey & Thrower 1977). Allen & Kilgore (1975) analyzed the AA composition of shucked tissue of *H. rufescens,* and determined the essential AAs (EAAs) as well. They found arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine to be essential. No conclusion about the essentiality of tyrosine could be drawn. Overall, the AA pattern was similar in the different organs and species (Table 2.2).

Florkin & Bricteux-Gr6goire (1972) estimated the pool of free AAs in *H. tuberculata* to be 0.8 % of the wet weight. This pool is probably subject to seasonal variations (011ey & Thrower

1977). Lange (1963) concluded that the mussel *Mytilus edulis* uses the AA taurine for osmoregulatory purposes. The concentration of free taurine varied from 0 - 50 nM, depending on ambient salinity. In fact, it is known for many molluscs that they achieve osmoregulation via adjustments in the solute content of their cytoplasma (for review see Burton 1983). In all molluscs free AAs such as taurine, glycine, alanine, proline and glutamic acid (Burton 1983, Someru & Bowlus 1983) are of importance in this respect. In *Haliotis* species, however, the pool of free AAs is probably not linked to osmoregulation as haliotids do not experience marked changes in salinity.

Fatty Acids

Fatty acid profiles have been obtained for the shucked tissue of *H. discus*, *H. japonica* (Shimma & Taguchi 1964), and *H. midae* (de Koning 1966a), the edible portion of *H. iris* (Bannatyne & Thomas 1969), the viscera (Hayashi & Yamada 1972, Uki *et al.* 1986c) and muscle of *H. discus hannai (Uki et al.* 1986c). The same authors found the co-3 and o)-6 polyunsaturated FAs (PUFAs) to be essential for *H. discus hannai.* They concluded that 1 % of the 5 % lipid in a diet should consist of w-3 PUFAs (Uki *et al.* 1986c).

The FA profiles have been summarized in Table 2.3, with the exception of the results of Uki *et al.* (1986c). They tested the influence of different diets on the FA profile of cultured *H. discus hannai* and not the profile of animals from the wild. Comparison of these profiles shows that the viscera and the foot are very similar in their FA composition. In general, haliotids appear to have an unusally high content of arachidonic acid (20:4) for marine animals. *H. midae* is unique in that it is the only abalone species with significant levels of docosahexaenoic acid (22:6).

Mineral Elements

Mineral element analyses have been undertaken in individual tissues or total soft parts of *H. fulgens* (Marks 1938), *H. rufescens* (Anderlini 1974, Stewart & Schulz-Baldes 1976, Martin *et al.* 1977), *H. tuberculata* (Bryan *et al.* 1977), *H. cracherodii* (Martin *et al.* 1977) and *H. discus*

(Ikuta 1987a,b). Only in one study on *H. diversicolor supertexta* (Chen 1989) has the shell been analyzed. The edible portion of *H. midae* (Van As *et al.* 1975) as well as individual tissues (Watling & Watling 1974) have been studied. Fourie (1976), in his study on *H. midae,* did not state which tissues were analyzed.

Mineral element profiles of fish are known to approximate their nutritional requirements (Steffens 1989), thus such data is of potential value to the abalone nutritionist. So far, however, only the

Table 2.3. Fatty acid profile of *Haliotis* species. Results are expressed as percentage of total fatty acids.

	Shucked tissue			Viscera	Foot
Fatty acid	*H. discus'*	*H. japonica'*	*H. midae*[2]	*H. d. hannai*[3]	*H. irls*[4]
12:0	--	--	--	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	²4.1	22.8
16:1	3.3	4.4	6.3	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	2.9	6.7
18:1	16.4	17.1	13.3	19.3	15.7
18:2	1.2	1.6	1.3[5]	2.8	05
18:3	--	--	0.3	3.4	1.2
18:4	--	0.9	--	3.6	--
19:0	--	--	—	trace	--
19:1	--	--	--	0.3	--
20:0	--	--	1.0	0.3	--
20:1	4.86	5.96	3.0	7.4	3.7
20:2	--	--	0.3	—	--
²0:4	10.7[7]	12.3[7]	7.3	5.1	13.4
²0:5	8.8	10.0	7.3	5.9	8.0
21:5	--	--	--	0.7	—
22:1	--	--	3.38	trace	5.3
22:2	5.58	3.98	--	—	--
22:4	2.0[8]	2.6[8]	0.3	--	3.²
22:5	73	8.4	2.0	1.4	10.4
22:6	--	--	3.3	trace	--
24:1	--	--	--	trace	--
24:4	35[8]	1.1[8]	--	--	--

[1] Shinuna & Taguchi (1964) [2] de Koning (1966a) [3] Hayashi & Yamada (1972) [4] Bannatyne & Thomas (1969)
[5] 18:2 and 19:0 [6] 20:1 and 18:3 [7] ²0:4 and ²2:1 [8] Identification uncertain

effect of a mineral mixture on the growth of juvenile *H. discus hannai* (Uki *et al.* 1985a) has been tested. Growth was significantly enhanced using a semi-purified diet supplemented with 8 % of the mineral mixture. However, the water stability of the diet was negatively affected by the high level of mineral supplementation. Consequently, the level has been reduced to 4 % (Uki *et at* 1985a).

CONCLUSIONS AND IMPLICATIONS FOR THE DEVELOPMENT OF ARTIFICIAL DIETS

Some aspects of the nutrition of abalone, such as the composition of the natural diet of adults, have received considerable attention, but overall there is a general lack of basic information. This applies specifically to the molecular processes of digestion and absorption, the function of bacteria in abalone nutrition, and to nutritional requirements in general. Unfortunately, these aspects are of particular importance regarding the development of artifical diets.

A reasonable amount of information on the chemical composition of haliotids has been obtained. However, very often the authors did not specify the season and location of sampling, the sample size, the size of the animals, or which specific tissues were analyzed. This is problematic as these factors influence the chemical composition of animals.

Studies on enzyme activities or specific activities suffer from a lack of standardization. Frequently, different pH and temperature regimes as well as different analytical methods have been used, making it impossible to compare results.

An ideal scenario for the development of an artificial diet can be described as follows. Firstly, the nutritional processes from feeding to defaecation at every life stage of an animal must be known. Secondly, the nutritional requirements of macro- and micro-nutrients should be determined and thirdly, the availability of the nutrients within an ingredient should be known.

Given this information, a diet can be formulated based on the chemical composition of ingredients and knowledge of the availability of the nutrients to the specific life stage of the animal (Hardy 1989, Wee 1992). This scenario certainly does not apply to abalone, although artificial diets have been formulated by simply mixing ingredients commonly used in the feed industry, and evaluating them in growth trials (Ogino & Ohta 1963, Ogino & Kato 1964, Sagara & Sakai 1974, Koike *et al.* 1979, Uki *et al.* 1985a, Britz *et al.* 1994). This has been possible as abalone, although herbivorous, readily feed on diets containing animal meals (e.g. fish or squid meal) or high fibre terrestrial plant meals (e.g. linseed meal or oats) (Maguire *et al.* 1993).

Knowledge of the nutritional requirements of abalone is currently limited to *H. discus hannai (Uki et al.* 1985a, 1985b, 1986b, Uki *et al.* 1986c, Uki & Watanabe 1986). Data on the availability of nutrients, as determined by digestibility studies, is also scarce. Crude protein and dry matter apparent digestibility coefficients of artificial diets have been determined for *H. midae* (Dixon 1992), *H. laevigata* and ***H.*** *rubra* (Wee *et al.* 1992).

Ceccaldi (1982) emphasized that nutritional requirements are often species-specific and can thus not simply be generalized within a genus. The same applies to different life stages, e.g., no studies on the nutritional requirements of juveniles at the weaning stage have been published. Likewise, information on the digestibility of feed in abalone at the weaning stage is non-existent. However, digestibility studies require the collection of faeces. It may be difficult to obtain a representative faecal sample, considering the size and the amount of faeces produced by such small animals.

In summary, a student of abalone nutrition is in the same situation as fish and crustacean nutritionists were 30 years ago, especially with regard to the development of a weaning diet.

However, it has been shown that there is a close correlation between the pattern of EAAs found in muscle or whole body tissue of an animal and its dietary requirements (Cowey & Tacon 1982).

Therefore, in the absence of experimentally defined requirements, the EAA pattern of body tissue can be used as a starting point in the formulation of the EAA pattern of a diet. Diets based on this approach have been developed for prawn (Deshimaru & Shigeno 1972, Colvin 1976) and fish species (Rumsey & Ketola 1975, Arai 1981, Cowey & Tacon 1982, Wilson & Poe 1985). An advantage of this method is that the approximate requirements of all ten EAAs can be determined in a single analysis (Tacon & Cowey 1985). In contrast, experimental determination of these requirements requires ten separate growth trials, each with varying concentrations of a single EAA (Tacon & Cowey 1985). Moreover, these trials are likely to be inexact if, as is the case with abalone, the animal is a messy feeder (Cowey & Tacon 1982). Since abalone are also slow feeders (Uki 1981), the leaching of nutrients from the artificial diet would be an additional problem in such studies.

It has also been shown that the concentrations of minerals in fish approximate their nutritional requirements (Steffens 1989). Mineral profiles are relatively easy to obtain, whereas the requirements of individual minerals are very difficult to determine, especially in aquatic animals.

To date, the relationship between the EAA pattern found in the body tissue and the dietary requirement pattern has not been used to develop a diet for abalone. As the nutritional requirements of *H. midae* are unknown, this approach appears to be useful especially in the initial formulation of a diet. It was therefore decided to develop an artificial weaning diet for *H. midae* based on the EAA pattern of its whole body tissue.

It is important to note, however, that neither of the methods to develop artificial diets exclude each other. Rather, the synthesis of information obtained with each method should increase the quality of the diets developed for abalone.

CHAPTER 3

CHEMICAL COMPOSITION OF JUVENILES

INTRODUCTION

The chemical composition of an animal provides a baseline regarding its nutritional requirements. This has been shown for essential amino acids (EAAs) (Cowey & Tacon 1982) and mineral elements (Steffens 1989).

Information on the chemical composition of *Haliotis midae* is however scant. De Koning (1966a,b) studied the lipid and fatty acid (FA) composition of shucked tissue, and Wading & Wading (1974) and Van As *et al.* (1975) analyzed the mineral element profile of several tissues of *H. midae.* It was therefore decided to undertake a thorough analysis of the whole body tissue of juvenile *H. midae.*

The objective of this study was firstly, to analyze the proximate composition of shucked tissue and total animal (shucked tissue and shell) of *H. midae.* As the proximate composition of haliotids is known to vary with size (011ey & Thrower 1977), two size classes (10 - 20 mm and 45 - 55 mm shell length (SL)) were analyzed for comparison. Secondly, the AA and FA composition of shucked tissue, and the mineral element profile (Ca, Cd, Co, Cu, Fe, K, Mg, Mn, Ni, Zn) of shucked tissue and shell of *H. midae* (5 - 20 mm SL) was analyzed.

MATERIALS AND METHODS

Sample Collection and Preparation

In March, April and May 1992 juvenile abalone were sampled at three localities along the eastern Cape coast of South Africa between 34°1'S 25°42'E and 33°31'S 27°6'E at Port Alfred (n = 116), Cape Recife (*n* = 109), and Great Fish River Point (n = 103). They were purged for 72 h at 18 °C. Following that, 5 animals from each sampling site were stored in

Table 3.1. Mean ± SD of length, total and shucked weight of *Ha[1]intis midae,* used for fatty acid analysis.

Sampling site	n	Length (mm)	Total weight (g)	Shucked weight *(g)*
Port Alfred	5	9.26 ± 129	0.10 ± 0.05	0.06 ± 0.03
Cape Recife	5	8.34 ± 1.42	0.08 ± 0.04	0.04 ± 0.03
Fish River	5	8.1[2] ± 0.80	0.08 ± 0.02	0.05 ± 0.02

liquid nitrogen for FA analysis (Table 3.1). The rest of the animals were shucked, and both shucked tissue and shells were oven-dried at 50 °C for 24 h. About 1 g of oven-dried shucked tissue of the size class 10 - 20 mm SL was stored in liquid nitrogen for AA analysis. The rest of the shucked tissue and the shells was kept at -30 °C for the analysis of the proximate composition (size class 1.0 - 20 and 45 - 55 mm SL) and the mineral element profile (size class 10 - 20 mm SL) (Table 3.2).

Proximate Composition

Moisture and Ash

Moisture content was determined by weighing samples before and after drying for 48 h at 100 °C.

To measure ash content, 3 x 0.500 g of each sample were powdered in a mortar and placed in an open crucible in a muffle furnace. Prior to use, the crucible was burnt at 550 °C for 8 h. The samples were burnt at 550 °C for 7 h (Crampton & Harris 1969, Montgomery & Gerking 1980) and cooled in a desiccator. Following that, the final weight was determined.

Protein

A modified form of the Folin-Lowry method (Clark & Switzer 1977, Montgomery & Gerking 1980) was used to calculate crude protein content. Three sets of each sample (0.100 g) were homogenized with 2 ml of 1 N NaOH and left to digest for 24 h at 4 °C. The samples were

Table 3,2. Mean length, total and shucked weight ± SD of two size classes of *Haliotis midae*. Both size classes were used for proximate analysis. The amino acid and mineral element profile of animals of the size class 10 - 20 mm SL was also analyzed.

Sample	Length (mm)	Total weight (g)	Shucked weight (g)
		Size class 10 - ²0 mm SL	
Port Alfred[t]			
S	15.62 ± 3.48	0.66 ± 0.47	0.4² ± 0.31
T	16.45 ± 433	0.81 ± 0.63	
Cape Recife			
S	16.96 ± 1.92	0.73 ± 0.22	0.46 ± 0.16
T	16.70 ± 1.85	0.67 ± 0.21	
Fish River Mouth			
S	16.30 ± 2.03	0.73 ± 0.²4	0.50 ± 0A7
T	16.25 ± 2.47	0.70 ± 0.31	
		Size class 45 - 55 mm SL	
Port Alfred[6]			
S	49.46t 2.68	27.02t 3.18	19.64 ± 1.63
T	51.54 ± 3.87	29.81 ± 4.95	
Cape Recife[5]			
S	52.73 ± 1.54	31.95 ± 4.58	22.38 ± 2.46
T	51.83 ± 2.28	30.99 ± 3.87	
Fish River Mouth[4]			
S	54.80 ± 1.87	35.00 ± 4.33	24.83 ± 2.61
T	50.76 ± 4.25	27.23 ± 7.14	

n = 101 [2] n = 95 [3] n = 88 [4] n = 10 [5] n = 9 S: shucked tissue T: total animal

subsequently centrifuged at 3000 x g for 5 min to remove particulate matter. A test tube was filled with 0.1 ml of sample and made up to 10 ml with distilled water. Out of this, 1 ml was taken and further processed. Alkaline copper reagent was freshly prepared, 5 ml of which was added and immediately mixed in. After 10 min at room temperature, 0.3 ml of Folin - Ciocalteu reagent were added and also immediately mixed in. After standing for a further 30 min the absorbance of each test tube was read three times on a spectrophotometer at 500 nm. A protein standard curve was generated, with samples identical to the experimental tubes, excepting that they contained a known amount (0, 50, 100, 150, 200, 250 pg/m1) of a 1 mg/ml bovine serum albumin (BSA) -protein standard solution. To test whether NaOH interfered with the Folin-Ciocalteu reagent, a standard curve using 1 N NaOH instead of distilled water was prepared in exactly the same manner. Regression analysis showed a very high correlation

between the two standard curves (r^2 = 0.9973).

Fat

The method used was modified from FoIch *et al.* (1957) and the method employed by the Fishing Industry Research Institute, Cape Town (G. N. Davis 1992, Fishing Industry Research Institute, Cape Town, South Africa, pers. comm.). Three sets (0.200 g) of each sample were placed in a centrifuge bottle, to which 3 ml distilled water, 6.25 ml methanol and 6.25 ml chloroform were added. The mixture was homogenized for 2 min, after **which a further** 6.25 ml of distilled water were added. The sample was homogenized for another minute. The blender head was washed with a minimum of distilled water into the centrifuge bottle. This was followed by centrifugation at 3000 x g for 10 min. An amount of 0.75 ml of the bottom layer (chloroform) was pipetted into a tared 5 nil conical flask (the flasks were dried out in an oven at 60 °C for 24 h prior to use) and evaporated to dryness at 60 °C on a hot plate. Following that, the beaker was placed in an oven set at 100 °C for 30 min. Finally, it was cooled in a desiccator for 15 min and weighed. Percentage fat was calculated according to the equation:

$$\% \text{ fat} = \frac{\text{mass of fat (g)} \times 25/15}{\text{mass of sample (g)}} \times 100$$

Carbohydrate

The percentage carbohydrate was determined by two methods. Firstly, it was calculated indirectly according to % carbohydrate = 100 - (% protein + % fat + % ash). Secondly, a modified version of the Somogyi-Nelson method (Clark & Switzer 1977, Plummer **1978)** was employed. Three sets (0.100 g) of each sample were homogenized in 1 ml **of a 1 M** HC1 solution. They were then heated in an oven at 100 °C for 4 h. Following that, the samples were centrifuged for 10 min at 3000 x g to remove particulate matter. Subsamples of 0.1 ml were mixed with 0.1 ml of **1 N NaOH** to neutralize HC1. Subsequently, 0.2 ml of 0.3 M BaOH and 0.2 ml of 0.17 M $ZnSo_4$ were added to precipitate the protein. A further 1.4 ml

of distilled water was added to make up a final volume of 2 ml. The samples were left standing for 5 min with occasional shaking on a vortex mixer. They were then centrifuged for 10 min at 3000 x g to remove the precipitated protein. One ml of alkaline copper sulphate was added to 1 ml of the supernatant and heated in a boiling waterbath for 15 min. The samples were cooled for 5 min on ice. Once again, they were centrifuged at 3000 x g for 10 min to remove any remnants of precipitated protein. Finally, I ml of arsenomolybdate was added to the supernatant and the samples diluted to 10 ml with distilled water. The absorbance was read three times at 510 nm. A glucose standard curve was prepared with distilled water and a known amount (0.0, 0.2, 0.4, 0.6, 0.8, 1.0 mg/ml) of a 1 mg/m1 glucose standard solution.

Amino Acid Profile

The AA content of shucked tissue of the three samples, except for tryptophan, was analyzed by the Department of Animal Science and Poultry Science, University of Natal, Pietermaritzburg. Tryptophan concentration was measured by the Faculty of Agricultural Sciences, University of Stellenbosch, Stellenbosch.

Fatty Acid Profile

Saponification, Esterification and Extraction of Fatty Acids

The method employed was modified from Skeef (1988). The shucked tissue of each animal was homogenized in 1 ml of sodium phosphate buffer (0.1 M, p1-1 8) for 2 min. The homogenate was then transferred into a vessel used for saponification. The beaker used for homogenization was rinsed with 2 ml of methanolic KOH (10 %) which was added to the homogenate. To saponify the lipids, the homogenate was heated with reflux and under nitrogen for 45 min at 85 °C. Acidification of the FAs was achieved by adding 1 ml of 7 N HCI. Three ml of petroleum ether was added to this, and the vessel was shaken for 2 min on a vortex mixer to extract the FAs. This procedure was repeated and the extracts were pooled. The latter were evaporated to dryness under nitrogen at 60 °C in a waterbath. The residual

FAs were methylated by heating with 0.3 ml of BF_3-methanol reagent with reflux and under nitrogen for 5 min at 85 °C. The FA esters were extracted twice, with 1 ml of petroleum ether, as described before. They were then evaporated to dryness with reflux under nitrogen at 60 °C and stored at -30 °C in the dark.

Gas-Liquid Chromatography

Prior to analyses, the FA esters were resuspended in 20 pl of petroleum ether and analyzed on a 5890A Hewlett Packard gas chromatograph. A fused silica capillary column (SP 2330) was used. The temperature program run was taken from Stoll & Duncan (1992). Standards were obtained from Nu Chek Prep, Inc., Minnesota, U.S.A. and run subjected to exactly the same conditions. The traces were analyzed, calculating percentage individual FAs of total percentage FAs.

Mineral Element Profile,

All containers, instruments and glassware used for this analysis were acid washed in a 1:1 solution of 55 % nitric acid and water. The glassware was borosilicate glass, which has low metal binding properties. All water used was ultrapurified **(18 0)** by the Millipore - Milli-Q system.

Samples were processed as modified from Bryan *et al.* (1977). Subsamples of 1.000 g and 0.500 g of shucked tissue and shell from each sample site were weighed into crucibles. They were ashed at 450 °C for 24 **h.** Following this, 5 ml of concentrated nitric acid were added twice to each sample and evaporated to dryness on a hot plate. Finally, the ash was redissolved in 1 ml of concentrated HCl and 9 ml of water. Stock solutions for each metal were prepared adding 2 % of concentrated nitric acid to prevent metal adsorption.

The methods for analysis were taken from Greenberg *et al.* (1980). All metals except for potassium were analyzed by atomic absorption using a Varian Techtron 1000 Atomic

Absorption Spectrophotometer. Potassium was analyzed by flame emission.

Statistical Analysis

Results of the three sample sites were combined, if they were found to be not significantly different using one way analysis of variance at the p 5 0.05 level of significance. To compare the proximate composition of the two size classes a Student T-test was used (p 5 0.05).

RESULTS

Results of the proximate analysis for both size classes (10 - 20 and 45 - 55 mm SL) are shown in Table 3.3. There were significant differences between the two size classes. An increase in size was accompanied by a significant decrease in fat content (shucked tissue and total animal p 5_ 0.025) and a significant increase in carbohydrate content (shucked tissue p 0.05, total animal p 5 0.01). The Somogyi-Nelson method produced consistently lower

Table 33. Proximate composition of the shucked tissue of *Haliotis midae* and total animal. Results are the mean ± SD of three samples. Significant differences between the two size classes are indicated by *.

		% Dry weight				
Sample	% Moisture	Fat	Protein	Ash	Carbohydrate Subtracted	Carbohydrate Absolute values
				Size class 10 - 20 mm SL		
Shucked tissue	8131 ± 1.52	2.40 ± 0.85	44.67 ± 4.16	11.96 ± 1.94	40.98 ± 3.80	38.93 ± 9.71
Total animal	68.69 ± 3.26	1.40 ± 0.47	22.87 ± 2.83	59.22 ± 5.66	16.51 ± 4.40	15.60 ± 1.54
				Size class 45 - 55 mm SL		
Shucked tissue	77.91 ± 1.23	0.76** ± 0.06	39.67 ± 1.33	11.88 ± ².²9	47.70* ± 3.51	42.19* ± 4.31
Total animal	62.56 ± 0.94	0.52** ± 0.26	31.33** ± 3.²0	34.91*** ± 4.02	33.04*** ± 457	2994*** ± 4.29

* p 5 0.05 ** p 5 0.025 *** p 5 0.01

values for carbohydrate concentration than obtained by subtraction. However, the results were not significantly different. Moreover, there was a significant increase in protein content (p 0.025) and a significant decrease in ash content (p 5_ 0.01) of the total animal.

The AA composition of shucked tissue of juvenile *H. midae* is presented in Table 3.4. Results are the mean ± SD of three samples. No statistical analysis could be done as results for each sampling site were only obtained in duplicate. However, the means from each sampling site were very similar and it is unlikely that there were any significant differences. The most abundant AAs in decreasing order (in percent of total animal) were glutamic acid (3.38 ± 0.33 %), aspartic acid (2.41 ± 0.29 %), glycine (1.91 ± 0.16 %), arginine (1.81 ± 0.20 %) and leucine (1.59 ± 0.18 %).

There was no significant difference in the FA composition of the abalone from the three sites. The results could therefore be pooled to calculate mean values (Table 3.5). Total saturated

Table 3.4. Amino acid composition of shucked tissue of *Palings midae.* Results are the mean of three samples ± SD.

Amino acid	% Shucked tissue	% Total animal[s]
ala	².81± 0.11	1.33± 0.15
arg	3.81± 0.14	1.81± 0.20
asp	5.08 ± 0.24	2.41 ± 0.29
cys	1.²3 ± 0.05	0.56 ± 0.14
glu	7.14 ± 0.²5	3.38 ± 0.33
gly	4.03 ± 0.14	1.91 ± 0.16
his	0.88 ± 0.09	0.42 ± 0.07
He	1.96 ± 0.11	0.93 ± 0.12
leu	3.34 ± 0.15	1.59 ± 0.18
lys	3.00 ± 0.17	1.42 ± 0.19
met	1.02 ± 0.03	0.48 ± 0.04
phe	1.88 ± 0.16	0.89 ± 0.14
pro	2.53 ± 0.11	1.20 ± 0.15
ser	2.47 ± 0.09	1.17 ± 0.12
thr	2.41 ± 0.15	1.15 ± 0.15
trp	0.36 ± 0.04	0.19 ± 0.02
vat	2.23± 0.15	1.06± 0.15

% protein in total animal ²².87 ± ².83

Table 33. Fatty acid composition of shucked tissue of *Hatiotis midae.* Results are the mean of three samples and expressed as % of total fatty acids.

Fatty acid	Mean %
12:0	1.80
14:0	ND
14:1	ND
15:0	ND
16:0	16.25
16:1	1.56
17:0	ND
18:0	4.70
18:1	7.27
18:2w-6[1]	10.86
18:3w-3	1.11
19:0	7.22
20:0	4.02
20:1	2.48
20:[2]	0.53
20:4w-30-6	3.[2]7
20:5w-3	9.55
22:0	2.95
22:1	2.47
22:4w-6	3.34
22:5w-3,w-6	1.12
22:6w-3	0.72
24:0	5.57
24:1*	4.91
24:4*	3.94

ND = non detectable [I] Essential fatty acids in *H. discus hannai (Uki et* al. 1986c) * Identification uncertain

FAs accounted for 42.51 %, monounsaturated FAs for 18.69 %, and polyunsaturated FAs for 34.44 %. The most abundant FAs, as a percentage of total FAs, were 1.6:0 (16.25 %), 18:2 (10.86 %), 20:5 (9.55 %), 18:1 (7.27 %) and 19:0 (7.22 %).

There were no significant differences in the mineral element concentrations of the animals from the three sites. The data were therefore pooled and mean ± SD could be calculated. The data are expressed either as pg/g dry weight for shucked tissue, shell and total animal, and as pg/g wet weight of total animal (Table 3.6).

Table 3.6. Mineral element composition of *Ha!Loris midae.* Results are the mean of three samples ± SD.

	ug/g Dry weight			ug/g Wet weight	
Mineral element	Shucked tissue	Shell	Total animal	Shucked tissue[s]	Total animal[2]
Ca	846.7 ± 378.2	243300.0 ± 53900.0	244180.0 ± 54040.0	187.0 ± 83.5	76452.7 ± 16920.0
Cd	1.1 t0.3	0.7 t0.1	1.7 ±0.4	0.2 t0.1	0.6 f0.1
Co	2.8 ± 0.6	8.0 ± 0.5	10.8 ± 0.6	0.6 t0.2	3.4 ± 0.2
Cu	32.5 ± 5.8	16.6 ± ².8	49.1 ± 7.8	7.2 ± 1.3	15.4 t2.4
Fe	1127.0 ± 258.0	18².8 ± 48.0	1309.5 t305.4	210.6 g18.2	410.0 ± 95.6
K	11200.3 ± 1200.7	1000.5 ± 100.²	12200.4 ± 1039.²	²100.0 ± 200.0	3800.0 ± 346.4
Mg	5100.0 ± 300.0	953.3 ± 318.8	6053.3 ± 617.0	1000.0 ± 100.0	1895.0 ± 193.2
Mn	4.4 ± 1.6	2.7 ± 0.6	7.I ± 2.1	0.8 ± 0.3	2.2 ± 0.7
Ni	10.3 ± 2.4	12.6 ± 4.1	22.9 ± 4.6	2.3 ± 0.5	7.2 ± 1.4
Zn	109.3 ± 1.2	37.5 ± 11.0	146.8 ± 11.0	20.5 t0.2	46.0 t3.5

[1] % moisture 81.31 [2] % moisture 68.69

DISCUSSION

In juvenile animals, body tissue is predominantly layed down in the form of protein-rich muscle (Covey & Tacon 1982, Steffens 1989, Wilson 1989). However, the protein concentration of shucked tissue did not change significantly with an increase in size. The increase in the protein concentration of the total animal therefore probably resulted from an increase in shell protein. This is contrary to expectation. As there were no significant differences between sample sites, the results seem to be reliable. However, further

investigations are required, including studies on other size classes.

Archaeogastropods are known to possess high glycogen stores (Giese 1966), and their energy metabolism has been shown to be carbohydrate based (Emerson 1967, Livingstone & de Zwaan 1983). An increase in size was associated with a significant increase in the carbohydrate content, probably as a result of a build up in the amount of stored glycogen.

The lower fat content of the larger size class could simply be the result of the increase in the protein and carbohydrate levels. The optimal level of lipid in a diet for juvenile *H. discus hannai* has been found to be 5 % (Uki *et al.* 1985a). This indicates that lipids do not play an important role regarding the energy metabolism of haliotids.

The decrease in the ash content of the total animal with increasing size shows that the soft tissue grows faster than the shell. The ash content of shucked tissue did not change with an increase in size.

As both of the size classes were sexually immature (Newman 1967, Wood 1993), gamete production could not account for the changes in the proximate composition. No other studies on the proximate composition of shucked tissue or total animal have been undertaken.

Information on the EAAs and their requirements in the nutrition of *H. midae* is lacking. However, the total and EAA profile of shucked tissue was determined for three juvenile *H. rufescens* of unknown size by Allen & Kilgore (1975). To compare their results to the ones of this study, the AA concentrations were converted from umoles/100 mg protein to a percentage of total protein (Table 3.7). The two AAs with the highest concentrations in both species were glutamic and aspartic acid. This could be expected as both are easily synthesized from the intermediates of the citric acid cycle and other major metabolic pathways (Stryer 1988). A Student T-test was used to compare the AA concentrations of the two species. There

were significant differences in the concentrations of alanine, leucine, methionine and tryptophan (p 5 0.05), glutamic acid (p 5 0.025), cysteine, glycine, proline and serine (p 0.01). No conclusions as to the significance of these differences between the two species could be drawn. Like in most other animals (Stryer 1988, Wilson 1989), the same ten AAs as for *H. rufescens* are probably also essential for *H. midae* (Table 3.7).

Palmitic acid (16:0), the endproduct of FA synthesis (Stryer 1988), makes up a major proportion of the total FAs of juvenile *H. midae.* Similar observations have been made for *H. discus, H. japonica* (Shimma & Taguchi 1964) and *H. midae* (de Koning 1966a). These are the only other studies on shucked tissue of abalone. All abalone species analyzed so far have a high percentage of arachidonic acid (20:4), which is unusual for marine species (011ey & Thrower 1977). In *H. discus* and *H. japonica,* 20:4 accounts for 10.7 % and 12.3 % of total

Table 3.7. Comparison of the amino acid composition of *Ha!jolts midae* and *H. rufescens*[1]. kesults are the mean of three samples ± **SD.** Significant differences between the two species are indicated by *.

Amino acid	% Protein	
	H. midae	*H. rufescens*[2]
ala[3]	5.82 ± 0.07*	5.45 ± 0.14
arg	7.91 ± 0.13	7.²6 ± 1.06
asp	10.54 ± 0.06	11.²0 ± 0.72
cys	2.41 ± 0.35***	0.69 ± 0.07
glu	14.8² ± 0.39**	13.50 ± 0.52
gly	8.37 ± 0.37***	4.96 ± 1.02
his[3]	1.82 ± 0.10	1.97 ± 0.26
ile[a]	4.11 ± 0.03	3.78 ± 0.49
Ieu[3]	6.93 ± 0.07*	7.84 ± 0.53
lys[3]	6.21 ± 0.03	5.99 ± 0.45
met	².09 ± 0.06*	2.60 ± 0.25
phe[3]	3.90 ± 0.14	4.07 ± 0.86
pro	5.24 ± 0.03***	2.68 ± 0.31
ser	5.13 ± 0.13***	4.29 ± 0.25
thr[3]	4.99 ± 0.05	4.63 ± 0.²5[4]
trP	0.8² ± 0.17*	0.38 ± 0.02
tyr	—[5]	3.81 ± 0.65
val[3]	4.61 ± 0.09	4.72 ± 0.42

[1] Allen & Kilgore (1975) [2] Converted from pinoles/100 mg protein [3] Essential amino acid (Allen & Kilgore 1975)
[4] Mean of two values [5] Not analyzed for * p 5 0.05
** p 5 0.025 *** p < 0.01

FAs, respectively (Shimma & Taguchi 1964). In comparison the percentage for 20:4 in *H. midae* is lower and ranges between 3.27 % (this study) and 7.3 % (de Koning 1966a).

As yet, docosahexaenoic acid (22:6) has only been recorded in *H. midae* (de Koning 1966a, this study). It is possible that the presence of 22:6 is specific for *H. midae. It* is unlikely that de Koning (1966a) also analyzed juvenile *H. midae* in the weaning phase. However, if he did so this would indicate that 22:6 was even specific to this stage of the animals life cycle. As neither de Koning (1966a) nor Shimma & Taguchi (1964) mention the size of the animals used in their study, no conclusion as to the significance of 22:6 can be drawn.

Another difference between *H. midae* and other haliotids is the ratio of stearate (18:0) and oleate (18:1). In *H. midae,* 18:1 accounts for less than double the 18:0 content, whereas in *H. discus* and *H. japonica,* the 18:1 content is three to four times higher than 18:0.

Bannatyne & Thomas (1969) pointed out that lipids of marine origin characteristically possess high levels of C_{20} and C_{22} FAs. In de Koning's (1966a) and this study the amount of these two FA classes in *H. midae* is 28 % and 30 %, respectively. On the other hand, Shimma & Taguchi (1964) reported higher values of 39 % for *H. discus* and 43 % for *H, japonica.*

Total polyunsaturated FAs (PUFAs) in juvenile *H. midae* account for 34.44 %, which falls within the range (30 - 35 %) typical for tissues or total soft body of *Haliotis* species (011ey & Thrower 1977). Uki *et al.* (1985a) established that the c0-3 and the to-6 PUFAs are essential for *H. discus hannai* (Table 3.5). It is very likely that these two classes of FAs are also essential for *H. midae.*

The concentrations of the mineral elements in individual tissues of *H. midae* have been determined by Watling & Watling (1974) and Van As *et al.* (1975). In the present study, however, the shucked tissue and the shell were analyzed. As expected, the results for shucked

tissue in pg/g wet weight were consistently higher than those reported for individual tissues of *H. midae* (Watling & Watling 1974, Van As *et al.* 1975). The absence of any abnormally high concentrations reflects an unpolluted environment (Fourie 1976).

Bryan *a* al. (1977) obtained similar concentrations for 10 mineral elements of shucked tissue of *H. tuberculata.* Chen (1989) measured the concentrations of Ca, Cu, Fe, Mg, and Zn for shells of *H. diversicolor supertexta.* The results are very similar to the ones of this study, with the exception of Ca and Mg (3 x lower and 3 x higher in *H. midae,* respectively). Although Chen (1989) did not provide the size range of shells analyzed, he referred to "young and juvenile abalones" (p. 279). It must be borne in mind however, that he analyzed animals reared under pond culture conditions in Taiwan and fed on artificial feed, *Ulva* spp. and *Gracilaria* spp. In the present study abalone were collected from the wild. Samples from each site have been shown to have a very varied diet, comprising diatoms, coralline algae and pieces of macroalgae (R. Anderson 1992, Sea Fisheries Research Institute, Cape Town, South Africa, pers. comm.). Abalone can take up minerals from sea water (Van As *et* a/. 1975), but probably obtain more metals from their food (Young 1977). Therefore, it is likely that the observed differences in Ca and Mg concentration were caused by the location of the animals and the different diets. As yet, there is no evidence that the mineral element profile of abalone approximates nutritional requirements as shown in fish (Steffens 1989).

H. midae is the first haliotid to be thoroughly analyzed regarding its chemical composition. The data, however, provides a baseline for nutritional requirements at the weaning stage only. The proximate composition of juveniles changed with an increase in size. Likewise, the concentration of micro-nutrients are known to change with size (011ey & Thrower 1977), sexual maturation and the seasonal reproductive cyle (Webber 1970). Therefore, the data cannot simply be extrapolated to other stages of the life cycle of *H. midae.*

CHAPTER 4

FORMULATION OF AN ARTIFICIAL WEANING DIET

INTRODUCTION

The aim of artificial feed formulation and preparation is to provide for optimal nutrition at the lowest possible cost. In reality, however, diet development is normally a compromise between this ideal situation and practical considerations, such as the cost of ingredients, pelletability, diet acceptability, water stability of the feed and handling requirements (Hardy 1989).

The principal aim of this project was to develop an artificial weaning diet for *Haliotis midae,* suitable for a commercial operation. In the initial phase of the development of such a diet, the nutritional composition and the acceptability of the feed to juveniles is of foremost importance. Therefore, at this stage the price of the ingredients was of a secondary nature.

Although artificial diets for several abalone species have been developed (Ogino & Ohta 1963, Ogino & Kato 1964, Sagara & Sakai 1974, Uki *et a/.* 1985a, Gorfine & King 1991, Morrison & Whittington 1991, Viana *et al.* 1993, Britz *et al.* 1994), there is a general lack of knowledge of the nutritional requirements of haliotids. So far, only the protein, lipid and mineral element requirements of juvenile *H. discus hannai (Uki et al.* 1985a, 1986b), as well as their essential fatty acid (EFA) requirements (Uki *et al.* 1986c) have been determined. Dixon (1992) studied the digestibility of a pellet developed for the grow-out phase of *H. midae. Wee et al.* (1992) and Maguire *et al.* (1993) have begun to establish a data base on the digestibility of potential feed ingredients for abalone. However, such information is currently very limited. Moreover, no data on the digestibility of feed ingredients in juveniles at the weaning stage have been published.

The major component needed for the growth of juvenile animals is protein (Steffens 1989). The composition of the protein is of particular importance, as the essential amino acids (EAAs) have to be supplied by the diet. It has been shown that in the absence of knowledge of the EAA

requirements, the EAA pattern of the whole body tissue of an animal provides a clue as to the required composition of the dietary protein (Cowey & Tacon 1982). The EAA profile of *H. midae* **was established (Chapter 3), and the EAA pattern of the weaning diet could therefore be developed based on this information.**

It is possible that nutritional requirements vary within a genus (Ceccaldi 1982), however, it is reasonable to assume that the gross requirements for protein, lipids and minerals do not differ significantly among haliotids. Therefore, in the absence of information on the nutritional requirements of *H. midae* **the data obtained on juvenile** *H. discus hannai* **(Uki** *et al.* **1985a, 19866) was used to supplement the weaning diet for** *H. .midae.*

The artificial weaning diet was formulated in the following manner. The AA profile of casein, fish meal, gelatine and *Spirulina* **spp. (Cyanophyceae) was analyzed. Casein, fish meal and** *Spirulina* **spp. were selected as they have been identified as good protein sources for** *H. midae* **(Britz** *et al.* **1994). Gelatine, on the other hand, has good binding properties and a high protein content of more than 88 % (Hardy 1989). Based on these analyses, a diet was formulated which reflected the EAA pattern of juvenile** *H. midae.* **Based on the findings of Uki** *et al.* **(1985a) the diet was supplemented with other ingredients to satisfy the lipid, carbohydrate, mineral element and the vitamin requirements.**

MATERIALS AND METHODS

Casein, gelatine, dextrin and the ingredients required for the vitamin and mineral mixtures were purchased from Saarchem (Pty.) Ltd., Krugersdorp. Chilean "low temperature" fish meal was obtained from South African Sea Products (Pty.) Ltd., Cape Town, sun-dried *Spirulina* **spp. from Western Tanning (Pty.) Ltd., Wellington, and fish oil from Marine Oil Refiners (Pty.) Ltd, Cape Town. "Helios" sunflower oil was purchased in a grocery store.**

The AA profile of casein, gelatine, fish meal and *Spirulina* spp. was analyzed as described in Chapter 3. The tryptophan content of each ingredient was taken from the literature. The EAA index, i.e. the concentration of each EAA, was calculated as a percentage of total protein. This information was computed and the percentage composition of the diet was derived by iteration (Simplex-method, "Quattro" spreadsheet). The EAA pattern of *H. midae* and the diet was calculated as percentage of total EAA content (Cowey & Tacon 1982):

$$\%EAA = \frac{[EAA]}{[\text{Total EAA}]} \times 100$$

The lipid, carbohydrate, mineral element and vitamin components of the diet were added using the information provided by Uki *et al.* (1985a) for juvenile *H. discus hannai.*

RESULTS

The EAA index of casein, gelatine, fish meal and *Spirulina* spp. is shown in Table 4.1 and compared to that of *H. midae.* Results for the four feed ingredients are the mean of two measurements and are expressed as percent protein. Casein has the highest nutritional value with only arginine, methionine + cysteine and threonine being limiting AAs. In fish meal and *Spirulina* spp. five and six AAs are limiting, respectively. Gelatine has the lowest nutritional value as every EAA is limiting.

Based on the EAA index of the protein sources, the weaning diet formulation consisted of 5 % casein, 15 % gelatine, 15 % fish meal and 10 % *Spirulina* spp., on a dry weight basis. The pattern of EAAs, expressed as percent of total EAA content of *H. midae* and the diet is compared in Table 4.2. The correlation coefficient between the two EAA patterns was $r^2 = 0.8989$.

Table 4.1. Comparison of the essential amino acid index (% protein) of *Haliotis midae* and four feed ingredients, used in the formulation of an artificial weaning diet. Results of the four feed ingredients are the mean of two measurements. Values underlined indicate limiting amino acids.

	% Protein				
Amino acid	Total animal'	Casein	Gelatine	Fish *meal*	*Spinlina* spp.
arg	7.91	3.53	6.98	5.12	5.71
his	1.82	3.19	0.82	2.90	1.21
ile	4.11	5.50	1.48	3.85	4.57
leu	6.93	9.68	2.94	7.13	7.44
lys	6.21	8.19	3.84	7.77	5.92
met + cys	4.50	3.46	1.02	3.75	2.5[2]
phe ÷ tyr	7.71[2]	11.21	2.38	7.07	7.01
thr	4.99	4.34	1.94	4.42	4.40
ITP	0.82	1.28'	0D5[4]	1.25[5]	1.18[6]
val	4.61	7.08	2.40	4.75	5.4.6
% Protein	22.87	88.22	98.94	70.10	57.10

Table 3.7
[3] Appendix (p. 730) in Halver (1989)
[5] S. Featherstone 1992, South African Sea Products (Pty.) Ltd., Cape Town, South Africa, pers. comm.
[2] Tyr content of *H. rufescens* (Table 3.7)
[4] Appendix (p. 733) in Halver (1989)
[6] Becker (1986)

Table 4.2. Essential amino acid pattern in *Haliatis midae* and the diet. Results are expressed as % of total essential amino acid content. Correlation coefficient between the two patterns is $r^2 = 0.8989$.

Amino acid	*H. midae*	Diet"
arg	15.93	14.72
his	3.70	4.55
ile	8.19	8.04
leu	14.00	14.51
lys	12.50	14.86
met + cys	9.07	5.97
phe + tyr	15.49	16.43
thr	10.12	8.46
trP	1.67	1.92
val	9.33	10.53

% composition: casein 5,0, gelatine 15.0, fish meal 15.0, *Spirulina* spp. 10.0

Table 4.3. Composition of the mineral and vitamin mixture of the formulated weaning diet for *Ha'lolls midae* (see [Ski *et al.* 1985a).

Mineral	%	'Trace element.	%
NaC]	1.0	$ZnSO_4.7H_2O$	35.3
$MgSo_47+1_2O$	15.0	$MnSO_4.4H_2O$	16.2
$NaH_2PO_4.2H_2O$	²5.0	$CuSO_4·5H_2O$	3.1
KH_2PO_4	32.0	$CoC1_2$-$6H_2O$	0.1
$Ca(H_2PO_4)_2+1_2O$	20.0	KIO_2	0.3
Fe-citrate	².5	Cellulose	45.0
Ca-lactate	3.5		
Trace elements'	1.0		
Vitamin	**mg**	**Vitamin**	**mg**
Thiamine HC1	6.0	Folic acid	1.5
Riboflavin	5.0	PABA	²0.0
Pyridoxine HC1	20.0	Menadione	4.0
Niacin	40.0	B12	0.009
Ca-pantothenate	10.0	C	200.0
Inositol	²00.0	A	1.5
Biotin	0.6	1)	0.0025

The composition of the mineral and vitamin mixture is listed in Table 4.3 and the final composition of the weaning diet is given in Table 4.4. The diet was supplemented with 2.5 % fish oil, 2.5 % sunflower oil, 44 % dextrin, 4.0 % of the mineral and 2.0 % of the vitamin mixture.

Table 4.4. Composition of the artificial weaning diet for *Hahods midae.*

Ingredient	%
Casein	5.0
Gelatine	15.0
Fish meal	15.0
Spirulina spp.	10.0
Fish oil	².5
Sunflower oil	2.5
Dextrin	44.0
Mineral mixture'	4.0
Vitamin mixture'	².0

For detailed composition see Table 4.3

DISCUSSION

The primary goal of the formulation of the weaning diet at this initial stage was to meet the EAA requirements of *H. midae.* The EAA index was taken as a direct indication of the nutritional value of a potential protein source. Comparison with the EAA index of ***H.*** *midae* showed casein to have a high nutritional value with three AAs being limiting. In fish meal there were five, in *Spirulina* spp. six, and in gelatine every EAA was limiting (Table 4.1). None of the feedstuffs could therefore be used as an exclusive protein source in the diet. However, a low nutritional value does not automatically exclude a protein source. Gelatine was still included in the diet as it has very good binding properties which is important for water stability (see Chapter 5).

There was a high correlation coefficient of $r^2 = 0.8989$ between the EAA pattern of the formulated diet and ***H.*** *midae* (Table 4.2). It was interesting to note that the EAA pattern of *H. midae* is very similar to that of *H. rufescens* (calculated from Allen & Kilgore 1975), and also to the ones reported for prawn (Deshimaru & Shigeno 1972, Colvin 1976) and fish species (Wilson *et al.* 1978, Robinson *et al.* 1980). This indicates similarities in the pattern of EAA requirements of vertebrates and invertebrates.

Although the pattern of EAAs found in the whole body of an animal forms a reasonable basis for the initial formulation of a diet, no account is taken of the maintenance requirements of EAAs and the digestibility of the protein sources (Cowey & Tacon 1982). Consequently, the dietary pattern can only be treated as an estimate of the nutritional requirements (Wilson & Poe 1985).

EAA requirements are influenced by interactions between themselves, or other AAs and nutrients (Cowey & Tacon 1982, Steffens 1989). Fish are capable of synthesizing the non-essential AAs cysteine from methionine and tyrosine from phenylalanine, if there is a short supply in the diet (Wilson 1989). These two non-essential AAs are therefore included in the EAA pattern, although it is not known whether abalone can interconvert these AAs as well. The relatively large difference between the percentage methionine + cysteine in *H. midae* and the diet is the result

of the inclusion of cysteine. It was not possible to reduce the difference using the available protein sources, as this would have changed the content of the other EAAs as well. However, cysteine in purified, crystalline form was not added, because it is not known to what extent abalone can utilize crystalline AAs.

The digestibility of the protein sources is also of importance. Dixon (1992) used calcium caseinate and dried kelp, *Ecklonia maxima,* as protein sources in a diet containing 25.52 % crude protein. He measured crude protein apparent digestibility coefficients (CPADCs) varying from 92.7 - 96.7 % in the 15 - 18 °C temperature range in *H. midae* (55 - 85 mm shell length (SL)). Nothing is known about the digestibility of any protein source in juvenile *H. midae.* Wee *et al.* (1992) obtained relatively low CPADCs (64.1 - 74.9 %) with artificial diets fed to *H. rubra.*

The percentage protein in the diet (35.48 %) resulted from the need to obtain a high correlation between the dietary EAA pattern and the one of *H. midae.* This is higher than the 20 - 30 % suggested by Uki et *al.* (1986b) for juvenile *H. discus hannai* (31.3 - 32.0 mm SL). However, the protein requirements of fish are known to decrease with an increase in size and age (Wilson 1989). Therefore, the protein requirement during the weaning stage of abalone is probably also higher than that of older animals.

Uki *et al.* (1986c) showed the w-3 and (0-6 polyunsaturated FAs (PUFAs) to be essential for *H. discus hannai.* Due to the nature of the food components used in the artificial weaning diet, FA levels in excess of the requirements can in any case be ensured (Fallu 1991). Fish meal, e.g., is commonly used in artificial diets for marine animals because of its suitable AA profile. Associated with it are easily digestible lipids that are high in PUFAs. This is also true for *Spirulina* spp. (Becker 1986). A dietary lipid content of 5 % was found to be optimal for *H. discus hannai* (Uki *et at.* 1985a). Thus, in the absence of a detailed PUFA profile of the fish meal and *Spirulina* spp. used, 5 % of a 1:1 mixture of fish and sunflower oil was added to the diet.

Uki *et al.* (1985a) did not find a clear relationship between carbohydrate content and growth. To date, nothing is known about optimal energy content or protein/energy ratios for abalone. Both fish meal and *Spirulina* spp. contributed an unknown quantity of carbohydrate to the diet. An amount of 44 % of dextrin was added to the weaning diet as it has been identified as a suitable carbohydrate source (Ogino & Ohta 1963, Ogino & Kato 1964, Uki *et al.* 1985a, Britz *et al.* 1994).

No work on the requirements of specific minerals or vitamins in haliotids has been done. The dietary requirements especially of minerals are very difficult to determine (Lail 1989). Although the use of feed ingredients such as fish meal is generally considered to adequately supply minerals in fish diets (Lail 1989), growth and survival of fish are known to improve with mineral supplementation (Cuplin 1969, Karagoz 1986, Lall 1989). Likewise, Uki *et al.* (1985a) highlighted the importance of mineral supplementation of a semi-purified diet. Thus, 4 % of a mineral as well as 2 % of a vitamin mixture was added to the weaning diet.

In summary, the formulation of the weaning diet was based on the EAA pattern of *H. midae*. Information on the lipid, carbohydrate, mineral element and vitamin requirements of juvenile *H. discus hannai* (Uki *et al.* 1985a, 1986a) was used to supplement the diet. This approach was chosen, as data on the nutritional requirements of juvenile *H. midae* and the digestibility of foodstuffs are lacking.

CHAPTER 5

DEVELOPMENT OF A WATER STABLE GEL AND PELLET FORM OF THE ARTIFICIAL WEANING DIET

INTRODUCTION

Abalone are nocturnal and relatively slow feeders, both in the wild (Sakai 1962a, Poore 1972a, Shepherd 1973, Barkai & Griffiths 1986, 1987, Tutschulte & Connell 1988) and under culture conditions (Uki 1981, Hayakama *et al.* 1987, Genade *et al.* 1988). Any artificial diet fed to abalone must therefore be water stable for at least 12 h. Inadequately water stable feeds result in poor growth due to the rapid leaching of nutrients (Chen 1989).

Various binding agents have been used to produce water stable feeds. Primary amongst these are alginates, which have been used in varying concentrations (Meyers *et al.* 1972, Meyers & Brand 1975, Farmanfarmaian & Lauterio 1979, Heinen 1981, Farmanfarrnaian *et al.* 1982, Storebakken & Austreng 1987). In fish feed pellets, alginates have been used at concentrations of 5 % (Storebakken & Austreng 1987), and in crustacean feeds concentrations vary from 0.5 % (Farmanfarmaian *et al.* 1982) to 3 % (Heinen 1981). For abalone weaning diets concentrations of up to 20 % have been used (Ogino & Kato 1964, Sagara & Sakai 1972, Koike *et al.* 1979). Uki *et al.* (1985a) cast abalone diets containing 20 - 30 % alginate in the form of cylindrical pastes.

Gelatine has also been widely used as a binder in semi-purified diets for fish. It serves both as a binding agent and a protein source (Hardy 1989). Starch at a concentration of 23 % (Britz *et al.* 1994) and agar at an inclusion level of 9 % (Dixon 1992) have also been identified as suitable binders for abalone feed pellets. However, no data on the water stability of any feed for abalone have been published, despite the importance of good water stability for the success of artificial diets.

Artificial diets suitable for juvenile abalone during the weaning stage have been developed as gels (Ogino & Ohta 1963, Ogino & Kato 1964, Sagara & Sakai 1974, Koike *et al.* 1979) and in pellet form (Hahn 1989e, Gorfine & King 1991). As both are acceptable as a means of presentation of a weaning diet, this study was aimed at developing a water stable gel as well as a pellet form of the formulated feed. The effects of five alginates using two different sequestrants (sodium hexametaphosphate and D-gluconic acid lactone), agar, gelatine, and a combination of agar and gelatine on 24 h water stability of the experimental weaning diet cast in gel form were investigated. At the same time, the 24 h water stability of the starch bound pellet form of the diet was also evaluated.

MATERIALS AND METHODS

The studies on water stability were undertaken prior to the dietary formulation work. The formulation of the experimental weaning diet used in this experiment (Table 5.1) was therefore different to the one developed in Chapter 4. With the exception of gelatine the same ingredients were used in both diets, although some of them in different concentrations_ However, it is reasonable to assume that the water stability of the formulations would not differ significantly. The diet used for the water stability trials was modified from Uki *et al.* (1985a). The dietary components were milled to 125 pm in a hammermill and oven dried at 50 °C for 24 h.

Table 5.1. Composition of the artificial weaning diet used for binder studies.

Ingredient	% Dry weight
Casein	18.6
Fish meal	14.7
Spirulina spp.	14.3
Fish oil	2,5
Sunflower oil	2.5
Dextrin	41.9
Mineral mixture[1]	4.0
Vitamin mixture	1.5

[1] For detailed composition see Table 4.3

Four of the alginates, Kelgin HV, Manucol DM, Manucol DMF and Manugel GMB were obtained in powdered form from Kelco International (Ltd.), London. Potassium alginate, in a paste form, was obtained from Kelp Products (Pty.) Ltd, Cape Town. The sequestrant sodium hexametaphosphate was obtained from Merck Inc., Johannesburg and D-gluconic acid lactone from Sigma Chemical Co., U.S.A. Agar was purchased from Lasec (Pty) Ltd., Johannesburg and gelatine from Davis Gelatine Industries (Pty.) Ltd, Krugersdorp. Starch was obtained from Sea Plant Products (Pty.) Ltd, Hermanus.

Initial tests were undertaken to determine the acceptability of the formulated feed. The feed was prepared with the different binders and offered to juvenile *Haliotis midae* (5 - 10 mm shell length (SL)) as described below. It was found that the animals readily accepted the diet, irrespective of the kind of binder used, whereapon the trials on water stability were undertaken.

Preparation of Gels

All percentages and weights refer to the dry weight of the feed. Once the mixtures had been prepared they were spread onto a screen (19 cm x 22.5 cm) made of inert fibre glass mosquito mesh (2 mm x 2 mm) fixed onto a Perspex frame, and allowed to set into a gel at room temperature, if not stated differently. The area occupied by each gel on the screen was approximately 22 cm x 5 cm and 1 -2 mm thick. A detailed description of gel preparation using the various binders follows.

Alginates

Initially, standard methods as described for binding crustacean feed pellets (Meyers *et al.* 1972, Farmanfarmaian & Lauterio 1979) were tested. Alginate concentrations of 2, 4, 10 and 20 %, with varying concentrations of sodium hexametaphosphate (1 - 5 %) did not result in proper gel formation. Therefore an alternative method was developed.

For each of the alginates, 0.11 g (2 %, corrected for 10 % moisture) and 0.05 g (1%) of either sodium hexametaphosphate or D-gluconic acid lactone were mixed for 30 sec in 8 ml of water. To this mixture 0.05 g of calcium citrate (1 %), dissolved in 2 ml of water, was added and mixed for 15 sec. Finally, 5.0 g of the weaning diet were added and mixed for 1 min. Each gel was allowed to set for 12 - 14 h prior to testing. Potassium alginate was mixed with the diet in exactly the same way, exccpt that a 2.03 g sample had to be added to include 2 % of alginate (dry weight of potassium alginate 4.92 %).

Agar

A 0.30 g sample (13.5 %, corrected for 10 % moisture) or a 0.20 g sample (9 %, corrected for 10 % moisture) of agar was boiled in 18 ml of water for 30 sec and then cooled to 45 °C. It was then mixed with 2.0 g of feed. The gels were allowed to set for 30 - 45 min before testing.

Gelatine

A 0.33 g sample of gelatine (15 %, corrected for **10 %** moisture) or a 0.13 g sample (6 %, corrected for 10 % moisture) was brought to the boil **in 10** ml of water. It was cooled to 45 °C and then mixed with 2.0 g of feed. A 0.44 g sample (corrected for 10 % moisture) of gelatine was prepared in exactly the same way and mixed with 2.0 g of feed to yield a 20 % concentration. The gels were allowed to set for 30 - 45 min before testing. The effect of setting at low temperature on water stability was tested by preparing gels containing 20 % gelatine, and allowing them to set in a refrigerator at 4 °C for 2 and 24 h, respectively.

Agar/Gelatine

Two agar/gelatine mixtures (1:1 and 1:3) were tested at a concentration of 20 %. Either 0.22 g (10 %, corrected for 10 % moisture) or 0.11 g of agar (5 %, corrected for 10 % moisture) were mixed with 0.22 g (10 %, corrected for 10 % moisture) or 0.33 g of gelatine (15 %, corrected for 10 % moisture). The mixtures were boiled in 15 ml of water for 10 sec and

cooled down to 45 °C. Finally, 2 g of feed were mixed in. Prior to testing, the gels were allowed to set in a refrigerator at 4 °C for 2 h.

Preparation of Pellets

The detailed production procedure of starch based pellets cannot be stated for proprietary reasons. The diet was mixed with 23 % starch, mixed, extruded and dried. A 5.0 g sample (6 % moisture content) of pellets varying from 1 - 2 cm in length, was tested for water stability.

Testing of Water Stability

Tests were performed in the absence of abalone. The pellets and screens were transferred to 15 1 glass aquaria simulating culture conditions (aeration with airstones at 4 1/min per tank, at a water temperature 18 °C, with a water flow rate of 0.4 limin per tank). Every preparation was tested five times. After 6, 12, 18 and 24 h the samples were removed from the tank and oven-dried at 70 °C for 48 h. The initial dry weight of all preparations was determined in the same way. The difference between final and initial weight, expressed as a percentage, was taken as a quantitative measure of loss of nutrients.

Statistical Analysis

The water stability results for each type of binding agent were analyzed using one way analysis of variance at the p 0.05 level of significance, followed by Tukey's multiple range test. For the comparison of two groups a Student T-test was used (p 5. 0.05).

RESULTS

The 24 h water stability trials of the weaning diet using different alginates as binders with two sequestrants produced very similar results (Table 5.2). The only significant difference in the water stability results between the two sequestrants was found after 24 h with the Kelgin

Table 52. Effect of alginates on 24 h water stability of the weaning diet. Results are expressed as mean % of 5 replications ± SD of initial weight. Significant differences for the same alginate using different sequestrants are indicated by *.

Alginate	% Dry weight			
	6 h	12 **h**	**18h**	**24 h**
		Sodium hexametaphosphate		
Kelgin ITV	49.7 ± 3.3	41.4 ± 6.0	349 ± 5.5	34.9 ± 5.1*
Manucol DM	48.2 ± 5.6	445 ± 3.7	40.5 ± 6.1	36.1 ± 4.2
Manucol DMF	49.0 ± 0.9	46.9 ± 4.1	36.6 ± 3.1	35.0 ± 8.7
Manugel GMB	49.8 ± 3.5	46.9 ± 3.9	42.8 ± 4,6	39.5 ± 25
Potassium alginate	50.2 ± 4.9	*473* ± 2.0	44.7 ± 3.3	37.5 ± 0.6
		D-Gluconic acid lactone		
Kelgin HV	40.2 ±10.8	37.7 ± 5.3	23.0 ± 8.0	14.1 ± 5.1
Manucol DM	49.0 ± 6.3	39.7 ± 1.9	37.0 ± 1.2	36.4 ± 05
Manucol DMF	46.8 ± ².6	41.4 ±11.6	39.7 ± 4.4	33.4 ± 1.7
Manugel GMB	47.1 ± 6.3	43.9 ± ².9	39.1 ± 1.2	37.1 ± 0.9
Potassium alginate	45.5 ± 59	45.3 ± 1.9	40.0 ± 55	38.6 ± 1.3

* p 0.01

HV alginate (sodium hexametaphosphate: 34.9 ± 5.1 % dry weight, D-gluconic acid lactone: 14.1 ± 5.1 % dry weight, p 5 0.01). The use of sodium hexametaphosphate as a sequestrant for alginate improved the water stability of the gels. The least amount of leaching of feed ingredients was obtained after 6, 12 and 18 h with potassium alginate and after 24 h with Manugel GMB. However, the water stability of the different alginates was not significantly different.

The gels containing 13.5 % agar had a significantly lower leaching rate than the 9 % agar gels after 6 (p 5 0.0025), 12 (p 5. 0.01), 18 (p 5 0.0025), and 24 h (p 5 0.001) (Table 5.3).

Gels bound with 15 % gelatine were significantly more water stable than the 6 % concentration after 6 h (p 5 0.05). After 12, 18 and 24 h, however, gels containing 6 % gelatine were more water stable than gels prepared with 15 % gelatine, with the only significant difference after 12 h (p 5 0.005) (Table 5.4).

Table 53. Effect of agar on 24 h water stability of the weaning diet. Results are the mean % of 5 replications ± SD of initial weight. Significant differences between the two concentrations are indicated by *.

	% Dry weight			
% Agar	6h	12h	18h	²4h
9.0	66.0 ± 0.9	63.6 ± 1.5	60.8 ± 1.4	57.1 ± 0.4
13.5	72.4 ± 1.²**	69.3 ± 1.0*	69.0 ± 5.0**	67.1 ± 1.2***

* p 5 0.01 ** p 0.00²5 *** p 0.001

The 20 % gelatine gels, which were allowed to set at 4 °C, were more water stable than the gels set at room temperature after 6 h (Table 5.4). However, only gels set at 4 °C for 2 h had a significantly better water stability (p 5_ 0.01). After 12, 18, and 24 h setting at 4 °C produced poorer results. After 18 h the gels set at room temperature were significantly more water stable than the gels set at 4 °C for 24 h (p 5_ 0.001). Moreover, gels set at 4 °C for 2 h were also significantly more water stable than the ones set at 4 °C for 24 h (p S 0.005).

There was no significant difference in water stability of the two agar/gelatine mixtures (1:1, 1:3) (Table 5.5), although the results of the 1:3 mixture were slightly better. The 1:3 agar/

Table 5.4. Effect of gelatine on 24 h water stability of the weaning diet. Results are the mean % of 5 replications **± SD** of initial weight. Significant differences between the 6 % and 15 % and within the 20 % gels are indicated by

	% Dry weight			
% Gelatine	6 h	1² h	18 h	24 h
6	5².1 ± 3.9	49.5 ± 2.7***	30.9 ± 2.5	27.6 ± 9.6
15	64.7 ± 6.3*	²6.9 ± 4.3	26.1 ±13.3	18.2 ± 5.6
20	72.9 ± ².1	58.9 ±10.1	44.7 ± ².3****[1]	²7.6 ± 9.5
²0[2]	81.² ± ².7**[3]	53.0 ± 5.4	4².1 ± 3.5***[4]	1E5 ± ².7
20[5]	76.8 ± 4.4	48.0 ± 4.3	28.3 ± 2.0	15.6 ± 5.1

[1] Comparison of ²0 and 20[5]
[2] Set for ² h at 4° C
[3] Comparison of 20 and 20[2]
[4] Comparison of 20[2] and ²0[5]
[5] Set for 24 h at 4 °C
* p 0.05
** p 0.01
*** p 0.005
**** p 0.001

Table 5.S. Effect of two 20 % agar/gelatine mixtures on 24 h water stability of the weaning diet. Results are the mean % of 5 replications * SD of initial weight.

Agar/gelatine ratio	% Dry weight			
	6h	12h	18h	24h
1:1	77.2t 4.1	70.8 ± 2.5	70.6t 5.7	69.4t 1.5
1:3	80.3 ± 1.9	73.4 ± 18	70.8 ± 2.2	70.7 ± 2.7

gelatine mixture resulted in the best water stability of all the gels after 12, 18, and 24 h.

While the water stability of the gels, particularly the agar/gelatine mixtures, were encouraging the pellets were far more water stable than the gels after 6 (91.8 ± 2.0 %), 12 (90.5 ± 4.1 %), 18 (89.9 ± 0.4 %) and 24 h (89.0 ± 0.6 %) (Table 5.6). The loss of nutrients after 24 h was not significantly different from that after 6 h.

DISCUSSION

The gels bound with alginates lost approximately 50 % of their dry weight after 6 h. This is in sharp contrast to other findings. Using the alginate Kelgin HV, Farmanfarmaian *et al.* (1982) coated a standard pellet for crustaceans with 0.5 %, 1 % and 2 % (% dry weight of feed). The pellets bound with 2 % alginate lost only 26 % of their dry weight after 24 h. Heinen (1981) did not present absolute values, but obtained "good 24 h water stability" (p. 144) with 2 % of alginate or 3 % of agar. Meyers et *al.* (1972) recorded water stability of up

Table 5.6. Water stability of a pelleted weaning diet over a 24 h period. Results are the mean % of 5 replications ± SD of initial weight.

% Starch	% Dry weight			
	6 *h*	12h	18h	24h
23	91.8 ± 2.0	90.5 ± 4.1	89.9 ± 0.4	89.0 ± 0.6

to 48 h (no absolute values given) of pellets bound with alginate.

Inadequate gel formation, and therefore poor water stability, of alginate bound gels has been ascribed to too little soluble Ca^{2+} ions in the feed (Heinen 1981). The diet used in the present study contained 14.7 % fish meal and 2.5 % fish oil, accounting for a total of 17.2 % of fish products. Moreover, Ca^{2+} was added in the form of calcium citrate. The diet used by Meyers *et al.* (1972) contained only 8 % fish meal and 2 % fish solubles. Therefore, it is unlikely that the poor water stability of the alginate gels resulted from a lack of multivalent cations to bridge the algin molecules. On the other hand, Ca^{2+} and other multivalent cations present in fish products can prematurely react with the alginate, which might also result in poor water stability (Meyers *et al.* 1972). However, in this investigation a sequestrant was added in order to control the release of Ca^{2+} and to prevent the interaction with other cations.

A loss of approximately 50 % of dry weight after 6 h using alginates as a binding agent is unsatisfactory, since good water stability for at least 12 h is required of abalone weaning diets. Alginates have been shown to produce good water stability in crustacean pellets (Meyers *et al.* 1972, Heinen 1981, Farmanfarmaian *et al.* 1982). Thus it has to be stressed that different concentrations of alginate and/or sequestrants, ingredients as well as different preparative methods might significantly increase water stability. However, in this study alginates required a setting period of more than 12 h, which is undesirable. Using other binders the preparation time of gels is reduced to 2 - 3 h.

Diets containing 20 % gelatine had a very good 6 h water stability (81.2 ± 2.7 %, when set at 4 °C for 2 h). The agar bound diet (13.5 %), on the other hand, lost 31 % of dry weight within 12 h. The 1:3 combination of a 20 % agar and gelatine binder produced an equally good result after 6 h (80.3 ± 1.9 %), and a better one after 12 h (73.4 ± 2.9 %) than either agar or gelatine alone. Therefore the combination of both binders at a 1:3 ratio is the most suitable binding agent for the gel form of the artificial weaning diet.

In terms of water stability the pellets bound with 23 % starch proved to be far superior to gels. Moreover, unlike gels, pellets can be produced in bulk and stored dry until required. This would be of great advantage in a commercial operation. A feeding trial was thus designed to evaluate the feeding behaviour and growth of abalone presented with diets in gel as well as pellet form.

CHAPTER 6

THE FEEDING BEHAVIOUR AND GROWTH OF JUVENILES FED ON A GEL AND PELLET FORM OF THE WEANING DIET

INTRODUCTION

Juvenile abalone at the weaning stage are known to feed on artificial diets in gel (Ogino & Ohta 1963, Ogino & Kato 1964, Sagara & Sakai 1972, Koike *et al*_ 1979) and pellet form (Hahn 1989e, Gorfine & King 1991). Artificial diets generally produce better growth than natural diets (Hahn 1989e,g), but gels and pellets have never been directly compared in a growth trial.

There is also no published information on the feeding behaviour of juveniles fed on artificial diets. The coating of a plate with a gel resembles the method used to offer diatoms to juveniles before and during the weaning stage (Hahn 1989e), while pellets resemble pieces of kelp. Therefore, it is possible that the feeding behaviour on the two forms of the weaning diet would be different.

In this study, the feeding behaviour of juvenile *H. rnidae* fed under culture conditions on the two forms of the artificial weaning diet was evaluated. At the same time, the most suitable form of presentation of the diet, either in pellet or gel form, was studied in a growth trial.

MATERIALS AND METHODS

Each presentation trial was undertaken in triplicate using glass tanks (29 cm x 22 cm x 30 cm), set up at the Old Harbour Marine Biological Laboratory in Hermanus (Figure 6.1). To inhibit the growth of diatoms, sea-water was filtered to 3 pm. Furthermore, all sides of the tanks were covered in black **plastic and the glass surfaces were cleaned daily. Ambient water temperature varied between 17.0 and 19.0 °C (mean 18.1 ± 0.6 °C). The flow rate through the experimental**

(a) **(b)**

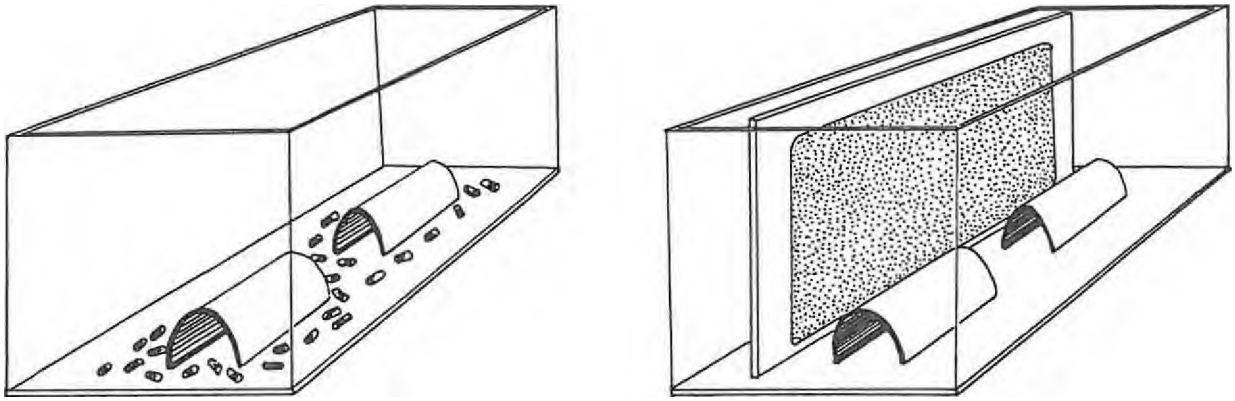

Figure 61. Design of the glass tanks used during the feeding trials using pellets (a) and gels (b).

tanks was maintained at 0.4 1/min and aeration was applied at a rate of 4 Natural photoperiod at the time of the experiment was ± 12 L: 12 D.

Juvenile abalone (5.00 - 8.54 mm shell length (SL)) were obtained from the Sea Plant Products Hatchery and from the Old Harbour Marine Biological Laboratory, both in Hermanus. All of the animals used had been settled and grown on diatoms. Each tank was stocked with 200 animals (= 3125 animals/m^2) and allowed to acclimatize for 4 days. They were immediately presented with artificial food in pellet or gel form. Mortalities during this period were replaced. Before and at the end of the 15 day experimental period a sample of 100 animals from each tank was weighed to the nearest 0.01 g and measured with a Mitutoyo Digimatic electronic vernier calliper to the nearest 0.01 mm.

The composition of the weaning diet (Chapter 4, Table 4.1) was altered in such a way that both the gel and the pellets contained equal amounts of the different binding agents (Table 6.1). The gels and the pellets were prepared as described in Chapter 5.

Table **6.1.** Composition of the artificial weaning diet for *Haliotis midae*, prepared either in gel or pellet form.

Ingredient	%
Casein	5.0
Gelatine	15.0
Fish meal	15.0
Spirulina spp.	10.0
Fish oil	².5
Sunflower oil	2.5
Agar	5.0
Dextrin	16.5
Starch	²10
Mineral mixture	4.0
Vitamin mixture	1.5

[1] For detailed composition see Table 4.3

A ration of 2.44 g (dry weight) of pellets or gel was offered to the juveniles daily at 17h00 and left in the tanks for 24 h. Pellets were broken into small pieces (0.5 - 1.0 cm in length) prior to feeding to increase their edge area. The water stability of the gels and broken pellets was determined as described in Chapter 5. Prior to the application of fresh food the left over feed and faeces were siphoned out.

The feeding behaviour of the animals was studied in terms of percentage appearance rate ((animals leaving their shelter/total number of animals) x 100) and percentage feeding frequency ((animals actively feeding/animals that had left their shelter) x 100). Observations on the behaviour of the animals in each tank were made hourly for 72 h on day 11 - 13 of the 15 day experimental period.

Replicate results of the feeding behaviour study were combined if they were not found to be significantly different using a one way analysis of variance at a significance level of $p < 0.05$. The feeding behaviour and the growth results observed on the two forms of the diet were tested for significant differences using a Student T-test (p S 0.05).

RESULTS

All the animals displayed an exclusively nocturnal feeding behaviour (Figure 6.2) and there were no significant differences in the overall appearance rate between those animals fed on pellets and those fed on the gel. However, those animals that were fed on gels had their highest appearance rate at 23h00 (75.56 ± 6.95 %), while the peak of appearance rate of those fed on pellets occurred at 01h00 (77.78 ± 6.25 %).

The percentage of animals actively feeding was always below 7 % (Figure 6.3). Feeding activity in the pellet groups peaked at 21h00 (5.03 ± 0.78 %) and in the gel groups at 01h00 (5.33 ± 1.23 %) and again at 5h00 (6.30 ± 4.14 %). After 20h00, the feeding activity in the gel groups was consistently higher than in the pellet groups, although this was only significant at 23h00 (pellets: 2.52 ± 0.33 %, gels: 3.69 ± 0.12 %, p 5 0.005).

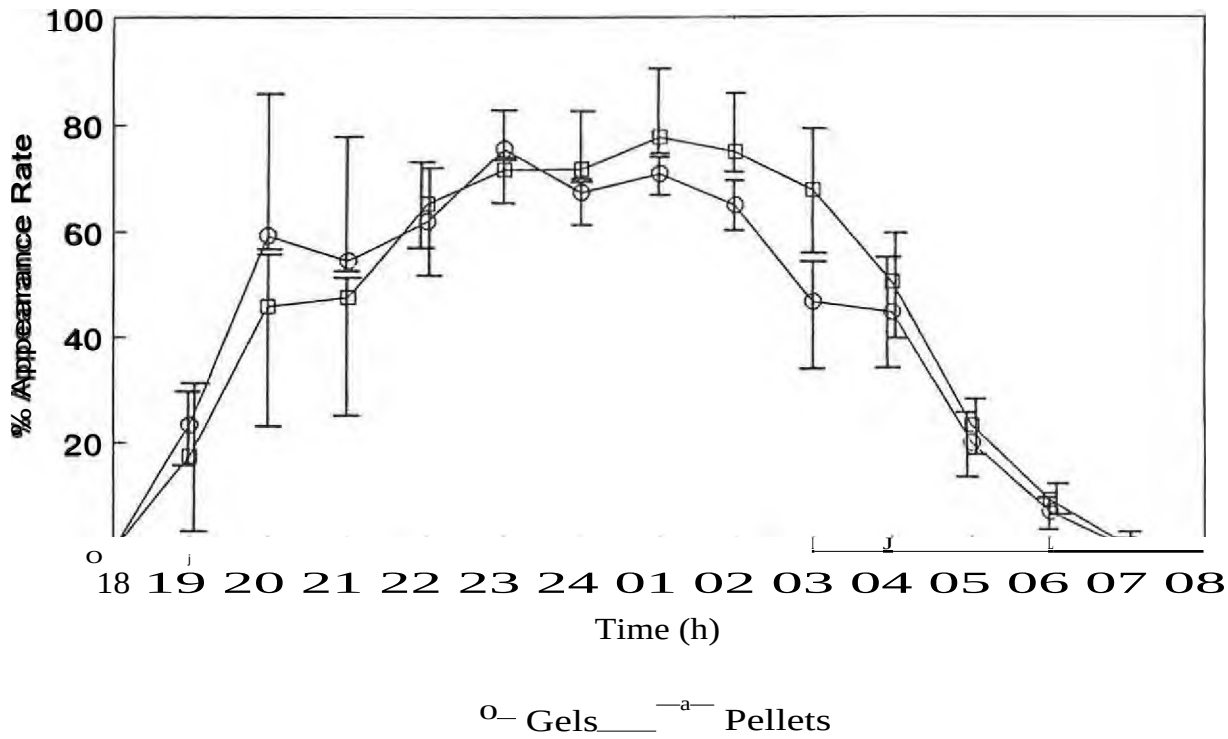

Figure 6.2. The 24 h appearance rate of *Haliatis midae,* fed on an artificial weaning diet. No animals appeared between 071100 and 18h00. Results are the mean ± SD of three tanks.

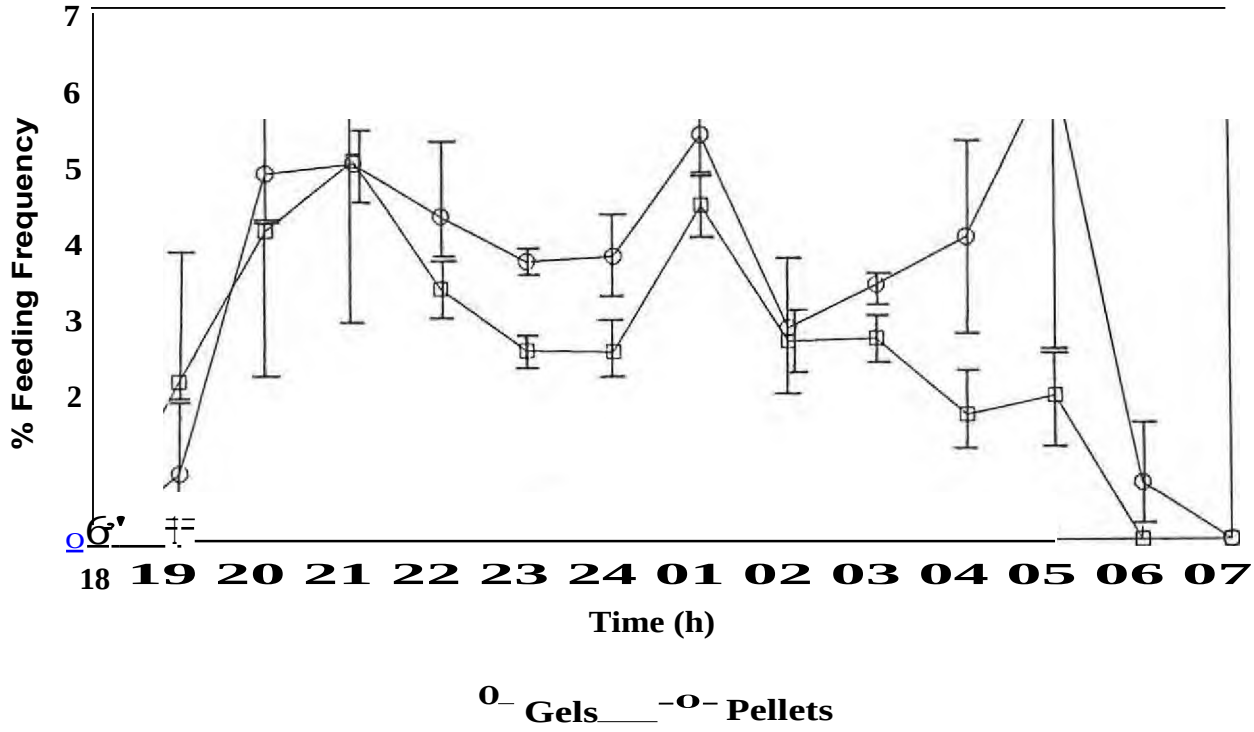

Figure 63. The ²4 h feeding frequency of *Haliotis midae,* fed on an artificial weaning diet. No feeding took place between 07h00 and 18h00. Results are the mean ± SD of three tanks (* p < 0.005).

The animals feeding on pellets had a significantly greater increase in SL (pellets: 1.52 ± 0.19 ram, gels: 1.10 ± 0.09 mm, p 5_ 0.05) and weight (pellets: 0.06 ± 0.01 g, gels: 0.03 ± 0.01 g, p 0.01) than those that were fed on gels (Table 6.2). Overall, the increase in total biomass was also significantly higher (p 5 0.02) for those animals fed on pellets (5.35 ± 0.75 g) than those fed on gels (3.52 ± 0.13 g) (Table 6.2).

The pellets were also significantly more water stable than the gels after 6 (pellets: 90.32 ± 3.01 %, gels: 78.83 ± 2.59 %, p _C 0.005), 12 (pellets: 87.09 ± 1.42 %, gels: 71.36 ± 2.21 %, p 0.001), 18 (pellets: 82.50 ± 2.70 %, gels: 69.67 ± 1.86 %, p 0.005) and 24 h (pellets: 80.80 ± 1.40 %, gels: 68.99 ± 2.02 %, p 0.005).

Mortality in both treatment groups was negligible (pellets: 0.50 ± 0.50 %, gels: 1.17 ± 0.29 %) and did not exceed 1.5 % in any one tank.

Table 6.2. Length, weight and total weight increase of *Haliotis midae,* fed on an artificial weaning diet for 15 days. Results are the meant SD of three tanks. Significant differences between the pellet and the gel diets are indicated by *.

Presentation method	Initial (range)	Final (range)	Increase
		Shell length (mm)	
Pellet	6.54 * 0.21 (5.01 - 8.54)	8.06 ± 0.11 (5.99 -10.14)	1.52 ± 0.19*
Gel	6.45 ± 0.16 (5.00 - 834)	7.55 ± 0.23 (5.42 - 9.75)	1.10 ± 0.09
		Weight (g)	
Pellet	0.04 ± 0.01 (0.01 - 0.11)	0.10 ± 0.00 (0,04 - 0.22)	0.06 ± 0.01***
Gel	0.04 ± 0.01 (0.01 - 0.09)	0.08 ± 0.01 (0.02 - 0.15)	0.03 ± 0.01
		Biomass Der tank (g)[1]	
Pellet	4.45 ± 0.85 (3.89 - 5.43)	9.81 ± 0.14 (9.66 - 9.94)	5.35 ± 0.75** :
Gel	4.21 ± 0.51 (3.62 - 4.55)	7.73 ± 0.64 (6.99 - 8.17)	3.52 ± 0.13

[1] n = 100 * p 0.05 ** p 0.02 *** p 5. 0.01

Table 63. Water stability of the artificial weaning diet over 24 h. Results are the mean t SD of five observations. Significant differences between gels and pellets are indicated by *.

Time (h)	% Dry weight	
	Pellets	Gels
6	90.32 ± 3.01*	78.83 ± 269
12	87.09 ± 1.42**	71.36 ± 2.21
18	82.50 ± ².70*	69.67 ± 1.86
24	80.80 ± 1.40*	68.99 ± 2.0²

* p .“1.005 ** p “1.001

DISCUSSION

Similar to natural populations (Barkai & Griffiths 1986, 1987), hatchery reared *H. midae* have been shown to be also nocturnal feeders (Genade *et al.* 1988, this study). The feeding behaviour of juvenile *H. midae* under culture conditions was found to be similar to that of juvenile *H. discus hannai* (mean SL 49.5 mm, Uki 1981). The appearance rate of *H. discus hannai* also increased from 18h00 and peaked at approximately 80 % between 19h00 and 23h00, whereafter the animals once again retreated into their shelters. Some of the juvenile *H. discus hannai*, however, also appeared during daylight hours (Uki 1981). The reason for this was that the algal fronds upon which the animals were feeding probably provided some protection against light. *H. midae,* on the other hand, had an exclusively nocturnal appearance rate, peaking between 23h00 and 02h00.

In the study on *H. discus hannai* feeding activity was measured over time as the accumulative amount of algae consumed (Uki 1981). From 17h00 onwards the amount consumed increased steadily and peaked at 07h00. Feeding activity in the present study was established by counting the number of actively feeding animals. The results were thus not directly comparable to those on *H. discus hannai.* However, a peak in feeding activity in the gel groups during the early morning hours was also observed. In both this study and the one on *H. discus hannai* (Uki 1981) the appearance rate never reached 100 %. This might indicate that animals possibly did not feed every night.

It is doubtful that the nearly consistently higher feeding activity in the groups fed on gels was a consequence of the difference in presentation of the weaning diet. Pellets were scattered around the shelters, whereas gels were vertically presented on one side of the tank only (Fig. 6.1). Therefore the likelihood to encounter food was greater in the tanks containing pellets. On the other hand, the higher feeding frequency in the tanks with gels would appear to indicate that animals feeding on pellets needed less time to satiate their hunger.

The superior growth obtained on pellets could have been a consequence of their better nutritional quality (due to their better water stability). However, as the pellets were broken into smaller pieces (0.5 - 1.0 cm in length) than in Chapter 5 (1 - 2 cm in length), their water stability was not as good as described earlier (Chapter 5, Table 5.6).

The nutritional profiles of pellets and gels were identical. However, to prepare a gel, the gelatine had to be boiled for 10 sec. It is not known if, or to what extent, this influenced the nutritional quality of gelatine. Uki & Watanabe (1986) found that extended heat treatment of protein sources (110 - 130 °C for 2 h) reduced the growth rates of juvenile H. *discus hannai.*

Similar to other studies on the growth rate of juvenile ***H. midae*** (Genade *et al.* 1988), the growth data also shows a wide range. This seems to be typical of haliotid growth (Day & Fleming 1992). Mortality in both the gel and pellet groups was very low_ This again is similar to other studies in which juvenile abalone were fed on artificial feeds (Hahn 1989d, Mozqueira 1992).

The animals fed on pellets showed a significantly better increase in SL and weight than the animals fed on gels after 15 days. This was probably due to the better water stability of pellets, and consequently better nutritional quality. However, long-term studies to confirm these very encouraging growth results with pellets are necessary_ In terms of hatchery management the use of pellets is advantageous. Unlike gels, pellets can be produced in bulk and stored. The tank design is simplified in comparison to tanks built to offer gels (Figure 6.1), as pellets can simply be scattered around a tank.

CHAPTER 7

THE EFFECT OF PHOTOPERIOD ON GROWTH AND NUTRITIONAL PARAMETERS OF JUVENILES

INTRODUCTION

The development of an artificial weaning diet, as described in the previous chapters, focused on the nutritional composition and the most suitable means of presentation of the diet.

A commercial hatchery aims to rear juveniles through the weaning stage as fast as possible. The growth of juveniles, however, is not only influenced by the composition and presentation of a weaning diet. Any information on growth enhancing effects of other biotic and abiotic factors is therefore of potential value to an abalone hatchery.

Under culture conditions, the growth of juvenile abalone, in particular, is very sensitive to factors such as stocking density (Koike *et al.* 1979, Chen 1984), temperature (Ino 1952, Leighton 1974, Uki 1981, McCormick & Hahn 1983, Chen 1984, Peck 1989), water quality (Ceccaldi 1982, Chen 1984) and photoperiod (Ebert & Houk 1984, Genade *et al.* 1988, Dixon 1992, Greenier & Takekawa 1992).

It has been found that an increase in the hours of darkness generally has a positive effect on the growth of abalone (Ebert & Houk 1984). Unlike water temperature, the change and control of photoperiod is a simple and cheap procedure. Therefore, it was the aim of this study to investigate if different photoperiods affect the nutritional physiology and the growth of abalone fed on the artificial weaning diet.

Hence, the effect of four light regimes (12, 16, 20 and 23 h of darkness) on growth, feed consumption, feed conversion ratio, protein efficiency ratio, protein deposition in the body and

digestive enzyme activity of juveniles was evaluated.

MATERIALS AND METHODS

Experimental Design

The experiment was conducted in the Abalone Hatchery of Sea Plant Products in Hermanus. Black PVC-containers (33 cm x 33 cm x 24 cm) were flushed with sea water and aerated for 4 days, whereafter each tank was stocked with 200 juvenile abalone (3.22 - 11.82 mm shell length (SL), 1835 animals/m^2). All of the animals used had been settled and grown on diatoms under a 12hL: 12hD light regime. A sample of 25 animals (mean SL 7.43 ± 0.29 mm, mean weight 0.07 ± 0.02 g) was frozen at - 30 °C to measure initial protein concentration as described in Chapter 3.

The effect of each photoperiod (12, 16, 20 and 23h of darkness) on the growth and the nutritional parameters of juveniles was tested in triplicate. For comparison, an experiment **in** which juveniles were fed on diatoms was set up under a 12hL: 12hD light regime. The animals were allowed to acclimatize for 4 days, during which time they were already fed on pellets or diatoms. The juveniles that died during this period were replaced. Water and air were supplied at a rate of **1.1** 1/min and 5 1/min, respectively. Water temperature was measured twice daily and varied from 14.9 °C to 17.1 °C, with a mean temperature of 15.8 ± 0.6 °C. Once the growth trial was started, mortalities were recorded daily, but juveniles were not replaced anymore. To inhibit the growth of diatoms, sea-water was filtered to 1 pm. Moreover, the surfaces of all tanks, except the ones supplied with diatoms, were cleaned daily.

The composition of the pelleted weaning diet is shown in Table 7.1. The diet was modified from the one used in Chapter 6 as agar, a binding agent for gels, could be excluded. Each tank was supplied with pellets daily, at a rate of 10 % of the **initial** total wet weight of animals in each tank. Pellets were broken into small pieces (0.5 - 1.0 cm in length) prior to feeding to increase

Table 7.1. Composition of the artificial weaning diet for *lialiota midae.*

ingredient	%
Casein	5.0
Gelatine	15.0
Fish mea]	15.0
Spirulina spp.	10.0
Fish oil	2.5
Sunflower oil	2.5
Dextrin	21.0
Starch	23.0
Mineral mixture[s]	4.0
Vitamin mixture[s]	2.0

For detailed composition see Table 4.3

their edge area. They were offered at 171100 and left in the tanks for 24 h. Prior to the addition **of** new food, the remaining food was siphoned from each tank and collected using a filter with a mesh size of 900 pm and dried for 24 h at 100 °C.

Diatoms were grown on sheets of corrugated plastic (30 cm x 20 cm). These sheets were replaced once ± 75 % of the diatoms had been grazed.

Growth Measurements

Before and at the end of the 30 day experimental period a sample of 50 animals from each tank was weighed to the nearest 0.01 g and measured with a Mitutoyo Digimatic electronic vernier calliper to the nearest 0.01 mm. For the final measurement, animals were collected between 07h00 and 08h00 and stored at 4 °C. After measuring, the animals were frozen and kept at - 30 °C for the analysis of enzyme activity.

Daily growth in SL was calculated according to:

$$\text{Daily growth (pm)} = \frac{\text{increase shell length}}{\text{days}}$$

Percentage daily growth rate in weight was determined using the formula of Focht (1984):

$$\% \text{ Daily Growth Rate} = \left(\frac{\text{final weight}}{\text{initial weight}}\right) 30 - 1) \times 100$$

Nutritional Parameters

The nutritional parameters listed below were only determined for animals fed on pellets. The percentage feed consumption (PFC) was calculated according to a formula modified from Uki *et al.* (1985a):

$$PFC = \frac{\text{dry weight of feed left after 24 h and corrected for leaching (g)}}{\text{dry weight of feed offered (g)}} \times 100$$

Percentage feeding rate (PFR) was determined according to the formula of Ebert & Houk (1984):

$$PFR = \frac{F_1 - F_2}{t \times w} \times 100$$

where F_i = initial food weight, F_2 = final food weight, t = number of days and w = mean abalone weight.

Feed conversion efficiency (FCR) protein efficiency ratio (PER), and percentage protein deposited (PPD) were calculated according to Hardy (1989) and Wilson (1989):

$$FCR = \frac{\text{dry weight of feed fed (g)}}{\text{wet weight gain (g)}}$$

$$PER = \frac{\text{wet weight gain (g)}}{\text{total protein fed (g)}}$$

$$PPD = \frac{\text{final body protein (g) - initial body protein (g)}}{\text{total protein fed (g)}} \times 100$$

To determine PPD, the animals from each tank were divided into two groups of 25 by weight. One of the groups was used to measure final protein concentration as described in Chapter 3, the other one was used for enzymatic analysis.

Enzyme Studies

Tissue Preparation

Enzyme activity was analyzed in all experimental groups, i.e. those fed on pellets as well as those fed on diatoms. As with the animals fed on pellets, 25 juveniles from each of the three groups fed on diatoms were separated by weight.

The shucked tissue of the 25 animals from each tank was pooled and homogenized with an Ultra Turrax T 25 homogenizer for 2 min **in** 15 ml of a 0.1 M citric acid- 0.2 M phosphate buffer (pH 5.2). This buffer was used as its **pH** corresponds to the pH of the gut of *H. midae* (P.J. Britz 1993, Department of Ichthyology and Fisheries Science, Rhodes University, Grahamstown, South Africa, pers. comm.). Subsequently, each sample was centrifuged for 45 min at 18,400 x g at 4 °C. The supernatant was then dialysed against the buffer using dialysis tubing (molecular cutoff weight of 12000 - 14000) at 4 °C for 48 h with two changes of buffer (Sweijd 1990). The extract was diluted 1:2 with buffer and stored at - 30 °C prior to assays.

To measure protein concentration in each sample, a test tube was filled with 0.1 ml of sample and made up to 1 ml with distilled water. This was then further processed as described in Chapter 3. A second standard curve using buffer instead of distilled water was prepared, to test whether the use of buffer would change the slope of the standard curve. However, regression analysis proved the slopes to be almost identical ($r^2 = 0.9945$).

Amylase Specific Activity

The assay for amylase activity was based on modified versions of the Bernfield-method (Plummer 1987, Uys 1989). The animals from each tank were tested in triplicate. One ml of a 1 % starch solution was added to 1 ml of sample solution and incubated at 20 °C in a waterbath. After 10 min, I ml of the colour reagent 3,5-dinitrosalicylic acid, made up as described by Uys (1989), was added. The resulting mixture was heated in boiling water for 5 min, then cooled down to 20 °C in the waterbath. After 10 min the absorbance was read on a spectrophotometer at 540 nm.

Blanks for each sample were prepared by boiling 1 ml of sample solution for 10 min and then following the same procedure as with the active sample. As the standard curve for the concentration range 0.1 - LO mg maltose/ml did not go through the origin, a second standard curve in the range 0.01 - 0.10 mg maltose/m1 was prepared. In that range, however, there was no linear relationship and a best fit curvc had to be drawn.

Amylase activity was expressed as mg maltose liberated from starch in 10 min at 20 °C. Specific activity was defined as amylase activity per mg protein.

Lipase Specific Activity

The activity of lipase was measured using a modification of the method employed by Tietz & Fiereck (1966) and Borlongan (1990). Results for the animals from each tank were obtained in triplicate. 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 6 h in a waterbath at 20 °C. A blank containing 1 rnt of sample, boiled for 10 min, was prepared and processed accordingly. The reaction was arrested by addition of 3.0 ml of 95 % ethanol. The mixture was then transferred to a 50 ml beaker. The test tubes used initially were 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 NaOH solution, each sample and blank were titrated to pH 10.5.

To measure lipase activity, 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 N NaOH required to titrate a sample to p1-1 10.5 (after correction by the appropriate blank) after 6 h at 20 °C. Lipase specific activity was expressed as activity per mg protein.

Protease Specific Activity

The method employed was based on modified versions of the Anson-method (Walter 1984, Uys 1989). Of each sample, 0.5 ml were added to 2.5 ml of haemoglobin substrate, prepared as described by Uys (1989). The animals from each tank were analyzed three times. Blanks for each sample were processed simultaneously, using 0.05 M HC1. The mixtures were incubated for 10 min in a waterbath at 20 °C. The reactions were stopped by addition of 5.0 ml of a 0.3 **M** trichloroacetic acid solution. Following that, 0.5 ml of 0.05 M HC1 were added to each sample and 0.5 ml of sample to the respective blank. After 5 min at room temperature, each test tube was centrifuged at 3000 x g for 20 min. Finally, 2.5 ml of the supernatant, 5.0 ml of a 1 N NaOH and 1.5 ml of Folin reagent (Walter 1984) 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 - **0.1** mg tyrosine/ml.

A unit activity was defined as mg tyrosine liberated from haemoglobin in 10 min at 20 °C. Specific activity was expressed as protease activity per mg protein.

Statistical Analysis

Replicate results were combined if they were not found to be significantly different using a one way analysis of variance at a significance level of p 0.05. Significant differences between treatments were tested by using a one way analysis of variance in combination with Tukey's multiple range test.

RESULTS

Shell length, weight and total abalone biomass per tank did not increase significantly with an increase in hours of darkness (Table 7.2). There were also no significant differences in the growth parameters between the animals fed on diatoms and those fed on pellets (Table 7.2). The increase in SL ranged from 50.00 ± 6.56 pm/day to 62.67 ± 3.51 pm/day. Percentage daily growth rate varied from 1.66 ± 0.24 % to 2.08 ± 0.12 %, and the increase in total abalone biomass per tank from 2.04 ± 0.53 g to 3.57 ± 0.87 g.

The different photoperiods also had no significant effect on any of the nutritional test parameters (Table 7.3). PFC, ranging from 15.03 ± 0.75 to 18.48 ± 1.01, increased with an increase in the hours of darkness ($y = el\ 94\ x0.32$, $r^2 = 0.9444$, Figure 7.1). PFR varied from 5.43 ± 0.64 to 6.06 ± 0.30. The FCRs were very low, ranging from 0.44 ± 0.04 to 0.60 ± 0.19. PERs, on the other hand, were very high and ranged from 5.06 ± 1.74 to 6.64 ± 0.77. PPD, varying from 23.91 ± 4.96 to 33.80 ± 5.58, decreased with an increase in the hours of darkness ($y = -0.94\ x + 45.35$, $r^2 = 0.7352$, Figure 7.2).

The specific activities (referred to as activity) obtained for the three principal digestive enzymes in the different groups are listed in Table 7.4. The activity of amylase in juveniles fed on diatoms is the result of the animals from two tanks only, as the third tank was significantly different ($p < 0.05$) from the two others. Amylase activity in the group fed on diatoms (0.188 ± 0.020) was significantly higher ($p < 0.05$) than in the pellet fed groups. There was no effect of photoperiod on amylase activity, with activity varying between 0.108 ± 0.009 and 0.114 ± 0.016. Lipase activity was very similar in all groups, with no marked difference between the groups fed on diatoms and pellets and ranged from 0.205 ± 0.045 to 0.296 ± 0.019. The activity of protease in the four groups fed on pellets, varying between 0.145 ± 0.023 and 0.159 ± 0.010 was significantly higher than in the group fed on diatoms (0.047 ± 0.013) ($p < 0.0002$). However, protease activity amongst the groups fed on pellets was not significantly different.

Table 7.2. Effect of photoperiod on the increase in length and weight of *Ha¹lo& midae* over 30 days. Animals were fed on diatoms or an artificial weaning diet. Results are the mean ± SD of three tanks.

Hours of darkness	Initial (range)	Final (range)	Increase	
	Shell length (mm)			um/day
12 (Diatoms)	7.43 ± 0.21 (3.52 -10.68)	8.94 ± 0.36 (4.54 -11.96)	131 ± 0.20	50.00 ± 6.56
12	7.94 ± 0.65 (3.22 -11.29)	9.69 ± 0.89 (4.50 -13.47)	1.75 ± 0.28	58.67 ± 9.29
16	7.62 ± 0.30 (4.16 -11.00)	9.34 ± 0.31 (5.56 -12.45)	1.72 ± 0.20	57.33 ± 6.35
20	7.63 ± 0.²6 (4.30 - 9.99)	9.15 ± 0.08 (6.05 -12.04)	1.51 ± 0.34	50.67 ±11.55
23	7.24 ± 0.53 (4.09 -11.82)	9.12 ± 0.61 (6.00 -13.08)	1.88 ± 0.10	62.67 ± 3.51
	Weight (g)			% Daily **growth rate**
12 (Diatoms)	0.07 ± 0.0I (0.01 - **0.19)**	0.11 ± 0.0² **(0.02 - 0.24)**	0.04 ± 0.01	1.66 ± 0.24
12	**0.10 ± 0.03 (0.01** - 0.25)	**0.17 ± 0.04** (0.02 - 0.41)	**0.07 ± 0.02**	**1.91 ± 0.27**
16	0.08 ± 0.01 (0.01 - 0.21)	0.13 ± 0.02 (0.03 - 0.28)	0.06 ± 0.01	1.92 ± 0.25
20	0.08 ± 0.01 (0.01 - 0.16)	0.13 ± 0.0² (0.03 - 0.28)	0.05 ± 0.02	1.80 ± 0.58
23	0.07 ± 0.01 (0.02 - 0.21)	0.13 ± 0.02 (0.04 - 0.33)	0.06 ± 0.01	2.08 ± 0.12
	Biomass per tank (g)[1]			
12 (Diatoms)	3.59 ± 0.45 (2.98 - 4.06)	5.32 ± 0.76 (4.49 - 6.92)	2.04 ± 0.53	
12	4.83 ± 126 (3.64 - 6.29)	8.29 ± 2.02 (630 -10.28)	3.57 ± 0.87	
16	338 ± 0.29 (3.54 - 4.66)	6.76 ± 0.29 **(5.82 - 7.28)**	2.83 ± 0.58	
²0	4.08 ± 0.52 (3.49 - 4.32)	6.67 ± 0.29 (6.42 - 7.08)	2.67 ± 0.76	
23	3.67 ± 0.58 (3.02 - 4.14)	6.83 ± 1.04 (5.61 - 7.43)	3.01 ± 0.45	

n = 50

Table 73. Effect of photoperiod on nutritional parameters of *Haliolis midae,* fed on an artificial weaning diet for 30 days. Results are the mean ± SD of three tanks.

Parameter	Hours of darkness			
	1²	16	²0	23
% Feed Consumption	15.03 ± 0.75	17.²4 ± ².0²	18.07 ± 1.53	18.48 ± 1.01
% Feeding Rate	5.55 ± 0.²0	5.43 ± 0.64	6.06 ± 0.30	5.89 ± 0.62
Feed Conversion Ratio	0.44 ± 0.04	0.48 ± 0.01	0.60 ± 0.19	0.47 ± 0.06
Protein Efficiency Ratio	6.64 ± 0.77	5.65 ± 0.16	5.06 ± 1.74	6.14 ± 0.89
% Protein Deposited	32.64 ± 3.7²	33.80 ± 5.58	²3.91 ± 4.96	²4.49 ±14.74

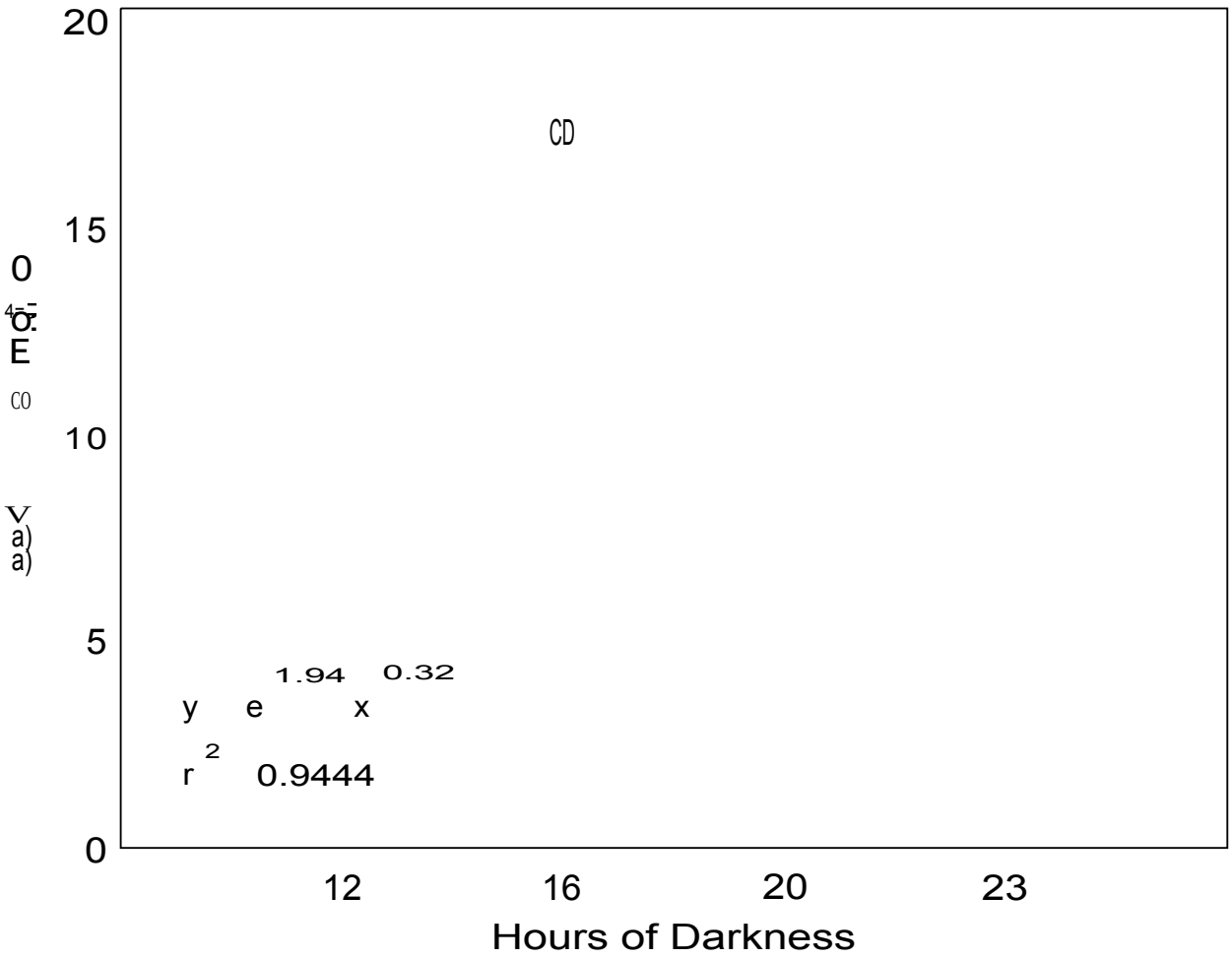

Figure *7.1*. The relationship between percentage feed consumption and photoperiod. Results are the mean ± SD of three tanks.

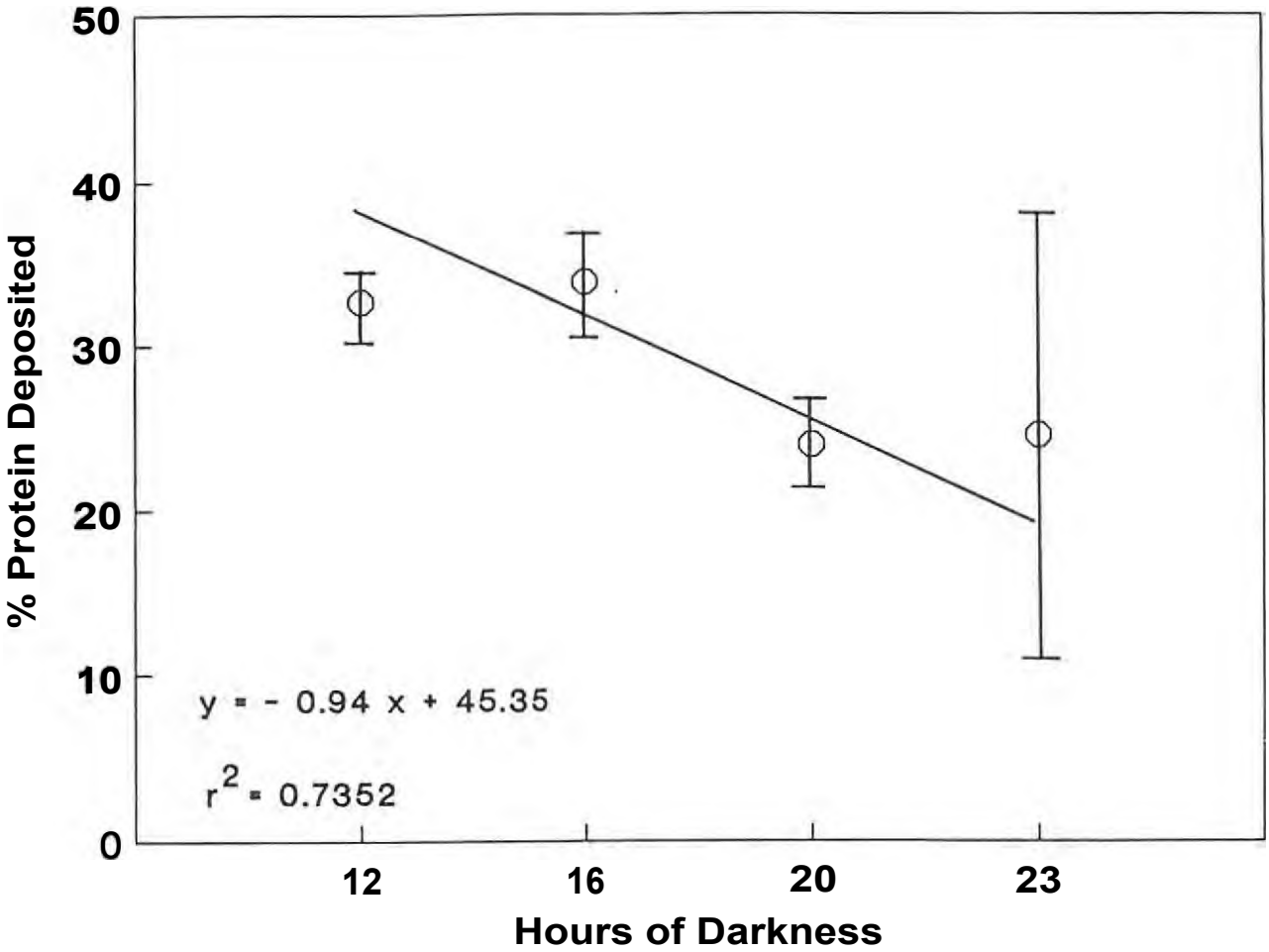

Figure 7.2. The inverse relationship ***between*** percentage protein deposited and photoperiod. Results are the mean ± SD of three tanks.

DISCUSSION

All the groups fed on pellets had a greater increase in SL and weight than the animals fed on diatoms, although these differences were not significant. The increase in biomass per tank in the groups fed on pellets was 24 - 43 % better than with diatoms, but again this was not significant (Table 7.2). The increase in SL and weight obtained in juveniles fed on pellets fall within the range reported for *H. rubra* (Mozqueira 1992), fed on artificial food as of 2.5 mm in SL.

These results show that juvenile *H. midae* can be directly weaned from diatoms to an artificial diet, and that the nutritional quality of pellets is as good as that of diatoms. It also demonstrates

Table 7.4. Specific activity of digestive enzymes of *Haliotis midae,* fed either on diatoms or an artificial weaning diet for 30 days. Results are the mean ± SD of three tanks. Significant differences between the group fed on diatoms and the ones fed on the diet are indicated by *.

	Hours of darkness				
Enzyme	1² (Diatoms)	1²	16	20	23
Amylase[s]	0.188[2] ± 0.020	0.111 ± 0.018*	0.110 ± 0.022*	0.114 ± 0.016*	0.108 ± 0.009*
LipRsd	0.207 ± 0.131	0.208 ± 0.072	0.205 ± 0.045	0.²34 ± 0.094	0.²96 ± 0.019
Protease[4]	0.047 ± 0.013	0.157 ± 0.028**	0.145 ± 0.0²3**	0.160 ± 0.0²3**	0.159 ± 0.010**

' specific activity: mg maltose liberated from starch in 10 min at ²0 °C per mg protein
2 mean ± SD of two tanks
³ specific activity: ml 0.05 N NaOH required to titrate a sample to pH 10.5 after 6 h at ²0 °C per mg protein
specific activity: mg tyrosine liberated from haemoglobin in 10 min at ²0 °C per mg protein
*p S0.05 ** p S 0.0002

that pellets can replace diatoms as the principal food of *H. midae* during the weaning stage. This is of particular importance for a commercial operation, where the control of the quality and quantity of diatoms is a key problem (McCormick & Hahn 1983, Ebert & Houk 1984, Hahn 1989d), which can now be overcome by the use of the artificial weaning diet.

Similar to this study, rearing juvenile *H. tuberculata* in the dark, in comparison to a natural photoperiod, did not have a noticeable effect on their growth (Hahn 1989d). Genade *et al.* (1988), on the other hand, detected that *H. midae* of 2 - 3 mm SL receiving "less" light were up to 50 % larger than those exposed to "bright" light. The effect was not quantified however, and the time period of this observation was not provided. Greenier & Takekawa (1992) evaluated the effect of a 24 h darkness treatment and two 12 h light regimes, using sunlight and artificial light on the growth of juvenile *H. rufescens* (mean SL 11.23 mm). The 24 h darkness treatment produced the best growth after 6 months. However, the growth of animals kept under the 12 h artificial light regime was nearly identical.

Numerous studies have shown that abalone growth rates show considerable variation in the wild (for review see Day & Fleming 1992). The same has been shown in the laboratory. Oba *et al.* (1968) found that in hatcheries some individuals grew only 20 - 30 % of the average. This is a common phenomenon even if individuals of the same age and parents are reared in the same tank under optimal conditions (Hahn 1989g). Genade *et al.* (1988) also observed that juvenile *H. midae* ranged in size from 5 - 30 mm after one year. Day & Fleming (1992) found the increase in weight in *H. rubra* over one year to vary between 30 mg/day and 117 mg/day. In all these studies, abalone were fed on natural food. The use of artificial feed, in comparison to a diatom diet, did not have an effect on the variability of growth results in this study.

The variability of the results, the short duration of the growth trial and maybe the sample size (n = 50, 25 % of each tank), might have masked the effects of the two different diets as well as the effects of photoperiod. This can, however, only be verified in long-term studies.

Mortality in the animals fed on diatoms ranged from 4 - 8 % over the experimental period, whereas in those groups fed on pellets it ranged from 2 - 5 %. These results confirm that mortality rates on artificial diets are low (Chapter 6), and generally less than on natural diets (Hahn 1989d, Mozqueira 1992).

Different photoperiods also had no significant effect on any of the nutritional parameters (Table 7.3). However, with an increase in hours of darkness PFC increased (Figure 7.1). The same trend was shown by adult *H. midae* fed on artificial food (Dixon 1992). As abalone in the laboratory have been shown to be nocturnal feeders (Uki 1981, Hayakama *et al.* 1987, Genade *et al.* 1988, Chapter 6), a correlation between feed consumption and an increase in hours of darkness was expected. Surprisingly, the same trend was not shown in the PFRs. The juveniles ate similar amounts of food a day, ranging from 5.43 to 6.06 % of their wet weight. These figures fall within the 2 - 7 % range reported for juveniles of 5 - 10 mm SL at a mean temperature of 15 °C (Anonymous 1981 in Hahn I989g).

The FCRs at all photoperiods were very low (0.44 ± 0.04 - 0.60 ± 0.19), indicating that juveniles at the weaning stage utilize food very efficiently. The mean FCR of all four groups was 0.50 ± 0.07. Corrected for leaching (20 % over 24 h) this means that 0.6 kg dry feed were required to produce 1.0 kg of wet weight of abalone. Britz (1993, pers. comm.) obtained FCRs ranging from 0.6 to 1.3 in juvenile *H. midae* (15 - 30 mm SL) fed on artificial food. Recalculation of the results of Uki *et al.* (1985a) showed that *H. discus hannai* of 28 - 47 mm SL had FCRs ranging between 0.66 and 1.35. Two conclusions can be drawn from these results. Firstly, the efficiency with which food is utilized seems to decrease with an increase in size. This applies generally to animals, and is well documented, e.g. in fish (Brett 1979, Lovell 1984, Henken *et al.* 1986, Hardy 1989, Steffens 1989). An increase in size is accompanied by a relatively higher requirement of energy for maintenance purposes (Brett 1979, Steffens 1989) and therefore a decrease in growth rate, as known for abalone (Forster 1967, Poore 1972h, Sainsbury 1982, Shepherd & Hearn 1983, Wood 1993). Secondly, artificial food is more efficiently utilized than natural food. **In** the absence of such data for diatoms, however, these results can only be compared to values obtained on macroalgae. Feeding giant kelp *(Macrocystis* spp.) to juvenile *H. rufescens* (mean SL 11.23 mm), Greenier & Takekawa (1992) obtained FCRs of 3.6 - 8.9, an order of magnitude less than the values obtained in this study.

In order to compare the results of this study to others the conversion of the present results into percentage feed conversion efficiency (% FCE = (I/FCR) x 100) on a wet weight basis was required. The mean FCR for the four groups fed on pellets was 0.50 and after correction for leaching (20 %) and the moisture content of the pellets (10 %) was found to be 0.66. Converted into % FCE this was 151.52 %. Ebert & Hoak (1984) fed *Macrocystis* spp. to *H. rufescens* of a mean SL of 42.6 mm. Over a year, % FCE per month ranged from 0.0 to 7.3 %. Han *et al.* (1986) obtained very similar results of 3.5 - 7.9 % with nine macroalgae fed to young *H. discus.* Uki *et al.* (1986a) evaluated the dietary value of 57 species of macroalgae for the growth of *H. discus hannai* (24.0 - 33.9 mm SL) and found % FCEs to vary from 0.0 to 14.9 %. All of the % FCE results obtained on macroalgae are at least an order of magnitude less than the ones

obtained on the artificial weaning diet. The validity of the results of this study is confirmed by the results of Uki *et al.* (1985a), who fed artificial food to *H. discus hannai* (28.8 ± 2.4 mm SL) and obtained % FCEs of between 46 and 123 %.

The reason as to why artificial food is more efficiently converted into tissue probably has to do with the lower moisture and higher protein content. Although the composition of macroalgae is highly variable, they have a mean moisture content of 88 %, and a mean protein content of 9 % (Hahn 1989g). In contrast, the weaning diet has a moisture content of 10 % and a protein content of 35.48 %. Currently, the role of digestibility with regard to feed conversion is not clear. On the one hand, Dixon (1992) found that artificial food is better digested than macroalgae in *H. midae,* while Wee *et al.* (1992), on the other hand, found the reverse in *H. rubra.* However, they used different artificial diets and macroalgae in the respective studies.

The PER values, ranging between 5.06 ± 1.74 and 6.64 ± 0.77, show that the dietary protein was used very efficiently by the juveniles. This could be expected, as casein, fish meal and *Spirulina* spp. are known to be good protein sources for *H. midae* (P.J. Britz 1993, pers. comm.). Uki *et al.* (1985a) also obtained very high PERs of 4.3 feeding a casein based diet to juvenile *H. discus hannai.*

The problem with PER values is that no allowance is made for maintenance requirements, size of the animal, experimental period or changes in protein concentration of the animal with time (Steffens 1989, Wilson 1989). Therefore, PPD is a more precise estimation of the amount of the dietary protein retained by an animal, as it measures the increase in tissue protein. It has to be mentioned, however, that in fish a test period of two to three months or even eight months is considered essential to obtain reliable results (Cho *et al.* 1974). Nevertheless, the PPD becomes less with an increase in hours of darkness (Figure 7.2). This is most likely a consequence of the increase in PFC with an increase in hours of darkness. It has been shown in carp and rainbow trout that the more feed is consumed, the less efficient the utilization of feed becomes in general,

and of protein in particular (Huisman 1976). Uki *et al.* (1986b), feeding a diet containing 32.5 % protein to juvenile *H. discus hannai,* obtained net protein utilization (NPU) values of 23 - 26, which falls within the range obtained in this study (PPD is a simplified method to measure NPU).

The two parameters that show trends with changes in photoperiod, namely PFC and PPD, are the only ones not defined in terms of individual weight gain as are PFR, FCR and PER. It is possible that trends in the latter three parameters are not obvious because of the great variation in individual growth as mentioned earlier. Trends and significant results would probably emerge if growth trials were conducted for much longer periods. Ebert & Houk (1984) grew *H. rufescens* (38 - 43 mm SL) under different light regimes on macroalgae. After a year, those abalone kept under continual darkness had the highest feeding rates, increase in SL, weight and FCEs. However, differences regarding SL between natural photoperiod and 24 h darkness only became apparent after 1 - 2 months, whereas differences in weight took 3 - 4 months to become evident.

The reason why changes in photoperiod take such a long time to exert an effect on growth probably has to do with the activity rhythms of abalone. Like many other marine invertebrates they display rhythmic behaviour in response to the day/night cycle (Naylor 1988), with a nocturnal activity and a diurnal resting phase (Hooker & Morse 1985, Barkai & Griffiths 1986, 1987, Tutschulte & Connel 1988, Wood 1993, Chapter 6). The underlying basis of this behaviour is a circadian rhythm, controlled by a biological clock (Reid & Naylor 1986). This biological clock is comprised of two components: an endogenous pacemaker, and an external synchronizer or Zeitgeber, which corrects the free-running endogenous element each day and keeps the rhythm in phase with prevailing environmental conditions (Schwassmann 1971, 1980, DeCoursey 1983). Only a few environmental factors, such as temperature or tides, are capable of functioning as Zeitgebers. However, light is by far the most important for aquatic animals such as fish (Eriksson 1978), and very likely also for abalone. A circadian rhythm has been characterized as being free-running and persistent even in the absence of the Zeitgeber, i.e. under constant conditions (Schwassmann 1980). The pacemaker has to be free-running to allow an environmental stimuli,

such as light, to adapt it to local conditions or annual changes (Eriksson 1978, Muller 1978a,b). The range of free-running is fairly narrow, however, generally spanning 23 - 26 h in most species (DeCoursey 1983). Therefore, a circadian rhythm continues with a free-running period of approximately 24 h, even if the Zeitgeber is eliminated due to constant conditions in the laboratory (Jones & Naylor 1970, Bregazzi & Naylor 1972, Reid & Naylor 1985). Generally, circadian rhythms are more stable under constant darkness than constant illumination (Kavaliers 1980).

It is not surprising, therefore, that juvenile *H. midae* did not show significant differences in growth and the nutritional parameters after only 30 days. Observations of the tanks subjected to more than 12 h of darkness showed that generally few animals were active and/or feeding outside the normal activity pattern. This deviation from the normal feeding behaviour, however, resulted in an increase of PFC with a longer period of darkness. This result can possibly be explained on the basis of the free-running pacemaker. A longer dark period slightly extended the feeding activity, as the Zeitgeber exerted its control action at a later stage.

The results of this study have implications for hatchery management. If the weaning period does not last for more than 1 - 2 months there is no need to control photoperiod. On the one hand, longer hours of darkness increase the PFC but, on the other hand, do not produce a significant effect on the growth of juveniles in such a short period of time (Ebert & Houk 1984, this study). However, photoperiod might have a positive effect on growth, if animals are reared under constant darkness from the post-settlement stage through to market size.

Animals fed on diatoms had a significantly higher amylase and a significantly lower protease activity than the groups fed on pellets. The activity of lipase, however, was very similar in all groups. Photoperiod also did not have an effect on the activity of the enzymes. This was to be expected, as photoperiod also had no effect on feeding behaviour to any significant extent.

The differences in amylase and protease activities between the group fed on diatoms and pellets can be explained on the basis of the different chemical composition of the diets. The protein, carbohydrate and fat concentrations of diatoms are known to be influenced by the culture medium used, the light intensity and wavelength, the stage of the life cycle, and the species composition (Parsons *et al.* 1961, Darley 1977). Not surprisingly, therefore, protein concentrations reported for diatoms range from 3.5 % (Werner 1971 in Darley 1977) to 47 % (Anse11 *et al,* 1964). **In** this study, three samples of diatoms were taken over the 30 day period (days 0, 15, and 30) and analyzed for their protein concentration. In all three, protein amounted to 5 % of the dry weight of the sample. This would explain the low protease activity in comparison to the pellet fed groups. That an increase in dietary protein content increases protease activity has been shown for carp (Kawai & Ikeda 1972). **Thus the high protease activity** of the pellet fed groups is probably simply a result of the much higher protein concentration in the pellets (35.48 %).

The carbohydrate concentration in the pellets was approximately 45 - 50 %, the majority being a-(1—)4) glucans. The main carbohydrate of diatoms, however, is chrysolaminarin, a l3-(1—)3) glucan, and not starch. The significantly higher amylase activity in the group fed on diatoms in comparison to the pellet fed groups is therefore surprising. It is possible that in the animals fed on diatoms, amylase acted non-specifically on other a-glycosidic bonds. The comparatively high *in vitro* activity could thus be an artefact of the experimental method, as starch was used as substrate.

Lipid concentration in pellets (approximately 5 - 10 %) and diatoms was probably very similar, as lipase activity was not significantly different. The animals fed on pellets did not show the marked difference in amylase and protease activity as did the group fed on diatoms. This was most likely due to the high levels of both protein and carbohydrates in the diet.

Comparable results on enzyme activities in juvenile abalone are currently lacking. Further studies should look at the effect of different nutrient concentrations on enzyme activity, and their change

over time as a function of digestive processes. For comparative reasons it would be essential that such studies employ the same methodology as in this study.

In conclusion, the study showed that juvenile *H. midae* can be weaned on pellets, as the growth results obtained on diatoms and pellets were not significantly different. Generally, the pellets were very efficiently utilized by the juveniles. Photoperiod did not have a significant effect on the growth rates or the feed utilization over the 30 day experimental period. This implies that the control of photoperiod is not necessary, if the weaning period does not last longer than 1 - 2 month. In comparison to the pellet fed groups, amylase activity in the animals fed on diatoms was significantly higher and protease activity significantly lower, respectively. Lipase activity did not differ significantly amongst the groups. The high amylase activity in the animals fed on diatoms was surprising, as starch is not the major carbohydrate in diatoms. The different protease and the similar lipase activities in animals fed on pellets and diatoms could be explained on the basis of the nutritional composition of the respective diet.

CHAPTER 8

THE EFFECT OF STOCKING DENSITY ON GROWTH: DEVELOPMENT OF A PRODUCTIVITY MODEL

INTRODUCTION

Similar to Chapter 7, this experiment evaluated the effect of an abiotic factor (stocking density) on the growth of juvenile *H. midae,* fed on the artificial weaning diet under culture conditions.

The costs of a commercial hatchery are made up of capital expenditures (e.g. land, buildings, rearing equipment) and operational costs (e.g. salaries, feed, utilities). A commercial operation aims to maximize productivity, which means that costs have to be reduced to a minimum. Hatchery productivity can be defined as the number of juveniles reared per unit space through to the grow-out stage per unit time.

Productivity is affected by stocking density (Koike *et al.* 1979, Chen 1984). Most studies on the effects of different stocking densities on growth have focused on size classes beyond the weaning stage (Chen 1984, Genade *et al,* 1988, Hahn 1989d, Greenier & Takekawa 1992, Singhagraiwan 1992 in Singhagraiwan & Doi 1993). However, Chen (1984) reported that juvenile *Haliotis diversicolor supertexta* in Taiwan are reared to 15 mm shell length (SL) at a density of 2500 juveniles/m^2.

The development of an artificial weaning diet allows for the rearing of juveniles at high densities, as the supply of food is not limiting. Currently, information on the effect of stocking density on the growth of ***H. midae*** is lacking, despite the economic implications. Therefore, juveniles, fed on the artificial weaning diet, were reared at different stocking densities (1250, 2500, 5000, 7500 and 10,000 juveniles/m^2) in an attempt to develop a model to predict hatchery production.

MATERIALS AND METHODS

The experimental set-up was the same as described in Chapter 7. Each stocking density of 1250, 2500, 5000, 7500 and 10,000 juveniles/m^2 was simulated in triplicate by stocking tanks with 136, 273, 545, 818 and 1090 juveniles, respectively. Juveniles were fed the same pellets as used in Chapter 7 (Table 7.1), supplied at the same rate. The experiment was conducted under a 12hL: 12hD light regime. The SL and weight of the juveniles was measured initially, and after the 30 day experimental period using the formulas for daily growth in SL (pm/day) and % daily growth rate (Chapter 7). The number of juveniles measured from each tank was 25 (1250/m^2), 50 (2500/m^2), 80 (5000/m^2), 90 (7500/m^2) and 100 (10,000/m^2). Mortalities were recorded daily in each tank, but not replaced during the growth trial.

Replicate results were combined if they were not found to be significantly different using a one way analysis of variance at a significance level of p 0.05. Different treatments were tested for differences using a one way analysis of variance in combination with Tukey's multiple range test.

RESULTS

The tanks stocked with 2500 juveniles/m^2 had a significantly higher increase in SL (76.33 ± 11.79 pm/day) than all the other stocking densities (p 5 0.002, Table 8.1). The increase in weight was also highest in the 2500 juveniles/m^2 groups (2.73 ± 0.77 % daily growth rate), but only significantly so when compared to the tanks stocked at 7500/m^2 (p 5 0.05, Table 8.1).

Percentage survival for all stocking densities was high, ranging from 92.33 ± 1.75 % to 96.96 ± 0.91 %. The survival rates amongst stocking densities were not significantly different (Table 8.2).

Table 8.1. Effect of stocking density on the increase in length and weight of *Haliotis midae*, fed on an artificial weaning diet for 30 days. Results are the mean ± SD of three tanks. Significant differences, when compared to the 2500 juveniles/ m^2 group, are indicated by *.

Stocking density (juveniles/m^2)	Initial (range)	Final (range)	Increase	
	Shell length (mm)			um/day
1250	6.99 ± 0.43 (3.96 - 9.99)	8.19 ± 0.53 (4.68 -11.76)	1.19 ± 0.27	39.78 ± 8.88**
2500	6.49 ± 0.15 (3.19 -12.20)	8.78 ± 0.26 (5.30 -14.63)	2.29 ± 0.35	76.33 ±11.79
5000	6.12 ± 0.32 (3.18 -10.57)	7.42 ± 0.41 (3.28 -12.23)	1.30 ± 0.18	43.22 ± 6.11**
7500	7.71 ± 0.71 (3.39 -11.79)	8.57 ± 0.79 (4.16 -13.51)	0.86 ± 0.13	28.67 ± 4.36**
10000	7.14 ± 0.62 (3.27 -10.62)	8.27 ± 0.60 (4.26 -12.43)	1.13 ± 0.44	37.67 ±14.68**
	Weight (g)			% Daily growth rate
1250	0.06 ± 0.01 (0.01 - 0.17)	0.11 ± 0.02 (0.02 - 0.31)	0.05 ± 0.01	2.12 ± 0.32
2500	0.06 ± 0.01 (0.01 - 0.34)	0.13 ± 0.01 (0.03 - 0.55)	0.07 ± 0.02	2.73 ± 0.77
5000	0.05 ± 0.01 (0.01 - 0.17)	0.09 ± 0.01 (0.01 - 0.32)	0.04 ± 0.01	2.16 ± 0.46
7500	0.08 ± 0.02 (0.01 - 0.24)	0.12 ± 0.03 (0.01 - 0.42)	0.04 ± 0.01	1.32 ± 0.28*
10000	0.07 ± 0.02 (0.01 - 0.20)	0.11 ± 0.03 (0.01 - 0.31)	0.04 ± 0.02	1.51 ± 0.62

* p 5 0.05 ** p 5 0.002

Based on these results, the following formula to measure hatchery productivity was developed:

$$P = S \times N$$

where P = number of juveniles produced/m^2/year, S = stocking density (juveniles/m^2), and N = number of batches that can be reared at a given stocking density during a year (12 months/T).

Table 8.2. Effect of stocking density on percentage survival of *Haliotis midae,* fed on an artificial weaning diet for 30 days. Results are the mean ± SD of three tanks.

Stocking density (juveniles/m^2)	survival
1250	9².39 ± 1.90
2500	96.96t 0.91
5000	94.37 ± 1.22
7500	95.27 ± 0.64
10000	92.33 ± 1.75

Rearing time (T) was defined as the number of months required to rear juveniles to a standard size:

$$T = \frac{LI}{G}$$

where LI = length increment required (mm), and G = growth in SL (ram/month).

It was assumed that juveniles would be weaned onto pellets at a SL of 5 mm, and reared through to 10 mm SL before transferring them to grow-out tanks. Unit time was defined as one year. As % survival was neither significantly different amongst stocking densities, nor did it show a trend it was not included in the model.

Hatchery productivity at the stocking densities tested is listed in Table 8.3. The least time (2.18 months) to grow juveniles to 10 mm was required by juveniles stocked at a density of 2500 juveniles/m^2. At the same stocking density, the highest number of batches could be reared per year (5.50). Productivity increased with an increase in stocking density ‹› --².72 x0.81, r² = 0.8194, Figure 8.1), and the highest productivity of 27,100 juveniles produced/m²/year resulted from a stocking density of 10,000 juveniles/m^2 (Table 8.3, Figure 8.1).

Table 83. Effect of stocking density on productivity of a hatchery for *Haliotis midae.*

Stocking density (juveniles/m²)	Growth (mm/month)	Time[2] (month)	Number of batches reared per year[3]	Productivity[4] (juveniles/m²/year)
1250	1.19	420	2.86	3575
2500	2.29	2.18	5.50	13750
5000	1.30	3.85	3.12	15600
7500	0.86	5.81	2.07	15525
10000	' .13	4.42	2.71	27100

Calculated from Table 8.1. (lun/day x 30/1000)
[2] Time: required length increment (5 mm)/ growth (mm/month)
[3] Number of batches: 1[2] month/ time (month)
[4] Productivity: stocking density x number of batches

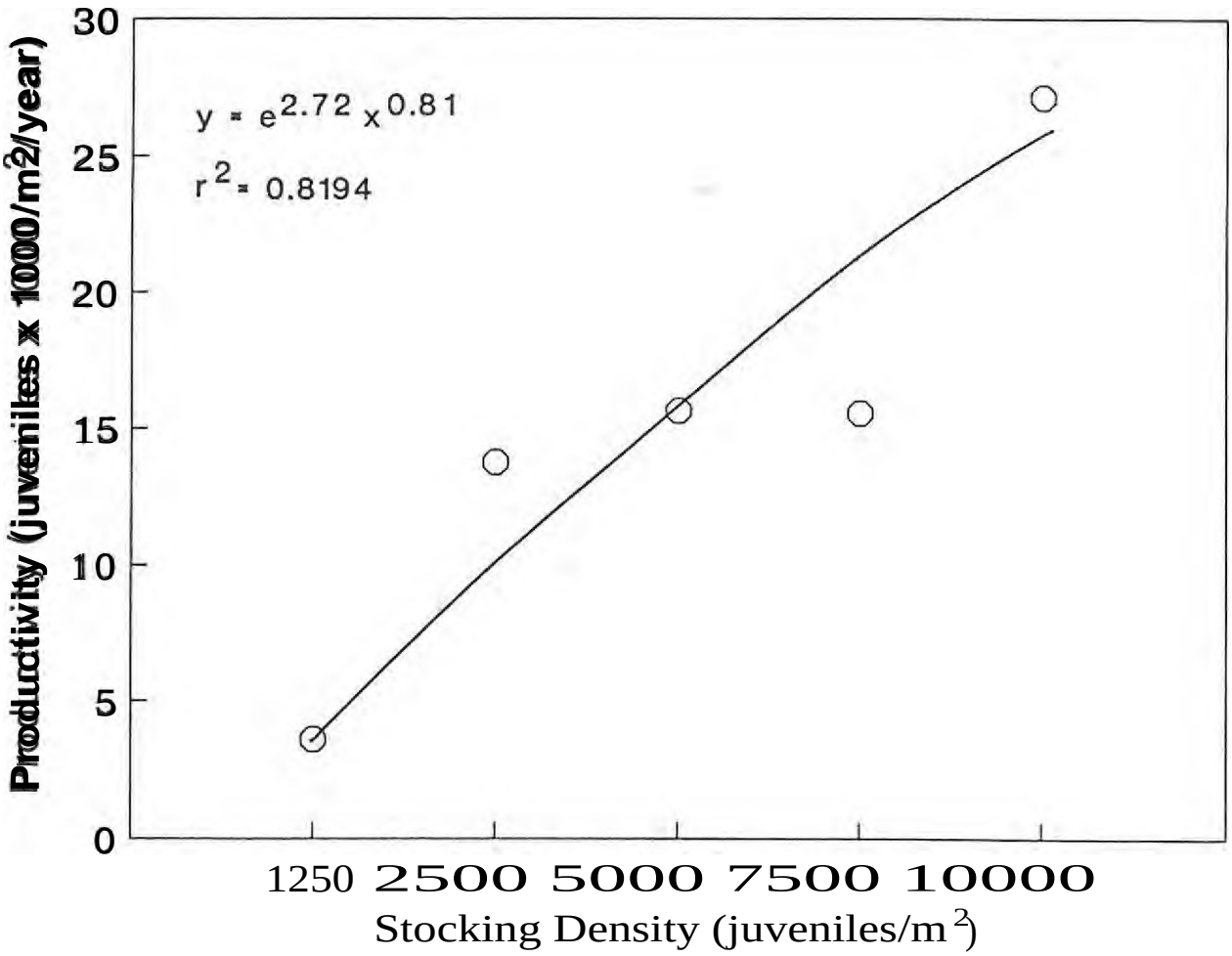

Figure 8.1. The predicted productivity per year of a hatchery for *Haliotis midae*, reared at different stocking densities.

DISCUSSION

A stocking density of 2500 juveniles/m^2 probably resulted in an optimal ratio of number of pellets per number of juveniles, and thus produced the best increase in SL and weight. At all other stocking densities access to food was most likely a problem. At the higher densities water quality could also have been affected, but this was not quantified. The result obtained in this study support the findings of Chen (1984), who also found the same density of 2500 juveniles/m^2 to produce the best growth in juveniles of less than 15 mm SL.

Although the growth of juveniles reared at a density of 10,000/m^2 was less than at a density of 2500/m^2, the productivity at the highest stocking density was the best with 27,100 juveniles produced/m^2/year. According to the trend shown in Figure 8.1, it is possible that productivity could be maximized by weaning juveniles at even higher stocking densities. However, the growth results, though significantly different, did not show a trend over the 30 day experimental period. Thus, it is not possible to extrapolate, and to calculate productivity at higher stocking densities, e.g. 20,000 juveniles/m^2. The control of photoperiod at a stocking density of 10,000 juVeniles/rn^2 might be beneficial, as longer hours of darknesss could have a significant effect on the growth of juveniles over a weaning period of more than four months (Chapter 7).

It is important to emphasize that a hatchery forms but one part of a farm. The productivity of a hatchery might therefore be constrained by the demands of other factors on a farm. On the one hand, it makes sense for a commercial hatchery to maximize productivity by weaning juveniles at as high a stocking density as possible. However, on the other hand, it might be advantageous for a farmer to transfer 2500 juveniles/m^2 to the grow-out tanks every 2.18 months, instead of 10,000 juveniles/m^2 every 4.42 months. During the grow-out phase the stocking density of the animals should be reduced to 731 - 1462/m^2 (Singhagraiwan 1992 in Singhagraiwan & Doi 1993), or even to 250/m^2 (Chen 1984). Therefore, if 10,000 juveniles/m^2 were transferred to the grow-out tanks, space could become a problem.

The productivity model developed here predicts rearing time as a function of density. Mortality, at least over the range studied, proved to be density-independent. It has to be emphasized, however, that other factors could influence productivity too. Most of them, such as temperature or photoperiod, would however affect all densities in the same way.

As a model is only as good as the assumptions and the data it is based on, growth trials in which juveniles are reared from 5 to 10 mm SL at different stocking densities are required. It is also necessary to investigate whether access to food or a decline in water quality causes a lower growth rate at higher densities, as observed in the present study. Juveniles of 9 - 12 mm SL have been shown to be extremely susceptible to hydrogen sulfide, which is formed by decomposition of food residues, faeces or dead animals. Concentrations as low as 0.3 ppm have been reported to retard the growth of the animals (Chen 1989). The toxicity of ammonia, which is formed in the same way as hydrogen sulfide, is less, but growth of juveniles can be retarded by concentrations of 0.5 ppm (Chen 1989).

The effect of feeding rate is another aspect worth studying. A higher feeding rate would be expected to benefit higher densities of animals, if water quality does not deteriorate. In that respect, an extensive investigation into the effect of temperature on water stability of the pellets is required. In Japan, e.g., artificial food is not fed at temperatures above 24 °C, as the food quickly disintegrates causing poor water quality (Hahn 1989g).

On the basis of the productivity a stocking density of 10,000 juveniles/m^2 is considered optimal for the weaning of *H. midae.* It might be possible, however, that maximal productivity would be obtained at even higher stocking densities. The productivity model developed here only takes one factor into account. Currently, it is not known whether factors besides stocking density influence productivity. If stocking density is the most significant factor, then the model can be directly applied to a commercial hatchery.

CHAPTER 9

GENERAL DISCUSSION

In this final discussion the experimental results obtained in the previous chapters are synthesized with regard to their commercial application, and future research needs in the nutritional and non-nutritional field are identified.

Certain aspects of the nutrition of juveniles studied in this project have proven to be advantageous with regard to the weaning of *H. midae* at a commercial level. Juveniles appear to feed readily on artificial food and their growth is as good as on a diatom diet. This allows for the replacement of diatoms as principal food source during the weaning stage by the artificial diet. Moreover, even at this early life stage they accept a diet in pellet form, which simplifies the feeding process as pellets can be stored in bulk and are easy to offer. The weaning diet developed is comprised of ingredients of animal and plant origin such as fish meal or *Spirulina* spp., respectively, showing that juveniles can digest a variety of ingredients. This confirms other findings (Dixon 1992, Maguire *et al.* 1993), and allows for the least-costing of the weaning diet as different feedstuffs can be included.

The efficiency with which artificial food is utilized and converted into body tissue, especially in comparison to fish, is very encouraging. The feed conversion ratios (FCRs) in juvenile *H. midae* vary from 0.44 to 0.60, while the FCRs in young *Oreochromis niloticus* range from 0.71 to 0.81 (Takeuchi *et al.* 1983), and in young *Acipenser transmontanus* they vary between 0.72 and 1.64 (Hung & Lutes 1988). The protein efficiency ratios (PERs) in *H. midae* range from 5.06 to 6.64. In contrast, typical PERs of fingerlings vary from 2.2 to 2.7 (Yu *et al.* 1977). There are two possible reasons as to why *H. midae* utilizes artificial food more efficiently than fish. Firstly, unlike fish abalone do not move around with the exception of the time spend searching for food and have therefore probably lower energy requirements. Energy requirements can be measured

by determining the consumption of 0_2. Uki & Kikuchi (1975 in Hahn 1989i) measured the uptake of 0_2 of *H. discus hannai* at different body weights and temperatures, and similar work has been done on many fish species (Steffens 1989). It is very difficult, however, to compare data even amongst different species of fish as factors such as size, diet, or different experimental conditions influence 0_2 consumption (Steffens 1989). No definite conclusion regarding differences in 0_2 consumption between abalone and fish can thus be drawn at this stage. Secondly, the energy metabolism of abalone appears to be based on carbohydrates (Emerson 1967, Livingstone & de Zwaan 1983), while in salmonid fish, e.g., fats and protein are the major fuels (Cho *et al.* 1977). Therefore, if the diet contains enough carbohydrates the protein component will not be allocated towards the synthesis of energy in abalone, whereas fish will always derive a certain portion of the energy required for maintenance purposes from the dietary protein component.

World-wide, the provision of food for juvenile abalone during the weaning stage has been identified as a major impediment in abalone nutrition (Mozqueira 1992). With the conclusion of the present study, this is no longer a problem at least for the culture of *H. midae.* However, there is still a lot of potential to improve the nutritional quality of the diet.

The essential amino acid pattern (EAA) pattern of the whole body tissue of juveniles was found to be an appropriate estimation of the dietary requirements of all ten EAAs. In fish, these requirements have traditionally been established in growth trials, feeding different levels of each AA within test diets (Ketola 1982). The "break point" in the resulting dose-response curve is usually taken as the level of dietary requirement. It is doubtful whether such feeding trials will provide more accurate information regarding the EAA needs of abalone, as there are many inherent problems. Firstly, the EAAs are often supplied in crystalline form. It has been found for fish that if crystalline AAs amount to a high proportion of the dietary protein, they generally display sub-optimal growth and feed conversion efficiency in comparison to animals fed on protein bound AAs. Requirements are thus measured under sub-optimal conditions (Wilson *et al.*

1978). Secondly, at least in test diets for fish, a wide range of different protein sources and reference proteins (the AA pattern of which is imitated) have been used. This makes it difficult to compare results (Tacon & Cowey 1985). Thirdly, the interpretation of the dose-response curve as to the exact position of the "break point" is likely to be inaccurate (Tacon & Cowey 1985).

The determination of the requirements of the major nutrients, as established for *H. discus hannai* (Uki *et al.* 1985a,b, 1986a, Uki & Watanabe 1986), is desirable not only for each haliotid species but also for each life stage. Although the results obtained for *H. discus hannai* proved to be applicable to *H. midae,* it is likely that the optimal concentrations of the major nutrients are species and life stage -specific.

A standardized procedure to study nutritional requirements is a prerequisite for the meaningful comparison of results. The lack of standardized growth trials in the relevant fish literature has been identified as a major problem (Tacon & Cowey 1985). This is most probably also a problem in abalone nutrition. In the test diets for *H. discus hannai* (for review see Uki & Watanabe 1992) casein or fish meal were used as protein sources, sodium alginate, dextrin and cellulose as sources of carbohydrates, soybean and cod liver oil as lipid sources, and mixtures of minerals and vitamins (Uki & Watanabe 1992). Obviously the experimental purpose dictates the nature of the nutrient sources and their content in a test diet. However, even if a different protein source is tested, such as squid meal, the rest of the diet should be kept constant. The use of a limited number of ingredients is advantageous as different ingredients are likely to differ in their digestibility and energy content (Maguire *et al.* 1993), thereby influencing growth results.

Besides information on the nutritional needs of abalone, studies on the digestibility of feed ingredients are required (Wee *et al.* 1992). To date, the only studies of this nature on abalone have been undertaken by Dixon (1992) and Wee *et al.* (1992). The investigations of Wee *et al.* (1992) and Maguire *et al.* (1993) to establish a data base on the digestibility of ingredients by abalone are therefore of extreme importance, particularly in view of the apparent similarities of

the nutritional requirements of different species. However, as with the studies on the nutritional requirements, species and life stage -specific investigations are necessary.

With respect to the digestibility of foodstuffs, information on the nutritional role of bacteria and the enzymology of abalone is desirable. Abalone are capable of hydrolizing a wide variety of carbohydrate substrates (for review see Chapter 2, Table 2.1). The determination of pH and temperature optima of key enzymes, such as amylase, will help to optimize the culture conditions, e.g. by adjusting the pH of the artificial diet (Ceccaldi 1982).

The emphasis of this project was laid on the development of an artificial weaning diet. Preliminary investigations into the effects of photoperiod (Chapter 7) and stocking density (Chapter 8) were undertaken in two 30 day growth trials. Stocking density produced significant differences in growth, but the effects of photoperiod seem to require much longer test periods (Ebert & Houk 1984, Chapter 7), as the activity of abalone appears to be controlled by a circadian rhythm. Ideally, different culture conditions should be tested over the whole weaning period.

The slow growth of abalone is one of the major problems for a commercial operation. Traditionally, the approaches to improve growth have been the development of more effective diets, and the determination of optimal culture conditions. However, translocation, hybridization and polyploid breeding experiments, as well as the biochemical manipulation of growth processes have recently emerged as promising new methods in abalone aquaculture.

The transfer of fast growing, non-indigenous species to South Africa has been proposed, because of the slow growth rate of *H. midae* (Hecht & Britz 1990). The cold water species *H. rufescens* would probably show superior growth to *H. midae* if cultured along the west coast. The warm water species *H. diversicolor supertexta,* on the other hand, might be suited for culture along the east coast (Hecht & Britz 1990). However, Hecht & Britz (1992) emphasized that the invasive

potential of the non-indigenous species and the organisms associated with it should be evaluated prior to its introduction and wherever possible, the farming of local species should be given priority (Hecht & Britz 1992).

In California, the hybridization of four abalone species has been achieved (H. *corrugate, H. fulgens, H. rufescens, H. sorenseni)* (Leighton & Lewis 1982). Cross-fertilization between *H. discus hannai* and the non-indigenous *H. fulgens* and *H. rufescens* has also been reported from China (Nie 1992) and in Tasmania, a cross between *H. rubra* and *H, laevigata* has been developed (T. Hecht 1993, Department of Ichthyology and Fisheries Science, Rhodes University, South Africa, pers. comm.).

The characteristics selected for in hybridization experiments are faster growth, adaptations to environmental culture conditions, and a better quality of meat than in either parent (Hahn 1989j). A key problem encountered in the production of hybrids is the facilitation of the entry of non-homologous sperm through the egg vitelline layer (Lewis *et al.* 1992). Leighton & Lewis (1982) found that in cross-fertilization experiments the sperm concentration had to be ten times higher than for homologous crosses. Furthermore, eggs had to be freshly spawned to obtain optimal fertilization rates (Leighton & Lewis 1982). The production of hybrids, therefore, is still frought with many difficulties.

Polyploid breeding experiments have been carried out on one species only. Triploid *H. discus hannai* display a marked temperature tolerance (Fujino *et al.* 1987) and show an increase in survival rate (Fujino 1992). However, problems exist regarding fertility and growth rate of the triploid animals (Fujino 1992). In experiments in China triploid *H. discus hannai* showed a low survival rate (Nie 1992).

There are also biochemical considerations to the problem of slow growth. The growth of juvenile abalone can be significantly accelerated by the exogenous application of the homologous peptide

hormones insulin and growth hormone (Morse 1984, Hooker & Morse 1985). In addition to the improvement in growth rate, these treatments reduce the variability in growth (Morse 1984) which is so typical for abalone, both in the wild and under culture conditions (Oba *et al.* 1968, Genade *et al.* 1988, Hahn 1989g, Day & Fleming 1992, this study). Hooker & Morse (1985) postulated that this variability, at least in a hatchery environment, is not genetically based but that it is a result of physiological deficiencies due to inadequate culture conditions. Application of hormones can overcome these problems to a certain extent (Hooker & Morse 1985). Moreover, in contrast to steroids, peptide hormones are rapidly degraded by enzymes and are therefore safe to use (Morse 1986).

From an aquaculture point of view it is also important to understand the hormonal control of nutrient utilization, protein and glycogen synthesis (the two major components of the foot) as this can also lead to major improvements regarding the growth of abalone.

An eventual commercial application of such hormones, however, depends on a regular and cheap supply. Since abalone produce only minute amounts of peptide hormones, the large-scale production of these growth factors will depend on genetic engineering techniques. A project to establish an abalone gene bank has already been started (Morse 1984). In combination with recombinant DNA technology this will allow to purify and clone specific genes, allowing for their mass production.

It is likely that the application of biotechnological techniques to produce growth-accelerating hormones will ultimately have a significant effect on the abalone industry, much in the way that agriculture has been affected (Walsh 1981).

In conclusion, the objective of this study has been met in that an artificial diet, suitable for the weaning of *Haliotis midae* at a commercial level, has been developed. The data have demonstrated that the EAA pattern of abalone can be successfully used as an estimate of the

dietary requirement pattern of the EAAs. It has also been shown that the gross protein, lipid, mineral and vitamin requirements of haliotids, in general, seem to be similar. A 30 day comparative growth trial showed that juvenile abalone grew equally well on the artificial weaning diet as they did on diatoms. Finally, an optimal stocking density for the weaning of *H. midae* was calculated based on a productivity model. The combination of traditional aquaculture methods, breeding experiments, and the application of biotechnological techniques will very likely set the basis for further expansion of the commercial culture of abalone on a global scale.

REFERENCES

Albrecht, P. 1921. Chemical study of several marine molluscs of the Pacific coast. *J. Biol. Chem.,* 45: 395-405.

Albrecht, P. 1923. Chemical study of several marine molluscs of the Pacific coast. The liver. *J. Biol. Chem.,* 57: 789-794.

Allen, W.V. & Kilgore, J. 1975. The essential amino acid requirements of the red abalone, *Haliotis rufescens. Comp. Biochem. Physiol.,* 50A: 771-775.

Anderlini, V. 1974. The distribution of heavy metals in the red abalone, *Haliotis rufescens,* on the California coast. *Arch. Environ. Contain.,* 2: 253-265.

Anonymous 1975. *Yearbook of Fisheries Statistics.* Vol. 40. United Nations Food and Agricultural Organization, Rome, Italy.

Anonymous 1989. *Yearbook of Fisheries Statistics.* Vol. 68. United Nations Food and Agricultural Organization, Rome, Italy.

Anonymous 1993. 1992/93 abalone quotas (kg whole mass). *SA. Shipp. News Fishg. Ind. Rev.,* 48: 29.

Ansell, A.D., Coughlan, J., Lander, K.F. & Coosmore, FA. 1964. Studies on the mass culture of *Phaeodactylum.* IV. Production and nutrient utilization in outdoor mass culture. *Limnol. Oceanogr.,* 9: 334-342.

Arai, S. 1981. A purified test diet for coho salmon, *Oncorhynchus kisutch,* fry. *Bull. Japan. Soc. Sci, Fish.,* 47: 547-550.

Avelar, P.M.F., Giacometti, D. & Bacila, M. 1978. Comparative levels of muscle glycolytic enzymes in mammals, fish, echinoderms and molluscs. *Comp. Biochem. Physiol.,* 60B: 143-148.

Avery, G. 1974. Open station shell midden sites and associated features from the Pearly Beach area, south-western Cape. *S. Afr. Archaeol. Bull.,* 29: 104-114.

Bannatyne, W.R. & Thomas, J. 1969. Fatty acid composition of New Zealand shellfish lipids. *N. Z. J. Sci.,* 12: 207-212.

Barber, A., Jordana, R. & Ponz, F, 1975. Active transport by the intestine of snail *(Cryptomphalus hortensis* Willer). *Rev. Esp. Fisiol.,* 31: 119-124.

Barber, A., Jordana, R. & Ponz, F. 1979. Competitive kinetics of sugar active transport in snail intestine. *Rev. Esp. Fisiol.,* 35: 243-248.

Barkai, R. & Griffiths, C.L. 1986. Diet of the South African abalone *Haliotis midae. S. Afr. J. Mar. Sci.,* 4: 37-44.

Barkai, R. & Griffiths, C.L. 1987. Consumption, absorbtion efficiency, respiration and excretion in the South African abalone *Haliotis midae. S. Afr. J. Mar. Sci.,* 5: 523-529.

Barkai, R. & Griffiths, C.L. 1988. An energy budget for the South African abalone *Haliotis midae* Linnaeus. *J. Mollusc. Stud.,* 54: 43-51.

Barlow, L.A. 1990. Electrophysiological and behavioral responses of larvae of the red abalone *(Haliotis rufescens)* to settlement inducing substances. *Bull. Mar. Sci.,* 46: 537-554.

Barnes, R.D. 1980. *Invertebrate Zoology.* 4[1h] ed. Holt-Saunders, Tokyo.

Becker, E.W. 1986. Nutritional properties of microalgae: potentials and constraints. In: *Handbook of Microalgal Mass Culture.* (Ed.) A. Richmond, pp. 339-419. CRC Press Inc., Boca Raton, Florida.

Becker, W. 1972. The glucose content **in** haemolymph of *Australorbis glabratus. Comp. Biochem. Physiol.,* 22A: 809-814.

Beis, I. & Newsholme, E.A. 1975. The contents of adenine nucleotides, phosphagens and some glycolytic intermediates in resting muscles from vertebrates and invertebrates. *Biochem. J.,* 152: 23-32.

Bennett, R.Jr. & Nakada, **H.I.** 1968. Comparative carbohydrate metabolism of marine Mollusca - I. The intermediary metabolism of *Myti!us californianus* and *Hallows rufescens. Comp. Biochem. Physiol.,* 24: 787-797.

Bennett, R.Jr., Thanassi, N. & Nakada, H. I. 1971. Hepatopancreas glycosidases of the abalone *(Haliotus rufescens). Comp. Biochem. Physiol.,* 408: 807-811.

Berrie, W.R. & Devereaux, M.W. 1964. Identification and location of carbohydrases in the intestinal tract of *Tegula funebralis. Veliger,* 6 (supplement): 20-24.

Bevelander, G. & Nakahara, H. 1966. Correlation of lysosomal activity and ingestion by mantle epithelium. *Biol. Bull.,* 148: 1-15.

Borlongan, I.G. 1990. Studies on the digestive lipases of milkfish, *Chanos chanos. Aquaculture,* 89: 315-325.

Bregazzi, P.K. & Naylor, E. 1972. The locomotor activity rhythm of *Talitrus saltator* (Montagu) (Crustacea, Amphipoda). *J. Exp. Biol.,* 57: 375-391.

Brett, J.R. 1979. Environmental factors and growth. In: *Fish Physiology. Vol. VIII: Bioenergetics and Growth.* (Eds.) W.S. Hoar, D. J. Randall & J.R. Brett, pp. 599-675. Academic Press, New York.

Britz, P.J., Hecht, T., Knauer, J. Sr. Dixon, M.G. 1994. The development of an artificial feed for abalone farming. *S. Afr. J. Sei.,* 90: 6-7.

Brough, C.N. & White, K.N. 1990. Functional morphology of the rectum in the marine gastropod *Littorina saxatilis* (Olivi) (Prosobranchia: Littorinoidea). *J. Mollusc. Stud.,* 56: 97-108.

Bruni, C.B., Auricchio, F. & Covelli, I. 1969. Acid a-D-glucosidase glucohydrolase from cattle liver. *J. Biol. Chem.,* 244: 4735-4742.

Bryan, G.W., Potts, G.W. & Forster, G.R. 1977. Heavy metals in the gastropod mollusc *Haliotis tuberculata* (L.). *J. Mar. Biol. Ass. U.K.,* 57: 379-390.

Burton, R.F. 1983. Ionic regulation and water balance. In: *The Mollusca, Vol. 2: Physiology, Part II.* (Eds.) A.S.M. Saleuddin & K.M. Wilbur, pp. 291-352. Academic Press, New York.

Butler, C. 1958. Nutritional value of fish in reference to artherosclerosis and current dietary research. *Commer. Fish. Rev.,* 20: 7-16.

Campbell, J.L. 1965. The structure and function of the alimentary canal of the black abalone, *Haliotis cracherodii* Leach. *Amer. Micros. Soc. Trans.,* 84: 376-395.

Carefoot, T.H. 1982. Gastropod nutrition. In: *Proceedings of the Second International Conference on Aquaculture Nutrition. Rehoboth Beuch, Delaware.* (Eds.) G.D. Pruder, C. Langdon & D. Conklin, pp. 321-337. World Mariculture Society, Special Publication No. 2, Louisiana State University, Louisiana.

Ceccaldi, H.J. 1982. Contributions of physiology and biochemistry to progress in aquaculture. *Bull. Japan. Soc. Sci. Fish.,* 48: 1011-1028.

Chen, H.-C. 1984. Recent innovations in cultivation of edible molluscs in Taiwan with special reference to the small abalone *Haliotis diversicolor* and the hard clam *Meretrix lusoria. Aquaculture,* 39: 11-27.

Chen, H.-C. 1989. Fanning the small abalone, Haliotis diversicolor supertexta, in Taiwan. In: *Handbook of Culture of Abalone and Other Marine Gastropods.* (Ed.) K.O. Hahn, pp. 265-283, CRC Press, Boca Raton, Florida.

Chen, S.-J. 1985. A formulated feed for rearing seed spats of the abalone, *Haliotis diversicolor* Reeve. *Haiyang Kexue,* 9: 39-40.

Chiang, P.K. 1977. Glycogen metabolism in the snail *Biomphalaria glabrata. Comp. Biochem. Physiol.,* 58B: 9-12.

Cho, C.Y., Bayley, H.S. & Slinger, S.J. 1974. Partial replacement of herring meal with soybean meal and other changes in a diet for rainbow trout *(Salmo gairdneri). J. Fish. Res. Board Can.,* 31: 1523-1528.

Cho, C.Y., Slinger, S.J. & Bayley, H.S. 1977. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. *Comp. Biochem. Physiol.,* 73B: 25-41.

Cho, -D.M., Pyeun, -J.H., Byun, -D.S & Kim, -C.Y. 1983. Proteolytic activity of the crude enzyme extracted from the digestive tract of marine gastropods. *Bull. Korean Fish. Soc.,* 16: 216-224.

Clark, A.G. & Jowett, D.A. 1978. Hydrolytic enzymes of the paua *Haliotis iris,* a marine gastropod. *N. Z. J. Mar. Freshw.* Res., 12: 221-222.

Clark, A.G. & Jowett, D.A. 1988. Purification and properties of an arylsulphatase from the digestive gland of *Haliotis iris. Comp. Biochem. Physiol.,* 89B: 409-417.

Clark, J.M.Jr. & Switzer, R.L. 1977. *Experimental Biochemistry.* 2nd ed. W.H. Freeman & Company, New York.

Clavier, J. 1992. The ormer *(Haliotis tuberculata)* fishery of France and the Channel Islands. In: *Abalone of the World. Biology, Fisheries and Culture.* (Eds.) S.A. Shepherd, M.J. Tegner & S.A. Guzman del Proo, pp. 454-460. Fishing News Books, Cambridge.

Coles, G.C. 1969. Isoenzymes of snail livers. II. Dehydrogenases. *Comp. Biochem. Physiol.,* 31: 1-14.

Colvin, P.M. 1976. Nutritional studies on penaeid prawns: protein requirements in compounded diets for juvenile *Penaeus indicus. Aquaculture,* 7: 315-326.

Coombs, T.L. 1977. Uptake and storage mechanisms of heavy metals in marine organisms. *Proc. Anal. Div. Chem. Soc.,* 14: 219-221.

Cowey, C.B. & Tacon, A.G.J. 1982. Fish nutrition - relevance to invertebrates. In: *Proceedings of the Second International Conference on Aquaculture Nutrition. Rehoboth Beuch, Delaware.* (Eds.) G.D. Pruder, C. Langdon & D. Conklin, pp. 13-30. World Mariculture Society, Special Publication No. 2, Louisiana State University, Louisiana.

Cox, K.W. 1960. Review of the abalone of California. *Calif. Fish Game,* 46: 381-406.

Cox, K.W. 1962. California abalones - family Haliotidae. *Fish. Bull. Calif.,* 118: 1-133.

Crampton, E.W. & Harris, L.E. 1969. *Applied Animal Nutrition. 2nd* ed. W.H. Freeman & Company, San Francisco.

Crofts, D.R. 1929. *Haliotis. Liverpool Mar. Biol. Comm. Mem.,* No. 29: 1-174.

Crofts, D.R. 1937. The development of *Haliotis tuberculata,* with special reference to organogenesis during torsion. *Phil. Trans. Roy. Soc. Land. Ser. B,* 228: 219-269.

Cuplin, **P.** 1969. Performance evaluation of chelated minerals in Idaho open-formula diets. *Trans. Am. Fish. Soc.,* 98: 772-776.

Darley, W.M. 1977. Biochemical composition. In: *The Biology of Diatoms.* (Ed.) D. Werner, pp. 198-223. University of California Press, Berkeley.

Day, R.W. & Fleming, A.E. 1992. The determinants and measurement of abalone growth. In: *Abalone of the World. Biology, Fisheries and Culture.* (Eds.) S.A. Shepherd, M.J. Tegner & S.A. Guzman del Pr6o, pp. 141-168. Fishing News Books, Cambridge.

DeCoursey, **P.J.** 1983. Biological timing. In: *The Biology of Crustacea. Vol. 7: Behavior and Ecology.* (Eds.) F.J. Vernberg & W.B. Vemberg, pp. 107-162. Academic Press, New York.

De Koning, A.J. 1966a. Phospholipids of marine origin IV. The abalone *(Haliotis midae). J. Sci. Fd. Agric.,* 17: 460-464.

De Koning, A.J. 1966b. Isolation of 2-aminoethylphosphonic acid from phospholipids of the abalone *(Haliotis midae). Nature,* 210: 113.

Dempsey, A.C. & Kitting, C.L. 1987. Characteristics of bacteria isolated from penaeid shrimp. *Crustacean,* 52: 90-94.

Deshimaru, D. & Shigeno, K. 1972. Introdution to the artificial diet for prawn *(Penaeus japonicus). Aquaculture, 1:* 115-133.

Dixon, M.G. 1992. *The Effect of Temperature and Photoperiod on the Digestive Physiology of the South African Abalone* Haliotis midae. MSc thesis, Rhodes University.

Ebert, E.E. 1992. Abalone aquaculture: a North America regional review. In: *Abalone of the World. Biology, Fisheries and Culture.* (Eds.) S.A. Shepherd, M.J. Tegner & S.A. Guzman del Pr6o, pp. 570-582. Fishing News Books, Cambridge.

Ebert, EE. & Houk, J.L. 1984. Elements and innovations in the cultivation of red abalone, *Haliotis rufescens Swainson. J. Fish Dis.,* 6: 111-128.

Ebert, E.E. & Houk, J.L. 1989. Abalone cultivation methods used at the California Department of Fish and Game's marine resources laboratory. In: *Handbook of Culture of Abalone and Other Marine Gastropods.* (Ed.) K.O. Hahn, pp. 239-254, CRC Press Inc., Boca Raton, Florida.

Elyakova, L.A., Shevchenko, N.M. & Avaeva, S.M. 1981. A comparative study of carbohydrase activities in marine invertebrates. *Comp. Biochem. Physiol.,* 69B: 905-908.

Emerson, D.N. 1967. Carbohydrate orientated metabolism of *Planorbis corneus* (Mollusca, Planorbidae) during starvation. *Comp. Biochem. Physiol.,* 22: 571-579.

Eriksson, L-0. 1978. Nocturnalism versus diumalism; dualism within fish individuals. In: *Rhythmic Activity of Fishes.* (Ed.) J.E. Thorpe, pp. 69-89. Academic Press, London.

Fallu, R. 1991. *Abalone Farming.* Fishing News Books, Oxford, England.

Farlinger, S. & Campbell, A. 1992. Fisheries management and biology of northern abalone, *Haliotis kamtschatkana,* in the northeast Pacific. In: *Abalone of the World. Biology, Fisheries and Culture.* (Eds.) S.A. Shepherd, M.J. Tegner & S.A. Guzman del Proo, pp. 395-406. Fishing News Books, Cambridge.

Farmanfarmaian, A. & Lauterio, T. 1979. Amino acid supplementation of feed pellets of the giant shrimp *(Macrobrachium rosenbergii). Proc. World Maricul. Soc.,* 10: 674-688.

Farmanfarmaian, A., Lauterio, T. & Ibe, M. 1982. Improvement of the stability of commercial feed pellet for the giant shrimp *(Macrobrachium rosenbergii). Aquaculture,* 27: 29-41.

Field, J.G., Jarman, N.G., Dieckmann, G.S., Griffiths, C.L., Velimimv, B. & Zoutendyk, P. 1977. Sun, waves, seaweed and lobsters: the dynamics of a West Coast kelp-bed. *S. Afr. J. Sci.,* 73: 7-10.

Florkin, M. & Bricteux-Gregoire, S. 1972. Nitrogen metabolism in molluscs. In: *Chemical Zoology. Vol.* 7. (Eds.) M. Florkin & B.T. Scheer, pp. 301-348. Academic Press, New York.

Focht, R.L. 1984. *Trout Growth in Aquaculture Systems: Methods of Estimating Daily Percent Weight Gain.* MSc thesis, University of Idaho.

Folch, J., Lees, M. & Sloane Stanley, G.Y. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.,* 226: 497-509.

Fong, W. & Mann, K.H. 1980. Role of gut flora in the transfer of amino acids through a marine food chain. *Can. J. Fish. Aquat. Sci.,* 37: 88-96.

Forster, G.R. 1967. The growth of *Haliotis tuberculata:* results of tagging experiments in Guernsey 1963 - 65. *J. Mar. Biol. Ass. U.K.,* 47: 287-300.

Fourie, H.O. 1976. Metals in organisms from Saldanha Bay and Langebaan Lagoon prior to industrialization. *S. Afr. J. Sci.,* 72: 110-113.

Fowler, B.A., Wolfe, D.A. & Hettler, W.F. 1975. Mercury and iron uptake by cytosomes in mantle epithelial cells of Quahog clams *(Mercenaria mercenaria)* exposed to mercury. *J. Fish. Res. Board Can.,* 32: 1767-1775.

Fretter, V. & Graham, A. 1962. *British Prosobranch Molluscs. Their Functional Anatomy and Ecology.* Ray Society, London.

Fretter, V. & Graham, A. 1976. A *Functional Anatomy of Invertebrates.* Academic Press, London.

Fujino, K. 1992. Review of genetics and stock management of the Pacific abalone. In: *Abalone of the World. Biology, Fisheries and Culture.* (Eds.) S.A. Shepherd, M.J. Tegner & S.A. Guzman del Proo, pp. 491-503. Fishing News Books, Cambridge.

Fujino, K., Kamakura, M., Mito, R. & Arai, K. 1987. Overdominance of survivals at phosphogluconate dehydrogenase locus in triploid Pacific abalone populations. *Nippon Suisan Gakkaishi,* 53: 1759-1764.

Furia, **M.,** Gianfreda, L. & Scardi, V. 1975. Cellulase and related enzyme activities in a carnivorous mollusc: *Octopus vulgaris* Lamarck. *Comp. Biochem. Physiol.,* 52B: 529-532.

Galli, D.R. & Giese, A.C. 1959. Carbohydrate digestion in a herbivorous snail, *Tegula funebralis. J. Exp. Zool.,* 140: 415-440.

Garland, C.G., Cooke, S.L., Grant, J.F. & McMeekin, T.A. 1985. Ingestion of the bacteria on and the cuticle of crustose (non-articulated) coralline algae by post-larval and juvenile abalone *(Haliotis ruber* Leach) from Tasmanian waters. *J. Exp. Mar. Biol. Ecol.,* 91: 137-149.

Genade, A.B., Hirst, A.L. & Smit, C.J. 1988. Observations of the spawning, development and rearing of the South African abalone *Haliotis midae. S. Afr. J. Mar. Sci.,* 6: 3-12.

George, S.G. & Coombs, T.L. 1975. A comparison of trace metal and metalloenzyme profiles in different molluscs and during development of the oyster. *Proc. Eur. Symp. Mar. Biol., 9th, Aberdeen, Scot., 1974,* pp. 433-449.

George, S.G. & Coombs, T.L. 1977. The effects of chelating agents on the uptake and accumulation of cadmium by *Mytilus edulis. Mar. Biol.,* 39: 261-268.

George, S.G., Pine, B.J.S. 1980. Metabolism of zinc in the mussel *Mytilus edulis.* **A** combined ultrastructural and biochemical study. *J. Mar. Biol. Assoc. U.K.,* 60: 575-590.

George, S.G., Pirie, B.J.S. & Coombs, T.L. 1976. The kinetics of accumulation and excretion of ferric hydroxide in *Mytilus edulis* (L.) and its distribution in the tissues. *J. Exp. Mar. Biol. Ecol.,* 23: 71-84.

George, W.C. 1952. The digestion and absorption of fat in lamellibranchs. *Biol. Bull.,* 102: 118-127.

Gianfreda, L., Imperato, A., Palescandolo, R. & Scardi, V. 1979a. Distribution of B-1,4-glucanase and B-glucosidase activities among marine molluscs with different feeding habits. *Comp. Biochem. Physiol.,* 63B: 345-348.

Gianfreda, L., Tosti, E. & Scardi, V. 1979b. A comparison of a- and B- glucanase activities in fourteen species of marine molluscs. *Biochem. Syst. Ecol.,* 7: 57-59.

Giese, A.C. 1966. Lipids in the economy of marine invertebrates. *Physiol. Rev.,* 46: 244-298.

Goddard, C.K. & Martin, A.W. 1966. Carbohydrate metabolism. In: *Physiology of Mollusca. Vol.* 2. (Eds.) K. Wilbur & C.M. Yonge, pp. 275-352. Academic Press, New York.

Godoy, C., Jerez, G. & Ponce, F. 1992. The introduction of abalone into Chile. In: *Abalone of the World. Biology, Fisheries and Culture.* (Eds.) S.A. Shepherd, M.J. Tegner & S.A. Guzman del Proo, pp. 485-488. Fishing News Books, Cambridge.

Gorfine, H. & King, R. 1991. New food for abalone. *Austasia Aquacult. Mag.,* 5: 40.

Goudsmit, E.M. 1972. Carbohydrates and carbohydrate metabolism in Mollusca. In: *Chemical Zoology. Vol.* 7. (Eds.) M. Florkin & B.T. Scheer, pp. 219-243. Academic Press, New York.

Graham, A. 1931. On the structure and function of the alimentary canal of the limpet. *Trans. Roy. Soc. Edinburgh.,* 57: 287-308.

Graham, A. 1949. The molluscan stomach. *Trans. Roy. Soc. Edinburgh,* 61: 737-778.

Grant, J.F. 1981. Abalone culture in Japan: development and current commercial practice. *Tasman. Fish. Res.,* No. 23: 2-17.

Greenberg, A.E., Conners, J.J., Jenkins, D. & Franson, M.A.H. 1980. *Standard Methods for the Examination of Water and Waste Water.* 5th ed. Pub. APHA, AWWA and WPCF.

Greenier, J.L. & Takekawa, J.Y. 1992. Growth models and food conversion of cultured juvenile red abalone *(Haliotis rufescens). In: Abalone of the World. Biology, Fisheries and Culture.* (Eds.) S.A. Shepherd, M.J. Tegner & S.A. Guzman del Proo, pp. 527-537. Fishing News Books, Cambridge.

Greer, M.L. & Lawrence, A.L. 1967. The active transport of selected amino acids across the gut of the chiton *(Cryptochiton stelleri). Comp. Biochem. Physiol.,* 22: 341-358.

Guzman del Proo, S.A. 1992. A review of the biology of abalone and its fishery in Mexico. In: *Abalone of the World. Biology, Fisheries and Culture.* (Eds.) S.A. Shepherd, M.J. Tegner & S.A. Guzman del Proo, pp. 341-360. Fishing News Books, Cambridge.

Hahn, K.O. 1989a. Survey of the commercially important abalone species in the world. In: *Handbook of Culture of Abalone and Other Marine Gastropods.* (Ed.) K.O. Hahn, pp. 3-11, CRC Press Inc., Boca Raton, Florida.

Hahn, K.O. 1989b. Abalone aquaculture in Japan. In: *Handbook of Culture of Abalone and Other Marine Gastropods.* (Ed.) K.O. Hahn, pp. 185-194, CRC Press Inc., Boca Raton, Florida.

Hahn, K.O. 1989c. Abalone aquaculture in New Zealand, Australia, and Ireland. In: *Handbook of Culture of Abalone and Other Marine Gastropods.* (Ed.) K.O. Hahn, pp. 295-299, CRC Press Inc., Boca Raton, Florida.

Hahn, K.O. 1989d. Culture of *Haliotis tuberculata* at the Argenton Experimental Station, France. In: *Handbook of Culture of Abalone and Other Marine Gastropods.* (Ed.) K.O. Hahn, pp. 285-294, CRC Press Inc., Boca Raton, Florida.

Hahn, K.O. 1989e. Japanese abalone culture techniques of the Oyster Research Institute. In: *Handbook of Culture of Abalone and Other Marine Gastropods.* (Ed.) K.O. Hahn, pp. 195-220, CRC Press Inc., Boca Raton, Florida.

Hahn, K.O. 1989f. Abalone aquaculture in California. In: *Handbook of Culture of Abalone and Other Marine Gastropods.* (Ed.) K.O. Hahn, pp. 221-225, CRC Press Inc., Boca Raton, Florida.

Hahn, K.O. 1989g. Nutrition and growth of abalone. In: *Handbook of Culture of Abalone and Other Marine Gastropods.* (Ed.) K.O. Hahn, pp. 135-182, CRC Press Inc., Boca Raton, Florida.

Hahn, K.O. 1989h. Larval development of abalone. In: *Handbook of Culture of Abalone and Other Marine Gastropods.* (Ed.) K.O. Hahn, pp. 71-99, CRC Press Inc., Boca Raton, Florida.

Hahn, K.O. 1989i. Biotic and abiotic factors affecting the culture of abalone. In: *Handbook of Culture of Abalone and Other Marine Gastropods.* (Ed.) K.O. Hahn, pp. 113-134, CRC Press Inc., Boca Raton, Florida.

Hahn, K.O. 1989j. Artificial induction of spawning and fertilization. In: *Handbook of Culture of Abalone and Other Marine Gastropods.* (Ed.) K.O. Hahn, pp. 53-70, CRC Press Inc., Boca Raton, Florida.

Halver, J.E. 1989. Appendix. In: *Fish Nutrition.* 2nd ed. (Ed.) J.E. Halver, pp. 715-753, Academic Press, San Diego.

Han, S.J., Lee, J.E., Kim, B.G., Kim, F.O. & Yang, K.Y. 1986. On the food values of nine kinds of marine algae on the young abalone *Haliotis discus* Reeve. *Bull. Fish. Res. Dev. Agency, Pusan,* 39: 127-133.

Harada, K. 1986. Feeding attraction activities of nucleic acid-related compounds for abalone, oriental weatherfish and yellowtail. *Bull. Japan. Soc. Sci. Fish.,* **52: 1961-1968.**

Harada, K. 1987. Feeding attraction activities in the combinations of amino acids and other compounds for abalone, oriental weatherfish and yellowtail. *Bull. Japan. Soc. Sci. Fish.,* **53: 1483-1489.**

Harada, K. 1989. Feeding attraction activities of L - dipeptides for abalone, oriental weatherfish and yellowtail. *Bull. Japan. Soc. Sci. Fish.,* **55: 1629-1634.**

Harada, K. 1991. Attraction activities of herbal crude drugs for abalone, oriental weatherfish and yellowtail. *Nippon Suisan Gakkaishi,* **57: 2083-2088.**

Harada, K. & Kawasaki, 0. 1982. The attractive effect of seaweeds based on the behavioral responses of young herbivorous abalone *Haliotis discus. Bull. Japan. Soc. Sci. Fish.,* **48: 617-621.**

Hardy, R.W. 1989. Diet preparation. In: *Fish Nutrition.* **2nd ed. (Ed.) J.E. Halver, pp. 476-548, Academic Press, San Diego.**

Hasegawa, S. & Nordin, J.H. 1969. Enzymes that hydrolyze fungal cell wall polysaccharides. I. Purification and properties of an endo-a-D(1,3)-glucanase from *Trichoderma viride. J. Biol. Chem.,* **244: 5460-5470.**

Hawkins, S.J., McCrohan, C.R. & Taylor, D. 1989. Introduction (to: Gastropod Feeding Biology - an Integrated View). *J. Mollusc. Stud.,* **55: 149.**

Hay, M.E. *&* **Fenical, W. 1988. Marine plant-herbivores interactions, the ecology of chemical defence.** *Ann. Rev. Ecol. Syst.,* **19: 111-145.**

Hayakama, Y., Watanabe, H. & Kittaka, J. 1987. Stochastic simulation of feeding behavior of abalone *Haliotis discus hannai. Nippon Suisan Gakkaishi,* **53: 1745-1753.**

Hayashi, K. & Yamada, M. 1972. Studies on the lipids of shellfish - I. On the visceral lipid composition of abalone, *Haliotis discus hannai* **(Ino).** *Nippon Suisan Gakkaishi,* **38: 255-263.**

Hecht, T. & Britz, P.J. 1990. *Aquaculture in South Africa. History, Status and Prospects.* **Aquaculture Association of South Africa, Pretoria.**

Hecht, T. & Britz, P.J. 1992. The current status, future prospects and environmental implications of mariculture in South Africa. *S. Afr. J. Sci.,* **88: 335-342.**

Heinen, J.M. 1981. Evaluation of some binding agents for crustacean diets. *Prog. Fish-Cult.,* **43: 142-145.**

Henken, A.M., Machiels, M.A.M., Dekker, W. & Hogendoorn, H. 1986. The effect of dietary protein and energy content on growth rate and feed utilization of the African catfish *Clarias gariepinus* (Burchell 1822). *Aquaculture,* 58: 55-74.

Hooker, N. & Morse, D.E. 1985. Abalone: the emerging development of commercial cultivation in the United States. In: *Crustacean and Mollusk Aquaculture in the United States.* (Eds.) J.V. Huner & E. Evan Brown, pp. 365-414. AVI Publishing Company, Inc., Westport, Connecticut.

Howard, B. & Simkiss, K. 1981. Metal binding by *Helix aspersa* blood. *Comp. Biochem. Physiol.,* 70A: 559-561.

Huisman, E.A. 1976. Food conversion efficiencies at maintenance and production levels for carp, *Cyprinus carpio* L., and rainbow trout, *Salmo gairdneri* Richardson. *Aquaculture,* 9: 259-273.

Hung, S.S.O. & Lutes, P.B. 1988. A preliminary study on the non-essentiality of lecithin for hatchery-produced juvenile white sturgeon *(Acipenser transmontanus). Aquaculture,* 68: 352-360.

Hylleberg-Kristensen, J. 1972. Carbohydrates of some marine invertebrates with notes on their food and on the natural occurrence of the carbohydrates studied. *Mar. Biol.,* 14: 130 - 142.

Hyman, H. 1967. *The Invertebrates. Vol. 6: Mollusca I.* McGraw Hill, New York.

Idler, D.R. & Wiseman, **P.** 1972. Molluscan sterols: a review. *J. Fish. Res. Board Can.,* 29: 385-398.

Ikuta, K. 1987a. Concentration thresholds in accumulation of heavy metals by *Haliotis discus* and *Batillus cornutus. Nippon Suisan Gakkaishi,* 53: 1673-1678.

Ikuta, K. 1987b. Localization of heavy metals in the viscera and the muscular tissues of *Haliotis discus* exposed to selected metal concentration gradients. *Nippon Suisan Gakkaishi,* 53: 2269-2274.

Ino, T. 1943. Feeding and growth of Japanese abalone, *Haliotis gigantea discus* Reeve. *Bull. Japan. Soc. Sci. Fish.,* 2: 171-174.

Ino, T. 1952. Biological studies on the propagation of Japanese abalone (genus *Haliotis). Bull. Tokai Reg. Fish. Res. Lab.,* 5: 1-102.

Intengan, C.L., Alejo, L.G., Concepcion, I., Corpus, V.A., Salud, R.D., Del Rosario, **I.,** Gomer, R. & Henson, J. 1956. Composition of Philippine foods. V. *Philipp. J. Sci.,* 85: 203-213.

Jaeckle, W.B. & Manahan, D.T. 1989. Feeding by a 'nonfeeding' larva: uptake of dissolved amino acids from seawater by lecithotrophic larvae of the gastropod *Haliotis rufescens. Mar. Biol.,* 103: 87-94.

Johnson, D.W., Al-Harassy, A. & Al-Harthy, M. 1992. The Sultanate of Oman abalone fishery. In: *Abalone of the World. Biology, Fisheries and Culture.* (Eds.) S.A. Shepherd, M.J. Tegner & S.A. Guzman del Proo, pp. 448-453. Fishing News Books, Cambridge.

Jones, D.A. & Naylor, E. 1970. The swimming rhythm of the sand beach isopod *Eurydice pulchra. J. Exp. Mar. Biol. Ecol.,* 4: 188-199.

Karagoz, H. 1986. Untersuchungen zu Wachstum and Korperzusammensetzung der Regenbogenforelle *(Salmo gairdneri* Rich.) in Netzgehegen im Schwarzwald. *Fischwirt,* 36: 25-26.

Kavaliers, M. 1980. The pineal organ and circadian rhythms of fishes. In: *Environmental Physiology of Fishes.* (Ed.) M.A. Ali, pp. 631-645. Plenum Press, New York.

Kawai, S. & Ikeda, S. 1972. Studies on digestive enzymes of fishes. II. Effect of dietary change on the activities of digestive enzymes in carp intestine. *Bull. Japan. Soc. Sci. Fish.,* 38: 265-270.

Ketola, H.G. 1982. Amino acid nutrition of fishes: requirements and supplementation of diets. *Comp. Biochem. Physiol.,* 73B: 17-24.

Kideys, A.E. & Hartnoll, R.G. 1991. Energetics of mucus production in the common whelk *Buccinum undatum* L. *J. Exp. Mar. Biol. Ecol.,* 150: 91-105.

Kimura, S. & Kubota, M. 1968. Properties of collagen from the abalone. *Bull. Japan. Soc. Sci. Fish.,* 34: 925-929.

Kohn, A.J. 1983. Feeding biology of gastropods. In: *The Mollusca. Vol. 2: Physiology, Part II.* (Eds.) A.S.M. Saleuddin & K.M. Wilbur, pp. 1-63. Academic Press, New York.

Koike, Y., Flassch, P. & Mazurier, J. 1979. Biological and ecological studies on the propagation of the ormer, *Haliotis tuberculata* Linnaeus. II. Influence of food and density on the growth of juveniles. *La Mer,* 17: 43-52.

Konosu, S. & Mori, T. 1959. Amino acid composition of shell fish proteins. *Bull. Japan. Soc. Sci. Fish.,* 25: 153-155.

Lall, S.P. 1989. The minerals. In: *Fish Nutrition.* 2nd ed. (Ed.) J.E. Halver, pp. 219-257, Academic Press, New York.

Lange, R. 1963. The osmotic function of amino acids and taurine in the mussel, *Mytilus edulis. Comp. Biochem, Physiol.,* 10: 173-179.

Lawrence, A.L. & Lawrence, D.C. 1967. Sugar absorption in the intestine of the chiton, *Ctyptochiton stelleri. Comp. Biochem. Physiol.,* 22: 341-358.

Leighton, D.L. 1961. Observations of the effect of diet on shell coloration in the red abalone, *Haliotis rufescens* Swainson. *Veliger,* 4: 29-32.

Leighton, D.L. 1966. Studies of food preference in algivorous invertebrates of Southern California kelp beds. *Pac. Sci.,* 20: 104-113.

Leighton, D.L. 1974. The influence of temperature on larval juvenile growth in three species of South California abalones. *Fish. Bull.,* 72: 1137-1145.

Leighton, D.L. & Boolootian, R.A. 1963. Diet and growth in the black abalone, *Haliotis chracherodii. Ecology,* 44: 227-238.

Leighton, D.L. & Lewis, C.A. 1982. Experimental hybridization in abalones. *Int. J. Invert. Reprod.,* 5: 273-282.

Lewis, C.L., Eickhoff, R., Stringham, C., Milhoan, K. & Tubbs, K. 1992. Abalone egg vitelline layers and hybrid gamete interactions. In: *Abalone of the World. Biology, Fisheries and Culture.* (Eds.) S.A. Shepherd, M.J. Tegner & S.A. Guzman del Pro°, pp. 59-74. Fishing News Books, Cambridge.

Lewis, R.W. 1962. Temperature and pressure effects on the fatty acids of some marine ectotherms. *Comp. Biochem. Physiol.,* 6: 75-89.

Lindberg, D.R. 1992. Evolution, distribution and systematics of Haliotidae. In: *Abalone of the World. Biology, Fisheries and Culture.* (Eds.) S.A. Shepherd, M.J. Tegner & S.A. Guzman del Pro°, pp. 3-18. Fishing News Books, Cambridge.

Livingstone, D.R. & de Zwaan, A. 1983. Carbohydrate metabolism of gastropods. In: *The Mollusca. Vol. 1: Metabolic Biochemistry and Molecular Biomechanics.* (Ed.) P.W. Hochachka, pp. 177-242. Academic Press, New York.

Lovell, R.T. 1984. Effect of size on feeding responses of channel catfish in ponds. *Aquaculture Mag., 11:* 35-36.

Maguire, G.B., Wee, K.L. & Hindrum, S.M. 1993. Digestibility studies - The "ins" and "outs" of abalone guts. *Austasia Aquacult. Mag.,* 7: 42-45.

Manahan, D.T. & Jaeckle, W.B. 1992. Implications of dissolved organic material in seawater for the energetics of abalone larvae *Haliotis rufescens:* a review. In: *Abalone of the World. Biology, Fisheries and Culture.* (Eds.) S.A. Shepherd, M.J. Tegner & S.A. Guzman del Prot), pp. 95-106. Fishing News Books, Cambridge.

Manahan, D.T., Jaeckle, W.B. & Nourizadeh, S.D. 1989. Ontogenic changes in the rates of amino acid transport from seawater by marine invertebrate larvae (Echinodermata, Echiura, Mollusca). *Biol. Bull.,* 176: 161-168.

Mann, R. 1982. Shipworm nutrition: a review of recent work with an unusual bivalve. *J. Shellfish Res.,* 2: 102.

Marks, G.W. 1938. The copper content and copper tolerance of some species of mollusks of the southern California coast. *Biol. Bull.,* 75: 224-237.

Marshall, I., McManus, D.P. & James, B.L. 1974. Glycolysis in the digestive gland of healthy and parasitized *Littorina saxatilis rudis* (Maton) and in the daughter sporocysts of *Microphallus similes* (Jorg) (Digenea: Microphallidae). *Comp. Biochem. Physiol.,* 49B: 291-299.

Martin, M., Stephenson, M.D. & Martin, J.H. 1977. Copper toxicity experiments in relation to abalone deaths observed in a power plant's cooling waters. *Calif. Fish Game,* 63: 95-100.

Mazon-Suastegui, J.M., Bazua-Sigre, L., Lucero-Martinez, G. & Rodriquez-Ramos, R. 1992. Produccian de semilla de abulon en el laboratorio: el metodo de Bahia Tortugas BCS, Mexico (Abalone seed production in the laboratory). In: *Abalone of the World. Biology, Fisheries and Culture.* (Eds.) S.A. Shepherd, M.J. Tegner & S.A. Guzman del Pro°, pp. 561-569. Fishing News Books, Cambridge.

McCormick III, T.B. & Hahn, K.O. 1983. Japanese abalone culture practices and estimated costs of juvenile production in the USA. *J. World Maricult. Soc.,* 14: 149-161.

McLachlan, A. & Lombard, H.W. 1980. Seasonal variations in energy and biochemical components of an edible gastropod, *Turbo sarmaticus* (Turbinidae). *Aquaculture,* 19: 117-125.

McLean, N. 1970. Digestion in *Haliotis rufescens* Swainson (Gastropoda: Prosobranchia). *J. Exp. Zool.,* 173: 303-318.

McMullen, J. & Thompson, T. 1989. Abalone culture in an urban environment. In: *Handbook of Culture of Abalone and Other Marine Gastropods.* (Ed.) K.O. Hahn, pp. 227-238, CRC Press, Boca Raton, Florida.

Meenakshi, V.R. & Scheer, B.T. 1968. Studies on the carbohydrates of the slug *Ariolimax columbianis* with special reference to their distribution in the reproductive system. *Comp. Biochem. Physiol.,* 26: 1091-1097.

Meyers, S.P. & Brand, C.W. 1975. Experimental flake diets for fish and crustacea. *Prog. Fish-Cult.,* 37: 67-72.

Meyers, S.P., Butler, D.P. & Hastings, W.H. 1972. Alginates as binders for crustacean rations. *Prog. Fish-Cult.,* 34: 9-12.

Migita, M., Matsumoto, J.J. & Aoe, N. 1959. A comparative study on the extractability of muscle proteins of some animals. *Bull. Japan. Soc. Sci. Fisk,* 24: 751-759

Montgomery, W.L. & Gerking, S.D. 1980. Marine macroalgae as foods for fishes: an evaluation of potential food quality. *Env. Biol. Fish.,* 5: 143-153.

Morrison, J. & Whittington, J. 1991. Development of an artificial food for abalone. *Austasia Aquacult. Mag.,* 5: 53-54.

Morse, A., Froyd, C.A. & Morse, D.E. 1984. Molecules from cyanobacteria and red algae that induce larval settlement and metamorphosis in the mollusc *Haliotis rufescens. Mar. Biol.,* 81: 293-298.

Morse, D.E. 1984. Biochemical and genetic engineering for improved production of abalones and other valuable molluscs. *Aquaculture,* 39: 263-282.

Morse, D.E. 1986. Biotechnology in marine aquaculture. *Aquacult. Eng.,* 5: 347-355.

Morse, D.E., Hooker, N., Duncan, H. & Jensen, L. 1979. y - aminobutyric acid, a neurotransmitter, induces planktonic abalone larvae to settle and begin metamorphosis. *Science,* 204: 407-410.

Morton, J.E. 1953. The functions of the gastropod stomach. *Proc. Linnean Soc. London,* 164: 240-246.

Mozqueira, A. 1992. Abalone growth rates on an artificial diet. *Austasia Aquacult. Mag.,* 6: 50-51.

Muller, S. 1984. *Studies on the Ecology, Feeding, Growth and Reproduction of* Haliotis spadicea *Donovan, 1808.* MSc thesis, University of Port Elizabeth.

Muller, S. 1986. Taxonomy of the genus *Haliotis* in South Africa. *Trans. Roy. Soc. S. Afr.,* 46: 69-77.

Mailer, K. 1978a. Locomotor activity of fish and environmental oscillations. In: *Rhythmic Activity of Fishes.* (Ed.) J.E. Thorpe, pp. 1-19. Academic Press, London.

Minter, **K.** 1978b. The flexibility of the circadian system of fish at different latitudes. In: *Rhythmic Activity of Fishes.* (Ed.) .J.E. Thorpe, pp. 91-104. Academic Press, London.

Nakada, H.I. & Sweeney, P.C. 1967. Alginic acid degradation by eliminases from abalone hepatopancreas. *J. Biol. Chem.,* 242: 845-852.

Nakagawa, T. & Nagayama, F. 1988. Distribution of glycosidase activities in marine invertebrates. *J. Tokyo Univ. Fish.,* 75: 239-245.

Naylor, E. 1988. Clock-contmlled behaviour in intertidal animals. In: *Proceedings of a NATO Advanced Research Workshop on Behavioural Adaptations to Intertidal Life, held May 19-24, 1987, in Castiglioncello, Italy.* (Eds.) G. Chelazzi & M. Vannini, pp. 1-14. Plenum Press, New York.

Newman, G.G. 1965. Abalone research in South Africa. *S. Afr. Shipp. News Fishg. Ind. Rev.,* 20: 93-101.

Newman, G.G. 1966. Movement of the South African abalone *Haliotis midae. Invest!. Rep. Div. Sea. Fish. S. Afr.,* 56: 1-19.

Newman, G.G. 1967a. Reproduction of the South African abalone *Haliotis midae. Invest!. Rep. Div. Sea. Fish. S. Afr.,* 64: 1-24.

Newman, G.G. 1967b. Abalone research in South Africa. *Fish. Bull. S. Afr.,* 4: 28-34.

Newman, G.G. 1968. Gmei van die Suid-Afrikaanse perlemoen *Haliotis midae. Invest!. Rep. Div. Sea. Fish. S. Afr.,* 67: 1-24.

Newman, G.G. 1969. Verspreiding van die perlemoen *Haliotis midae* en die uitwerking van temperatuur op produktiwiteit. *Investl. Rep. Div. Sea. Fish. S. Afr.,* 74: 1-7.

Nie, Z.Q. 1992. A review of abalone culture in China. In: *Abalone of the World. Biology, Fisheries and Culture.* (Eds.) S.A. Shepherd, M.J. Tegner & S.A. Guzman del Pr6o, pp. 592-602. Fishing News Books, Cambridge.

Nie, Z.Q., Wang, P., Wang, Z. & Yan, J. 1986. Experiments on preparing of formulated feed and feeding efficiency of young abalone, *Haliotis discus hannai* 'no. *Mar. Fish. Res.,* 7: 53-64.

Norman-Boudreau, K. 1988. Abalone nutrition and the potential role of purified diets. *J. Shellfish Res.,* 7: 564.

Norman-Boudreau, K. 1989a. A new tool for nutritional studies of young abalone. Abstract of talk delivered at the First International Symposium on Abalone Biology, Fisheries, and Culture, La Paz, Mexico, 21-25 November 1989, p_ 40.

Norman-Boudreau, K. 1989b. Concerns and considerations in formulating diets for abalone. Abstract of talk delivered at the First International Symposium on Abalone Biology, Fisheries, and Culture, La Paz, Mexico, 21-25 November 1989, p. 41.

Norman-Boudreau, K., Burns, D., Cooke, C.A. & Austin, A. 1986. A simple technique for detection of feeding in newly metamorphosed abalone. *Aqu.aculture,* 51: 313-317.

Oba, T., Sato, H. & Tanaka, T. 1968. Studies on the propagation of an abalone, *Haliotis diversicolor supertexta.* III. On the size of the one-year-old specimen. *Bull. Japan. Soc. Sci. Fish.,* 34: 457-46L

Ogino, C. & Kato, N. 1964. Studies on the nutrition of abalone - II. Protein requirements for growth of abalone, *Haliotis discus. Bull. Japan. Soc. Sci. Fish.,* 30: 523-526.

Ogino, C. & Ohta, E. 1963. Studies on the nutrition of abalone - I. Feeding trials of abalone, *Haliotis discus* Reeve, with artificial diets. *Bull. Japan. Soc. Sci. Fish.,* 29: 691-694.

011ey, J. & Thrower, S.J. 1977. Abalone - an esoteric food. In: *Advances in Food Research.* Vol. 23. (Eds.) C.O. Chichester, I.M. Mark & G.F. Stewart, pp. 144-186. Academic Press, New York.

Olsen, D.A. 1968. Banding patterns in *Haliotis* - IL Some behavioral considerations and the effect of diet on shell coloration for *Haliotis rufescens, Haliotis corrugata, Haliotis sorenseni,* and *Haliotis assimilis. Veliger, it:* 135-139.

Orive, E., Berjon, A. & Otero, M.P.F. 1980. Metabolism of nutrients during intestinal absorption in *Helix pomatia* and *Anion empiricorum. Comp. Biochem. Physiol.,* 66B: 155-158.

Orzel, R. 1967. Aryl sulfatase and 13-glucuronidase activity in various species of tropical mollusc. *Can. J. Zool.,* 45: 134-135.

Oshima, K. 1931. Enzymes in the intestine of *Haliotis giganteus* Gm. *J. Agriculr. Chem. Soc. Japan,* 7: 332-339.

Owen, G. 1966a. Feeding. In: *Physiology of Mollusca. Vol. 2.* (Eds.) K. Wilbur & C.M. Yonge, pp. 1-52. Academic Press, New York.

Owen, G. 1966b. Digestion. In: *Physiology of Mollusca. Vol.* 2. (Eds.) K. Wilbur & C.M. Yonge, pp. 53 - 96. Academic Press, New York.

Paradis, M. & Ackman, R.G. 1975. Occurrence and chemical structure of nonmethylene interrupted dienoic fatty acids in American oyster *Crassostrea virginica. Lipids,* 10: 12-16.

Parker, D.O., Haaker, P.L. & Togstad, H.A. 1992. Case histories for three species of California abalone, *Haliotis corrugara, H. fulgens* and *H. cracherodii.* In: *Abalone of the World. Biology, Fisheries and Culture.* (Eds.) S.A. Shepherd, M.J. Tegner & S.A. Guzman del Proo, pp. 384-394. Fishing News Books, Cambridge.

Parsons, T.R., Stephens, K. & Strickland, J.D.H. 1961. On the chemical composition of eleven species of marine phytoplankters. *J. Fish. Res. Board Can.,* 18: 1001-1016.

Paul, A.J., Paul, J.M., Hood, D.W. & Neve, R.A. 1977. Observations on food preferences, daily ration requirements and growth of *Haliotis kamtschatkana* Jonas in captivity. *Veliger,* 19: 303-309.

Peck, L.S. 1989. Feeding, growth and temperature in the ormer, *Haliotis tuberculata* (L.) *Prog. Underwater Sci.,* 14: 95-107.

Peck, LS., Culley, M.B. & Helm, M.M. 1987. A laboratory energy budget for the ormer *Haliotis tuberculata* L. *J. Exp. Mar. Biol. Ecol.,* 106: 103-123.

Piavaux, A. 1977. Distribution and localization of the digestive laminarinases in animals. *Biochem. Syst. Ecol.,* 5: 231-239.

Plummer, D.T. 1978. *An Introduction to Practical Biochemistry.* 2nd ed. McGraw - Hill Book Company, London.

Poggenpoel, C.A. & Robertshaw, P.T. 1981. The excavation of Smitswinkelbaai Cave, Cape Peninsula. *S. Afr. Archaeol. Bull.,* 36: 29-35.

Poore, G.C.B. 1972a. Ecology of New Zealand abalones, *Haliotis* species (Mollusca: Gastropoda) 1. Feeding. *N. Z. J. Mar. Freshw. Res.,* 6: 11-22.

Poore, G.C.B. 1972b. Ecology of New Zealand abalones, *Haliotis* species (Mollusca: Gastropoda) 3. Growth. *N. Z. J. Mar. Freshw. Res.,* 6: 534-559,

Prim, P. & Lawrence, J.M. 1975. Utilization of marine plants and their constituents by bacteria isolated from the gut of echinoids (Echinodermata). *Mar. Biol.,* 33: 167-173.

Prince, J.D., Sellers, T.L., Ford, W.B. & Talbot, S.R. 1987. Experimental evidence for limited dispersal of haliotid larvae (genus *Haliotis;* Mollusca: Gastropoda). *J. Exp. Mar. Biol. Ecol.,* 106: 243-263.

Prince, ID. & Shepherd, S.A. 1992. Australian abalone fisheries and their management. In: *Abalone of the World. Biology, Fisheries and Culture.* (Eds.) S.A. Shepherd, M.J. Tegner & S.A. Guzman del Pro°, pp. 407-426. Fishing News Books, Cambridge,

Purchon, R.D. 1977. *The Biology of the Mollusca.* e^d ed. Pergamon Press, Oxford.

Pyeun, J.H., Hashimoto, K. & Matsuura, F. 1973. Isolation and characterisation of abalone paramyosin. *Bull. Japan. Soc. Sci. Fish.,* 39: 395-402.

Reid, D.G. & Naylor, E. 1985. Free-running, endogenous semilunar rhythmicity in a marine isopod crustacean. *J. Mar. Biol. Ass. U.K.,* 56: 85-91.

Reid, D.G. & Naylor, E. 1986. An entrainment model for semilunar rhythmic swimming behaviour in the marine isopod *Eurydice pulchra* (Leach). *J. Exp. Mar. Biol. Ecol.,* 100: 25-35.

Robertshaw, P.T. 1977. Excavations at Paternoster, south western Cape. *S. Afr. Archaeol. Bull.,* 32: 63-73.

Robinson, E.H., Wilson, R.P. & Poe, W.E. 1980. Total aromatic amino acid requirement, phenylalanine requirement and tyrosine replacement value for fingerling channel catfish. *J. Num,* 110: 1805-1812.

Ruddell, C.L. & Rains, D.W. 1975. The relationship between zinc, copper and the basophils of two crassostreid oysters *Crassostrea gigas* and *Crassostrea virginica. Comp. Biochem. Physiol.,* 51A: 585-591.

Rumsey, G.L. & Ketola, H.G. 1975. Amino acid supplementation of casein diets of Atlantic salmon *(Salmo salar)* fry and of soybean meal for rainbow trout *(Salmo gairdnerii)* fingerlings. *J. Fish. Res. Board Can.,* 32: 422-426.

Ryther, J.H. & Goldman, J.C. 1975. Microbes as food in mariculture. *Annu. Rev. Microbiol.,* 29: 429-441.

Sagara, J. & Sakai, K. 1974. Feeding experiment of juvenile abalones with four artificial diets. *Bull. Tokai Reg. Fish. Res. Lab., 77:* 1-7.

Sainsbury, K.J. 1982. Population dynamics and fishery management of the paua, *Haliotis iris.* I. Population structure, growth, reproduction, and mortality. *N. Z. J. Mar. Freshw. Res.,* 16: 147-161.

Saito, K. 1981. The appearance and growth of 0-year-old Ezo abalone. *Bull. Japan. Soc. Sci. Fish.,* 47: 1393-1400.

Saito, K. 1984. Ocean ranching of abalones and scallops in northern Japan. *Aquaculture,* 39: 361-373.

Sakai, S. 1962a. Ecological studies on the abalone, *Haliotis discus hannai* In° - I. Experimental studies on the food habit. *Bull. Japan. Soc. Sci. Fish.,* 28: 766-779.

Sakai, S. 1962b. Ecological studies on the abalone, *Haliotis discus hannai* 'no - IV. Studies on the growth. *Bull. Japan. Soc. Sci. Fish.,* 28: 899-904.

Sakata, K. 1989. Feeding attractants and stimulants for marine gastropods. In: *Bioorganic Marine Chemistry. Vol.* 3. (Ed.) P.J. Scheuer, pp. 115-129. Springer Verlag, Berlin.

Sakata, K. & Ina, K. 1992. Algal feeding stimulants for abalone. In: *Abalone of the World. Biology, Fisheries and Culture.* (Eds.) S.A. Shepherd, M.J. Tegner & S.A. Guzman del Prot), pp. 182-192. Fishing News Books, Cambridge.

Sakata, K., Sakura, T. & Ina, K. 1988. Algal phagostimulants for marine herbivorous gastropods. *J. Chem. Ecol.,* 14: 1405-1416.

Salas Garza, A.E. & Searcy Bernal, R. 1992. Desarollo y estado actual del cultivo de abulon en Mdxico (Development and present status of abalone culture in Mexico). In: *Abalone of the World. Biology, Fisheries and Culture.* **(Eds.) S.A. Shepherd, M.J. Tegner & S.A. Guzmim del Pn5o, pp. 538-546. Fishing News Books, Cambridge.**

Salvini-Plawen, L.v. 1988. The structure and function of molluscan digestive systems. In: *The Mollusca. Vol. 2: Form and Function.* **(Eds.) E.R. Trueman & M.R. Clarke, pp. 301-380. Academic Press, San Diego.**

Schiel, D.R. 1992. The paua (abalone) fishery of New Zealand. In: *Abalone of the World. Biology, Fisheries and Culture.* **(Eds.) S.A. Shepherd, M.J. Tegner & S.A. Guzman del Pro°, pp. 427-437. Fishing News Books, Cambridge.**

Schwassmann, H.O. 1971. Biological rhythms. In: *Fish Physiology. Vol. VI: Environmental Relations and Behaviour.* **(Eds.) W.S. Hoar & DJ. Randall, pp. 371-428. Academic Press, New York.**

Schwassmann, H.O. 1980. Biological rhythms: their adaptive significance. In: *Environmental Physiology of Fishes.* **(Ed.) M. Ali, pp. 613-630. Plenum Press, New York.**

Seki, T. & Kan-no, H. 1981. Induced settlement of the Japanese abalone, *Haliotis discus hannai,* **veliger by the mucus of the juvenile and adult abalones.** *Bull. Tohoku Reg. Fish. Res. Lab.,* **43: 29-36.**

Shepherd, S.A. 1973. Studies on southern Australian abalone (Genus *Haliotis). 1.* **Ecology of five sympatric species.** *Aust. J. Mar. Freshwater Res.,* **24: 217-257.**

Shepherd, S.A. 1976. Breeding, larval development and culture of abalone. *Aust. Fish.,* **35: 1-4.**

Shepherd, S.A. & Hearn, W.S. 1983. Studies on Southern Australian abalone (Genus *Haliotis).* **IV. Growth of** *H. laevigata* **and** *H. ruber. Aust. J. Mar. Freshwater Res.,* **34: 461-475.**

Shepherd, S.A. & Steinberg, P.D. 1992. Food preferences of three Australian abalone species with a review of the algal food of abalone. In: *Abalone of the World. Biology, Fisheries and Culture.* **(Eds.) S.A. Shepherd, M.J. Tegner & S.A. Guzman del Prop, pp. 169-181. Fishing News Books, Cambridge.**

Shepherd, S.A. & Turner, J.A. 1985. Studies on Southern Australian abalone (genus *Haliotis).* **VI. Habitat preference, abundance and predators of juveniles.** *J. Exp. Mar. Biol. Ecol.,* **93: 285-298.**

Shimma, Y. & Taguchi, H. 1964. A comparative study on fatty acid composition of shellfish. *Nippon Suisan Gakkaishi,* **30: 153-160.**

Sidwell, VD., Foncannon, P.R., Moore, N.S. & Bonnet, J.C. 1974. Composition of the edible portion of raw (fresh or frozen) crustaceans, finfish, and mollusks. I. Protein, fat, moisture, ash, carbohydrate, energy value, and cholesterol. *Mar. Fish. Rev.*, 36: 21-35.

Simkiss, K. & Mason, A.Z. 1983. Metal ions: metabolic and toxic effects. In: *The Mohasco. Vol. 2: Environmental Biochemistry and Physiology.* (Ed.) P.W. Hochachka, pp. 102-164. Academic Press, New York.

Simon, G. & Rouser, G. 1969. Species variations in phospholipid class distribution of organs. H. Heart and skeletal muscle. *Lipids,* 4: 607-614.

Singhagraiwan, T. & Doi, M. 1993. *Seed Production and Culture of a Tropical Abalone,* Haliotis asinina *Linne.* EMDEC, Department of Fisheries, Ministry of Agriculture and Cooperatives, Thailand.

Sirenko, B.I. & Kas'yanov, V.C. 1976. An abalone from Moneron Island (Sea of Japan). *Soviet J. Mar. Biol.,* 2: 358-363.

Skeef, N.S. 1988. *Zinc Inhibition of Cell Division: its Tolerance to Cancer Cells and Possible Mechanisms of Action.* PhD thesis, Rhodes University.

Smith, A.B. 1981. An archaeological investigation of Halocene deposits at Rooi Els Cave, south western Cape. *S. Afr. Archaeol. Bull.,* 36: 75-83.

Somero, G.N. & Bowlus, R.D. 1983. Osmolytes and metabolic end products of molluscs: the design of compatible solute systems. In: *The Mollusca. Vol. 2: Environmental Biochemistry and Physiology.* (Ed.) P.W. Hochachka, pp. 77-100. Academic Press, New York.

Spaulding, D.C. & Morse, D.E. 1991. Purification and characterization of sulfatases from *Haliotis rufescens:* evidence for changes in synthesis and heterogeneity during development. *J. Comp. Physiol. B,* 161: 498-515.

Stauber, L.A. 1950. The fate of india ink injected intracardially into the oyster *Ostrea virginica* (Gmelin). *Biol. Bull.,* 98: 227-241.

Steffens, W. 1989. *Principles of Fish Nutrition.* Halsted Press, New York.

Stein, J.L. 1984. Subtidal gastropods consume sulfur-oxidizing bacteria: evidence from coastal hydrothermal vents. *Science,* 223: 696-698.

Steinberg, P.D. 1984. Algal chemical defense against herbivores: allocation of phenolic compounds in the kelp *Alaria marginata. Science,* 223: 405-407.

Steinberg, PD. 1988. The effects of quantitative and qualitative variation in phenolic compounds on feeding in three species of marine invertebrate herbivores. *J. Exp. Mar. Biol. Ecol.,* 120: 221-237.

Stephenson, T.A. 1924. Notes on *Haliotis tuberculata 1. J. Mar. Biol. Ass. U.K.,* 13: 480-495.

Stewart, M.G. 1978. The uptake and utilization of dissolved amino acids by the bivalve *Mya arenaris. Physiol. Beh. Mar. Org., Proc. Eur. Symp. Mar. Biol., 12th, Stirling, Scot. 1977,* pp. 165-176.

Stewart, M.G. & Bamford, D.R. 1975. Kinetics of alanine uptake by the gills of the left shelled *clam Mya arenaria. Comp. Biochem, Physiol.,* 52A: 67-74.

Stewart, M.G. & Bamford, D.R. 1976. The effects of environmental factors on the absorption of amino acids by isolated gill tissue of the bivalve *Mya arenaria* (L.). *J. Exp. Mar. Biol. Ecol.,* 24: 205-212.

Stewart, J. & Schulz-Baldes, M. 1976. Long-term lead accumulation in abalone *(Haliotis spp.)* fed on lead treated brown algae *(Egregia laevigata). Mar. Biol.,* 36: 19-24.

Stoll, K.E. & Duncan, J.R. 1992. The effect of ascorbic acid on arachidonic acid and prostaglandin E_2 metabolism in B16 murine melanoma cells. *Prostagl. Leulcotr. Ess. Fatty Acids,* 47: 307-314.

Storebakken, T. & Austreng, E. 1987. Binders in fish feeds. II. Effect of different alginates on the digestibility of macronutrients in rainbow trout. *Aquaculture,* 60: 121-131.

Stryer, L. 1988. *Biochemistry.* 3rd ed. W.H. Freeman & Company, New York.

Suyama, M. & Sekine, Y. 1965. Studies on the amino acid composition of shell-fish proteins. *Bull. Japan. Soc. Sci. Fish.,* 31: 634-637.

Sweijd, N.A. 1990. *The Digestive Mechanisms of an Intertidal Grazer, the Sea Urchin* Parechinus angulosus *(Leske).* MSc thesis, Rhodes University.

Tacon, A.G.J. & Cowey, C.B. 1985. Protein and amino acid requirements. In: *Fish Energetics. New Perspectives.* (Eds.) P. Tytler & P. Calow, pp. 155-183. The Johns Hopkins University Press, Baltimore, Maryland.

Takeuchi, T., Satoh, S. & Watanabe, T. 1983. Requirement of *Tilapia nilotica* for essential fatty acids. *Bull. Japan. Soc. Sci. Fish.,* 49: 1127-1134.

Tanaka, K., Nakano, T., Naguchi, S. & Pigman, W. 1968. Purification of a-L-fucosidase of abalone livers. *Arch. Biochem. Biophys.,* 126: 624-633.

Tanaka, K. & Sarai, S. 1970. Hydrolysis of fucoidan by abalone liver a-L-fucosidase. *F.E.B.S. Lett.,* 9: 45-48.

Tarr, R.J.Q. 1989. Abalone. In: *Oceans of Life off Southern Africa.* (Eds.) A.I.I. Payne & R.J.M. Crawford, pp. 62-69. Vlaeberg, Cape Town.

Tarr, R.J.Q. 1992. The abalone fishery of South Africa. In: *Abalone of the World. Biology, Fisheries anti Culture.* (Eds.) S.A. Shepherd, M.J. Tegner & S.A. Guzman del PrOo, pp. 438-447. Fishing News Books, Cambridge.

Tegner, M.J. 1989. The California abalone fishery: production, ecological interactions, and prospects for the future. In: *Marine Invertebrate Fisheries. Their Assessment and Management,* (Ed.) J.F. Caddy, pp. 401-420. John Wiley & Sons, New York.

Tegner, M.J. & Butler, R.A. 1985. The survival and mortality of seeded and native red abalones, *Haliotis rufescens,* on the Palos Verdes Peninsula. *Calif. Fish Game,* 73: 150-163.

Tegner, M.J. & Butler, R.A. 1989. Abalone seeding. In: *Handbook of Culture of Abalone and Other Marine Gastropods.* (Ed.) K.O. Hahn, pp. 157-182, CRC Press, Boca Raton, Florida.

Tegner, M.J., Demartini, J.D. & Karpov, K.A. 1992. The California red abalone fishery: a case study in complexity. In: *Abalone of the World. Biology, Fisheries and Culture.* (Eds.) S.A. Shepherd, M.J. Tegner & S.A. Guzman del Prcio, pp. 370-383. Fishing News Books, Cambridge.

Teshima, S.I. & Kanazawa, A. 1974. Biosynthesis of sterols in abalone *Haliotis gurneri,* and mussel *Mytilus edulis. Comp. Biochem. Physiol.,* 47B: 555-561.

Thanassi, N.M. & Nakada, H.I. 1967. Enzymic degradation of fucoidan by enzymes from the hepatopancreas of abalone, *Haliotus* species. *Archs. Biochem. Biophys.,* 118: 172-177.

Tietz, N.W. & Fiereck, E.A. 1966. A specific method for serum lipase determination. *Clin. Chico. Acta,* 13: 352-358.

Tomita, K. & Tazawa, N. 1971. On the stomach contents of young abalone, *Haliotis discus hannai* Ino, in Rebun Island, Hokkaido. *Sci. Rep. Hokkaido Fish. Exp. Stn.,* 13: 31-38.

Tong, L.J. & Moss, G.A. 1992. The New Zealand culture system for abalone. In: *Abalone of the World. Biology, Fisheries and Culture.* (eds.) S.A. Shepherd, M.J. Tegner & S.A. Guzman del Pro°, pp. 583-591. Fishing News Books, Cambridge.

Tong, L.J., Moss, G.A. & Illingworth, J. 1987. Enhancement of a natural population of abalone, *Haliotis iris,* using cultured larvae. *Aquaculture,* 62: 67-72.

Tripp, M.R. 1960. Mechanism of removal of injected micro-organisms from the American oyster *Crassostrea virginica* (Gmelin). *Biol. Bull.,* 119: 273-282.

Tutschulte, T.C. & Connel, J.H. 1988. Feeding behaviour and algal food of three species of abalones *(Haliotis)* in southern California. *Mar. Ecol. Prog. Ser.,* 49: 57-64.

Ueda, S. & Okada, Y. 1939. Studies on the food of useful Gastropoda in Japan. 1. Two species of Japanese abalone, *Haliotis gigantea* Gmelin and *H. kamtschatkana* Jonas. *Bull. Japan. Soc. Sci. Fish.,* 8: 51-57.

Uki, N. 1981. Feeding behaviour of experimental populations of the abalone, *Haliotis discus hannai. Bull. Tohoku Reg. Fish. Res. Lab.,* 43: 53-58.

Uki, N., Kemuyama, A. & Watanabe, T. 1985a. Development of semipurified test diets for abalone. *Bull. Japan. Soc. Sci. Fish.*. 51: 1825-1833.

Uki, N., Kemuyama, A. & Watanabe, T. 1985b. Nutritional evaluation of several protein sources in diets for abalone *Haliotis discus hannai. Bull. Japan. Soc. Sci. Fish., 51:* 1835-1839.

Uki, N., Kemuyama, A. & Watanabe, T. 1986b. Optimum protein level in diets for abalone. *Bull. Japan. Soc. Sci. Fish.,* 52: 1005-1012.

Uki, N. & Kikuchi, S. 1979. Food value of six benthic micro-algae on growth of juvenile abalone, *Haliotis discus hannai.* Tohoku Reg. Fish. Res. Lab., 40: 47-52.

Uki, N., Sugiura, M. & Watanabe, T_ 1986a. Dietary value of seaweeds occurring on the Pacific coast of Tohoku for growth of the abalone *Haliotis discus hannai. Bull. Japan. Soc. Sci. Fish., 52: 257-266.*

Uki, N., Sugiura, M. & Watanabe, T. 1986c. Requirement of essential fatty acids in the abalone *Haliotis discus hannai. Bull. Japan. Soc. Sci. Fish., 52: 1013-1023.*

Uki, N. & Watanabe, T. 1986. Effect of heat treatment of dietary protein sources on their protein quality for abalone. *Bull. Japan. Soc. Sci. Fish.,* 52: 1199-1204.

Uki, N. & Watanabe, T. 1992. Review of the nutritional requirements of abalone *(Haliotis* spp.) and development of more efficient artificial diets. In: *Abalone of the World. Biology, Fisheries and Culture.* (Eds.) S.A. Shepherd, M.J. Tegner & S.A. Guzman del Pro°, pp. 504-517. Fishing News Books, Cambridge.

Uys, W. 1989. *Aspects of the Nutritional Physiology and Dietary Requirements of Juvenile and Adult Sharptooth Catfish,* Clarias gariepinus *(Pisces: Clariidae).* PhD thesis, Rhodes University.

Van As, D., Fourie, H.O. & Vleggaar, C.M. 1975. Trace element concentrations in marine organisms from the Cape West coast. *S. Afr. J. Sci.,* 71: 151-154.

Van Noten, F. 1974. Excavations at the Gordons Bay shell midden, south western Cape. *S. Afr. Archaeol. Bull.,* 29: 122-142.

Viana, M.T., Lopez, L.M. & Salas, A. 1993. Diet development for juvenile abalone *Haliotis fulgens.* Evaluation of two artificial diets and macroalgae. *Aquaculture,* 117: 149-156.

Voigt, E.A. 1982. The molluscan fauna. In: *The Middle Stone Age at Klaasies River Mouth in South Africa.* (Eds.) R. Singer & J. Wymer, pp. 155-186. University Press, Chicago.

Voogt, P.A. 1972. Lipids and sterol components and metabolism in Mollusca. In: *Chemical Zoology. Vol.* 7. (Eds.) M. Florkin & B.T. Scheer, pp. 245-300. Academic Press, New York.

Voogt, P.A. 1983. Lipids: their distribution and metabolism. In: *The Mollusca. Vol. 1: Metabolic Biochemistry and Molecular Biomechanics.* (Ed.) P.W. Hochachka, pp. 329-370. Academic Press, New York.

Walker, G. 1972. The digestive system of the slug *Agriolirnax reticulatus* (Muller): Experiments on phagocytosis and nutrient absorption. *Proc. Malacol. Soc. London,* 40: 33-43.

Walsh, J. 1981. Biotechnology boom reaches agriculture. *Science,* 213: 1339-1341.

Walter, HE. 1984. Proteinases: methods with hemoglobin, casein and azocoll as substrates. In: *Methods of Enzymatic Analysis. Vol. V.* (Ed.) H.U. Bergmeyer, pp. 270-277. Verlag Chemie, Weinheim.

Watling, R.J. & Watling, H.R. 1974. *Environmental Studies in Saldanha Bay, and Langebaan Lagoon. I. Trace Metal Concentrations in Selected Molluscs and Algae.* C.S.I.R. Report FIS 70, Pretoria.

Webber, H.H. 1970. Changes in metabolic composition during the reproductive cycle of the abalone *Haliotis cracheroidii* (Gastropods: Prosobranchiata). *Physiol. Zool.,* 43: 213-231.

Wee, K.L. 1992. An overview of fish digestive physiology and the relevance to the formulation of *artificial* feeds. In: *Proc. Aquaculture Nutrition Workshop, Salamander Bay, NSW, 15-17 April, 1991.* (Ed.) G.L. Allan & W. Dail, pp. 17-24, NSW Fisheries, Brackish Water Fish Culture Research Station, Salamander Bay, Australia.

Wee, K.L., Maguire, G.B. & Hindrum, S.M. 1992. Methodology for digestibility studies with abalone. I. Preliminary studies on the feeding and defaecatory behaviour of blacklip abalone, *Haliotis rubra,* fed natural and artificial diets. In: *Proc. Aquaculture Nutrition Workshop, Salamander Bay, NSW, 15-17 April, 1991.* (Ed.) G.L. Allan & W. Dall, pp. 192-196, NSW Fisheries, Brackish Water Fish Culture Research Station, Salamander Bay, Australia.

Welborn, J.R. & Manahan, D.T. 1990. Direct measurement of sugar uptake from seawater into molluscan larvae. *Mar. Ecol. Prog. Ser.,* 65: 233-239.

Wells, F.E. & Kiesing, J.K. 1989. Reproduction and feeding in the abalone *Haliotis roes* Gray. *Aust. J. Mar. Freshwater Res.,* 40: 187-197.

Wilson, R.P. 1989. Amino acids and proteins. In: *Fish Nutrition.* 2nd ed. (Ed.) J.E. Halver, pp. 112-151, Academic Press, San Diego.

Wilson, R.P., Allen, 0.W., Robinson, E.H. & Poe, W.E. 1978. Tryptophan and threonine requirements of fingerling channel catfish. *J. Nutr.,* 108: 1595-1599

Wilson, R.P. & Poe, W.E. 1985. Relationship of whole body and egg essential amino acid patterns to amino acid requirement patterns in channel catfish, *Ictalurus punctatus. Comp. Biochem. Physiol.,* 80B: 385-388.

Wood, A.D. 1993. *Aspects of the Biology and Ecology of the South African Abalone* Haliotis midae *Linnaeus, 1758 (Mollusca: Gastropoda) along the Eastern Cape and Ciskei Coast.* MSc thesis, Rhodes University.

Wurzinger, K.H. & Hartenstein, R. 1974. Phylogeny and correlations of aldehyde oxidase, xanthine oxidase, xanthine dehydrogenase and peroxidase in animal tissues. *Comp. Biochem. Physiol.,* 49B: 171-185.

Yamaguchi, K., Araki, T., Aoki, T., Tseng, C.-H. & Kitamikado, M. 1989. Algal cell wall - degrading enzymes from viscera of marine animals. *Nippon Suisan Gakkaishi, 55:* 105-110.

Yoo, S.K. 1989. Abalone farming in Korea. In: *Handbook of Culture of Abalone and Other Marine Gastropods.* (Ed.) K.O. Hahn, pp. 255-263, CRC Press, Boca Raton, Florida.

Young, M.L. 1975. The transfer of ^{65}Zn and ^{59}Fe along a *Fucus serrates* (L.) *Littorina obtusata* (L.) food chain. *J. Mar. Biol. Assoc. U.K.,* 55: 583-610.

Young, M.L. 1977. The roles of food and direct uptake from water in the accumulation of zinc and iron in the tissues of the dogwhelk *Nucella lapillus* (L.). *J. Exp. Mar. Biol. Ecol.,* 30: 315-325.

Yu, T.C., Sinnhuber, R.O. & Putnum, G.B. 1977. Use of swine fat as an energy source in trout rations. *Prog. Fish-Cult.,* 39: 95-97.

Zammit, V.A., Beis, I. & Newsholme, E.A. 1978. Maximum activities and effects of fructose biphosphate and pyruvate kinase from muscles of vertebrates and invertebrates in relation to the control of glycolysis. *Biochem. J.,* 174: 989-998.

Zammit, V.A. & Newsholme, E.A. 1976. The maximum activities of hexokinase, phosphofructokinase, glycerol phosphate dehydrogenase, lactate dehydrogenase, octopine dehydrogenase, phosphoenol-pyruvate carboxykinase, nucleoside diphosphatekinase, glutamate-oxaloacetate transaminase and arginine kinase in relation to carbohydrate utilization in muscles from invertebrates. *Biochem. J.,* 160: 447-462.

PUBLICATIONS

Parts of the work presented in this thesis have either been published or accepted for publication:

Knauer, J., Brady, D., Duncan, J.R. & Hecht, T. 1994. Amino acid, fatty acid and mineral element profile of juvenile South African abalone, *Haliotis midae*. *Aquacult. Fish. Manage.:* in press.

Knauer, J., Britz, P.J. & Hecht, T. 1993. The effect of seven binding agents on 24-hour water stability of an artificial weaning diet for the South African abalone, *Haliotis midae* (Haliotidae, Gastropoda). *Aquaculture*, 115: 327-334.

Knauer, J., Hecht, T. & Britz, P.J. 1994. A note on the feeding behaviour and growth of juvenile South African abalone, *Haliotis midae*, fed on an artificial weaning diet. *S. Afr. J. Sci.:* in press.

Knauer, J., Hecht, T. & Duncan, J.R. 1994. Proximate composition of the South African abalone, *Haliotis midae* (Haliotidae: Gastropoda). *Aquacult. Fish. Manage.:* in press.