

THE EFFECT OF TEMPERATURE AND PHOTOPERIOD ON THE DIGESTIVE
PHYSIOLOGY OF THE SOUTH AFRICAN ABALONE
Haliotis midae.

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MARK GEOFFREY DIXON
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ABSTRACT

Inadequate information of the nutritive physiology and the dietary requirements of abalone are the principle factors that currently limit the development of a formulated feed for the commercial culture of *Haliotis midae*. The need to develop a method to determine apparent digestibility co-efficient's for abalone in order to facilitate further applied nutritional research was identified. Animals between 50 and 80 mm were collected from natural stocks along the east Cape coast of South Africa at Port Alfred and Great Fish Point, and acclimated to laboratory conditions. Initial trials demonstrated that *H. midae* accepted and preferred a semi-purified diet to the seaweed *Plocamium corallorhiza*, one of the main components of it's natural diet. A technique of determining apparent digestibility co-efficient's (ADC) using the indirect method with chromic oxide as an inert marker was developed. Digestibility trials yielded higher dry matter (DMADC) and crude protein apparent digestibility co-efficient's (CPADC) for the semi-purified diet than for two species of algae, *Gelidium amanzii* and *P. corallorhiza* (83.7% and 95.6%, 70.7% and 80.0%, and 29.9% and 57.3% respectively). The ability of the animals to utilize terrestrial animal and plant ingredients efficiently makes it feasible to use conventional feed ingredients in formulated feeds for *H. midae*. Trials to determine the effect of different temperatures (15°C, 18°C and 22°C) on DMADC and CPADC of the semi-purified diet showed that peak digestibility occurred at 18°C.

There was also a positive relationship between temperature and consumption rate. Although no enzyme studies with *H. midae* have been conducted, the peak ADC's at 18°C is attributed to an increase in enzyme activity at this temperature. Transit time, an inverse function of temperature and consumption, is considered to be responsible for the decrease in the ADC's at 22°C in conjunction with a possible decrease in enzyme activity at this temperature. A photoperiod trial to investigate the effect of darkness on DMADC and CPADC of the semi-purified diet revealed that digestive efficiency decreased with increasing hours of darkness. There was also a positive relationship between duration of darkness and the rate of consumption. The decrease in ADC's is attributed to decreased transit times as the duration of darkness increased. The contribution of this project to the understanding of abalone nutrition, the development of a formulated abalone feed and systems design for abalone farms is discussed.

CHAPTER 1. Introduction

Abalone are large marine gastropod snails, of the genus *Haliotis* which comprises some 100 species world wide (Hahn, 1989). The history of mans relationship with abalone, documented by Cox (1962), began with observations by Aristotle who referred to "the other *Patella major*" under the name "Aporrhias" in the 4th century B.C. These prosobranch gastropods belong to the order Archaeogastropoda and are considered to be one of the most primitive molluscan genera in form and structure (Cox 1960). Fossil forms of abalone are represented in rocks as old as the Oligocene (30 to 50 million years) (Cox, 1960) and appear to have changed little since then. Abalone are highly prized for the delicately flavoured white meat of their large muscular foot (Hooker and Morse, 1985; Chen, 1989), and have been exploited as food by man since prehistoric times. Abalone shells are common in South African and other middle stone age cave deposits dating back about 125 000 years (Olley and Thrower, 1977; Voigt, 1982; Barkai and Griffiths, 1986). The oldest record of a fishery for abalone by sub-tidal diving is from Japan, in which a diver lost his life in an attempt to collect an abalone pearl for an island Emperor in the year 425 A.D. (Cox, 1962).

Since the early 1950's the demand for abalone has stimulated research into their biology in an attempt to develop culture techniques in order to supplement the supply from the globally declining abalone fishery.

Ecological studies have been conducted on a number of commercially important abalone species and the distribution, feeding biology, reproduction seasonality and growth of these species is fairly well documented (Booolootain, Farmanfarmanian and Giese, 1962; Leighton and Booolootain, 1963; Newman, 1968; Poore, 1972a, b, c; Leighton, 1972; Shepherd, 1973; Barkai and Griffiths, 1986; Tegner, Breen and Lennert, 1989).

Abalone are found along the rocky shores of all the major continents and many of the islands of the Pacific, Atlantic and Indian Oceans (Cox, 1960, 1962). The greatest number of species are found in the central and south pacific and regions of the Indian ocean, however the larger, and economically more important, species occur in the temperate waters (Cox, 1962) of California, Japan, South Australia, New Zealand and South Africa. The largest, *H. rufescens*, occurs off the coast of California and reaches a shell diameter of 294 mm (Haaker, Henderson and Parker, 1986).

The principle characteristics of the family Haliotidae are the auriform and approximate circular outline of the shell, it's convex profile (ranging from highly arched to flattened), a row of rounded perforations overlying the respiratory cavity, and the enormous shell aperture (Cox, 1962). The pores also function as excurrent channels for excretion and the release of gametes. As the shell grows larger, new respiratory pores develop along the growing edge of the shell above the head of the animal. The number of pores and the colour, texture, and shape of the shell

are often used to identify different species of abalone (Cox, 1960).

Abalone have a very large foot that fills the ventral side of the shell. The ventral surface of the foot is used for locomotion and adhesion to the substratum by means of suction. The abalone can quickly clamp fast to the substrate by means of powerful muscular contractions if threatened. The small head is attached anteriorly to the foot and has a ventral mouth, two stalked eyes, and two long retractable sensory cephalic tentacles. The edge of the foot is surrounded by the epipodium, a ruffled flap of tactile sensory tissue. The epipodium bears numerous short, tentacles which are used for chemical and tactile sensing of the local environment (Cox, 1962).

All abalone species are herbivorous (Cox, 1962; Hooker and Morse, 1985; Barkai and Griffiths, 1986) and inhabit rocky substrata in sub-tidal waters to depths of up to 10 m (Poore, 1972a; Shepherd, 1973; Tarr, 1989). Both adults and juveniles have a diel activity rhythm being dormant during the day, and feeding at night (Wells and Keesing, 1989; Barkai and Griffiths, 1987). Juvenile abalone seek protection from predators in crevices, under sea urchins and ledges, while larger specimens are found on exposed surfaces (Hooker and Morse, 1985). Larger abalone are more sedentary and feed by raising the anterior portion of the shell and foot to face the current. When an algal frond drifts against the abalone, the foot folds inwards and the abalone clamps down onto the substratum, thereby trapping the frond whereupon feeding

commences (Shepherd, 1973).

Abalone Fisheries.

Worldwide demand for this valuable resource is centred in Japan and other Asian countries, and has risen steadily, driving retail prices in the mid-1980's to US\$44-66/kg (Hooker and Morse, 1985). The product is purchased in fresh, frozen, canned, and dried forms. The muscular foot is sliced and can be eaten raw or cooked (FAO, 1975). The shells of several species have a high value and are used for decorative purposes, jewellery, and in traditional medicines in Asia (Hooker and Morse, 1985).

Approximately 15 abalone species are commercially exploited world wide (Hooker and Morse, 1985). The global fishery for abalone has declined from 20 000 MT in 1975 to 16 000 MT in 1985 (FAO, 1975; FAO, 1985). The principle fishing countries include Australia (47.9%), Japan (28.5), Mexico (7.1%), South Africa (6%) and New Zealand (4.7%); smaller amounts are also harvested in Canada, Korea and the Philippines (the percentages indicate the % of the total world harvest in 1985) (FAO, 1985).

The South African abalone fishery.

There are six species of abalone in Southern Africa (Kilburn and Rippey, 1982), but only one, *Haliotis midae*, is fished commercially (Tarr, 1989; Barkai and Griffiths, 1986, 1987). *H. midae*, locally known as perlemoen, is found on shallow

sublittoral rocky shores between St Helena Bay ($32^{\circ}45'S$, $18^{\circ}5'E$), on the west coast, and southern Transkei ($31^{\circ}58'S$, $29^{\circ}10'E$), on the east coast (Newman, 1966; Day, 1974; Field, Jarman, Dieckman, Griffiths, Velimirov and Zoutendyk, 1977). Perlemoen are most abundant on the S.W. Cape coast between Cape Hangklip ($34^{\circ}23'S$, $18^{\circ}45'E$) and Cape Agulhas ($34^{\circ}50'S$, $20^{\circ}5'E$) (see Figure 1), where they are found in depths of less than 10 m, in kelp (*Ecklonia maxima*) beds (Tarr, 1989).

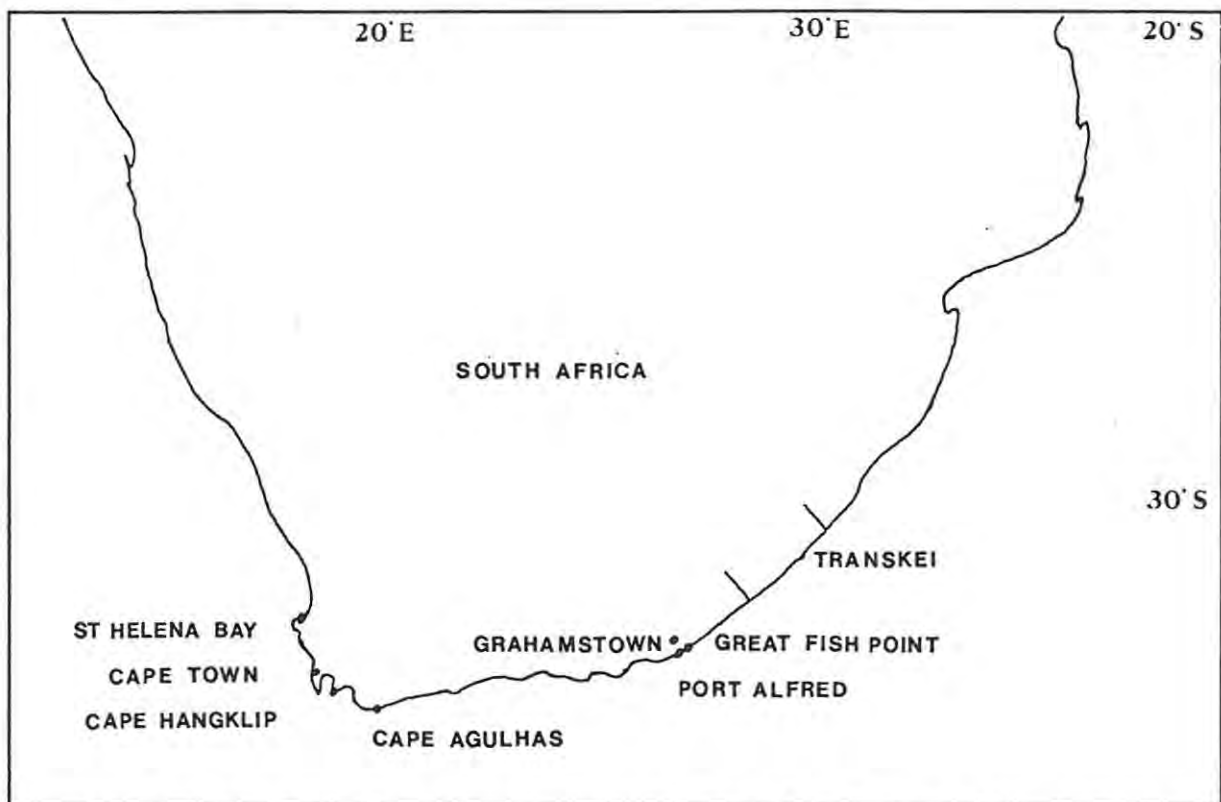


Figure 1. Map showing the range of *H. midae* along the coast of South Africa.

The South African abalone fishing industry began in the early 1950's and the export market grew rapidly, especially to Japan and South East Asia. Generally the South African perlemoen is considered superior to the Australian and other species and demand always exceeds supply (Diemont, Barrie, Stoops, Ramsay and

Goldschmidt, 1986). Harvesting of the South African abalone is concentrated between Cape Hangklip and Cape Agulhas. When the fishery began there was no control and production was indirectly limited only by demand. When production peaked in the mid 1950's it became apparent that control measures were necessary. Size limits were first introduced in 1953 and adjusted to the current limit 11.43 cm shell width in 1954. The same year also saw the introduction of permits to restrict the number of divers. In 1963 the innovation of freezing abalone led to rapid growth of the industry and resulted in further over-exploitation. Production quotas of processed meat were introduced in the same year. The quotas were reduced from 386 MT to 181 MT in 1971 as a result of fluctuating catches. In 1983 a global whole mass receiving quota of 660 tons replaced the old production quota system which had been open to abuse. Over exploitation of natural stocks of abalone has resulted in a substantial decline in production of abalone. Production dropped from 810 MT whole mass in 1976 to 660 MT in 1986 (Diemont et al., 1986) and to 629 tons in 1991 (Tarr, Sea Fisheries Research Institute, pers. comm.).

Declining global yields from abalone fisheries, together with the escalating price of the product, have stimulated interest in abalone aquaculture to enhance depleted natural stocks by reseedling and to provide a direct supply to meet the market demand. Presently, South Africa is initiating abalone aquaculture with *H. midae* by transferring existing technology from other countries and were necessary developing new techniques to suit local conditions.

Abalone aquaculture.

The successful propagation of abalone in Japan facilitated the initiation of abalone aquaculture (Ino, 1952). Since then the technology has been improved and transferred to a number of countries. Consequently, abalone aquaculture has developed rapidly, particularly in Japan, California and Taiwan (Hooker and Morse, 1985; Chen, 1989; Hahn, 1989). For example abalone culture in Taiwan has resulted in an increase in production from 38 MT in 1976 to peak of 500 MT in 1981. This production has rendered catches from natural stocks unimportant (Chen, 1984).

The successful culture of any species relies primarily on the ability to control reproduction. Abalone are dioecious, and the sexes can be determined by forcing the foot and mantle away from the right side of the shell and inspecting the gonad which is closely associated with the digestive gland. In males the colour of the gonad is cream or beige and in females it is dark green (Cox, 1960, 1962; Genade, Hirst and Smit, 1988).

All species have distinct spawning peaks. Depending on the species, abalone spawn either once or twice per year (Poore, 1972b; Newman, 1967; Boolootain *et al.*, 1962). Gonad conditioning may be controlled by temperature and nutrition (Uki and Kikuchi, 1984). Artificial spawning can be induced by ultra violet irradiation of sea water (Uki and Kikuchi, 1984) or treatment with hydrogen peroxide (Morse, Duncan, Hooker and Morse, 1977). Both methods produce a hydroperoxy free radical, or peroxy

diradical which activates the enzymatic reaction of prostaglandin endoperoxide synthesis (Morse *et al.*, 1977; Uki and Kikuchi, 1984). Sperm and eggs are released simultaneously into the water where fertilization takes place. After approximately 24 hours free swimming planktonic trocophore larva hatch from the fertilized eggs. During the first 24 hours after hatching the larva develops into a veliger larva. After development of the veliger stage is complete (4-10 days), the larval abalone settle on a suitable substratum and metamorphose into juvenile abalone (Morse, Hooker, Jensen and Duncan, 1979).

The economic viability of abalone culture depends on the growth rate and feed conversion efficiency of the species (Hahn, 1989). When juvenile abalone settle on a substratum they begin to feed on diatoms. At about six months of age, their diet changes and thus begin to feed on macroalgae. Abalone are generalist herbivores and consume a wide range of algal species, however each species of abalone appears to have a preference for particular algal species (Leighton and Boolootain, 1963; Leighton, 1966; Poore, 1972a; Shepherd, 1973; Barkai and Griffiths, 1986). The preferred species of algae are however, not necessarily the species that produce the best growth (Leighton, 1966). Two species of algae, *E. maxima* along the south coast and *Plocamium corallorhiza* along the east coast, have been identified as the major algal species in the diet of *H. midae* (Barkai and Griffiths, 1986; Tarr, 1989).

To effectively conduct applied nutritional research on abalone,

it is important to understand the movement of ingested material through the alimentary tract. From a detailed description of the structure and function of the alimentary tract of *H. cracherodii* (Campbell, 1965), and a study of digestion in *H. rufescens* (McLean, 1970), it can be assumed that the digestive process is similar in all abalone species. The alimentary tract of abalone is long and complex, which is characteristic of herbivores. The digestive tract is similar to that of other gastropods, consisting of a mouth, oesophagus, crop, stomach, digestive gland, and intestine. The digestive gland, also known as the hepatopancreas (Owen, 1966; McLean, 1970) makes up the bulk of the prominent conical appendage (Hooker and Morse, 1985).

Food is brought into the mouth by the raspings of the long toothed radula (Hooker and Morse, 1985), and passed into the buccal region where it is mixed with liberal quantities of mucus. The ingested food then passes openings of the salivary gland ducts and enters the oesophagus, after which it passes into the thin walled muscular crop, where it is thoroughly mixed with digestive fluids. In *H. rufescens* the crop fluid has strong proteolytic, amylolytic and lipolytic activity (McLean, 1970). Below the crop, separated by fleshy fold, are several large ducts of the digestive gland. Several smaller ducts connect the digestive gland to the stomach, through which small particles of food are directed into the digestive gland by muscular contractions and ciliary current's (McLean, 1970; Hughes, 1986). In the Gastropoda, fine food particles in the digestive gland are phagocytosed and digested intracellularly (Hughes, 1986), however

this process does not appear to be present in *H. rufescens* (McLean, 1970). Tissue samples of the digestive gland showed strong protease and amylase activity with weak lipase activity, but there was no evidence of phagocytosis (McLean, 1970). In *H. rufescens* and other gastropoda, larger and undigested food particles are directed away from the digestive gland openings towards the intestine. Digestive waste products excreted from the digestive gland are transported along the ducts to the stomach, where they are mixed with the rejected particles entering the intestine. At the junction of the stomach and the intestine this rejected material and excreta are bound with mucus and rotated by ciliary action into a faecal rod. The faecal rod is fragmented by muscular contraction as it passes through the intestine to the anus (McLean, 1970; Hughes, 1986).

Abalone have slow growth rates of between 20 and 30 mm per year (Hooker and Morse, 1985; Newman, 1967). *H. midae* has a growth rate of about 27 mm/year in the first two years, and it takes about 14 years to reach the legal size limit of 11.43 cm shell width, and 30 years to reach a maximum size of 200 mm shell width (Newman, 1968). Due to their slow growth rate it is impractical to attempt to culture the large species of abalone to their legal fishery size. Therefore, to reduce production time cultured abalone are harvested as "cocktail" sized abalone when they are between two and a half and five years old and have reached a size of 50 to 80 mm shell length (Hooker and Morse, 1985).

There are two principle options available for abalone culture.

The first, known as ocean ranching, is an extensive form of abalone farming that requires the release of cultured spat into a suitable natural habitat. The abalone are left to grow in the wild till they are large enough to be harvested. Though this form of abalone farming does not have high running costs, it is risky with potentially high losses through predation and poaching (Saito, 1984; Tegner and Butler, 1985; Tong, Moss and Illingworth, 1987). This method is practised in Japan (Hahn, 1989), and has been conducted experimentally in New Zealand and the United States (Tegner and Butler, 1985; Hooker and Morse, 1985; Tong et al., 1987).

The second option involves the intensive captive spawning and rearing of abalone to marketable size. Hatchery reared spat are grown out to between 50 and 80 mm. There are two options for rearing abalone intensively. Juveniles can either be kept in shore based tanks which requires a large investment in land and equipment, with concomitant high operating costs. Alternately, abalone may be reared in containment systems in sheltered waters. In the latter option abalone are placed in cages suspended from buoys, piers, or anchored to the sea bottom. The disadvantages of this system include difficulties of feeding the abalone, continual maintenance, and potential damage from rough seas and stormy weather (Hooker and Morse, 1985).

The first attempts at culturing *H. midae* in South Africa were made in 1981 when Genade et al., (1988) successfully spawned captured specimens to produce spat and juvenile abalone. A more

dedicated research effort on the culture of *H. midae* was initiated in 1988/9. Research into growth, reproduction and the nutritional requirements of *H. midae* is now being undertaken at Rhodes University in Grahamstown, The University of Cape Town, Sea Fisheries Research Institute in Cape Town, and the CSIR in Stellenbosch.

One of the major limiting factors for successful abalone aquaculture is the ability to secure a viable and cost effective source of feed. The choice of available diets include harvested algae, cultured algae and formulated feeds.

Though algal diets are widely used for shore based abalone culture, the recent expansion of the Japanese abalone culture industry is attributed to the extensive use of artificial diets which allows the development of abalone culture sites away from sources of abundant algae (Hahn, 1989). Further advantages of artificial diets are ease of storage, lower costs than algae (frozen or dried) (Hahn, 1989), better growth rates (Uki, Kemuyama and Watanabe, 1985a; Nie, Wang, Wang and Yan, 1986) and the facilitation of the use of automatic feeders (Hahn, 1989). The disadvantages of algal diets include geographical and seasonal variations in nutritional value (Paine and Vadas, 1969; Carefoot, 1980; Uki, Sugiura and Watanabe, 1986b), difficulties of harvesting, and the high transport cost of wet algae from the harvest site to the abalone culture site. Use of an artificial diets provides the abalone culturist with a reliable and convenient food source that has a consistent nutrient quality.

Therefore as abalone culture develops, artificial diets are likely to become the main feed source.

Previous abalone nutritional research has been limited to growth studies to evaluate the potential use of various ingredients in artificial diets (Ogino and Ohta, 1963; Uki *et al.*, 1985a; Uki, Kemuyama and Watanabe, 1985b; Uki and Watanabe, 1986), and the phagostimulatory effects of several agents (Harada, Eguchi and Kurosaki, 1987; Sakata, Itoh and Ina, 1984). Satisfactory growth rates have been achieved with protein levels ranging between 20% and 30% (Ogino and Kato, 1964; Uki, Kemuyama and Watanabe, 1986a), and total lipid levels of about 5%, of which omega 3 lipids make up about 1% (Uki, Sugiura and Watanabe, 1986c). Comparisons of growth rates of abalone fed algal diets and artificial diets show that artificial diets yield better growth rates (Nie *et al.*, 1986).

The economic viability of utilizing harvested algae depends on the location and availability of sufficient quantities of algae (Norman-Boudreau, 1988). In the event that algae is used as the principle diet for cultured *H. midae*, the abundance of the giant kelp, *E. maxima*, compared to other algal species available, make it a logical species to be used. In South Africa, the kelp beds are limited to the south west coast and if used as the principle diet the development of abalone culture along the east Cape coast of South Africa will be inhibited. In addition the successful harvesting of kelp is dependent on sea conditions. The exposed conditions of the South African coast limit the number of days

of possible harvesting. This will obviously present problems with respect to obtaining a regular supply. Furthermore, utilizing algae as the principle source of nutrition is costly and labour intensive when the expenses of harvesting, drying and storage are taken into account (Hahn, 1989).

The production costs of kelp as a diet for *H. midae* are estimated to be more expensive than using a formulated feed. A formulated feed containing fishmeal as the main ingredient has an estimated cost of R2500/ton dry weight, compared to R400/ton for wet kelp. When the respective food conversion ratios (FCR) for the two diets are considered the final production costs of using kelp are more expensive. A fishmeal based dry pellet and wet kelp have FCR's of 1.2:1 and 11:1 respectively (P.J. Britz, Rhodes University, unpublished). Therefore, compared to a fishmeal based diet, the actual amount of kelp needed to produce the same growth is about ten times the amount of the fishmeal based diet. Thus the production costs using kelp will be approximately one and a half times that of using an artificial diet. Furthermore the growth rates of *H. midae* are higher on a fishmeal based diet than a wet kelp diet (P.J. Britz, Rhodes University, unpublished).

Nutrient availability.

Feed costs constitute between 50% and 70% of the operating costs of rearing fish and shellfish from stocking size to market size (Brown, Robinson, Clark and Lawrence, 1989; NRC, 1983). This emphasises the importance of a correct balance of nutrients in

the diet to reduce grow-out time. The objective of artificial diets is to supply the correct nutrient concentration in a readily available form to enable optimum utilization of nutrients for somatic growth (Hastings and Dickie, 1972; Phillips, 1972).

Overall, the success of intensive animal husbandry can be attributed to the use of balanced feeds to enhance growth rates (Flatt, 1988). Although many forms of aquaculture rely on natural food sources, there is an increasing demand for complete aquatic animal feeds particularly for intensive forms of aquaculture (NRC, 1983). In the aquaculture industry, as stocking densities increase the animals become more dependent on supplemental feeds for all their nutrients (Lovell, 1989). In abalone culture, the requirement for maximum yield per unit volume will necessitate concentrated, nutritionally complete and cost effective feeds, which is the case in other established aquaculture practices (Lovell, 1989, Brown *et al.*, 1989). The use of manufactured feeds for cultured fish (Hilton and Slinger, 1981; Lovell, 1989; Uys, 1989) and crustaceans (Bordner, D'Abramo and Conklin, 1983; Brown, Williams, Robinson, Akiyama and Lawrence, 1986) has led to significant increases in production. This is due to the efficient conversion of complete feeds into somatic growth (Hastings and Dickie, 1972).

Feed ingredients must initially be evaluated to assess their nutritional value for each cultured species before the nutritionist is able to formulate a feed and calculate rations (Flatt, 1988). This information combined with the cost of the

ingredients will enable a balanced and economically viable diet to be formulated (Morrison, 1954).

A selection of methods exist to evaluate the nutritive value of ingredients, though not all of them are appropriate for the purposes of diet formulation. Commercial culture of abalone in South Africa will most probably be practised in intensive onshore culture systems. Due to the disadvantages of using harvested algae, cultured *H. midae* will most probably be fed a formulated feed. The present lack of knowledge of the nutritional value of potential ingredients, combined with the lack of knowledge of the nutritional requirements of abalone in general is the major impediment for the development of a commercial diet. The nutritional requirements of an animal can be established by a series of deletion trials using a semi-purified diet formulated with chemically pure ingredients. The potential nutritive value of a feedstuff can be determined chemically, however, the actual availability of nutrients can only be determined after allowances have been made for inevitable losses that occur during digestion, absorption and metabolism (McDonald, Edwards and Greenhalgh, 1984; Crampton and Harris, 1969; Morrison, 1954). A method of evaluating the biological availability of nutrients that is extensively used in livestock and aquaculture nutrition, is the determination of the digestibility of a feedstuff. The digestibility of a diet accounts for the loss of nutrients in the faeces, and can be defined as that proportion of the diet which is not excreted in the faeces and thus assumed to be absorbed by the animal (Morrison, 1954; McDonald et al., 1984).

Aims.

For the development of an economically viable abalone aquaculture industry in South Africa it is important to establish whether *H. midae* accepts, and is able to utilise, formulated diets. Although techniques are available to conduct digestibility trials with livestock and most existing aquaculture species, no standard methods exist for abalone. One of the principle aims of the study was thus to develop a technique for determining digestibility coefficient's for abalone.

Environmental factors, particularly temperature and photoperiod, have been shown to influence the behaviour and metabolism of abalone. Thus, it was considered important to determine the effect of temperature and photoperiod on nutrient utilization by *H. midae*. Temperature is the primary controlling factor of metabolic rate in poikilotherms (Fry, 1971) and by implication is one of the most important environmental factors affecting feed consumption and growth rates of aquaculture species. Photoperiod is a directive factor (Brett, 1979) which influences feeding periodicity and food intake in abalone (Leighton and Boolootian, 1963; Poore, 1972a; Shepherd, 1973; Ebert and Houk, 1984). The effect that temperature and photoperiod might have on growth are therefore vitally important for abalone production under intensive culture conditions. Establishing their possible effects on digestive efficiency will provide valuable information for formulating feeds and designing culture systems for *H. midae*. The objectives of this project were thus to:

- 1) establish whether *H. midae* would accept a semi-purified diet,
- 2) compare apparent digestibility co-efficient's of a semi-purified diet and two species of algae, *Plocamium corallorhiza* and *Gelidium amanzii*,
- 3) determine the effect of temperature on apparent digestibility co-efficient's,
- 4) determine the effect of photoperiod on apparent digestibility co-efficient's.

CHAPTER 2. The role of digestibility studies in applied nutritional research.

This chapter considers the methods available to the applied nutritionist in the development of artificial diets. It also considers in depth the importance of digestibility studies as a major tool in determining the availability of nutrients in feeds.

The present project forms part of a programme to develop a commercial diet for abalone. To formulate a diet, information regarding the nutrient requirements of abalone in conjunction with the nutrients supplied by the constituent ingredients of the diet are required.

To establish the nutritional requirements of abalone and other cultured species, semi-purified diets are used in deletion trials (Conklin, D'Abramo, Bordner and Baum, 1980; Carefoot, 1984). A semi-purified diet is one that contains well defined ingredients and is used principally in nutritional studies to determine nutrient requirements of the species under investigation (Conklin, Devers and Bordner, 1977; Hardy, 1989). Once the nutrient requirements have been established, various ingredients must be evaluated to enable the formulation of a well balanced diet. There are several methods available for evaluating the nutritional value of dietary ingredients. The different methods available can be broadly categorised into two groups, namely biological and chemical methods (Hardy, 1989; McDonald et al., 1984).

Chemical tests are conducted *in vitro*, and are used for quality control and to quantify the nutrient content of an ingredient. The advantage of chemical tests is that no feeding trials need be conducted (Hardy, 1989). However they fail to convey information on the biological value of the ingredient for the animal through digestion (Hastings and Dickie, 1972; McDonald et al., 1984).

An obvious initial analysis of a potential ingredient is to determine which nutrient compounds are present and in what proportion. Proximate analysis is the partitioning of a feed into six categories based on the chemical properties of the compounds (Hardy, 1989; Morrison, 1954; McDonald et al., 1984). It must be noted that proximate analysis is not a nutrient analysis, but a apportionment of nutrients and non-nutrients into similar chemical groups. Ingredient samples are analyzed for moisture, crude protein, fat (ether extract), crude fibre, ash and carbohydrate (nitrogen-free extract). The latter is determined indirectly by subtracting from 100% the total percentages of the other five categories (Hardy, 1989).

For a more detailed analysis of an ingredient, a nutrient analysis can be done to quantify the specific essential nutrients (amino acids, fatty acids, mineral elements and vitamins) present (Hardy, 1989). Nutrient analysis is used to determine whether a particular ingredient or diet contains sufficient essential nutrients to meet the animals nutrient requirements.

Simple chemical tests and nutrient tests are only able to show the quantity of nutrients present in a feed or feed ingredient, and are therefore not a good measure of the quality of the nutrients, or the amounts that are available to the animal.

Various factors during the processing and storage stages can also affect nutrient availability and quality. For example during processing, excessive heat can have either a positive or a negative effect on the protein quality. Lysine in the presence of reducing sugars forms an indigestible complex that is unavailable to the animal. Heat treatment of soya bean products on the other hand destroys trypsin inhibitors which are anti-nutritional factors.

During storage, lipid quality is affected by exposure to moisture and microorganisms, or oxygen, resulting in hydrolytic and oxidative rancidity, lowering the acceptability and quality of the feedstuff. A range of specific quality tests exist to determine the effect of processing and storage on a diet (Hardy, 1989).

Although these quality tests can be used to evaluate ingredients, they do not inform the researcher of the amounts of the nutrients available to the animal. Chemical analysis must therefore be complemented with a knowledge of the biological availability of ingredients to the organism.

The biological availability of feed ingredients and formulated feeds is determined in feeding trials and by recording some aspect of animal growth and/ or diet digestibility. There are three general categories:

- (1) retention studies, in which the deposition of a nutrient in a carcass over a short time is measured,
- (2) performance studies, where growth is used to evaluate and compare feeds,
- (3) loss studies, in which the loss of various nutrients via the faeces, urine and gills of a particular ration are measured.

The data obtained in biological tests are closer to reality than those obtained by chemical evaluation, and can be used to predict the nutritional value of a formulated diet by summing up the nutritional value of the ingredients in the formulated feed (Hardy, 1989). A shortcoming of biological tests is the inability to predict the associative effects of different ingredients on each other (Brown *et al.*, 1989).

To date, most information used in the formulation of feeds for abalone has been gained from biological studies, in particular performance and retention studies (Uki *et al.*, 1985a, 1986a, 1986c; Nie *et al.*, 1986).

A common and efficient method of evaluating diets is to conduct growth trials which evaluate finished diets. Simple growth trials involve feeding the animals and measuring wet body weight or length gain over a specified time interval (Hardy, 1989). Growth parameters that quantify the growth produced per unit mass of food ingested are food conversion ratio and food conversion efficiency. Both are widely used expressions of food utilization, and indicate the quantity of food required to produce a unit mass growth in the experimental animal. Food conversion ratio is the ratio of food consumed per unit of time/ weight gain per unit time. Food conversion efficiency is the reciprocal of the food conversion ratio. Ideally the values of food and growth must be in equivalent terms such as calories, protein or dry weights to provide relevant data. In practice however food weight is often expressed in terms of dry weight and growth in wet body weight (Hastings and Dickie, 1972). In fish, values between 1 and 2 are most common for the feed conversion ratio.

Biological value, protein efficiency ratio and net protein retention are retention studies that are used specifically for protein evaluation. These evaluation methods are useful to compare the protein quality of an ingredient that has been subjected to different processing techniques. They are calculated by measuring the nitrogen retention, or weight gain per unit protein fed, and are good methods to compare quality of different protein sources (McDonald *et al.*, 1984). The usefulness of retention studies in applied nutrition is limited to comparative evaluation of completed diets, and is thus not an important

instrument for diet formulation.

To formulate a successful commercial diet for abalone, ingredients have to be evaluated for nutrient utilization and availability. Loss studies are trials that measure the loss of ingested nutrients via the faeces, urine and gills. Digestibility trials use a combination of chemical analysis and biological feeding studies to quantify the difference of nutrients in the diet and the faeces. The difference of nutrients between the food and faeces is understood to represent the nutrients absorbed by the animal, and is thus considered to be the amount of available nutrients in the diet. Animals are fed on a diet for a time period, during which the faeces are collected. Both the diet and faecal samples are chemically analyzed to determine the levels of the nutrients present. The difference in the amounts of nutrients are then used to calculate the digestibility coefficient's (Maynard and Loosli, 1969; McDonald *et al.*, 1984). Digestibility studies have been successfully applied in aquatic animals such as fish (Spyridakis, Metailler, Gabuadan and Riaza, 1989) and crustaceans (Bordner and Conklin, 1981). It was one of the objectives of this study to develop a method for performing digestibility studies on abalone to facilitate the development of an artificial diet. The values from digestibility trials express the proportion of the total amount of nutrients available to the animal in an ingredient. This information is essential for the least costing of a diet. Digestibility co-efficient's, which are widely used in nutritional studies, are the focus of this project and are reviewed in depth below.

For this project a method of diet evaluation was required that would determine the amount of nutrients that were absorbed by the animal, and that would reflect any change in digestion and absorption induced by temperature or photoperiod. Thus, chemical, performance and retention tests, which do not quantify the nutrients digested and absorbed, were inappropriate for the present study. The method of ingredient evaluation that was most suitable for the project was the determination of digestibility co-efficient's. Any change in digestibility co-efficient's under the different experimental conditions would theoretically have been caused by differential digestion and absorption rates.

Review of apparent digestibility determination of ingredients and diets.

Digestibility is best defined as that proportion of the food that is not excreted in the faeces and is thus assumed to be absorbed by the animal. This makes allowances for the losses occurring during digestion, absorption and metabolism of feed sources (McDonald *et al.*, 1984). An accurate knowledge of the digestibility of commonly used feedstuffs is of prime importance in fish feed formulation (Spyridakis *et al.*, 1989; De la Noue and Choubert, 1986). Digestibility studies are also useful as a measure of the efficiency of utilization of complete diets (Choubert, De la Noue and Luquet, 1979) by informing the nutritionist of the exact amounts of protein, lipids and carbohydrates available in the ingredients and the final diet (Crampton and Harris, 1969). Calculation of digestibility co-

efficient's (DC) make allowances for incomplete digestion, which represents the greatest loss between the quantity of the nutrient present in the diet and the amount finally utilized by the animal (Crampton and Harris, 1969; Jobling, 1983; McDonald et al., 1984). There are two general methods of measuring nutrient digestibility (De la Noue and Choubert, 1986).

The first method is the "direct method" (Post, Shanks and Smith, 1965; Ogino, Kakino and Chen, 1973; McDonald et al., 1984), which requires the quantitative collection of faeces corresponding to a specific meal. The nutrient DC is calculated using the equation:

$$DC = 100 \left\{ 1 - \frac{[(\text{nutrient in faeces} * \text{faeces dry weight})]}{(\text{Nutrient in diet} * \text{food dry weight})} \right\}$$

(De la Noue and Choubert, 1986).

An alternative method, the "indirect method" omits the quantitative collection of faeces by using an inert internal marker such as chromic oxide. The marker is incorporated in the feed at a constant concentration and is analyzed in the faeces. The DC is calculated by comparing the concentrations of the nutrient and the marker in both the feed and in the faeces (Maynard and Loosli, 1969; Choubert et al., 1979; McDonald et al., 1984; Hardy, 1989). The nutrient DC for the indirect method is calculated using the equation:

$$DC = 100 \left\{ 1 - \frac{[(\text{nutrient in faeces} / \text{nutrient in diet})]}{(\%Cr203 \text{ in diet} / \%Cr203 \text{ in faeces})} \right\}$$

(De la Noue and Choubert, 1986).

The most widely used marker in aquaculture nutritional studies is chromic oxide (Jobling, 1983), as it is not digestible or absorbed in the alimentary canal. The quantity recovered in the faeces then serves as a marker of the amount of feed digested (Epko and Bender, 1989). Conover (1966), demonstrated that for biological studies the ash content of natural feeds and the resultant faeces can also be used as a marker for the indirect method. To accurately calculate a DC, and to minimise error when using the indirect method, the marker must be evenly mixed throughout the diet, pass through the digestive tract at the same rate as the nutrients, remain unabsorbed, and be evenly distributed in the faeces (Calow and Fletcher, 1972). The marker should also not influence the rate of ingestion (Leavitt, 1985), as the ingestion rate plays a role in the digestion of nutrients (Condrey, Gosselink and Bennett, 1972).

In a comparison of the direct and indirect method of measuring digestibility using chromic oxide as an inert marker, De la Noue and Choubert (1986) found that higher DC's were obtained using the direct method. This could have been a result of the faecal sample in the indirect method not being representative of the faeces. In addition, the effect of the marker leaching from the faeces into the water would reduce the DC for the indirect method. A third factor to consider is the form of the food. Live food for example, is difficult to mark with an inert marker. It must however be noted that for the direct method to be effective the total amount of faeces must be collected. If the total amount of faeces is not collected, elevated DC values will be obtained

(De La Noue and Choubert, 1986).

The DC of a nutrient is measured by the difference (expressed as a percentage) between the nutrient ingested and the nutrient recovered in the faecal matter (Spyridakis et al., 1989). Since over 30 % of the faecal nitrogen is highly soluble in water (Lied, Julshamn and Braekkan, 1982), it is technically difficult to collect faeces without any leaching of the soluble compounds taking place. Thus sampling methods may affect the results of DC measurements (Spyridakis et al., 1989). Windell, Foltz and Sarokon (1978) and Vens-Cappell (1985), working on trout, showed clearly that the method of faeces collection resulted in a variation of DC. Spyridakis et al. (1989), using sea bass (*Dicentrarchus labrax*), tested six different methods of faeces collection to determine the difference in protein and lipid DC.

The methods included:

1. Dissection
2. Stripping
3. Anal suction
4. Immediate pipetting from the tank
5. Continuous filtration
6. Decantation

For both protein and lipid digestibility, the lowest values were obtained with the stripping and dissection methods. Protein and lipid DC differed according to the method used to collect the faeces. The lowest values obtained for lipids were with the stripping and dissection methods. The samples collected by these

two methods contained gametes, mucus, blood and other endogenous products which would be measured as crude protein in the faeces and lower the DC. Protein DC measurement was more sensitive to the method of collection, particularly stripping and dissection, than lipid DC due to the ability of enterocytes in the lower intestine to ingest intact proteins by endocytosis. DC of nutrients can also be affected by excessive handling during stripping which may induce a sudden defecation and acceleration of the intestinal transit, thereby reducing the contact time between the nutrients and digestive enzymes. Faecal collection by dissection caused variation in DC, depending on which region of the intestine the sample was removed from (Spyridakis *et al.*, 1989). Therefore, the sample obtained by sucking, stripping or dissection may contain exaggerated levels of crude protein and may not be representative of naturally egested faeces.

The problems of intestinal sampling can be overcome by collecting the faeces as soon as they are naturally released. However they must be collected rapidly to prevent an overestimation of DC should the soluble material leach (Spyridakis *et al.*, 1989). Popma (1982) confirmed that there is a negative nutrient leaching from the faeces in the first 30 min after defecation. Removal of faeces from the water within 30 sec using immediate pipetting or continuous filtration will prevent any significant variation in protein DC. In choosing one of the two methods, the researcher must consider the scale of the trial. Pipetting is labour intensive, but is suitable for small scale trials, while continuous filtration involves high costs that are only justified

if extensive work on digestibility is planned (Spyridakis *et al.*, 1989).

In the present study the daily faecal production rendered small samples, and total faecal collection could not be guaranteed. The indirect method of apparent digestibility determination was therefore chosen and faeces were collected within thirty minutes of defecation.

Considering the scale of the operation, and the total faecal material egested by a single animal, the continuous faecal collection method was impractical. Furthermore, the size of animals used in the trials and the anatomy of the abalone made faecal collection by means of dissection, anal suction or stripping impractical. The method of faecal collection used in the trials was an adaption of the pipetting method, whereby the abalone in each tank were transferred to a five litre beaker placed in the experimental tanks at the start of the daily collection period. Water was allowed to flow through the beaker at a rate which supplied the abalone with adequate fresh sea water, without flushing out the faeces.

Despite the importance of determining digestibility coefficient's for use in diet formulation studies there are several limitations which have to be considered (Morrison, 1954; Crampton and Harris, 1969; Maynard and Loosli, 1969; McDonald *et al.*, 1984). The determination of DC relies on the assumption that all faecal matter represents that proportion of the diet that is not

digested and absorbed. However, this is not entirely correct as a portion of the faecal matter is composed of waste metabolites from the body itself in the form of unabsorbed digestive enzymes, epithelial cells abraded from the intestinal wall and mucus secretions, referred to as the metabolic faecal nitrogen (Morrison, 1954; Crampton and Harris, 1969; McDonald *et al.*, 1984). The metabolic secretions result in underestimating the digestibility co-efficient. The co-efficient should thus be referred to as the apparent digestibility co-efficient. This distinguishes it from the true digestibility which is difficult to determine accurately (Maynard and Loosli, 1969; Calow and Fletcher, 1972; McDonald *et al.*, 1984; Leavitt, 1985). However, from a practical point of view determining endogenous losses is not considered to be critical (NRC, 1983).

Digestibility co-efficient's are further affected by the method of ration preparation, the quantity consumed per meal, the proportion of protein and energy in the ration, the quality of the protein source, the physical form of the ration when presented and inherent factors specific to the animals (Morrison, 1954; NRC, 1983; McDonald *et al.*, 1984).

Despite the potential inaccuracies, digestibility trials are extensively used in aquaculture nutritional studies with fish (Austreng, 1978; De la Noue and Choubert, 1985, 1986; Kirchgessner, Kurzinger and Schwarz, 1986; Epko and Bender, 1989; Pongmaneerat and Watanabe, 1991) and crustaceans (Bordner *et al.*, 1983; Brown *et al.*, 1986; Brown *et al.*, 1989; Brown, Tazik, Hooe

and Blythe, 1990). Use of digestibility trials in marine gastropods has been limited to ecological studies on *Aplysia juliana* and *A. dactylomela* (Carefoot, 1970) and *Haliotis midae* (Barkai and Griffiths, 1987). The latter were the first attempts to use the indirect method with ash content as a marker.

In the development of a model of applied nutrition for abalone, digestibility studies using semi-purified diets will be an essential tool in the development of practical diets. They can be conducted to compare nutrient availability of several ingredients and to investigate factors that can affect nutrient utilisation. Digestibility trials are also used to ascertain the nutritive value of various ingredients so that effective substitution of ingredients can be accomplished in the least costing process of diet formulation (NRC, 1983).

CHAPTER 3. Materials and Methods.

The first objective of this study was to determine if *H. midae* would accept a semi-purified diet. If it did accept the semi-purified diet, trials would be conducted to ascertain if *H. midae* preferred it to seaweed. Should *H. midae* prefer the semi-purified diet, it was important to compare the apparent digestibility coefficient's of the semi-purified diet to those attained with two species of seaweed, *Plocamium corallorhiza* and *Gelidium amanzii*. Finally the main objective of the study was to establish the effect of temperature and photoperiod on the apparent digestibility co-efficient's of the semi-purified diet.

Experimental systems.

Abalone were held in a 8000 l recirculating system in Grahamstown, which consisted of a header tank (3000 l), holding tanks (total of 900 l), and four biological filters in series (total of 4100 l). Water was pumped from the biological filters to the header tank, from which it was gravity fed to the holding tanks. Water temperature was controlled by a heating element and a chiller unit in the header tank and was maintained at $18\pm 1^{\circ}\text{C}$. Salinity was maintained at 35 ‰ using deionised water to compensate for evaporative losses. All experiments were performed in glass aquaria (500mmx300mmx315mm) with two compartments, one of which contained a biological filter. Details of the experimental tank design are shown in Figure 2. The experimental tanks were housed in a constant temperature room with a constant

ambient air temperature of $15 \pm 0.5^\circ\text{C}$.

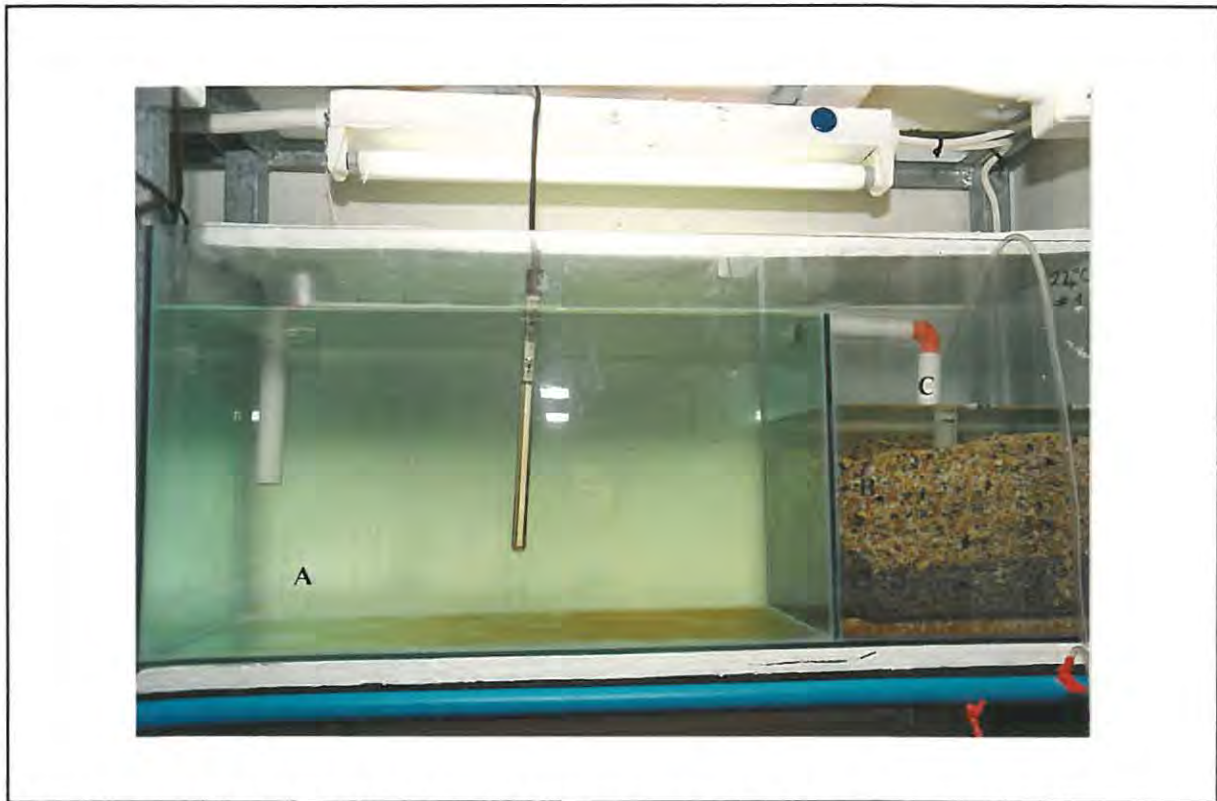


Figure 2. Photograph of experimental aquaria. A = holding area; B = biological filter; C = water uplift pipe.

In the experimental tanks water temperature was controlled with 200 W thermostatically controlled submersible heaters. Lighting was supplied by individual 20 W Lascon Lighting Biolux fluorescent lights, producing 1100 lumin, with a 97 RA index and a Kelvin rating of 6500 K. Light intensity ranged from 0.67 - 0.71 and 0.49 - 0.53 $\text{Qs}^{-1}\text{cm}^{-2}$ at a depth of one and thirty centimetres respectively. The photoperiod for each trial was regulated by an in-line timer switch.

Experimental animals.

Animals between 55 and 85 mm were collected by free diving on

rocky outcrops in water 0.5 to 2 meters deep at Great Fish Point and Port Alfred (33°35'S 26°52'E) on the East Cape coast of South Africa (see Figure 1). The abalone were transported to our marine laboratory in Grahamstown in plastic bins packed with wet seaweed, and held in the recirculating system until required. Prior to placing the animals in the glass tanks their shells were cleaned to remove any attached seaweed and invertebrates. For the digestibility experiments 10 abalone were placed in each of the six glass aquaria and were allowed to acclimatise for a period of one week. At the start of each digestibility trial a period of 16 days was allowed for adaptation to the experimental conditions prior to a faecal collection period of 5 days.

Description of the diets fed to the experimental abalone.

The composition of the semi-purified diet is based on the formulation used by Uki *et al.* (1985b) and is shown in Table I.

Table I Ingredients of the semi-purified diet.

<u>Ingredients</u>	<u>Percent</u>
Calcium caseinate	30%
Dextrin	50%
Dried kelp (Ecklonia maxima)	5%
Fats (1:1 mix of sunflower & marine oil)	4.5%
Vitamins and minerals mix	0.5%
Agar	9%
<u>Chromic oxide (Cr₂O₃)</u>	<u>1%</u>
Total	100%

The vitamin, mineral, trace element and the fatty acid composition are also given (Table II and Table III). The diet contained 25.52% crude protein and 9% agar as a binder. The dry

ingredients, excluding the agar, were premixed and homogenized in a hammer mill and stored at 3°C until used.

Table II. Vitamin, mineral and trace element formulations (after Uki *et al.*, 1985b).

<u>Composition of vitamin mixture (mg).</u>			
Thiamine HCL	6 mg	Folic acid	1.5 mg
Riboflavin	5	PABA	20
Pyridoxine HCL	2	Menadione	4
Niacin	40	B ₁₂	0.009
Ca pantothenate	10	Ascorbic acid	200
Inositol	200	Vitamin A	5000 I.U.
Biotin	0.6	Vitamin D	100 I.U.
<u>Mineral mixture</u>		<u>Trace element mixture</u>	
NaCl	1.0 g	ZnSO ₄ .7H ₂ O	1.5 mg
MgSO ₄ .7H ₂ O	15.0	MnSO ₄ .4H ₂ O	16.2
NaH ₂ PO ₄ .2H ₂ O	25	CuSO ₄ .5H ₂ O	3.1
KH ₂ PO ₄	32	KIO ₃	0.3
Fe-citrate	2.5	<u>Cellulose</u>	<u>45.0</u>
Trace element mixture	1.0	<u>Total</u>	<u>100.0g</u>
<u>Ca-lactate</u>	<u>3.5</u>		
<u>Total</u>	<u>100.0g</u>		

Table III. Fatty acid (FA) composition of the sunflower and oil mixture.

<u>Fatty acid composition of fats (lipid)</u>					
<u>Sunflower</u>		<u>Marine oil</u>			
<u>FA</u>	<u>%</u>	<u>FA</u>	<u>%</u>	<u>FA</u>	<u>%</u>
14:0	0.1	10:0	0.15	18:1	14.0
16:0	5.5	12:0	0.1	18:2	5.0
16:1	0.1	14:0	7.0	20:0	1.5
18:0	4.7	14:1	0.4	20:1	4.0
18:1	19.5	15:0	0.5	20:2	2.25
18:2	68.6	15:1	0.1	20:5	18.0
18:3	0.5	16:0	16.0	22:0	0.25
20:0	0.3	16:1	9.0	22:1	4.5
20:1	0.1	17:0	2.0	22:2	1.0
22:0	0.9	17:1	0.05	22:5	1.5
24:0	0.2	18:0	3.0	22:6	8.5
				24:0	0.5
				24:1	0.45

The semi-purified diet was prepared by boiling the agar (1.5g/100ml water) for five minutes, allowing the solution to cool to 40°C and then adding the premixed ingredients and the oil mixture. The solution was mixed to an even consistency and then poured into petri dishes to a depth of two millimetres and allowed to set. The moist diet was cut up into five by two centimetre strips. In the experiments in which seaweed was used rations of fresh *P. corallorhiza* and *G. amanzii* were collected from Port Alfred twice a week, rinsed off in clean seawater, and fed to the abalone.

Digestibility trials.

The indicator method was used to determine the dry matter and crude protein apparent digestibility co-efficient's for the semi-purified diet and the two seaweeds *Gelidium amanzii* and *Plocamium corallorhiza*. Ash content and chromic oxide were used as indicators in the algal and semi-purified diets respectively. Chromic oxide was included at a level of 1% of the total dry mass of the semi-purified diet. Samples of fresh algae and faeces were ashed at 600°C for 24 hours, cooled in a desiccator and then weighed to four decimal places and the ash content calculated by subtraction.

The organic apparent digestibility co-efficient (OADC) of the algal species was calculated using the equation:

$$\text{OADC}\% = \frac{F - E}{(1-E)F} * 100$$

where F is the fraction lost (organic matter) by ashing the food

and E is the fraction lost (organic matter) by ashing the faeces (Conover, 1966).

The dry matter apparent digestibility co-efficient (DMADC) for the semipurified diet and the algal species was calculated using the equation:

$$\text{DMADC} = \frac{\% \text{faecal indicator} - \% \text{diet indicator}}{\% \text{faecal indicator}}$$

(McDonald *et al.*, 1984).

The crude protein apparent digestibility co-efficient (CPADC) for the semipurified diet and the algal species was calculated using the equation:

$$\text{CPADC} = 100 - \left(100 * \frac{\% \text{diet indicator}}{\% \text{faecal indicator}} * \frac{\% \text{faecal nutrient}}{\% \text{diet nutrient}} \right)$$

(Crampton and Harris, 1969)

Faecal collection.

For each digestibility trial there were three sets of duplicate tanks. Faeces was collected over a five day collection period by transferring the abalone of each tank into a five litre beaker placed in each of the experimental tanks. Collection commenced at the start of the light phase. Faecal strands in the beakers were pipetted onto 4.5cm Whatman GF/C filters in a Buchner funnel attached to a vacuum pump. Before use, the filters were pre-dried, stored in a desiccator and then weighed to four decimal places. The combined filter and faecal matter were then dried to constant weight at 70°C for 24 hrs in a convection oven after

which they were placed in a glass desiccator to cool off before being weighed to four decimal places. The faecal mass was determined by subtraction. Because of the small quantities collected the faeces collected from each tank were pooled together for the total collection period of five days and analyzed.

Samples of the pooled faeces were analyzed for crude protein content using the Kjeldhal method, which determines the nitrogen content of the samples. Based on the assumption that protein contains 16% nitrogen the value obtained was then multiplied by 6.25 to give the percentage crude protein in each sample. Faecal samples from the semi-purified diet were analyzed for chromic oxide content using the chromic oxide determination method described by Bolin, King and Klosterman (1952).

Experiment I. Acceptability and preference trials.

Twenty trials were undertaken to determine whether the abalone would accept the semi-purified diet. Two tanks containing ten abalone each were used. Abalone in the first tank were offered the semi-purified diet and those in the second tank were offered a sample of *P. corallorhiza*. The abalone were then timed to determine how long they took to respond to the presence of the respective rations.

For the preference trials samples of both the semi-purified diet and fresh *P. corallorhiza* were placed equi-distant (15cm) from

the abalone in the same tank. The trials were repeated 20 times and the choice made by the animals was recorded.

Experiment II. Apparent digestibilities of the semi-purified diet and two species of algae.

To establish the suitability of the diet for further digestibility trials the crude protein and dry matter apparent digestibility co-efficient's of the semi-purified diet, *P. corallorhiza* and *G. amanzii* were compared. Abalone in each duplicate pair of tanks were fed an *ad lib* ration of one of the three respective diets. The abalone fed on the semi-purified diet were provided with fresh daily rations at the start of the dark phase, while those abalone fed the two seaweed species received fresh rations twice a week. In all the experimental tanks the temperature was kept constant at $18 \pm 0.5^{\circ}\text{C}$, and photoperiod was maintained at 10L/14D.

The organic apparent digestibility of both algal species was calculated using the Conover (1966) ratio. Crude protein and dry matter digestibility co-efficient's were calculated using the respective equations shown above.

Experiment III. Effect of temperature on the apparent digestibility of the semi-purified diet.

To quantify the effect of temperature on crude protein and dry

matter apparent digestibility co-efficient's of abalone, duplicate tanks were maintained at three different temperatures. These were 15°C, 18°C and 22°C respectively. A constant photoperiod of 10L/14D was maintained in all the experimental tanks.

Abalone in each tank were fed fresh rations daily at the start of the dark phase. After the adaptation period of 16 days, the faeces were collected over a period of 5 days. Crude protein and dry matter digestibility co-efficient's were calculated using the respective equations shown above. Consumption rates, expressed as a percentage of body weight eaten per day, were calculated gravimetrically on a wet weight basis, by measuring the difference in the mass of the food before and after feeding. The uneaten food was dried using paper towel to remove excess moisture before the mass was measured.

Experiment IV. Effect of photoperiod on the apparent digestibility of the semi-purified diet.

To quantify the effect of photoperiod on crude protein and dry matter apparent digestibility co-efficient's, duplicate tanks were kept at three different photoperiod's. These were 14L/10D, 10L/14D and 6L/18D respectively. Water temperature in all the experimental tanks was constant at 18°C.

The ten abalone in each tank were fed fresh rations daily at the start of the dark phase. As in the temperature experiments the

abalone were also adapted for 16 days, were after the faeces was collected over a five day period. Crude protein and dry matter digestabilities were calculated using the respective equations shown above. Consumption rates were calculated in the manner described above.

CHAPTER 4. RESULTS.

Experiment 1. Acceptability and preference trials.

The results of the diet acceptability and preference trials are shown in Table IV. It is evident that *H. midae* readily accepted and preferred the semi-purified diet. The time the abalone took to respond to the presence of the semi-purified diet ranged from 15 to 52 seconds with a mean of 34.3 seconds, while abalone offered the seaweed did not respond within a two minute period. Abalone presented with both diets moved towards the semi-purified diet on each occasion.

Table IV. Response time to the presence of the semi-purified diet or *P. corallorhiza* when offered individually (acceptability), and % orientation of abalone when presented with both the semi-purified diet and *P. corallorhiza* simultaneously (preference).

	<u>Diet</u>	
	Semi-purified diet	<i>P. corallorhiza</i>
<u>Acceptability</u>		
Mean response time and S.D.	33.6±10.3 sec	> two min
<u>Preference</u>		
Percentage orientation towards preferred diet	100%	0%

The response the semi-purified diet evoked from the abalone was their characteristic "feeding position". This consisted of the lifting of the anterior part of the foot with extended feelers, accompanied by vigorous rotation to orientate themselves in the direction of the ration. This suggests that detection was by means of chemoreception. Once the abalone had moved forward and the ration was secured, it was positioned under the foot with the

mouth close to the edge of the feed portion and feeding commenced. The method of feeding is shown in the photographs in Plate 1.

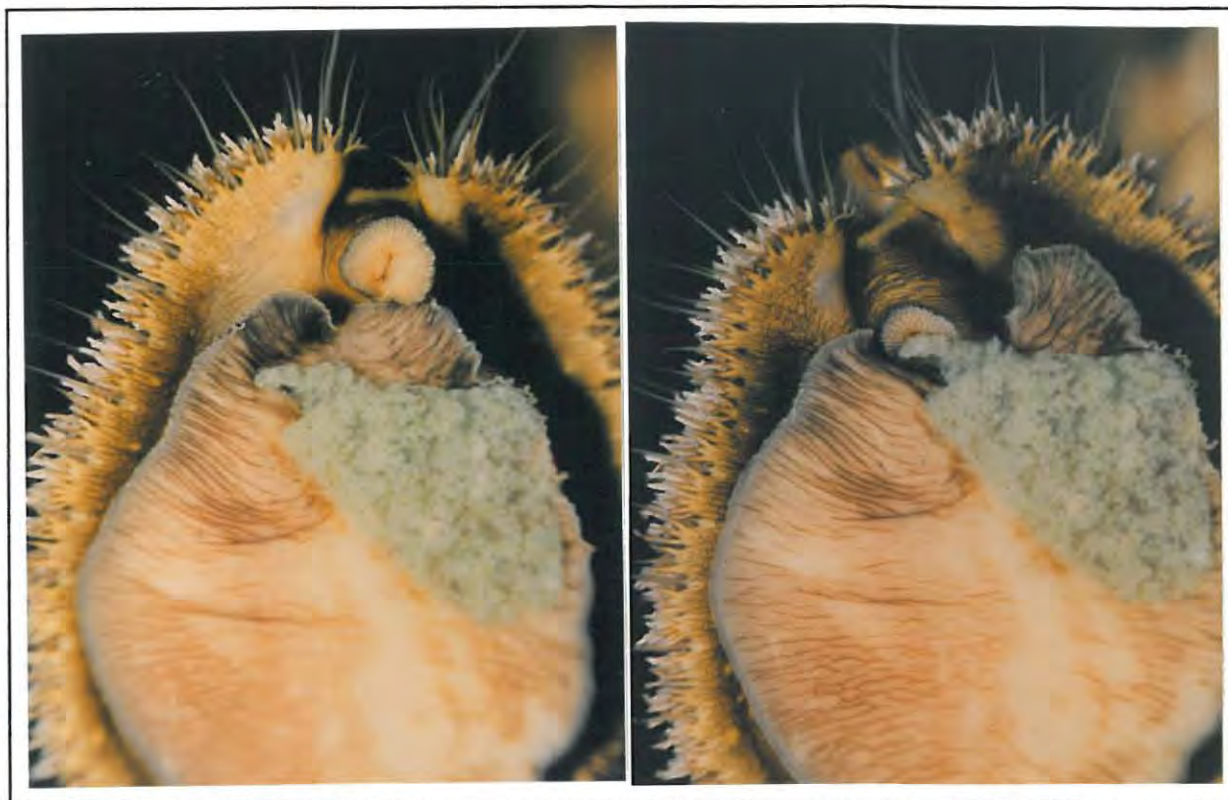


Plate 1. Photographs depicting *H. midae* holding onto, and eating a piece of the semi-purified diet.

This method of feeding is similar to that of *Aplysia* eating an artificial diet (Carefoot, 1980). The remains of the feed portion would either be stored under the foot for later consumption or be discarded.

The faecal strands produced by abalone that were fed on the semi-purified diet were a homogenous dark green to black colour. The faeces were firm and remained intact when pipetted from the tank. The uniform colour was taken to indicate a uniform concentration of chromic oxide throughout the faeces, which is a prerequisite

for indirect digestibility studies (Calow and Fletcher, 1972). On the basis of the acceptability and preference of the semi-purified diet, and the consistency and even colour of the faeces the semi-purified diet was considered suitable for further studies.

Experiment 2. Comparison of apparent digestibility co-efficient's of the semi-purified diet and two species of algae.

The dry matter content, ash content and crude protein content of the two macroalgae fed to the experimental animals, *Plocamium corallorhiza* and *Gelidium amanzii*, are shown in Table V.

Table V Mean percentages of dry matter, ash and crude protein content of *Plocamium corallorhiza* and *Gelidium amanzii*. DM = dry matter; CP = crude protein; n = number of samples analyzed.

	n	DM (%)	Ash (%)	C P (%)
<i>Plocamium corallorhiza</i>	10	15.45%	34.27%	20.49%
<i>Gelidium amanzii</i>	10	32.96%	13.95%	22.68%

P. corallorhiza has an ash content that is within the range (20.7% - 51.5%) found for other red fleshy algae (Montgomery and Gerking, 1980). The ash content of *G. amanzii* was found to be similar to the ash content of *Gelidium cartilagineum* (Paine and Vadas, 1969). The protein content of both *P. corallorhiza* and *G. amanzii* were higher than the average for red fleshy algae (Montgomery and Gerking, 1980). This difference could be attributed to seasonal, geographical and species differences

(Paine and Vadas, 1969).

The crude protein and the dry matter apparent digestibility co-efficient's for the semi-purified diet, *P. corallorhiza* and *G. amanzii* are shown in Table VI. There was a significant difference between respective crude protein (F-ratio = 164.165; $p \leq 0.05$) and dry matter (F-ratio = 999.999; $p \leq 0.05$) apparent digestibility co-efficient's of the semi-purified diet, *P. corallorhiza* and *G. amanzii*.

Table VI. Dry matter (DMADC) and crude protein apparent digestibility co-efficient's (CPADC) for the three diets offered. Different letters denote a significant difference, while similar letters denote no significant difference between groups ($p \leq 0.05$).

	DMADC mean \pm S.D.	CPADC mean \pm S.D.	Organic ADC mean \pm S.D.
<i>P. corallorhiza</i>	29.9% \pm 1.55 a	57.3% \pm 5.41 a	45.5% \pm 2.39
<i>G. amanzii</i>	70.7% \pm 1.99 b	80.0% \pm 2.07 b	82.1% \pm 2.11
Semi-purified diet	83.7% \pm 0.98 c	95.6% \pm 0.25 c	

Crude protein and the dry matter apparent digestibility co-efficient's for the semi-purified diet (95.7% and 83.3% respectively) were higher than either values for *G. amanzii* (80.0% and 70.4% respectively) which in turn were higher than the values obtained for *P. corallorhiza* (57.9% and 30.3% respectively). These trends are illustrated in Figure 3.

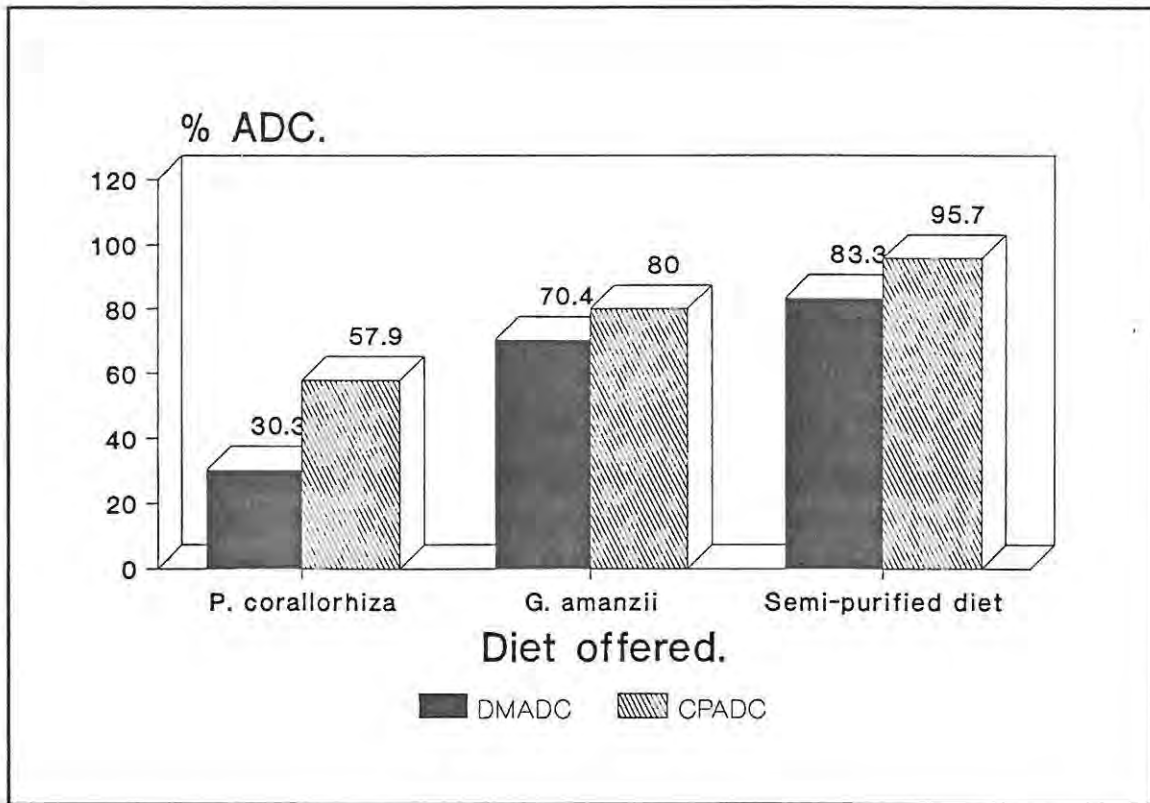


Figure 3. Dry matter and crude protein apparent digestibility co-efficient's of *P. corallorhiza*, *G. amanzii* and the semi-purified diet.

Experiment 3. The effect of temperature on crude protein and dry matter apparent digestibility co-efficient's.

The crude protein and dry matter apparent digestibility co-efficient's recorded at different temperatures are shown in Table VII.

Both crude protein and dry matter apparent digestibility co-efficient's at 18°C (96.7% and 69.9% respectively) were significantly higher than at 15°C and 22°C. The relationship between temperature and dry matter apparent digestibility (DMADC) and crude protein apparent digestibility (CPADC) is shown in

Table VII. The effect of temperature on apparent digestibility co-efficient's (ADC). Different letters denote a significant difference between groups, while similar letters denote no significant difference between groups when $p \leq 0.05$.

Temperature	Dry matter ADC mean \pm S.D.	Crude protein ADC mean \pm S.D.
15°C	69.5 \pm 3.46 a	93.9 \pm 0.69 c
18°C	86.7 \pm 1.00 b	96.7 \pm 0.47 d
22°C	61.2 \pm 4.19 a	92.7 \pm 0.67 c

Figure 4. There were significant differences for respective crude protein (F-ratio = 33.730; $p \leq 0.05$) and dry matter (F-ratio = 41.025; $p \leq 0.05$) apparent digestibility co-efficient's between all three temperatures.

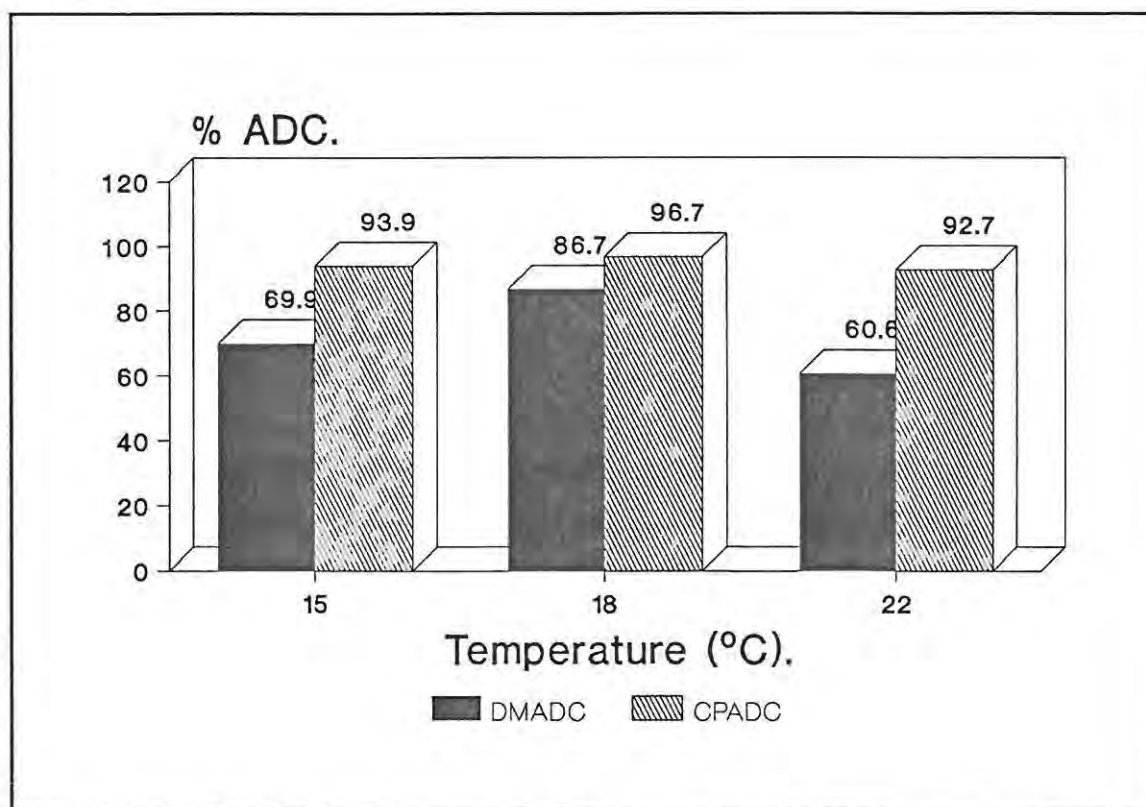


Figure 4. The relationship between temperature and apparent digestibility co-efficient's for dry matter and crude protein.

There was also a significant increase (F-ratio = 44.846; $p \leq 0.05$) in consumption rate with increasing temperatures. The intake

doubled from 15°C to 18°C and at 22°C was two and a half times that at 15°C (See Figure 5).

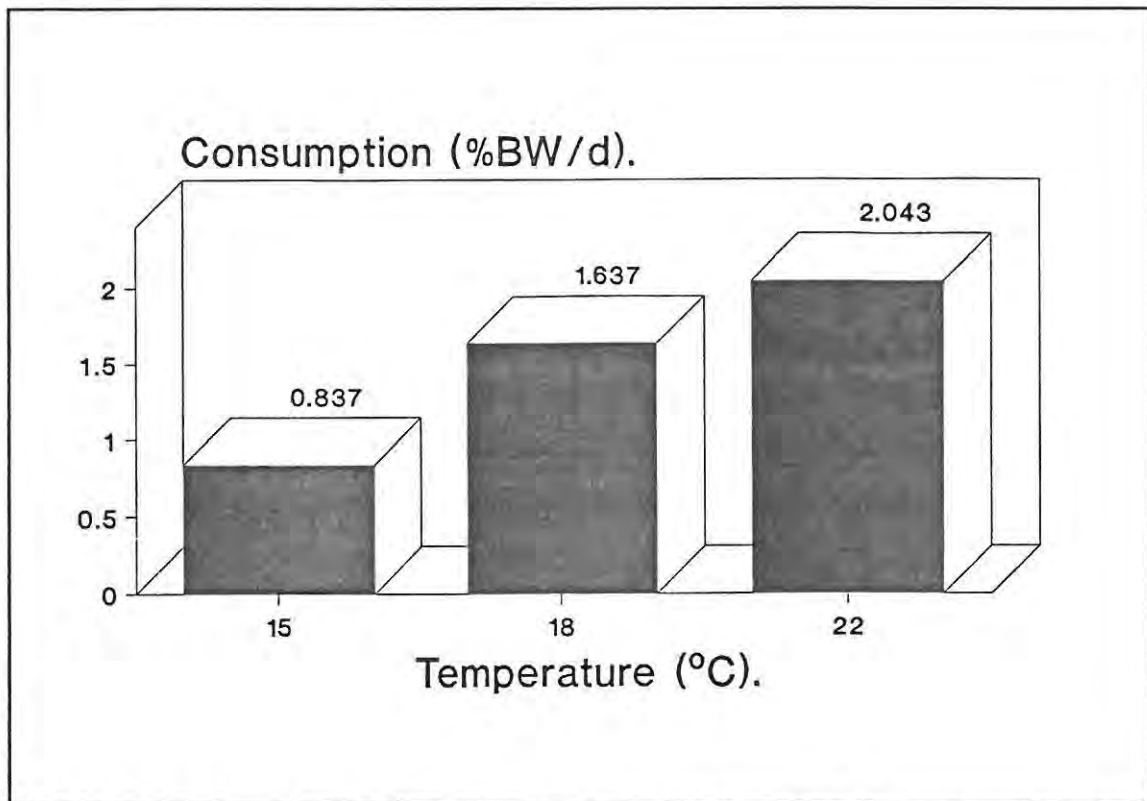


Figure 5. Relationship between temperature and consumption of the semi-purified diet by *H. midae*.

Experiment 4. The effect of photoperiod on crude protein and dry matter apparent digestibility co-efficient's.

The CPADC and the DMADC for the different periods of darkness are shown in Table VIII.

With longer periods of darkness there was a significant decrease in crude protein (F-ratio = 316.750; $p \leq 0.05$) and in dry matter (F-ratio = 273.633; $p \leq 0.05$) apparent digestibility co-efficient's. The relationship between increasing darkness and apparent digestibility is illustrated in Figure 6. From this it

Table VIII. The effect of photoperiod on apparent digestibility co-efficient's (ADC). Different letters denote a significant difference between groups, while similar letters denote no significant difference between groups when $p \leq 0.05$.

Photoperiod	Dry Matter ADC mean±S.D.	Crude Protein ADC mean±S.D.
14L/10D	86.7%±0.67 a	96.7%±0.23 d
10L/14D	75.6%±1.15 b	95.8%±0.36 e
6L/18D	66.5%±1.42 c	91.4%±0.25 f

can be seen that the CPADC and the DMADC decreased from 96.8% and 86.8% respectively to 93.1% and 66.5% respectively with a longer dark period. There was also a positive relationship between photoperiod and consumption rate, with a significant increase (F-ratio = 40.306; $p \leq 0.05$) as hours of darkness increase (Figure 7).

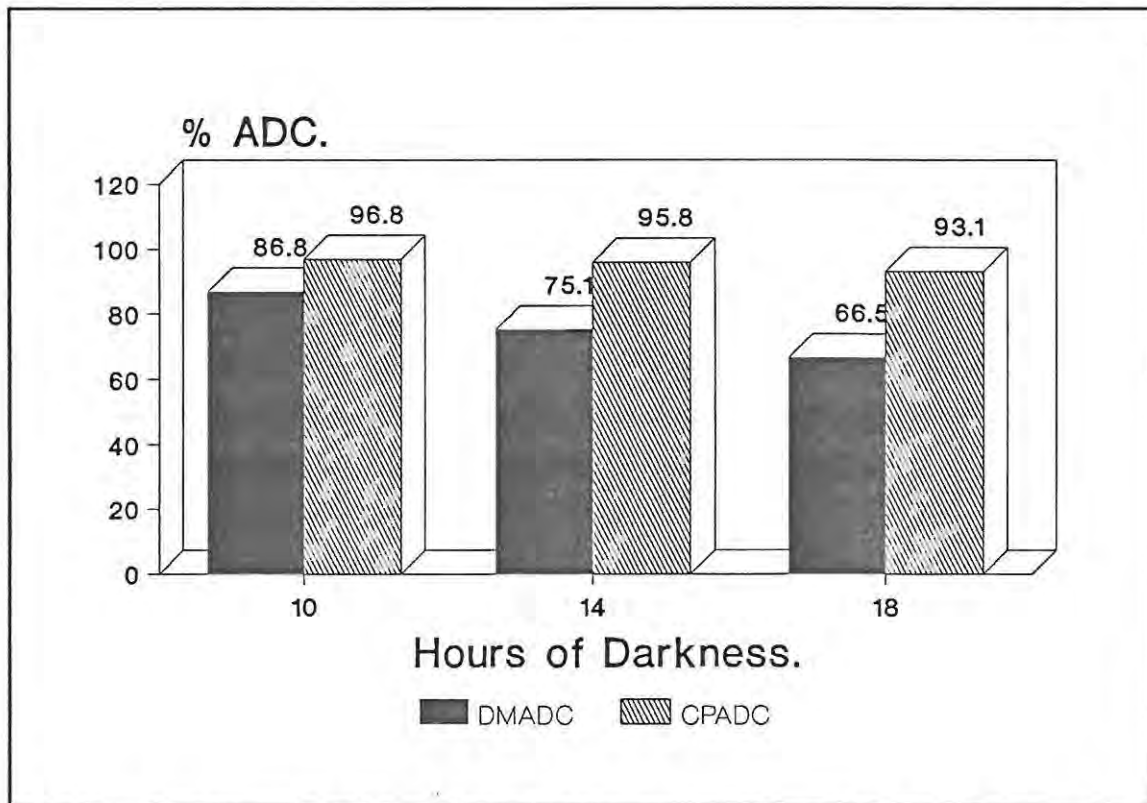


Figure 6. The relationship between photoperiod and apparent digestibility co-efficient's for dry matter and crude protein.

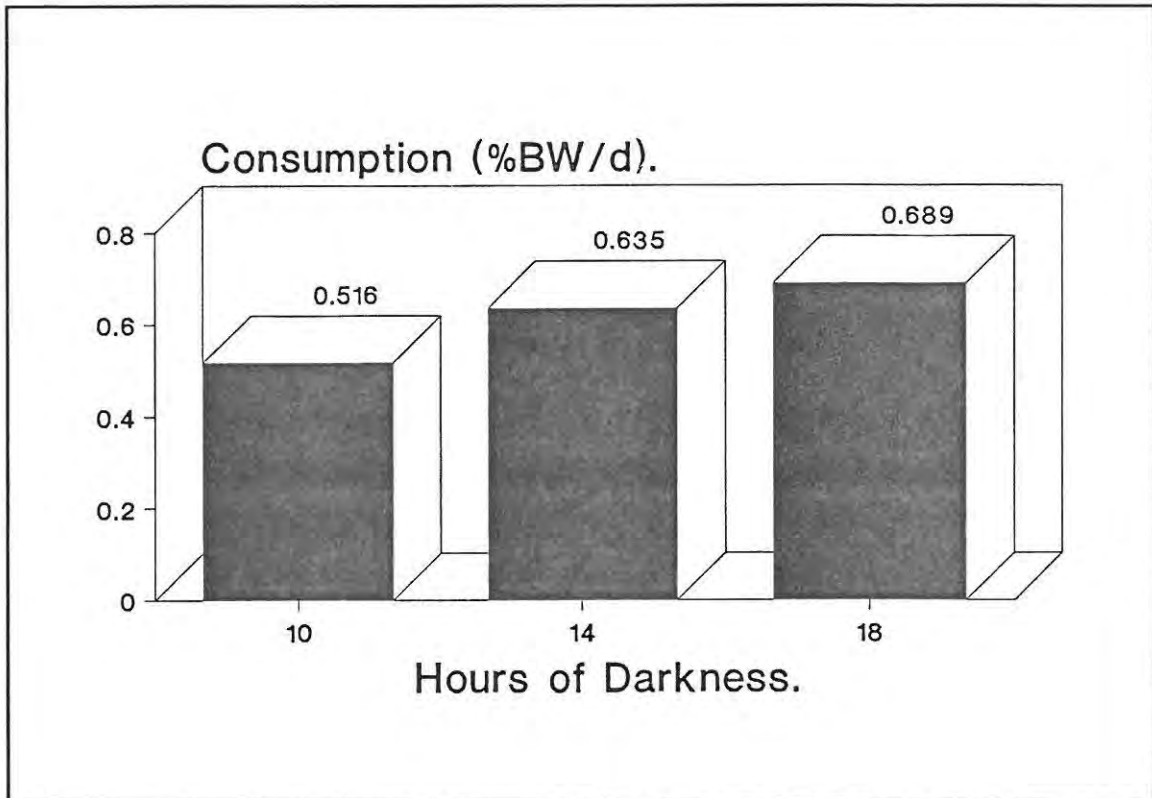


Figure 7. Relationship between photoperiod and food consumption by *H. midae*.

CHAPTER 5. Discussion.

The results of this project contribute towards the understanding of the digestive physiology of abalone, and are an important the development of a practical commercial diet. It has been shown that *H. midae* accepts a semi-purified diet and in fact prefers it to macroalgae. A method to conduct indirect digestibility trials with abalone was also developed. Results from the temperature and photoperiod trials showed that both these variables have significant effects on dry matter and crude protein apparent digestibilities.

Acceptance and preference of the semi-purified diet by *H. midae*.

The acceptance of and preference for the semi-purified diet indicates that, despite being a marine herbivore, *H. midae* has a predilection for ingredients of animal and terrestrial plant origin. The Japanese abalone, *H. discus hannai*, has also been found to accept artificial diets that contain various ingredients of animal and plant origin (Ogino and Ohta, 1963; Uki et al., 1985a; Uki and Watanabe, 1986).

In the present study the ability of abalone to utilize such ingredients has facilitated the development of a semi-purified diet with which it was possible to evaluate the effects of temperature and photoperiod on apparent digestibility coefficient's. The use of semi-purified diets in the development of formulated feeds for prospective aquaculture species is

extremely important for determining specific nutrient requirements (Conklin *et al.*, 1977; Conklin, Goldblatt, Bordner, Baum and McCormick, 1978; Conklin *et al.*, 1980). Semipurified diets have also been developed for deletion and augmentation trials to determine the optimum dietary protein and lipid levels for *H. discus hannai* (Uki *et al.*, 1985b, 1986a, 1986c).

Comparative investigations have also indicated that abalone are able to utilize ingredients of animal origin (casein and fishmeal) more efficiently than ingredients of plant origin (soya bean meal and seaweed) (Uki *et al.*, 1985a; Nie *et al.*, 1987; P.J. Britz, Rhodes University, unpublished), a trend that is confirmed by the present study.

Previous studies have shown olfaction and chemoreception to be important in abalone feeding (Leighton, 1966; Harada and Kawasaki, 1982; Sakata and Ina, 1985; Harada *et al.*, 1987). In the present study the response of *H. midae* to the semi-purified diet is attributed largely to leaching of feeding attractants from it. The formulation of the semi-purified diet used in this study included dried kelp at a level of 5% as a feeding attractant. Later formulations that excluded dried kelp nevertheless still elicited a feeding response. However, for continuity the diet used in the digestibility trials included dried kelp. Previous investigations with abalone have shown that combinations of amino acids and lipids (Harada *et al.*, 1987), algal extracts (Sakata *et al.*, 1984) and isolated digalactosyldiacylglycerols and phosphatidylcholines (Sakata and

Ina, 1985) evoke a definite chemoreceptive feeding reaction. Similar results have also been found for other marine gastropods (Carefoot, 1980, 1984; Sakata, Masahiro, Kamiya and Ina, 1985; Sakata, Sakura, Kamiya and Ina, 1988). Small amounts of chemicals are known to diffuse from seaweeds (Khailov and Burlakova, 1969; Sieburth, 1969), however, diffusion from algae is probably slower than that from the agar based diets (Carefoot, 1980), which were used in this study. This would in part explain the slower response times of *H. midae* to *P. corallorhiza* in comparison to the semi-purified diet. Since nitrogenous bases, particularly pyrrolidine, do not act as feeding attractants for abalone (Harada et al., 1987), it is suggested that the leaching of either the free amino acids or lipids, or a combination of both in the semi-purified diet, accounted for the evoked chemoreceptive response.

In any deletion studies using semi-purified diets it is important to know if the nutrient being tested is itself a feeding attractant or not. The use of unpalatable ingredients can depress intake and thus growth rates, thus giving erroneous results for the nutrient under investigation (Carefoot, 1984). For example, nutritional studies with sea hares, *Aplysia dactylomela*, necessitated the identification and inclusion of feeding attractants into an artificial diet before satisfactory results were obtained (Carefoot, 1980). However, a wide range of ingredients of animal and terrestrial plant origin, (casein, fishmeal, corn gluten meal, soya meal, torula yeast, dextrin and *Spirulina*) elicit a feeding response and are accepted by abalone

(Ogino and Ohta, 1963; Uki et al., 1985a; P.J. Britz, Rhodes University, unpublished). The relatively wide choice of ingredients available for abalone diets facilitates least cost formulation and manufacture of cosy effective commercial diets made from conventional feed ingredients.

Digestibility

To date nutrient evaluation trials on abalone have largely been limited to performance and retention trials, which have provided comparative nutritional values for the various ingredients. However, the information gleaned from such studies does not quantify specific nutrient availability of the ingredients and therefore does not facilitate the formulation of a nutritionally balanced diet. It was therefore considered necessary to develop a dietary evaluation technique that would provide suitable information on nutrient availability which is necessary for feed formulation.

An exploratory feeding trial with *H. midae* using the semi-purified diet indicated that the indirect digestibility method is a feasible technique for nutrient evaluation. The faecal strands produced by *H. midae* which had been fed on the semi-purified diet had a uniform dark green-black appearance indicating an even concentration of chromic oxide in the faeces. The consistent dry matter and crude protein apparent digestibility co-efficient's obtained in this study also confirmed that a homogenous concentration of chromic oxide must

have been present in the faeces. This demonstrates that chromic oxide can be used with confidence in indirect digestibility trials, thereby eliminating the quantitative collection of faecal matter required for the direct method, which is difficult to accomplish with aquatic animals. In the event that the inert marker is not homogenously distributed in the faeces the researcher will be restricted to the direct method. For example, Brown *et al.* (1986), found the incorporation of chromic oxide unsuitable for indirect digestibility trials with crayfish on account of its uneven concentration in the faeces. This was attributed to possible selective consumption and digestion.

A comparison of the digestibilities of *P. corallorhiza* and *G. amanzii*, and the semi-purified diet revealed that *H. midae* had higher dry matter (DMADC) and crude protein apparent digestibility co-efficient's (CPADC) for the latter. The high DMADC and CPADC values for the semi-purified diet demonstrated that it was suitable for further use in digestibility trials. This was in fact expected as casein is a high quality protein with a well balanced essential amino acid composition. Of several protein sources tested in experimental diets for *H. discus hannai*, casein was found to be superior to white fishmeal (Uki *et al.*, 1986a), egg albumin and whole egg (Uki *et al.*, 1985a). Though casein is suitable as a protein source in semi-purified diets, its inclusion in a commercial diet for abalone is impractical because of its high price. The mean CPADC and DMADC values obtained in the present study compare well with values for prepared diets fed to other species (Table IX).

Table IX. Comparison of crude protein (CPADC) and dry matter apparent digestibility co-efficient's of different protein sources.

<u>Dietary protein source</u>	<u>Species</u>	<u>CPADC</u>	<u>Author</u>
Casein	crayfish	95.4%	Brown et al., 1986
Casein	carp	93.2%	Eid and Matty, 1989
Fishmeal	carp	91.2%	Eid and Matty, 1989
Fishmeal	trout	92%	De la Noue and Choubert, 1986
Fishmeal	carp	87%	Pongmaneerat and Watanabe, 1991
Casein	<i>H. midae</i>	95.6%	This study
		<u>DMADC</u>	
Fishmeal	trout	82%	De la Noue and Choubert, 1986
Casein	crayfish	73.4%	Brown et al., 1986
Casein	<i>H. midae</i>	83.7%	This study

It was concluded that indirect digestibility trials are possible with *H. midae* using chromic oxide as a marker.

The acceptance of and the high utilization efficiency of the semi-purified diet by *H. midae* suggests the possibility of using conventional protein ingredients in formulated feeds. Animal tissue is usually more digestible than plant tissue and herbivores thus have to process food faster and in larger quantities than carnivores to absorb the same quantity of nutrients (Hughes, 1986). However, it would be incorrect to assume that all herbivorous species are able to utilize ingredients of animal origin efficiently. For some species, such as *Procambarus clarkii*, a herbivorous crustacean, higher apparent digestibilities were obtained for diets containing plant ingredients (Brown et al., 1986; Brown et al., 1989). Such

inconsistencies in the acceptance of various ingredients by different herbivorous species highlights the need to evaluate all prospective ingredients for use in commercial diets of proposed culture species.

Abalone are opportunistic feeders, consuming a large variety of algal species (Leighton and Boolootian, 1963; Poore, 1972a; Uki *et al.*, 1986b). *H. midae* has been shown to consume 33 species of algae of which *Ecklonia maxima* and *Plocamium corallorhiza* constitute the bulk (Barkai and Griffiths, 1986). Comparing the organic digestibility co-efficient's of the two algae, *G. amanzii* was found to be more efficiently utilized than *P. corallorhiza*, despite the latter being one of the main dietary components of *H. midae* on the east coast of South Africa (Barkai and Griffiths, 1986; Tarr, 1989). Both the organic digestibility co-efficient's of *G. amanzii* (81.9%) and *P. corallorhiza* (46.7%) were higher than for *E. maxima* (the main dietary constituent of *H. midae* along the southern South African coast). *E. maxima* has an approximate organic digestibility co-efficient of 37% (Barkai and Griffiths, 1987).

Algal quality may affect digestion rates and apparent digestibility, both of which will affect absorption efficiency (Hughes, 1986). The low protein content (10.8%) and tough nature of the *E. maxima* fronds (Barkai and Griffiths, 1988) probably account for its relatively low apparent digestibility. Despite having similar protein contents, *P. corallorhiza* had a lower digestibility than *G. amanzii* which may be due either to the

differences in protein quality or the structure or composition of the cell walls.

The higher ingestion rate of *E. maxima* (3.2 % body weight/day) compared to *P. corallorhiza* (1.2 % body weight/day) (P.J. Britz, Rhodes University, unpublished) is most likely a compensation by *H. midae* for the lower organic digestibility of the former. A similar inverse relationship between food quality and ingestion rate was also found in two freshwater gastropods, with increased intake compensating for the poorer dietary quality (Calow, 1975). Conversely, it has also been shown that instead of increasing intake when fed with a high proportion of indigestible material in the diet, sea urchins simply improve their digestive efficiency (Lawrence, Regis, Delmas, Gras and Klinger, 1989). Although *E. maxima* makes up the bulk of the diet in certain habitats, the poor weight gain and low food conversion ratio (FCR) of *H. midae* fed on it in culture systems (P.J. Britz, Rhodes University, unpublished), suggest that from a nutritional point of view it is probably not the most suitable diet for intensive abalone culture.

The effect of temperature on intake and dry matter digestibility.

The importance of environmental effects on metabolism and physiology have been demonstrated in aquaculture management (Brett, 1979). In contrast to homiothermic animals (Lovell, 1989), temperature influences basal and active metabolic rates in poikilotherms (Bullock, 1955). Temperature is also considered

to be one of the most important environmental factors that controls food utilization at all levels and all stages of growth in aquatic poikilothermic animals (Kaushik, 1986).

The variation in basal metabolic rates associated with changing temperatures is usually manifested in growth rates and the maximum size obtained by fish (Prosser, 1955; Bullock, 1955; Ricker, 1979), gastropods (Newman, 1969), and crustaceans (Serfling and Ford, 1975; Bordner and Conklin, 1981). However, higher temperatures will only produce a positive effect on growth rate if the nutrient intake exceeds the metabolic demand of the animal (Brett and Groves, 1979; Hughes, 1986). Poikilotherms exposed to higher temperature have the choice of two strategies in order to maintain an energetic intake that exceeds basal requirements. They can either increase their rate of ingestion, or increase their digestive efficiency of the food, or a combination of both (Newell and Branch, 1980).

The data obtained in this study clearly showed that temperature has a significant influence on the dry matter apparent digestibility co-efficient's (DMADC) and the crude protein apparent digestibility co-efficient's (CPADC). Both values were highest at 18°C. There were significant differences in both the DMADC and the CPADC values between 15°C and 18°C, and 18°C and 22°C respectively.

In addition to altering digestive efficiencies, *H. midae* showed a significant increase in consumption rate with increasing

temperature. These increased from 0.84% of body mass/day at 15°C to 2.05% of body mass/day at 22°C. The increasing consumption rates associated with temperature can be regarded as an attempt by *H. midae* to increase the energetic intake to meet the temperature related rise in metabolic costs.

A positive relationship between temperature and food intake has also been observed for *H. midae* by Barkai and Griffiths, (1987) and in other gastropods (Edwards and Huebner, 1977; Lee, Yoo, Rho and Kim, 1988; Gao, Liu, Liu and Liu, 1990), fish (Luquet, 1979; Wax and Pote, 1990; Horn and Gibson, 1990), and crustaceans (Serfling and Ford, 1975; Bordner and Conklin, 1981). Under culture conditions the importance of this relationship and the effect it has on increasing feed costs has long been appreciated (Brett, 1979), since feed costs constitute as much as 70% of the running costs of an aquaculture venture (NRC, 1983). It is important, therefore, to determine the effect of temperature on nutrition to facilitate calculation of the amount and cost of food required for a prospective or new aquaculture species (Bordner and Conklin, 1981).

Key factors that may have influenced the DMADC and CPADC values at the different temperatures are ingestion rate, digestive enzyme activity, and the transit time of the ingested material through the gut. A hypothetical representation of the relationship between intake, transit time and ADC's is illustrated in Figure 8.

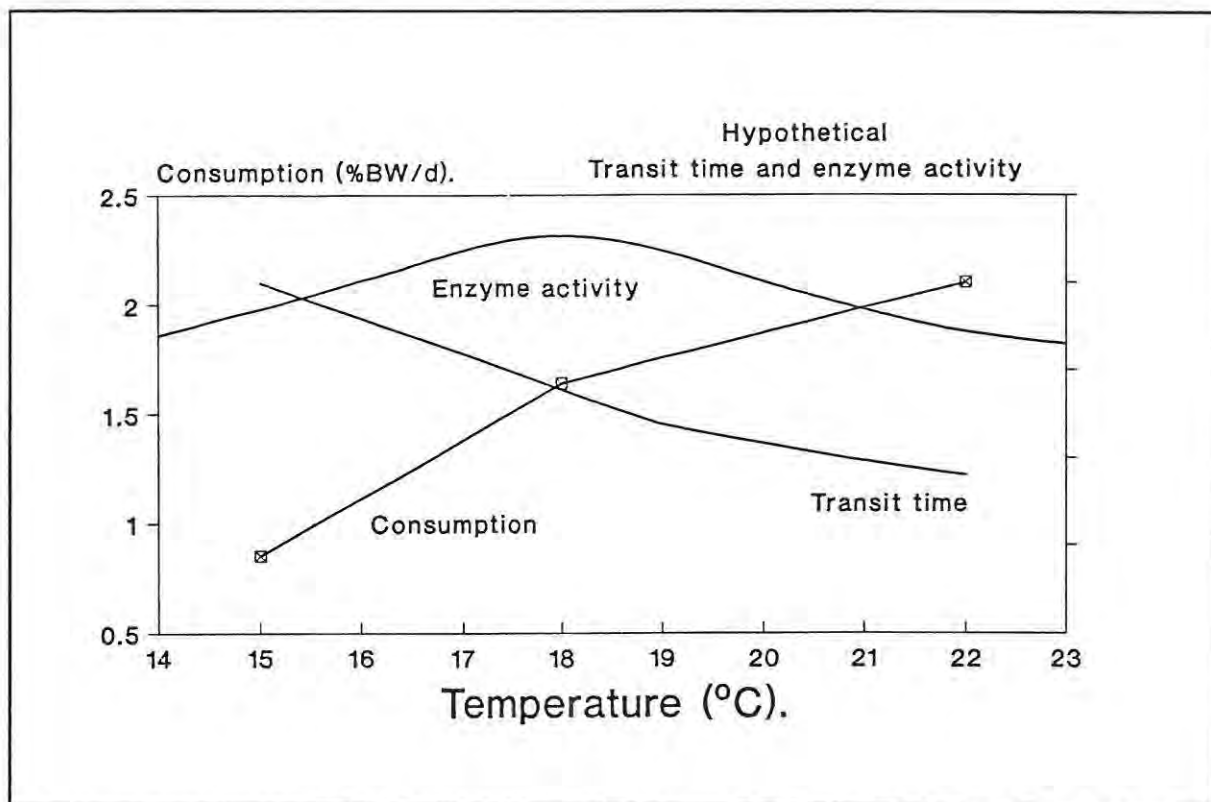


Figure 8. Hypothetical representation of the relationship between intake, transit time and apparent digestibility co-efficient's.

Gut transit time, which is an inverse function of both temperature (Horn and Gibson, 1990) and ingestion rate (Elliot and Persson, 1978; Fänge and Grove, 1979; Jobling, 1980; Fauconneau, Choubert, Blanc, Breque and Luquet, 1983), would have tended to decrease with increasing temperature.

Despite the doubling in food consumption rate observed between 15°C and 18°C, and a probable concomitant reduction in transit time, the higher ADC's observed at 18°C indicate that digestive efficiency was higher at this temperature. Although thermal optima of the digestive enzymes of *H. midae* have not been determined it is probable that enzyme activity was more efficient at 18°C than at 15°C. Enzyme activity at 18°C could have been

enhanced by two possible mechanisms:

1. an increased secretion of enzymes (Luquet, 1979; Hepher, 1988) and/or
2. a decrease in the reaction time of the enzyme on the substrate (Hepher, 1988).

Raising the temperature from 18 to 22°C resulted in a decrease in apparent digestibility co-efficient's (ADC). The theoretical reduction of gut transit time would greatly reduce the contact time between the digestive enzymes and food particles (Hepher, 1988) at 22°C, thus reducing the proportion of food that is digested. An additional factor that may have contributed to reduced digestive efficiency at 22°C is a possible decrease in enzyme activity due to the temperature exceeding the optimal activity range (Hoffman, 1983).

From an economic perspective, these results imply that food utilization is most efficient at 18°C and that feed costs will be lowest at this temperature. At the higher temperature of 22°C the positive relationship between temperature and intake, and the depressed ADC's would undoubtedly lead to increased feed costs. However, abalone growth rates display a positive relationship with temperature and for *H. midae* higher growth rates are obtained at 22°C than at 18°C (T. Hecht, Rhodes University, unpublished). Thus the savings in feed costs at 18°C would have to be weighed against those from a reduced production time at 22°C. Because the running costs of pump ashore abalone farms are very high and abalone are slow growers, the economic gains from

a reduction in production time at 22°C may well outweigh the savings in feed costs at 18°C.

The effect of photoperiod on dry matter and crude protein digestibility co-efficient's.

Investigations by several authors into the effect of light on growth have resulted in diverse, complex and ambiguous results (Brett, 1979). This can be attributed to the diversified ways in which light can affect an organism, the main influences being photoperiod, light intensity and light quantity (Brett, 1979). The influence of photoperiod on behaviour patterns and physiology deserves attention when fundamental aquaculture research for abalone is undertaken. An understanding of the established diel behavioral activity of abalone could be of vital importance to culturists endeavours to maximise their growth rate and survival.

In the present investigation *H. midae* showed a positive relationship between intake and period of darkness. Ebert and Houk (1984) reported that abalone exposed to continuous darkness had higher growth rates, feeding rates and food conversion efficiencies, which would suggest that increased darkness would be beneficial for abalone culture.

Observations on different *Haliotis* spp. under natural conditions have shown that feeding is a function of photoperiod. Abalone have a distinct diel pattern of activity, feeding predominantly at night (Poore, 1972; Shepherd, 1972; Barkai and Griffiths,

1986; Wells and Keesing, 1989). This diel pattern of activity in *H. midae* continues in experimental tanks (personal observation). In the present study the increased period of darkness would have provided *H. midae* with more opportunity to feed, thereby increasing its intake. Such a relationship has also been observed in *H. rufescens* (Ebert and Houk, 1984) and in lobsters, which are also nocturnal feeders (Bordner and Conklin, 1981).

Under natural conditions, the longer duration of the dark period in winter would induce *H. midae* to consume more during this time of year, which would theoretically result in seasonal differences in growth. In fact, Newman (1967) found that *H. midae* grows faster in winter than in summer. Newman (op. cit) also pointed out that these differences could not be related to temperature or the reproductive cycle. However, because other variables were not constant, seasonal growth spurts cannot conclusively be ascribed to photoperiod.

In addition to the positive relationship between period of darkness and intake, a negative relationship between the ADC's and period of darkness was also observed indicating that the digestive efficiency of *H. midae* decreased. The principle factors, intake, transit rate and ADC's, are illustrated in a hypothetical representation in Figure 9.

Food transit time would have tended to decrease with increasing duration of darkness, reducing the contact time between enzymes

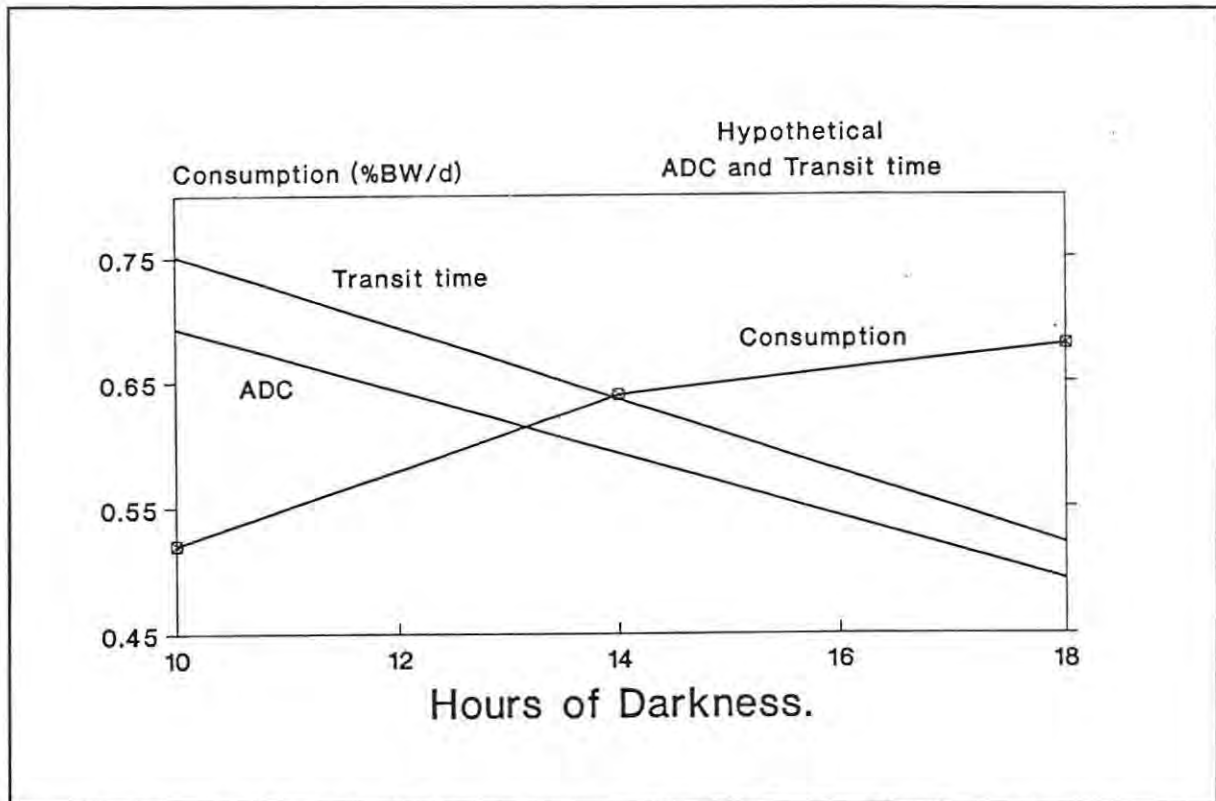


Figure 9. Hypothetical representation of the relationship between intake, transit time and apparent digestibility co-efficient's (ADC).

and ingested food and thereby effecting a decrease in the digestive efficiency of *H. midae*. Although no investigations have been undertaken to determine the effect of photoperiod on digestive enzyme activity of *H. midae* this may also have influenced the observed ADC's. The effects of photoperiod on abalone metabolism, as reflected by oxygen consumption rates (Uki and Kikuchi, 1975; Jan et al., 1981; Barkai and Griffiths, 1987), are inconsistent. Barkai and Griffiths (op. cit.) observed no increase in oxygen consumption while Uki and Kikuchi (1975) and Jan et al. (1981) found a significant increase in oxygen consumption when animals were exposed to darkness. However, the latter authors only observed a significant difference in starved animals. The observation that locomotory activity was responsible

for the increase (Barkai and Griffiths, 1987) and not a direct influence of photoperiod on metabolism makes it doubtful that enzyme activity would increase when the period of darkness increased.

The lower DMADC and CPADC values during longer periods of darkness seem to be inconsistent with the improved feed conversion efficiencies obtained with longer periods of darkness by Ebert and Houk (1984). However, the lower ADC's are not necessarily a disadvantage to nutrient acquisition by *H. midae*. In the presence of abundant food the net gain of nutrients would be positive despite a decrease in apparent digestibility (Newell and Branch, 1980; Hughes, 1986; Peck, 1989).

From a food utilization perspective, the most economical photoperiod for intensive abalone culture, using a formulated feed would be 14L/10D. At this regime the digestive efficiency would be at it's highest and intake at it's lowest, which would reduce feed costs. However, the positive relationship between darkness and growth (Ebert and Houk, 1984) would shorten production time, reducing running costs for abalone exposed to increased hours of darkness. Thus, it is probable that the lower feed costs of abalone exposed to less darkness will be outweighed by lower running costs when the duration of darkness is increased.

A review of the results reveals substantial differences in consumption rate between the temperature and photoperiod

experiments. Whereas the consumption rate more than doubles for an increase in temperature (15°C to 22°C), the increase in hours of darkness only results in a 25% increase in consumption rate, which indicates that temperature has a greater influence on the digestive physiology of *H. midae*.

Comparison of CPADC and DMADC in both the temperature and photoperiod experiments shows that there is a proportionately greater variation for DMADC. For both experiments DMADC varied between 61% and 86% whereas CPADC varied in a narrower range between 91% and 96%, implying that protein is efficiently utilized over a range of environmental conditions. This differential variation between CPADC and DMADC has cost implications with respect to commercial diets. Since protein makes up the bulk of the cost of commercial diets, feeding costs under different temperature and photoperiod regimes will vary more in proportion to the CPADC values than the DMADC values.

Conclusions.

The results of this study will assist nutritionists in developing a formulated feed for *H. midae*. It was shown that *H. midae* accepted a formulated diet consisting primarily of ingredients of terrestrial plant and animal origin. The inclusion of ingredients of animal origin is beneficial for use in deletion trials to ascertain specific nutrient requirements for *H. midae*. A technique to conduct indirect digestibility trials with abalone using chromic oxide as a marker has been developed, which lays

a foundation for further applied nutritional work on abalone. Temperature was found to have a significant effect on dry matter and crude protein apparent digestibilities. There was also a significant increase in consumption rate with rising temperature. Both DMADC and CPADC were higher at 18°C than at 15°C and 22°C suggesting that *H. midae* has a maximum digestive efficiency close to this temperature.

The photoperiod experiments showed that longer periods of darkness resulted in significant increases in consumption rates and significant decreases in DMADC and CPADC. The extended hours of darkness afforded *H. midae*, a nocturnal feeder, the opportunity of consuming more. It is proposed that the higher consumption rates reduced digestive efficiency by reducing the contact time between digestive enzymes and the food in the digestive tract.

Although this study has provided some information that has a bearing on abalone nutrition, additional investigations are required before a balanced feed can be formulated. Concurrent deletion, growth and digestibility trials evaluating various ingredients must still be conducted. The resulting information on nutrient requirement, food conversion ratios and nutrient availability will provide nutritionists with comprehensive information to accurately formulate a diet that supplies adequate nutrients.

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