Evaluating Namibian macrophytic algae as dietary source for South African abalone (*Haliotis midae*)

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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the owner of the copyright thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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ABSTRACT

An 84-day study was conducted to find a suitable diet and feeding level for the culture of South African abalone (*Haliotis midae*) in Namibia. Two experimental diets, namely, a seaweed diet (SWD) *Laminaria pallida* (macrophytic algae) and a formulated diet (FD) (macro-algae), for use in abalone (*Haliotis midae*) feed development, were evaluated. The animals used in this study were juveniles (24.33 ± 3.14 mm shell length; 2.72 ± 0.83 g live weight, mean $\pm$ SE) and sub-adults (58.07 ± 10.33 mm shell length and 41.96 ± 20.61 g live weight, mean $\pm$ SE). The nutrient profile of the SWD and FD displayed no differences in the protein and carbohydrate levels. Crude protein levels ranged from 4.91 to 17.68% (dry matter (DM) basis). The lipid levels in the FD (0.25%) were almost 0.56% lower than that in the SWD (0.76%).

The feed conversion ratio (FCR) and protein efficiency ratio (PER) for the sub-adult abalone ranged from 2.80 to 10.90 and 0.10 to 0.40, respectively. The juvenile abalone fed on the FD yielded significantly lower ($P < 0.05$) FCRs (0.8) and higher PERs (1.20) than their counterparts fed on the SWD. A similar trend was observed for the sub-adult abalone although the differences were not significant ($P > 0.05$).

The relative growth rate (RGR) of juvenile fed on the FD was 25% lower compared to those fed on the SWD, while that of the sub-adult abalone fed on the FD was 29% lower compared to the abalone fed on the SWD.

From the daily growth rate (DGR) in terms of daily body weight (DGR_{BW}) calculated after the 84-day period, repeated-measures ANOVA (RANOVA) indicated no interaction between time period and diet. Although slightly lower, the DGR_{BW} for the juvenile abalone fed on the SWD diet (0.033 g/day) did not differ significantly from the DGR_{BW} of abalone fed on the FD (0.079 g/day). In contrast, sub-adult abalone fed on the SWD exhibited significantly higher DGR_{BW} compared to those fed on the FD.

Although the abalone fed on the FD was slightly higher in nutritional content, there was no significantly difference ($P > 0.05$) in the nutritional profile of the abalone soft body tissue fed on either the SWD or FD. There was no significant ($P > 0.05$) difference in preference when comparing the aroma of the abalone meat samples fed on either the SWD or FD. However, there was a significant difference ($P < 0.05$) in the consumers' preference in terms of flavour for the abalone sample fed on the FD. The trained taste panel results indicated that there was no difference in the aroma and flavour of the abalone fed on the different diets ($P > 0.05$).

This study showed that cultured juvenile *H. midae*, readily accepted a FD, producing high consumption and survival rates. The FD still warrants further refinement and testing for it to become a more effective mariculture feed with commercial potential.

UIITREKSEL

'n Studie is oor 84 dae uitgevoer om 'n geskikte dieet en vlakke van voeding vir die kweek van Suid-Afrikaanse perlemoen (*Haliotis midae*) in Namibië te vind. Twee eksperimentele diëte, naamlik 'n seewier dieet (SWD) (*Laminaria pallida,* makrofitiese alge) en 'n geformuleerde dieet (FD) (makro-alge) vir die gebruik in die onwikkeling van 'n perlemoen- (*Haliotis midae*) voer, is geëvalueer. Die diere wat in die studie gebruik is was jong (24.33 ± 3.14 mm doplengte; 2.72 ± 0.83 g lewendige gewig, gemiddeld ± SE) en sub-volwasse perlemoen (58.07 ± 10.33 mm doplengte; 41.96 ± 20.61 g lewendige gewig, gemiddeld ± SE). Die voedingsprofiel van die SWD en FD het geen verskil in die proteïen- en koolhidraatvlakke getoon nie. Proteïenvlakke het gewissel van 4.91 tot 17.68% (droë materiaal (DM) basis). Die vlakke van lipiede in die FD (0.25%) was byna 0.56% laer as dié in die SWD (0.76%).

Die voer omskakelingsverhouding (FCR) en proteïen effektiwiteitsverhouding (PER) vir die sub-volwasse perlemoen het gewissel van 2.80 tot 0.90 en 0.10 tot 0.40, onderskeidelik. Die jong perlemoen op die FD, het betekenisvolle ($P < 0.05$) laer FCRs (0.8) en hoër PERs (1.20) as die ooreenstemmende perlemoen op die SWD, gelewer. Dieselfde neiging is vir die sub-volwasse perlemoen waargeneem; die verskille was egter nie betekenisvol nie ($P > 0.05$).

Die relatiewe groeikoers (RGR) van die jong perlemoen op die FD, was 25% laer in vergelyking met dié op die SWD, terwyl die RGR van die sub-volwasse perlemoen wat met die FD gevoer is 29% laer was in vergelyking met dié op die SWD.

Van die berekende daaglikse groeikoers (DGR) in terme van daaglikse liggaamsmassa (DGR$_{BW}$), het herhaalde-meting ANOVA (RANOVA) geen interaksie tussen tyd en dieet na 84 dae aangedui nie. Alhoewel effens laer, het die DGR$_{BW}$ vir die jong perlemoen op die SWD (0.033 g/dag) nie betekenisvol verskil van die DGR$_{BW}$ van die perlemoen op die FD (0.079 g/dag) nie. In teenstelling was die DGR$_{BW}$ van die sub-volwasse perlemoen op die SWD, betekenisvol hoër as dié van die perlemoen op die FD.

Alhoewel die voedingsinhoud van die perlemoen op die FD effens hoër was, is geen betekeinsvolle verskille ($P > 0.05$) in die voedingsprofiel van die vleis van die perlemeon geien wat op óf die SWD óf die FD was nie. Daar was geen betekenisvolle verskil ($P > 0.05$) in voorkeur toe die aroma van die perlemoenvleismonsters op óf SWD óf FD vergelyk is nie. Daar was egter 'n betekenisvolle verskil ($P < 0.05$) in verbruikersvoorkeur in terme van die geur van die perlemoenmonsters wat op die FD was. Die resultate van die opgeleide proepaneel het geen verskille ($P > 0.05$).in aroma en geur van die perlemoen wat op enige van die diëte was, getoon nie. Die studie het getoon dat gekweekte jong *H. midae*, die FD geredelik aanvaar het en hoê verbruik

en oorlewingskoerse is waargeneem. Die FD het egter verdere verfyning en toetsing nodig voordat dit 'n effektiewe voer met kommersiële potensiaal sal wees.

ACKNOWLEDGEMENTS

DEDICATION

This work is dedicated to my beloved wife Linda and children, *Sweetie*, John-Neville and Mizpah for being there for me during trying times.

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Language and style used in thesis are in accordance with the requirements of the International *Journal of Food Science and Technology*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

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LIST OF ABBREVIATIONS

AD[F, L]	Acid detergent [fibre, lignin]
ANOVA	Analysis of variance
BW, LW	Body weight, live weight
c	Rate constant at which b is degraded
Ca	Calcium
CF	Crude fibre
CP	Crude protein
DDM	Digestible dry matter
DM	Dry matter
DW	Dry weight
EE	Ether extract
EAA	Essential amino acid
EFA	Essential fatty acids
MCRC	Marine and Coastal Research Centre, Hentiesbay
ME (expressed in MJ)	Metabolizable energy (expressed in Mega Joules)
N	Nitrogen
NDF	Neutral detergent fibre
NFE	Nitrogen free extract
OMD	Organic Matter Digestibility
P	Phosphorus
P	Significance level
r	Correlation
R^2	Coefficient of determination
RGR	Relative Growth Rate
SEM	Standard error of mean
ZAR	South African Rand, on par with Namibian Dollar (N$)

CHAPTER 1

INTRODUCTION

Seaweeds, collecting on the Namibian coastline, have become a menace or unwanted waste to the local municipal authorities in terms of cleaning up costs. These naturally abundant seaweed resources are often wrongly regarded as 'weed', thus being referred to as a menace rather than an opportunity (Anon., 2006). On average, more than 1700 cubic meters of seaweed are cleared annually (Anon., 2004a; b). The brown seaweed or kelp (*Laminaria pallida* var Greville) is one of the most abundant natural resources found along the Namibian coastline. Its economic potential, however, remains untapped. These Namibian derived macro-algae, however, have potential to benefit the booming mariculture industry in Namibia.

In most aquaculture operations feeds account for more than 60% of the variable operating costs (Du & Niu, 2003). It therefore remains a high priority for abalone growers to find solutions to enhance their demand for economically viable artificial diets that have ingredients not derived from the marine environment (Jones *et al*., 1996). This would also ensure the development of a sustainable and profitable mariculture industry.

Seaweed do not seem to be efficiently utilised in Namibia (Anon., 2006). *Gracilaria* spp. is the only seaweed species that is exploited on an economical scale for production of agar-agar, which is exported to the Asian markets. Namibian seaweed farming can therefore support the abalone industry if production of feeds could be based on locally available seaweed resources. Studies on beneficial use of seaweed showed that freshly harvested macro-algae can be used as an alternative and economical source of protein for both marine and domestic animals (Levitt *et al*., 2002; Molloy, 1990). The efficiency and cost effectiveness of feed formulations based on different combinations of ingredients being either animal or seaweed based (protein sources) for abalone culture has been studied (Simpson & Cook, 1998). A study by Barkai and Griffiths (1986) demonstrated that wild abalone are opportunistic feeders, thus feeding on a broad selection of algae. The natural diet for *Haliotis midae* is brown and red seaweed, i.e. *Gracilaria gracilis* or *Ecklonia maxima* (Stepto & Cook, 1993; Fleming, 1995). *E. maximum* reportedly has an ideal food conversion ratio (FCR) profile. This ideal food conversion ratio obtained was, however, never experimentally confirmed nor compared when abalone was fed with Namibian derived *Laminaria* spp.

Due to increasing growth of the abalone industry the amount of fresh seaweed available to abalone growers is insufficient to supply the demands in both South Africa and Namibia. Abalone require seaweed, equivalent to about 7% of their body mass per day to produce 100 tons of abalone

of size 50-70 mm in diameter. This requires five tons of freshly harvested seaweed daily (Levitt *et al*., 2002).

A study by Kandjengo and Tjipute (2004) revealed some limitations of *Laminaria* biomass, indicating that lack of supply of fresh or dried kelp will not withstand the anticipated demand. The amount of fresh kelp fronds harvested for abalone feed in South Africa also increased exponentially from less than one ton (wet) in 1992 to more than 6000 tons (wet) in 2003 (Rothman *et al*., 2006). Similar findings by Molloy (1990) also predicted harvesting of *Laminaria* fronds could yield around 300 tons, if harvested in a sustainable way, i.e. if cutting occurs at least 20-30 cm above the base of the secondary fronds. It is estimated that 300 ton could be viable to sustain one medium sized abalone enterprise per year. Harvesting of seaweed for abalone feed involves cutting the fronds during low tides, thus leaving the primary blade and stipe undamaged according to the non-lethal harvesting method developed by Levitt *et al*. (2002). The steepness of the Namibian coastline presents an additional challenge of optimal harvesting of *Laminaria* resources. As a result of these limitations, envisaged abalone farming will suffer greatly because it will have to rely on artificial feed, which could become even more expensive as the demand increases.

Although natural feeds are widely used for abalone farming globally, development of pelleted diets is still regarded as being fundamental for growth of the abalone industry (Britz, 1996a). Therefore over the past decade, there was a trend moving away from natural diets (seaweeds) towards the use of pelleted feeds. The need for nutritionally complete feeds is becoming more essential mainly due to shortage of seaweed supply and logistical problems in harvesting, transportation and storage of seaweed. The artificially developed diet is a compact product, which is more stable and constant, compared to seaweed (Sales & Britz, 2001) and handling and storage is easier. It is therefore vital for artificial feeds to be explored, as the wild stocks of seaweeds will not be able to sustain the abalone industry (Kandjengo & Tjipute, 2004).

In South Africa a range of feeding regimes has been developed for *H. midae*, which indicated that some abalone farms in South Africa relied on harvested seaweed, especially *Gracilaria gracilis*. Sales and Britz (2001) demonstrated that South African abalone growers fed larger abalone either on fish-meal based artificial diets such as ABfeed[TM] (Marifeed, Pty Ltd., South Africa) or relied on the harvested fresh fronts of *Laminaria* in combination with harvested (*Ecklonia maxima*) or cultured seaweed (*Gracilaria*) for certain stages of culture (Barkai & Griffiths, 1986; Knauer *et al*., 1995; Britz, 1996b; Wood & Buxton, 1996; Simpson & Cook, 1998). Fishmeal based diets such as ABfeed[TM], developed for the abalone industry, have consistently proved to achieve the fastest growth rates and food conversion rates from 1.0-6.2 for large abalone under commercial culture. Its continuous usage is limited by price and availability, which make its usage prohibitive for large-scale commercialisation. Similarly satisfactory growth rates were achieved using fishmeal and

silage (Britz, 1996b; Fleming *et al*., 1996; López *et al*., 1998; Shipton & Britz, 2001), soya, spirulina (Britz, 1996b; Shipton & Britz, 2001) and casein (Uki & Watanabe, 1992; Britz, 1996b) as suitable protein sources for inclusion in commercial abalone diet formulations. The high raw material costs, however, make these ingredients economically undesirable. Britz (1996b) outlined some of the advantages of fishmeal based artificial feeds over their natural macro-algae counterparts. The study revealed that artificial feeds are ideal, but not sustainable. Leading workers on *H*. *midae* nutrition stressed the need for further research towards the development of an effective artificial feed based on inexpensive natural raw materials that can be obtained locally (Britz *et al.,* 1994; Fleming *et al.,* 1996). Naidoo *et al*. (2006) tested the effects on growth of mixed diets consisting of combinations of green seaweed (*Ulva* spp.) and red seaweed (*Gracillaria* spp). It was shown that fresh kelp (*Laminaria* spp.) fortified with protein–enriched (farm grown) *Gracilaria* or *Ulva* performed better even when compared to the fishmeal-based commercial diet ABfeed[TM].

To add value to the 'problem' seaweed biomass along the Namibian coastal towns, research and development on potential inter-linkages between the seaweed sector and abalone industry was embarked on by the University of Namibia (Sam Nujoma Marine and Coastal Management Resource Research Centre, Hentiesbay, Namibia) and Stellenbosch University in collaboration with a private company, Taurus Products (Pty) Ltd. (Rivonia, South Africa). The latter company is the largest supplier of fresh seaweed to most abalone farmers both in Namibia and South Africa.

The study aims to determine the viability of feeding farmed abalone (under recirculation system) with *Laminaria* spp., freshly collected under Namibian environmental conditions. It also aims to evaluate an artificial diet prepared from seaweeds [*Laminaria* spp., *E. maxima* (stipes and fronds), *Gracillaria* spp., *Gelidium* spp. (including the epiphyte *G. vittatum*) and *Phorphyra capensis*]. The suitability and efficacy of *Laminaria* and the artificial diet was tested via feeding trials of South African abalone (*H. midae*) at two size classes, i.e. a juvenile (24.33 ± 3.14 mm shell length; 2.72 ± 0.83 g in weight) and sub-adult (58.07±10.33 mm shell length and 41.96 ± 20.61 g in weight) group, respectively.

Specific objectives for this study were therefore to:
   a) **Chemically characterise** the freshly harvested *Laminaria* as well as the artificial diet;
   b) Assess the **suitability** of the Laminaria and artificial diet as abalone feed as well as **leaching and pellet stability;**
   c) Conduct a **feeding trial** at Sam Nujoma Marine and Coastal Management Resource Research Centre (Hentiesbay, Namibia) to evaluate growth rate, feed conversion ratio (*FCR*) and protein efficiency ratio (*PER*);

d) Examine the **effect of dietary nutrients** on live weight gain, protein gain and carcass composition; and

e) **Evaluate sensory** preferences in terms of the abalone flavour and aroma.

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CHAPTER TWO

LITERATURE REVIEW

1. Introduction

Abalone are large algivorous marine mollusks of the genus *Haliotis (Gastropoda, Prosobranchia, Archaeogastropoda and Haliotidae)*. Abalone is distinguished from other gastropods by its gill structure, which draws water through a characteristic pattern of apertures in the shell (Killburn & Rippey, 1982). Haliotids have been recorded as providing a favoured source of protein to humans for nearly 4000 years; especially in Asia (Iwama, 1991). In southern Africa, *Haliotis midae* locally known as 'perlemoen' is the only haliotid that is exploited on commercial scale for export purposes to the Far East as a live, canned, frozen or dried product (Brink, 2002). Worldwide it is one of the most prized seafood delicacies (Mai *et al*., 1995).

As global market prices for abalone rapidly increased over the past two decades, interest was stimulated in South Africa in the aquaculture of *H. midae* (Cook, 1998). Multi-stakeholder processes and partnerships enabled rapid commercialisation of abalone farms in South Africa in the 1990s (Sales & Britz, 2001). However, a steady decline of interest in abalone farming was observed (Attwood *et al*., 2000). This may be due to the fact that both the Namibian and South African marine environment is showing symptoms of degradation or due to competition from other cultured abalone producers.

2. Occurrence and distribution

The largest number of haliotids occurs throughout the world's temperate oceans; living on near shore substrates, reefs and rocky crevices (Leighton, 2000). There are six known endemic South African haliotids that occur along the southern coastal waters, i.e. *H. parvum*; *H. spadicea*; *H. queketti*; *H. speciosa*; *H. pustulata* and *H. midae* (Tarr, 1992). In southern Africa premium green-lipped abalone *H. midae,* is the most important and valuable species for food.

Cape Agulhas, confluence of the Atlantic (minimum of 12-13°C) and Indian Oceans (maximum of 21°C), forms part of the natural habitat of *H. midae* (Britz *et al*., 1997). Thus, Cape Agulhas is regarded as convergence between east and west coast *H. midae* stocks (**Figure 1**) along the South African coast (De Waal *et al*., 2003). *Haliotis midae* populations in the Eastern Cape Province are patchily distributed with slower growth rates and smaller size ranges (Wood & Buxton, 1996) compared to their south-western Cape counterparts (Fielding *et al*., 1995). As abalones are sedentary animals with a relatively short larval dispersal phase, their natural distribution is unlikely to change rapidly (Godfrey, 2003). The distribution of adults is perhaps mainly dictated by the

availability of suitable substrates such as algal food. The concentration of most *H. midae* populations on the south-western coast of South Africa seems to coincide with concentration of the *Gracillaroid* spp. (Iyer *et al*., 2005).
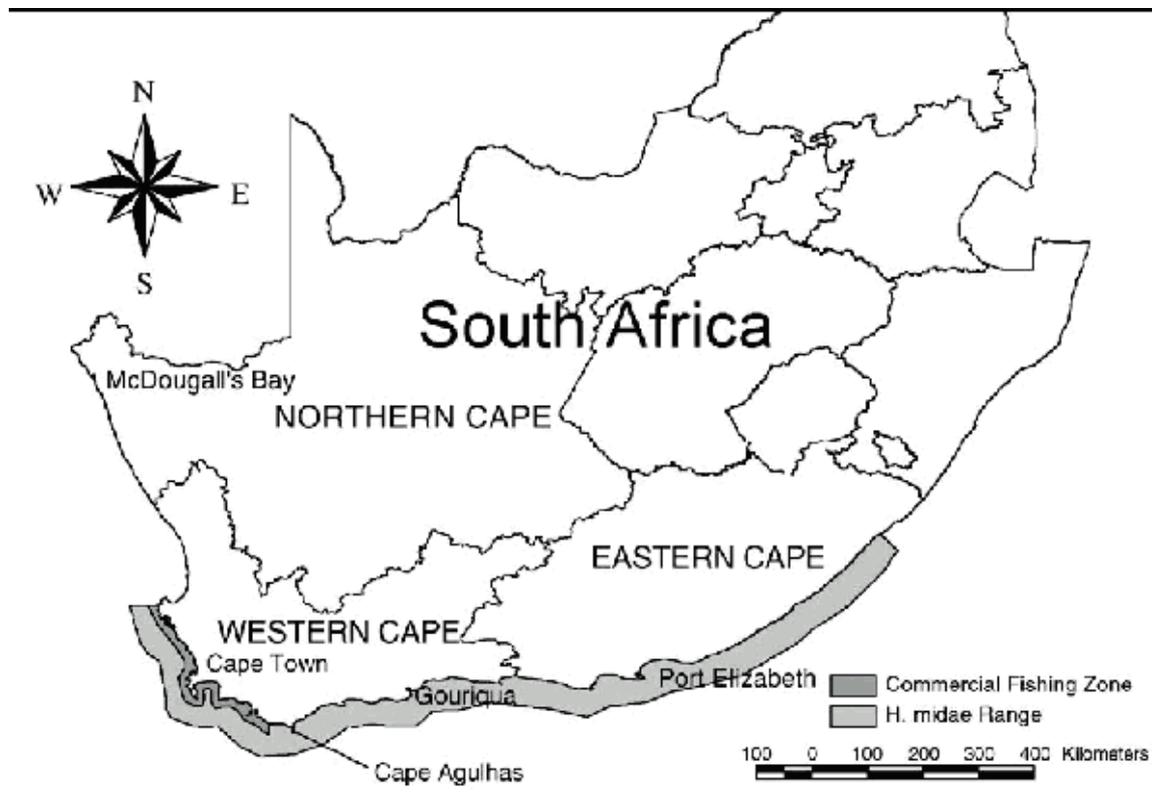
Figure 1 Natural range of *H. midae* along the South African coast. Cape Agulhas is regarded as confluence between east coast (Indian Ocean) and west coast (Atlantic Ocean) *H. midae* stock (De Waal *et al*., 2003).

3. Potential of abalone culture in Namibia

Strong export markets have stimulated great interest in the development of abalone culture in Namibia (Smith, 1949; Mperdempes, 2000). Although *Haliotis midae* does not occur naturally in Namibian coastal waters, conditions are similar to those found in its natural range; and the proximity for transport of brood stock and juveniles have meant that it is the most viable species for mariculture development in Namibia (Anon., 2004a). Development of mariculture in Namibia should take advantage of the country's natural assets, while also taking into consideration the limitations. Presently South African abalone production is depressed, primarily due to poaching, the freshwater aquaculture focus, and prevailing competitive markets that already exist from main aquaculture producing nations such as China, Japan and USA. These trends could be advantageous to the Namibian mariculture sector, particularly if niche markets can be developed.

The only abalone farm currently operating in Namibia imports its entire supply of spat from Hermanus, South Africa (Anon., 2004b). The temperature of the water (12-20°C) along the

Namibian coast line is optimum for growth and culture of *H. midae* species (Sales & Britz, 2001). Although favourable conditions exist to culture the native South African abalone (*H. midae*) along the Namibian coast, the challenge of adopting a viable abalone aquaculture model that suits the Namibian conditions remains.

The culture of *H. midae* has been evaluated extensively in South Africa (Britz *et al*., 1997; Sales, 2001; Shipton, 2000). This is not the case in Namibia, but the aquaculture knowledge developed on culture of *H. midae* in South Africa could be used to develop the technical systems for abalone culture in Namibia. Slinde *et al*. (2002) in his feasibility study also concluded that a potential exists for abalone culture in the Oranjemund ponds (sheltered lagoons created by diamond mining) and possibly sea-ranching in Namibia. Currently, there is limited scientific information (unpublished data) on basic nutrition and micronutrient requirements of abalone in Namibia.

Abalone has a naturally slow growth rate and high costs are involved in holding them in culture systems to harvest size. Generally, artificial diets are seen as crucial to successful abalone farming by those countries whose abalone do not readily grow on brown algae. However, even those who have historically harvested kelp (*Laminaria* spp.) as a food source are now keen to supplement this with artificial diets to enhance growth rates and/or during times of kelp shortage (Fleming *et al*., 1996). Therefore, the identification of cheap, local sources of protein that promote commercial growth rates is essential (Britz, 1995; Viana *et al*., 1993). The abalone feed industry will be demanding high quality raw materials to manufacture feeds, which will meet the optimal nutritional requirement of the animal under culture.

4. Factors affecting the quality of abalone feed

Despite some initial investigations by various researchers on the broad nutritional requirements of abalone, such as protein, lipid, vitamins and minerals (Uki & Watanabe, 1992, Britz, 1995; Coote *et al*., 1996; Shipton, 2000), available information focuses more specifically on the micronutrient needs and amino acid requirements of artificially developed diets for abalone. The latter is, however, still somewhat fragmented and limited. It has been suggested that the development of nutritionally complete artificial feeds is fundamental to the growth of the abalone industry (Fallu, 1991; Britz *et al*., 1994; Allan *et al*., 2000).

4.1 Nutrient requirements of abalone

4.1.1 Proteins and amino acids

Protein is a key ingredient in artificial abalone diets as it is generally expensive. Thus, it must contain the correct balance of amino acids to ensure efficient utilisation and minimum wastage. Abalone, like marine teolosts, that have been investigated for culture, does not only have a total

protein requirement, but requires a well balanced mixture of amino acids (Hecht *et al*., 2003). Generally, optimum dietary crude protein requirement is dictated primarily by the animal's physiological status. It is, however difficult to compare experimental values directly due to different diets, experimental animals and management regimes (Mai *et al*., 1995). Sales *et al*. (2003) suggested that 28–36% dietary protein is needed for optimum growth and feed conversion for *H. midae*. In contrast, Britz (1996) suggested 47% dietary protein for *H. midae* to be optimum. However, various studies in the Far East, albeit on other species, agree well with the former author's findings, suggesting optimum values of 22-35% for both *H. tuberculata* and *H. discus hannai* (Mai *et al*., 1995). Sales & Janssen (2004) illustrated in their review on artificial diets for abalone, in agreement with Mai *et al*. (1995), that contradictory results were reported in studies investigating optimum dietary crude protein levels for certain size classes of some abalone species. Different modes of expression also affect how the information on both optimum crude protein and essential amino acid (EAA) requirements is applied towards practical formulation of cost-effective diets. This could result in completely opposite interpretations (Cowey & Cho, 1993). Thus, a need for further information on optimum crude protein exists; especially for formulated diets derived from fishmeal free ingredients.

Abalone, like any other species, responds best to protein sources of high biological value, primarily those proteins with balanced EAA content. Essential amino acids are those amino acids that cannot be synthesized *de novo*, but must be obtained solely from dietary protein (Lovell, 1989). Shipton (2000) established the limiting amino acid in *H. midae* formulated feeds to be lysine opposed to arginine as the first limiting amino acid.

Factors such as protein source and the protein to energy ratio (P:E) in a diet as well as the size and age of the animal and ambient temperature, amongst others, influence the optimal dietary protein requirement of an animal (Sargent *et al*., 1989). Protein retention in crustaceans is known to be affected by the carbohydrate/lipid balance in the feed (Ackefors, 1992; Kanazawa & Koshio, 1994). Therefore, the only energy source that can be altered significantly for experimental diets is carbohydrate.

4.1.2 Lipids and fatty acids

In a review on the use of feed ingredients in artificial diets for abalone, Sales & Janssen (2004) found that more than three percent lipids might have a negative effect on the digestibility of amino acids. They also reported that supplementation with long-chain fatty acids or essential fatty acids (EFA) such as linoleic (18:2 n-6 LOA) and linolenic (18:3 n-6 LNA) acid may be needed for effective growth of different species of abalone. Australian workers in contrast found that eicosapentanoic acid (20:5 n-3, EPA) and docosahexaenoic acid (20:6 n-3, DHA) are the fatty acids

of greater significance in abalone diets. Thus, both the latter lipids should be included in a diet at more than 0.3 % dry weight.

Lipids play a vital role both in marine and terrestrial animals as high-energy storage molecules and as components of cell membranes (De Silva & Anderson, 1995). According to Hardy (1996, 2003) fatty acid content of marine teolost tissue reflects the fatty acid content of their diet. This presents some challenges for the aquaculture industry when plant and animal lipid sources are used to replace fish oils in diets of farmed fish.

4.1.3 Carbohydrates

Haliotids are naturally herbivorous animals and their digestive systems possess various types of polysaccharide hydrolases (Fleming *et al*., 1996), and bacteria (Erasmus *et al*., 1997) for processing the variety of complex polysaccharides present in plant materials. According to Mai *et al*. (1995) abalone can utilise high levels of dietary carbohydrates (40-50%) to satisfy their energetic requirements. Thus replacing dietary fishmeal with plant protein sources in prepared diets is highly feasible (Sales *et al*., 2003). Usually the least expensive ingredients in the diets supply carbohydrates. As suggested by Monje & Viana (1998) some cellulose may be available to the abalone through hydrolysis of complex polysaccharides present in algae.

4.1.4 Vitamins and minerals

To date little is known about the composition and levels of vitamin requirements for *H. midae,* although there is data recommended by the National Research Council (NRC, 1993) for general fish vitamin requirements. Vitamins are normally added to compound diets in excess to ensure minimum requirements are met, as well as compensating for vitamin losses through deterioration in storage and leaching (Viana *et al*., 1993; Mai, 1998). Such a formulation does not necessarily result in maximum efficiency of nutrient utilisation and profit. The mineral and vitamin requirements of another abalone species, juvenile *H*. *discus hannai* are summarised in **Table 1**.

The vitamin C requirement for haliotids, as with other vitamins, is the amount of vitamin activity required per kilogram of body weight per day to achieve specific physiological responses (NRC, 1993). Vitamin C deficiency leads to abnormal or physiological stresses and diseases. Vitamin C is essential for numerous biochemical and physiological functions such as collagen formation, wound healing, hematopoiesis, and detoxification of compounds in both plant and animal metabolism (Tolbert, 1979). It is assumed that *H. midae* cannot synthesise vitamin C *de novo*, like some primates, fishes and shrimps, therefore it should be supplied in diets. Absence or insufficiency of L-gulunolactone oxidase could attribute to this phenomenon (Lehninger, 1979). L-

gulunolactone oxidase is required for biosynthesis of ascorbic acid from glucose or other simple precursors.

Table 1 Mineral and vitamin requirements (dry matter basis) of juvenile *H. discus hannai* (Sales & Janssen, 2004)

Nutrient	Initial size (g)	Recommended dietary level
Minerals		
Phosphorus	0.62	1.15% available (1.25% total)
Iron	0.70	65-70 ppm
Zinc	0.74	16-18 ppm
Vitamins		
Vitamin K	1.18	10 ppm (as menadione sodium bisulfite)
Thiamin	0.55	51-61 ppm

A required dietary phosphorus level has been established for *H. midae* (Sales *et al*., 2003). When dietary phosphorus leaching and apparent phosphorus digestibility were taken into account, calcium phosphate mono dibasic (72.56%) seemed to be the most promising inorganic phosphorus source for inclusion in abalone diets. Validated results on mineral requirement for *H. midae* are still not yet available and therefore warrants further investigation.

4.2 Digestibility

The determination of the digestibility is the first step in evaluating the potential of an ingredient for use in the diet for aquaculture species (Allan *et al*., 2000). According to De Silva (1989), digestibility studies of naturally ingested foodstuff proved to be useful to evaluate and understand the success or failure of the species under culture. In haliotids, direct digestibility determination is difficult due to problems associated with the accurate measurements of feed ingested and faeces voided (Wee *et al*., 1992). If these problems can be overcome, dry matter coefficients are reliable indicators for energy digestibility for all ingredients (Allan *et al*., 1999).

4.3 Water stability

4.3.1 Pellet integrity

The feed must be stable when immersed, resist leaching of essential water-soluble nutrients into the surrounding seawater, and amenable to consumption. The properties and the performance of two pellet binding agents, alginate and gluten, have been examined to determine the benefits and limitations of moist and dry pellets as a means of consolidating the artificial diet into a stable, cohesive solid form that facilitates ease of consumption by the Haliotids (Knauer *et al*. 1993). Dry,

gluten-bound pellets resisted mass and nutrient leaching better than the moist, alginate-bound pellets; the alginate bound pellets were leached at over twice the rate of the dry pellets. However, this benefit may have been countered by the destruction of heat labile nutrients in the dry pellets because of the high temperatures required during the manufacturing process to gelatinise the starch component of the gluten and provide exterior case hardening.

4.3.2 Nutrient leaching

Nutrient leaching is detrimental to the rearing system and water quality. Thus, consideration must be given to water stability of feed pellets during formulation of the feed (Sales & Janssen, 2004). One of the requirements of abalone feed is that the water-soluble nutrients remain in the feed and the feed particles remain bound together for at least two days (Fleming *et al*. 1996). Knauer *et al*. (1993) proved that hydrocolloids derived from macroalgae, when used as effective binders, were impractical and too costly to be used in commercial abalone diets.

4.4 Production performance parameters

4.4.1 Growth rate

Fishmeal based diets developed for abalone have consistently proved to achieve the fastest growth rates and food conversion rates for large abalone under commercial culture. Its continuous usage is, however, offset by price and availability, which makes its usage prohibitive for large-scale commercialisation. Similarly satisfactory growth rates were achieved using fishmeal and silage (Britz, 1996; Fleming *et al*., 1996; López *et al*., 1998; Shipton, 2000), soya, spirulina (Britz, 1996; Shipton, 2000) and casein (Uki & Watanabe 1992; Britz, 1996) as protein sources for inclusion in commercial abalone diet formulations. Still the high raw material costs make these ingredients economically undesirable. Leading researchers on *H*. *midae* nutrition stressed the need for further research towards development of an effective artificial feed, based on inexpensive natural diets that can be obtained locally (Britz *et al.,* 1994; Fleming *et al.,* 1996).

4.4.2 Feed intake

The large volumes of seaweed required to commercially produce abalone inhibits the use of fresh naturally occurring feeds. Consequently, the majority of abalone farms use artificial feeds, with a proper balance of amino acids, minerals, vitamins and calcium, to replace seaweeds. The feed should, however, hold together in the water for several days and be palatable to the abalone. The feed also has to have a good texture that the abalone will find attractive. The method of nutrient delivery to the target animal is an important component of abalone aquaculture feed development.

The feed must be stable when immersed, resist leaching of essential water-soluble nutrients into the surrounding seawater, and be amenable to consumption by abalone.

5. Potential utilisation of Namibian algal resources in abalone culture

5.1 Algae as natural abalone food source

As a mature animal (Shipton, 2000) *H. midae* subsists entirely on *Laminaria* (kelp) and other seaweeds such as red algae i.e., *Gracilariaceae* and *Rhodophyta*. Similar to most animal species *H. midae* absorb only a fraction of the algal nutrients into its body. The post-larval juvenile haliotids live primarily on diatoms and flagellate micro-algae (Sakata, 1989). Most preferred diatoms are *Cocconeis sublittoralis* (Hendey), *Amphora proteoides* (Hustedt) and *Achnanthes brevipes* (Agardh) (Matthews & Cook, 1995). Diatoms thus remain the principal food until abalone reaches a length of 2-3 mm, which is usually in about 50 days, before changing onto larger seaweeds (Bardach *et al.*, 1990). In South Africa both juvenile sub-adults and adult abalones are fed on harvested *(Ecklonia maxima)* or cultured macroalgae (*Gracillaria*).

5.2 Algae as ingredient in artificial abalone feeds

As the abalone markets are expanding, so does the demand for feeds. The production of compound aqua feeds, particularly feeds for carnivorous finfish species, marine shrimp and abalone species has so far been dependent upon the use of fishmeal and fish oil as cost-efficient sources of dietary protein and fat. Due to the stability of fish meal supply, market forces have acted to reallocate the use of this commodity between competing production systems (poultry, swine, beef cattle and aquaculture (Tidwell & Allan, 2001). However, various pressure groups have recently questioned the use of fish meal in aqua feeds on ethical grounds. This has encouraged the abalone feed industry to more seriously examine the use of alternate protein sources during the formulation process. Currently fishmeal is the main protein source in the pelleted, South African abalone commercial diets (Fleming *et al.*, 1996). Although promising results were obtained for abalone growth when fishmeal was used as ingredient, substantial leaching of protein occurred too (Fleming *et al.*, 1996; Shipton & Britz, 2001). Sales & Britz (2001) indicated that formulated pelleted feeds are often offered in combination with probiotics to aid digestion.

5.3 Distribution of Namibian algae resources

According to Mshigeni (1991) seaweed beds can be defined as heterogeneous assemblages of simple, chlorophyllous, aquatic, macrophytic algae, which grow attached to various objects at varying depths in the sea. Very rich beds of seaweeds can be found along the 1500 km stretch of the Namibian coast (17°30'S and 28°20'S latitudes on the south western coast of Africa), despite being an extension of west coast seaweed biodiversity (flora) of South Africa from Cape Agulhas north-westwards into Namibia (Engledow *et al.*, 1992). Some Namibian species are endemic to south west African waters namely, *Laminaria pallida* var. *schinzii* formerly referred to as *L. schinzii* Foslie (Stegenga *et al.*, 1997) and the macrophyte *Ecklonia maxima*. Both the former and latter species are not found on the east coast of Africa (Mshigeni, 1991).

5.4 Availability of materials

Commercially formulated feeds are available in several countries for culturing abalone. These feeds are principally developed from locally available materials in the respective countries. The Taurus group: Taurus Products (Pty) Ltd. (Rivonia, South Africa) has developed a pelleted abalone feed (FD) that exclusively utilises materials of a beach-cast seaweed origin without any fishmeal, fish oil, or non-natural abalone feed. Such utilisation of natural products is in line with an environmentally friendly production initiative that aims to promote sustainable use of natural resources and prevent environmental pollution. This diet aims to combine the features of a natural diet with the convenience and cost benefits to farm management of a pelleted feed.

A study on New Zealand abalone (*Haliotis iris*) fed on a mixture of beach-cast, aged seaweeds, i.e. *Gracilaria chilensis* and *Macrocystis pyrifera*, respectively, yielded positive results (Gray, 1998) in terms of growth and diet stability. On the contrary Naidoo *et al.* (2006) reported that dried beach cast seaweed does not promote the growth rate of *H. midae* species. Although abalone possesses the ability to consume dried macro-algae quantitative data on its effect on growth rates and quality of the product are not readily available.

6. Conclusion

Historically *Laminaria* spp. was harvested and used as a feed source for abalone. To enhance growth rates and/or during times of kelp shortage, supplementation with artificial diets has become more relevant. Artificial diets are seen as crucial to successful abalone farming. The identification of cheap, local sources of protein for abalone feed is therefore necessary. There is therefore a need for high quality raw materials to manufacture feeds, which will meet the optimal nutritional requirement of abalone.

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NUTRITIONAL PROFILE AND DIGESTIBILITY OF MACROPHYTIC ALGAE (*Laminaria pallida*) OR ON A FORMULATED MACRO-ALGAE DIET FOR SOUTH AFRICAN ABALONE (*Haliotis midae*)

Abstract

This study was conducted to evaluate the nutritional profile of two experimental diets, namely, a seaweed diet (SWD) *Laminaria pallida* (macrophytic algae) and a formulated diet (FD) (macro-algae) for use in abalone (*Haliotis midae*) feed development. The formulated diet was exclusively produced using seaweed and seaweed extracts. Additionally, the suitability of both diets was tested by examining daily feed intake and apparent feed digestibility. Results revealed relatively high variations in nutritional composition in both the SWD and the FD. The FD contained significantly ($P < 0.05$) higher crude protein (17.15%) and total ash (43.13%) compared to the SWD (6.67% and 36.42% for crude protein and total ash, respective). Both diets were low in fat content. It was not possible to determine the apparent digestibility accurately due to difficulties in quantitative faecal collection.

Introduction

Development of nutritionally balanced and cost-effective feeds is dependent on information on the nutritional requirements and feed utilisation of the species. Recently the sole commercial abalone farm in Namibia conducted an experiment, feeding *Haliotis midae* with freshly collected seaweed (*Laminaria pallida*), and obtained positive growth results (Anon., 2004). However, despite the good consumption rates obtained by *H. midae*, there is no quantitative data on the effect on growth rates or quality of the product. Additionally, other studies also clearly demonstrated the significant role played by freshly collected seaweeds and formulated feeds in enhancing growth of cultured abalone (Fleming *et al*., 1996, Sales & Britz, 2001). Nutritional quality of a feed is critical, amongst many other factors affecting growth and survival of abalone. It is hence, a prerequisite for proximate analysis and determination of nutrient digestibility of dietary components to be performed to allow formulation of biologically and economically optimised diets (Alarcón *et al.,* 2002; Sauer *et al*., 2000).

As reported by Akiyama (1991), the feeding value of a nutritional component of a feedstuff depends not only on the quantity of the nutrient in the feedstuff, but also on the ability of the animal to absorb and utilise the nutrient. A nutrient may be present in an ingredient, but nutritionally worthless if it is unavailable to the animal. Serviere-Zaragoza *et al*. (2001) showed that the

nutritional value of diet rations depends on many factors including nutrient composition, bio-availability, palatability and digestibility.

Knowledge on the digestibility of the basic dietary nutrient is necessary for evaluation of the value of foodstuffs and feed ingredients (De Silva, 1989). Feed digestibility, or true digestibility, is the difference between the portion of the feed that is absorbed by the animal and the portion lost in the processes of ingestion and digestion (Bayne & Newell, 1983). The losses are classified as metabolic faecal nitrogen losses, which refer to protein digestibility and metabolic energy losses for energy, which refer to energy, i.e. digestibility (Bayne & Newell, 1983). Apparent digestibility is usually determined by collecting all the faeces and comparing the dry matter consumed with the dry matter in the faeces. Another way to quantify apparent digestibility is by measuring the caloric content or the amount of any single nutrient in the feed and faeces in order to calculate the amount of unabsorbed material (Montaño *et al*., 2002). According to De Silva (1989) digestibility studies of naturally ingested foodstuff proved to be useful to evaluate and understand the success or failure of cultivation of the species under culture.

In haliotids, direct digestibility determination is difficult due to problems associated with the accurate measurements of feed ingested and faeces voided (Wee *et al*., 1992). However, studies by Sales (2000), Sales and Britz (2001) and Knauer *et al*. (1995) established a reliable and replicable technique to determine digestibility for *H. midae*. Apparent protein digestibility of feedstuffs was evaluated for *H. midae* using chromic oxide as internal marker with in vitro techniques (Shipton, 2000; Shipton & Britz, 2001) as well as the acid insoluble ash method as reliable internal marker for digestibility studies (Sales & Britz, 2001). The chromic oxide markers and the total collection methods are the most commonly used methods for digestibility in aquatic species, but have their limitations (Lee & Lawrence, 1997). Since it is difficult to measure the proportion of feed lost by the gut in the process of ingestion, apparent digestibility is more commonly used for determining digestibility (Montaño *et al*., 2002). A study by Britz (1995) also indicated that digestibility trials have achieved little success, hence the requirement to intensify research in areas of repeatable digestibility techniques. Although the total collection method is ideal for digestibility assays (Fleming *et al*., 1996), its use has been eliminated in abalone studies due to difficulties in faecal collection. Haliotids produce faeces that are often not discrete pellets. Artificial diets must also remain bound together for at least two days so that the uneaten portion can be recovered and measured (Fleming *et al*. 1996). Therefore due to difficulties associated with the collection of all the faecal material produced during total collection studies in an aquatic environment, an alternative methodology was developed to validate the results obtained from the chromic oxide recovery study (Shipton, 2000; Shipton & Britz, 2001).

The purpose of this study was to determine and compare the chemical composition of both the seaweed diet (SWD) and formulated diet (FD) as a source of protein and other nutrients for growing abalone (*H. midae)* under Namibian conditions. Furthermore, the apparent digestibility coefficients (ADCs) (dry matter, organic matter and energy) were evaluated in *H. midae*. The study also attempted to validate the most suitable method of measuring digestibility in abalone, taking into account difficulties in handling the animals and, where possible, aimed to reduce the number and size of faecal samples needed. Therefore, the protocol developed by Shipton (2000) was tested to verify the faecal collection and leaching for possible determination that could aid food intake in *H. midae*. A simple and inexpensive method by Helland *et al*. (1996) for determining daily feed intake was partially incorporated into the protocol developed by Shipton (2000) for efficiency. Although the FD was not expected to be an optimal diet for abalone culture, enhancement of a defined and reproducible formula was envisaged for establishment of a reference diet for future trials under local Namibian conditions.

Materials and methods

Experimental diets

Stipes and fronds from macrophytic algae, the brown seaweed *Laminaria pallida* (Greville) and *L. pallida* var *schinzi*, were collected freshly every morning as wash-ups or beach casts and constituted the SWD. The FD was an all-seaweed (macro-algae), commercially formulated diet in the form of dried dark grey pellets (20-30 mm in length, 18 mm wide and 2 mm thick). The main ingredients of the FD were the farmed species, *Gracilaria gracilis* (*ca*. 80%), *Laminaria* spp. (low in crude protein), *Ecklonia maxima*, *Gelidium* spp. (including the epiphyte *Gelidium vittatum*, formerly known as *Suhria vittata*), *Phorphyra capensis* and agar-agar (high in crude protein). (K. Laufer, Taurus Products (Pty) Ltd., personal communication). The FD was manufactured and provided by Taurus Seaweed Company (Pty) Ltd. The detailed formula and production is subjected to a patent application.

Nutritional profile of experimental diets

The proximate analysis of the respective feed samples (SWD and FD) was performed according to official methods (AOAC, 1983); ash by incineration at 550ºC for 16 h; moisture content by oven drying at 72 ºC for 16 h; crude protein (N x 6.25) by Kjeldahl method after acid digestion; crude fat after extraction with petroleum ether by the Soxhlet method (James, 1996); crude fiber by the Weende method; and organic matter calculated as dry matter minus ash. Gross energy was determined by calorimetry (IKA Adiabatic Calorimeter C4000A). Specific amino acid content was determined by hydrolysing a sample after which the constituent amino acids were isolated by means

of HPLC under gradient conditions and purified by partition chromatography (Cowey *et al*., 1970). Micro-minerals were quantified by the ICP method.

Faecal collection system

Experimental system and water quality

A 14 day experiment was conducted at the Sam Nujoma Marine and Coastal Research Centre, Hentiesbay Laboratory using an indoor recirculation system consisting of a series of 15 glass holding tanks (40 L capacity) (**Figure 3.1**). A settlement tank of 300 L capacity, as well as primary and secondary biological filters, was used for maintenance of water quality. Approximately 25% of water replacement of the system volume per day occurred. Flow rate in the tanks was maintained at 1 L/min while water-flow through the jars (PVC holding container) was maintained by the use of air-stones. Water temperature was recorded twice daily (morning and afternoon) while water quality parameters were measured on a daily basis. Temperatures were recorded using a HOBO H8 data logger. Mean water temperatures and mean salinity were maintained at $17.31 \pm 0.87^{o}C$ (mean $\pm$ SE) and $33.11 \pm 1.59\%$ (mean $\pm$ SE), respectively. Water quality parameters such as free ammonia and nitrite were determined according to official methods (AOAC, 1995) while pH was measured using a calibrated pH meter (Jenway, U.K). Ammonia, nitrite and nitrate levels were found to be negligible (<0.01 mg/L) while the pH remained consistent at 8.05 ± 0.06 (mean $\pm$ SE). Dissolved oxygen (Oxygen meter, YSI, model 58) was 7.55 ± 0.11 mg/L (mean $\pm$ SE). A 'natural lighting' photo-period was used throughout the experimental period. Lights were only turned on during cleaning, feeding and sampling, which occurred during the normal hours of daylight.

Experimental animals

All animals (n=12) used in this study were juveniles (n=6) (24.33 ± 3.14 mm shell length; 2.72 ± 0.83 g live weight, mean $\pm$ SE) and sub-adults (n=6) (58.07 ± 10.33 mm shell length and 41.96 ± 20.61 g live weight, mean $\pm$ SE). Lüderitz commercial abalone farm imported the spawned abalone from commercial abalone hatcheries (AbaSeed, Hermanus, South Africa). At the Lüderitz farm, the imported abalone were acclimatised for nearly one month and weaned and fed a maintenance diet comprising natural fresh seaweeds (*Gracilaria* spp*., Ecklonia maxima and Laminaria* spp.) (K. Laufer, Taurus Products (Pty) Ltd., personal communication). These abalone were then transferred to the University of Namibia (UNAM) Sam Nujoma Marine and Coastal Research Center at Hentiesbay, Namibia. Six abalone per treatment (juvenile and sub-adult) were then placed in the PVC holding containers and housed in 3 fibre-glass experimental containers (30x50x26 cm, 40 L volume). These two containers were then placed into the glass holding tanks. Slight modifications were done, compared to experimental protocol as described by Shipton (2000). Within the

experimental containers, three replicate groups of animals were housed in perforated 2 L plastic jars. Tanks were randomly allocated to treatments; 3 replicates per diet, i.e. SWD and FD,
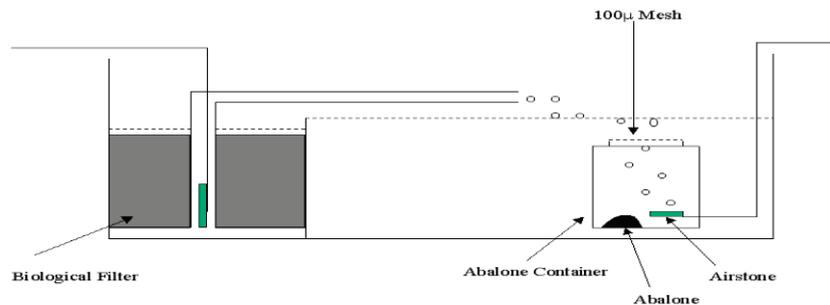
respectively.

Figure 3.1 Experimental system in which faecal collections were performed (Shipton, 2001).

Faecal collection

Quantitative collection of faeces was undertaken in perforated 2 L plastic jars that were placed in a water bath, maintained at 18°C. As it was necessary to collect all the faecal material produced during these trials, there was no movement of water between the jars, containing the abalone, and the water bath; and in addition, to prevent the loss of faecal material via filtration, there was no water filtration within the jars. Faecal samples were obtained every 12 h for a period of 84 h. Faecal samples were collected by lifting the glass jar that housed the abalone from the water and allowing the water to drain through the mesh which retained the faecal material. The abalone was then washed twice with a small amount of seawater to remove any faecal material that may have become attached to the shell of the animal. The washing water was filtered through the mesh, thus ensuring that all the faecal material was collected. The mesh was placed on a piece of paper towel to absorb excess water. The faeces were placed on aluminium foil and oven dried at 60°C for 24 h, stored at -20°C, and the weights of each faecal type produced over the 84 h period recorded. Frozen faeces were stored until the end of the 14-day collection period where after they were freeze-dried and analysed according to official methods (AOAC, 1983). Faeces weight was recorded to the nearest 0.01 g using an electronic balance.

Dry matter digested and apparent digestibility

The dry matter digested was determined as the daily feed intake. Daily approximation of feed intake was done using an estimate of dry matter content of waste feed as obtained in the recovery test according to the formula from Helland *et al*. (1996) (equation 3.1).

Air-dry feed eaten (g)

$$= [(A \times A_{DM} /100) - (W \times W_{DM} /R)] / A_{DM} / 100 \qquad \text{… equation 3.1}$$

where

A = initial air-dry feed (g)

A_{DM} = dry matter content of air-dry feed (%)

W = weight of waste feed collected (g)

W_{DM} = dry matter content of waste feed

R = the recovery of dry matter of waste feed (equation 3.2)

Recovery (R) (%) $\qquad \text{… equation 3.2}$

$$= (W \times WDM) / (A \times ADM) \times 100$$

where

$(W \times WDM)$ = final dry weight of feed (g)

$(A \times ADM)$ = initial dry weight of feed (g)

Correction factors for total solids and protein leaching were determined for each diet by placing 4 g of feed in a control container containing no abalone and placing it in the aquaria for a 12 h period. Total solids leached were calculated as dry weight loss over this period. Correction factors for total solids leaching from the diets were 0.76 and 0.77 for the SWD and FD diets, respectively. Correction factors were used where appropriate during the quantitative trials.

Statistical analyses

Results for dry matter leaching of diets over time and thus dry matter digestibility were subjected to one-way analysis of variance. Differences between means were evaluated by the Tukey's HSD test ($P < 0.05$). Unpaired *t*-tests were used to compare dry matter and nutrient leaching at different times.

Results

Nutritional profile of experimental diets

Nutritional, mineral and amino acid composition of the SWD and FD on a dry matter (DM) basis are shown in **Table 3.1**. The nutritional composition of the SWD and FD displayed marked differences

in their protein and carbohydrate levels. The protein content of the FD was 17.15% compared to 6.67% in the SWD; the difference being significant ($P < 0.05$). In contrast the lipid content of the FD diet (0.24%) was 0.09% lower ($P > 0.05$) than that of the SWD feed (0.33%). The carbohydrate levels were significantly higher (40.32%) in the SWD diet ($P < 0.05$) in comparison to the FD (24.17%). A high ash content for both FD and SWD was observed (43.13% and 36.42%, respectively). The amino acid profile of the two feed diets (FD and SWD) were similar ($r^2 = 89\%$). The exact formulation and production process of the FD is subjected to patent application and can therefore not be revealed.

Faecal collection

The faecal production, from both the SWD and FD over the 84 h time period, is shown in **Table 3.2**.

Dry matter digestibility

The dry matter digestibility of the SWD and FD on a dry matter (DM) basis is shown in **Table 3.1**. The dry matter digestibility of the SWD (92.57%) was significantly lower ($P < 0.05$) than that of the FD (82.37%).

Discussion

The nutritional profile of both the diets (SWD and FD), in spite of the differences observed, corresponds to values reported for other species of seaweeds used as feed for abalone (Viena *et al*., 2005). The protein content (17.15% DM basis) of the FD was within the reported range for red seaweeds (10-47% DM basis) (Fleurence, 1999) and similar to that found in the red seaweed, *Palmaria palmata* (18.4%). *Palmaria palmata* has been found to be a good algal diet for *H. tuberculata* (Mercer *et al*., 1993). The protein content of the SWD (6.67% DW) was within the range reported for other species of *Hypnea* (4.2-19% DM basis) (Wong & Cheung, 2000). However, the protein content of both the SWD and FD were lower than the protein requirements described for several species of Haliotids such as *H. discus hannai* (20-30%) (Uki *et al*., 1986) and *H. midae* (47%) (Britz, 1996). Various studies (Britz, 1996; Mai *et al*., 1995; Sales *et al*., 2003) investigated the optimum dietary protein level for certain different of certain abalone species and suggested that 28-36% dietary protein is needed for optimum growth for *H. midae*. Both SWD and FD contained less than 20% protein, which is considered insufficient. This requirement for dietary protein is not yet conclusive for *H. midae* thus warrants further investigation.

Table 3.1 Nutritional, mineral and amino acid composition of the seaweed diet (SWD) and formulated diet (FD) on a dry matter (DM) basis

Components	SWD (Control)	FD
Crude protein (%)	6.67 ± 1.59 a[*#]	17.15 ± 1.21 b
Gross energy (MJ/kg)	10.18 ± 0.26 a	10.06 ± 1.08 a
Crude fat (%)	0.33 ± 0.53 a	0.24 ± 0.02 a
Ash (%)	36.42 ± 2.01 a	43.13 ± 2.83 b
Carbohydrates (%)	40.32 ± 1.86 a	24.17 ± 2.37 b
Moisture (%)	8.77 ± 1.87 a	10.57 ± 0.49 a
Fiber (%)	7.45 ± 0.21 a	4.74 ± 0.72 a
Digestible dry matter (%)	92.57 ± 6.34 a	82.37 ± 13.95 b
Vitamin C (mg/100 g)	7.3	0.9
Minerals		
Calcium (%)	1.70	2.22
Phosphorus (%)	0.38	0.30
Cobalt (%)	4.50	ND
Zinc (ppm)	28.70	30.80
Copper (ppm)	10.75	10.60
Manganese (ppm)	8.75	40.75
Iron (ppm)	645	368
Sodium (ppm)	4.10	2.54
Magnesium (ppm)	1.33	1.17
Amino acids (% of total protein)		
Arginine	1.30	1.10
Histidine	3.30	4.50
Isoleucine	5.60	9.00
Leucine	4.20	4.50
Lysine	3.50	4.50
Phenylalanine	ND	2.20
Methionine	4.60	6.70
Threonine	3.50	6.70
Valine	3.50	6.70
Aspartic acid	9.90	2.20
Serine	4.90	6.70
Glutamic acid	3.70	5.60
Proline	5.70	7.90
Glycine	5.50	12.3
Alanine	3.50	4.50
Tyrosine	ND	1.10

[*]mean ± SE

[#]Values in the same row with different superscripts differ significantly ($P < 0.05$)

Table 3.2 *H. midae* faecal production over 84 h time period (g/100 g).

Time (h)	Formulated diet (g/100 g) (FD)	Seaweed diet (g/100 g) (SWD)
12	$0.09 \pm 0.16^{*}$	0.48 ± 0.22
24	0.03 ± 0.03	0.52 ± 0.18
36	0.34 ± 0.03	0.54 ± 0.58
48	0.60 ± 0.29	0.31 ± 0.12
60	0.61 ± 0.39	0.54 ± 0.50
72	0.49 ± 0.16	0.38 ± 0.25
84	0.49 ± 0.18	0.48 ± 0.15

[*]Mean $\pm$ SE, n=6 replicates

The significantly higher carbohydrate values for the SWD compared to the FD (40.32% and 24.17%, respectively) were comparable to those obtained for other algal species (Viena *et al*., 2005). Dietary carbohydrates levels should be substantial for slow eaters such as abalone which can utilise high levels of dietary carbohydrates (40-50%) to satisfy their energetic requirements (Mai *et al*., 1995). Carbohydrates enhance growth (Thongrod *et al*., 2003) of abalone. Haliotids have various enzymes capable of hydrolysing complex carbohydrates (Fleming *et al*., 1996) and a good capacity to synthesise non-essential lipids from carbohydrates.

Lipid levels in the FD (0.24% DM basis) and SWD (0.33 % DM basis) were low compared to other species of red seaweeds (2-4% DW). High levels of dietary lipid may negatively affect abalone growth. Various studies on lipid inclusion levels from 1-11% (as is basis) in Australian abalone diets suggested that levels in excess of 4% would inhibit growth (Anon., 2001). Variations in fatty acid compositions were noted indicating use of oils varying from purely vegetable sources to those from marine origin. It was shown that very low lipid content (<2% DM) in seaweed *Gracillaria ramulosa* cf *cliftonii* yielded positive growth rates when fed to abalone (Anon., 2001). Lipid content higher than 4% (DM) would suppress growth and increase the fat content of the abalone (Anon., 2001). The main significance of this finding is that additional lipid could be included in the FD if deemed necessary to provide essential unsaturated fatty acids.

According to Hay *et al*. (1994) high ash content in algae results from the calcium carbonate, which limits the other nutrients' presence and reduces nutrient digestibility. A high ash content was observed in the present study for both the FD and SWD (43.13% and 36.42%, respectively).

The lower dry matter digestibility of the FD compared to the SWD could be due to its high ash content (43.13%). According to Wee (1992) nutrient digestibility decreases with an increase in carbohydrate structural complexity. Dry matter digestibility coefficients are reliable indicators for energy digestibility for all ingredients (Allen *et al*., 2000).

Of particular importance, in the present study, was the similarity in the amino acid profile (R^2 = 89%) of the two diets (SWD and FD). Similarities between protein and energy efficiency were low (R^2 = 0.37 and 0.27, respectively). With regards to the essential amino acids the two most important sulphur-containing amino acids were observed to be lacking in the SWD. The FD diet had adequate levels of both methionine (2.2 g/100 g) and cystine (1.1 g/100 g), respectively. Wilson (1989) considered cystine to be a non-essential amino acid as it could be synthesised from the essential amino acid, methionine. Therefore if methionine is fed without cystine, a portion of the methionine is used for protein synthesis, while a portion is converted to cystine. Converted cystine is then incorporated into protein as cystine. In accordance with the present results, it is evident that due to the lack of a cystine-methionine relationship in the SWD, the test animal will also lack the total sulphur amino acid requirement. If cystine was present in the SWD, then the amount of methionine present could have been reduced. Evidently lower vitamin C levels were detected in FD (0.9 mg/100 g) compared to that in SWD (7.3 mg/100 g). This could be due to drying or the hot pelleting process used during manufacture as vitamin C is heat labile. Therefore it could be corrected quite easily by adding a small amount of vitamin pre-mix to the pellets. This vitamin can not be synthesised *de novo* by most mollusks, humans, primates, insects and fish due to absence of L-gulunolactone-oxidase that converts L-gulunolactone into ascorbic acid. Therefore supplementation with high levels (320 ppm) of vitamin C (NRC, 1993) is essential for normal growth and immune system enhancement. The data on mineral and vitamin content in the present study could not be used to validate the composition and levels of vitamin and mineral requirements for *H. midae*, the values seems to be higher than that published (NRC, 1993). As the rule of thumb, vitamins are often added in excess (Mai, 1998; Viana *et al.*, 1993), but this does not result in maximum efficiency of nutrient utilisation.

Faecal collection

The major constraint in formulating cost-effective diets is the lack of information on nutritional requirements of slow feeders like Haliotids and the digestibility of suitable feed ingredients. The approach followed in the present study did not yield sufficient representative quantitative faecal material to obtain reliable results. Quantitative faecal collection is required (Maynard & Loosli 1969) for valid interpretation of the results. Unfortunately, insufficient amounts of faeces were available to perform nutrient analyses. Difficulties associated with weighing were experienced after the collected faeces were freeze-dried. Some studies on Haliotid faecal material also experienced difficulties during collection of faecal material. Extremely high ash contents (>56%) were attributed to the failure to obtain gross energy values (Sales & Britz, 2002). Thus, the protocol by Sales & Britz (2001) allowed accurate intake measurements for research purposes and for commercial

operations, without ascertaining biomass in tanks or temperature regimes, but still warrants alternatively adjustments of procedures suited for Namibian culture conditions.

Conclusion

It appears from the nutritional profile of the FD that it can be classified as adequately having a suitable profile for successful abalone culture under Namibian conditions. In addition the advantage of the low raw material costs, all season availability and a favourable amino acid profile suggest that the FD has potential to become an important protein source in commercial abalone feed formulations. Reduced levels of vitamin C, which could have adverse effects on the health and nutrition of both juvenile and sub-adult abalone could be corrected by adding a vitamin premix. Thus the FD offers positive encouragement for the potential of successful formulated feed development; more specifically for rearing small abalone. However, the main challenge still remains for determining the nutritive value of most of the locally derived seaweed in a cost effective manner and to collect efficient feedstuffs for rearing abalone in Namibia. The present study failed to define the digestibility of the experimental diets primarily due to insufficient faecal material. Standardised precise and correct protocols aided with markers need to be applied for future digestibility studies under local environmental conditions.

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CHAPTER FOUR

WATER STABILITY OF MACROPHYTIC ALGAE (*Laminaria pallida*) OR ON A FORMULATED MACRO-ALGAE DIET FOR SOUTH AFRICAN ABALONE (*Haliotis midae)*

Abstract

The 24 h water stability trial demonstrated that the formulated diet (FD) had an excessive leaching rate (66.71% $\pm$ 4.32, mean $\pm$ SD) compared to the seaweed diet (SWD) (9.14% $\pm$ 1.67, mean $\pm$ SD). A pH nutrient leaching experiment also showed a similar rapid decline in pH values over the first 24 h period. The SWD decreased with 1.6 (mol/L) compared to 2.04 (mol/L) for the FD diet. A preliminary study has revealed the FD diet as a promising juvenile abalone feed.

Introduction

Natural feeds for feeding haliotids are constrained by factors such as seasonality and cost in terms of harvesting, storage and handling (Britz, 1995). Thus to maintain a productive and cost-effective abalone culture operation, a cost-effective and reliable feed must be developed which is both acceptable and enhances growth and survival of the abalone under culture. Pelleting is increasingly becoming the most economical and efficient method of producing mariculture feeds (Flores & Valdes Martinez, 1993). Formulated and pelletised feeds were shown to offer convenience and cost benefits to abalone farms (Britz *et al*., 1994) as opposed to using only seaweed sources. Conklin *et al*. (1983) and Kanazawa (1994) stressed the need of further refinement of effective formulated diet development. Most formulated diets are still not as successful as natural (seaweed) diets mainly due to higher manufacturing cost per unit compared to seaweed harvesting (Britz *et al*., 1994).

Despite the advantages, understanding the availability of nutrients in a feed ingredient is an essential requirement for formulating least-cost diets. Therefore a way of minimising feed costs is through improved diet development by more effective use of available nutrients in feed ingredients to satisfy the nutritional requirements of the animal (Sales, 2001). Abalone can consume seaweed at a rate close to 35% of its body weight per day (Tahil & Juinio-Menez, 1999). It therefore requires a large amount of fresh macro-algae to sustain abalone growth. Haliotids is known to readily consume a wide variety of formulated diets (Britz *et al*., 1994; Britz, 1995; Fallu, 1991; Fleming *et al*., 1996) with superior feed conversion ratios (*ca.* 1-1.5:1) compared to seaweed (*ca.* 12-15:1). Studies by Britz *et al*. (1994) and Shipton (2000) suggested that pelletised formulated diets are additionally free of the problems associated with natural foods (seasonality, rancidity, harvesting, transport and storage), ABFEED (a natural abalone feed developed in South Africa) is to date the

most advanced, cost-effective and reliable feed for abalone under intensive culture (Sales & Britz, 2001). However, it contains fishmeal ingredients which is costly and may become unsustainable.

An important requirement of abalone feed is that the water-soluble nutrients must remain in the feed and the pellet must remain intact for at least two days (Fleming *et al*. 1996). Leaching of nutrients from feed (Allan *et al*., 1999) may lead to over-estimation of feed digestibility. Nutrient leaching is also damaging to the rearing system and water quality. Hence consideration should be given to methods of reducing leaching of nutrients. The present study investigated the leaching of nutrients of two diets in seawater: a seaweed diet (SWD) and a formulated diet (FD).

Materials and methods

Experimental diets

Experimental diets were as described in Chapter 3.

Nutritional profile

Nutritional profiles were determined as described in Chapter 3.

Water stability test (dry matter leaching)

Water stability of the SWD and FD was determined at 4, 8, 12, 16, and 24 h, respectively (Anon., 1994). Three replicates samples (2-3 grams) of each of the SWD and FD were placed inside oyster netting baskets (net floor with 8 x 6 mm mesh aperture) and submerged in separate 300 L glass-fibre tanks in a recirculating system. There was one basket for each treatment per tank. A seawater flow rate of 1 L/min was maintained at a constant temperature of 18 ± 2°C. After each of the respective submersion periods the samples were gently removed from the tanks and rinsed with deionised water to remove any salt residue. The samples were subsequently dried according to AOAC (1983) procedures. Percentage weight loss (water stability) was calculated using differences between the respective initial and final weights (equation 4.1).

Weight loss = final dry weight (g) of feed / initial dry weight (g) of feed x 100   … equation 4.1

pH measurements

Two gram samples of each of the SWD and FD were placed in 50 mL of seawater at 18 ± 2°C (**Figure 4.1**), while pH changes (Microcomputer pH meter, Jenway, UK) were monitored at 12 hourly intervals for 72 h. Measurements were performed in triplicate.

Statistical analyses

The results for both water stability (dry matter leaching) of the SWD and FD and effect of diets on pH of the water were reported as the average mean ± standard deviation (SD). The average mean values were compared using Student *t*-tests, and differences at $P < 0.05$ were considered significant.

Results

Nutritional profile

The nutritional profile of the diets (SWD and FD) is reported in detail in Chapter 3.

Water stability test (dry matter leaching)

The SWD diet was found to be extremely water stable retaining 87.08% of dry matter (DM) after being submerged in the water for 24 h (**Figure 4.2**). The pellets of the FD lost their shape after 24 h, with excessive crumpling or disintegration whereas the SWD diet retained its shape with minimal crumbling or disintegration. Approximately 58.63% of the DM for the FD was thus lost after being submerged in the water for 24 h, whereas only 12.92% loss was observed for the SWD.

pH measurements

Water quality can be monitored by means of measuring ammonia, nitrite and nitrate contents and change in pH. These can also be used as possible indicators of DM leaching from diets. Both diets (SWD and FD) showed a significant decrease ($P < 0.05$) in pH values over the first 24 h (**Figure 4.3**). The pH values of the water in which the SWD pellets were submerged did not decrease to the same extent (pH value = *ca.* 6.8) as the FD diet (pH value = *ca.* 5.9). The pH of the latter was significantly lower ($P < 0.05$) as the SWD after 24 h. Between the 24 to 36 h time period, the pH values of both diets reached a plateau after which the values for the SWD decreased whereas that of the FD increased. As from 60 h submersion both diets stabilised at a constant pH value of *ca.* 6.5. This stabilisation at 60 h interval could suggest that almost all the dietary nutrients might have leached out of the diets by this time.
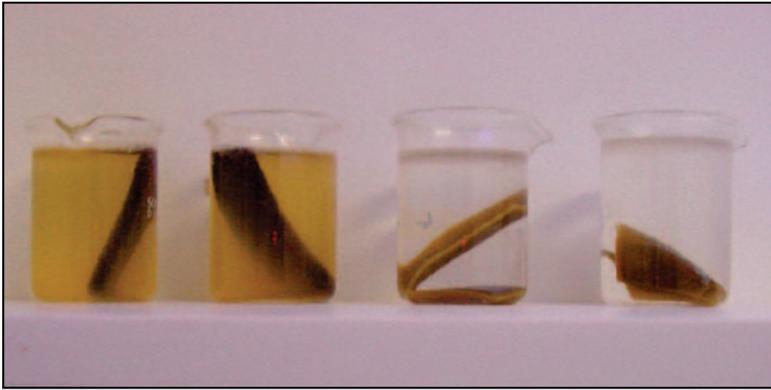
Figure 4.1 Dry matter leaching over time of the formulated diet (FD) (darker solution, left) and the seaweed diet (SWD) (transparent solution, right) tested at 12 h interval.
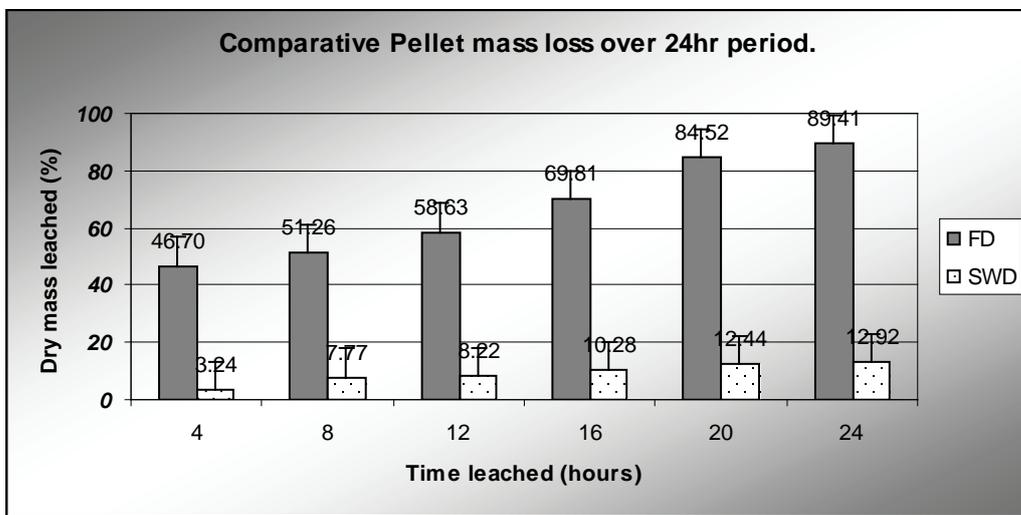
Figure 4.2 Dry matter leaching of SWD and FD feed samples over 24 h submersion in seawater.
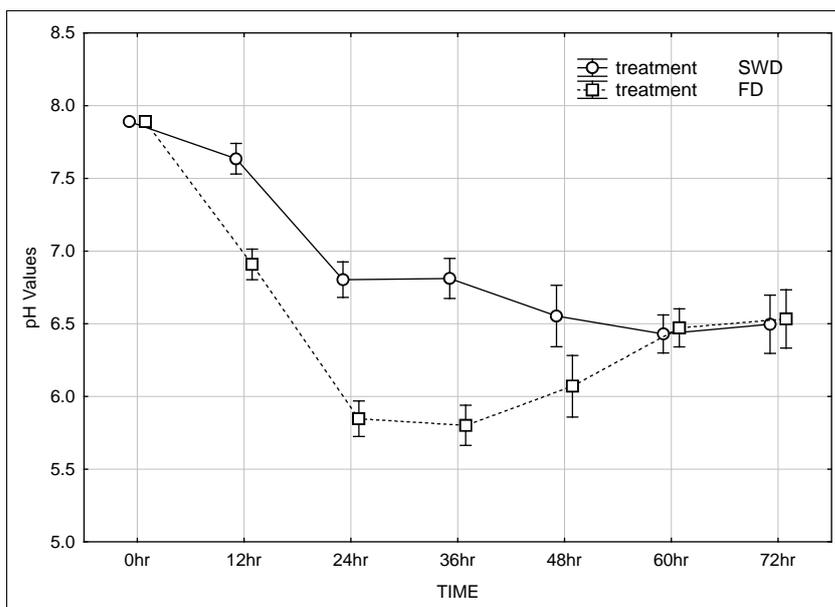
Figure 4.3 Difference in average pH values over 72 h period of the leaching water of the formulated diet (FD) and the seaweed diet (SWD). Vertical bars indicate 95% confidence intervals ($P < 0.01$).

Discussion

The pellets of the FD leached dry matter at more than five times the rate of the the SWD. As expected the dry matter leaching (rate of weight loss) of both diets increased as a function of time. With slow feeders like abalone the stability of feed pellets in water for prolonged periods is crucial.

Monitoring of pH values indicated that the water quality was not significantly affected by the presence of disintegrating feed pellets of the FD. The rate of dietary nutrient leaching, indicated by the differences in the pH of the leachate, revealed that essential dietary nutrients leached rapidly during the first 24 h of immersion, causing a pH decrease proportionally (**Figure 4.1**). Basic amino acids such as lysine have an isoelectric point of 10.7, thus if leaching occurs, an increase in pH should occur (Shipton *et al*., 2002). Thus based on the latter principle, change in pH could be used as a good indication of possible leaching of nutrients such as the basic amino acids. A study by Britz *et al*. (1997), to obtain an indication of the rate of arginine leaching by monitoring pH change, proved to be effective. Arginine is a strong basic amino acid (isoelectric point = pH 11.1) and thus causes a pH rise when it leaches into water (Britz *et al*., 1997). The difference between the pH of the leachate, control (no supplemental arginine) and test diets was thus used as a measure of arginine leaching. The isoelectric point is the pH at which a particular molecule or surface carries no net electrical charge. Thus, in the present study it could be assumed that seawater (control), while any change observed in pH values of test diets (FD and SWD) could imply potential loss in dietary nutrients.

It is, however, noteworthy to emphasise that the pelleting process needs to be re-evaluated to determine the optimal manufacturing technique/s required to extrude a cohesive formulated diet. Testing of various pellet sizes of the same feed could also be done to determine optimum pellet size in terms of dry matter leaching. Smaller pellet sizes might pose problems for feeding larger abalone classes, due to rapid wastage.

Conclusion

It is suggested that this research be extended to also include a detailed nutrient analysis of pelleted diets after being submersed in water to determine not only the extent of nutrients lost during manufacturing process, but also after subsequent immersion in seawater over an extended period of time.

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CHAPTER FIVE

GROWTH RATES AND CHEMCIAL COMPOSITION OF SOUTH AFRICAN ABALONE (*Haliotis midae*) RAISED ON MACROPHYTIC ALGAE (*Laminaria pallida*) OR ON A FORMULATED MACRO-ALGAE DIET

Abstract

The effect of two experimental diets, a seaweed diet (SWD) and a formulated diet (FD) on growth rates and chemical composition of juvenile and sub-adult South Africa abalone (*Haliotis midae*) was evaluated over an 84-day trial period. There were no significant differences observed in the efficiency of protein (PER) and feed conversion (FCR) for all treatments within the same treatment but marked distinction among the treatments ($P < 0.05$). At the end of the 84-day feeding trial, the abalone were weighed and six abalone per treatment randomly sampled which were euthanized for body composition analysis. The relative growth rate of juvenile (24.33 ± 3.14 mm; shell length; 2.72 ± 0.83 g in weight) fed on the test diet (FD) was 25% lower; while that of sub-adult class (58.07 ± 10.33 mm shell length and 41.96 ± 20.61 g) was 29% lower compared to the SWD. From the DGR_{BW} calculated after the 84-day period, repeated-measures ANOVA (RANOVA) indicated no interaction between time period and diet. Although slightly lower, the DGR_{BW} for the juvenile abalone fed on the SWD diet (0.033 g/day^{-1}) did not differ significantly from the DGR_{BW} of abalone fed on the FD (0.079 g/day^{-1}). The proximate composition of the SWD and FD feed diets showed no differences in the protein and carbohydrate levels. Crude protein levels ranging from 4.91 - 17.68% respectively on a dry matter basis, were fed to six tanks (two treatments per tank) containing five abalones per treatment each in a continuous flow system. The lipid levels in the FD diet (0.25%) were almost 0.56% lower than in the SWD feed (0.76%). Whole body composition (moisture, crude protein, crude fat, crude fiber, nitrogen free-extracts and ash content) of abalone soft-body tissue fed SWD and FD diet was not significantly different ($P > 0.05$). Body composition analysis showed that abalone had more fat (lipid) when fed FD than SWD diets. EAA profiles of SWD ($r^2 = 0.82$) and FD ($r^2 = 0.72$) were positively correlated, respectively. Present study showed that cultured juvenile *Haliotis midae*, readily accepted a formulated ration (FD), producing high consumption rates and survival. The artificial diet FD still warrants further refinement and testing for it to become a more effective mariculture feed with commercial potential.

Introduction

Beach cast seaweed (*Laminaria pallida*) has become a menace and unwanted waste to the Namibian authorities due to cleaning up costs. This macrophytic algae, however, has potential to benefit the

booming mariculture industry in Namibia. In most aquaculture operations feed costs accounts for more than 60% of the variable operating costs (Du & Niu, 2003). Abalone growers' need for economically viable abalone diets, comprising ingredients not derived from the marine environment, remains a high priority to ensure the development of a sustainable and profitable mariculture industry (Jones *et al*., 1996).

Natural abalone feeds are constrained by factors such as seasonality and cost in terms of harvesting, storage and handling. Thus to maintain a productive and cost-effective mariculture operation, a feed must be found which is both cost-effective and enhances growth and survival of the cultured abalone. High growth rates of abalone at least cost is highly desirable. Growth is defined as a change in magnitude (Azevedo *et al*., 1998) where the change can be in size (weight and/or length) or in chemical composition. In South Africa, ABFEED®, the only natural abalone feed, has been successful at fulfilling these criteria (Sales & Britz, 2001). However, it contains fishmeal ingredients, which are costly and unsustainable.

Development of an effective formulated diet for South African abalone, *Haliotis midae,* relies on a better understanding of the nutrient requirements of this species. Hence, the present study evaluated a formulated feed for abalone consisting mainly of natural ingredients (seaweed) that could benefit the abalone culture both in Namibia and South Africa in comparison to a seaweed diet. Integrating seaweeds as feeds into mariculture result in reduced cost and waste, while increasing productivity (Anon., 2004).

This present study compared production performance parameters in South African abalone (*H. midae*) raised on a seaweed diet (SWD), consisting of macrophytic algae (*Laminaria pallida*), and a formulated diet (FD), comprising macro-algae as ingredients, respectively. The FD was prepared by a Lüderitz company, and formulated entirely from natural seaweed, of which 80% was the farmed species *Gracilaria gracilis*. Feeding trials are the most dependable method for determining the nutritive value of novel protein sources (Eid & Matty, 1989). Therefore, the effect of the two respective diets on growth rate (length and weight), survival rate, rate of feed consumption, feed conversion and efficiency, protein efficiency ratio, and the chemical composition as well as the fatty acid and essential amino acid contents in the tissue of juvenile and sub-adult *H. midae,* grown under commercial culture conditions, was evaluated over a 84-day trial period. The aim of this study was to be able to provide information which would enhance the development of locally available, economical alternative feeds to fulfil the needs of the envisaged growth of the abalone farming industry in Namibia. This study formed part of a larger research project initiated by Hentiesbay Coastal Research Center of the University of Namibia which evaluates the development of formulated feeds comprising of natural ingredients for abalone culture.

Materials and methods

Experimental diets

The experimental diets (SWD and FD) used during this study were as described in Chapter 3.

Nutritional profile of experimental diets

The nutritional profiles of the experimental diets (SWD and FD) were determined as described in Chapter 3.

Experimental abalone, rearing facilities and feeding trial

Abalone samples

Animals (n = 70) used in this study were divided into juveniles (n=40) (24.33 ± 3.14 mm shell length; 2.72 ± 0.83 g live weight, mean ± SE) and sub-adults (n=30) (58.07 ± 10.33 mm shell length; 41.96 ± 20.61 g live weight, mean ± SE). Lüderitz commercial abalone farm imported the spawned abalone from commercial abalone hatcheries (AbaSeed in Hermanus, South Africa). At the Lüderitz farm, the abalone were acclimatised for nearly one month before being weaned and fed a maintenance diet comprising natural fresh seaweeds (*Gracilaria* spp*., Ecklonia maxima and Laminaria* spp.) (K. Laufer, Taurus Products (Pty) Ltd., personal communication). These samples were then transferred to University of Namibia (UNAM) Marine and Coastal Research Center at Hentiesbay, Namibia.

Rearing facilities

A continuous flow system, comprising two rows of six 250 L GIP fiberglass tanks was used (**Figure 5.1**). Seawater was filtered through a 20 μm cartridge filter using a sand filter that goes through a biological filter. Water flow rate of 1 L/min (48.6 cm³/sec) was maintained throughout the experiment. Constant water temperature was maintained at 17.31 ± 0.87°C during the experiment. One air line in each tank was used to aerate the water. Water salinity was 33.11 ± 1.59 parts per thousand (ppt) and pH was maintained at 8.05 ± 0.06 (pH meter, Jenway, U.K). Dissolved oxygen (Oxygen meter, YSI, model 58) was 7.55 ± 0.11 mg/L and negligible levels of free ammonia and nitrite (AOAC, 1995) was present.

Feeding trial

The two respective *H. midae* classes, i.e. juvenile and sub-adult were assigned to tanks using a randomised block design with three blocks, two treatments per diet and three replicates per treatment consisting of 5 animals per treatment. All abalone were starved for two days prior to commencement of the trial and subsequently fed 10% of their body weight either every second day,

if fed on the FD, or every fourth day, if fed on the SWD, until completion of the trial at 84 days. Uneaten feed and faeces were removed from the tanks once every second week to maintain the quality of the water. The abalone was anaesthetised using 5-10% magnesium sulphate before being weighed and measured.

Figure 5.1 The continuous flow set-up used during the abalone feeding trial.

Abalone growth performance and nutrient utilisation

The shell length was measured with a vernier calliper to the nearest 0.02 mm and the abalone weighed to the nearest 0.1 g using an electronic balance. All growth performance measurements were made when the trail commenced (day 1) and on days 28, 56 and 84, respectively. The condition factor of the respective animals was, however only calculated on days 1 and 84 according to Britz (1996) (equation 5.1).

Condition factor

The condition factor (equation 5.1) is a concept that was developed to account for the relationship between the weight of abalone per unit shell length (Britz, 1996).

$$\text{Condition factor} = \text{body weight (g)} / \text{shell length (mm)}^{2.99} \times 5575 \qquad \text{… equation 5.1}$$

Weight gain and survival rate

The diet efficiency was evaluated and determined as the percentage weight gain (equation 5.2) and survival rate (equation 5.3).

$$\% \text{ weight gain} = ((\text{final weight (g)} - \text{initial weight (g)}) / \text{initial weight (g)}) \times 100 \qquad \text{… equation 5.2}$$
$$\text{Survival rate} = (\text{final number of abalone} / \text{initial number of abalone}) \times 100 \qquad \text{… equation 5.3}$$

Daily growth rate and shell length

The daily growth rate (DGR) in terms of daily body weight (DGR_{BW}) and shell length (DGR_{SL}) was calculated according to equations 5.4 and 5.5.

$$DGR_{BW} = (W_1 - W_0) / t \qquad \text{… equation 5.4}$$

$$DGR_{SL} = (L_1 - L_0) / t \qquad \text{… equation 5.5}$$

where

W_0 = mean initial weight (g);

W_1 = mean final weight (g);

L_0 = mean initial length (mm);

L_1 = mean final length (mm); and

t = time in days.

Food conversion ratio and protein efficiency ratio

In addition the food conversion ratio (FCR) and protein efficiency ratio (PER) over the 84 day trail period were calculated (equations 5.6 & 5.7).

$$FCR = \text{dry weight feed consumed (g) / abalone wet weight gain (g)} \qquad \text{… equation 5.6}$$

$$PER = \text{wet weight increase (g) / protein intake per day (g)} \qquad \text{… equation 5.7}$$

Daily feed consumption and relative growth rate and shell length increase

The daily feed consumption (expressed as % of body weight) per day was determined using the formula of Shipton *et al.* (2002) (equation 5.8).

$$\text{Daily feed consumption (\% of body weight)} = (C_g / W_t) \times 100 \qquad \text{… equation 5.8}$$

where

C_g = the mean daily feed consumption corrected for leaching (g); and

W_t = the mean abalone weight at time t (days) (equation 9).

$$W_t = W_0 \times (RGR/100) + 1)^{t-1} \qquad \text{… equation 5.9}$$

where

W_0 = mean initial weight; and

RGR = relative growth rate (% body weight increase per day) (equation 5.10).

$$RGR = \{\ln(W_f) - \ln(W_i) / t\} \, 100 \qquad \text{… equation 5.10}$$

where

ln(W_f) = the natural log of the mean final weight;

ln(W_i) = the natural log of the mean initial weight; and

t = the time in days.

Nutritional profile of abalone meat

Proximate analysis

Six animals from each treatment were randomly selected at the end of the feeding trial and kept frozen at -20°C until chemical analyses were performed. For comparison purposes abalone representing wild abalone was also analysed. The samples were slightly thawed before shells and soft-bodies were separated. A representative sample of the soft-body tissue (comprising of mantle, foot muscle and viscera) was ground using a meat mincer. Moisture was determined by drying each sample at 105°C for 24 h, ash determination was performed at 550°C for 12 h and crude fibre by the Weende method. The remaining soft-body sample was freeze-dried for 24 h and ground into a fine powder for analyses of crude protein and crude fat. Crude protein content was determined using the Dumas method (AOAC, 1995) using a protein analyser (Leco Corporation, St Joseph, MI, USA) and crude fat lipid was performed after extraction with petroleum ether using the Soxhlet method. Crude fibre was determined using the Weende method. All measurements were performed in duplicate. Carbohydrate content was determined by difference. The fatty acid and amino acid profiles of the abalone meat samples were determined (single measurements) and analysis was done at the Nutritional Laboratory, Ministry of Agriculture, Water and Forestry, Windhoek, Namibia.

Fatty acid profile

Fatty acid composition was determined by gas chromatography (GC). Fatty acid methyl ester analysis (FAME) was done by means of a modified procedure of Folch *et al*. (1957). This approach uses direct acid catalysed trans-esterification, without prior extraction of total fat, on dry sample amounts ranging from 10-150 mg. Ten percent of internal standard 20:2 (n-6) was added prior to the reaction. The freeze dried abalone soft-body tissue was spread on filter paper (5 cm diameter), to further reduce the water content and 2 g weighed and transferred to an extraction tube. Extraction was carried out according to a modified method of Folch *et al*. (1957). The extraction solvent was added, 30 mL chloroform:methanol (C:M 2:1; v/v). All extraction solvents contained 0.01% butylated hydroxytoluene (BHT) as an antioxidant. Heptadecanoic acid (C17:0; 500 uL; 10 mg/mL) was used as an internal standard to quantify the individual fatty acids. A polytron mixer was used to homogenise the sample within the extraction solvent. The solution was transferred through an extraction funnel into a 50 mL volumetric flask, using a glass microfibre filter. The extraction tube

was rinsed with 10 mL C:M (2:1; v/v) and the solution transferred through the extraction funnel. The extraction funnels were placed under a vacuum to dry the remaining filtrate. The volumetric flask was filled to 50 mL with C:M (2:1; v/v), mixed and 100 uL was transferred to a spotting tube and dried under nitrogen.

The lipids isolated were transmethylated adding 2 mL transmethylating reagent (methanol/sulphuric acid; 19:1; v/v) for 2 h at 70°C. After cooling, the resulting fatty acid methyl esters (FAME) were extracted with 1 mL H_2O and 2 mL hexane. The top hexane phase was transferred to a spotting tube and dried under nitrogen.

Thin layer chromatography (TLC) was performed by re-dissolving the extract in 60 uL saline solution saturated with chloroform/methanol/saline (0.9% C:M:S (86:14:1; v/v/v); m/v in H_2O) and spotting 30 μL onto a silica gel 60 plate (10 x 10 cm, Merck) using the solvent system petroleum ether:diethyl ether:acetic acid (90:30:1; v/v/v). The diethyl ether was filtered through aluminium oxide. The plate was sprayed with chloroform:methanol (1:1; v/v) containing BBOT (2,5-bis-(5'tert.-butylbenzoxazolyl-[2']) thiophene; 10 mg/100ml). The fatty acid band observed under longwave ultraviolet light was scraped off into a spotting tube and 2 mL transmethylating reagent was added. The contents were vortexed, after which 1 mL H_2O and 2 mL hexane were added and vortexed again. The top phase was transferred to a glass stoppered tube. After being dried under nitrogen, 20 μL CS_2 was added and 1 μL analysed by gas liquid chromatography (GLC) (Varian Model 3300 equipped with flame ionisation detection) using a 60 m BPX70 capillary column of 0.25 mm internal diameter (SGE, Australia). Gas flow rates were: hydrogen, 25 mL/min and hydrogen carrier gas, 2-4 mL/min. Temperature programming was linear at 3°C/min: initial temperature 150°C; final temperature 220°C; injector temperature 240°C and detector temperature 250°C. The FAME were identified by comparison of the retention times to those of a standard FAME mixture (Nu-Chek-Prep Inc., Elysian, Minnesota) and the mg fatty acid/g of tissue sample was calculated.

Amino acid profile

Amino acid content was determined by hydrolysing a sample of protein. From this, the amino acids were isolated by means of HPLC under gradient conditions and purified by partition chromatography (Cowey & Cho, 1993). The analyses were conducted at the Department of Animal Science, Stellenbosch University. Samples were weighed and hydrolysed for 24 h at 110°C in 6 N HCl and 1.5% phenol. Hydrolysed samples were stored at -20°C. Before use, samples were thawed to room temperature. Each sample was mixed on a vortex for 5-10 seconds and centrifuged at 15 000 g for 5 minutes (Hermle bench centrifuge). The supernatant (25 uL) was measured with a Hamilton syringe and placed into a glass hydrolysis tube. These were dried under vacuum for 1 h,

and derivatised. Re-dry solution (methanol:water:triethylamine 2:2:1) was added (20 uL) to adjust pH and dried for 1 h. The sample was derivatised by adding 20 uL derivatising solution (methanol:water:triethylamine:phenylisothiocyanate 7:1:1:1) and allowed to react for 10 min at room temperature, and dried under vacuum for a minimum of 1 h and a maximum 3 h till dry.

The dried sample was re-suspended in 400 μl of Picotag sample diluent (Waters, Milford, MA, USA). A portion of the sample (16 μL) was separated by HPLC under gradient conditions, where buffer A was a sodium acetate buffer (pH 6.4) containing 5000 ppm EDTA, 1:2000 triethylamine and 6% acetonitrile and buffer B a 60% acetonitrile buffer containing 5000 ppm EDTA. The obtained data was analysed using Breeze software (Waters, USA).

Statistical analysis

Percentage data for survival were square-root arcsine transformed prior to analysis. Data from each treatment were subjected to two-way repeated-measures ANOVA (RANOVA). Tukey's test was used to compare mean values between individual treatments where appropriate.

Results

Nutritional profile of experimental diets

The nutritional profile of the two respective experimental diets (SWD and FD) is described in detail in Chapter 3.

Abalone growth performance and nutrient utilisation

<u>Condition factor</u>

The body condition of *H. midae* (juvenile and sub-adult) was evaluated to determine any possible changes in overall condition of the abalone over the 84-day trial period. The mean condition factors of both the juvenile and sub-adult abalone fed on the SWD and FD, respectively, at the beginning and completion of the trial period are presented in **Table 5.1**. No significant changes in condition were observed, although the condition of the juvenile class fed on the FD declined slightly while the sub-adult class fed on the SWD showed a small improvement in overall body condition.

<u>Weight gain, survival rate and daily growth rate</u>

The mean abalone wet weight, daily growth rate in terms of body weight (DGR_{BW}) and body weight:shell length ratios (BW:SL) of the juvenile and sub-adult *H. midae* fed on either the SWD and FD over the 84-day trial period are shown in **Table 5.2**. The mean shell length and daily growth rate in terms of shell length (DGR_{SL}) of the juvenile and sub-adult *H. midae* fed on either the SWD or FD over the 84-day trial period are shown in **Table 5.3**.

Table 5.1 Mean body condition factors of both juvenile and sub-adult abalone classes fed on the seaweed diet (SWD) and formulated diet (FD), respectively, over a period of 84 days

	Condition factor	
Diet	**Day 1**	**Day 84**
Juvenile		
SWD	0.60	0.60
FD	0.61	0.60
Sub-adult		
SWD	0.67	0.71
FD	0.72	0.72

A significant increase in weight, over the 84-day trial period, of the sub-adult abalone fed on both the respective diets (**Figure 5.2**) was observed. However, the final weight of the sub-adults fed on the SWD was significantly higher ($P < 0.05$) than that of the sub-adult abalone fed on the FD. There was no significantly difference ($P > 0.05$) in the increase in weight or final weights of the juvenile abalone fed on either the SWD or FD. At completion of the 84-day feeding trial, the highest DGR_{BW} of 0.127 g/ day^{-1} was attained with the sub-adult abalone fed on the SWD (**Table 5.2**).

For shell length there was a significant increase ($P < 0.05$), over the 84-day trial period, for the sub-adult abalone fed on the SWD in contrast to those fed on the FD where no significant difference ($P > 0.05$) was observed (**Figure 5.3**). In the case of the juvenile abalone a significant increase ($P < 0.05$) in shell length, over the 84-day trial period, was observed when fed on either the SWD or the FD (**Figure 5.3**). At completion of the 84-day feeding trial, the highest DGR_{SL} of 0.82 mm/day^{-1} was attained with the juveline abalone fed on the FD (**Table 5.3**).

Body weight:shell length ratio (BW:SL), which indicates the mass of abalone per unit shell length (mean final weight:mean final length), was calculated for both the juvenile and sub-adult abalone fed on the SWD and FD, respectively (**Table 5.2**). The final abalone BW:SL ratio was significantly higher ($P < 0.05$) for the sub-adult abalone fed on the SWD diet (3.221) compared to those fed on the FD (1.788) ($P > 0.05$) (**Table 5.2**). No significant difference ($P > 0.05$) in BW:SL ratio was observed for the juvenile abalone fed on either diet (0.455 and 0.427, respectively for FD and SWD).

Table 5.2 Abalone initial and final wet weights, % weight gain, growth rate per day and year and body weight:shell length ratios (BW:SL) of the juvenile and sub-adult *H. midae* fed on either the seaweed diet (SWD) or formulated diet (FD) over the 84-day trial period

Diet	Mean initial weight (g)	Mean final weight (g)	% Weight gain	Growth rate $(g.day^{-1})$	Growth rate (g/year)	BW:SL (g/mm^{-1})
Juvenile						
SWD	$2.64 \pm 0.16^{*}\,a^{\#}$	5.38 ± 0.41 a	103.79	0.033 a	11.93 a	0.427 a
FD	3.17 ± 0.16 a	6.31 ± 0.41 a	99.05	0.037 a	13.64 a	0.455 a
Sub-adult						
SWD	44.21 ± 4.85 a	54.84 ± 5.90 b	24.04 a	0.127 a	46.19 a	3.221 a
FD	39.69 ± 4.85 a	44.77 ± 5.90 c	22.02 a	0.060 b	22.04 b	1.788 b

*Mean $\pm$ SE

$^{\#}$Values in the same column, within each class, with different superscripts differ significantly ($P < 0.05$)

Table 5.3 Abalone initial and final shell length and daily and yearly growth rates of the juvenile and sub-adult *H. midae* fed on either the seaweed diet (SWD) or formulated diet (FD) over the 84-day trial period

Diet	Mean initial length (mm)	Mean final length (mm)	% Length gain	Growth rate (mm/day)	Growth rate (mm/year)
Juvenile					
SWD	$24.26 \pm 2.23^{*}\,a^{\#}$	30.70 ± 2.49 b	26.55	0.077 a	27.96 a
FD	25.60 ± 2.32 a	32.50 ± 2.49 a	26.95	0.082 b	29.98 b
Sub-adult					
SWD	59.80 ± 2.32 a	63.10 ± 2.49 b	5.52	0.040 a	14.34 a
FD	56.33 ± 2.32 a	59.17 ± 2.49 c	5.04	0.034 b	12.32 b

*Mean $\pm$ SE

$^{\#}$Values in the same column, within each class, with different superscripts differ significantly ($P < 0.05$)
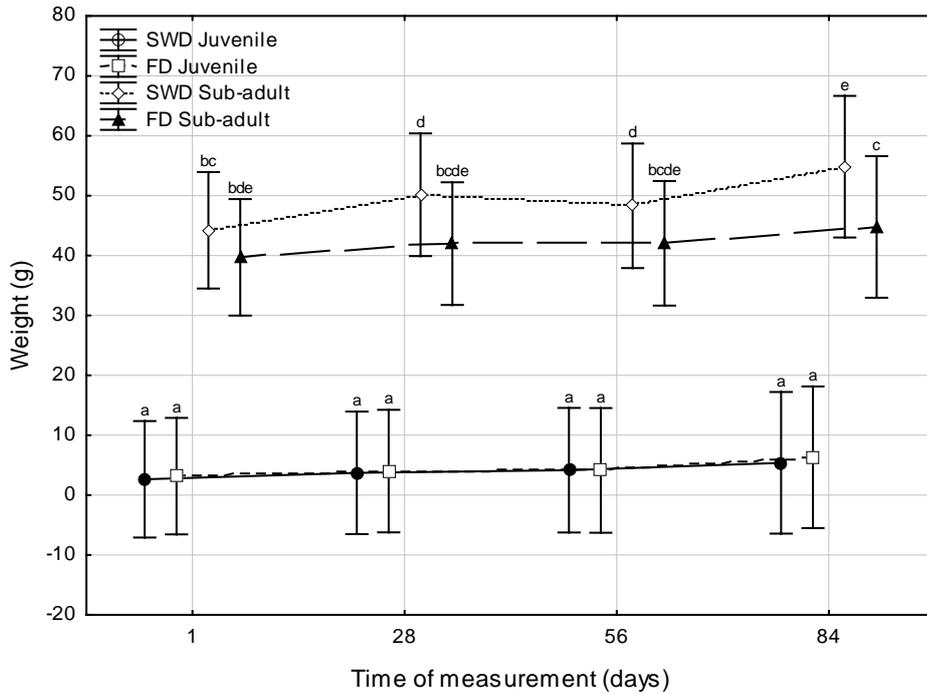
Figure 5.2 Differences between the average weights (g) obtained for the the juvenile and sub-adult *H. midae* fed on either the seaweed diet (SWD) or formulated diet (FD) over the 84-day trial period obtained with analysis of variance (ANOVA). Error bars denote 0.95 confidence intervals. Different letters indicate significant differences obtained from Bonferroni post-hoc analyses.
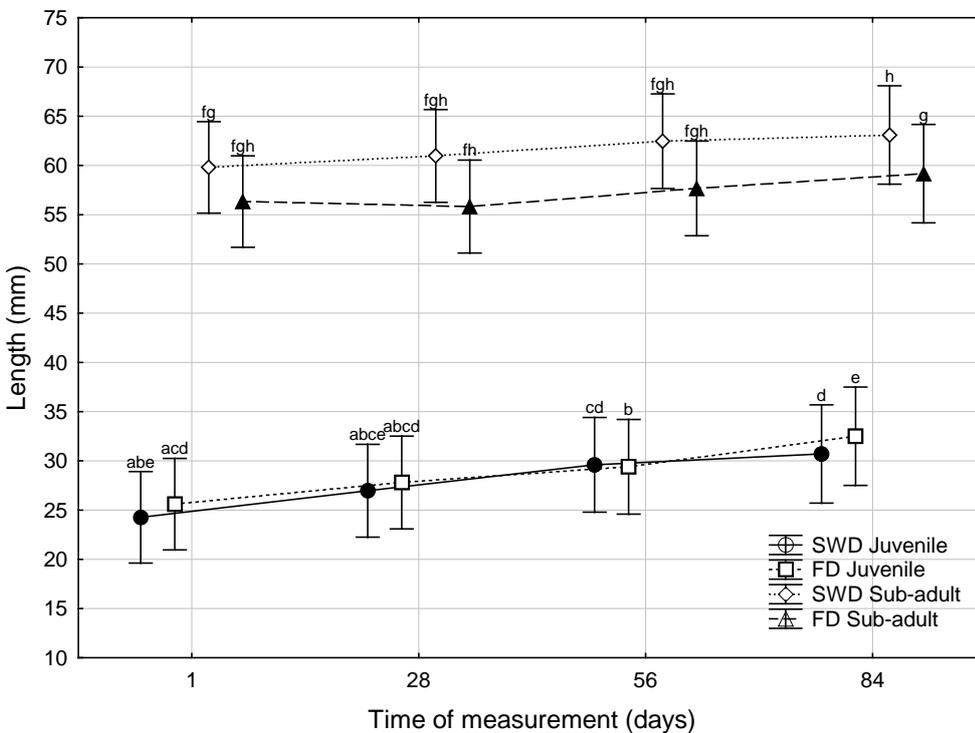
**Figure 5.3** Differences between the average shell lengths (mm) obtained for the the juvenile and sub-adult *H. midae* fed on either the seaweed diet (SWD) or formulated diet (FD) over the 84-day trial period obtained with analysis of variance (ANOVA). Error bars denote 0.95 confidence intervals. Different letters indicate significant differences obtained from Bonferroni post-hoc analyses.

<u>Feed conversion ratio and protein efficiency ratio</u>

The feed conversion ratio (FCR) and protein efficiency ratio (PER) for the sub-adult abalone ranged from 2.80-10.90 and 0.10-0.40, respectively (**Table 5.4**). The juvenile abalone fed on the FD yielded significantly lower ($P < 0.05$) FCRs (0.8) and higher PERs (1.20) than their counter parts fed on the SWD. This indicates the more efficient feed conversion in the case of the FD and the low biological value of the SWD. A similar trend was observed for the sub-adult abalone although the differences were not significant ($P > 0.05$).

<u>Daily feed consumption and relative growth rate</u>

The juvenile abalone consumed significantly less ($P < 0.05$) of the SWD, expressed as percentage body weight, in comparison to the FD (**Table 5.4**). The corresponding relative growth rate (RGR) was thus also significantly different. No significant difference (P > 0.05) was observed in feed consumption and corresponding RGR for the sub-adult abalone fed on the two respective diets.

Table 5.4 Feed conversion ratio (FCR), and protein efficiency ratio (PER), feed consumption and relative growth rate (RGR) of the juvenile and sub-adult abalone (*H. midae*) fed on either a seaweed diet (SWD) or a formulated diet (FD) over the 84-day trial period

Diet	FCR	PER	Feed consumption[*]	RGR
Juvenile				
SWD	3.90 a[#]	0.30 acd	2.09 a	0.95 a
FD	0.80 b	1.20 b	11.66 c	0.65 b
Sub-adult				
SWD	10.90 c	0.10 c	2.92 c	0.30 c
FD	2.80 c	0.40 ca	6.31 c	0.20 c

[*]Total dry weight feed consumed (g) in 84 days

[#]Values in the same column with different superscripts differ significantly ($P < 0.05$)

Nutritional profile of abalone meat

<u>Proximate analysis</u>

The soft-body nutritional profile (crude protein, crude fat, crude fiber, ash content expressed on a dry matter basis) of abalone soft-body tissue fed on either the SWD or the FD was not significantly different ($P > 0.05$). A slight increase in fat was, however, observed for the abalone fed on the FD 2.28 ± 0.01% DM basis, mean ± SE) compared to the SWD (2.14 ± 0.08% DM basis, mean ± SE) (**Table 5.5**). The nutritional profile of the abalone fed on the two respective diets was also compared with wild abalone.

<u>Amino acid profile</u>
The amino acid composition of abalone soft-body tissue fed on the SWD and the FD as well as that of wild abalone is presented in **Table 5.6**. The amino acid composition of abalone tissue fed on both diets (SWD and FD) was similar to that of wild abalone. A positive correlation (Pearson correlation) was observed between the amino acid composition of the wild abalone and that of both the SWD ($r^2 = 0.82$) and FD ($r^2 = 0.72$), respectively.

<u>Fatty acid profile</u>
The fatty acid composition of abalone soft-body tissue fed on the SWD and the FD as well as that of wild abalone is presented in **Table 5.7**. A positive correlation (Pearson correlation) was observed between the fatty acid composition of the wild abalone and that of both the SWD ($r^2 = 0.94$) and FD ($r^2 = 0.99$) was observed, respectively.

Table 5.5 Chemical composition (dry matter basis) of the soft-body tissue of juvenile and sub-adult abalone fed on either the seaweed diet (SWD) or the formulated diet (FD) in comparison to wild abalone

	Wild abalone (n=4)	SWD (n=5)	FD (n=5)
Moisture (%)[*]	82.30 ± 0.49	76.09 ± 1.67	76.77 ± 0.21
Crude fat (%)	3.06 ± 0.37	2.14 ± 0.08	2.28 ± 0.01
Crude protein (%)	60.61 ± 0.32	66.16 ± 0.23	66.50 ± 0.09
Ash (%)	2.20 ± 0.57	1.86 ± 2.46	2.19 ± 0.90
Crude fiber (%)	0.31 ± 0.01	0.35 ± 0.06	0.42 ± 0.02
Carbohydrate by difference (%)	33.82	29.49	28.61

[*]Moisture content after freeze drying for: Wild abalone (3.85%); SWD (3.77%) and FD (3.7%)

Table 5.6 Essential amino acid (EAA) profile (% of protein on DM basis) of soft-body tissue of juvenile and sub-adult *H. midae* (n = 3 samples from 6 pooled animals per treatment) in comparison to wild abalone

Amino acid composition (g/100g)	Wild abalone (n=3)	SWD (n=3)	FD (n=3)
Essential amino acids			
Arginine	4.70	1.90	4.63
Histidine	1.25	0.79	1.19
Isoleucine	1.42	0.75	0.86
Leucine	2.18	2.16	3.30
Lysine	2.79	1.43	3.34
Phenylalanine	1.40	1.07	1.43
Methionine	0.81	0.78	0.65
Threonine	2.82	1.30	2.20
Valine	2.46	1.02	1.49
Non-essential amino acids			
Aspartic acid	2.74	4.63	5.62
Serine	1.87	3.44	4.16
Glutamic acid	3.43	6.23	7.68
Glycine	3.88	7.45	9.46
Alanine	2.37	4.26	5.50
Tyrosine	0.94	1.27	1.47

Table 5.7 Fatty acid profile of the whole soft body tissue of juvenile and sub-adult *Haliotis midae* (fed SWD and FD), compared to the fatty acid (FA) profile of wild abalone. Fatty acid values are presented as mg/g (% dry weight)

Fatty acid composition (mg/100 g)	Common name	Systemic name	Wild abalone (n=3)	SWD (n=3)	FD (n=3)
6:0			0.11	3.06	1.77
8:0			ND	ND	0.09
10:0			ND	0.144	ND
11:0			0.123	1.027	ND
12:0			0.16	0.12	0.13
13:0			0.09	0.10	0.09
14:0			4.13	2.63	4.45
14:1			0.27	0.19	0.15
15:0			0.90	0.67	1.24
15:1			0.21	0.17	0.21
16:0	Palmitic	Hexadecanoic	31.51	27.43	30.80
16:1	Palmitelaidic	Transhexadecenoic	2.06	1.27	3.06
18:0	Stearic	Octadecanoic	10.99	10.38	9.18
18:1n-9c	Oleic	cis-9-Octadecenoic	10.01	8.25	10.05
18:1n-9t	Elaidic		0.30	0.25	0.38
18:2n-6t	Linolelaidic		0.09	0.16	0.18
18:2n-6c	Linoleic	9,12-Octadecadienoic	2.13	1.84	1.32
18:3n-6	y-Linolenic	6,9,12-Octadecatrienoic	0.65	0.13	0.11
18:2n-3	a-Linolenic	9,12,15-Octadecatrienoic	1.59	1.40	1.01
20:0	Arachidic	Eicosanoic	0.52	0.38	0.25
20:1	Gondoic	11-Eicosanoic	2.06	1.88	1.15
20:2		11,14-Eicosadienoic	0.36	0.48	0.32
20:3n-6	Dihomo-y-linolenic	11,14-Eicosadienoic	0.26	0.31	0.27
20:3n-3	Mead	5,8,11-Eicosatrienoic	12.54	15.23	12.33
20:4n-6	Arachidonic	5,8,11,14-Eicosatetraenoic	0.13	0.21	0.14
20:5n-3	Eicosapentaenoic	5,8,11,14,17-Eicosapentaenoic	5.44	5.42	6.23
21:0			0.12	0.19	0.34
22:0	Behenic	Docosanoic	0.11	0.48	0.09
22:1n-9	Erucic	13-Docosenoic	1.21	1.09	0.96
22:2			0.09	0.29	0.37
22:5n-3		7,10 13,16,19-Docosapentaenoic	8.31	10.73	9.58
22:6n-3	Docosahexaenoic	4,7,10,13,16,19-Docosahexaenoic	0.16	0.24	0.10
24:0	Lignoceric	Tetracosenoic	2.98	3.41	3.38
24:1	Nervonic	15-Tetracoseanoic	0.37	0.33	0.29

Discussion

Nutritional profile of experimental diets

The marked differences observed in the nutritional composition of the SWD and FD and especially in their protein and carbohydrate levels have been discussed in detail in Chapter 3.

Abalone growth performance and nutrient utilisation

Weight gain, survival rate and daily growth rate

Feeding trials are the most dependable method for determining the nutritive value of new protein sources (Eid & Matty, 1989). Despite being expensive and time-consuming it is essential for assessment of novel protein sources for slow growers such as Haliotids. Live weight gain is a reliable indicator for growth as long as the experimental variables are not expected to be affected by the composition of weight gain in the animal (Lovell, 1978). In contrast to what was expected abalone fed on the SWD resulted in significantly higher weight gain compared to those fed on the FD. The weight increase of sub-adult abalone fed on FD was also lower than that of the abalone fed on SWD. The poor weight gain achieved with FD in the sub-adult abalone could be due to a decrease in activity and specialisation of proteases with age (Britz & Hecht, 1997).

From the DGR_{BW} calculated after the 84-day period, repeated-measures ANOVA (RANOVA) indicated no interaction between time period and diet. Although slightly lower, the DGR_{BW} for the juvenile abalone fed on the SWD diet (0.033 g/day) did not differ significantly from the DGR_{BW} of abalone fed on the FD (0.079 g/day). In contrast, sub-adult abalone fed on the SWD exhibited significantly higher DGR_{BW} compared to those fed on the FD. The SWD diet consists of *Laminaria pallida* as sole protein source. For the juvenile abalone, differences between the two diets became less distinct as the experiment progressed than for the sub-adult abalone. A significant interaction ($P < 0.05$) was found between diet, animal size and feed conversion indicating that the FCR response to a given diet is dependent upon animal size. It was also observed that gonad development was less pronounced in sub-adult abalone fed on the FD. In this study the juvenile abalone performed better when fed on the FD compared to the sub-adult abalone fed on the same diet. The BW:SL ratio results obtained in the present study are comparable to the findings of Naidoo *et al*. (2006) when juvenile *H. midae* was fed fresh kelp (0.420) and Abfeed® (0.463). This is an important measurement to determine the marketable size (80-100 mm) of abalone (Naidoo *et al*., 2006).

Contrasting results in terms of shell length were obtained for the abalone fed on the SWD and FD, respectively. The juvenile abalone showed significantly higher DGR_{SL} when fed the FD where as the sub-adult abalone showed significant higher DGR_{SL} when fed the SWD. Juvenile abalone of 1-2 cm shell length can actively feed on macro-algae or formulated diets until they reach market

size. From the present results it could be expected that it would take juvenile abalone, fed solely the FD, 8 years to reach a marketable size of 100 mm whereas it would take them 7 years if fed the SWD. Similarly, it would take sub-adult abalone 3 years, when fed the SWD and 3.6 years when fed the FD (calculations based on average shell length growth (mm) per year (**Table 5.3**).

In a study conducted by Britz (1996) *H. midae* fed on fish meal and *Spirulina* spp. based diets produced a higher shell length increment and growth rate increase compared to the present study. This was also observed when *H. midea* was fed on diets containing casein and S*pirulina* spp. (Shipton, 2000). In the present study it was only for the juvenile abalone fed on both respective diets that no significant difference in weight gain over the 84-day trial period was observed. Similarly no significant increase in shell length was observed for the sub-adult abalone fed on the FD. It is possible that a significant increase in shell length could have been observed if the study would have continued for a longer period of time.

Food conversion ratio and protein efficiency ratio

There was a high efficiency of feed conversion in the juvenile abalone fed on the FD. The present study thus suggests that the feed conversion (FCR = 0.08) is more efficient in juvenile abalones, which has also been found to be the case in many cultured fish species (Hardy, 1989). Haliotids readily consume a wide variety of formulated diets (Britz *et al*., 1994; Britz 1995; Fleming *et al*. 1996), with superior FCRs ranging *ca*. 1-1.5 compared to those fed on seaweed diets having FCRs ranging *ca*. 12-15. The less efficient feed conversion observed for the juvenile abalone fed on the SWD (FCR = 3.9 for juvenile and 10.9 for sub-adult abalone) agrees with the results by Day and Fleming (1992) who also reported less efficient FCR for *H*. *rubra* fed on single algal diets.

Nutritional profile of abalone meat

Proximate analysis

Body composition analysis showed that *H. midae* fed on FD had slightly more fat (3.16%) compared to SWD fed abalone, although it was not significant (**Table 5.4**). This was in spite of the SWD having had a slightly higher crude fat content (0.76% DM) compared to that of the FD diet (0.25% DM). A study by Mercer *et al*. (1993) showed that to achieve high growth rates for abalone under culture, macro-algae diets with 3-5% fat and formulated diets with 5% fat are required. Both experimental diets in the present study contained less than 1% crude fat which is below the minimum amount of dietary fat required for Haliotids (NRC, 1993). This could explain the differences in poor growth rates for sub-adults fed on the FD and juveniles fed on the SWD. However, it should be noted that these growth differences may involve nutritional factors other than

fats. A study by Mercer *et al*. (1993) demonstrated that macro-algae with the lowest fat content produced the lowest growth rates in *H. discus hannai*.

Amino acid profile

A positive correlation was observed between the amino acid and fatty acid profiles of the abalone fed on the SWD or FD, respectively. However, a slightly lower correlation ($r^2 = 0.49$) was found when the amino acid and fatty acid profile of the soft-body tissue of abalone fed on the SWD was compared to wild abalone. There was no methionine present in the abalone fed on the SWD compared to the abalone fed on the FD diet which contained 2.2 mg per 100 g (DM). Britz & Hecht (1997) demonstrated that although the amino acid requirements of the animals may be the same, their response to a given protein source will vary resulting in qualitative differences in their protein requirements.

The amino acid profile is expected to simulate the tissue muscle of the animal being tested (Bautista-Teruel *et al*., 2003). It is generally accepted that the amino acid profile of the lean tissue of an animal gives a good indication of the balance of amino acids present in the diet (Wilson, 1989). Therefore in order to promote maximal growth, sub-adult abalone require a higher dietary protein content than juvenile abalone.

Fatty acid profile

The fatty acid profile of *Haliotis midae* fed on the two respective experimental diets (SWD and FD) revealed that certain essential fatty acids in the shucked abalone meat were properly assimilated into the abalone soft-body tissue. Both the SWD, FD and wild abalone contained the following major fatty acids: 16:0, 18:0, 18:l(n-9c), 20:3(n-3), 20:5(n-3) and 22:5(n-3). The fatty acid profiles of the abalone muscle (SWD, FD and wild abalone) were similar, thus reflecting similarities in the biochemical and nutritional composition. The degree of similarity (r^2) in the fatty acid profiles of the abalone meat (fed on the SWD and FD) where high with r^2 values of 0.94 and 0.99, respectively, when meat fed on SWD and FD, respectively, were compared to wild abalone.

The (n-6):(n-3) ratios were 0.31:0.11 and 0.06:0.11 for the SWD and FD, respectively. The slightly larger (n-6):(n-3) ratio in the SWD diet can be attributed to the high levels of linoleic acid present in the diet. A study by Dunstan *et al*. (1996) demonstrated difference in composition of abalone compared to that of other marine animals which have 20:5(n-3) and 22:6(n-3) as the main (polyunsaturated fatty acids) PUFAs. Neither of the PUFAs 22:4n-6 and 20:4n-3 was detected in any of the soft-body tissue of the abalone fed on the SWD and FD, respectively. It is generally accepted that animals cannot produce (n-6) PUFA from (n-9) monoenoic fatty acids nor (n-3) from (n-6) PUFAs. An Australian dietary study on abalone (*H. laeoigata* and *H. rubra*) showed that both

20:5(n-3) and 22:6 (n-3) should be included in the diet at a level of more than 0.3% (DM). Abalone fed on FD yielded extremely low levels of 22:6n-3 (0.103 mg/100 g DM) compared to SWD (0.241 mg/ 100 g DM). While high levels (20:5n-3) in the FD fed *H. midae* were reported (6.23 mg per 100 dry weight) compared to the abalone fed on SWD (5.42 mg per 100 g). However, the SWD could be improved by adding a small amount of fish oil which would provide these fatty acids.

Poor growth rates for FD could also be attributed foremost, due to imbalances of some nutrients such as vitamin C and carbohydrate/fat ratio's amongst other factors. Evidently lower vitamin C levels detected in FD (775 ppm) which could be attributed due to drying or hot pelleting process used during manufacture. Vitamin C is heat labile.

Conclusion

It was evident that the FD had nutritional inadequacies, compared to the SWD. The FD thus needed to be further refined before being released as an effective mariculture feed with commercial potential both in Namibia and South Africa. The importance of this work was to show the significant impact of the different diets on especially the fatty acid and amino acid profiles of the soft-body tissue.

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CHAPTER SIX

SENSORY AND NUTRITIONAL CHARACTERISTICS OF SOUTH AFRICAN ABALONE (*Haliotis midae*) FED ON MACROPHYTIC ALGAE (*Laminaria pallida)* OR ON A FORMULATED MACRO-ALGAE DIET

Abstract

Difference in chemical composition, flavour and degree of liking of cooked (steamed at 95°C for 45 min) *Haliotis midae* meat fed either a seaweed diet (SWD) or a formulated diet (FD) was tested using chemical analyses, as well as sensory evaluation. Abalone fed on the FD did not differ significantly ($P > 0.05$) in its chemical composition, sensory characteristics, as well as degree of liking from the SWD fed abalone.

Introduction

In the consumption of seafood, the sensory characteristics are generally the most important factors affecting consumer acceptance (Chiou *et al*., 2004). Information derived from sensory evaluation will reduce risks involved in decision making in terms of product development and strategies for meeting consumer needs.

Globally abalone, with its unique flavour and texture, is regarded as a highly valued seafood product with prime demand in Asian countries where it is part of the traditional cuisine (Britz, 1996). Odour, colour, appearance and size are very important quality factors of abalone and related products. Asian consumers' perceptions are mainly focused on aroma, flavour and colour (Britz, 1996). These attributes should thus be taken into consideration when evaluating consumer perception of cultured abalone fed on formulated diets.

Diet will also influence meat quality in cultured abalone (Haard, 1992). Various studies (Watanabe *et al*., 1993; Mercer *et al*., 1993; Fleming *et al*., 1996) reported on the chemical composition of natural feeds such as macrophytic algae and formulated diets and the effect thereof on the nutrient content of abalone. Sales & Britz (2001) reported on the variation in the chemical composition of cultured abalone meat. Abalone muscle contains, amongst others, high proportions of the health-promoting omega-3 polysaturated fatty acids (PUFA) (Dunstan *et al*., 1996).

In this study cooked meat of cultured abalone (*Haliotis midae*), fed on either a seaweed diet (SWD) or a formulated diet (FD), was evaluated for difference in chemical and sensory characteristics as well as consumer acceptability.

Materials and methods

Nutritional profile of experimental diets and abalone meat

The nutritional profiles of the respective diets were determined as described in Chapter 3. The nutritional profile of the abalone (*H. midae*) meat was determined as described in Chapter 5.

Abalone meat samples

Live cultured juvenile abalone (*Haliotis midae*) (24.33 ± 3.14 mm shell length; 2.72 ± 0.83 g live weight, mean ± SE), fed either a SWD or a FD were collected from culture tanks from Sam Nujoma Marine and Coastal Research Centre, Hentiesbay, Namibia. Five animals, representative of the population from each treatment, were randomly selected and euthanised by immersion in ice water. The abalone was placed in ziplock-type polyethylene bags before being transported on ice to Windhoek, Namibia for further analyses. The abalone was shucked and de-gutted before being cooked. The two abalone treatments were placed in separate stainless steel trays, covered with polyethylene film, and thereafter cooked at 95°C for 45 min in a steam cooker. After cooling, the meat was removed and evaluated for sensory characteristics and degree of liking.

Sensory analysis

A consumer test was conducted on the abalone fed on the two respective diets (SWD and FD) to determine whether the respective treatments differed in consumer preference. Fifteen consumers, eight male and seven female participated. Participants were requested to indicate a difference in preference using the paired preference test. This technique is used when one wants to look at the preference of one product directly against a second product (Lawless & Heymann, 1998). The cooked samples were coded with 3-digit codes and two samples were served in a complete randomised order to each panellist. This test was done for aroma as well as flavour. Participants worked individually in tasting booths. Data were analysed using the Roessler Tables for Paired Preference Tests (Roessler *et al*., 1978) and a significant difference in preference was calculated at the 5% level.

Additionally, a trained taste panel was used to assess eating quality differences in the respective treatments of cultured abalone (*H. midae*). The trained panel results could be used to explain the findings of the consumer panel. Three male panelists, aged 40-54 years, participated. These panelists were selected on the basis of having formal training in sensory evaluation. The triangle test (Lawless & Heymann, 1998) was used and replicated five times. In the triangle test, three samples are presented simultaneously to the panelists, two samples are identical and one is odd or different. This test technique was used to determine whether a sensory difference existed between the abalone fed on the respective test diets. The sample presentation was randomised and each

sample was coded with a 3-digit code. Distilled water was provided for panelists to rinse their mouths between samples. The abalone meat samples were presented to the panelists in 1 cm^{-3} blocks at room temperature. The number of correct responses (odd sample identified correctly) was calculated and the Roessler Table for Triangle Tests was used to determine whether the two treatments differed significantly in aroma or flavour at the 5% level of significance (Roessler *et al.,* 1978). The latter was thus done in order to determine whether a significant difference ($P < 0.05$) in aroma and flavour existed between abalone grown on the two diets (SWD and FD).

Results

Nutritional profile of experimental diets and abalone meat

The nutritional profile of the diets (SWD and FD) is reported on in detail in Chapter 3. The nutritional profile (moisture, crude protein, crude fat, crude fiber, ash and carbohydrates by difference) of the abalone fed on the SWD and FD is presented in **Table 6.1**. Although the abalone fed on the FD was slightly higher in nutritional content, there was no significantly difference ($P > 0.05$) in the nutritional profile of the abalone soft body tissue fed on either the SWD or FD.

Table 6.1 Nutritional profile (on a dry matter basis) of abalone soft-body tissue fed on either of the seaweed diet (SWD) or formulated diet (FD)

	Treatments	
	FD (n=5)	SWD (n=5)
Moisture (%)	76.77 ± 0.21[*]	76.09 ± 1.67
Crude fat (%)[$]	2.28 ± 0.01	2.14 ± 0.08
Crude protein (%)	66.50 ± 0.09	66.16 ± 0.23
Ash (%)	2.19 ± 0.90	1.86 ± 2.46
Crude fiber (%)	0.42 ± 0.02	0.35 ± 0.06
Carbohydrate by difference (%)	28.61	29.49

[*]Mean ± SE

[$]All measurements reported on a dry matter basis

Sensory analysis

There was no significant ($P > 0.05$) difference in preference when comparing the aroma of the abalone meat samples fed on either the SWD or FD. However, there was a significant difference ($P < 0.05$) in the consumers' preference in terms of flavour for the abalone sample fed on the FD. The trained taste panel results indicated that there was no difference in the aroma and flavour of the abalone fed on the different diets ($P > 0.05$).

Discussion

The chemical composition (crude protein, crude fat, crude fiber and ash content, calculated on a dry matter basis, as well as the moisture) of abalone soft-body tissue fed either the SWD and FD did not differ significantly ($P > 0.05$). However, the composition analysis showed that abalone meat fed on the FD had a slightly higher percentage of crude fat ($2.28 \pm 0.01\%$ DM) compared to meat of abalone fed on the SWD ($2.14 \pm 2.46\%$ DM). There could thus be a possible tendency for the SWD to result in a slightly lower percentage fat.

The abalone fed on the respective diets (SWD and FD) did not differ significantly in sensory aroma and flavour. However, the consumer panel indicated that they preferred the flavour of the abalone fed on the FD ($P < 0.05$). The latter result can be questioned. When a trained sensory panel cannot detect a difference, it is usually assumed that a consumer panel will not indicate a difference in preference (Lawless & Heymann, 1998). The consumer panel consisted of only 15 consumers. It is also possible that the small sample size (n = 5) may have affected the results. Future studies should definitely include increased sample sizes and this would most probably result in a difference in meat characteristics of cultured abalone fed on different diets. Hence, the findings of this research should be viewed as being only indicative and warrants further investigation.

Conclusion

The results obtained during this study suggest that abalone fed on a SWD and a FD, respectively, are similar in terms of chemical composition, sensory attributes and degree of liking. This study should be extended using a larger number of samples, as well as more consumers for testing the difference in preference.

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CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

The objective of this study was to test the suitability of a formulated dry, pelleted diet produced as a trial feed for South African abalone (*Haliotis midae*). The formulated diet (FD) was manufactured by a Lüderitz company and made entirely from 'natural' seaweed of which 80% was the farmed species *Gracilaria gracilis*. The aim was to produce a natural feed that would yield abalone meat with the same characteristics as that fed on fresh seaweed. This diet was therefore compared with a seaweed diet (SWD). The time and costs involved in the collection or culture of large amounts of macrophytic-algae for abalone culture is often high. Supply of macrophytic-algae can be unreliable and the environmental consequences of removing it are also a concern. Thus, the focus of research has moved to the development of convenient and nutritious artificial or formulated diets that produce high growth rates (Fleming *et al.*, 1996).

The nutritional profiles of both the diets were determined as well as that of the abalone meat fed on these diets for 84 days. The FD was the least preferred diet by the sub-adult compared to the SWD diet that consists solely from *Laminaria pallida* (Greville). Despite a reduced growth rate compared to the SWD, the FD diet proved to be more stable and readily acceptable by the juvenile abalone. Unfortunately, high consumption did not translate into wet weight growth as efficiently as the SWD diet in the sub-adult abalone class. The presence of non-nutritional compounds in most plant derived proteins, if not removed or processed, may attribute to lower growth rates of animals under culture (Bautista-Teruel *et al.*, 2003).

Fish meal based diets are highly digestible and contain ingredients that make them readily acceptable to an animal. It could be speculated that the FD had high phenolic levels making it less acceptable to abalone. The FD contained *Ecklonia maxima* which is suggested to contain high phenolic levels (Stepto & Cook, 1993). Therefore the inclusion of *E. maxima* needs to be avoided in abalone feeds (Simpson & Cook, 1998). Another reason that could also account for poor feed intake and growth rate in the sub-adult group fed on the FD is the inefficiency of feed intake when larger abalone are fed small pellets. Also, due to their increased surface area to size ratio; small pellets suffered a higher rate of mass and nutrient leaching into the water column. Subsequently, fewer nutrients are available for the abalone to consume over time.

It is generally accepted that balanced levels of protein (15%), lipid (3-5%) and carbohydrate (20-30%), with no toxic substances in natural algae, are essential for optimal growth performance of abalone (Mercer *et al.*, 1993). The crude protein content of the FD was higher than that of the SWD. The balance of essential amino acids was, however, satisfactory. On the other hand, the FD had

much lower levels of vitamin C; probably as a result of the drying/ hot pelleting process used during manufacture. It was also found that levels of long chain highly unsaturated omega three fatty acids were low in the test diet, especially 20:5(n-3) and 22:6(n-3). This was also reflected in low levels of these important unsaturated fatty acids in the meat of the abalone fed on the FD diet. From this point of view, FD diets tested appeared to be lacking the criteria to match the requirements for a abalone feed.

In the present study it was observed that the variations in feeding efficiency relative to abalone growth were slightly higher. It probably is a combination of biological variation and experimental error in growth measurements. During the experiment it was observed that gonad development was less pronounced in the sub-adult abalone groups fed on the FD. This could also be regarded as biological variations. The pH, salinity and dissolved oxygen remained at stable and acceptable levels throughout the trial and hence should not have influenced the results significantly. A three-to four fold increase in body weight was found over the course of the 84-day trial period with juvenile abalone. Final body weight and feed intake decreased progressively and significantly with the increase of time.

In conclusion the present research showed that cultured juvenile *H. midae* (24.33 ± 3.14 mm, shell length; 2.72 ± 0.83 g, weight) readily accepted a FD with high consumption and survival rates. Despite providing a good reference for future abalone nutrition studies, the FD will need to be refined further and tested to become an effective mariculture feed with commercial potential.

As the FD was found to be deficient in vitamin C and long chain omega three fatty acids it is recommended that it be supplemented with a vitamin premix and a small percentage of fish oil. A further growth trial should then be carried out to determine whether this corrects the problem causing reduced growth rates. Secondly, the preliminary findings from this study should be confirmed using a wider range of abalone classes and feed pellet sizes. This would assist in optimisation of pellet size for specific abalone sizes. The diet effectiveness on the growth rates of the range of the abalone ages and sizes and sexes should be tested using extended growth trials. Thirdly, the digestibility of the SWD should also be researched to determine its effectiveness at facilitating nutrient assimilation. This could be achieved by using the results of the present study to identify components of the diet that require modification to improve digestibility and feed conversion efficiency over an extended period of time. Fourthly, the pelleting process needs to be re-evaluated critically to determine the optimal manufacturing techniques required to extrude a cohesive FD pellet that will maintain its shape, consistency and water stability for at least 24 h. Finally, in terms of sensory analysis, further work is necessary to investigate the effects of seaweed derived FD on marketability of the product and acceptance by the consumer.

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