THE PROTEIN AND ENERGY REQUIREMENTS OF THE SOUTH AFRICAN ABALONE, *HALIOTIS MIDAE*

A thesis submitted in fulfillment of the
requirements for the Degree of
MASTER OF SCIENCE
Rhodes University

by
Alistair John Green
January 2009

CONTENTS

ACKNOWLEDMENTS

I would like to thank my supervisors Dr Cliff Jones and Prof. Peter Britz for all their guidance, support, advice and patience during the course of my studies. Many of the research objectives were developed by them. Cliff, your assistance during the building of the experimental system, your patience during the editing of chapters and your friendship are much appreciated. Pete, thanks for taking the time to edit chapters, guide me in the right direction with some of my weird and wonderful nutrition ideas and lastly for always managing to find a bit of extra funding to keep the lab going.

I am grateful for the support I received from my colleagues at the Port Alfred Marine Research Laboratory. Special thanks must go to Justin Kemp who helped me turn "legoland" back into a fully functioning efficient research facility. Your advice went a long way, from turning me into someone who didn't have a clue about building into someone who now quite enjoys it. I'll never forget the many tea time chats or afternoon beers on the lawn. Thank you. Mention must also go to Dave and his team, Bernard Mackenzie and Bruce Donovan for their assistance with the construction of the abalone system. Then to all my happy weighing and measuring abalone helpers: Paula Pattrick, Lindsey Woolley and Jessica Escobar-Porras a very big thank you. To our neighbours at Keryn's Dive School, Andre and Keryn thank you so much for helping us keep fish alive and the generator running during the countless power failures.

I am indebted to the various sponsors of the project: Funding was provided by the "Mariculture Frontier Programme" of the branch, Marine and Coastal Management of the Department of Environmental Affairs and Tourism, South Africa, the National Research Foundation (NRF) and THRIP. Feed ingredients and manufacturing equipment were made available by Marifeed (Pty) Ltd and I'm very grateful to Kurt Mätschke, Denzil and the Marifeed staff for all their assistance with manufacturing diets. Special thanks must go to Gavin Johnson, Louise Jansen, Bertus van Oordt, Greg Tutt, Mvaba Maho, Mike Gray and the rest of the staff at HIK for all their support during the running of growth trials on the farm. Experimental abalone were kindly donated by Marine Growers Abalone Farm (Pty) Ltd, Roman Bay Sea Farm (Pty) Ltd and HIK Abalone Farm (Pty) Ltd.

To Ernst Thompson and Albert Esterhuizen thank you for the many discussions on nutrition and for all your patience and guidance with the biochemical analyses.

To my parents, family and friends a very big thank you for all the love and support during the course of my studies. Lastly, to Pets, words cannot explain the gratitude that I feel for all your help from building abalone tanks to spending whole weekends weighing and measuring abalone, I wouldn't have managed without all your love, support and your positive attitude towards life. Thank you my love.

ABSTRACT

The abalone *(Haliotis midae)* culture industry in South Africa is becoming increasingly dependent on the use of formulated feeds, due to limitations in the supply of kelp. The bulk of the feeds that are currently available were developed based on the requirements of juvenile abalone cultured within the optimal temperature range for growth (18 – 20 °C). However, most abalone farming facilities are land-based pump ashore operations and are thus mostly exposed to temperatures outside of this range. In addition, these feeds have been found to be unsuitable for abalone cultured at elevated water temperatures (> 20 °C). The aim of the study was to develop size and temperature specific diets for *H. midae* through optimisation of dietary protein, energy and lipid levels.

Abalone were cultured under farm-like conditions in three partially recirculating temperature controlled systems at either 18, 22 or 24 °C and fed formulated diets containing graded levels of protein (18, 22 and 26 %) and energy (11.6, 13.5 and 16.2 $MJ.kg^{-1}$). Abalone were stocked into baskets at 5 % of available of surface area (n=36) and each diet (n=9) was fed to four baskets of abalone at each of the three temperature regimes for ten weeks. Abalone growth was temperature dependent, with growth declining from 4.33 $g.month^{-1}$ for abalone cultured at 18 °C to 0.77 $g.month^{-1}$ at 24 °C. Dietary protein could be reduced from 26 to 18 % provided dietary energy levels were maintained at 13.5 $MJ.kg^{-1}$. A dietary energy level of 11.6 $MJ.kg^{-1}$ was insufficient to meet the energetic requirements of *H. midae* regardless of the protein content of the diet.

The effects of water temperature and body size on the protein requirements of *H. midae* were investigated by culturing abalone at temperatures within the optimal range for abalone farming (i.e. 14, 16 and 18 °C). Three size classes of abalone (15, 50 and 80 mm) were fed formulated feed containing graded levels of dietary protein (20, 26, 32, 38 and 44 %) under controlled laboratory conditions for 12 weeks, and, in a separate experiment, under commercial farm conditions for 24 weeks. It was not possible to convincingly define the optimal protein levels for abalone of different sizes in this experiment because growth rates fell below average commercial growth rates obtained on farms. Growth was temperature dependent in the laboratory trial, with the rate of weight gain of the 15 mm (ANOVA: $p=0.002$) and 50 mm abalone (ANOVA: $p=0.02$) increasing significantly with an increase in temperature from 14 to 18 °C. In the farm trial, dietary protein content did not affect the growth rate of the 10-15 or 80 mm abalone (ANOVA: $p>0.05$), however, the 50 mm abalone displayed significantly higher weight gain on the 32 % (4.72 ± 0.20 $g.month^{-1}$) and 38 % (5.01 ± 0.34 $g.month^{-1}$) protein diets compared to those fed the 20 % protein diet (3.75 ± 0.13 $g.month^{-1}$) (ANOVA: $p=0.01$). Although definition of optimal dietary protein levels were not possible, the effects of dietary protein content and water temperature on the growth of *H. midae* were independent signifying that the protein requirements of abalone are temperature independent. In addition, there was no evidence to indicate that abalone of the different sizes tested here had different dietary protein requirements.

The size specific dietary lipid and protein requirements of *H. midae* were investigated by feeding two size classes of abalone (30 and 60 mm initial shell length) diets containing

graded levels of dietary lipid (4, 7, 10, 13 and 16 %) and protein (34 – 39 %) for 12 weeks. The 30 and 60 mm abalone were stocked at 7 (n=200) and 9 % (n=36) of the available basket surface area respectively and each diet was fed to four baskets of abalone of each size class. The protein requirements of *H. midae* are influenced by the amount of available dietary energy and thus it is possible that the ability of abalone to utilise lipids as a source of energy differs in the presence of varying levels of dietary protein. High levels of dietary lipid negatively affected the growth, condition factor and soft tissue glycogen content of both size classes of abalone. This negative effect was greater in the 30 mm size class compared to the 60 mm abalone. The corresponding increase in feed consumption and feed conversion ratio in response to increasing levels of dietary protein also provides evidence that abalone are unable to utilise dietary lipids as an energy source and high levels of dietary lipid probably inhibit the uptake of carbohydrates and protein. High dietary lipid levels did however appear to promote gonad maturation. It was possible to reduce dietary protein from 34 to 20 % without negatively affecting growth through the maintenance of dietary energy levels and thus it is recommended that future experiments on the energy content of formulated feeds should focus on the improved use of carbohydrates.

Reductions in the protein portion of formulated feeds for *H. midae* are possible provided the diet contains sufficient levels of energy supplied from carbohydrates. As the ability of abalone to utilise dietary lipid is limited, lipids are unlikely to play a significant role as an energy source in abalone feeds. Further investigations should focus on the utilisation of various carbohydrate sources in abalone feeds.

CHAPTER 1.

INTRODUCTION

Abalone is one of the highest valued seafoods in the world fetching an average market price of $ 40/kg (Limin, 2006). The high market value coupled with a decline in natural stocks worldwide set the stage for the development of abalone farming technology and farming operations are now established in a number of countries including China, Korea, South Africa, Taiwan, Australia, Chile, United States of America, Mexico, Iceland, Peru and New Zealand (FAO, 2005). The success of the industry has led to farmed abalone accounting for 8600 metric tonnes of the total world abalone production of 22 600 metric tonnes in 2002 (Gordon and Cook, 2004).

The Japanese set the stage for the culture of abalone worldwide as their pioneering work on artificial spawning, animal husbandry, culture techniques and feeding strategies provided a foundation for other countries to initiate research on their respective species through the adoption and adaptation of these techniques (Hahn, 1989a; Britz, 1995). Genade et al. (1988) laid the foundation for abalone farming in South Africa by successfully spawning the local abalone *Haliotis midae* in captivity. The successful spawning of *H. midae* coupled with the high market value and decline in natural stocks led to several fishing companies initiating research projects into the development of abalone farming in the early 1990's (Hecht, 1994; Cook, 1998; Sales and Britz 2001a; Troell et al., 2006). The farming of abalone is now the largest and fastest growing sector in the local mariculture industry, and South Africa is currently the third largest producer of cultured abalone in the world with an annual production of 830 $t.y^{-1}$ (FAO, 2005; Jones and Britz, 2006).

A number of factors have led to the success of the local abalone culture industry, such as the close working relationship established between fishing companies and research institutions into the development of culture techniques, the availability of suitable farming sites and favourable water quality conditions (Cook, 1998; Sales and Britz 2001a; Troell et al., 2006). However, the development of a water stable nutritionally

complete formulated feed was one of the crucial breakthroughs in the development of abalone farming technology in South Africa, since formulated feeds offer a number of nutritional, economic and convenience benefits (Britz et al., 1994; Sales and Britz 2001a). The South African abalone was found to readily accept an artificial feed which produced superior growth rates compared to those reared on natural seaweed diets and growth rates obtained indicated that abalone farming was economically feasible (Britz et al., 1994).

Feed costs account for a large proportion of operational costs on South African abalone farms and it is for this reason that the bulk of published research on *H. midae* has focused on the nutritional requirements and digestive capabilities of the animal (Sales and Britz 2001a). This was done in an effort to develop economically viable formulated diets that contain a suitable balance of feed ingredients that are nutritionally available to the animal in order to maximize growth rates (Sales and Britz 2001a). Abalone farmers in South Africa currently make use of either cultured or harvested algal diets, formulated feeds or a combination of the two (Cook, 1998; Troell et al., 2006). Kelp (*Ecklonia maxima*) and Abfeed® a formulated feed manufactured by Marifeed (Pty) Ltd. (Hermanus, South Africa), constitute the two major feed sources used on South African abalone farms (Naidoo et al., 2006; Troell et al., 2006). However, the maximum sustainable harvest of kelp was reached in 2003 and thus the continued use of kelp as a feed source is limited (Loubser, 2005; Troell et al., 2006). In addition to this, there are currently twenty two registered abalone permits with a further five in the developmental stages (Troell et al., 2006). Thus, further expansion of the abalone culture industry has always been viewed as being reliant on the use of cultured algal or formulated feeds (Britz et al., 1994; Troell et al., 2006). However, about half by weight of the abalone produced in 2006 were produced using Abfeed® and thus the use of formulated feeds is likely to increase (Britz and Jones, 2006).

Haliotis midae has a natural distribution spanning the convergence of the Indian and Atlantic Oceans, from the cold waters of the Benguela upwelling system on the west coast to the warmer waters of the Agulhas current on the east coast (Britz et al., 1997).

Thus, natural populations are exposed to mean monthly sea temperatures ranging from 12 – 13 °C along the west coast to 21 – 22 °C on the east coast (Greenwood and Taunton-Clark, 1994; Schumann et al., 1995; Britz et al., 1997). Temperature is the principal environmental factor influencing the metabolic rate of poikilotherms (Fry, 1971) and *H. midae* has been shown to conform with this trend (Britz et al., 1997). Britz et al. (1997) demonstrated that water temperatures in the range of 12 to 20 °C were physiologically optimal for *H. midae* in terms of growth, condition factor and feed conversion efficiency. Temperatures above 20 °C resulted in a decline in growth and a sharp increase in mortality and feed conversion ratio (FCR) (Britz et al., 1997). Therefore, most formulated feeds currently available in South Africa have been developed to promote the growth of abalone cultured within the physiological optimal temperature range 18 – 20 °C (Britz et al., 1997) i.e. the bulk of research conducted to optimise Abfeed® was carried out within this narrow range. However, the majority of abalone farming operations make use of land-based pump ashore single pass technology and thus are mostly exposed to temperatures outside of the optimal range (Cook, 1998; Troell et al., 2006). Attempts at regulating on-farm water temperatures are not economically viable and thus it was hypothesised that it may be possible to improve the growth of abalone cultured at temperatures outside the optimal range by means of development of temperature specific dietary formulations.

While a number of studies have demonstrated the effects of temperature on abalone growth (Britz et al., 1997; Lopez et al., 1998; Searle et al., 2006; Garcia-Esquivel et al., 2007), none of these studies have considered the effect of temperature on the nutritional requirements of abalone. The energetic requirements of an animal are determined primarily by temperature (Smith, 1989), and the protein requirements of certain fish species have been found to be altered at different water temperatures (Wilson, 2002). Dixon (1992) found that protein digestibility in *H. midae* was significantly higher at 18 °C compared to either 15 or 22 °C. Since protein digestibility (Dixon, 1992) and feed consumption (Britz et al., 1997) have been found to be influenced by water temperature it is likely that the protein and energy requirements of *H. midae* are affected by water temperature and thus improvements in growth for abalone grown outside of the optimal

temperature range may be possible through the provision of sufficient levels of dietary protein and energy.

Protein has received the most attention in abalone nutritional studies as it is the principal dietary ingredient responsible for growth and is the most expensive cost component in formulated feeds (Mai et al., 1995a; Fleming et al., 1996). Feed formulators continually strive to define the optimal dietary protein level of formulated feeds for different species i.e. the level at which maximal growth occurs with the minimum amount of dietary protein (Wilson, 2002). Early attempts at defining optimal dietary protein levels of abalone involved feeding abalone incremental levels of dietary protein often provided from a single dietary protein source with little consideration of dietary energy levels (Mai et al., 1995a; Britz, 1996a). However, when attempting to define optimal dietary protein levels it is vital to consider the digestibility, amino acid profile of the test ingredients as well as the energy level of the test diet as a deficiency in one or more of these factors may lead to an overestimation of optimal dietary protein levels (Wilson, 2002). In addition, provision of dietary protein from a single source is likely to lead to an overestimation of dietary protein requirements (Mai et al., 1995a; Coote et al., 2000). Consideration of dietary energy levels is vital to ensure that sufficient energy is available for the energetic costs of physical activity and maintenance, thus allowing the protein portion of the diet to be made available exclusively for growth (Smith, 1989). The use of protein as an energy source should be avoided not only due to the costs associated with protein but also because it leads to the deamination of amino acids and the excretion of excess ammonia, which can create water quality complications (Smith, 1989). Therefore, beyond the requirements for essential amino acids for growth, the energetic requirements of abalone should as far as possible be satisfied through the use of non-protein sources of energy.

Carbohydrates and lipids serve as the potential energy sources for use in abalone feeds. Carbohydrates have been found to be highly digestible by abalone and are therefore the preferred energy source in abalone feeds (Mai et al., 1995b; Knauer et al., 1996; Monje and Viana, 1998; Gomez-Montes et al., 2003; Thongrod et al., 2003; Durazo-Beltran et

al., 2004; Montano-Vargas et al., 2005; Viana et al., 2007). Abalone have been found to have a limited ability to digest and utilise high levels of dietary lipid which is believed to be linked to the low lipid content of natural algal diets and the low levels of lipases present in the abalone gut (Mercer et al., 1993; Mai et al., 1995b; Britz et al., 1996; Fleming et al., 1996; Knauer et al., 1996; Britz and Hecht, 1997; Durazo-Beltran et al., 2003; Thongrod et al., 2003; Montano-Vargas et al., 2005; Garcia-Esquivel and Felbeck, 2006). In addition to this high levels of lipid have been found to reduce the uptake of other nutrients in the diet (Van Barneveld et al., 1998). However, previous research on lipid utilisation has not considered the relationship between dietary energy and protein and it is possible that the ability of abalone to utilise lipids as a source of energy differs in the presence of varying levels of dietary protein.

Recent trends in abalone nutritional studies have focused on the identification of alternative dietary protein sources as well as reductions in the protein portion of formulated feeds. Identification of alternative dietary protein sources have been undertaken for *Haliotis fulgens* (Guzman and Viana, 1998), *Haliotis midae* (Shipton and Britz, 2001a), *Haliotis asinina* (Bautista-Teruel et al., 2003) and *Haliotis discus hannai* (Cho et al., 2008). Shipton and Britz (2001a) found that fishmeal could be replaced with up to 30 % of sunflower meal, soya or torula yeast without significantly affecting the growth of *H. midae*. Reductions in the protein portion of formulated feeds for abalone have been demonstrated for species including: *H. asinina* (Bautista-Teruel and Millamena, 1999), *H. laevigata* (Coote et al., 2000), *H. midae* (Sales et al., 2003; Jones and Britz, 2006). These reductions have been achieved through the provision of sufficient levels of dietary energy supplied from carbohydrates. It is likely that further reductions in the protein portion of formulated feeds may be possible through manipulation of dietary protein to energy ratios.

Efforts to optimise the dietary protein content of formulated feeds are not focused exclusively on the potential economic benefits but also on improvements on factors such as culture environment water quality and animal health. The use of high protein diets has been associated with outbreaks of the sabellid worm *Terebrasabella heterouncinata* on

abalone farms (Simon et al., 2004). In addition to this, abalone fed high protein diets and exposed to additional stressors such as handling or elevated water temperatures have been found to be susceptible to "bloat". This is a condition which is believed to be caused by the proliferation of gut bacteria which leads to the fermentation of the gut contents and the accumulation of gas in the digestive tract (Macey and Coyne, 2005; Godoy et al., 2006). Optimisation of dietary protein to energy ratios may lead to economic savings as well as reductions in water quality complications associated with formulated feeds.

The relationship between the dietary protein and energy requirements of *H. midae* have not been addressed since the findings of Britz and Hecht (1997). A dietary lipid content of 10 % was found to negatively affect growth, and juvenile abalone (10 mm shell length) were found to have a lower dietary protein requirement (34 %) compared to young adult abalone (40 mm shell length) (44 %) (Britz and Hecht, 1997). Differences in the nutritional requirements of abalone of different sizes were also detected by Shipton and Britz (2001b). The protein requirements of fish generally decrease with an increase in age and size (Wilson, 2002). Based on the findings of Britz and Hecht (1997) abalone may not conform to this trend. However, more recently Jones and Britz (2006) found that it was possible to reduce the protein content of formulated feeds for adult *H. midae* (> 50 mm shell length) from 34 to 26 % without negatively affecting growth. Clarification of the nutritional requirements of abalone of different sizes may lead to the development of size specific dietary formulations thus optimising current dietary formulations.

The South African abalone culture industry is now considered to have entered a mature phase with an estimated total investment in excess of ZAR 190 million (Loubser, 2005). However, the rise in abalone production worldwide from countries such as Australia and Chile threatens to reduce the average market price (Loubser, 2005). It is therefore vital for South African abalone farmers to reduce operational costs in order to remain competitive on the world's markets. The optimisation of dietary protein and energy levels in formulated feeds as well as the potential development of temperature and size specific feeds will not only result in economic benefits but will also help take pressure off natural kelp reserves and improve on-farm water quality conditions and animal health.

Aims and objectives

The overall aim of the study was to investigate the protein and energy requirements of abalone of different sizes cultured within a range of different water temperatures.

The objectives were to:

1. Investigate the protein and energy requirements of *H. midae* cultured at optimal (18 – 20 °C) and elevated (22 – 24 °C) water temperatures.
2. Investigate the size specific dietary protein requirements of *H. midae* cultured within the optimal temperature range (14 – 18 °C) under laboratory and farm conditions.
3. Investigate the size specific dietary lipid requirements of *H. midae* and determine whether abalone are able to utilise dietary lipids as a source of energy.

CHAPTER 2.

THE PROTEIN AND ENERGY REQUIREMENTS OF FARMED SOUTH AFRICAN ABALONE *HALIOTIS MIDAE* CULTURED AT OPTIMAL AND ELEVATED WATER TEMPERATURES

2.1 Introduction

Abalone farms in South Africa are becoming increasingly dependent on the use of formulated feeds due to limitations in the supply of harvested seaweed (Britz et al., 1994; Loubser, 2005; Troell et al., 2006), and formulated feeds promote superior growth rates compared to natural diets (Viana et al., 1993; Britz et al., 1994; Britz, 1996b; Capinpin and Corre, 1996; Bautista-Teruel and Millamena, 1999). Farmers make use of either cultured or harvested algal diets, formulated feeds or a combination of the two (Cook, 1998; Troell et al., 2006). However, the use of formulated feeds is avoided at temperatures in excess of 20 °C (Britz et al., 1997), due to the perception that the abalone are susceptible to "bloat", a fatal condition caused by proliferation of bacteria in the gut resulting in the fermentation of the gut contents and accumulation of gas in the digestive tract (Macey and Coyne, 2005; Godoy et al., 2006). Britz et al. (1997) demonstrated that temperatures in the range of 12 to 20 °C were physiologically optimal for *H. midae* and growth, feed consumption and condition factor declined and feed conversion ratio (FCR) and mortality increased sharply at temperatures in excess of 20 °C. Since the currently available commercial feeds were formulated to promote maximal growth of abalone cultured within the physiologically optimal temperature range, it was hypothesised that the dietary protein and energy requirements of abalone cultured within the stressful temperature range of (20-25 °C) might differ significantly.

The overall aim of this study was thus to investigate the effect of dietary protein and energy on the growth and survival of abalone cultured at optimal and elevated water temperatures in order to try and develop a diet that can safely be fed to abalone cultured at elevated water temperatures. The growth, consumption and survival of adult *H. midae* (> 50 mm initial shell length) fed formulated diets containing graded levels of protein

(18, 22 and 26 %) and energy (11.6, 13.5 and 16.2 $MJ.kg^{-1}$) and cultured at 18, 22 and 24 °C were compared.

2.2 Materials and methods

2.2.1 Experimental system

The experiment was conducted at the Rhodes University Port Alfred Marine Research Laboratory (33°45'S; 26°00'E) in three identically designed partially recirculating culture systems, each comprised of four 1500 L canvas tanks (2.2 m x 1 m x 0.8 m; length, width and depth) and a 1000 L header tank (Figures 2.1, 2.2 and 2.3). Three of the tanks in each system were used to hold abalone while the fourth tank acted as a sedimentation tank and held the biological filter. The biological filter included submerged oyster shells and a shredded plastic trickle filter. Water was recirculated within each system so that the entire volume of each of the three tanks in each system was exchanged every two hours. A protein skimmer (Ultrazap®, Johannesburg, South Africa) was included inline and 10 % of the entire system's volume was replaced daily with seawater (35 $g.L^{-1}$) from the Kowie River estuary.

Each holding tank contained 12 oyster mesh baskets (36 cm x 48 cm x 60 cm), and each basket contained six vertical Acrylonitrile butadiene styrene plastic plates (total surface area: 14 040 cm^2) and a horizontal "feeding plate" positioned eight cm below the surface of the water.

The water temperature in each of the systems was set at either 18, 22 or 24 °C. Water temperatures were raised by 1 °C every 24 h at the start of the trial until the experimental systems reached the required temperatures. The temperatures were maintained using thermostatically controlled submerged heaters (three kW) housed in each header tank and thermostatically controlled in-line water chilling units (Aqua medic® Titan 4000, Guangzhou, China). In addition to this Isotherm® insulation was placed around the tanks and header tanks and corrugated plastic sheeting was also placed around the sides of the tanks and lids were placed over the top of the tanks.

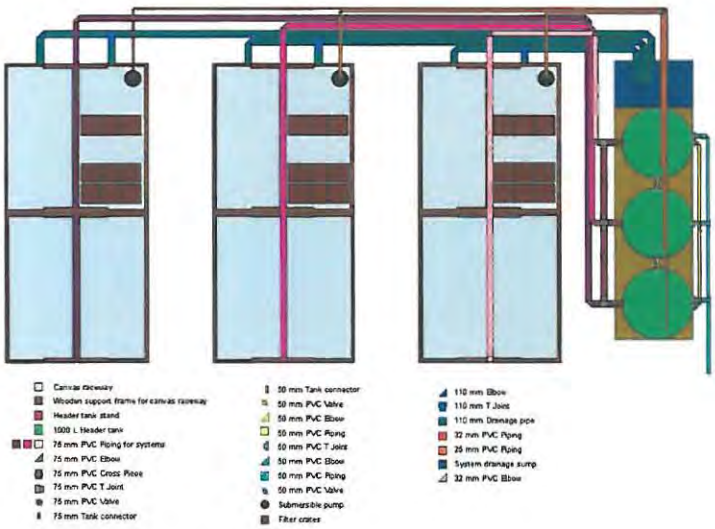

Figure 2.1: Aerial plan of the experimental abalone system constructed at the Rhodes University Port Alfred Marine Research Laboratory.

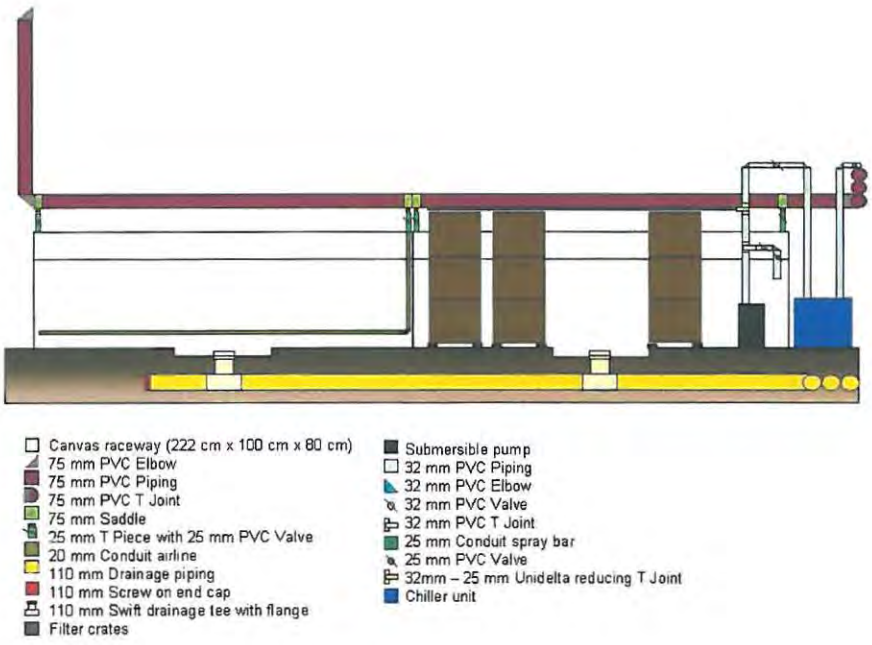

Figure 2.2: Section plan of the experimental abalone system constructed at the Rhodes University Port Alfred Marine Research Laboratory.

Figure 2.3: The experimental abalone system at the Rhodes University Port Alfred Marine Research Laboratory.

Aeration was provided by airlines installed along the length of the bottom of each tank. The tanks were drained, cleaned and refilled with water of the required temperature from the same system once a week. The systems were exposed to a natural photoperiod regime, which was approximately 12:12 (L:D).

2.2.2 Experimental diets

Nine experimental diets were formulated to contain graded levels of crude protein (18, 22 and 26 %) and energy, in order to produce a range of protein to energy ratios (1.10 – 2.14 g protein/ $MJ.kg^{-1}$ energy) (Table 2.1). This resulted in three dietary energy contents, high (16.2 $MJ.kg^{-1}$), medium (13.5 $MJ.kg^{-1}$) and low (11.6 $MJ.kg^{-1}$) per dietary protein content. Low temperature steam dried, formaldehyde free, mackerel fishmeal (Oceana

(Pty) Ltd, South Africa) and soya-oil cake were used as the primary protein sources and the diets had a constant dietary lipid content of 5.5 %. Fresh kelp (*Ecklonia. maxima*), a vitamin and mineral mix (Vitamin mineral premix, BASF Animal Nutrition (Pty) Ltd, South Africa) and starch carbohydrates were also included based on a proprietary commercial formulation (Marifeed Pty Ltd, South Africa). Diatomaceous earth replaced starch as a non-nutritive filler in order to obtain the desired protein:energy ratios.

The digestible energy coefficients of fishmeal, soya-oil cake and carbohydrates were calculated using the digestibility coefficients determined by Sales and Britz (2001b). While, the value for kelp (*E. maxima*) was not available so the value was estimated based on the digestible energy coefficients of other seaweeds that were established for the sea urchin *Paracentrotus lividus* (Schlosser et al., 2005).

The proximate composition of the experimental diets were measured using the standard methods of the AOAC (2003). Crude protein was determined using the semi-micro Kjeldahl method (N x 6.25), moisture by oven drying samples at 95 °C for 72 h, ash as the residue remaining following the combustion of samples at 550 °C for 18 h, fat by the Soxhlet extraction method, phosphorus content by digesting samples with sulphuric acid, hydrogen peroxide and a selenium catalyst using a block digestor at 360 °C and gross energy was determined by combustion of samples in a adiabatic calorimeter.

2.2.3 Experimental animals, acclimation and feeding

Abalone with a mean starting shell length of 54.90±0.08 mm and weight of 28.99±0.16 g were obtained from Marine Growers Abalone Farm (Pty) Ltd situated on the east coast of South Africa where they had previously been fed a combination of cultured algal diets and an artificial feed (Table 2.2). They were stocked into the baskets at 5 % of available surface area (i.e. 36 abalone per basket) and acclimated to the experimental systems for five weeks before the start of the trial. They were fed a locally produced commercial feed Abfeed®-K26 (26% crude protein, 1% lipid, Marifeed (Pty) Ltd) and kept at 18 °C during the acclimation period.

Table 2.1: The nine formulated diets containing graded levels of dietary protein and energy. The values displayed are the values formulated and the values obtained from nutritional analyses.

	Diet								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Formulations									
Crude protein (%)	26	26	26	22	22	22	18	18	18
Digestible energy ($MJ.kg^{-1}$)	16.01	13.80	12.13	16.20	13.60	11.70	16.38	13.20	11.10
Protein:energy ratio (g protein/$MJ.kg^{-1}$ energy)	1.62	1.88	2.14	1.36	1.62	1.88	1.10	1.36	1.62
Lipid (%)	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
Proximal analyses									
Crude protein (%)	25.04	25.42	25.7	22.33	21.88	21.70	18.04	18.30	17.04
Gross energy ($MJ.kg^{-1}$)	18.09	16.15	14.23	17.86	15.45	13.60	17.61	14.88	12.52
Lipid (%)	3.80	4.36	5.31	3.72	5.08	5.12	2.94	5.21	4.28
Phosphorus (%)	0.67	0.69	0.74	0.65	0.58	0.58	0.44	0.46	0.47
Moisture (%)	7.30	5.70	4.20	7.60	5.30	4.10	8.40	5.20	4.10
Ash (%)	5.31	18.03	28.72	5.31	19.87	31.27	4.10	21.78	34.87

Table 2.2: Mean initial shell length (ANOVA: $F_{(8,99)}=0.98$; $p=0.46$), weight (ANOVA: $F_{(8,99)}=0.98$; $p=0.46$) and condition factor (ANOVA: $F_{(8,99)}=0.71$; $p=0.68$) of the abalone at the start of the trial. Significant differences are indicated by different alphabetical superscripts in each column (ANOVA: $p<0.05$).

Diet	Mean initial shell length (mm)	Mean initial weight (g)	Mean initial condition factor
1	55.05 ± 0.28^{a}	29.59 ± 0.54^{b}	1.03 ± 0.01^{c}
2	54.94 ± 0.22^{a}	29.35 ± 0.42^{b}	1.03 ± 0.01^{c}
3	54.72 ± 0.23^{a}	28.84 ± 0.51^{b}	1.02 ± 0.01^{c}
4	54.97 ± 0.19^{a}	29.21 ± 0.40^{b}	1.02 ± 0.01^{c}
5	54.53 ± 0.23^{a}	28.17 ± 0.36^{b}	1.01 ± 0.01^{c}
6	54.73 ± 0.26^{a}	28.58 ± 0.49^{b}	1.01 ± 0.01^{c}
7	54.77 ± 0.20^{a}	28.57 ± 0.39^{b}	1.01 ± 0.01^{c}
8	55.03 ± 0.29^{a}	29.27 ± 0.63^{b}	1.02 ± 0.01^{c}
9	55.31 ± 0.19^{a}	29.37 ± 0.49^{b}	1.01 ± 0.01^{c}

The diets were assigned to baskets of abalone using a randomised block design to ensure that each diet was fed to four baskets of abalone at each temperature regime and that there was at least one replicate in each of the three tanks in each system. Thus each diet was replicated four times at each of the three temperature regimes. Each basket was assumed to be a unit of measure, since it was not feasible to build nine isolated systems, i.e. one for each of the three temperature regimes, at the commercial-like scale at which the experiment was run. The reliability of this assumption was strengthened by block-design described here.

Abalone were fed to apparent satiation daily at 16h00 six days a week. Food consumption and uneaten food was recorded for each basket of abalone during the course of the trial. Any uneaten food was removed within in 24 hours and frozen at - 4 °C. It was later oven dried at 100 °C for 24 h and then weighed at the end of the trial.

2.2.4 Solid leaching of diets

Leaching rates were determined for each diet at the three temperature regimes by placing 10 g of feed on a "feeding plate" in a basket containing no abalone with four replicate samples per diet at each temperature regime. The feed was removed after 24 hours and

oven dried at 100 °C for a further 24 hours. The leaching rates were calculated as the dry weight loss during the 24 h period in the water.

2.2.5 Data collection

The abalone were purged for 24 h prior to being weighed and measured. They were then anaesthetised with a 10 % magnesium sulphate solution and excess water was removed from the shell of the abalone using paper towel. All the abalone in each basket were weighed (0.01 g) using an electronic balance and measured (0.01 mm) with digital vernier calipers at the start and end of the 10 week growth trial.

Abalone shell length gain, weight gain and survival were calculated for all treatments. Abalone condition factor was calculated according to Britz (1996b) using the equation:

$$\text{Condition factor} = \text{weight (g)/length (mm)}^{\wedge 2.99} \times 5575 \quad (1)$$

Feed conversion ratio (FCR) and daily feed consumption (% body weight per day) were corrected for solids leaching and calculated for all treatments according to Britz (1996b) using the equations:

$$\text{FCR} = \text{dry feed consumed (g)/wet weight gain (g)} \quad (2)$$

$$C_{\%\ b.\ wt} = C_g / W_t \times 100 \quad (3)$$

where C_g is the mean daily feed consumption corrected for leaching and W_t is the mean abalone weight at time t (days). W_t was calculated using the equation:

$$W_t = W_o \times ((SGR/100) + 1)^{t-1} \quad (4)$$

where W_o is the mean initial abalone weight and SGR is the specific growth rate.
The SGR was calculated using the equation:

$$SGR = ((\ln(W_f) - \ln(W_i))/ t\ )100 \quad (5)$$

where SGR is the specific growth rate (% body weight increase per day), $\ln(W_f)$ is the natural log of the mean final weight of abalone, $\ln(W_i)$ is the natural log of the mean initial weight of abalone , and t is the time in days.

Baskets were checked for mortalities every second day and any dead abalone were removed and recorded.

2.2.6 Water chemistry

Temperature and pH readings were recorded twice a day using a digital temperature and pH probe (Hanna Instruments, HI 98128, Woonsocket, USA). Dissolved oxygen was monitored daily with an oxygen meter (Hanna Instruments HI 9143, Woonsocket, USA). Salinity was monitored using a refractometer (Extech Instruments, RF20, Waltham, USA). Ammonia and nitrite were measured twice a week using colourmetric titration kits (Red Sea Fish Pharm, Houston, USA). Free unionised ammonia (FAN) was calculated based on total ammonia nitrogen readings (TAN), temperature, pH and salinity values according to Bower and Bidwell (1978). Water chemistry data for the 10 week trial is summarised in Table 2.3.

Table 2.3: Summary of the environmental parameters recorded during the 10 week growth trial. Values shown are means with standard deviations.

Parameter	**System 1**	**System 2**	**System 3**
Temperature (°C)	17.97±0.44	21.91±0.55	23.45±0.98
pH	7.90±0.10	7.89±0.08	7.96±0.07
Dissolved oxygen ($mg.L^{-1}$)	8.30±0.85	7.34±1.02	7.34±0.68
Ammonia ($mg.FAN.L^{-1}$)	0.0077±0.00	0.0091±0.00	0.012±0.00
Nitrite ($mg.L^{-1}$)	0.05±0.00	0.05±0.00	0.05±0.00
Salinity (‰)	35	35	35

2.2.7 Statistical analysis

The effect of dietary protein, energy and water temperature on abalone shell length gain, weight gain, final condition factor and feed consumption were compared using a multifactor analysis of variance (multifactor ANOVA) and a Tukey's multiple range analysis at $p<0.05$. If there were no interactions between the three factors, i.e. the effect of one factor did not influence the effect of another factor, then a one-way analysis of variance (ANOVA) and a Tukey's multiple range analysis ($p<0.05$) were used to compare the means within each factor. When data did not meet the assumptions of an ANOVA a non parametric Kruskal-Wallis ANOVA ($p<0.05$) was used.

2.3 Results

Water temperature alone had a significant effect on abalone shell length gain (ANOVA: $F_{(2,105)}=39.60$; $p<0.00$) and weight gain (ANOVA: $F_{(2,105)}=58.43$; $p<0.00$) (Table 2.4). Abalone cultured at 18 °C grew fastest (2.23±0.10 mm.month^{-1}, 4.33±0.25 g.month^{-1}) followed by those cultured at 22 and 24 °C. Similarly, the final condition factor of abalone was significantly effected by water temperature (ANOVA: $F_{(2,105)}=65.89$; $p<0.00$), and was highest in abalone cultured at 18 °C (1.03±0.01) decreasing significantly with an increase in water temperature (Table 2.4).

Table 2.4: The effect of water temperature on mean (± standard error) abalone shell length gain (ANOVA: $F_{(2,105)}=39.60$; $p<0.00$), weight gain ANOVA: $F_{(2,105)}=58.43$; $p<0.00$), final condition factor (ANOVA: $F_{(2,105)}=65.89$; $p<0.00$) and feed conversion ratio (Kruskal-Wallis: $H_{(2,95)}=28.83$; $p<0.00$) after 10 weeks. Significant differences are indicated by different alphabetical superscripts within each row (ANOVA; $p<0.05$).

	Temperature (°C)		
	18.0±0.44	**22.0±0.55**	**24.0±0.98**
Mean shell length gain (mm.month^{-1})	2.23±0.10^{a}	1.77±0.08^{b}	1.13±0.05^{c}
Mean weight gain (g.month^{-1})	4.33±0.25^{a}	2.53±0.20^{b}	0.77±0.14^{c}
Final condition factor	1.03±0.01^{a}	1.00±0.01^{b}	0.93±0.01^{c}
FCR	1.82±0.13^{a}	2.45±0.20^{b}	3.47±0.34^{c}

There were no interactions between the effects of the three factors combined, i.e. the effect of water temperature, dietary protein and energy on abalone shell length gain (multifactor ANOVA: $F_{(8,81)}=1.68$; $p=0.12$) (Figure 2.4), weight gain (multifactor ANOVA: $F_{(8,81)}=1.04$; $p=0.41$ (Figure 2.5) or final condition factor (multifactor ANOVA: $F_{(8,81)}=0.66$; $p=0.73$). However, there were significant interactions between the effects of dietary protein and dietary energy on shell length gain (multifactor ANOVA: $F_{(4,81)}=8.51$; $p=0.01\text{x}10^{-3}$), weight gain (multifactor ANOVA: $F_{(4,81)}=17.05$; $p=0.04\text{x}10^{-3}$) and final condition factor (multifactor ANOVA: $F_{(4,81)}=7.17$; $p=0.05\text{x}10^{-3}$). The protein content of the diet had no significant effect on abalone shell length gain or weight gain provided the energy content of the diet did not drop below 13.5 MJ.kg^{-1} (Figures 2.6 and 2.7). Abalone fed the low protein low energy diet (i.e. 18 % protein; 11.6 MJ.kg^{-1} energy) had significantly lower shell length gain (0.81±0.08 mm.month^{-1}), weight gain (0.20±0.23 g.month^{-1}) and final condition factor (0.92±0.01) than those fed the higher protein (22

and 26 %) low energy diets with combined means of 1.38±0.15 mm.month^{-1}, 1.83±0.43 g.month^{-1} and 0.98±0.02 (Figures 2.6, 2.7 and 2.8).

Water temperature, dietary protein and dietary energy each had a significant effect on abalone mortality, with no interactions between the three factors (multifactor ANOVA: $F_{(8,81)}$=1.28; p=0.26) (Figure 2.9). The rate of mortality was similar at 18 and 22 °C (8.1±2.25 and 10.1±2.27 % respectively) but increased significantly at 24°C (17.4±2.63 %) (Kruskal-Wallis: $H_{(2,108)}$=13.04; p=0.002). Abalone in treatments fed the 22 and 26 % protein diets had similar mortality rates (8.6±1.77 and 7.6±1.60 % respectively) while mortalities were significantly higher at 19.4±3.21 % in treatments fed the 18 % protein diets (Kruskal-Wallis: $H_{(2,108)}$=9.80; p=0.01). Similarly, mortalities were significantly higher for abalone fed the low energy diets (11.6 MJ.kg^{-1}) (27.1±2.59 %) compared to those fed the 13.5 and 16.2 MJ.kg^{-1} diets with means of 4.8±1.00 and 3.8±0.91 % respectively (Kruskal-Wallis: $H_{(2,108)}$=56.41; p<0.00).

There were significant interactions between dietary protein and energy in terms of feed consumption (multifactor ANOVA: $F_{(4,81)}$=30.12; p<0.00) (Figure 2.10). Abalone fed the low protein low energy diet (i.e. 18 % protein, 11.6 MJ.kg^{-1} energy) had significantly lower feed consumption rates (0.26±0.03 % bd.wt.day^{-1}) compared to those fed the other diets (combined mean of 0.49±0.03 % bd.wt.day^{-1}). Abalone fed the low energy (11.6 MJ.kg^{-1}) 22 and 26 % protein diets had significantly higher feed consumption rates (0.53±0.04 and 0.55±0.04 % bd.wt.day^{-1} respectively) compared to those fed the high energy (16.2 MJ.kg^{-1}) 22 and 26 % diets (combined mean of 0.45±0.04 % bd.wt.day^{-1}). Abalone fed the 26 % protein 13.5 MJ.kg^{-1} diet had significantly higher feed consumption (0.53±0.03 % bd.wt.day^{-1}) compared to those fed the 18 % protein 13.5 MJ.kg^{-1} diet (0.43±0.03 % bd.wt.day^{-1}). Feed consumption decreased significantly with an increase in water temperature from 0.56±0.02 % bd.wt.day^{-1} at 18 °C to 0.33±0.02 % bd.wt.day^{-1} at 24 °C (ANOVA: $F_{(2,105)}$=47.74; p<0.00) (Figure 2.10).

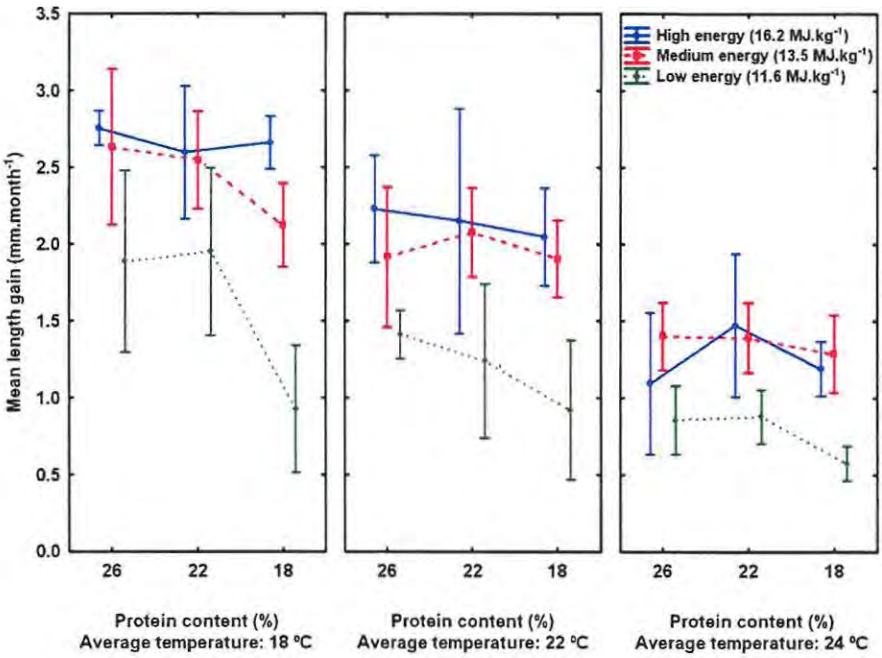

Figure 2.4: Mean shell length gain (± 95 % confidence intervals) (multifactor ANOVA: $F_{(8,81)}$=1.68; p=0.12) of abalone fed formulated diets containing graded levels of protein and energy and cultured at three different water temperatures for 10 weeks.

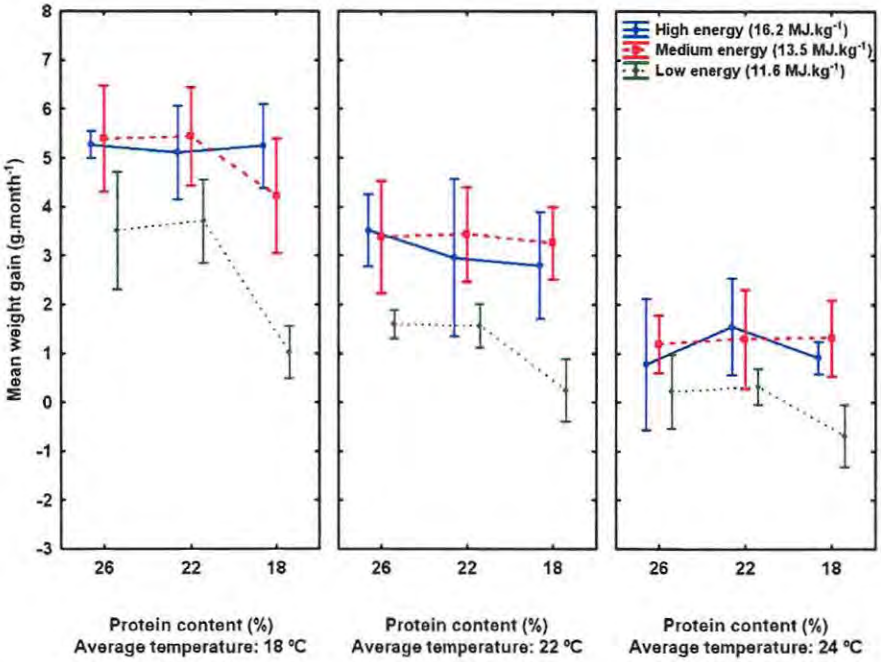

Figure 2.5: Mean weight gain (± 95 % confidence intervals) (multifactor ANOVA: $F_{(8,81)}$=1.04; p=0.41) of abalone fed formulated diets containing graded levels of protein and energy and cultured at three different water temperatures for 10 weeks.

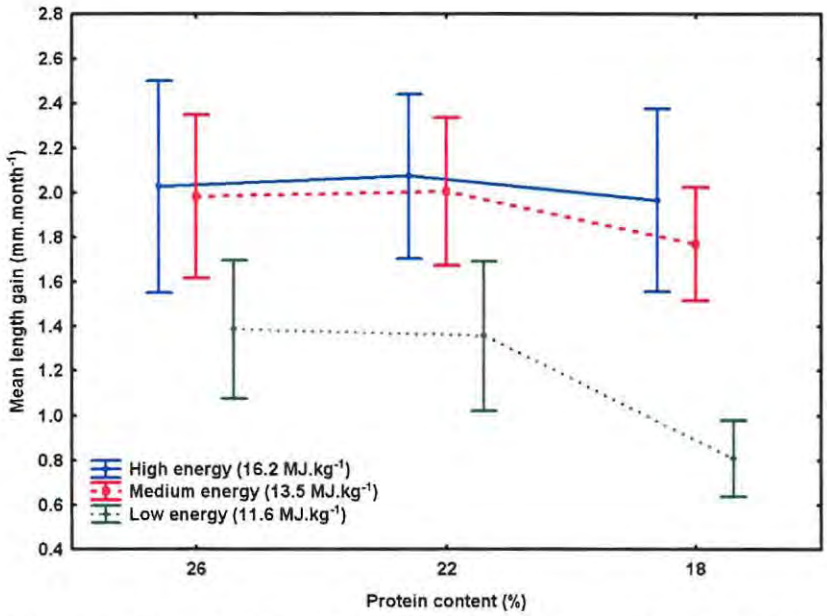

Figure 2.6: Mean shell length gain (± 95 % confidence intervals) (multifactor ANOVA: $F_{(4,81)}$=8.51; p<0.00) of abalone fed formulated diets containing graded levels of protein and energy.

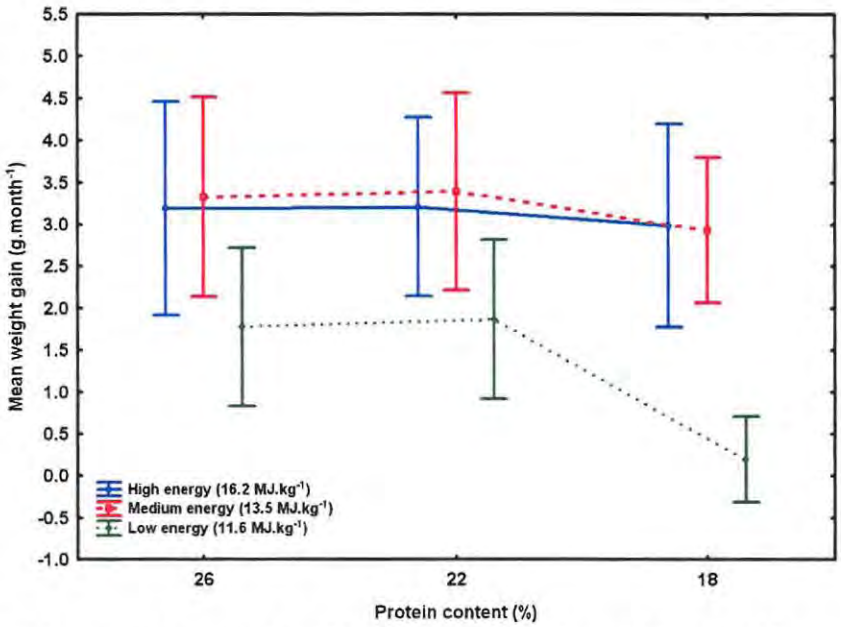

Figure 2.7: Mean weight gain (± 95 % confidence intervals) (multifactor ANOVA: $F_{(4,81)}$=17.05; p<0.00) of abalone fed formulated diets containing graded levels of protein and energy.

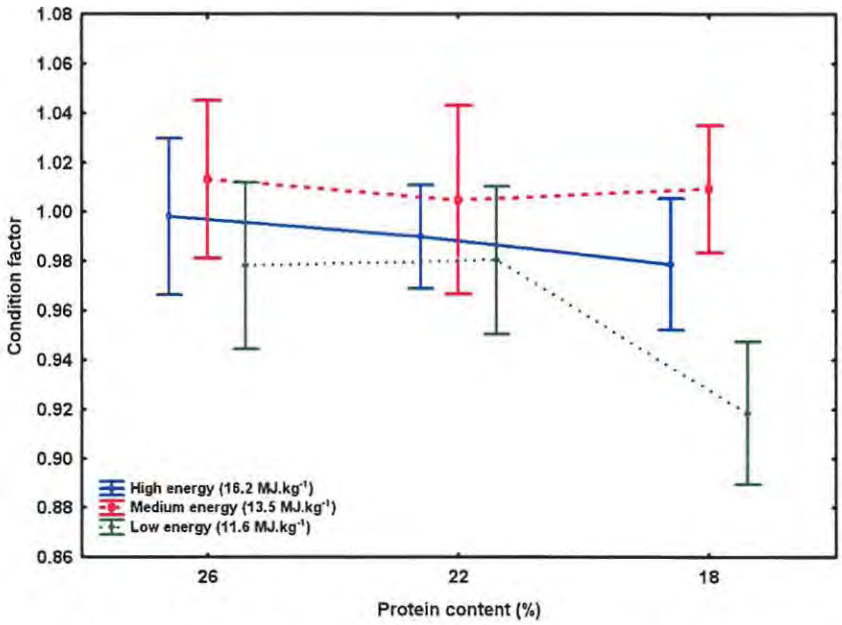

Figure 2.8: Final condition factor (± 95 % confidence intervals) of abalone fed formulated diets containing graded levels of protein and energy (multifactor ANOVA: $F_{(4,81)}$=7.68; p<0.00). The mean condition factor of the abalone at the start of the trial was 1.02.

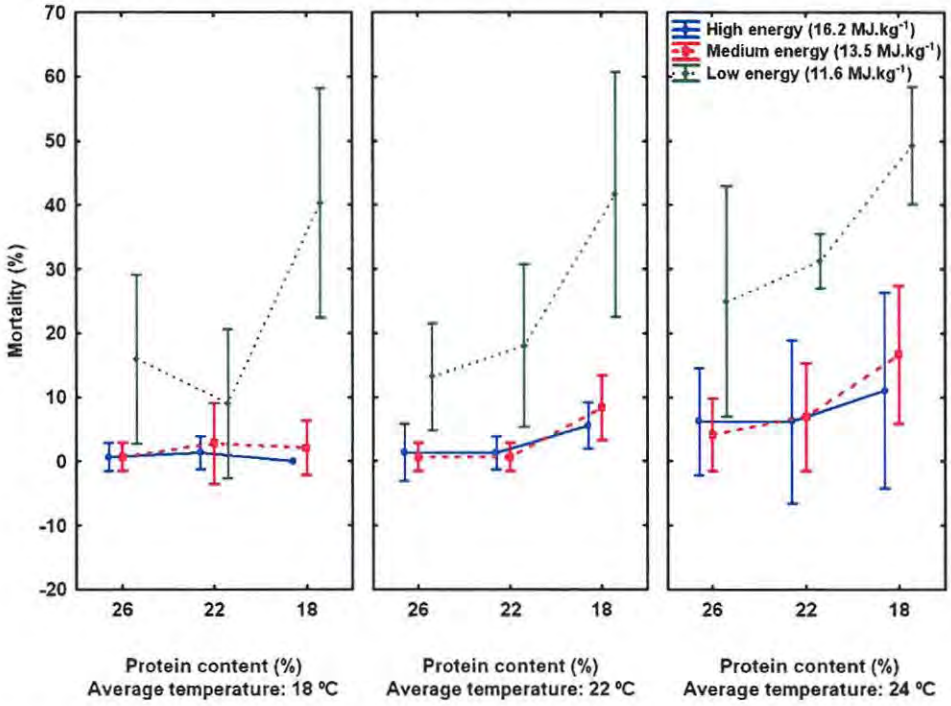

Figure 2.9: Mean mortality (± 95 % confidence intervals) of abalone cultured at three water temperatures and fed formulated diets containing graded levels of protein and energy (multifactor ANOVA: $F_{(8,81)}$=1.28; p=0.26).

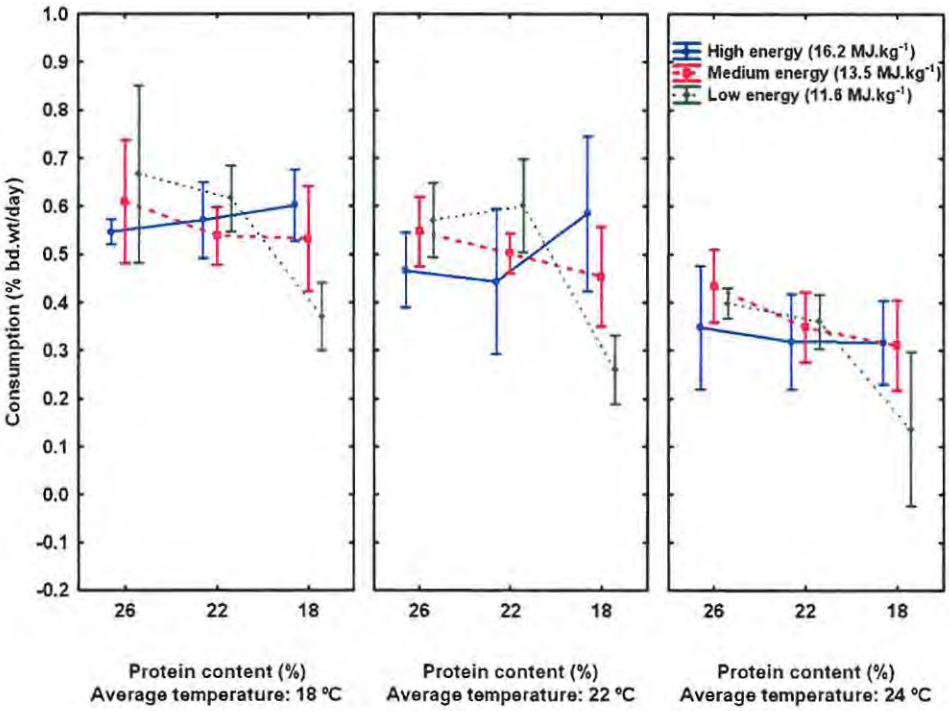

Figure 2.10: Abalone feed consumption (± 95 % confidence intervals) expressed as percentage of body weight per day of abalone cultured at three water temperatures and fed formulated diets containing graded levels of protein and energy (multifactor ANOVA: $F_{(8,81)}$=1.45; p=0.18).

Water temperature and dietary energy each had a significant effect on FCR, although there were no interactions between the three factors (multifactor ANOVA: $F_{(7,69)}$=1.18; p=0.32). FCR increased significantly with an increase in water temperature from 18 to 24 °C (Kruskal-Wallis: $H_{(2,95)}$=28.83; p<0.00) (Table 2.4). FCR values were similar for abalone fed the high (16.2 MJ.kg^{-1}) and medium (13.5 MJ.kg^{-1}) energy diets (1.98±0.13 and 2.16±0.21 respectively) but increased significantly when dietary energy was reduced to 11.6 MJ.kg^{-1} (3.63±0.31) (Kruskal-Wallis: $H_{(2,95)}$=25.50; p<0.00). Interestingly, dietary protein content had no effect on FCR with a combined mean of 2.48±0.24 for the three dietary protein levels (Kruskal-Wallis: $H_{(2,95)}$=1.12; p=0.57).

2.4 Discussion

The observed rise in mortality coupled with the decline in abalone growth, food conversion efficiency, condition factor and feed consumption with an increase in water temperature from 18 to 24 °C confirmed the trends observed by Britz et al. (1997) who reared abalone on a fishmeal/starch diet.

While some studies have attempted to define optimal protein:energy ratios for cultured abalone (Britz and Hecht, 1997; Bautista-Teruel and Millamena, 1999; Gomez-Montes et al., 2003; Montano-Vargas et al., 2005), the present results support the suggestion by Fleming et al. (1996) that the energy contents of commercial abalone feed formulations are over-specified. Published dietary energy values for formulated feeds for abalone range from 12.94 to 21.62 $MJ.kg^{-1}$ (Mai et al., 1995a; Mai et al., 1995b; Bautista-Teruel and Millamena, 1999; Coote et al., 2000; Durazo-Beltran et al., 2003; Gomez-Montes et al., 2003; Sales et al., 2003; Thongrod et al., 2003; Montano-Vargas et al., 2005). There were no differences in growth or mortality between abalone fed the high (16.2 $MJ.kg^{-1}$) or medium energy (13.5 $MJ.kg^{-1}$) diets. However, the poor growth and survival of abalone fed the low energy (11.6 $MJ.kg^{-1}$) diets suggests that this dietary energy level is insufficient to meet the energetic requirements of *H. midae* in the size range tested here, or the diets were unpalatable. The three low energy diets (11.6 $MJ.kg^{-1}$) had relatively high ash contents (30 %) in comparison to the medium (20 %) and high (5 %) energy diets. High ash levels in algae are due to the presence of calcium carbonate which has been found to limit the presence of other nutrients and reduce their uptake (Hay et al., 1994; Viera et al., 2005). It is possible that the low energy diets contained high levels of calcium carbonate and this may explain the poor growth and survival observed.

It is possible to reduce the protein content of formulated feeds for *H. midae* to as low as 18 % provided the diet contains sufficient energy (13.5 $MJ.kg^{-1}$ or more). This value is lower than the optimal protein levels reported by other authors, for example, Bautista-Teruel and Millamena (1999) reduced the protein content of diets for *H. asinina* from 32 to 27 % by increasing the energy content of the diet from 12.94 to 13.19 $MJ.kg^{-1}$ with no significant effect on abalone growth. Sales et al. (2003) fed juvenile *H. midae* diets with

protein contents ranging from 5.5 to 48 %, with gross energy values of 16.0 to 17.0 $MJ.kg^{-1}$, and found weight gain to peak at a dietary protein content of 22 %. Coote et al. (2000) found that young *H. laevigata* grew fastest when fed a semipurified diet with a protein and energy content of 27.4 % and 18 $MJ.kg^{-1}$ respectively, but these growth rates were not significantly different to diets containing less protein but similar energy levels, further supporting the notion that the dietary protein level of formulated feeds for abalone may be reduced provided there is a sufficient supply of dietary energy.

The feed consumption rates fell within the range reported for *H. midae* of similar sizes (Britz and Hecht, 1997; Shipton and Britz, 2001b). Abalone fed the 26 % protein 13.5 $MJ.kg^{-1}$ diet showed higher feed consumption rates and FCR compared to those fed the 18 % protein diet with the equivalent energy level. The explanation provided by Mai et al. (1995b) and Coote et al. (2000) may explain the observed trend. As the protein content of the diet increases the energy content of the diet does not always increase proportionately and thus protein in the diet is catabolised into energy in order to breakdown and excrete the excess protein (Mai et al., 1995a; Coote et al., 2000). This may explain the higher feed consumption rate of abalone fed the 26 % protein diet compared to those fed the 18 % protein diet at a similar energy level, which further emphasises the importance of balancing the amount of dietary protein and energy in abalone diets.

The higher consumption rate of abalone fed the higher protein (22 and 26 %) low energy (11.6 $MJ.kg^{-1}$) diets suggests that the abalone were consuming more food in order to satisfy their energetic requirements. Bautista-Teruel and Millamena (1999) observed a similar trend with *H. asinina* where abalone fed a low energy natural diet (17.56 % protein; 9.21 $MJ.kg^{-1}$ energy) displayed the highest feed conversion ratio. Similarly, Gomez-Montes et al. (2003) attributed differences in caloric intake for *H. fulgens* fed diets ranging from 26 to 44 % protein and 16.89 to 17.26 $MJ.kg^{-1}$ energy to a requirement for dietary energy.

Abalone cultured at 22 and 24 °C in the current study achieved average growth rates of 2.09 mm.month^{-1}; 3.33 g.month^{-1} and 1.34 mm.month^{-1}; 1.21 g.month^{-1} respectively, provided they received a sufficient balance of dietary protein (22 – 26 %) and energy (13.5 – 16.2 MJ.kg^{-1}). While the growth rates at 24 °C fall below the desired rate of 2-3 mm.month^{-1} required by farmers (Hahn, 1989b; Fleming et al., 1996), the use of these diets would allow farmers to safely feed their stock during periods of elevated water temperatures in order to prevent abalone weight loss and mortalities that normally occur during these periods.

2.5 Conclusion

The efficiency of feed conversion, growth rate and condition factor of *H. midae* fed the experimental diets decreased, and mortality increased, with increasing water temperature from 18 to 24 °C. A dietary energy content of at least 13.5 MJ.kg^{-1} for *H. midae* in the size range tested (50-60 mm shell length) is required for optimal growth performance. A dietary energy level of 11.6 MJ.kg^{-1} was insufficient to meet the energetic requirements of *H. midae* regardless of the protein content of the diet. The protein content of the diet may be reduced to 18 % without significantly reducing growth, provided dietary energy is maintained at 13.5 MJ.kg^{-1} or more.

CHAPTER 3.

THE INFLUENCE OF BODY SIZE AND WATER TEMPERATURE ON THE DIETARY PROTEIN REQUIREMENTS OF FARMED SOUTH AFRICAN ABALONE *HALIOTIS MIDAE*

3.1 Introduction

The protein requirements and digestive capabilities of abalone have been the primary focus of abalone nutritional studies as protein is the principal dietary component responsible for growth, and is the highest cost component in formulated feeds (Mai et al., 1995a; Fleming et al., 1996). Investigations into the protein requirements of abalone have been undertaken for *Haliotis tuberculata* and *Haliotis discus hannai* (Mai et al., 1995a), *Haliotis midae* (Britz, 1996a; Sales et al., 2003) and *Haliotis laevigata* (Coote et al., 2000), but none have considered the effects of water temperature on the protein requirements of abalone.

Aquaculture nutritional studies continually strive to define optimal dietary protein levels, i.e. the minimum dietary protein level at which growth is maximised (Wilson, 2002). However, in defining optimal dietary protein levels consideration of the amino acids profile, digestibility and energy level of the test ingredients is vital as a deficiency in one or more of these factors may lead to an overestimation of dietary protein requirements (Wilson, 2002). In addition, provision of protein from a single dietary ingredient is likely to lead to an overestimation (Mai et al., 1995a; Coote et al., 2000). Provision of sufficient levels of dietary energy allows the energetic requirements for maintenance and physical activity to be satisfied thus allowing the protein portion of the diet to be utilised exclusively for growth (Smith, 1989). The use of protein as an energy source should be avoided not only due to the expense associated with protein but because it leads to deamination of the amino acids and the excretion of excess ammonia which can reduce water quality (Smith, 1989) and lead to complications such as outbreaks of the sabellid worm *Terebrasabella heterouncinata* (Simon et al., 2004) or "bloat". This usually fatal condition is believed to be caused by proliferation of bacteria in the digestive tract

resulting in the fermentation of the gut contents and formation of gas bubbles (Macey and Coyne, 2005; Godoy et al., 2006). Thus, definition of optimal dietary protein levels will not only result in potential economic savings through the reduction of high cost protein rich ingredients such as fishmeal and soya but may also produce beneficial secondary effects such as improvements in culture environment water quality and animal health.

It is generally accepted that the protein requirements of fish decrease with an increase in body size and age (Wilson, 2002) and there is evidence to suggest that the nutritional requirements of abalone differ with age (Britz and Hecht, 1997; Shipton and Britz, 2001b). The most recent evidence for this was presented by Jones and Britz (2006) who found that it was possible to reduce the dietary protein content of formulated feeds for *H. midae* (> 50 mm shell length) from 34 to 26 % protein. It is likely that further reductions in the protein portion of formulated feeds for abalone of different sizes may be possible.

Studies on the effects of water temperature on the protein requirements of cultured abalone are limited and no clear trends are evident, for example, the protein requirements of certain fish species have been found to be influenced by water temperature, while the requirements of other species are unaffected (Wilson, 2002). Temperature is the primary environmental factor governing the metabolic rate of poikilotherms (Fry, 1971) and *H. midae* has been found to conform to this trend (Britz et al., 1997). Britz et al. (1997) found the optimal temperature for the growth, condition factor and feed conversion ratio of *H. midae* to lie in the range of 18 to 20 °C with feed consumption rates increasing significantly with an increase in water temperature from 12 to 20 °C. In addition, Dixon (1992) determined that crude protein digestibility in *H. midae* was significantly influenced by water temperature, being higher at 18 °C compared to 15 and 22 °C. Since protein digestibility (Dixon, 1992) and feed ingestion (Britz et al., 1997) in *H. midae* are influenced either by water temperature or body size or both it is likely that the protein requirements of *H. midae* may be affected by these factors.

The bulk of formulated feeds currently available for *H. midae* were developed based on the results of studies conducted within the optimal temperature range (18-20 °C) for

growth (Sales and Britz 2001a). However, the bulk of abalone farming operations in South Africa are situated on the south coast, make use of land based pump ashore single pass technology, and are exposed to mean sea water temperatures of 15 °C (unpublished data, HIK Abalone Farm (Pty) Ltd; Cook, 1998; Troell et al., 2006). Farmers therefore have very little control over on-farm water temperatures which predominantly fall below the optimal temperature for growth. The development of temperature and size specific diets may assist in improving the growth of abalone cultured at cooler water temperatures.

The overall aim of the study was to investigate the size and temperature specific protein requirements of *H. midae* cultured under laboratory and on-farm conditions in an attempt to reduce feed production costs and complications associated with high protein diets.

The objectives of the study were to compare the growth, survival and nutritional indices of *H. midae* of different sizes (15 mm, 50 mm and 80 mm) cultured at temperatures that are predominantly experienced on land-based abalone farming operations situated in temperate regions (i.e. 14, 16 and 18 °C), and fed formulated feeds containing graded levels of dietary protein (20, 26, 32, 38 and 44 %) under laboratory and farm conditions.

3.2. Materials and methods

3.2.1 Experimental diets

Two suites of experimental diets were formulated to contain graded levels of crude dietary protein for three size classes of abalone. Three diets with protein contents of either 32, 38 or 44 % were formulated for the small abalone (15 mm shell length), while four diets with protein contents of either 20, 26, 32 and 38 % were formulated for the larger size classes (i.e. 50 and 80 mm shell length) (Table 3.1). Low temperature steam dried, formaldehyde free, mackerel fishmeal (Oceana (Pty) Ltd, South Africa) was the primary protein source in the diets for the small abalone. The same fishmeal, as well as soya-oil cake were the primary protein sources in the diets formulated for the larger abalone. The diets for the larger abalone also included fresh kelp (*Ecklonia maxima*). Each suite of diets was formulated to contain similar amounts of dietary energy and lipid.

The two suites of diets contained the same inclusion level of a vitamin and mineral mixture (Vitamin mineral premix, BASF Animal Nutrition (Pty) Ltd, South Africa) and starch carbohydrates based on a proprietary commercial formulation (Marifeed (Pty) Ltd, South Africa). Diatomaceous earth replaced starch as a non-nutritive filler in order to balance the energy levels of the diets.

The digestible energy coefficients of the dietary ingredients were calculated according to the methods described in Chapter 2.

Table 3.1: The two suites of experimental diets formulated to contain graded levels of dietary protein and fed to three size classes of abalone. Nutrient composition and nutritional analyses of the dietary formulations.

Diet	**L38**	**L32**	**L26**	**L20**	**S44**	**S38**	**S32**
Abalone size class		**50 and 80 mm**				**10-15 mm**	
Formulations							
Crude protein (%)	38	32	26	20	44	38	32
Digestible energy ($MJ.kg^{-1}$)	15.05	15.05	15.05	15.05	14.85	14.85	14.85
Lipid	3.33	3.33	3.33	3.33	6.65	6.65	6.65
Proximal analyses							
Crude protein (%)	37.18	31.57	25.81	19.27	43.00	36.78	30.49
Gross energy ($MJ.kg^{-1}$)	17.43	17.15	16.67	16.22	18.10	17.86	17.22
Lipid (%)	2.26	2.46	1.90	1.79	5.72	5.78	5.33
Phosphorus (%)	1.34	1.10	0.81	0.61	2.12	1.72	1.43
Moisture (%)	6.90	6.70	7.10	7.70	6.30	5.90	6.70
Ash (%)	9.42	9.44	9.63	9.62	14.00	14.27	14.02

The proximal composition of the experimental diets were measured using the standard methods of the AOAC (2003) using the methods described in Chapter 2.

3.2.2 Experiment 1: Laboratory growth trial

3.2.2.1 Experimental system, animals, acclimation and feeding

The experiment was conducted at the Rhodes University Port Alfred Marine Research Laboratory (33°45'S; 26°00'E) in the same partially recirculating experimental systems described in Chapter 2. The water temperature in each of the experimental systems was set at either 14, 16 or 18 °C. Tanks were cleaned once a week by siphoning settled

sediment from the tank bottom and 10 % of the entire system's volume was replaced daily with seawater (35 $g.L^{-1}$) pumped from the Kowie River estuary.

The three size classes of abalone (i.e. 15, 50 and 80 mm) were obtained from two commercial abalone farms (Table 3.2) situated on the south coast of South Africa where they had previously been fed artificial feeds. The abalone were stocked into the baskets at size dependent stocking densities (Table 3.2) and allowed to acclimate to the experimental systems for five weeks before the start of the trial. They were fed size specific locally produced commercial abalone feeds and kept at 18 °C during acclimation (Table 3.2). There were no differences in the shell length, weight or condition factor of the abalone in the different treatments within each size class (i.e. 15, 50 and 80 mm) at the start of the trial (ANOVA: $p>0.05$) (Tables 3.4, 3.6 and 3.8). The water temperatures were lowered by 1 °C every 24 h at the start of the trial until experimental systems reached the required temperatures.

Table 3.2: The size specific stocking density, conditioning diets and sources of the three size classes of abalone used in the growth trial conducted at the Rhodes University Port Alfred Marine Research Laboratory.

Abalone size class (mm)	**Stocking density (%) (no. per basket)**	**Conditioning diet**	**Source**
15	3 % (250)	Abfeed®-S34	HIK Abalone Farm (Pty) Ltd
50	4 % (29)	Abfeed®-K26	HIK Abalone Farm (Pty) Ltd
80	4 % (12)	Abfeed®-K26	Roman Bay Sea Farm (Pty) Ltd

The experimental diets were assigned to baskets using a randomised block design to ensure that each diet was fed to three baskets of abalone in each of the three size classes at each of the three temperatures. Thus each diet was replicated three times for each size class at each of the three temperature regimes. In addition, the block design ensured that each diet was positioned in each of the three tanks in each system run at the different temperatures.

Abalone were fed to apparent satiation at 16h00 six days a week. Food consumption was recorded for the abalone in each basket for the duration of the trial. Uneaten food was removed within 24 h and frozen at -4°C. It was later oven dried at 100 °C for 24 h and then weighed at the end of the trial.

3.2.2.2 Solid leaching of diets

Leaching rates were calculated according to the methods described in Chapter 2. There were four replicate samples for each diet at each of the three temperatures.

3.2.2.3 Data collection

Animals were purged for a 24 h prior to the weigh and measure processes. The abalone were subsequently anaesthetised and weighed and measured according to the methods described in Chapter 2. All the 50 and 80 mm abalone in each basket were individually weighed and measured at the start and end of the 12 week growth trial. The total biomass of the abalone in each basket containing the 15 mm abalone was recorded and a random sample of 75 abalone were individually weighed and measured at the start and end of the trial.

Mean abalone shell length gain and weight gain were calculated for all treatments. Condition factor, feed conversion ratio (FCR) and feed consumption were calculated according to Britz (1996b) using the equations 1-5 in Chapter 2. Protein efficiency ratio (PER) was calculated according to Britz (1996b) using the equation:

$$\text{Protein efficiency ratio} = \text{grams wet weight gain/grams protein consumed} \qquad (6)$$

The baskets were checked for mortalities three times a week and any dead abalone were recorded and removed.

3.2.2.4: Water chemistry

Temperature and pH readings were recorded twice a day using a digital temperature and pH probe (Hanna Instruments, HI 98128, Woonsocket, USA). Salinity was monitored

using a refractometer (Extech Instruments, RF20, Waltham, USA). Ammonia and nitrite were measured twice a week using colourmetric titration kits (Red Sea Fish Pharm, Houston, USA). Free unionised ammonia (FAN) was calculated based on total ammonia nitrogen readings (TAN), temperature, pH and salinity values according to Bower and Bidwell (1978). Water chemistry data for the 12 week trial is summarised in Table 3.3.

Table 3.3: Mean (±standard deviation) of temperature (n=152), pH (n=152), ammonia (n=24), nitrite (n=24) and salinity (n=12) recorded during the 12 week laboratory growth trial.

Parameter	**System 1 (18 °C)**	**System 2 (16 °C)**	**System 3 (14°C)**
Temperature (°C)	18.12±0.42	16.13±0.60	14.44±0.63
pH	7.97±0.06	7.97±0.09	7.98±0.08
Ammonia ($mg.L^{-1}$ FAN)	0.0079±0.00	0.0071±0.00	0.0063±0.00
Nitrite ($mg.L^{-1}$)	0.05±0.00	0.05±0.00	0.05±0.00
Salinity ($g.L^{-1}$)	35±0.00	35±0.00	35±0.00

3.2.2.5: Statistical analysis

The effect of dietary protein content and water temperature on abalone shell length gain, weight gain, final condition factor, mortality, FCR, feed consumption and PER were compared using a two-way analysis of variance (multifactor ANOVA) and a Tukey's multiple range analysis at $p<0.05$. If there were no interactions between these factors a one-way analysis of variance (ANOVA) and a Tukey's multiple range analysis ($p<0.05$) were used to compare means within each factor. Data that did not meet the assumptions of an ANOVA were analysed using a non parametric Kruskal-Wallis ANOVA ($p<0.05$).

3.2.3 Experiment 2 On-farm growth

3.2.3.1: Experimental system, animals and feeding

The trial was carried out HIK Abalone Farm (Pty) Ltd situated on the south coast of South Africa. Abalone were cultured in eleven 3000 L canvas tanks (4 m x 1 m x 0.8 m; length, width and depth). Each tank contained six oyster mesh baskets (95 cm x 55 cm x 60 cm; length, width and depth), and each basket included a vertical Acrylonitrile butadiene styrene plastic rack (total surface area: 3.28 $m^2.basket^{-1}$) and a horizontal feeding plate positioned 5 – 10 cm below the water surface. The total volume of each

tank was exchanged every 2.5 h and aeration was provided by airlines installed below the baskets along the length of each tank. The tanks were cleaned once a week by siphoning settled sediment from the tank bottom. All the tanks were positioned outside and exposed to a natural photoperiod of approximately 12:12 (L:D).

All abalone used in the growth trial had previously been fed Abfeed®-S34 (34 % protein, 2 % lipid). The abalone were stocked into baskets according to standard farm stocking methods: the 10-15 mm abalone were stocked at 2200 animals per basket (0.001 $kg.L^{-1}$ per tank), while the 50 and 80 mm abalone were stocked at 13 % (0.01 $kg.L^{-1}$ per tank) and 18 % (0.02 $kg.L^{-1}$ per tank) of the available surface area in the basket respectively. There were no differences in the shell length, weight or condition factor of the abalone in the different treatments within each size class at the start of the trial with the exception of the initial condition factor of the 80 mm abalone (ANOVA: $p>0.05$) (Tables 3.10, 3.11 and 3.12). Abalone in the 38 % protein diet treatment had a higher initial condition factor (1.11±0.01) compared to those in the 26 and 32 % dietary protein treatments (1.08±0.01) (ANOVA: $F_{(3,20)}=3.39$; $p=0.04$).

Abalone were fed the same diets used in the laboratory growth trial (Table 3.1). They were fed to apparent satiation at approximately 16h00 every day according to standard farm feeding methods. Each diet was assigned to six baskets of abalone using a randomised block design. Thus there were six replicates of each diet for each abalone size class. The diets were assigned to the baskets to ensure that each diet was represented at least once in each of the tanks in each size class.

3.2.3.2: Data collection

The abalone were anaesthetised prior to all the weigh and measure processes according to standard farm procedures. The 15 and 50 mm abalone were anaesthetised with a 10 % magnesium sulphate solution and the 80 mm abalone were anaesthetised using carbon dioxide gas which was bubbled into solution. Twelve 10-15 mm abalone were randomly selected from each basket during the weigh and measure at the start of the trial and were individually marked with numbered bee tags. These were attached to the shell of each

abalone using super glue. Each tagged abalone was then individually weighed (0.01 g) and measured (0.01 mm). Similarly, ten 50 mm and ten 80 mm abalone were randomly selected from each basket at the start of the trial and marked by placing a piece of epoxy putty (Pratley's Putty® Original, Krugersdorp, South Africa) onto the shell of each abalone. These abalone were also individually weighed (0.01 g) and measured (0.01 mm). In addition, the total weight of each basket was recorded.

All three size classes of abalone were weighed and measured after 12 and 24 weeks using the same methods described above. The stocking densities in the 50 mm and 80 mm baskets were adjusted during this period in order to maintain a stocking density of 13 and 18 % respectively, which is standard farm practice. This was done by randomly removing non-tagged animals from the baskets. The tagged abalone were weighed and measured and the basket weights recorded after 24 weeks which marked the end of the trial.

Food weights were recorded for each diet fed to each size class of abalone. A single weight was obtained for each diet fed to the six replicates of each abalone size class.

Mean abalone shell length gain and weight gain were calculated for all treatments. Condition factor was calculated using Equation 1 in Chapter 2. FCR and PER were calculated for the 10-15 and 50 mm size class treatments using equation 2 in Chapter 2 and equation 6.

3.2.3.3: Water chemistry

The temperature, dissolved oxygen and pH of the incoming farm seawater was recorded daily using digital dissolved oxygen (YSI Incorporated 55D, Yellow Springs, USA), pH and temperature (YSI Incorporated 60/10FT, Yellow Springs, USA) probes. The mean (± standard deviation) temperature of the incoming farm seawater during the period of the trial was 14.7±1.65 °C ranging from 10.3 to 17.3 °C. Dissolved oxygen and pH averaged 8.16±0.56 $mg.L^{-1}$ and 7.62±0.25 respectively.

3.2.3.4: Statistical analysis

The effect of dietary protein content on abalone shell length gain, weight gain, condition factor, FCR and PER were compared using a one way analysis of variance (ANOVA) and a Tukey's multiple range analysis ($p<0.05$).

3.3 Results

3.3.1.1 Experiment 1: Laboratory growth: Interaction of dietary protein content and water temperature on abalone growth and nutritional indices

There were no interactions between dietary protein content and water temperature on abalone shell length gain, weight gain, final condition factor, mortality, FCR, feed consumption or PER for all three size classes of abalone (multifactor ANOVA: $p>0.05$).

3.3.1.2 Experiment 1: 15 mm Abalone

Dietary protein did not affect the shell length gain (ANOVA: $F_{(2,24)}=1.78$; $p=0.19$), weight gain (ANOVA: $F_{(2,24)}=1.47$; $p=0.25$) or final condition factor (ANOVA: $F_{(2,24)}=0.11$; $p=0.90$) of the abalone in the different treatments (Table 3.4). Abalone cultured at 18 °C had higher weight gain (0.13±0.0.01 g.month^{-1}) compared to those cultured at 14 °C (0.09±0.01 g.month^{-1}) (ANOVA: $F_{(2,24)}=8.31$; $p=0.002$) (Table 3.5). However, abalone cultured at 14 °C had a higher final condition factor compared to those cultured at 18 °C (ANOVA: $F_{(2,24)}=3.87$; $p=0.04$) (Table 3.5).

Dietary protein content did not affect abalone mortality (Kruskal-Wallis: $H_{(2,27)}=1.52$; $p=0.47$) (Table 3.4). However, the rate of mortality was significantly higher at 14°C (4.1±0.8 %) compared to 18 °C (1.5±0.3 %) (Kruskal-Wallis: $H_{(2,27)}=8.22$; $p=0.02$) (Table 3.5).

FCR and feed consumption rates were unaffected by the independent effects of dietary protein content (Kruskal-Wallis: $H_{(2,27)}=1.41$; $p=0.49$ and ANOVA: $F_{(2,24)}=2.10$; $p=0.14$ respectively) and water temperature (Kruskal-Wallis: $H_{(2,27)}=4.17$; $p=0.12$ and ANOVA: $F_{(2,24)}=1.86$; $p=0.18$ respectively) (Tables 3.4 and 3.5). However, PER decreased significantly with an increase in dietary protein content from 1.94±0.17 for abalone fed

the 32 % protein diet to 1.46±0.11 and 1.44±0.12 for those fed the 38 and 44 % protein diets respectively (ANOVA: $F_{(2,24)}$=4.35; p=0.02) (Table 3.4). The PER values were similar for abalone cultured at the different temperatures (ANOVA: $F_{(2,24)}$=1.32; p=0.28) (Table 3.5).

Table 3.4: Mean (± standard error) shell length gain, weight gain, condition factor, survival and nutritional indices of 15 mm abalone fed formulated diets containing graded levels of dietary protein and cultured in the laboratory. Different superscripts indicate significant differences within each row (ANOVA: p<0.05).

Dietary protein content (%)	**32**	**38**	**44**
Mean initial shell length (mm)	15.99±0.12^{a}	15.90±0.12^{a}	16.10±0.17^{a}
Mean initial weight (g)	0.58±0.01^{a}	0.57±0.02^{a}	0.59±0.01^{a}
Mean initial condition factor	0.79±0.02^{a}	0.79±0.02^{a}	0.79±0.01^{a}
Mean shell length gain (mm.month^{-1})	0.69±0.04^{a}	0.71±0.04^{a}	0.81±0.06^{a}
Mean weight gain (g.month^{-1})	0.10±0.01^{a}	0.11±0.01^{a}	0.12±0.01^{a}
Mortality (%)	2.00±0.35^{a}	3.07±0.85^{a}	3.47±0.80^{a}
Feed conversion ratio	1.74±0.18^{a}	1.90±0.16^{a}	1.74±0.25^{a}
Consumption (% bd.wt.d^{-1})	0.64±0.03^{a}	0.73±0.03^{a}	0.72±0.04^{a}
Protein efficiency ratio	1.94±0.17^{a}	1.46±0.11^{b}	1.44±0.12^{b}

Table 3.5: Mean (± standard error) shell length gain, weight gain, condition factor, survival and nutritional indices of 15 mm abalone cultured in the laboratory at different water temperatures and fed formulated diets. Different superscripts indicate significant differences within each row (ANOVA: p<0.05).

Average temperature (°C)	**14**	**16**	**18**
Mean shell length gain (mm.month^{-1})	0.66±0.04^{a}	0.77±0.05^{a}	0.78±0.06^{a}
Mean weight gain (g.month^{-1})	0.09±0.01^{a}	0.11±0.01^{ab}	0.13±0.01^{b}
Final condition factor	0.86±0.00^{a}	0.84±0.01^{ab}	0.83±0.01^{b}
Mortality (%)	4.09±0.83^{a}	2.98±0.66^{ab}	1.47±0.27^{b}
Feed conversion ratio	2.10±0.26^{a}	1.56±0.09^{a}	1.72±0.19^{a}
Consumption (% bd.wt.d^{-1})	0.68±0.04^{a}	0.67±0.03^{a}	0.75±0.03^{a}
Protein efficiency ratio	1.42±0.17^{a}	1.75±0.11^{a}	1.67±0.16^{a}

3.3.3 Experiment 1: 50 mm Abalone

Shell length gain (ANOVA: $F_{(3,32)}$=3.83; p=0.02) and weight gain (ANOVA: $F_{(3,32)}$=7.28; p=0.001) increased significantly with an increase in dietary protein content. Abalone fed the 38 % protein diet had significantly higher weight gain (2.13±0.15 g.month^{-1}) compared to those fed the 20 and 26 % protein diets (1.33±0.14 and 1.54±0.12 g.month^{-1} respectively) (Table 3.6). Water temperature had a significant effect on shell length gain (ANOVA: $F_{(2,33)}$=9.24; p=0.001) and weight gain (ANOVA: $F_{(2,33)}$=4.69; p=0.02). Abalone cultured at 18 °C had significantly higher weight gain (1.99±0.14 g.month^{-1}) compared to those cultured at 14 °C (1.43±0.11 g.month^{-1}) (Table 3.7). However, the final condition factor of abalone cultured at 14 and 16 °C was significantly higher than those cultured at 18 °C (ANOVA: $F_{(2,33)}$=5.70; p=0.01) (Table 3.7).

Neither dietary protein content (Kruskal-Wallis: $H_{(3,36)}$=1.48; p=0.69) nor water temperature (Kruskal-Wallis: $H_{(2,36)}$=4.18; p=0.12) affected the abalone mortality rate (Tables 3.6 and 3.7).

FCR's were not affected by dietary protein content (Kruskal-Wallis: $H_{(3,36)}$=2.31; p=0.51) (Table 3.6). However, temperature did have an affect, with significantly lower FCR's recorded at 18 °C (1.38±0.06) compared to 14 °C (1.86±0.16) (Kruskal-Wallis: $H_{(2,36)}$=7.14; p=0.03) (Table 3.7). Feed consumption rates increased significantly with an increase in dietary protein content from 0.29±0.02 % for abalone fed the 20 % protein diet to 0.38±0.01 % for those fed the 38 % protein diet (ANOVA: $F_{(3,32)}$=3.74; p=0.02) (Figure 3.1). PER was significantly higher for abalone fed the 20 % protein diet (3.09±0.23) compared to those fed the 32 and 38 % protein diets (2.24±0.11 and 1.81±0.12 respectively) (ANOVA: $F_{(3,32)}$=6.76; p=0.001) (Table 3.6). While feed consumption (ANOVA: $F_{(2,33)}$=0.11; p=0.89) and PER (ANOVA: $F_{(3,33)}$=1.58; p=0.22) values were similar for abalone cultured at the different temperatures (Table 3.7).

Table 3.6: Mean (± standard error) shell length gain, weight gain, condition factor, survival and nutritional indices of 50 mm abalone fed formulated diets containing graded levels of dietary protein and cultured in the laboratory. Different superscripts indicate significant differences within each row (ANOVA: $p<0.05$).

Dietary protein content (%)	**20**	**26**	**32**	**38**
Mean initial shell length (mm)	52.15 ± 0.26^{a}	51.76 ± 0.27^{a}	52.07 ± 0.25^{a}	51.85 ± 0.21^{a}
Mean initial weight (g)	23.40 ± 0.39^{a}	22.70 ± 0.25^{a}	23.15 ± 0.26^{a}	23.31 ± 0.27^{a}
Initial condition factor	0.95 ± 0.01^{a}	0.95 ± 0.01^{a}	0.95 ± 0.01^{a}	0.97 ± 0.00^{a}
Mean length gain ($mm.month^{-1}$)	0.85 ± 0.09^{a}	0.97 ± 0.09^{ab}	1.14 ± 0.09^{ab}	1.25 ± 0.09^{b}
Mean weight gain ($g.month^{-1}$)	0.33 ± 0.14^{a}	0.54 ± 0.12^{ab}	1.93 ± 0.13^{bc}	2.13 ± 0.15^{c}
Final condition factor	0.96 ± 0.01^{a}	0.97 ± 0.01^{a}	0.98 ± 0.01^{a}	0.99 ± 0.01^{a}
Mortality (%)	14.94 ± 2.44^{a}	22.61 ± 5.01^{a}	16.09 ± 2.87^{a}	17.62 ± 3.61^{a}
Feed conversion ratio	0.71 ± 0.15^{a}	0.73 ± 0.21^{a}	1.42 ± 0.07^{a}	1.51 ± 0.11^{a}
Protein efficiency ratio	3.09 ± 0.23^{a}	2.51 ± 0.30^{ab}	2.24 ± 0.11^{b}	1.81 ± 0.12^{b}

Table 3.7: Mean (± standard error) shell length gain, weight gain, condition factor, survival and nutritional indices of 50 mm abalone cultured in the laboratory at different water temperatures and fed formulated diets. Different superscripts indicate significant differences within each row (ANOVA: $p<0.05$).

Average temperature (°C)	**14**	**16**	**18**
Mean length gain ($mm.month^{-1}$)	0.83 ± 0.07^{a}	1.05 ± 0.08^{ab}	1.27 ± 0.07^{b}
Mean weight gain ($g.month^{-1}$)	1.43 ± 0.11^{a}	1.78 ± 0.15^{ab}	1.99 ± 0.14^{b}
Final condition factor	0.99 ± 0.01^{a}	0.99 ± 0.01^{a}	0.95 ± 0.01^{b}
Mortality (%)	18.68 ± 2.34^{a}	21.55 ± 3.26^{a}	13.22 ± 2.39^{a}
Feed conversion ratio	1.86 ± 0.16^{a}	1.54 ± 0.11^{ab}	1.38 ± 0.06^{b}
Consumption (% $bd.wt.d^{-1}$)	0.33 ± 0.02^{a}	0.35 ± 0.02^{a}	0.34 ± 0.02^{a}
Protein efficiency ratio	2.13 ± 0.26^{a}	2.44 ± 0.18^{a}	2.67 ± 0.20^{a}

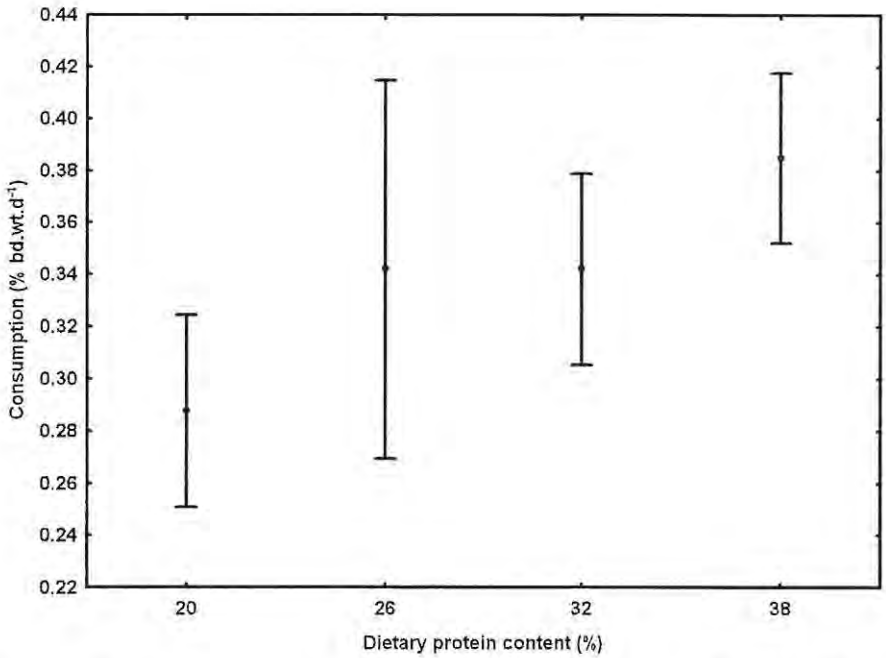

Figure 3.1: Feed consumption rates as a percent of body weight (±95 % confidence intervals) of 50 mm abalone fed formulated diets containing graded levels of dietary protein and cultured at the Rhodes University Port Alfred Marine Research Laboratory. Non-overlapping confidence intervals indicate significant differences (ANOVA: $F_{(3,32)}$=3.74; p=0.02).

3.3.1.3 Experiment 1: 80 mm Abalone

Dietary protein content had no effect on shell length gain (ANOVA: $F_{(3, 32)}$=1.65; p=0.20), weight gain (ANOVA: $F_{(3, 32)}$=1.27; p=0.30) or final condition factor (ANOVA: $F_{(3, 32)}$=0.42; p=0.74). Although not significant growth was lower for abalone fed the 20 % protein diet (Table 3.8). Abalone cultured at 18 °C had significantly higher shell length gain (1.26±0.07 mm.month^{-1}) compared to those cultured at cooler temperatures with a combined mean of 1.02±0.07 mm.month^{-1} (ANOVA: $F_{(2,33)}$=4.51; p=0.02) (Table 3.9). Weight gain did not differ significantly between temperature treatments (ANOVA: $F_{(2,33)}$=1.62; p=0.21), and there were no significant differences in the final condition factor of the abalone cultured at the different temperatures (ANOVA: $F_{(2,33)}$=3.38; p=0.05) (Table 3.9).

Neither dietary protein content (Kruskal-Wallis: $H_{(3,36)}$=3.19; p=0.36) nor water temperature (Kruskal-Wallis: $H_{(3,36)}$=2.58; p=0.27) affected the rate of mortality (Tables 3.8 and 3.9).

Dietary protein content did not affect FCR (Kruskal-Wallis: $H_{(3,36)}$=4.51; p=0.21) or feed consumption rates (ANOVA: $F_{(3,32)}$=1.27; p=0.30) (Table 3.8). Abalone cultured at 14 °C displayed significantly higher FCR's (1.61±0.14) compared to those cultured at 16 °C (1.21±0.12) (Kruskal-Wallis: $H_{(2,36)}$=6.37; p=0.04). Abalone feed consumption rates were similar at all temperatures (ANOVA: $F_{(2,33)}$=1.23; p=0.31). PER decreased significantly with an increase in dietary protein content from 3.89±0.37 and 3.44±0.44 for the 20 and 26 % protein diets respectively to 1.79±0.16 for the 38 % protein diet (ANOVA: $F_{(3,32)}$=8.21; p=0.0003) (Table 3.8). PER values were unaffected by differences in water temperature (ANOVA: $F_{(2,33)}$=1.80; p=0.18) (Table 3.9).

Table 3.8: Mean (± standard error) shell length gain, weight gain, condition factor, survival and nutritional indices of 80 mm abalone fed formulated diets containing graded levels of dietary protein and cultured in the laboratory. Different superscripts indicate significant differences within each row (ANOVA: p<0.05).

Dietary protein content (%)	**20**	**26**	**32**	**38**
Mean initial shell length (mm)	79.34±0.40^{a}	79.56±0.36^{a}	79.96±0.34^{a}	79.65±0.29^{a}
Mean initial weight (g)	84.09±1.35^{a}	84.65±1.54^{a}	86.74±1.51^{a}	86.38±1.66^{a}
Initial condition factor	0.98±0.01^{a}	0.98±0.01^{a}	0.99±0.01^{a}	0.99±0.01^{a}
Mean length gain (mm.month^{-1})	0.97±0.07^{a}	1.22±0.09^{a}	1.12±0.08^{a}	1.09±0.08^{a}
Mean weight gain (g.month^{-1})	3.28±0.22^{a}	4.25±0.40^{a}	4.21±0.46^{a}	3.80±0.47^{a}
Final condition factor	0.98±0.01^{a}	0.98±0.02^{a}	1.00±0.01^{a}	1.00±0.02^{a}
Mortality (%)	3.70±2.82^{a}	10.19±4.56^{a}	4.63±2.02^{a}	2.78±1.96^{a}
Feed conversion ratio	1.38±0.13^{a}	1.34±0.23^{a}	1.26±0.13^{a}	1.57±0.15^{a}
Consumption (% bd.wt.d^{-1})	0.17±0.01^{a}	0.19±0.02^{a}	0.18±0.01^{a}	0.21±0.01^{a}
Protein efficiency ratio	3.89±0.37^{a}	3.44±0.44^{a}	2.68±0.25^{ab}	1.79±0.16^{b}

Table 3.9: Mean (± standard error) shell length gain, weight gain, condition factor, survival and nutritional indices of 80 mm abalone cultured in the laboratory at different water temperatures and fed formulated diets. Different superscripts indicate significant differences within each row (ANOVA: $p<0.05$).

Average temperature (°C)	**14**	**16**	**18**
Mean length gain (mm.month^{-1})	1.02±0.06^{a}	1.02±0.07^{a}	1.26±0.07^{b}
Mean weight gain (g.month^{-1})	3.42±0.34^{a}	4.29±0.44^{a}	3.95±0.23^{a}
Final condition factor	1.00±0.01^{a}	1.00±0.01^{a}	0.97±0.01^{a}
Mortality (%)	9.72±3.82^{a}	3.47±1.61^{a}	2.78±1.57^{a}
Feed conversion ratio	1.61±0.14^{a}	1.21±0.12^{b}	1.35±0.14^{ab}
Consumption (% bd.wt.d^{-1})	0.20±0.01^{a}	0.18±0.01^{a}	0.19±0.01^{a}
Protein efficiency ratio	2.41±0.26^{a}	3.25±0.28^{a}	3.18±0.46^{a}

3.3.2.1 Experiment 2: On-farm growth trial

3.3.2.2 Experiment 2: 10-15 mm Abalone

Dietary protein content had no effect on abalone shell length gain (ANOVA: $F_{(2,15)}=0.43$; $p=0.66$), weight gain (ANOVA: $F_{(2,15)}=1.01$; $p=0.39$), final condition factor (ANOVA: $F_{(2,15)}=0.69$; $p=0.52$) or FCR (ANOVA: $F_{(2,15)}=0.64$; $p=0.54$) (Table 3.10). PER decreased significantly with an increase in dietary protein content from 2.16±0.13 for abalone fed the 32 % protein diet to 1.78±0.05 and 1.63±0.03 for those fed the 38 and 44 % protein diets respectively (ANOVA: $F_{(2,15)}=11.50$; $p=0.001$) (Table 3.10).

Table 3.10: Mean (± standard error) shell length gain, weight gain, condition factor and nutritional indices of 10-15 mm abalone fed formulated diets containing graded levels of dietary protein and cultured at HIK Abalone Farm (Pty) Ltd for 24 weeks. Different superscripts indicate significant differences within each row (ANOVA: $p<0.05$).

Dietary protein content (%)	**32**	**38**	**44**
Mean initial shell length (mm)	12.10±0.26^{a}	11.65±0.33^{a}	12.30±0.32^{a}
Mean initial weight (g)	0.24±0.02^{a}	0.22±0.02^{a}	0.26±0.02^{a}
Initial condition factor	0.73±0.02^{a}	0.78±0.02^{a}	0.76±0.02^{a}
Mean shell length gain (mm.month^{-1})	2.01±0.15^{a}	1.96±0.06^{a}	1.89±0.05^{a}
Mean weight gain (g.month^{-1})	0.35±0.03^{a}	0.31±0.01^{a}	0.31±0.01^{a}
Final condition factor	0.92±0.02^{a}	0.94±0.03^{a}	0.90±0.01^{a}
Feed conversion ratio	1.47±0.09^{a}	1.48±0.04^{a}	1.40±0.03^{a}
Protein efficiency ratio	2.16±0.13^{a}	1.78±0.05^{b}	1.63±0.03^{b}

3.3.2.3 Experiment 2: 50 mm Abalone

Dietary protein content significantly affected shell length gain (ANOVA: $F_{(3,20)}$=5.71; p=0.01) and weight gain (ANOVA: $F_{(3,20)}$=5.85; p=0.01). Abalone fed the 32 and 38 % protein diets had significantly higher shell length gain (2.12±0.08 and 2.20±0.13 mm.month^{-1} respectively) than those fed the 20 % protein diet (1.71±0.09 mm.month^{-1}) (Table 3.11). However, there were no significant differences in the final condition factor of abalone fed the different diets (ANOVA: $F_{(3,20)}$=1.15; p=0.35). FCR increased significantly with an increase in dietary protein content from 0.93±0.03 for abalone fed the 26 % protein diet to 1.12±0.03 for those fed the 38 % protein diet (ANOVA: $F_{(3,20)}$=8.24; p=0.001) (Figure 3.2). PER declined significantly with an increase in dietary protein content from 4.68±0.07 for the 20% protein diet to 2.36±0.07 for the 38 % protein diet (ANOVA: $F_{(3,20)}$=106.55; p<0.00) (Table 3.11).

Table 3.11: Mean (± standard error) shell length gain, weight gain, condition factor and nutritional indices of 50 mm abalone fed formulated diets containing graded levels of dietary protein and cultured at HIK Abalone Farm (Pty) Ltd for 24 weeks. Different superscripts indicate significant differences within each row (ANOVA: p<0.05).

Dietary protein content (%)	**20**	**26**	**32**	**38**
Mean initial shell length (mm)	53.67±0.92^{a}	53.10±0.65^{a}	52.68±0.81^{a}	52.65±1.00^{a}
Mean initial weight (g)	25.88±1.66^{a}	25.25±0.92^{a}	23.73±1.16^{a}	24.33±1.55^{a}
Initial condition factor	0.96±0.01^{a}	0.98±0.01^{a}	0.93±0.01^{a}	0.96±0.01^{a}
Mean shell length gain (mm.month^{-1})	1.71±0.09^{a}	1.89±0.06^{ab}	2.12±0.08^{b}	2.20±0.13^{b}
Mean weight gain (g.month^{-1})	3.75±0.13^{a}	4.25±0.18^{ab}	4.72±0.20^{b}	5.01±0.34^{b}
Final condition factor	1.07±0.02^{a}	1.10±0.01^{a}	1.07±0.01^{a}	1.10±0.01^{a}
Protein efficiency ratio	4.68±0.07^{a}	4.15±0.15^{b}	3.16±0.09^{c}	2.36±0.07^{d}

3.3.2.4 Experiment 2: 80 mm Abalone

Dietary protein had no affect on abalone shell length gain (ANOVA: $F_{(3,20)}$=0.72; p=0.55), weight gain (ANOVA: $F_{(3,20)}$=1.39; p=0.28) or final condition factor (ANOVA: $F_{(3,20)}$=2.36; p=0.10) of abalone in the different treatments (Table 3.12).

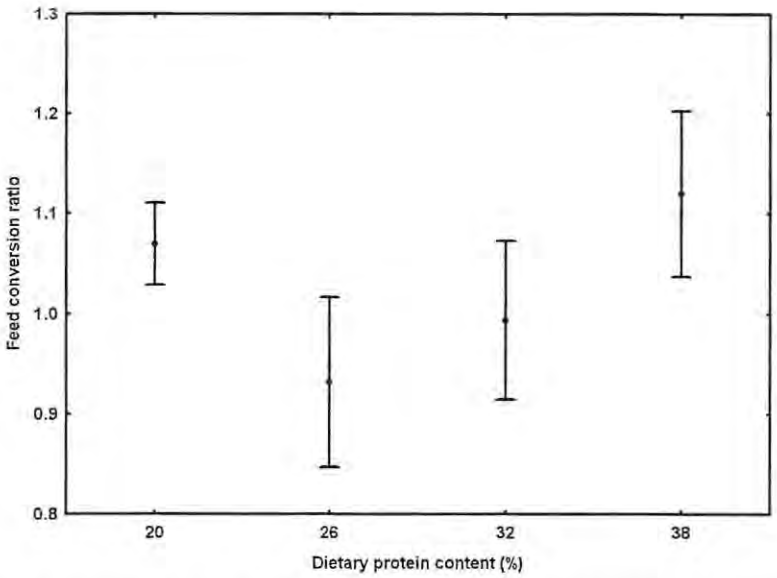

Figure 3.2: Feed conversion ratios (±95 % confidence intervals) of 50 mm abalone fed formulated diets containing graded levels of dietary protein and cultured at HIK Abalone Farm (Pty) Ltd for 24 weeks. Non-overlapping confidence intervals indicate significant differences (ANOVA: $F_{(3,20)}=8.24$; $p=0.001$).

Table 3.12: Mean (± standard error) shell length gain, weight gain and condition factor of 80 mm abalone fed formulated diets containing graded levels of dietary protein and cultured at HIK Abalone Farm (Pty) Ltd for 24 weeks. Different superscripts indicate significant differences within each row (ANOVA: $p<0.05$).

Dietary protein content (%)	**20**	**26**	**32**	**38**
Mean initial shell length (mm)	79.54±0.39^{a}	78.76±1.14^{a}	79.71±0.23^{a}	79.18±0.25^{a}
Mean initial weight (g)	95.25±2.01^{a}	90.58±4.13^{a}	93.79±0.73^{a}	94.34±0.94^{a}
Initial condition factor	1.10±0.01^{ab}	1.08±0.01^{a}	1.08±0.01^{a}	1.11±0.01^{b}
Mean shell length gain (mm.month^{-1})	1.48±0.06^{a}	1.48±0.10^{a}	1.54±0.04^{a}	1.40±0.05^{a}
Mean weight gain (g.month^{-1})	5.36±0.42^{a}	5.58±0.51^{a}	6.37±0.27^{a}	5.72±0.16^{a}
Final condition factor	1.10±0.02^{a}	1.06±0.01^{a}	1.10±0.01^{a}	1.12±0.01^{a}

3.4 Discussion

The study attempted to determine the size and temperature specific protein requirements of *H. midae* based on the response of abalone cultured at different temperatures to increasing levels of dietary protein. The growth of the 50 mm abalone increased significantly in response to increasing levels of dietary protein while the growth of the 15 and 80 mm abalone was independent of dietary protein content which is contrary to the

results of a number protein based studies on abalone (Mai et al., 1995a; Britz, 1996a; Coote et al., 2000; Sales et al., 2003).

The growth rates obtained during both the laboratory and farm trials were well below average which indicates that the abalone were not realising the maximum growth potential possible under the prevailing water temperature conditions. Thus, recommendations on defining "optimal" dietary protein levels were not possible. The growth rates obtained during the farm trial (i.e. 1.5 to 2.0 mm.month^{-1}) fell below the average rates required by commercial farming operations (2-3 mm.month^{-1}) (Fleming et al., 1996). This did not appear to be linked to the experimental conditions, as it was established that abalone farms were experiencing a period of below average growth rates over the same period as the trial (G. Johnson, HIK Abalone Farm (Pty) Ltd, personal communication). The growth rates obtained from the laboratory trial were particularly poor, suggesting that the abalone used were from a poor batch of animals. It has been found that abalone that grow poorly initially do not recover later in life (Haaker et al., 1998). Slower growing abalone would have a lower rate of protein deposition and thus the increases in dietary protein did not result in an increase in growth as protein was not limiting in terms of protein deposition rates. Definition of an optimal dietary protein level is the point at which growth is maximised with the minimum amount of dietary protein (Wilson, 2002). Since growth rates in the current study were below average, the definition of optimal dietary protein levels for *H. midae* of different sizes were not possible.

Growth and feed consumption rates of the 50 mm abalone in the laboratory trial increased in response to increasing levels of dietary protein. It is possible that the lower consumption and resultant lower growth rate of abalone fed the 20 % protein diet could be linked to a protein/energy imbalance or an attractiveness/palatability complication.

The increase in growth in response to increasing water temperature for abalone cultured in the laboratory trial was expected since temperature is the principle environmental factor governing the metabolic rate of poikilotherms (Fry, 1971). A similar finding was

reported by Britz et al. (1997), where abalone growth rates increased with an increase in water temperature from 12 to 20 °C. Similarly, the growth of *H. iris* was found to increase with an increase in water temperature from 14 to 22 °C (Searle et al., 2006).

The higher condition factor of abalone cultured at cooler water temperatures in the laboratory growth trial is evidence that abalone cultured at cooler water temperatures were investing more energy into body growth in relation to shell growth. Britz et al. (1997) reported a similar trend and suggested that the maintenance requirements of *H. midae* are reduced when they are cultured cooler temperatures and a greater proportion of energy is channelled into glycogen reserves. However, further studies on the effect of water temperature on the nutritional content of abalone tissue are required before this can be confirmed.

The effects of dietary protein content and water temperature on abalone growth were independent of one another. Thus the protein requirements of *H. midae* were not affected by water temperature within the ranges of water temperatures and dietary protein contents tested in this study. Although the protein requirements of some fish species have been found to be influenced by water temperature (Wilson, 2002), it seems that the dietary protein requirements of abalone are independent of water temperature within the ranges tested here. This is a promising result as it indicates that land-based farms exposed to temperate water conditions (14 – 18 °C) need not adjust the protein content of abalone feeds in response to changing water temperatures.

Protein efficiency ratio (PER) measures the efficiency of abalone to convert dietary protein into body weight. Interestingly, PER values were similar at different temperatures further supporting the possibility that water temperatures within the range tested here do not affect the protein requirements of *H. midae*. A similar trend was observed by Britz et al. (1997) for juvenile *H. midae* cultured at temperatures in the ranges of this study. PER values decreased significantly with an increase in dietary protein content for all the size classes of abalone in the range tested here. This is consistent with past research where PER values of juvenile *H. midae* declined from 3.2 on a 27 % protein diet to 2.3 on a 47

% protein diet (Britz, 1996a). Although higher levels of dietary protein increased growth it is vital in an aquaculture context to weigh the costs of increasing dietary protein against the resultant increase in growth (Britz, 1996a).

5. Conclusion

The effects of dietary protein content and water temperature on the growth of *H. midae* are independent indicating that the protein requirements of abalone are temperature independent within the ranges tested in this study. Definition of optimal dietary protein levels for *H. midae* of different sizes was not possible due to the below average growth rates obtained from both the laboratory and farm trials.

CHAPTER 4.

SIZE SPECIFIC DIETARY LIPID AND PROTEIN REQUIREMENTS OF FARMED SOUTH AFRICAN ABALONE *HALIOTIS MIDAE*

4.1 Introduction

Protein has been the primary focus of abalone nutritional studies since it is principal dietary ingredient responsible for growth, and it is the highest cost component formulated feeds (Mai et al., 1995a; Fleming et al., 1996). However, recent evidence suggests that the protein requirements of abalone are influenced by the amount of available dietary energy (Bautista-Teruel and Millamena, 1999; Coote et al., 2000; Sales et al., 2003; Jones and Britz, 2006), and recent research showed that it was possible to reduce the protein content of a formulated feed for *H. midae* from 26 to 18 % provided the energy content of the diet was maintained at 13.5 $MJ.kg^{-1}$ (Chapter 2). Further reductions in the protein portion of formulated feeds may be possible provided there is a sufficient supply of energy in the diet.

Lipids have the potential to play a key role as an ingredient in formulated feeds as they offer a highly concentrated source of energy, are vital for the provision of essential fatty acids and provide a number of precursors required for growth and gonad maturation (Mai et al., 1995b; Nelson et al., 2002). Dietary lipid studies have been carried out on species including *Haliotis tuberculata* and *Haliotis discus hannai* (Mai et al., 1995b), *Haliotis midae* (Britz and Hecht, 1997), *Haliotis asinina* (Thongrod et al., 2003), *Haliotis fulgens* (Durazo-Beltran et al., 2003; Durazo-Beltran et al., 2004) and *Haliotis corrugata* (Montano-Vargas et al., 2005). However, high levels of dietary lipid have been found to negatively affect growth and reduce the uptake of other nutrients in the diet (Van Barneveld et al., 1998). The inability of abalone to utilise high levels of dietary lipids has been linked to the low levels of lipases detected in the digestive tracts of abalone (Mercer et al., 1993; Britz et al., 1996; Fleming et al., 1996; Knauer et al., 1996; Garcia-Esquivel and Felbeck, 2006). However, recent findings have detected the presence of lipases in the digestive tracts of juvenile abalone and high levels of lipid are present in many diatoms, which suggests that lipids may play a role in the nutrition of juvenile abalone as an

energy source (Johnston et al., 2005; Esterhuizen, et al. unpublished data). In addition to this, the nutritional requirements of *H. midae* have been found to be influenced by size (Britz and Hecht, 1997; Shipton and Britz, 2001). It is possible that lipids may serve as an important role as an energy source in abalone feeds. Therefore, it was hypothesised that lipids have a role to play as an energy source in abalone diets, and it is possible that the ability of abalone to utilise lipids as a source of energy differs in the presence of varying levels of dietary protein.

The overall aim of the study was to investigate the utilisation of dietary lipid and protein of two size classes of *H. midae* (30 and 60 mm mean initial shell length), in order to determine the extent to which abalone are able to utilise dietary lipids as an energy source and whether further reductions in the protein portion of formulated feeds are possible.

The objectives of the study were addressed through three hypotheses:

1. Abalone growth and survival can be improved through the provision of increased dietary lipid levels at a constant P:E (protein: energy) ratio.
2. The ability of abalone to utilise dietary lipids is influenced by the interaction between high and low dietary protein levels in relation to high and low lipid levels.
3. Dietary protein content can be reduced provided that the energy content of the diet is maintained, through the manipulation of lipid levels.

4.2 Materials and methods

4.2.1 Experimental system

The experiment was conducted at the Rhodes University Port Alfred Marine Research Laboratory (33°45'S; 26°00'E) in two of the temperature controlled partially recirculating experimental systems described in Chapter 2. The delivery and drainage plumbing of the two systems were joined to allow water to mix between the systems to ensure similar water chemistry conditions.

The water temperature in the experimental system was maintained at 18 °C using thermostatically controlled submerged heaters (three kW). The tanks were cleaned once a week by siphoning settled sediment from the tank bottom. Water was recirculated within each of the experimental systems so that the entire volume of the three abalone holding tanks was exchanged every two hours and 10 % of the entire system's volume was replaced daily with sea water (35 $g.L^{-1}$) pumped from the Kowie River estuary.

4.2.2.1 Experimental diets

Three suites of experimental diets were formulated to contain graded levels of dietary lipid and protein. Low temperature steam dried, formaldehyde free, mackerel fishmeal (Oceana (Pty) Ltd, South Africa) and calcium caseinate (Chempure (Pty) Ltd, South Africa) were the primary protein sources in all the experimental diets. Fish oil (Oceana (Pty) Ltd, South Africa) served as the dietary lipid source. Each diet contained the same inclusion level of a vitamin and mineral mixture (Vitamin mineral premix, BASF Animal Nutrition (Pty) Ltd, South Africa) and fresh kelp (*Ecklonia maxima*). The diets also contained starch carbohydrates based on a proprietary commercial formulation (Marifeed (Pty) Ltd, South Africa).

The digestible energy values of fishmeal, carbohydrates and kelp were calculated using the digestible energy coefficients described in Chapter 2. The digestibility energy coefficient of casein for *H. midae* was not available so the value was estimated based on an average of the digestibility coefficients determined for *H. rubra* and *H. laevigata* by Vandepeer and Van Barneveld (2003).

4.2.2.2 Experiment 1: Abalone growth and survival can be improved through the provision of increased dietary lipid levels at a constant P:E ratio

Five diets were formulated to contain graded levels of dietary lipid (4, 7, 10, 13 and 16 %) and protein (34, 35, 36, 38 and 39 %) (Table 4.1). The protein contents of the diets were increased as the levels of dietary lipid increased in order to maintain a constant P:E ratio (Table 4.1).

4.2.2.3 Experiment 2: The ability of abalone to utilise dietary lipids is influenced by the interaction between high and low dietary protein levels in relation to high and low lipid levels

Four diets were formulated to contain a combination of either 34 or 39 % dietary protein, each with a dietary lipid content of either 4 or 16 % (Table 4.2).

Table 4.1: The five diets formulated to contain graded levels of dietary lipid and protein.

Diet	**L4**	**L7**	**L10**	**L13**	**L16**
Formulations					
Lipid (%)	4.00	7.00	10.00	13.00	16.00
Total protein (%)	34.00	35.23	36.46	37.69	38.92
Digestible energy ($MJ.kg^{-1}$)	15.36	15.92	16.47	17.03	17.59
P:E Ratio (g protein/$MJ.kg^{-1}$ energy)	2.213	2.213	2.213	2.213	2.213
Proximal analysis					
Crude protein (%)	34.40	36.28	37.80	39.26	36.47
Gross energy ($MJ.kg^{-1}$)	18.69	19.44	20.21	21.12	21.37
Lipid (%)	2.81	5.31	8.75	12.48	16.09
Phosphorus (%)	0.94	0.94	0.90	0.97	1.18
Moisture (%)	6.67	5.67	5.60	5.40	6.00
Ash (%)	5.72	5.93	5.91	6.18	7.91

Table 4.2: The four diets formulated to contain either 34 or 39 % dietary protein each with a lipid content of 4 or 16 %.

Diet	**P34L4**	**P34L16**	**P39L4**	**P39L16**
Formulations				
Total protein (%)	34.00	34.00	38.92	38.92
Digestible energy ($MJ.kg^{-1}$)	15.36	17.59	15.36	17.59
Lipid (%)	4.00	15.37	3.45	16.00
P:E Ratio (g protein/$MJ.kg^{-1}$ energy)	2.213	1.933	2.534	2.213
Proximal analysis				
Total protein (%)	34.30	35.34	40.36	36.47
Gross energy ($MJ.kg^{-1}$)	18.69	21.23	19.01	21.37
Lipid (%)	2.81	13.91	2.99	16.09
Phosphorus (%)	0.94	0.88	0.97	1.18
Moisture (%)	6.67	5.73	6.40	6.00
Ash (%)	5.72	5.38	6.31	7.91

4.2.2.4 Experiment 3: Reductions in dietary protein content through the maintenance of dietary energy levels

Two isoenergetic (17.59 $MJ.kg^{-1}$) diets were formulated to contain dietary protein contents of either 20 or 34 % (Table 4.3). Starch carbohydrates and fish oil were used to balance the energy content of the two diets.

Table 4.3: The two diets formulated to similar amounts of dietary energy and a dietary protein content of either 20 or 34 %.

Diet	**P20**	**P34**
Formulations		
Total protein (%)	20.00	34.00
Digestible energy ($MJ.kg^{-1}$)	17.59	17.59
Lipid (%)	13.60	15.37
P:E Ratio (g protein/$MJ.kg^{-1}$ energy)	1.137	1.933
Proximal analysis		
Total protein (%)	22.59	35.34
Gross energy ($MJ.kg^{-1}$)	20.04	21.23
Lipid (%)	9.83	13.91
Phosphorus (%)	0.55	0.88
Moisture (%)	5.90	5.73
Ash (%)	3.67	5.38

4.2.4 Proximate analysis

The proximal composition of the experimental diets were measured using the standard methods of the AOAC (2003) using the methods described in Chapter 2.

4.2.5 Experimental animals, acclimation and feeding

The 30 mm abalone were obtained from HIK Abalone Farm (Pty) Ltd which is situated on the south coast where they had previously been fed Abfeed® S-34 (Marifeed (Pty) Ltd, South Africa). The 60 mm abalone were obtained from Marine Growers Abalone Farm (Pty) Ltd which is situated on the south east coast where they had previously been fed a combination of cultured algal and formulated diets. The abalone were stocked into the baskets at 7 and 9 % of available basket surface area (i.e. 200 and 36 abalone per basket for the 30 and 60 mm abalone respectively) and allowed to acclimate to the experimental systems for five weeks before the start of the trial, at a water temperature of

18 °C. Both size classes of abalone were fed Abfeed®-S34K during acclimation. The mean initial shell length, weight and condition factor were similar for the abalone within the two size classes allocated to each of the three experiments at the start of the trial ($p>0.05$) with the exception of the condition factor of the 30 mm abalone allocated to Experiment 3 (Students *t*-test=3.12; $p=0.02$) (Tables 4.4, 4.6 and 4.8).

Table 4.4: Mean (± standard error) shell length, weight and condition factor of the two size classes of abalone allocated for use in Experiment 2 at the start of the 12 week growth trial. Significant differences are indicated by different alphabetical superscripts in each row (ANOVA: $p<0.05$).

Dietary protein content (%)	**34**		**39**	
Dietary lipid content (%)	**4**	**16**	**4**	**16**
Mean initial sizes – small abalone				
Shell length (mm)	25.93±0.36[a]	26.03±0.23[a]	26.05±0.22[a]	25.79±0.26[a]
Weight (g)	2.70±0.12[a]	2.75±0.06[a]	2.60±0.10[a]	2.69±0.06[a]
Condition factor	0.87±0.01[a]	0.89±0.01[a]	0.85±0.01[a]	0.85±0.01[a]
Mean initial sizes – large abalone				
Shell length (mm)	66.02±0.47[a]	66.13±0.58[a]	66.83±0.29[a]	66.77±0.15[a]
Weight (g)	50.41±1.02[a]	49.53±1.75[a]	51.62±1.17[a]	51.70±0.83[a]
Condition factor	1.01±0.01[a]	0.99±0.01[a]	1.00±0.01[a]	1.00±0.01[a]

The diets were assigned to the abalone in the baskets using a randomised block design to ensure that each diet was fed to four baskets of abalone of each size class. The block design ensured that the four replicate samples of each treatment were positioned in a different tank and the two size classes of abalone were divided evenly among the tanks.

Abalone were fed to apparent satiation at 16h00 every day except Sundays. Food consumption was recorded within each basket for the duration of the trial. Uneaten food was removed within 24 h and stored at -4 °C until the end of the trial. The uneaten food was then oven dried at 100 °C for 24 h and then weighed.

4.2.6 Solid leaching of diets

Leaching rates were calculated according to the methods described in Chapter 2.

4.2.7 Data collection

Abalone were purged for 24 h prior to being weighed and measured. The abalone were subsequently anaesthetised and weighed and measured using the methods described in Chapter 2. All the 60 mm abalone in each basket were individually weighed and measured at the start and end of the 12 week trial. The total biomass of the abalone in each basket containing the 30 mm abalone was recorded and a random sample of 50 abalone were individually weighed (0.01 g) and measured (0.01 mm) at the start and end of the trial.

Mean abalone shell length gain, specific growth rate (SGR), condition factor, feed conversion ratio (FCR), protein efficiency ratio (PER) and feed consumption were calculated for all treatments according to Britz (1996b) using the equations 1-6 in Chapters 2 and 3.

The baskets were checked for mortalities three times a week and any dead abalone were recorded and removed.

4.2.8 Biochemical composition of body tissues

The soft tissue glycogen content of the two size classes of abalone was measured at the end of the trial. Soft tissue glycogen content was measured in quadruplet (i.e. a tissue sample was taken from one abalone from each of the four baskets fed each of the test diets). The soft tissue was removed from the shell by cutting the abductor muscle away as close to the shell as possible. The whole body of the 30 mm abalone was retained while the organs and excess tissue of the 60 mm abalone was trimmed away from the foot muscle and only the foot muscle was retained. The tissue samples were then frozen in liquid nitrogen and then stored at -4 °C until required. These samples were subsequently thawed and homogenised with triple distilled water (1:1) using a homogeniser (Ika Ultra Turrax ® T25, Staufen, Germany).

Soft tissue glycogen content was measured using a method adapted from (Woodcock and Benkendorff, 2008). Soft tissue (200 mg) was reacted with 1.0 ml of 0.6 M perchloric acid for thirty min in a 1.5 ml reaction tube. The samples were then centrifuged at 10 000 rpm for 10 min. The supernatant (600 µl) was then decanted into a new 1.5 ml reaction tube and centrifuged at 10 000 rpm for a further five min. The supernatant (77 µl) was then reacted with 500 µl (I_2KI) reagent for 20 min in a new 1.5 ml reaction tube. Samples (200 µl) were subsequently decanted in duplicate into a micro titre plate. Absorbance of the solution was recorded using a 96 microtitre spectrophotometer (PowerWave X, Biotek Instruments, USA) at 460 nm. Glycogen concentration was determined using a standard curve prepared with oyster glycogen (Sigma Chemicals, USA).

4.2.9 Water chemistry

Water temperature, pH and dissolved oxygen were measured once a day using a digital temperature and pH probe (Hanna Instruments, HI 98128, Woonsocket, USA) and a dissolved oxygen probe (Hanna Instruments HI 9143, Woonsocket, USA). Salinity was monitored once a week using a refractometer (Extech Instruments, RF20, Waltham, USA). Ammonia and nitrite were measured twice a week using colourmetric titration kits (Red Sea Fish Pharm, Houston, USA). Free unionised ammonia (FAN) was calculated based on total ammonia nitrogen (TAN), temperature, pH and salinity according to Bower and Bidwell (1978). Water chemistry data for the 12 week trial is summarised in Table 4.5.

Table 4.5: Mean (± standard error) temperature (n=75), pH (n=41), dissolved oxygen (n=33), ammonia (n=19), nitrite (n=19) and salinity (n=12) readings recorded in the two experimental systems during the 12 week growth trial. Significant differences are indicated by different alphabetical superscripts in each row (Mann-Whitney *U*: $p<0.05$).

Parameter	**System 1**	**System 2**
Temperature (°C)	18.21 ± 0.03^a	18.18 ± 0.03^a
pH	7.92 ± 0.01^a	7.92 ± 0.01^a
Dissolved oxygen ($mg.L^{-1}$)	8.56 ± 0.10^a	8.29 ± 0.26^a
Ammonia ($mg.L^{-1}$ FAN)	0.002 ± 0.00^a	0.002 ± 0.00^a
Nitrite ($mg.L^{-1}$)	0.08 ± 0.01^a	0.08 ± 0.01^a
Salinity ($g.L^{-1}$)	35 ± 0.00^a	35 ± 0.00^a

4.2.10 Statistical analysis

A multifactor analysis of variance (multifactor ANOVA) was used to determine if there were interactions between factors ($p<0.05$). If there were no interactions between factors a one way analysis of variance (ANOVA) and a Tukey's multiple range analysis or a Student's *t*-test were used to compare the treatment means within each factor ($p<0.05$). Data that did not meet the assumptions of an ANOVA were analysed using either a Mann-Whitney U test or a Krukal-Wallis ANOVA ($p<0.05$). Multiple linear regression analysis was used to correlate condition factor to dietary lipid content.

4.3 Results

4.3.1 Experiment 1: Abalone growth and survival can be improved through the provision of increased dietary lipid levels at a constant P:E ratio

The response in shell length gain (multifactor ANOVA: $p=0.84$), SGR (multifactor ANOVA: $p=0.74$), final condition factor (multifactor ANOVA: $p=0.92$), soft tissue glycogen content (multifactor ANOVA: $p=0.06$), mortality (multifactor ANOVA: $p=0.52$), feed consumption (multifactor ANOVA: $p=0.20$) , FCR (multifactor ANOVA: $p=0.62$) and PER (multifactor ANOVA: $p=0.70$) was similar for both size classes.

Abalone shell length gain decreased significantly with an increase in dietary lipid content from 4 to 16 % for both the 30 mm (ANOVA: $F_{(4,15)}=3.16$; $p=0.05$) and 60 mm abalone (ANOVA: $F_{(4,15)}=8.16$; $p=0.001$; Table 4.6). Although not significant the SGR of the 30 mm abalone decreased with an increase in dietary lipid content (ANOVA: $F_{(4,15)}=2.77$; $p=0.07$). However, the SGR of the 60 mm abalone decreased significantly from 0.18±0.02 % body weight per day (bd.wt.d^{-1}) for abalone fed the 4 % lipid diet down to 0.06±0.03 % bd.wt.d^{-1} for those fed the 16 % lipid diet (ANOVA: $F_{(4,15)}=3.40$; $p=0.04$; Table 4.6). Mortality was unaffected by dietary lipid content for both the 30 mm (Kruskal-Wallis: $H_{(4,20)}=2.97$; $p=0.56$) and 60 mm abalone (Kruskal-Wallis: $H_{(4,20)}=4.94$; $p=0.29$; Table 4.6).

Table 4.6: Growth (mean ± standard error) and nutritional indices of 30 and 60 mm abalone fed formulated diets containing graded levels of dietary lipid and protein. Significant differences are indicated by different alphabetical superscripts in each row (ANOVA: $p<0.05$).

Parameter	Dietary lipid content (%)				
	4	7	10	13	16
Mean initial size – small abalone					
Shell length (mm)	25.93±0.36^{a}	25.36±0.54^{a}	25.84±0.22^{a}	25.70±0.33^{a}	25.79±0.26^{a}
Weight (g)	2.70±0.12^{a}	2.48±0.16^{a}	2.64±0.05^{a}	2.61±0.09^{a}	2.60±0.10^{a}
Condition factor	0.87±0.01^{a}	0.85±0.01^{a}	0.86±0.01^{a}	0.86±0.01^{a}	0.85±0.01^{a}
Mean initial size – large abalone					
Shell length (mm)	66.02±0.47^{a}	66.76±0.52^{a}	65.78±0.20^{a}	65.78±0.28^{a}	66.77±0.15^{a}
Weight (g)	50.41±1.02^{a}	51.21±1.17^{a}	50.12±0.54^{a}	49.28±0.94^{a}	51.70±0.83^{a}
Condition factor	1.01±0.01^{a}	0.99±0.00^{a}	1.02±0.00^{a}	1.00±0.02^{a}	1.00±0.01^{a}
Mean shell length gain (mm.month^{-1})					
Small abalone	1.95±0.09^{a}	1.93±0.07^{ab}	1.76±0.09^{ab}	1.70±0.22^{ab}	1.38±0.13^{b}
Large abalone	1.05±0.07^{a}	1.14±0.07^{a}	1.11±0.10^{a}	0.98±0.09^{a}	0.58±0.06^{b}
Specific growth rate (% bd.wt.d^{-1})					
Small abalone	0.70±0.03^{a}	0.68±0.05^{a}	0.62±0.02^{a}	0.59±0.08^{a}	0.48±0.07^{a}
Large abalone	0.18±0.02^{ab}	0.19±0.02^{a}	0.14±0.03^{ab}	0.14±0.03^{ab}	0.06±0.03^{b}
Final condition factor					
Small abalone	0.86±0.01^{a}	0.83±0.03^{a}	0.84±0.01^{a}	0.83±0.01^{a}	0.82±0.01^{a}
Large abalone	1.02±0.01^{a}	1.00±0.01^{a}	0.99±0.01^{a}	0.99±0.01^{a}	0.98±0.01^{a}
Glycogen content (%)					
Small abalone	0.65±0.13^{a}	0.66±0.08^{a}	1.24±0.35^{a}	0.54±0.09^{a}	0.58±0.04^{a}
Large abalone	4.00±0.12^{a}	3.25±0.40^{ab}	3.33±0.16^{ab}	3.55±0.18^{ab}	2.14±0.76^{b}
Mortality (%)					
Small abalone	0.13±0.13^{a}	0.25±0.14^{a}	0.38±0.24^{a}	0.25±0.25^{a}	0±0.00^{a}
Large abalone	1.39±0.80^{a}	2.78±1.60^{a}	2.78±2.78^{a}	4.17±1.39^{a}	0±0.00^{a}
Consumption (% bd.wt.d^{-1})					
Small abalone	0.43±0.02^{ab}	0.42±0.03^{a}	0.50±0.02^{ab}	0.50±0.02^{ab}	0.56±0.05^{b}
Large abalone	0.25±0.01^{ac}	0.23±0.01^{a}	0.28±0.01^{bc}	0.27±0.01^{ac}	0.28±0.01^{bc}
Feed conversion ratio					
Small abalone	0.93±0.04^{a}	0.92±0.07^{a}	1.19±0.04^{ab}	1.36±0.04^{ab}	2.02±0.21^{b}
Large abalone	1.56±0.17^{a}	1.36±0.10^{a}	2.88±1.14^{b}	3.10±1.41^{b}	4.19±1.06^{b}
Protein efficiency ratio					
Small abalone	3.14±0.14^{a}	3.04±0.23^{a}	2.24±0.08^{b}	1.87±0.05^{bc}	1.41±0.15^{c}
Large abalone	1.92±0.20^{a}	2.06±0.16^{a}	1.26±0.29^{ab}	1.22±0.29^{ab}	0.61±0.26^{b}

Dietary lipid content did not affect the final condition factor (ANOVA: $F_{(4,15)}=0.82$; $p=0.53$) or soft tissue glycogen (ANOVA: $F_{(4,15)}=2.61$; $p=0.08$) content of the 30 mm abalone. In addition, there was no trend between the effect of increasing levels of dietary lipid on the final condition factor of the 30 mm abalone ($T_{(1,18)}=-1.42$; $r^2=0.10$; $p=0.17$).

The final condition factor of the 60 mm abalone did not differ significantly (ANOVA: $F_{(4,15)}$=2.19; p=0.12), however, there was a significant trend of decreasing condition factor with an increase in dietary lipid content ($T_{(1,18)}$=-2.88; r^2=0.31; p=0.01). There was a corresponding significant reduction in the soft tissue glycogen content of the foot muscle from 4.00±0.12 % for abalone fed the 4 % lipid diet down to 2.14±0.76 % for those fed the 16 % lipid diet was recorded (ANOVA: $F_{(4,15)}$=2.89; p=0.05; Table 4.6).

Feed consumption rates and FCR increased significantly with an increase in dietary lipid content for both the 30 mm (ANOVA: $F_{(4,15)}$=3.58; p=0.03 and Kruskal-Wallis: $H_{(4,20)}$=17.39; p=0.002 respectively) and 60 mm abalone (ANOVA: $F_{(4,15)}$=4.77; p=0.01 and Kruskal-Wallis: $H_{(4,19)}$=10.82; p=0.03 respectively). PER decreased significantly as the protein content of the diet increased from 34 to 39 % for both the 30 mm (ANOVA: $F_{(4,15)}$=26.35; p<0.00) and 60 mm abalone (ANOVA: $F_{(4,15)}$=5.72; p=0.005; Table 4.6).

4.3.2 Experiment 2: The ability of abalone to utilise dietary lipids is influenced by the interaction between high and low dietary protein levels in relation to high and low lipid levels

There were no interactions between the effects of the three factors combined (i.e. abalone size, dietary protein content and dietary lipid content) for shell length gain (multifactor ANOVA: p=0.08), SGR (multifactor ANOVA: p=0.28), final condition factor (multifactor ANOVA: p=0.96), soft tissue glycogen content (multifactor ANOVA: p=0.21), mortality (multifactor ANOVA: p=0.84), feed consumption (multifactor ANOVA: p=0.66), FCR (multifactor ANOVA: p=0.08) and PER (multifactor ANOVA: p=0.06).

Shell length gain and SGR were significantly lower for both size classes fed the 16 % lipid diets compared to those fed the 4 % lipid diets. However, the increase in dietary lipid content had a greater negative effect on the shell length gain (multifactor ANOVA: $F_{(1,24)}$=6.66; p=0.02) and SGR (multifactor ANOVA: $F_{(1,24)}$=19.02; p=0.0002) of the 30 mm abalone compared to the 60 mm abalone (Figures 4.1 and 4.2). High levels of dietary

lipid had a negative effect on the shell length gain of abalone fed both the 34 and 39 % protein diets, however, this negative effect was more prominent for abalone fed the 39 % protein diet compared to those fed the 34 % protein diet (multifactor ANOVA: $F_{(1,24)}$=9.52; p=0.005; Figure 4.3). SGR was unaffected by dietary protein content for both the 30 and 60 mm abalone (Students *t*-test: $t_{(1,14)}$=-0.93; p=0.37 and Students *t*-test: $t_{(1,14)}$=0.38; p=0.71 respectively; Table 4.7), and this response was similar for both size classes (multifactor ANOVA: $F_{(1,24)}$=4.34; p=0.05).

Table 4.7: Growth (mean ± standard error) and nutritional indices of the 30 and 60 mm abalone fed formulated diets containing a dietary protein content of either 34 or 39 %. Significant differences are indicated by different alphabetical superscripts in each row (p<0.05).

Size class (mm)	**30**		**60**	
Dietary protein content (%)	**34**	**39**	**34**	**39**
Specific growth rate (% bd.wt.d^{-1})	0.55±0.06^{a}	0.64±0.07^{a}	0.15±0.02^{z}	0.14±0.03^{z}
Final condition factor	0.84±0.01^{a}	0.84±0.01^{a}	1.00±0.01^{z}	1.00±0.01^{z}
Glycogen content (%)	0.80±0.24^{a}	0.48±0.05^{a}	3.76±0.13^{z}	3.03±0.49^{z}
Mortality (%)	0.06±0.06^{a}	0.63±0.63^{a}	1.39±0.74^{z}	0.35±0.35^{z}
Consumption (% bd.wt.d^{-1})	0.53±0.05^{a}	0.50±0.03^{a}	0.28±0.01^{z}	0.27±0.01^{z}
Feed conversion ratio	1.52±0.22^{a}	1.46±0.23^{a}	2.19±0.32^{z}	2.53±0.71^{z}
Protein efficiency ratio	2.24±0.35^{a}	2.08±0.27^{a}	1.50±0.20^{z}	1.27±0.28^{z}

Increases in dietary protein and lipid produced similar trends in the final condition factor of both size classes (multifactor ANOVA: p>0.05). Both the 30 mm (Students *t*-test: $t_{(1,14)}$=3.39; p=0.004) and 60 mm abalone (Students *t*-test: $t_{(1,14)}$=4.06; p=0.001) fed the 4 % lipid diets (0.85±0.01 and 1.02±0.01 respectively) had significantly higher final condition factors compared to those fed the 16 % lipid diets (0.82±0.01 and 0.98±0.01 respectively). Dietary protein content had no effect on the final condition factor of the 30 mm (Students *t*-test: $t_{(1,14)}$=0.11; p=0.92) or 60 mm abalone (Students *t*-test: $t_{(1,14)}$=0.17; p=0.87; Table 4.7). Increases in dietary lipid content from 4 to 16 % had a greater negative effect on the soft tissue glycogen content of the 60 mm abalone compared to the 30 mm abalone (multifactor ANOVA: $F_{(1,24)}$=8.56; p=0.007) (Figure 4.4). Dietary protein content had no effect on the soft tissue glycogen content of the 30 mm (Students *t*-test: $t_{(1,14)}$=1.31; p=0.21) or 60 mm abalone (Students *t*-test: $t_{(1,14)}$=1.42; p=0.18) (Table 4.7).

Trends in the mortality rates of the two size classes were similar (multifactor ANOVA: $p>0.05$). There were no differences in mortality between the two size classes fed the diets containing either 4 or 16 % lipid with values of 0.7±0.62 and 0.00 % respectively for the 30 mm abalone (Mann-Whitney U=24.00, p=0.84, df=1) and 1.0±0.51 and 0.7±0.69 % respectively for the 60 mm abalone (Mann-Whitney U=25.50, p=0.68, df=1). Mortality of the 30 mm (Mann-Whitney U=31.50, p=0.96, df=1) and 60 mm abalone (Mann-Whitney U=23.50, p=0.37, df=1) was also independent of dietary protein content (Table 4.7).

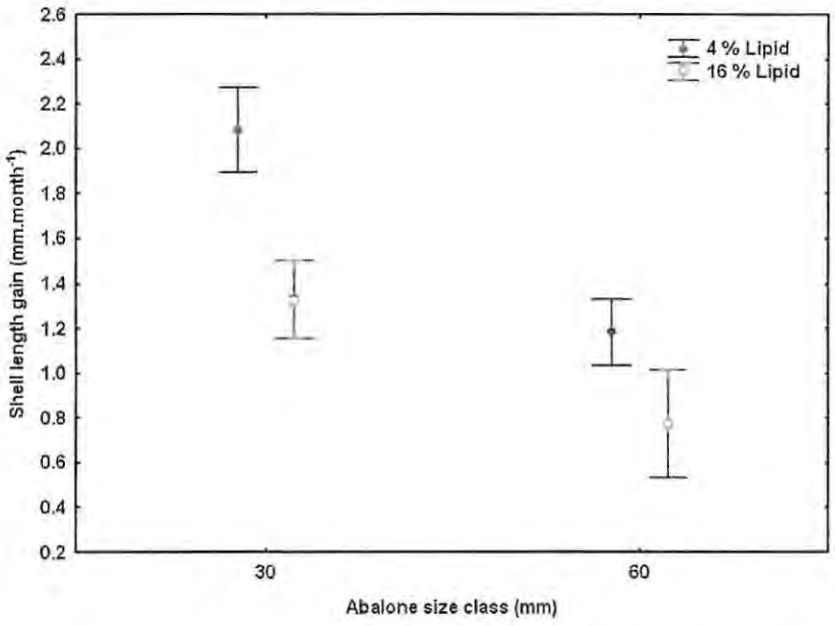

Figure 4.1: Mean shell length gain (± 95 % confidence intervals) of two size classes of abalone fed formulated feeds with dietary lipid contents of either 4 or 16 %. Non-overlapping confidence intervals indicate significant differences (multifactor ANOVA: $F_{(1,24)}$=6.66; p=0.02).

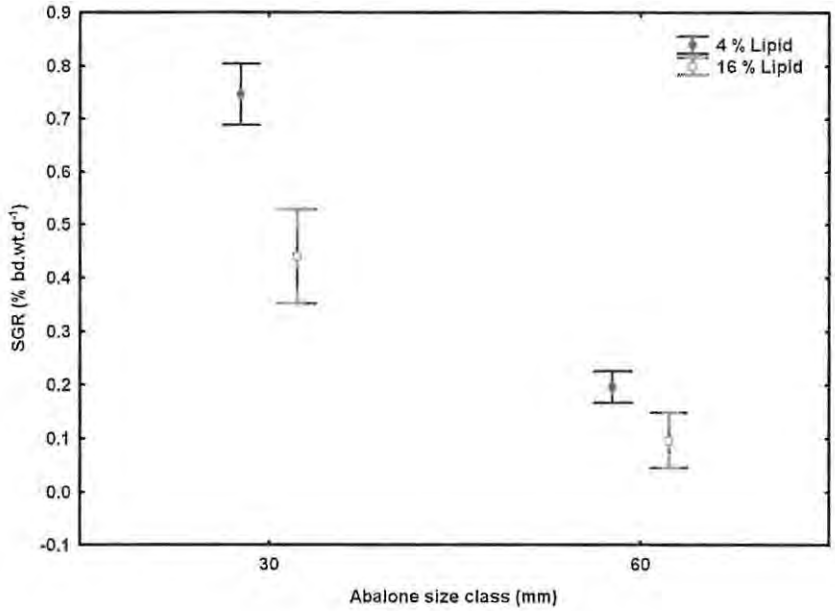

Figure 4.2: Specific growth rate as a percent of body weight per day (± 95 % confidence intervals) of two size classes of abalone fed formulated feeds with dietary lipid contents of either 4 or 16 %. Non-overlapping confidence intervals indicate significant differences (multifactor ANOVA: $F_{(1,24)}$=19.02; p=0.0002).

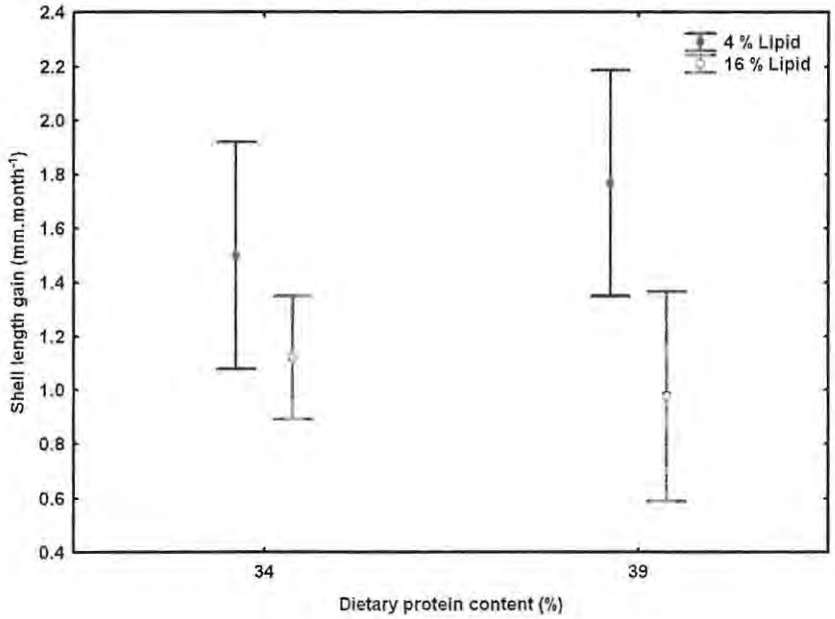

Figure 4.3: Mean shell length gain (± 95 % confidence intervals) of two size classes of abalone fed formulated feeds with protein contents of either 34 or 39 % each with a corresponding dietary lipid content of 4 or 16 %. Non-overlapping confidence intervals indicate significant differences (multifactor ANOVA: $F_{(1,24)}$=9.52; p=0.005).

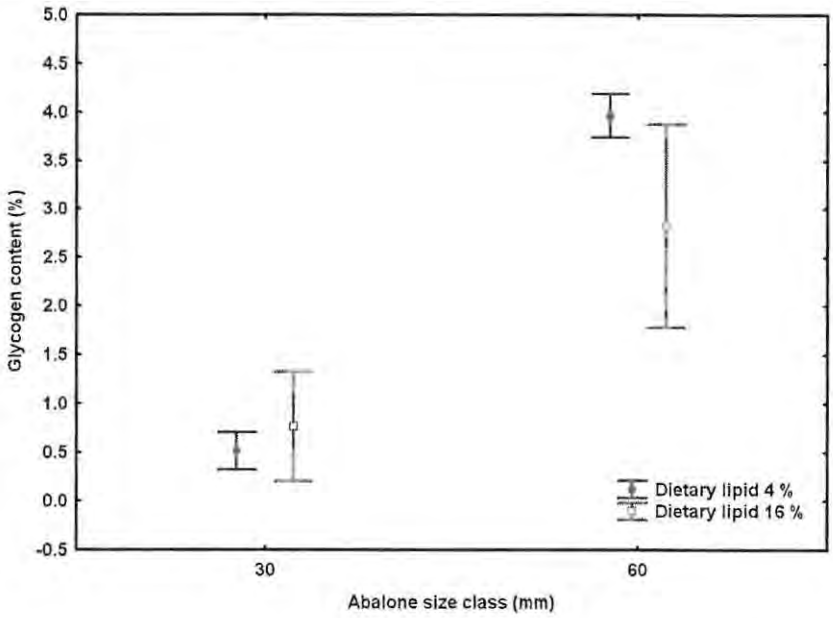

Figure 4.4: Glycogen content of soft body tissue (± 95 % confidence intervals) of two size classes of abalone fed formulated feeds with dietary lipid contents of either 4 or 16 %. Non-overlapping confidence intervals indicate significant differences (multifactor ANOVA: $F_{(1,24)}$=19.02; p=0.0002).

Feed consumption rates were significantly higher for both size classes of abalone fed the 16 % lipid diets. However, the 30 mm abalone consumed significantly more food when dietary lipid was increased from 4 to 16 % compared to the 60 mm abalone (multifactor ANOVA $F_{(1,24)}$=7.77; p=0.01) (Figure 4.5). Increases in dietary lipid and protein produced similar FCR trends for both size classes (multifactor ANOVA: p>0.05). However, FCR's were significantly higher for both the 30 mm (Mann-Whitney U=0.00, p=0.001, df=1) and 60 mm abalone (Mann-Whitney U=1.00, p=0.002, df=1) fed the 16 % lipid diets (2.06±0.10 and 3.41±0.53 respectively) compared to those fed the 4 % lipid diets (0.92±0.02 and 1.43±0.10 respectively). Feed consumption rates and FCR's were independent of dietary protein content for both the 30 mm (Students t-test: $t_{(1,14)}$=0.49; p=0.63 and Mann-Whitney U=32.00, p=1.00, df=1 respectively) and 60 mm abalone (Students t-test: $t_{(1,14)}$=0.68; p=0.51 and Mann-Whitney U=24.00, p=0.64, df=1 respectively) (Table 4.7). PER decreased significantly for both size classes when dietary lipid was increased from 4 to 16 %. However, the increase in dietary lipid content had a greater negative effect on the PER of the 30 mm abalone (multifactor ANOVA:

$F_{(1,24)}$=5.39; p=0.03) (Figure 4.6). In addition, the 30 mm abalone utilised dietary protein more efficiently than the 60 mm abalone. The PER was not affected by dietary protein content for both the 30 mm (Mann-Whitney *U*=26.00, p=0.53, *df*=1) and 60 mm abalone (Mann-Whitney *U*=26.00, p=0.53, *df*=1) (Table 4.7).

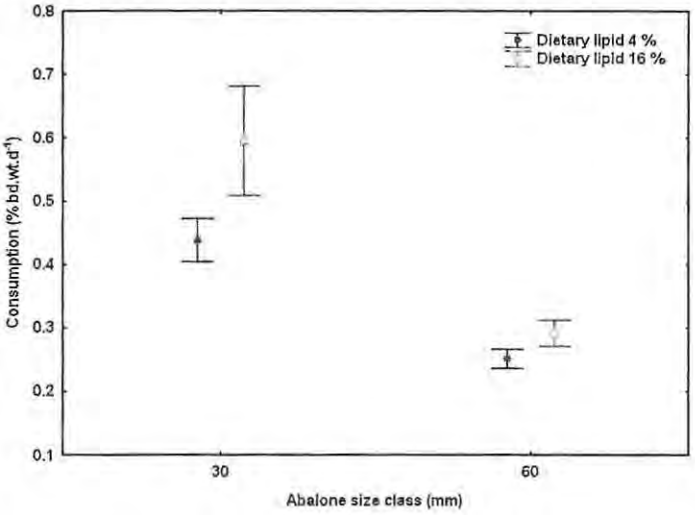

Figure 4.5: Mean daily feed consumption (± 95 % confidence intervals) expressed as a percentage of body weight consumed per day of two size classes of abalone fed formulated feeds with dietary lipid contents of either 4 or 16 %. Non-overlapping confidence intervals indicate significant differences (multifactor ANOVA: $F_{(1,24)}$=7.77; p=0.01).

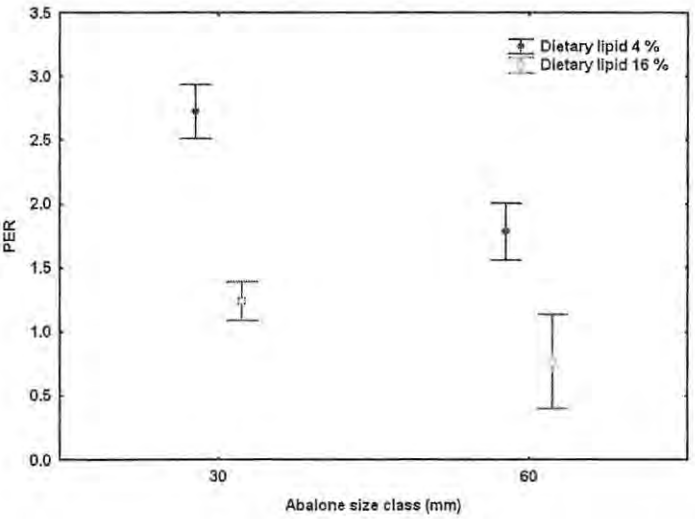

Figure 4.6: Protein efficiency ratio (PER) of two size classes of abalone fed formulated feeds with dietary lipid contents of either 4 or 16 %. Non-overlapping confidence intervals indicate significant differences (multifactor ANOVA: $F_{(1,24)}$=5.39; p=0.03).

4.3.3 Experiment 3: Dietary protein can be reduced if the energy content of the diet is maintained

The two size classes of abalone had similar responses to an increase in dietary protein content reflected in their shell length gain (multifactor ANOVA: p=0.13), SGR (multifactor ANOVA: p=0.14), final condition factor (multifactor ANOVA: p=0.50), mortality (multifactor ANOVA: p=0.65), feed consumption (multifactor ANOVA: p=0.81), FCR (multifactor ANOVA: p=0.06) and PER (multifactor ANOVA: p=0.06).

Dietary protein content did not affect the shell length gain, SGR or final condition factor of the two size classes (Students *t*-test: p>0.05) (Table 4.8). However, the soft tissue glycogen content of the 30 mm abalone decreased significantly with an increase in dietary protein content from 20 to 34 % (multifactor ANOVA: $F_{(1,12)}$=5.71; p=0.03; Figure 4.7). Dietary protein content had no effect on the mortality of the 30 mm (Mann-Whitney *U*=2.00, p=0.08, *df*=1) or 60 mm abalone (Mann-Whitney *U*=7.00, p=0.77, *df*=1) (Table 4.8).

The feed consumption rates of the 30 mm abalone were independent of dietary protein content (Students *t*-test: $t_{(1,6)}$=0.37; p=0.73), while the 60 mm abalone fed the 34 % diet (0.30±0.01 % bd.wt.d^{-1}) consumed significantly more compared to those fed the 20 % protein diet (0.26±0.01 % bd.wt.d^{-1}) (Students *t*-test: $t_{(1,6)}$=2.52; p=0.05) (Table 4.8). The 30 mm abalone fed the 20 % protein diet (2.55±0.08) had significantly higher FCR's compared to those fed the 34 % protein diet (2.10±0.05) (Students *t*-test: $t_{(1,6)}$=-4.77; p=0.003), while dietary protein content had no effect on the FCR's of the 60 mm abalone (Students *t*-test: $t_{(1,6)}$=1.17; p=0.28) (Table 4.8). PER decreased significantly with an increase in dietary protein for both the 30 mm (Students *t*-test: $t_{(1,6)}$=-6.10; p=0.001) and 60 mm abalone (Students *t*-test: $t_{(1,6)}$=-3.79; p=0.009) (Table 4.8).

Table 4.8: Growth (mean ± standard error) and nutritional indices of 30 mm and 60 mm abalone fed isoenergetic formulated diets containing either 20 or 34 % dietary protein. Significant differences are indicated by different alphabetical superscripts in each row ($p<0.05$).

	Dietary protein content (%)	
	20	**34**
Mean initial sizes – small abalone		
Shell length (mm)	25.75±0.24[a]	26.03±0.23[a]
Weight (g)	2.57±0.06[a]	2.75±0.06[a]
Condition factor	0.84±0.01[a]	0.89±0.01[b]
Mean initial sizes – large abalone		
Shell length (mm)	66.11±0.59[a]	66.13±0.58[a]
Weight (g)	49.89±1.36[a]	49.53±1.75[a]
Condition factor	1.00±0.01[a]	0.99±0.01[a]
Mean shell length gain ($mm.month^{-1}$)		
Small abalone	1.71±0.31[a]	1.28±0.08[a]
Large abalone	0.82±0.06[a]	0.97±0.14[a]
SGR (% $bd.wt.d^{-1}$)		
Small abalone	0.48±0.03[a]	0.40±0.03[a]
Large abalone	0.12±0.01[a]	0.13±0.03[a]
Final condition factor		
Small abalone	0.82±0.02[a]	0.81±0.01[a]
Large abalone	1.00±0.01[a]	0.97±0.01[a]
Mortality (%)		
Small abalone	0.75±0.32[a]	0.00[a]
Large abalone	1.39±0.80[a]	1.39±1.39[a]
Consumption (% $bd.wt.d^{-1}$)		
Small abalone	0.60±0.04[a]	0.62±0.06[a]
Large abalone	0.26±0.01[a]	0.30±0.01[b]
Feed conversion ratio		
Small abalone	2.55±0.08[a]	2.10±0.05[b]
Large abalone	2.27±0.21[a]	2.82±0.42[a]
Protein efficiency ratio		
Small abalone	1.74±0.05[a]	1.35±0.03[b]
Large abalone	2.00±0.18[a]	1.08±0.17[b]

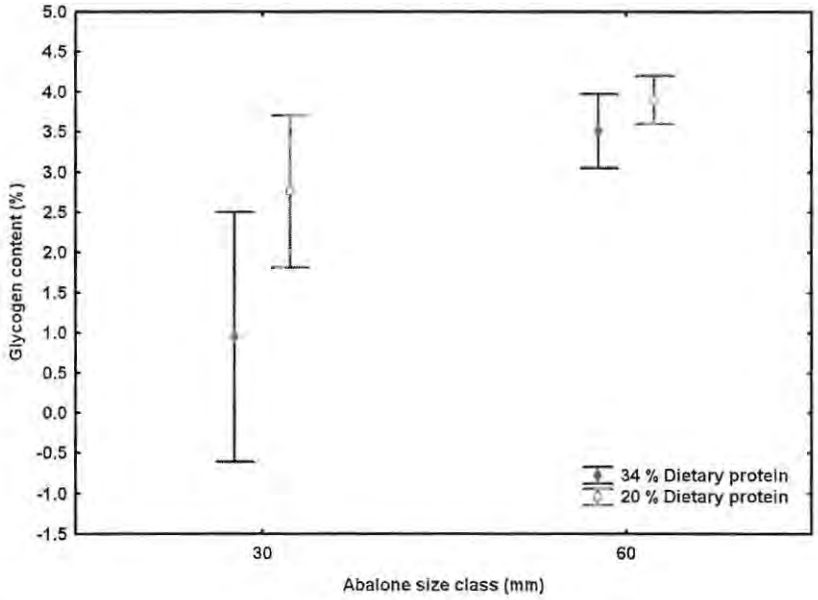

Figure 4.7: Glycogen content of soft body tissue (± 95 % confidence intervals) of two size classes of abalone fed formulated diets with dietary protein contents of either 20 or 34 %. Non-overlapping confidence intervals indicate significant differences (multifactor ANOVA: $F_{(1,12)}=5.71$; p=0.03)

4.4 Discussion

The decline in abalone growth in response to the increasing levels of dietary lipid supported the findings of a number of other lipid based abalone nutritional studies on species including *H. tuberculata* and *H. discus hannai* (Mai et al., 1995b), *H. midae* (Britz and Hecht, 1997), *H. fulgens* (Durazo-Beltran et al., 2003), *H. asinina* (Thongrod et al., 2003) and *H. corrugata* (Montano-Vargas et al., 2005). Interestingly, the increasing levels of dietary protein did not mitigate the negative effects of high dietary lipid levels on growth. Abalone growth rates generally increase in response to increasing levels of dietary protein (Mai et al., 1995a; Britz, 1996a; Coote et al., 2000; Gomez-Montes et al., 2003; Sales et al., 2003; Montano-Vargas et al., 2005). The limited ability of abalone to digest high levels of dietary lipids has been attributed to the low levels of lipases present in the digestive gland (Britz et al., 1996; Knauer et al., 1996; Garcia-Esquivel and Felbeck, 2006) which in turn is believed to be linked to the low lipid content of natural algal diets and because abalone store energy as glycogen (Webber, 1970; Mercer et al., 1993).

Feed consumption and FCR increased significantly with an increase in dietary lipid content for both size classes. Feed consumption in abalone is believed to be linked to their requirement for energy (Bautista-Teruel and Millamena, 1999; Gomez-Montes et al., 2003). Therefore, feed consumption and FCR should decrease as the energy content of the diet increases. However, the increase in feed consumption and FCR in response to increasing levels of lipid is evidence that the energetic requirements of the abalone were not being met by the increasing levels of dietary lipid. Thus, abalone fed the high lipid diets were probably consuming more food in order to satisfy their energetic requirements from non-lipid sources of energy i.e. the protein and carbohydrate portion of the diets. In addition, Van Barneveld et al. (1998) found that a dietary oil inclusion level of three percent reduced gross energy digestion in juvenile *H. laevigata* and levels of six percent reduced the digestion of dietary nitrogen and amino acids. Thus, the increasing levels of dietary lipid may have reduced the uptake of other nutrients in the diet.

The inhibiting effect of lipids on the uptake of other dietary nutrients may explain the observed decrease in condition factor and soft tissue glycogen content in response to the increasing levels of dietary lipid, signifying that less energy was available for storage as glycogen. Mai et al. (1995a) showed that soft tissue protein content was negatively correlated to increasing levels of dietary lipid in *H. tuberculata* and *H. discus hannai.*

The results demonstrate that the abalone were unable to utilise increasing levels of dietary lipid as a source of energy, and it is likely that the abalone were satisfying their energetic requirements from dietary proteins and carbohydrates. Protein and carbohydrates have been identified as the principal energy sources in abalone nutrition (Carefoot et al., 1993; Knauer et al., 1996; Durazo-Beltran et al., 2004; Montano-Vargas et al., 2005; Viana et al., 2007). Durazo-Beltran et al. (2004) investigated the effects of starvation on *H. fulgens* for 60 days. As expected the abalone were observed to lose body weight during the starvation period. However, soft tissue lipid concentrations increased during the starvation period and thus the weight loss was associated with the use of bodily protein and carbohydrate stores as energy sources (Durazo-Beltran et al., 2004).

The inability of abalone to utilise dietary lipids as a source of energy was linked to a condition that occurs in certain muscle types (Durazo-Beltran et al., 2003). For example, rat heart and chondrichthian muscles lack the ability to utilise free fatty acids as an energy source and thus anaerobic metabolism making use of carbohydrates and ketonic bodies serves as an energy source (Moyes and West, 1995 cited by (Durazo-Beltran et al., 2003). However, free fatty acids inhibit the use of carbohydrates and thus cut off the energy pathway (Moyes and West, 1995 cited by (Durazo-Beltran et al., 2003), and it is possible that a similar mechanism may occur in abalone tissue (Durazo-Beltran et al., 2003). The inability of abalone to utilise dietary lipids as a source of energy has also been ascribed to their low metabolic rate (Thongrod et al., 2003).

The growth rates of the 60 mm abalone (i.e. mean shell length gain of 1 $mm.month^{-1}$) fell below the average growth rates required by commercial farming operations (2-3 $mm.month^{-1}$) (Fleming et al., 1996). However, when dissected the abalone were observed to be sexually mature. This may explain the below average growth rates as it is possible that the abalone were investing energy into gonad maturation rather than growth. Reductions in growth in response to the onset of sexual maturity is well documented in abalone and has been ascribed to energy being channelled into gonad development rather than growth (Shepherd and Hearn, 1983; Mercer et al., 1993; Capinpin and Corre, 1996). It is possible that the high lipid diets stimulated the development of the gonads leading to the subsequent reduction in growth rates. Mercer et al. (1993) found algal diets with high levels of lipid stimulated the development of gonads and resulted in a reduction in growth, while abalone fed diets with low lipid contents had no such reduction. Although abalone have limited ability to utilise lipids as a energy source they are able to store high levels of lipid in their body tissues (Webber, 1970; Mercer et al., 1993; Mai et al., 1995b; Dunstan et al., 1996; Britz and Hecht, 1997; Nelson et al., 2002; Gomez-Montes et al., 2003; Thongrod et al., 2003; Durazo-Beltran et al., 2004). The storage of lipids in abalone tissues is unlikely to be linked to energy storage but rather as a source of lipid for gonad maturation and larval development as there is evidence to suggest that lipids pass from the hepatopancreas to the gonads (Nelson et al., 2002). Further studies are required to investigate the effects of dietary lipid levels on gonad development.

It was possible to reduce dietary protein content of the diet from 34 to 20 % without negatively affecting the growth of the two size classes. Reductions in the protein portion of formulated feeds through the provision of sufficient levels of dietary energy have been demonstrated in a number of species including: *H. asinina* (Bautista-Teruel and Millamena, 1999), *H. laevigata* (Coote et al., 2000) and *H. midae* (Sales et al., 2003; Jones and Britz, 2006; Chapter 2). Bautista-Teruel and Millamena (1999) reduced the protein content of a formulated feed for *H. asinina* from 32 to 27 % by increasing the energy content of the diet from 12.94 to 13.19 $MJ.kg^{-1}$. However, there is a large amount of evidence, including the present results, to suggest that energy should be provided by carbohydrates not lipids (Mai et al., 1995b; Britz et al., 1996; Knauer et al., 1996; Britz and Hecht, 1997; Van Barneveld et al., 1998; Bautista-Teruel and Millamena, 1999; Durazo-Beltran et al., 2003; Thongrod et al., 2003; Durazo-Beltran et al., 2004; Montano-Vargas et al., 2005; Viana et al., 2007; Chapter 2).

The significant reduction in growth of the 30 mm abalone in response to increasing levels of dietary lipid mirrored the findings of Britz and Hecht (1997). However, Britz and Hecht (1997) suggested that young adult abalone (40 mm) have a higher dietary protein requirement (i.e. 44 %) than juvenile abalone (10 mm) (i.e. 34 %). There present study provided no evidence to suggest that the two size classes had different dietary protein requirements. However, the 30 mm abalone had higher PER's compared to the 60 mm abalone, which suggests that the 30 mm abalone made more efficient use of dietary protein compared to the 60 mm abalone. This may be linked to a higher degree of digestive plasticity in juvenile abalone compared to older abalone (Shipton and Britz, 2001b). The channelling of energy into gonad development in the 60 mm abalone may have resulted in the poorer PER values.

4.5 Conclusion

High levels of dietary lipid negatively affected the growth, condition factor and soft tissue glycogen content of both size classes of abalone. This negative effect was greater in the 30 mm size class compared to the 60 mm abalone. The corresponding increase in feed consumption and FCR in response to increasing levels of dietary protein also

provides evidence that abalone are unable to utilise dietary lipids as an energy source and high levels of dietary lipid probably inhibit the uptake of carbohydrates and protein. High dietary lipid levels did however appear to promote gonad maturation. It was possible to reduce dietary protein from 34 to 20 % without negatively affecting growth through the maintenance of dietary energy levels and thus it is recommended that manipulations in the energy content of formulated feeds should be approached through the use of carbohydrates.

CHAPTER 5.

Concluding Discussion

It was possible to reduce the protein portion of formulated feeds for *Haliotis midae* through the provision of sufficient levels of dietary energy supplied from carbohydrates. This result is consistent with the findings of previous studies (Coote et al., 2000; Bautista-Teruel et al., 2003; Sales et al., 2003; Jones and Britz, 2006) but is based on a larger, more quantitative dataset covering a range of temperatures, abalone sizes, protein levels and energy levels. In summary, the results demonstrated that it was possible to reduce dietary protein from 26 to 18 % provided dietary energy was maintained at 13.5 $MJ.kg^{-1}$ or more. In addition to the reduction in dietary protein it was possible to reduce dietary energy from 16.2 to 13.5 $MJ.kg^{-1}$ which is suggested to be an "optimal" energy level for formulated feeds for *H. midae* (Chapter 2). These results support the notion of (Fleming et al., 1996) that dietary energy levels are often over-specified in formulated abalone feeds. The result is useful to feed manufacturers and abalone farmers as there are potential economic savings associated with reductions in the protein and/or carbohydrate portions of formulated feeds. Further investigations into the manipulation of dietary carbohydrate levels may also allow for further reductions in the protein portion of formulated feeds for *H. midae*.

A number of abalone nutritional studies have investigated the protein and energy requirements of abalone in order to determine optimal dietary protein to energy ratios (Britz and Hecht, 1997; Bautista-Teruel and Millamena, 1999; Gomez-Montes et al., 2003; Montano-Vargas et al., 2005). Protein to energy ratios (P:E) were a poor predictor of diet performance in this study. For example, in Chapter 2 abalone fed diet treatments with P:E ratios ranging from 1.36 to 1.86 (Diets 1, 2, 4, 5, 7 and 8; $\geq$ 13.2 $MJ.kg^{-1}$ energy) grew at similar rates, whereas diets with similar P:E ratios (Diets 6 and 9; < 13.2 $MJ.kg^{-1}$ energy) displayed poor growth (Table 5.1). Thus, provision of sufficient levels of dietary energy is probably a more important consideration in abalone feed formulations, as opposed to maintaining a fixed dietary protein to energy ratio.

Table 5.1: The nine experimental diets used in Chapter 2 and formulated to contain graded levels of protein and energy to produce five protein:energy (P:E) ratios. The values shown are the values obtained from the original dietary formulations.

		Protein (%)			
		26	22	18	
P:E ratio	1.1	-	-	(7) 16.4	Digestible energy ($MJ.kg^{-1}$)
	1.36	-	(4) 16.2	(8) 13.2	
	1.62	(1) 16.0	(5) 13.6	(9) 11.1	
	1.88	(2) 13.8	(6) 11.7	-	
	2.14	(3) 12.1	-	-	

The protein sparing effect observed in the trials described in Chapters 2 and 4 were not evident in the feeding trials described in Chapter 3, where the growth rates of the abalone were well below the expected commercial growth rate, despite all diets having energy levels of approximately 15 $MJ.kg^{-1}$ energy. The suppressed growth rates were probably due to a non-nutritional factor. The trends observed between the dietary treatments thus need to be interpreted with caution, and cannot be regarded as representative of farmed abalone growing at maximal rates.

There is limited information on the minimum nutritional requirements of abalone. Abalone growth and survival were significantly compromised when dietary energy levels were reduced to 11.6 $MJ.kg^{-1}$ regardless of the protein content of the diet (18 – 26 %) (Chapter 2). It seems that the diet has to contain a minimum amount of dietary energy supplied from carbohydrate and protein in order for the abalone to efficiently utilise the protein portion of the diet, as even abalone fed the 26 % protein 11.6 $MJ.kg^{-1}$ energy diet had poor growth and survival. This finding may have important implications for farmers making use of natural algal diets as a feed source, since seasonal fluctuations in the nutritional contents of a number of seaweed species have been reported (Mercer et al., 1993). Seasonal variations in the carbon content of the local kelp *Ecklonia maxima* have also been detected (Smith, 2008) and thus it is possible that kelp contains insufficient levels of carbohydrates during certain periods of the year. It may be beneficial for farmers making use of kelp as a feed source to supplement the abalone diet with formulated feeds during these periods. Abalone eat to satisfy their energetic requirements (Bautista-Teruel and Millamena, 1999; Gomez-Montes et al., 2003; Chapters 2, 3 and 4).

This principle is a useful tool for formulating diets for *H. midae* as it allows formulators to ensure that the diet contains sufficient energy but also that the diet does not contain excessive energy that may result in the abalone's energetic requirements being met before the requirements for other nutrients are satisfied (Fleming et al., 1996).

The growth, survival and feed conversion efficiency were highest for abalone cultured at 18 °C (Chapters 2 and 3) but were significantly reduced at water temperatures of 22 and 24 °C (Chapter 2) which is consistent with the results of Britz et al. (1997). Thus, once abalone receive an optimal dietary formulation and are stocked under optimal conditions with favourable water quality, any further improvements in growth will be reliant on maintaining optimal temperatures in the culture environment. The bulk of abalone farming operations in South Africa are land-based making use of pump ashore single pass technology and thus have very little control over culture environment water temperatures (Cook, 1998; Troell et al., 2006). Famers could potentially explore the use of recirculating aquaculture systems or heating incoming water, as abalone cultured under optimal temperature conditions in the experiment described in Chapter 2 achieved growth rates of 2 - 3 $mm.month^{-1}$.

The nutritional requirements of *H. midae* were unaffected by water temperature in the ranges tested in this study. This has promising implications for feed management regimes as farmers need not adjust dietary formulations in response to changing water temperatures. The feeds developed in Chapter 2 will not only allow farmers to safely feed their stock during periods of elevated water temperatures but also potentially allow the abalone culture industry to expand into the warmer Eastern Cape region of South Africa, which has been identified as the most suitable area for expansion of the industry (Hecht, 1994; Britz et al., 1997).

Carbohydrates and protein serve as the primary sources of energy in abalone nutrition (Carefoot et al., 1993; Knauer et al., 1996; Durazo-Beltran et al., 2004; Montano-Vargas et al., 2005; Viana et al., 2007; Chapters 2, 3 and 4), and the results from Chapter 4 confirm that lipids probably play a minimal role as an energy source. However, the

inclusion of lipids is vital for the provision of essential fatty acids and other precursors required for growth and gonad maturation (Mai et al., 1995; Nelson et al., 2002). It is likely that the dietary lipid requirements of abalone are being met by the lipid content of the dietary ingredients supplied in formulated feeds (Fleming et al., 1996).

There was no empirical evidence in the present experiments to support the hypothesis that abalone of different sizes have differing dietary protein and energy requirements (Britz and Hecht, 1997; Shipton and Britz, 2001b), although smaller abalone appeared to have a lower tolerance to high levels of dietary lipid (Chapter 4). The tolerance of the larger abalone to high levels of dietary lipid may be linked to the sexual maturity of larger animals and the channelling of lipids into gonad maturation (Chapter 4). Nonetheless, further studies are required to address the nutritional requirements of abalone of different sizes.

To conclude, reductions in the protein portion of formulated feeds for *H. midae* are possible provided the diet contains sufficient levels of energy supplied from carbohydrates. A "high temperature maintenance" diet based on the current research is now commercially available to farms exposed to periods of elevated water temperatures. As the ability of abalone to utilise dietary lipid is limited, lipids are unlikely to play a significant role as an energy source in abalone feeds. Further investigations should focus on the utilisation of various carbohydrate sources in abalone feeds.

REFERENCES

AOAC, 2003. *Official methods of analysis of the Association of Official Analytical Chemists*. Association of analytical communications, Gaithersburg, MD, USA.

Bautista-Teruel, M.N., Fermin, A.C., Koshio, S.S., 2003. Diet development and evaluation for juvenile abalone, *Haliotis asinina*: animal and plant protein soutrces. *Aquaculture* 219, 645-653.

Bautista-Teruel, M.N., Millamena, O.M., 1999. Diet development and evaluation for juvenile abalone, *Haliotis asinina*: protein/energy levels. *Aquaculture* 178, 117-126.

Bower, C.E., Bidwell, J.P., 1978. Ionisation of ammonia in seawater: effects of temperature, pH and salinity. *J. Fish. Res. Board Can.* 35, 1012-1016.

Britz, P.J., 1995. *The nutritional requirements of Haliotis midae and development of a practical diet for abalone aquaculture.* Ph.D Dissertation, Rhodes University, Grahamstown, South Africa, 150 pp.

Britz, P.J. 1996a. Effect of dietary protein level on growth performance of South African abalone, *Haliotis midae*, fed fishmeal-based semi-purified diets. *Aquaculture* 140, 55-61.

Britz, P.J., 1996b. The suitability of selected protein sources for inclusion in formulated diets for the South African abalone, *Haliotis midae. Aquaculture* 140, 63-73.

Britz, P.J., Hecht, T., 1997. Effect of dietary protein and energy level on growth and body composition of South African abalone, *Haliotis midae. Aquaculture* 156, 195-210.

Britz, P.J., Hecht, T., Knauer, J., 1996. Gastric evacuation time and digestive enzyme activity in abalone *Haliotis midae* fed a formulated diet. *S. Afr. J. Mar. Sci.* 17, 297-303.

Britz, P.J., Hecht, T., Knauer, J., Dixon, M.G., 1994. The development of an artificial feed for abalone farming. *S. Afr. J. Sci.* 90, 7-8.

Britz, P.J., Hecht, T., Mangold, S., 1997. Effect of temperature on growth, feed consumption and nutritional indices of *Haliotis midae* fed a formulated diet. *Aquaculture* 152, 191-203.

Britz, P.J., Jones, C.L.W., 2006. Review of abalone feeding options in South Africa. *Book of Abstracts. The Sixth International Abalone Symposium, Puerto Varas, Chile*, February 19 – 24 2006, 152.

Capinpin, E.C., Corre, K.G., 1996. Growth rate of the Philippine abalone, *Haliotis asinina* fed an artificial diet and macroalgae. *Aquaculture* 144, 81-89.

Carefoot, T.H., Quian, P.Y., Taylor, B.E., West, T., Osbourne, J.M., 1993. Effect of starvation on energy reserves and metabolism in the Northern abalone, *Haliotis kamtschatkana*. *Aquaculture* 118, 315-325.

Cho, S.H., Park, J., Kim, C., Yoo, J.H., 2008. Effect of casein substitution with fishmeal, soybean meal and crustacean meal in the diet of the abalone *Haliotis discus hannai*. *Aquacult. Nutr.* 14, 61-66.

Cook, P.A., 1998. The current status of abalone farming in South Africa. *J. Shellfish Res.* 17, 601-602.

Coote, T.A., Hone, P.W., Van Barneveld, R.J., Maguire, G.B., 2000. Optimal protein level in a semipurified diet for juvenile greenlip abalone *Haliotis laevigata*. *Aquacult. Nutr.* 6, 213-220.

Dixon, M.G., 1992. *The effect of temperature and photoperiod on the digestive physiology of the South African abalone Haliotis midae*. MSc Thesis, Rhodes University, Grahamstown, 85p.

Dunstan, G.A., Baillie, H.J., Barrett, S.M., Volkman, J.K., 1996. Effect of diet on the lipid composition of wild and cultured abalone. *Aquaculture* 140, 115-127.

Durazo-Beltran, E., D'Abramo, L.R., Toro-Vazquez, J.F., Vasquez-Pelaez, C., Viana, M.T., 2003. Effect of triacylglycerols in formulated diets on growth and fatty acid composition in tissue of green abalone (*Haliotis fulgens*). *Aquaculture* 224, 257-270.

Durazo-Beltran, E., Viana, M.T., D'Abramo, L.R., Toro-Vazquez, J.F., 2004. Effects of starvation and dietary lipid on the lipid and fatty acid composition of muscle tissue of juvenile green abalone (*Haliotis fulgens*). *Aquaculture* 238, 329-341.

FAO, 2005. The State of the World Fisheries and Aquaculture 2005. FAO, Rome. FishStat Plus, 2007. Universal Software for Fishery Statistical Time Series 1950-2005. FAO Fisheries Department, Fisheries informations, Data and Statistics Unit.

Fleming, A.E., Van Barneveld, R.J., Hone, P.W., 1996. The development of artificial diets for abalone: A review and future directions. *Aquaculture* 140, 5-53.

Fry, F.E.J., 1971. The effect of environmental factors on the physiology of fish. In: Hoar, W.S., Randall, D.J. (Eds.), *Fish Physiology*, Academic Press, New York, pp. 1-98.

Garcia-Esquivel, Z., Felbeck, H., 2006. Activity of digestive enzymes along the gut of juvenile red abalone, *Haliotis rufescens*, fed natural and balanced diets. *Aquaculture* 261, 615-625.

Garcia-Esquivel, Z., Montes-Magallon, S., Gonzalez-Gomez, M.A., 2007. Effect of temperature and photoperiod on the growth, feed consumption, and biochemical content of juvenile green abalone, *Haliotis fulgens*, fed on a balanced diet. *Aquaculture* 262, 129-141.

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

Godoy, M., Aviles, F., Flores, R., Aedo, I., 2006. Gastric dilation syndrome in red abalone (*Haliotis rufescens*). *Book of Abstracts. The Sixth International Abalone Symposium, Puerto Varas, Chile*, February 19 – 24 2006, 152.

Gomez-Montes, L., Garcia-Esquivel, Z., D'Abramo, L.R., Shimada, A., Vasquez-Pelaez, C., Viana, M.T., 2003. Effect of dietary protein:energy ratio on intake, growth and metabolism of juvenile green abalone *Haliotis fulgens*. *Aquaculture* 220, 769-780.

Gordon, H.R., Cook, P.A., 2004. World abalone fisheries and aquaculture update: supply and market dynamics. *J. Shellfish Res.* 23, 935-939.

Greenwood, C., Taunton-Clark, J., 1994. Time series of monthly mean sea temperatures around the South African coast. *Sea Fisheries Research Institute Internal Report No. 124A*. Department of Environmental Affairs and Tourism, Cape Town, -36p.

Guzman, J.M., Viana, M.T., 1998. Growth of abalone *Haliotis fulgens* fed diets with and without fish meal, compared to a commercial diet. *Aquaculture* 165, 321-331.

Haaker, P.L., Parker, D.O., Barsky, K.C., Chun, C.S.Y., 1998. Growth of red abalone, *Haliotis rufescens* (Swainson), at Johnson Lee, Santa Rosa Island, California. *J. Shellfish Res.* 17, 747-753.

Hahn, K.O., 1989a. Survey of commercially important abalone species in the world. In: Hahn, K.O. (Ed.), *Handbook of culture of abalone and other marine gastropods*, CRC Press, Florida, pp. 3-11.

Hahn, K.O., 1989b. Nutrition and growth of abalone. In: Hahn, K.O. (Ed.), *Handbook of Culture of Abalone and other Marine Gastropods*, CRC Press, Florida, pp. 135-156.

Hay, M.E., Kappel, Q.E., Fenical, W., 1994. Synergism in plant defences against herbivores: interactions of chemistry, calcification and plant quality. Ecology, 75, 1714-1726.

Hecht, T., 1994. Behavioural thermoregulation of the abalone, *Haliotis midae*, and the implications for intensive culture. *Aquaculture* 126, 171-181.

Johnston, D., Moltschaniwskyj, N., Wells, J., 2005. Development of the radula and digestive system of juvenile blacklip abalone (*Haliotis rubra*): Potential factors responsible for variable weaning success on artificial diets. *Aquaculture* 250, 341-355.

Jones, C.L.W., Britz, P.J., 2006. Development of a low-protein, water stable kelp diet for the South African abalone culture industry. *Book of Abstracts. The Sixth International Abalone Symposium, Puerto Varas, Chile*, February 19 – 24 2006, 152.

Knauer, J., Britz, P.J., Hecht, T., 1996. Comparative growth performance and digestive enzyme activity of juvenile South African abalone, *Haliotis midae*, fed on diatoms and a practical diet. *Aquaculture* 140, 75-85.

Limin, S., 2006. The current situation of abalone (*Haliotis discus hannai*) production in northern China. *Book of Abstracts. The Sixth International Abalone Symposium, Puerto Varas, Chile*, February 19 – 24 2006, 152.

Lopez, L.M., Tyler, P.A., Viana, M.T., 1998. The effect of temperature and artificial diets on growth rates of juvenile *Haliotis tuberculata* (Linnaeus, 1758). *J. Shellfish Res.* 17, 657-662.

Loubser, N.C., 2005. Abalone farming in South Africa. Book of Abstracts. *Aquaculture for Africa – Unlocking the potential. 7th Bi-annual conferences of the Aquaculture Association of South Afica, Grahamstown, South Africa*, September 12 – 16 2005 150.

Macey, B.M., Coyne, V.E., 2005. Improved growth rate and disease resistance in farmed *Haliotis midae* through probiotic treatment. *Aquaculture* 245, 249-261.

Mai, K., Mercer, J.P., Donlon, J., 1995a. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino. IV. Optimum dietary protein level for growth. *Aquaculture* 136, 165-180.

Mai, K., Mercer, J.P., Donlon, J., 1995b. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino. III. Rsponse of abalone to various levels of dietary lipid. *Aquaculture* 134, 65-80.

Mercer, J.P., Mai, K., Donlon, J., 1993. Comparative studies on the nutrition of two psecies of abalone, *Haliotis tuberculata* Linnaeus and *Haliotis discus hannai* Ino I. Effects of algal diets on growth and biochemical composition. *Invert. Reprod. Dev.* 23, 75-88.

Monje, H., Viana, M.T., 1998. The effect of cellulose on the growth and cellulolytic activity of the abalone *Haliotis fulgens* when used as an ingredient in formulated artificial diets. *J. Shellfish Res.* 17, 657-662.

Montano-Vargas, J., Viana, M.T., D'Abramo, L.R., Shimada, A., Vasquez-Pelaez, C., 2005. Growth and energy utilization of juvenile pink abalone *Haliotis corrugata* fed diets containing different levels of protein and two starch:lipid ratios. *J. Shellfish Res.* 24, 1179-1185.

Naidoo, K., Maneveldt, G., Ruck, K., Bolton, J.J., 2006. A comparison of various seaweed-based diets and formulated feed on growth rate of abalone in a land-based aquaculture system. *J. Appl. Phycol.* 18, 437-443.

Nelson, M.M., Leighton, D.L., Phleger, C.F., Nicholas, P.D., 2002. Comparison of growth and lipid composition in the green abalone, *Haliotis fulgens*, provided specific macroalgal diets. *Comp. Biochem. Physiol.* Part B 131, 695-712.

Sales, J., Britz, P.J., 2001a. Research on abalone (*Haliotis midae* L.) cultivation in South Africa. *Aquacult. Res.* 32, 863-874.

Sales, J., Britz, P.J., 2001b. Evaluation of different markers to determine the apparent nutrient digestibility coefficients of feed ingredients for South African abalone (*Haliotis midae* L.). *Aquaculture* 202, 113-129.

Sales, J., Truter, P.J., Britz, P.J., 2003. Optimum dietary crude protein level for growth in South African abalone (*Haliotis midae* L.). *Aquacult. Nutr.* 9, 85-89.

Schlosser, S.C., Lupatsch, I., Lawrence, J.M., Lawrence, A.L., Shpigel, M., 2005. Protein and energy digestibility and gonad development of the European sea urchin *Paracentrotus lividus* (Lamarck) fed algal and prepared diets during spring and fall. *Aquacult. Res.* 36, 972-982.

Schumann, E.H., Cohen, A.L., Jury, M.R., 1995. Coastal sea surface temperature variability along the south coast of South Africa and the relationship to regional and global climate. *J. Mar. Res.* 53, 231-248.

Searle, T., Roberts, R.D., Lokman, P.M., 2006. Effects of temperature on growth of juvenile blackfoot abalone, *Haliotis iris* Gmelin. *Aquacult. Res.* 37, 1441-1449.

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

Shipton, T.A., Britz, P.J., 2001a. The partial and total replacement of fishmeal with selected plant protein sources in diets for the South African abalone, *Haliotis midae* L. *J. Shellfish Res.* 20, 637-645.

Shipton, T.A., Britz, P.J., 2001b. The effect of animal size on the ability of *Haliotis midae* L. to utilize selected dietary protein sources. *Aquacult. Res.* 32, 393-403.

Simon, C.A., Kaiser, K., Britz, P.J., 2004. Infestation of the abalone, *Haliotis midae*, by the sabellid, *Terebrasabella heterouncinata*, under intensive culture conditions, and the influence of infestation on abalone growth. *Aquaculture* 232, 29-40.

Smith, M., 2008. Seasonal variations in the nutritional content of the kelp *Ecklonia maxima* on the west and southwest coasts of South Africa, with reference to its use as abalone feed. *A Frontier Programme for Mariculture Research and Development. Marine and Coastal Management, Cape Town*, 253 pp.

Smith, R.R., 1989. Nutritional Energetics. In: Halver, J.E. (Ed.), *Fish Nutrition*, Academic Press, San Diego, Ca, pp. 1-29.

Thongrod, S., Tamtin, M., Chairat, C., Boonyaratpalin, M., 2003. Lipid to carbohydrate ratio in donkey's ear abalone (*Haliotis asinina*, Linne) diets. *Aquaculture* 225, 165-174.

Troell, M., Robertson-Andersson, D., Anderson, R.J., Bolton, J.J., Maneveldt, G., Halling, C., Probyn, T., 2006. Abalone farming in South Africa: An overview with perspectives on kelp resources, abalone feed, potential for on-farm seaweed production and socio-economic importance. *Aquaculture* 257, 266-281.

Van Barneveld, R.J., Fleming, A.E., Vandepeer, M.E., Kruk, J.A., Hone, P.W., 1998. Influence of dietary oil type and oil inclusion level in manufactured feeds on the digestibility of nutrients by juvenie greenlip abalone (*Haliotis laevigata*). *J. Shellfish Res.* 17, 649-655.

Vandepeer, M.E., Van Barneveld, R.J., 2003. A comparision of the digestive capacity of blacklip (*Haliotis rubra*) and greenlip (*Haliotis laevigata*) abalone. J. Shellfish Res. 22, 171-175.

Viana, M.T., D'Abramo, L.R., Gonzalez, M.A., Garcia-Suarez, J.V., Shimada, A., Vasquez-Pelaez, C., 2007. Energy and nutrient utilization of juvenile green abalone (*Haliotis fulgens*) during starvation. *Aquaculture* 264, 323-329.

Viana, M.T., Lopez, L.M., Salas, A., 1993. Diet development for juvenile abalone *Haliotis fulgens*. Evaluation of two artificial diets and macroalgae. *Aquaculture* 117, 149-156.

Viera, M.P., Gomez Pinchetti, J.L., Courtois de Vicose, G., Bilbao, A., Suarez, S., Haroun, R.J., Izquierdo, M.S., 2005. Suitability of three red macroalgae as a feed for the abalone *Haliotis tuberculata coccinea* Reeve. Aquaculture 248, 75-82.

Webber, H.H., 1970. Changes in metabolite composition during the reproductive cycle of abalone *Haliotis cracheroidii* (Gastropoda: Prosobranchiata). *Physiol. Zool.* 43, 213-231.

Wilson, R.P., 2002. Amino acids and proteins. In: Halver, J.E., Hardy, R.W. (Eds.), *Fish Nutrition*, Academic Press, New York, pp. 143-179.

Woodcock, S.H., Benkendorff, K., 2008. The impact of diet on the growth and proximate composition of juvenile whelks, *Dicathais orbita* (Gastropoda: Mollusca). *Aquaculture* 276, 162-170.