Growth and gonad size in cultured South African abalone, *Haliotis midae*

A thesis submitted in fulfilment of the requirements for the degree of

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By
Nicholas Alwyn Riddin

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ABSTRACT

According to farm records, cultured *Haliotis midae* (50-70 g.abalone^{-1}) were growing 10 % slower in winter when compared to summer. This reduction in growth rate also coincided with enlarged gonads. Initial trials showed that there were differences in mean monthly growth rates ranging from 1.97 – 5.14 g abalone^{-1} month^{-1}, and gonad bulk index (GBI) also varied between months (GBI range: 26.88 ± 12.87 to 51.03 ± 34.47). The investment of energy into gonad tissue growth did not compromise whole body growth as the abalone continued to gain weight throughout the reproductive periods, probably due to gonadal growth. Growth of this size class of abalone was not influenced by water temperature or day length, suggesting favourable on-farm culture conditions (regression analyses, $p > 0.05$). There is no need to implement a seasonal dietary regime.

Cultured *H. midae* were fed artificial diets with different protein sources, including only soya, only fishmeal, a combination of soya and fishmeal, and these were compared to kelp-fed abalone. Kelp-fed abalone grew slower than those fed artificial feeds ($p>0.05$). Gonad growth was the greatest when soya meal was included in the diet (average GBI: 74.91 ± 23.31), while the average gonad size of abalone fed the fishmeal-based diet had gonads which were 38 % smaller, and kelp-fed abalone had gonads which were 75 % smaller than those of the abalone fed on diets containing soya meal. The increased gonad mass in abalone fed on diets including soya meal could be attributed to phytoestrogenic activity, as a result of the presence of isoflavones found in the soya plant; this remains to be tested. The use of soya in brood stock diet development is advised.

The influence of dietary protein to energy ratio (1.41 – 2.46 g MJ^{-1}) on growth and gonad size was tested. Protein and energy levels within the ranges tested (22 and 33 % protein; 13.5 and 15.6 MJ kg^{-1}) did not interact to influence growth rates of cultured *H. midae*. GBI increased from 50.67 ± 4.16 to 83.93 ± 9.35 units as a function of dietary protein to energy ratio ($y = 42.02\ x^{0.81}$; $r^2 = 0.19$; regression analysis: $F_{1.38} = 8.9$; $p = 0.005$). In addition, protein level influenced gonad size, with gonad growth being greater in abalone fed the high protein diet (factorial ANOVA: $F_{1, 32} = 7.1$, $p = 0.012$). Canning yields were reduced by 7 % when the protein content was increased, while increasing the quantity of dietary energy improved canning yields by ~ 6 % (one-way ANOVA: $F_{1,28} = 14.4$, $p= 0.001$).

The present study provided evidence that although growth rates are varying seasonally, reproductive investment is not hindering weight gain. Gonad growth can be influenced if desired by farms, depending on the level of soya inclusion, as well as the protein to energy ratio in the diet. Monthly variation in growth and gonad size, as well as the influence of diet on gonad growth were highlighted, and the implications for farm application and further research were discussed.

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CHAPTER 1

General introduction

Abalone culture in South Africa

The natural fish stocks worldwide are in decline (FAO, 2010). The demand for both food and ornamental fish is increasingly being supplemented with commercially produced fish, crustaceans, molluscs and other aquatic organisms. Worldwide, abalone population sizes declined late in the 20th century with South Africa showing reduced yields from wild fisheries (Sales & Britz, 2002). This may be attributed to high fishing pressure (Sloan & Breen, 1988; Tegner, 1993) or disease of wild animals (Friedman *et al.*, 2000) resulting in an increased distance between spawning adults (Allee *et al.*, 1949). The decrease in the wild fishery combined with an increase in consumer demand has led to the focus on abalone culture in South Africa (Britz, 1995).

The contribution of aquaculture to the global supply of fish and other aquatic animals has increased from 3.9 % of total supply in 1970 to 27.1 % in 2000, 32.4 % in 2004 and 37.8 % in 2009 (FAO, 2010). Aquaculture is the fastest growing food production sector globally. China accounted for 62.3 % of the total global production in 2008. Sub-Saharan Africa produced only 0.5 % of the total global produce in 2004, making up 0.8 % of the global aquaculture production value (FAO, 2010). This increase in South African aquaculture production can be accredited to the farming of the high-value abalone species *H. midae* Linnaeus, 1758 (Gastropoda: Haliotidae), with an approximate market price of US$ 40.00 / kg live weight (G Johnston, HIK Abalone Farm (Pty) Ltd, pers. comm.). The high consumer demand for cultured *H. midae* has placed increased importance on research collaborations with the aim of optimising abalone growth rate, while reducing production costs and providing farms with a cheaper protein source at an optimal combination of dietary protein and energy level. By addressing

these objectives research and development can increase production volume and improve the financial status of the industry.

The abalone farming industry in South Africa began in 1981 after the successful spawning of wild-caught South African abalone, *H. midae* (Sales & Britz, 2001). Subsequently, twelve abalone farms have been established, with an estimated investment value of ZAR 190 million and projected annual production of 500-800 t (Sales & Britz, 2001; Loubser, 2005). South Africa is currently the largest producer of abalone outside of Asia (FAO, 2010). Factors such as on-going partnership between industry and research institutions, favourable water quality conditions, and the availability of suitable farming sites have contributed to the increase in production (Sales & Britz, 2001; Troell *et al.*, 2006). Research and development programs were initiated in 1990 by the Council for Scientific and Industrial Research (CSIR), Rhodes University, University of Cape Town and three fishing companies (Sales & Britz, 2001) resulting in the availability of artificial diets for farmed abalone.

The South African abalone Haliotis midae

Haliotis midae is a marine gastropod inhabiting shallow coastal waters from the cold western cape coast to the warmer eastern cape coast of South Africa (Newman, 1965). Their natural diet consists of micro- and macro-algae (Erasmus *et al.*, 1997), which are consumed either by grazing on plants or by trapping pieces of drifting algae (Wood & Buxton, 1996a). Ocean water temperature throughout their distribution range varies from 12°C in the Western Cape up to 21°C in the Eastern Cape (Greenwood &

Taunton-Clark, 1994). Male and female *H. midae* exist as separate sexes unlike many terrestrial gastropods. The gonad develops on the right side of the body (Bevelander, 1988). When ripe, the female gonad has a green colouration while the male gonad appears creamy-yellow (Bevelander, 1988). The abalone gonad is a racemose gland which occupies the space between the external integument and the outer surface of the digestive diverticulum (Bevelander, 1988). Strands of underlying connective tissue, as well as the epithelium, grow outwards forming trabeculae, which form either sperm or ova (Bevelander, 1988). *Haliotis midae* is a dioecious broadcast spawner (Newman, 1967). Their gonad surrounds the digestive gland, comprising the bulk of the visceral mass (Newman, 1967).

Trade-off between somatic and reproductive growth

Abalone (*Haliotis midae*) farmers in South Africa that rely on the locally produced formulated feed, i.e., Abfeed® (Marifeed Pty. Ltd, Hermanus), reported a periodic drop in abalone growth. This reduction in growth appeared to only affect abalone above a certain size and during certain times of year. In addition, abalone that were fed kelp did not appear to show this drop in growth. The farmers also suggested that gonad growth may be greater during the periods of reduced somatic growth. Since the abalone that were fed formulated feeds only showed an increase in gonadal growth, it should be tested whether kelp provides abalone with some compounds that inhibit gonadal development, or whether the artificial abalone food contains compounds that enhance

gonadal development. This research was designed to study the difference in growth and gonadal development between abalone fed the natural diet (kelp) and Abfeed.

Prior to the present study, I conducted some baseline analyses on data collected on one of the commercial abalone farms in Hermanus, Western Cape, South Africa, between 2004 and 2009. The results suggested that growth was varying seasonally. When combining the data for the six year period, this growth appeared to be affecting abalone of a certain size range more than others. Individual *H. midae* of 60 - 80 g abalone^{-1} showed a decrease in growth from 2.6 g month^{-1} during summer to 1.83 g month^{-1} during the winter period, whereas this seasonal difference was not as pronounced among the smaller size classes of < 20 g and 20 - 40 g abalone^{-1} (Figure 1.1). The large standard deviation in the summer samples for animals greater than 60 g abalone^{-1} was likely due to the small sample sizes for these two size classes during summer (60 – 80 g abalone^{-1}: n = 16, > 80 g abalone^{-1}: n = 7).

Juvenile abalone invest available energy into somatic growth, however, as they mature, an increasing amount of energy is invested into reproductive growth (Barkai & Griffiths, 1988). Spawning events had been observed on two commercial farms in August, when the water turned milky as a result of gamete excretion. The energy expenditure building up to and during this release is high with investment into gonad tissue being more than three times higher prior to spawning (Newman, 1967). The gonad bulk index (GBI) of wild *H. midae* was estimated to reach > 70 mm^3 prior to spawning, while after excretion of gametes, the GBI volume was < 20 mm^3 (Newman, 1967). Excretion of gametes in wild *H. midae* accounted for the loss of up to 10 % of

total body mass, and it was suggested that this loss in mass and subsequent recruitment of gametes reduced somatic growth (Newman, 1968).

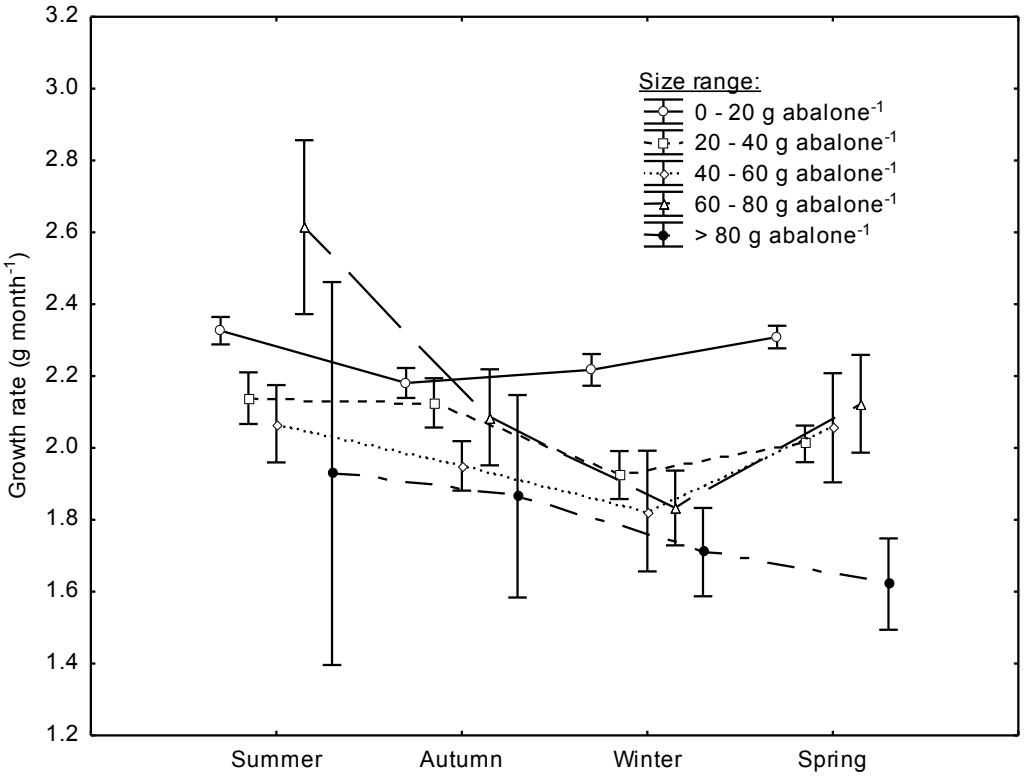

Figure 1.1: Mean (± standard error) of monthly growth rates for *H. midae* on a farm highlighting the effect of size and season on weight gain on a commercial abalone farm in Hermanus (total n = 764, between 2004 and 2009).

Potential factors which may influence somatic growth and gonadal development

The importance of water temperature, food type and food availability in influencing reproductive periodicity has been highlighted (Newman, 1967; Lin *et al*.,

2006; Azad *et al.*, 2011). *Haliotis cracheroidii* gonad development was thought to be influenced by changes in environmental conditions (Webber & Giese, 1969). Both temperature and diet influenced growth and gonad development of *H. tuberculata* (Lopez & Tyler, 2006), while temperature was suggested as the primary cause for changes in seasonal growth rates in *H. midae* (Newman, 1968). It was therefore assumed that artificial diets improve growth rates and that seasonality would affect the variability of somatic and gonadal growth in farmed *H. midae*.

Diet development

The success of abalone culture in South Africa is partly due to a long-standing partnership and co-operation between government-funded institutions and the private sector (Sales & Britz, 2001). Abalone farmers in South Africa prefer pelleted feeds to the macro-algae, *Ecklonia maxima*, due to kelp's seasonal variation in chemical composition and deficiency in many essential nutrients (Simpson, 1994; Sales & Britz, 2001). Development of a water-stable and nutritionally complete artificial feed propelled South African abalone farming forward by offering convenience and economic benefits (Britz, 1994; Sales & Britz, 2001). The maximum sustainable harvest of kelp was reached in 2003, suggesting that the continued use of *E. maxima* as a feed source is limited (Loubser, 2005; Troell *et al.*, 2006). In addition, Britz *et al.* (1994) indicated that the development of pelleted food for abalone was fundamental to the growth of the industry since pelleted feeds offer reliability, convenience and cost benefits.

To date, work on *H. midae* has concentrated on reducing the feed conversion ratio, protein level, and quantity of fishmeal used by inclusion of cheaper protein sources (Britz, 1996a; Britz, 1996b; Knauer *et al.*, 1996; Britz & Hecht, 1997; Britz *et al.*, 1997; Green *et al.*, 2011a; Green *et al.*, 2011b). This work has focussed on optimizing growth rate, but there is a paucity of data on the relative allocation of ingested energy to somatic and reproductive growth. Altering the diet of *H. midae* may help to determine how much energy is allocated to somatic growth and reproductive growth, and it is important to assess how *H. midae* allocates energy, and how abalone diet influences these processes.

Feed comprises the bulk of variable production costs and protein makes up as much as 60 % of feed costs in aquaculture. As a result, research has focused on replacing fishmeal-based proteins with alternative readily available, environmentally sustainable and cheaper protein sources (Britz, 1996a). Protein sources vary in amino acid composition, and it is important to provide the abalone with the required quantity and combinations of amino acids (Wilson, 2002).

Bacteria in the abalone intestine can improve digestive efficiency (El-Shanshoury *et al.*, 1994) by breaking down alginate, laminarin and cellulose, which are prominent polysaccharides in the natural diet of *H. midae* (Erasmus *et al.*, 1997). Assays conducted on *H. midae* showed that this species produces its own cellulase, alginate lyase, laminarinase, agarase and carrageenase (Erasmus *et al.*, 1997), but the expression of these endogenous polysaccharidases is controlled by diet. This ability to break down cellulose is an uncommon trait in eukaryotes (Erasmus *et al.*, 1997). It is

possible that these naturally occurring bacteria are not able to digest and utilize alternative protein sources as effectively as those present in kelp, resulting in less efficient use of the available nutrients. Testing the impact or role of gut flora was not a research objective in the present study.

Long-chain polyunsaturated fatty acids (PUFAs) are major structural components of cell membranes and the concentration of PUFAs can vary during sexual maturation (Rodriguez *et al.*, 1993; Brazão *et al.*, 2003). Thus, PUFAs can influence reproductive development of specific tissues (Innis, 1991). In abalone, the C_{20} and C_{22} PUFAs have been associated with somatic growth (Uki *et al.*, 1986; Mai *et al.*, 1995b). It is possible that the fatty acid profiles of various protein sources in the artificial diet will influence reproductive development in *H. midae.*

Aims and objectives

The aim of this study was to investigate seasonal changes in total and gonadal growth in farmed *H. midae* on two commercial abalone farms in Hermanus, South Africa. Literature suggests that temperature and diet are the main factors influencing growth and gonadal development. Thus, the present study aimed to determine whether environmental conditions influenced growth or gonad size. In addition, this study aimed to evaluate the influence of diet (protein source; and dietary protein and energy levels) on growth and gonad size in farmed *H. midae.*

The research objectives were to:

1. monitor monthly length and weight gain;
2. monitor changes in mean monthly gonad size in relation to total body mass between months;
3. determine whether growth and gonad size correlate with each other or with selected environmental variables;
4. to determine the influence of dietary protein source on growth and gonad size in male and female *H. midae*; and
5. to determine the influence of dietary protein and energy level on growth and gonad size in male and female *H. midae*.

CHAPTER 2

Monthly changes in growth and gonad size

2.1 Introduction

Haliotids can be divided into three groups according to spawning periodicity: summer spawners, non-summer spawners and those that spawn throughout the year (Shepherd & Laws, 1974). Haliotids are predominantly summer breeders (Boolootian *et al.*, 1962) while *Haliotis midae* showed spawning peaks during spring and autumn along the Western Cape coastline of South Africa (Newman, 1967). In a further study in this region, high water temperatures (14 – 18.5 ºC) in summer correlated with periods of slow growth, with fast growth occurring during periods of water temperatures ranging from 13 - 15.5 ºC in winter (Newman, 1968). Shepherd & Laws (1974) showed that falling sea temperature initiated spawning in *Haliotis ruber*. Newman (1968) suggested that seasonal variation in growth rate of natural abalone stocks was associated with spawning, with periods after spawning resulting in recruitment of gonad tissue and resultant slow somatic growth. Similarly, faster somatic growth was observed during winter and spring when little gamete recruitment was taking place (Newman, 1968).

Haliotis midae which consumed a natural diet of seaweed excreted 63 % of ingested gross energy as faeces and 32 % was used during respiration (Barkai & Griffiths, 1988). This leaves 5 % of gross dietary energy for growth and reproduction. In juveniles, this portion can be invested in somatic growth, but as the abalone reach maturity, they allocate an increasing amount of energy to reproduction (Barkai & Griffiths, 1988). Spawning in *H. midae* resulted in the loss of as much as 10 % of the total body weight, and this rapid loss in gonad mass and recruitment of gametes may result in interrupted or reduced somatic growth (Newman, 1968).

Haliotid ovaries contain two batches of eggs with the first batch being released during the first spawning, while the second batch undergoes final maturation thereafter and is released during the second spawning (Newman, 1967). This allows for a reduced time between spawning events. Investment in gonad growth uses metabolic energy that could have been available for somatic growth. It is hypothesised therefore that preparation for spawning will reduce growth as gametes are being recruited for gonadal growth. This has implications for farm management if periods of reduced growth are the result of gonadal growth.

A combination of diet and temperature influenced growth and development of juvenile *Haliotis tuberculata*. When cultured at 15 ºC, there was no difference in growth between dietary treatments (fishmeal-based diet, a commercial abalone feed and fresh seaweed), however growth was better in abalone fed the artificial diet at 18 and 22 ºC (Lopez & Tyler, 2006). When cultured at 22 ºC, *H. tuberculata* invested 22 % less energy in growth, but instead the abalone invested nearly 2.5 times as much energy in reproduction than individuals cultured at 18 ºC (Lopez & Tyler, 2006).

Abalone allocate metabolic energy to either somatic or gonadal growth. No published research has identified seasonal variation in the proportional allocation of metabolic energy to either total or gonadal growth. To address this topic, this study describes the changes in monthly growth of that size class of abalone that, according to a preliminary analysis of farm data, appeared to be the most affected (45 – 65 g abalone^{-1}), of farmed South African abalone *H. midae*. In addition, growth and gonad size were correlated with water temperature and photoperiod.

The objectives of this study were to:

1) quantify growth of 45 – 65 g abalone^{-1} over 12 months;
2) quantify gonad size of farmed *H. midae* in four size classes, i.e., < 45, 45 – 65, 65-85 and > 85 g abalone^{-1} over 12 months;
3) determine if growth correlated with gonad size of 45 – 65 g abalone^{-1}; and
4) determine whether *H. midae* growth or gonad size is correlated with water temperature or photoperiod.

Four research hypotheses were proposed at the start of the study:

H_{o1}: There is no difference in the growth of abalone between months.

H_{a1}: Abalone growth differs between at least two months.

H_{o2}: There is no difference in gonad size between months in farmed *H. midae.*

H_{a2}: There is a difference in gonad size between at least two months in farmed *H. midae.*

H_{o3}: In farmed *H. midae*, growth is not correlated with gonad size.

H_{a3}: In farmed *H. midae*, growth is correlated with gonad size.

H_{o4}: Abalone growth and gonad size of farmed *H. midae* is not correlated with water temperature.

H_{a4}: Abalone growth and gonad size of farmed *H. midae* is correlated with water temperature.

2.2 Materials and Methods

Study site

This study was conducted on two farms situated adjacent to each other in Hermanus, Western Cape, South Africa: HIK Abalone Farm (Pty) Ltd (Farm 1) and Aquafarm Development (Pty) Ltd (Farm 2) (34º26'04.35"S; 19º13'12.51"E).

Experimental system

Farm 1

Farm 1 used canvas tanks of length 3.9 m, width 0.85 m and depth 0.65 m, with a volume of 2.16 m^3 (Yearsley *et al.,* 2009). The tanks were supported by wooden frames and shielded from the sun by shade cloth (Figure 2.1a). Each tank had six oyster mesh baskets (Yearsley *et al*., 2009). Each basket contained seven vertical acrylonitrile butadiene styrene plastic plates with a total surface area of 3.2 m^2, and a horizontal feeder plate (Figure 2.1b) submerged just below the water surface to provide shading from the sun and to hold the artificial feed (Green, 2009).

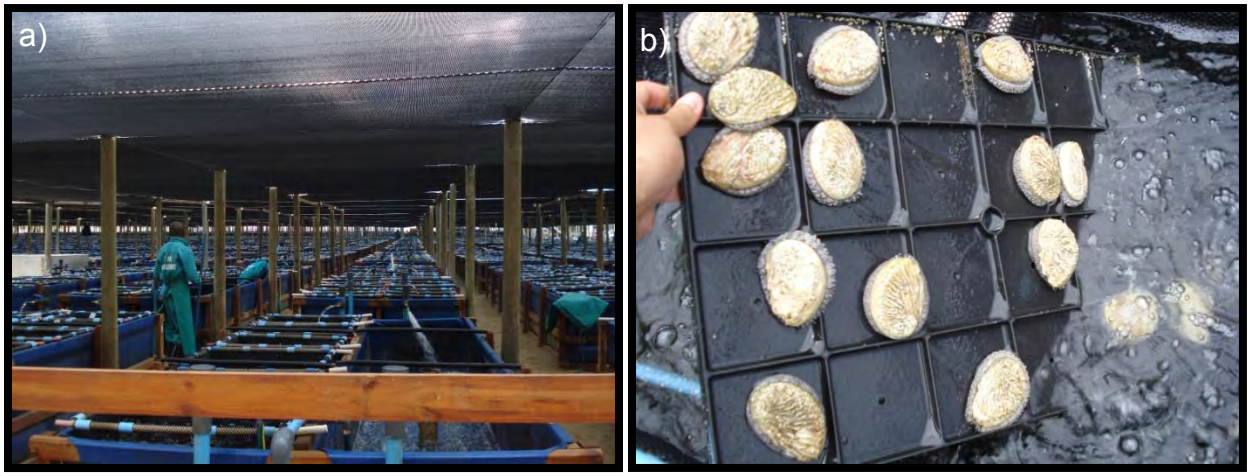

Figure 2.1: a) Shade cloth-covered canvas tank system supported by wooden frames and a water inlet on one end and an outlet on the opposite end of the tank. Each tank contained six baskets. Each basket had a feeder plate (b) onto which feed was placed. The feeder plate was briefly lifted out of the water in the image for the sake of demonstration, but while abalone were in the baskets, the feeder plate remained secured just below the water surface.

Ambient temperature sea water (35 g L^{-1}) was pumped into a header tank. The inflowing water was treated in a micro-screen drum filter (85 µm), after which it flowed by gravity through the farm at a flow rate to achieve 1.5 exchanges of the tank volume per hour before being discharged into the ocean.

Aeration was provided by blowers via 20 mm polyvinylchloride (PVC) tubing raised 50 mm off the tank bottom and running horizontally along the length of the tank

(Green *et al.*, 2011a; Naylor *et al.*, 2011). Each week the baskets containing abalone were moved to clean tanks, while the dirty tanks were cleaned.

Farm 2

Farm 2 used a combination of canvas tanks (Figure 2.2) and black fiberglass tanks both with the same dimensions as those described for Farm 1 (Yearsley *et al*., 2009). Each tank contained six oyster mesh baskets with vertical racks as described for Farm 1. Farm 2 used asbestos feeder plates. Ambient temperature sea water was pumped into a header tank. The water was then filtered through a micro-screen drum filter (85 μm), after which it flowed by gravity through the farm before being discharged into the ocean.

Aeration was provided as described for Farm 1. The same cleaning procedure and abalone handling routine was followed as will be described for Farm 1.

Figure 2.2: Blue canvas tanks used on Aquafarm Development (Pty) Ltd. Each tank contained six baskets. Water flowed by gravity from a raised header tank and entered the tank at one end and left through an outlet pipe at the opposite end.

Experimental animals and feed

Farm 1

All animals were spawned from stock kept on the farm, and thus no acclimation to the system was required. Animals of age 32 months and an average size of 49 g abalone^{-1}, based on farm records, were used. The abalone were fed a formulated diet (Abfeed® S34, Marifeed (Pty) Ltd, Hermanus, South Africa; 34.7 % protein, 2.4 % lipid,

57.3 % carbohydrate, 1.6 % fibre, 5.6 % dry mass) once daily (Yearsley *et al*., 2009). Feeding was done once daily by placing pellets (average pellet mass of 33.7 ± 0.2 g cup^{-1} from a sample of 30 cups) from a feeding cup onto the feeder plate. Baskets were stocked at a biomass of 8.1 kg $basket^{-1}$ according to the industry standard stocking density for this size class of abalone (Naylor *et al.*, 2011).

Farm 2

All animals were from stock spawned and kept on the farm. Abalone of 32 months and of the same size class as described for Farm 1 were used. These abalone were fed the same diet at the same feeding regime as described for Farm 1. The abalone used in this experiment were stocked at the same density as described for Farm 1.

Abalone growth

Each month, the same number, size class and age of abalone per farm as described in "*Experimental animals and feed*" were marked by sticking individually numbered bee-tags to the blot-dried abalone shell using waterproof adhesive (BOSTIK© blits stik gel super glue, Bostik, Cape Town, South Africa). Each month, the same number of new animals was tagged in order to measure the same individuals after one month of growth. Out of this sample of 60 animals, 10 abalone were randomly assigned to each of the six experimental baskets. Each basket was located in a

separate tank, and in a separate raceway, hereby making the basket the unit of measurement and making each basket independent of the others. Abalone were kept amongst untagged abalone subject to farm management conditions and stocking density. One month after being tagged these animals were removed from the basket and blot-dried and the whole body mass was weighed (0.01 g) on an electronic balance (Snowrex BBA-600, Snowrex International, Taipei, Taiwan), and the length of the shell was measured to a precision of 0.1 mm using Vernier calipers. This process was repeated on each farm after 30 days to determine the weight gain and length gain of each abalone over that period. This procedure was repeated every month for one year on both farms using new abalone each month. The growth rate of each abalone was calculated using Equations 1 and 2:

$$M_g = \frac{M_f - M_i}{t} 30 \quad (1)$$

where M_g is the weight gain of each abalone (g 30 days^{-1}), M_f and M_i are the final and initial weights (g abalone^{-1}) and *t* is time in days. This was done to ensure that monthly growth was calculated over a 30-day period and months were comparable to each other. The condition factor of each abalone was calculated according to Equation 2 (Britz, 1996a):

$$CF = \frac{W}{L^{2.99}} 5575 \quad (2)$$

where W is the weight (g abalone^{-1}) and L is shell length (mm).

Gonad size

Four size classes of abalone were used: < 45, 45-65, 65-85 and > 85 g abalone^{-1}. Five male and five female abalone per size class were collected. Each individual was randomly selected from a separate basket, and from a separate raceway on each farm. This ensured that animals of a range of ages and sizes, all with 'average' historical farm growth rates up until this point, ensuring no stunted individuals were used. This procedure was repeated each month for twelve months on both farms. Animals were purged for 72 h. The abalone from both farms were taken to SPP Canning (Pty) Ltd processing plant and placed into a chilling room (-10 ºC) for three hours prior to processing. Animals were individually weighed (0.01 g) and shell length was measured (0.1 mm) using Vernier calipers. The animals then had their shells removed (shucked) to obtain the soft tissue mass (M_{st}) according to the following equation:

$$M_{st} = M_t - M_s \quad (3)$$

where M_{st} is the soft tissue mass (g) of an individual abalone, M_t is the total mass of each abalone (g) and M_s is the mass of the shell (g). Shell mass was obtained by subtracting the soft tissue mass from the total mass according to the following equation:

$$M_s = M_t - M_{st} \quad (4)$$

The visceral mass (M_v) was calculated by subtracting the meat mass from the soft tissue mass according to the following equation:

$$M_v = M_{st} - M_m \quad (5)$$

where M_v is the visceral mass (g), M_{st} is the soft tissue mass (g) and M_m is the meat mass (g) of each individual abalone. The sex of each abalone was recorded.

The visceral complex of each abalone was placed into a labelled 40-ml sample bottle. The bottle was topped up with saline Davidson's fixative (20 % formalin, 10 % glycerol, 10 % acetic acid, 30 % absolute ethanol, 30 % salt water) according to methods developed by Shaw & Battle (1957). This solution was replaced with 70 % ethanol after 48 hours for post-fixation of the gonad tissue.

A cross-section was cut through the mid-point of the arc length of the conical appendage (Figure 2.3). The length of the conical appendage and length and width of the cross-sectional view of the gonad and digestive gland (DG) were measured using photographic measuring software (Sigmascan® PRO 5, Systat Software, San Jose, CA, USA). This digital method of measuring was used to determine abalone shell length (Naylor *et al.* 2011). Using these measurements, a lower bound estimate of the effective gonad volume (EGV) was made according to the following equation (Tutschulte & Connell, 1981; Wood & Buxton, 1996b):

$$EGV = \frac{\mathrm{Lca}\,\pi}{96}\left[\, 8(x+y)^2 \; - \frac{(x+y+a+b)^3}{x+y} \,\right] \qquad (6)$$

where EGV is the effective gonad volume (mm^3), L_{ca} is the curved length (mm) of the conical appendage, *a, b, x* and *y* are the length and width of the gonad and digestive gland, respectively (Figure 2.4).

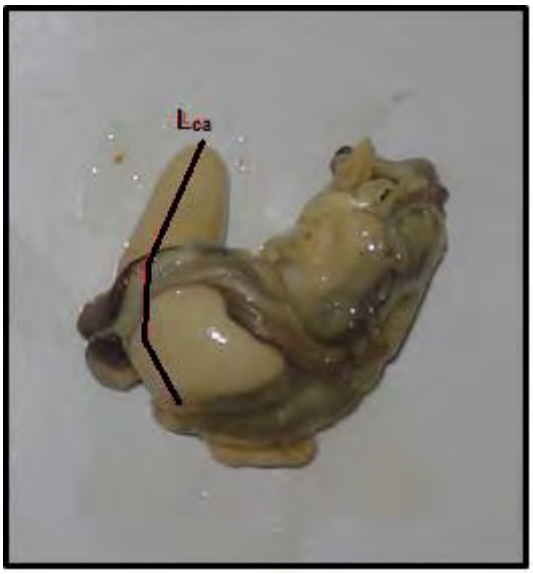

Figure 2.3: An example to show how a digital image was used to measure the curved length of the conical appendage in order to calculate the gonad bulk index, GBI.

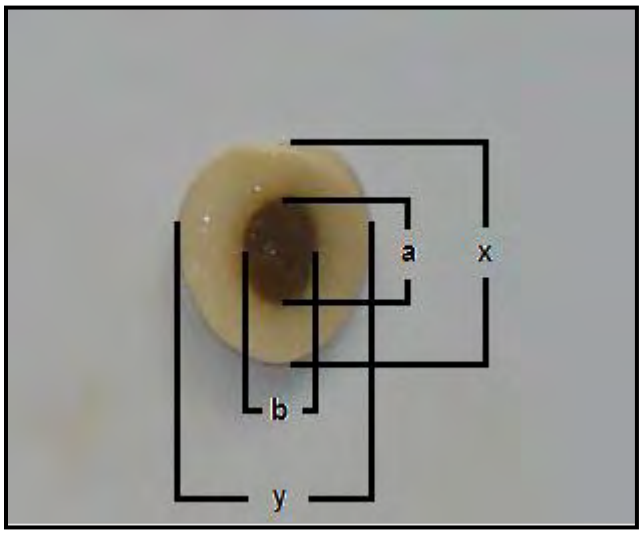

Figure 2.4: The linear dimensions of the gonad section (x and y) and the digestive gland (a and b) used for the calculation of the effective gonad volume.

The EGV was divided by the abalone's soft tissue mass (g) to present gonad size as a proportion of the abalone's shucked weight. The gonad bulk index ($mm^3\ g^{-1}$ soft tissue) was calculated using Equation 7 (Tutschulte & Connell, 1981; Wood & Buxton, 1996b):

$$GBI = \frac{EGV}{W_s} \quad (7)$$

where GBI is the gonad bulk index, EGV is the effective gonad volume ($mm^3\ g^{-1}$ soft tissue) and W_s is the shucked weight of the abalone (g).

Environmental variables

Water quality variables temperature (°C), pH, salinity, dissolved oxygen (DO) level ($mg\ L^{-1}$) and total ammonia nitrogen (TAN, $\mu g\ L^{-1}$) were measured in the header tank of each farm between 10:00 am and 11:00 am every Monday, Wednesday and Friday. Water temperature was recorded every three hours using submerged pendant data loggers (Hobo, Onset® Computer Corporation, MA, USA). The pH was measured twice weekly using a pH meter (YSI Model # 60 / 10 FT; Yellow Springs, OH, USA) and dissolved oxygen was measured twice weekly using an oxygen meter (YSI Model # 55 D, Yellow Springs, OH, USA). While the DO and pH measurements were being recorded, 50 ml water samples were collected in acid-washed glassware. The samples were processed according to the phenol hypochlorite method (Solórzano, 1969) to determine the concentration of total ammonia nitrogen (TAN). The colour absorbance was read using a spectrophotometer (Prim Light, Secomam, Ales, France) at 360 nm.

The TAN concentration was determined using standard curves made of known concentrations of TAN. Photoperiod data were obtained from the online weather service, *www.wunderground.com/hermanus*.

Correlation of growth and gonad size

The gonad tissue is a racemose gland surrounding the digestive gland (Bevelander, 1988). Thus a direct measure of the gonad tissue mass was not deemed accurate due to the difficulty of dissecting the visceral mass. In addition, the moisture content of the gonad tissue may vary and could be influenced by diet, hereby altering the specific density of the tissue. Here, gonad development was quantified using the established equations for gonad bulk index (Tutschulte & Connell, 1981; Wood & Buxton, 1996b), and abalone growth was quantified as whole body growth.

Statistical analysis

Data for the two farms were analysed separately. Thus, “farm” was not analysed as an independent variable as conditions between farms differed in several ways. The assumptions for the equality of variance were tested using Levene’s test (Levene, 1960) and the assumptions for the normal distribution of residuals was tested using the Shapiro-Wilk test (Shapiro & Wilk, 1965). The outcome of the tests for equality of variance and distribution of residuals determined which analysis of variance was used. For example, if the tests confirmed assumptions, analysis of variance (ANOVA; Fisher,

1928) was used, but if the assumptions were not confirmed, the non-parametric Kruskal-Wallis test was used. The dependent variables weight gain for Farm 1 and weight and length gain for Farm 2, respectively, were compared using a one-way analysis of variance (one-way ANOVA). A Kruskal-Wallis analysis of variance (Kruskal-Wallis ANOVA; Kruskal & Wallis, 1952) was used to compare treatment medians for the dependent variable length gain on Farm 1 and to test the effect of the independent variable “month” on the dependent variables weight and length gain. A factorial analysis of variance (factorial ANOVA) was used to test for interactions between the independent variables sex, size class and month on GBI. If there were no interactions between main effects at an error level of 5 %, treatment means were compared within each main effect. Tukey’s *post-hoc* test (Tukey, 1960) was used to identify where significant differences occurred between treatments. Environmental variables were pooled to obtain a monthly mean (± standard deviation). Correlations between dependent variables weight gain, length gain and GBI and the environmental variables water temperature and photoperiod were done using least squares regression analyses. Analyses were conducted using the Statistica 10® software package. All data presented in tables are means ± standard deviation, while figures show means ± 95 % confidence intervals.

2.3 Results

Abalone growth

Farm 1

There was a significant difference in mean monthly weight gain of farmed *H. midae* between months (one-way ANOVA: $F_{11, 59} = 2.4$, $p = 0.015$). Mean monthly weight gain in November 2010 (1.63 ± 1.41 g abalone^{-1}) was significantly lower than the mean monthly weight gain for April and May 2011 (5.57 ± 1.58 g abalone^{-1} and 5.17 ± 1.96 g abalone^{-1}, respectively; Figure 2.5), while all other mean monthly weight gains were not different from each other.

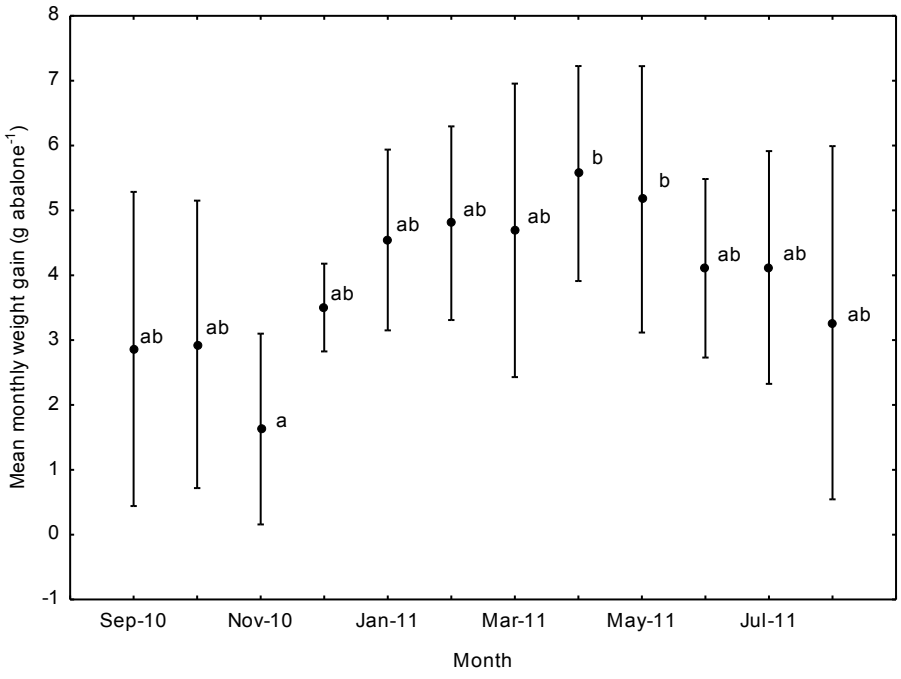

Figure 2.5: Mean monthly weight gain (± 95 % confidence interval) of tagged abalone (45 – 65 g abalone^{-1}) on Farm 1. Different letters show significantly different averages (one-way ANOVA: $F_{11, 59} = 2.4$, $p = 0.015$; Tukey's *post-hoc* test).

There was a significant difference in the mean monthly length gain of the tagged abalone (Kruskal-Wallis ANOVA on ranks: $H_{11, 71} = 44.1$, $p = 0.001$; Figure 2.6). Mean monthly length gain was lower in September 2010 and November 2010 than in January, February and March 2011. Length gain in January and February 2011 were also higher than in August 2011.

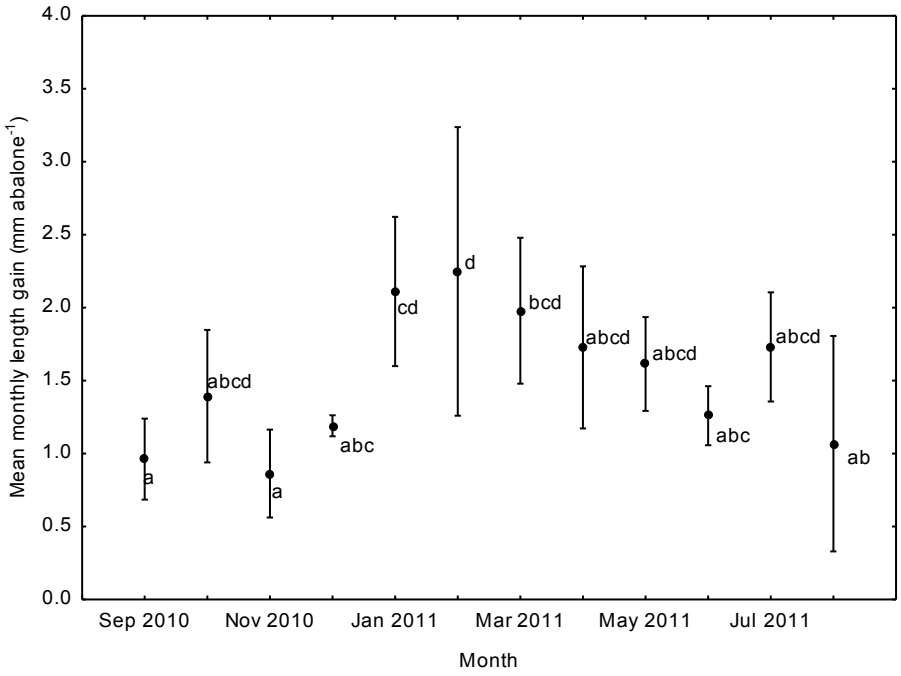

Figure 2.6: Mean (± 95 % confidence interval) monthly length gain of the tagged abalone on Farm 1. Different letters show significantly different means (Kruskal-Wallis ANOVA on ranks: $H_{11, 71} = 44.1$, $p = 0.001$; Dunn's *post-hoc* test).

Farm 2

There was no significant difference between months in the mean monthly weight gains of tagged *H. midae* (one-way ANOVA: $F_{11, 58} = 1.23$, $p = 0.291$). The average monthly weight gain of all tagged abalone was 4.33 ± 0.89 g abalone^{-1}. There was a significant difference in mean monthly length gain between months (one-way ANOVA: $F_{11, 58} = 3.6$, $p = 0.001$; Figure 2.7). *Haliotis midae* showed a slower mean monthly length gain in September 2010 (4.33 ± 0.89 mm abalone^{-1}) than in October 2010 (1.79 ± 0.32 mm abalone^{-1}), January to April 2011 (average for the period: 2.04 ± 0.98 mm

abalone^{-1}) and July 2011 (1.75 ± 0.58 mm abalone^{-1}). The other monthly values were not significantly different from each other.

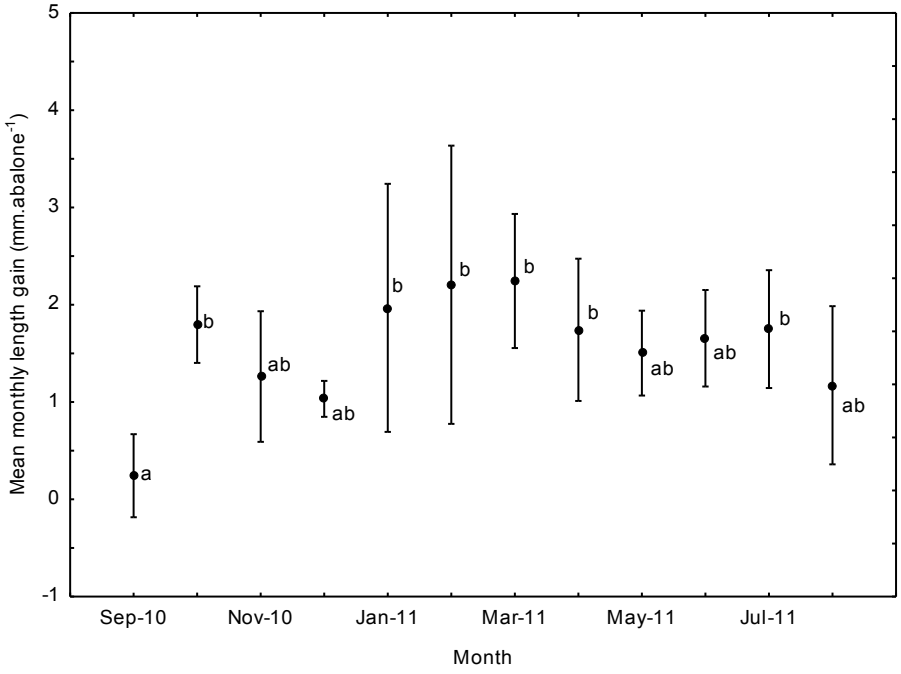

Figure 2.7: Mean monthly length gain (± 95 % confidence interval) of *H. midae* on Farm 2. Different letters show significantly different means (one-way ANOVA: $F_{11, 58} = 3.6$, $p = 0.001$; Tukey's *post-hoc* test).

Gonad size

Farm 1

There was no interaction between the main effects sex, size class and month for GBI in *H. midae* (factorial ANOVA: $F_{32, 491} = 1.3$, $p = 0.162$). There was also no

significant interaction between the factors sex and size class (factorial ANOVA: $F_{2, 491}$ = 0.4, p = 0.651) or month and sex (factorial ANOVA: $F_{10, 491}$ = 1.2, p = 0.301) for gonad size in *H. midae*. There was a significant interaction between the effects month and size class (factorial ANOVA: $F_{32, 491}$ = 1.7, p = 0.011) for gonad size. In the larger abalone (> 85 g abalone^{-1}) gonad size increased from September 2010 to November 2010. This increase was not apparent in the other size classes (Figure 2.8). There was a second increase from May 2011 to July 2011 for both size classes of abalone > 65 g abalone^{-1}, whereas there was no significant increase over this period in the smaller size classes (< 65 g abalone^{-1}).

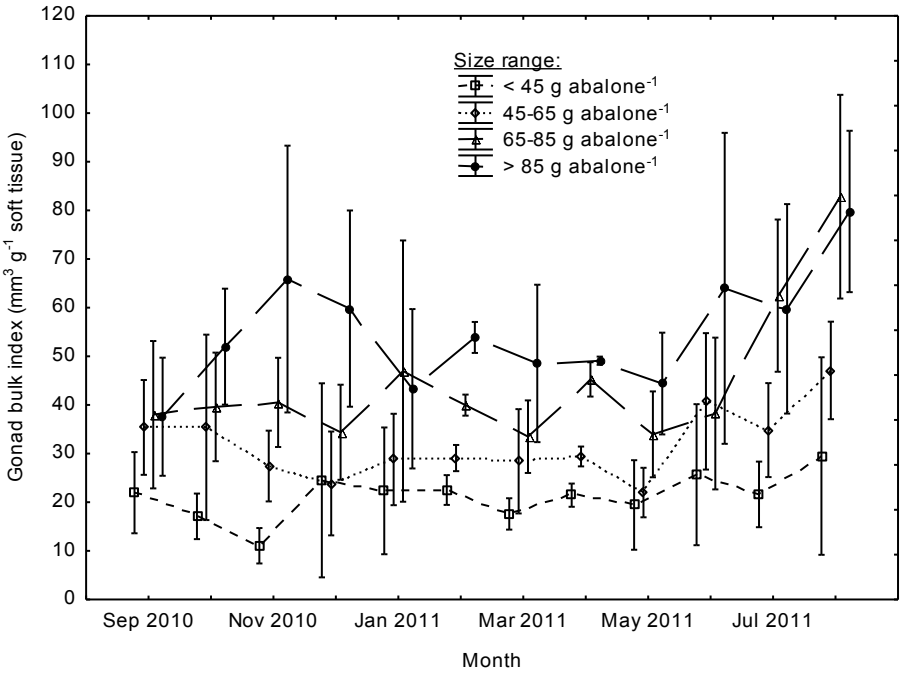

Figure 2.8: Mean monthly gonad bulk index (mm^3 g^{-1} soft tissue; ± 95 % confidence interval) for male and female *H. midae* combined in separate size classes (factorial ANOVA: $F_{32,491}$ = 1.7, p = 0.011).

Farm 2

There was no interaction between the factors sex, size class and month for *H. midae* GBI (factorial ANOVA: $F_{32, 492} = 1.1$, $p = 0.326$). There was also no interaction between sex and size class for average GBI (factorial ANOVA: $F_{2, 492} = 0.6$, $p = 0.536$). There was a significant interaction between the main effects month and sex on GBI (factorial ANOVA: $F_{10, 492} = 2.1$, $p = 0.031$). The GBI among females did not differ between September and November 2010, while that of males increased between September and October 2010 and dropped by November 2010 (Figure 2.9). There was also a significant interaction between main effects month and size class on gonad size (factorial ANOVA: $F_{32, 492} = 1.8$, $p = 0.004$). The GBI of the larger size classes (65 – 85 and > 85 g abalone^{-1}) increased between September and October 2010, whereas that of the smaller size classes did not (Figure 2.10).

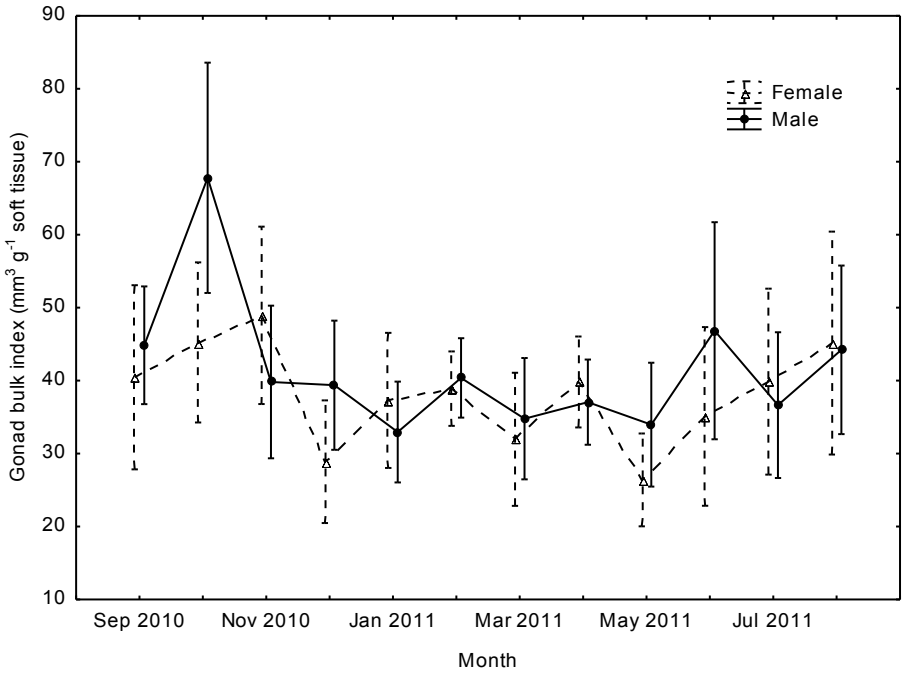

Figure 2.9: Mean monthly gonad bulk index (mm^3 g^{-1} soft tissue; ± 95 % confidence interval) for male and female *H. midae* of all size classes (factorial ANOVA: $F_{10, 492}$ = 2.1, p = 0.031).

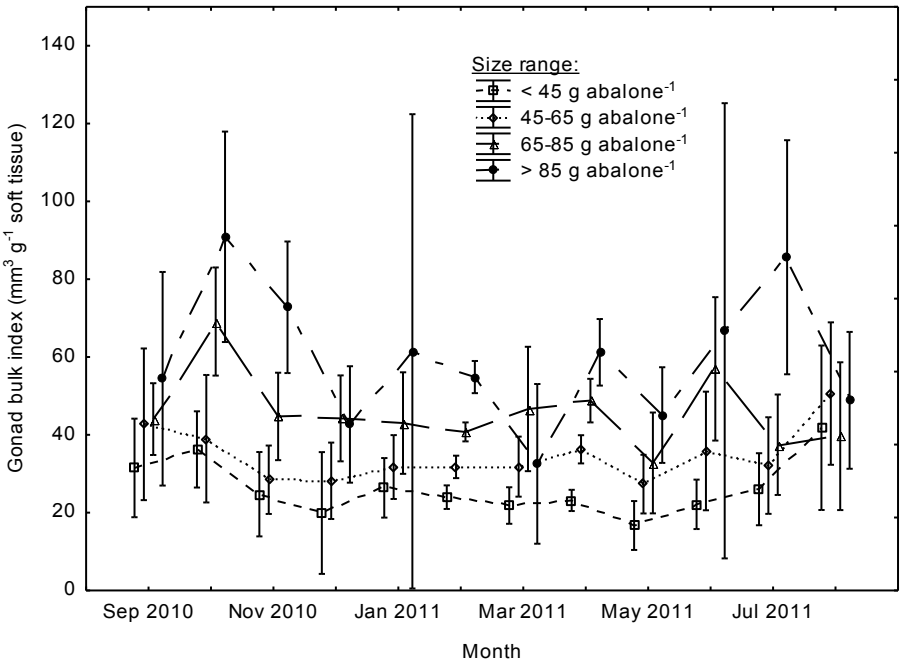

Figure 2.10: Mean monthly gonad bulk index (mm^3 g^{-1} soft tissue; ± 95 % confidence interval) for each size class of *H. midae* (factorial ANOVA: $F_{32, 492} = 1.8$, $p = 0.004$).

Environmental variables

The trial was conducted at ambient water temperatures under natural light conditions and photoperiod (Table 2.1). Water temperature ranged from 10.6 – 19.1 ºC and 10.5 – 18.7 ºC on Farm 1 and Farm 2, respectively. Day length ranged from 9.88 – 14.43 hours. The mean monthly pH was similar for both farms (7.84 ± 0.28). Total ammonia nitrogen values were low, averaging 8.3 – 126.3 µg L^{-1} and 6.3 – 98.3 µg L^{-1} on Farm 1 and Farm 2, respectively. Mean monthly dissolved oxygen content was 7.35 ± 0.63 mg L^{-1} O_2 on Farm 1 and 7.39 ± 0.71 mg L^{-1} O_2 on Farm 2.

Table 2.1: Mean (± standard deviation), minimum and maximum values for environmental variables pH, dissolved oxygen (DO), total ammonia nitrogen (TAN), water temperature and day length on each of the two farms. Months in brackets indicate the month during which the minimum or maximum value was recorded.

	Minimum (month)	Mean	Maximum (month)
FARM 1			
pH	7.18 (Jul 2011)	7.84 ± 0.28	9.86 (Sep 2010)
DO (mg L^{-1})	6.35 (Apr 2011)	7.35 ± 0.63	9.29 (Aug 2011)
TAN (µg L^{-1})	8.30 (May 2011)	47.2 ± 23.24	126.3 (Jul 2011)
Water temperature (°C)	10.60 (Feb 2011)	14.50 ± 1.70	19.10 (Nov 2010)
Day length (h)	9.88 (Jun 2011)	12.10 ± 0.22	14.43 (Dec 2010)
FARM 2			
pH	7.34 (Jul 2011)	7.84 ± 0.28	9.56 (Sep 2010)
DO (mg L^{-1})	5.77 (Feb 2011)	7.39 ± 0.71	9.44 (Aug 2011)
TAN (µg L^{-1})	6.30 (Jun 2011)	45.1 ± 17.68	98.3 (Aug 2011)
Water temperature (°C)	10.50 (Feb 2011)	14.30 ± 1.60	18.70 (Nov 2010)
Day length (h)	9.88 (Jun 2011)	12.10 ± 0.22	14.43 (Dec 2010)

Correlation between abalone growth and gonad size

In the 45 – 65 g abalone^{-1} size class, the mean monthly GBI was not correlated with weight gain (regression analysis: $p = 0.339$ and $p = 0.558$, for Farm 1 and Farm 2, respectively) or length gain (regression analysis: $p = 0.095$ and $p = 0.231$, for Farm 1 and Farm 2, respectively) of *H. midae* on either farm.

There was no significant interaction between condition factor of the abalone and gonad size on either of the farms (Farm 1- Regression analysis: $F_{1,585} = 0.78$, $p = 0.38$; Farm 2: $F_{1,585} = 0.49$, $p = 0.48$). The size of the gonad was independent of animal condition. In addition, there was no difference in the condition factor of sexes. The average condition factor of abalone on Farm 1 was 1.09 ± 0.01 (ANOVA: $F_{1,585} = 0.03$, $p = 0.86$;), with the abalone on Farm 2 having a mean condition factor of 1.08 ± 0.01 (ANOVA: $F_{1,585} = 0.01$, $p = 0.92$), with both sexes combined.

Correlation between abalone growth or gonad size and environmental variables

Abalone growth was not correlated with water temperature or day length on either farm (Table 2.2). Gonad size was not influenced by water temperature (Table 2.3). Mean monthly day length only influenced gonad size of female abalone smaller than 45 g abalone^{-1} on Farm 1 (Figure 2.11), while it was not affected in males, or females in any other size class. The mean monthly GBI ($mm^3\ g^{-1}$ soft tissue) of female (< 45 g abalone^{-1}) *H. midae* decreased as a function of mean monthly day length (regression analysis: $F_{1,\ 10} = 7.1$; $y = 49.32 – 56.93x$, $r^2 = 0.41$, $p = 0.024$; Figure 2.11). There was no correlation between growth or gonad size and water temperature or photoperiod in any of the other size classes.

Table 2.2: The p-values for the regression analyses used to test whether weight gain or length gain were correlated with water temperature or day length. P-values indicate no significant differences between any factors ($p > 0.05$).

	Temperature (°C)	Day length (h)
FARM 1		
Weight gain (g abalone^{-1})	0.466	0.284
Length gain (mm abalone^{-1})	0.455	0.813
FARM 2		
Weight gain (g abalone^{-1})	0.351	0.711
Length gain (mm abalone^{-1})	0.181	0.945

Table 2.3: The p-values to test the hypothesis that gonad size (mm^3 g^{-1} soft tissue) was correlated with the environmental variables water temperature or photoperiod, for males and females in four size classes of *H. midae,* on two adjacent farms. The bold p-value indicates a significant correlation ($p < 0.05$).

	Size class	Temperature	Day length
FARM 1			
Male	*< 45 g*	0.265	0.879
	45 - 65 g	0.778	0.138
	65 - 86 g	0.781	0.979
	> 85 g	0.942	0.361
Female	*< 45 g*	0.466	**0.024**
	45 - 65 g	0.652	0.293
	65 - 86 g	0.925	0.088
	> 85 g	0.368	0.914
FARM 2			
Male	*< 45 g*	0.08	0.991
	45 - 65 g	0.546	0.599
	65 - 86 g	0.734	0.421
	> 85 g	0.459	0.743
Female	*< 45 g*	0.416	0.919
	45 - 65 g	0.275	0.307
	65 - 86 g	0.872	0.294
	> 85 g	0.506	0.433

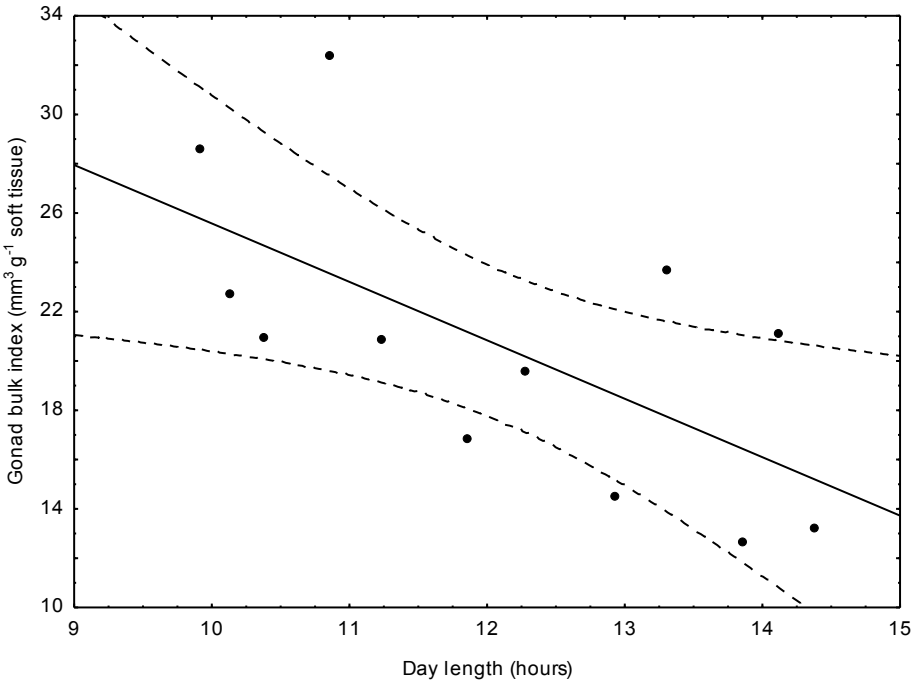

Figure 2.11: Decrease in mean monthly GBI (mm^3 g^{-1} soft tissue) of female (< 45 g $abalone^{-1}$) *H. midae* as a function of mean monthly day length (regression analysis: $F_{1, 10} = 7.1$; $y = 49.32 – 56.93x$, $r^2 = 0.41$, $p = 0.024$) on Farm 1. Dotted lines represent 95 % confidence bands.

2.4 Discussion

Abalone growth

Growth rates varied between months for cultured *H. midae*. The growth rates recorded in the present study fell within the range of published values for *H. midae* fed on artificial diets (Britz, 1996b; Knauer *et al*., 1996; Britz and Hecht, 1997; Britz *et al*., 1997; Shipton, 2000; Naylor *et al*., 2011). Stuart & Brown (1994) suggested differences

in growth rates may be attributed to the palatability, digestibility and utilization of the diet. The present trial was conducted using the same diet, fed using the same regime throughout the 12-month period. Thus, since a standard feed type and quantity were administered daily, a dietary shift was not responsible for the observed changes in growth rates. The present study differs from recent studies on cultured abalone which indicate that diet influences growth significantly (Britz, 1996b; Capinpin & Corre, 1996; Knauer *et al*., 1996; Britz and Hecht, 1997; Britz *et al*., 1997; Shipton, 2000; Naylor *et al.*, 2011). Studies suggest that, under culture conditions, the provision of a suitable diet for abalone farming is important in generating suitable growth rates, and that it is possible to manipulate growth rate by changing the diet (Mercer *et al.*, 1993; Fleming, 1995; Mai *et al.*, 1995a; Britz, 1996b; Capinpin & Corre, 1996; Britz and Hecht, 1997; Nelson *et al.*, 2002; Montáno-Vargas *et al.*, 2005 ; Naidoo *et al.*, 2006; Dlaza *et al.*, 2008; Green *et al.*, 2011a; Tung & Alfaro, 2011; Tung & Alfaro, 2012). It is possible that seasonal variation in the availability and ingestion of diatoms present in the tanks supplemented the artificial diet. Micro-algal concentration showed seasonal variation, with greater abundance being observed in winter compared to summer (Jenkins *et al.*, 2001). A mixture of diatoms in the diet improved the growth of *H. discus hannai.*

The size of abalone used in the growth component of the present study (45 – 65 g abalone^{-1}) was chosen since abalone during this trial were being observed over a two month period. Abalone were initially tagged and left for a month, then weighed and measured, left under experimental conditions for a further month and weighed and measured once again. The choice of the initial size of the animals was so that during the trial they would enter the desired size class during the trial.

The growth results for the two farms differed. This may be as a result in a number of differences in farm layout and management procedures. The tanks on Farm 1 were covered by shade cloth, while those on Farm 2 were not covered, but rather exposed to direct sunlight. The difference in light intensity in the tanks might have influenced the abundance of benthic diatoms, hereby resulting in a difference in nutrient availability and abundance between the two farms. In addition, the two farms stocking densities varied slightly, which may play a major role in influencing growth rates. This is currently under evaluation by a student at the Department of Ichthyology and Fisheries Science, Rhodes University.

Gonad size

Allocation into gonad size varied between months with abalone of different sizes allocating resources to gonad growth differently. Gonad morphology in *H. midae* is typical of that known in haliotids (Wood & Buxton, 1996b) and the lack of a difference in gonad size between male and female abalone could be attributed to the spawning behaviour of haliotids, since they are broadcast spawners (Crofts, 1929; Boolootian *et al.*, 1962; Newman, 1967). Thus, both sexes are likely to invest similarly in reproduction.

Since a standardized quantity and formulation of feed was administered to abalone gonad size was not related to a shift in feeding regime. Webber & Giese (1969) suggested that food availability was probably not responsible for directly regulating gonad growth in *Haliotis cracheroidii.* In the present study, abalone were fed at the

same diet, using the same feeding regime throughout the trial. This suggests that there was a non-nutritional factor influencing gonad growth.

There were two peaks in gonad size in the present study. Maximal gonad size occurred just prior to spawning events (Webber & Giese, 1969). Thus, it is likely that peaks in gonad size in the present study indicate spawning peaks. Abalone possess two batches of oocytes at different levels of development, with the second cohort of oocytes starting to develop only once the first cohort has been spawned or reabsorbed (Newman, 1967; Hahn, 1994; Fukazawa *et al.*, 2007). Mature wild *H. midae* had no resting stage after spawning events and gonadal recrudescence began once spawning was completed (Wood & Buxton, 1996b). This results in the female abalone holding separate batches of oocytes which are used in separate spawning events, as was observed in a study conducted on wild *H. midae* near Hermanus which spawned in autumn and spring (Newman, 1967). The same cues for spawning cause similar spawning patterns for both wild (Newman, 1967) and farmed *H. midae* which thus explains the presence of bimodal peaks in gonad index of farmed abalone.

Correlation between abalone growth and gonad size

The investment of energy and nutrients into gonad tissue growth did not compromise whole body growth as the abalone continued to gain weight throughout the reproductive periods, probably due to gonadal growth. This finding conflicts with other studies which suggest that growth rate can be reduced when abalone channel energy

into gonad development (Shepherd & Hearn, 1983; Capinpin & Corre, 1996). Gamete atresia could be converting nutrients from unused gametes to produce new gametes (Wood & Buxton, 1996), along with the lack of correlation between growth and gonad size in this study suggest that farmed *H. midae* may be allocating energy and nutrients into growth and gonads simultaneously during reproductive periods.

Correlation between abalone growth or gonad size and environmental variables

Gonad growth was not influenced by water temperature or day length. Water temperatures recorded during the study were largely within the optimal temperature range for *H. midae* (Britz *et al.*, 1997). *Haliotis cracheroidii* gonad growth was also not correlated with temperature (Webber & Giese, 1969). Only gonad size in the smallest size class of female abalone was affected by day length, with no correlation between gonad size and day length in any of the other size classes of females, or males of any size class. These smaller animals were also younger individuals, and not stunted animals. Abalone have been identified as being reproductively mature, but retained their gametes in the first season of maturity and then only spawned the following season (Shepherd & Laws, 1974). Gonad size of the smallest size class of female abalone increased at the end of winter, when day length was short. It is possible that, since the gonads of *H. midae* are typical of haliotids, these small females did not release their first batch of oocytes but rather underwent gamete atresia and built up gametes from their second batch of oocytes in preparation for a larger spawning during early autumn.

Variations in whole body weight gain and shell growth were not influenced by environmental variables. The changes in growth rate were not as a result of water temperature, day length or gonad growth, suggesting that growth is independent of water temperature, day length and reproductive seasonality under the conditions tested here. Despite mature *Haliotis diversicolor* being exposed to variation in seawater temperature, tidal and lunar phases, spawning was strongly associated with typhoon events, with gonad index and gonadosomatic index both decreasing significantly only after typhoons (Onitsuka *et al.*, 2007). Gonad growth varied between months, showing similar trends to wild *H. midae*. Water temperature had no influence on gonad size, while day length only influenced gonad size in the smallest size class of females, suggesting that gonad growth is independent of water temperature and day length.

It is possible that dietary supplementation took place as a result of the presence of diatoms on the feeder plates. Although growth was not correlated to any of the environmental parameters measured during the trial, micro-algal abundance has been correlated with maximum monthly air temperature and monthly sunshine hours (Jenkins *et al.*, 2001). *Haliotis discus hannai* showed superior growth when fed diets with diatom mixtures (Gordon *et al.*, 2006). The diatoms present on the feeder plates may have provided the abalone with an alternative or supplementation of nutrients which weren't present in the artificial diets. In addition, the abundance of these diatoms may have varied seasonally with the environmental conditions, hereby supplementing the artificial diet to varying degrees throughout the trial.

Difference between protein sources

Care should be taken in attributing changes to differences in protein source, where the influence is more likely to be as a result of the amino acid source as abalone do not have a requirement for the protein source *per se.* As this was a first of its kind study into understanding and evaluating the influences that different dietary protein sources have on growth and gonad development in *H. midae*, the term protein source was retained. It would be useful for future work to isolate differences in amino acid profiles in the respective protein sources, taking the work one step further and evaluating the influence of various amino acids on growth and gonad development in *H. midae.*

Conclusion

The conditions under which cultured *H. midae* are maintained are providing them with an optimal environment, and it appears that animals are not having to trade-off between investing nutrients into either growth or reproduction, but rather are able to invest in both since whole body growth and gonad size were not correlated. Therefore, this component of the study suggests that it would not be beneficial to manipulate water temperature or day length, within the ranges observed during the trial, in an attempt to reduce gonad growth or improve whole body growth. Since growth rates varied monthly and the food supply was constant during this component of the study, the present study suggests that there is no need for the farms to implement a seasonal dietary change in order to inhibit reproductive growth. However, the following chapters, which were

designed as a result of previous literature suggesting the important role that diet plays in influencing growth and gonad development, highlight and discuss the role that diet plays in the culture of *H. midae* on two farms in Hermanus, South Africa.

CHAPTER 3

The influence of dietary protein source on growth and gonad size

3.1 Introduction

Protein provides the essential amino acids for growth, maintenance and reproduction (Unuma *et al.*, 2003). Dietary protein source influenced growth rate of the snail *Semisulcospira gottschei*, with animals that were fed on diets containing only soybean meal as the protein source, or a combination of cottonseed meal and casein growing faster than treatments fed on diets with other protein sources, such as blood meal, fish meal, meat meal or corn gluten meal (Lee & Pham, 2010). The proximate composition of these gastropods varied between experimental diets with treatments that were fed a soybean meal diet having the highest average crude protein and lowest crude ash content when compared with the other treatments (Lee & Pham, 2010).

Abalone farms use either naturally occurring algal diets, artificial feeds, or a combination of the two. Due to their high water content, algal diets contain low concentrations of micro- and macro-nutrients such as protein and carotenoids (Simpson, 1994; de Jong-Westman *et al.*, 1995; Cook *et al.*, 2007). As a result, algal diets have produced lower growth rates and gonad sizes in sea urchins than artificial feeds containing animal products (Fernandez & Caltagirone, 1994; Cook *et al.*, 1998). Abalone fed on artificial feeds grew faster than those fed on seaweed (Lopez & Tyler, 2006). *Haliotis midae* fed on diets containing fishmeal or *Spirulina spp.* as the protein source grew faster than those fed on *Ecklonia maxima* (Britz, 1996a). Similarly, *H. midae* fed on fishmeal-based protein feeds grew better than those fed on seaweed-based protein diets (Dlaza *et al.*, 2008). This reduced performance on algal diets has been attributed to the concentrations of micro- and macronutrients, such as proteins,

carotenoids and fatty acids (Cook *et al.*, 2000; Fernandez & Boudouresque, 2000; Hammer *et al.*, 2006). *Haliotis rubra* ingested the algae based on their nitrogen and digestible nitrogen content as opposed to the energy content, and this had a direct impact on growth (Fleming, 1995).

The use of *E. maxima*, in general, produced low growth and it is labour-intensive for *H. midae* farming. On South African abalone farms the use of seaweed as a feed is absent, scarce or insufficient to sustain fast growth (Troell *et al.*, 2006; Dlaza *et al.*, 2008). As a result, artificial feeds have been developed to fulfil the nutritional requirements of *H. midae* (Britz, 1996a; Knauer *et al.*, 1996; Britz *et al.*, 1997; Dlaza *et al.*, 2008; Green *et al.*, 2011a; Green *et al.*, 2011b; Naylor *et al.*, 2011) in collaborative research between academic institutions and local feed manufacturers. Fishmeal, casein, soybean, and *Spirulina* have all been recommended as high-quality and nutritive dietary components for abalone diet formulation (Tung & Alfaro, 2012). A feed produced by Marifeed (Pty) Ltd in Hermanus, South Africa, comes close to meeting the nutritional requirements of *H. midae* (Dlaza *et al.*, 2008). Such a formulated diet was used on a commercial scale on the farms where the research for this thesis was conducted.

Increasing cost with an increasing demand and decreasing supply of fishmeal that is used in artificial feeds resulted in fish nutritionists focusing on finding alternative protein sources in artificial feeds (Lim *et al.*, 1979; Mai *et al.*, 1995a; Britz, 1996b). The use of plant-based protein sources such as soybean meal, cottonseed meal, linseed meal and sunflower meal has been at the centre of such research (Koumi *et al.*, 2011).

Soybean meal is one of the most frequently used substitute protein sources in fish feeds (Yigit *et al.*, 2010).

Soybean meal has been investigated as a replacement for fishmeal in fish feeds as it has a high crude protein content and a favourable amino acid balance (Carter & Hauler, 2000). It is cheaper than fishmeal (K Matschke, SPP Canning Ltd, pers. comm.). Research conducted on the potential for the use of artificial feeds for *H. midae* suggested that animal feed science models are applicable to Haliotid nutrition (Britz, 1995). Technology developed for the culture of other marine species has since been used as a basis for the development of abalone feeds.

The protein to energy ratio, amino acid composition, lipid profile, ash content and mineral content of soybean meal differs from that of fishmeal (Koumi *et al.*, 2011). Depending on the recipient species, these differences in nutrient composition can influence growth, feed utilization and fish carcass composition (Tomás *et al.*, 2005). No research has evaluated the influence of protein source on gonadal development in *H. midae.*

Reproductive impacts of grazing on animals has been recorded since the 1940's, with hormone-like substances causing reproductive difficulties in sheep (Bennetts *et al.*, 1946). Phytochemicals such as those found in soybean meal or kelp can mimic oestrogens both structurally and functionally (Dixon, 2004). Phytoestrogens are present in many plants and vegetables and can be categorized into three classes: isoflavones, lignans and coumestans (Cederroth & Nef, 2009). Isoflavones are found in the soybean plant, and are the most important source of phytoestrogens for humans, cattle and

rodents (Cederroth & Nef, 2009). These isoflavones are non-steroidal in structure but have a phenol ring which facilitates binding to oestrogen receptors and allows the phytoestrogen to act as an oestrogen antagonist (Makela *et al.*, 1994; Makela *et al.*, 1995). No research has evaluated the possible influence of these compounds in haliotids.

Since soybean meal is present in the artificial feeds administered to abalone on certain commercial farms, it is suggested that presence of soybean meal in the feed may influence gonadal development, while reproduction in abalone that were fed on soybean meal-free diets would not be affected in the same way.

Phytoestrogens were recognized as bioactive compounds in the 1940s when formononetin was isolated in red clover (*Trifolium pratense*) and was identified as the cause for infertility syndrome in sheep, which were grazing on this clover (Bennetts *et al.*, 1946). Similarly, desert annual plants showed stunted growth during dry years, when they had high levels of phytoestrogens. These high levels lead to impaired reproduction in the California quail (*Lophortyx californicus*). In wet years, when drought had not stunted the growth and elevated the phytoestrogen levels in the herb, quails bred normally (Leopold *et al.*, 1976).

Isoflavonous compounds in soybean meal had numerous oestrogenic effects in fish. Genistein has induced vitellogenin production in the liver and promoted oocyte growth in a manner similar to that of oestradiol-17β in the yellow perch, *Perca flavescens* (Malison *et al.*, 1985; Malison *et al.*, 1988). No literature to date has provided evidence that isoflavonous compounds present in soybean meal influence gonadal

development in haliotids, and the present study aimed to gain some understanding on the topic. This study aims to lay a foundation for further research in this field.

Haliotis midae fed a fishmeal-based diet grew faster and had a better feed conversion ratio than those fed diets with soya oil cake, torula yeast, casein or *E. maxima* as the protein source (Britz, 1996a). Thus, since protein source has been documented to influence growth, reproduction and proximate composition of abalone (Mai *et al.*, 1995b; Britz, 1996a, 1996b; Coote *et al.*, 2000; Dlaza *et al.*, 2008; Green, 2009; Green *et al.*, 2011b), the aim of this study was to determine whether the source of dietary protein influenced farmed abalone growth through effects on gonad development. The specific objectives of this study were to determine growth, feed conversion ratio, cookout yields, gonad size and gonad tissue proximate composition of abalone fed *E. maxima* or artificial diets containing fishmeal, soya meal or a combination of fishmeal and soya meal.

The following hypotheses were proposed:

H_{o1}: Dietary protein source does not influence growth of farmed *H. midae*.

H_{a1}: Growth of *H. midae* differs between at least two dietary protein sources.

H_{o2}: Dietary protein source does not influence gonad size of farmed *H. midae*.

H_{a2}: Gonad size differs between *H. midae* fed on diets with at least two different dietary protein sources.

3.2 Materials and methods

Experimental system

This experiment was conducted on farm 1 as described in Chapter 2, Experimental system, Farm 1. The same tanks and basket system were used. This trial was run concurrently with the trial described in Chapter 4. These two trials comprised of three tanks with 36 baskets, 12 baskets per tank. Treatments were randomly allocated to baskets, to ensure that basket position (proximity to inlet / outlet) was not a confounding factor (Figure 3.1). There was a single basket position within tank 3 which was not required for the experiments, and as such was rather stocked with a standard farm basket to ensure that total stocking density within the tank was standardized.

Tank 1

Inlet

Kelp	Fishmeal
Ch 4	Ch 4/ combo
Soya	Ch 4
Fishmeal	Ch 4
Ch 4	Kelp
Ch 4/ combo	Ch 4

Outlet

Tank 2

Inlet

Soya	Kelp
Ch 4	Ch 4/ combo
Ch 4	Ch 4
Fishmeal	Soya
Kelp	Ch 4
Ch 4	Ch 4

Outlet

Tank 3

Inlet

Ch 4	Kelp
Ch 4/ combo	*farm feed
Soya	Ch 4
Fishmeal	Ch 4/ combo
Ch 4	Soya
Ch 4	Fishmeal

Outlet

Figure 3.1: Placement of treatment baskets within each of the three tanks. The diets used in the present trial are described below in the *Experimental animals and feed*

section. The trials for Chapter 3 and Chapter 4 (Ch4) were run concurrently.

Experimental animals

A total biomass of 180 kg of 33 month-old abalone from a single batch with an average starting weight of 48.67 ± 3.88 g abalone^{-1} were stocked into 20 oyster mesh baskets at the farm-assigned stocking density for this size class of 8.90 ± 0.27 kg basket^{-1}. Thus, abalone were maintained under farm conditions.

Experimental diets and feeding regime

The trial comprised of four dietary treatments, i.e., ocean harvested kelp, and three treatments of formulated feeds, each with five replicate baskets. For the purpose of a comparison between treatments, the values for kelp mass are expressed as dry mass. The artificial diets were formulated based on proprietary formulations (Abfeed® - S34, Marifeed Pty Ltd; Table 3.1). They were manufactured under industrial conditions (Abfeed® - S34, Marifeed Pty Ltd). The artificial feed formulations were designed so that all treatments were isonitrogenous (Protein = 34 % dry mass) and with a protein to energy ratio of 2.13 g MJ^{-1}. One formulated diet had a combination of soya and fishmeal as the protein source (combo), as used by the industry. Another formulated diet contained soya as the sole protein source (soya meal), while the third dietary treatment contained only fishmeal as the source of protein (fishmeal). A proximate analysis was

conducted on the formulated feeds, while the values for *E. maxima* were taken from Francis *et al.* (2008) (table 3.1).

Table 3.1: Diet formulations for the three pelleted diets that were fed to the abalone during the trial, and the proximate analysis of each diet, where P : E is the protein to energy ratio.

Dietary treatment:	**Kelp**	**Soya**	**Combo**	**Fishmeal**
Protein source				
Kelp (%)	100	-	-	-
Fishmeal (%)	-	-	68	100
Soya (%)	-	100	32	-
Formulation				
Protein (%)	9.05	33.20	33.20	33.20
Lipid (%)	1.12	1.18	3.03	4.16
Energy (MJ kg^{-1})	-	15.59	15.59	15.59
P : E (g MJ^{-1})	-	2.13	2.13	2.13

Data collection

Abalone growth

The abalone were purged for 48 hours prior to handling. They were anaesthetized with a 10 % magnesium sulphate solution (Green *et al*., 2011a). A randomly selected sample of 35 animals per basket was weighed to obtain an average

abalone weight for the basket. The total shell length of each animal was measured to the nearest 0.01 mm using Vernier calipers.

A container with feed was assigned to each basket so that the mass of pellets administered to each basket during the trial could be recorded. Fresh kelp was harvested locally and stored submerged in canvas tanks with aeration and flowing seawater. Kelp was then collected from the kelp-holding tank to feed the experimental animals. The wet weight of the kelp to be placed into each basket was recorded before feeding. Uneaten kelp was removed from the kelp treatment baskets before adding fresh kelp, at which point the wet weight of the uneaten kelp was recorded. This was done twice weekly, with some variation depending on whether sea conditions allowed for the safe harvest of kelp. Feed conversion ratio was calculated using Equation 8 (Britz *et al*., 1997):

$$FCR = \frac{M_f}{WG} \tag{8}$$

where *FCR* is the feed conversion ratio, M_f is the dried matter (DM, 0 % moisture) of the feed fed and *WG* is the total abalone biomass gained per basket.

Abalone in all baskets were size-graded after 147 days, according to standard farm procedure. Grading involved reducing the biomass of baskets to the farm standard stocking density for a larger size class (9.86 ± 0.44 kg basket^{-1}). The trial ended after 262 days when a sample of 35 randomly selected animals was weighed to obtain an average animal weight (0.01 g). The total length of these 35 abalone was measured with a precision of 0.01 mm.

Gonad size

Three male and three female abalone from each basket were randomly selected and placed into marked purging bags for 72 hours. These animals were taken to the same factory and processed as described in Chapter 2, *Gonad size*. The gonad tissue was preserved in Davidson's fixative (Chapter 2, *Gonad size*). The conical appendage was photographed and measured according to the methods described above (Chapter 2, *Gonad size)* in order to obtain an effective gonad volume (EFV) estimate and a gonad bulk index (GBI) value for each abalone.

Cookout yield

At the end of the trial, five male and five female abalone per diet were randomly selected and taken to SPP Canning (Pty) Ltd. The whole mass of each individual was measured (0.01 g) on an electronic balance (Snowrex BBA-600, Snowrex International, Taipei, Taiwan). These animals were shucked to obtain their soft tissue. The meat was separated out of the soft tissue and a hole was punched through each foot muscle. A numbered tag was attached to each piece of muscle. This meat was processed and cooked according to factory procedure. After cooking, each abalone's foot muscle was weighed. The percentage cookout yield was calculated according to Equation 9 (adapted from Chiou *et al.,* 2004):

$$CY = 100 \times \frac{M_{cm}}{M_t} \quad (9)$$

where *CY* is the percentage mass of whole abalone that is converted into cooked meat, M_{cm} is the wet mass of the meat after cooking and M_t is the blot-dried body mass of the live abalone. All units are in grams.

Gonad tissue proximate composition

While meat was taken from those abalone that had been used to determine cookout yield, the gonad tissue was kept for further analysis. The gonad tissue from three male and three female abalone per basket was placed individually into labelled containers and frozen in labelled containers. These were then sent for proximate analysis at the Department of Animal Science and Poultry Science, University of Kwa-Zulu Natal, Pietermaritzburg, South Africa.

Statistical analysis

Each basket containing abalone was considered an experimental unit. Thus, to avoid the error committed by pseudo-replication, the average values of the dependent variables of all abalone per experimental unit were used for the analyses. The assumption of equality of variance was tested using Levene's test (Levene 1960) and the assumptions for the normal distribution of residuals was tested using the Shapiro-Wilk test (Shapiro & Wilk 1965). The effect of the independent variable diet on the dependent variable average abalone size was analysed using a repeated measures analysis of variance (repeated measures ANOVA) as each experimental unit was

measured at the start of the trial (t_0), after 147 days (t_1) and after 262 days (t_2). Since the gonads of abalone were only collected at the end of the trial, the presence of interactions between the independent variables sex and diet on GBI was analysed using a factorial analysis of variance (factorial ANOVA). In addition, a factorial ANOVA was also conducted to test for the interaction between sex and diet on cookout yield. An α-error level of 5 % was used for all analyses. Presence of a significant interaction between the main effects time and diet on the dependent variable abalone size was tested using a factorial repeated measures ANOVA. The effect of treatment on FCR was tested using a one-way analysis of variance (One-way ANOVA). Tukey's *post-hoc* test was used to identify where significant differences occurred between treatments. Analyses were conducted using the Statistica 10® software package. All data presented in tables are means ± standard deviation, while figures show means ± 95 % confidence intervals.

3.3 Results

Abalone growth

There was no significant difference in the average abalone mass between treatments at the start of the trial (one-way ANOVA: $F_{3, 15} = 0.2$, $p = 0.876$). There was a significant interaction between main effects diet and time on average animal mass (g abalone^{-1}) with average mass increasing over time depending on the dietary treatment (Figure 3.2). The average mass at the start of the trial was 48.67 ± 3.88 g abalone^{-1},

with an increase to 64.33 ± 5.83 g abalone^{-1} after 147 days and a final mean mass of 82.17 ± 7.86 g abalone^{-1} at the end of the trial. Kelp-fed abalone grew slower than those from the other treatments (factorial repeated measures ANOVA: $F_{8,\ 12}$ = 14.24, p = 0.001; Figure 3.2).

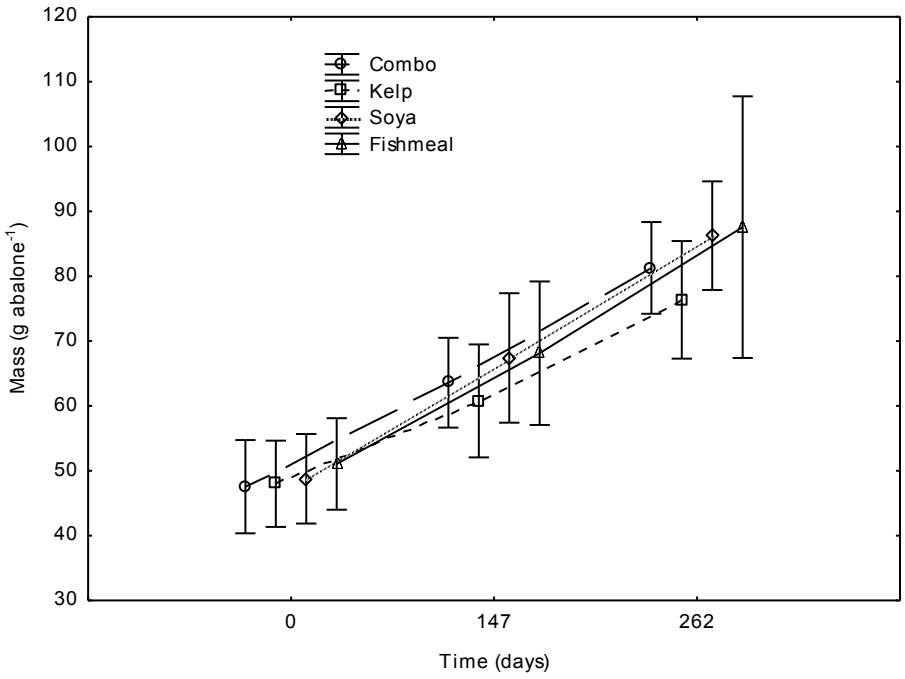

Figure 3.2: Mean abalone mass (± 95 % confidence interval) of abalone fed four diets for 262 days (factorial repeated measures ANOVA: $F_{8,12}$ = 14.24, p = 0.001). All measurements were conducted on the same day, but for the sake of graphic representation, mean and error bars were offset.

There was a significant difference in FCR between treatments after 262 days (one-way ANOVA: $F_{3,\ 16}$ = 12.9, p = 0.001). Kelp-fed animals had an FCR (2.85 ± 0.66,

based on dry matter values) which was not different from those fed the soya only diet, but was significantly higher than in the abalone fed the two diets containing fishmeal. Animals fed the combo diet had an FCR which was not different from those fed the fishmeal diet, but significantly lower than for animals fed kelp or the soya diet (Figure 3.3).

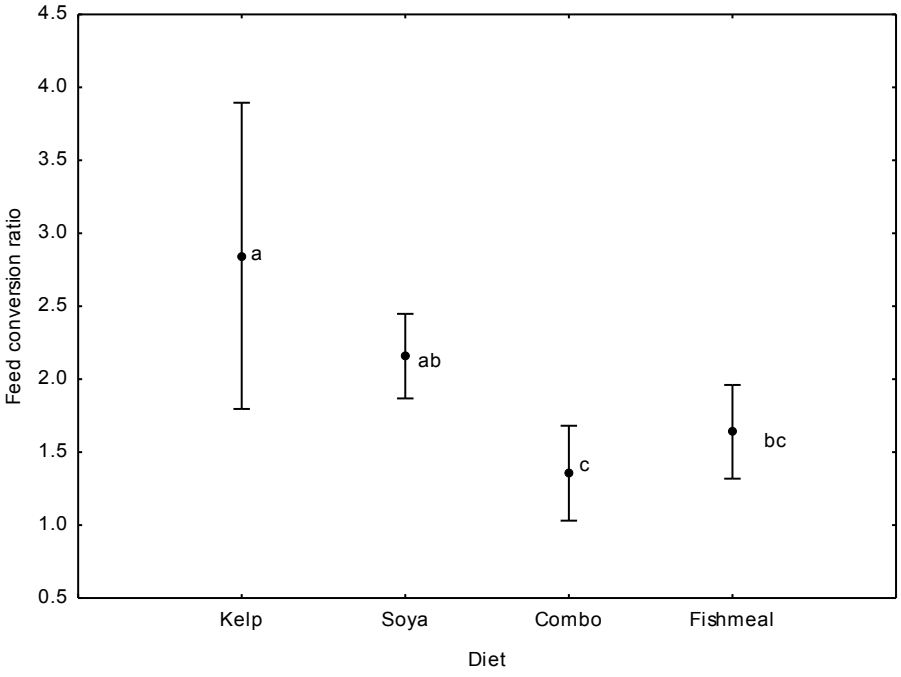

Figure 3.3: Mean feed conversion ratio (± 95 % confidence interval) for *H. midae* fed *E. maxima* (dry mass) and three artificial diets (one-way ANOVA: $F_{3, 16} = 13.6$, $p = 0.001$; Tukey's *post-hoc* test). Different letters indicate significant differences between treatments ($p < 0.05$).

Gonad size

There was no significant interaction between the main effects sex and diet on GBI in farmed *H. midae* fed the four experimental diets (factorial ANOVA: $F_{3, 32} = 1.7$, $p = 0.179$). There was no significant effect of sex on GBI. Gonad size was the largest when soya was present in the diet (69.91 ± 24.34 and 79.91 ± 22.28 mm^3 g^{-1} for the soya and combo diets, respectively), whereas the kelp-fed animals had the lowest GBI (19.15 ± 9.01 mm^3 g^{-1}; ANOVA: $F_{3, 36} = 19.7$, $p = 0.001$; Figure 3.4).

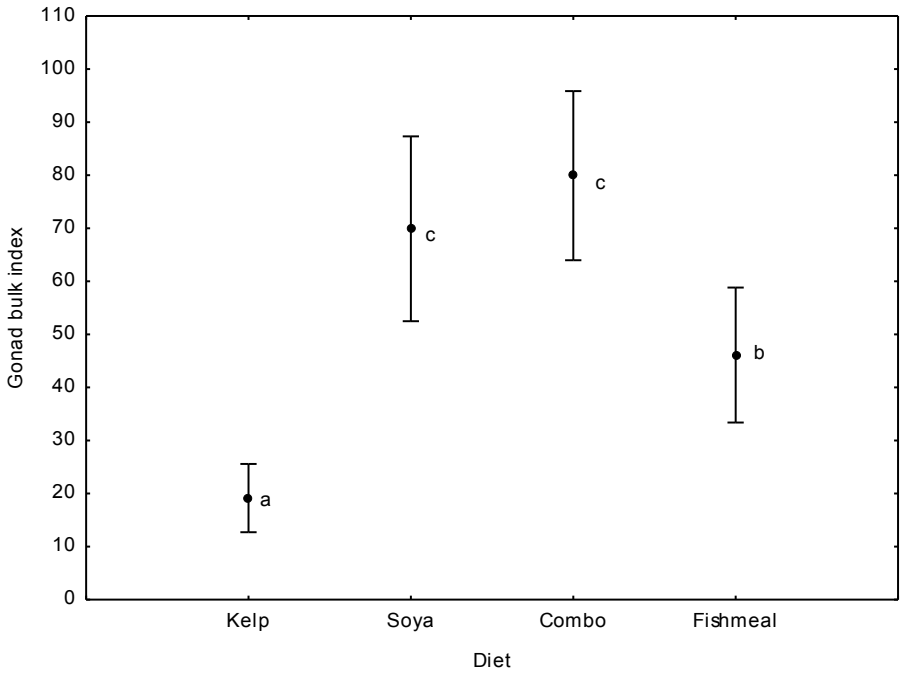

Figure 3.4: Mean gonad bulk index (± 95 % confidence interval) for *H. midae* fed four experimental diets for 262 days. Different letters indicate significant differences between treatments (ANOVA: $F_{3, 36} = 19.7$, $p = 0.001$; Tukey's *post-hoc* test).

Cookout yield

There was no significant interaction between the main effects sex and diet on the average percentage cookout yield (factorial ANOVA: $F_{3, 32} = 0.8$, $p = 0.523$; Table 3.3). There was no significant effect of sex on cookout yield ($F_{1, 34} = 0.1$, $p = 0.977$). There was a significant difference in meat yield between dietary treatments ($F_{3, 32} = 20.9$, $p < 0.001$; Figure 3.5), with the animals that were fed the artificial diets having higher cookout yields (23.62 ± 2.59, 27.09 ± 1.96 and 25.81 ± 1.81 % body mass for the soya, combo and fishmeal diets, respectively) than those fed kelp (19.73 ± 1.91 % body mass).

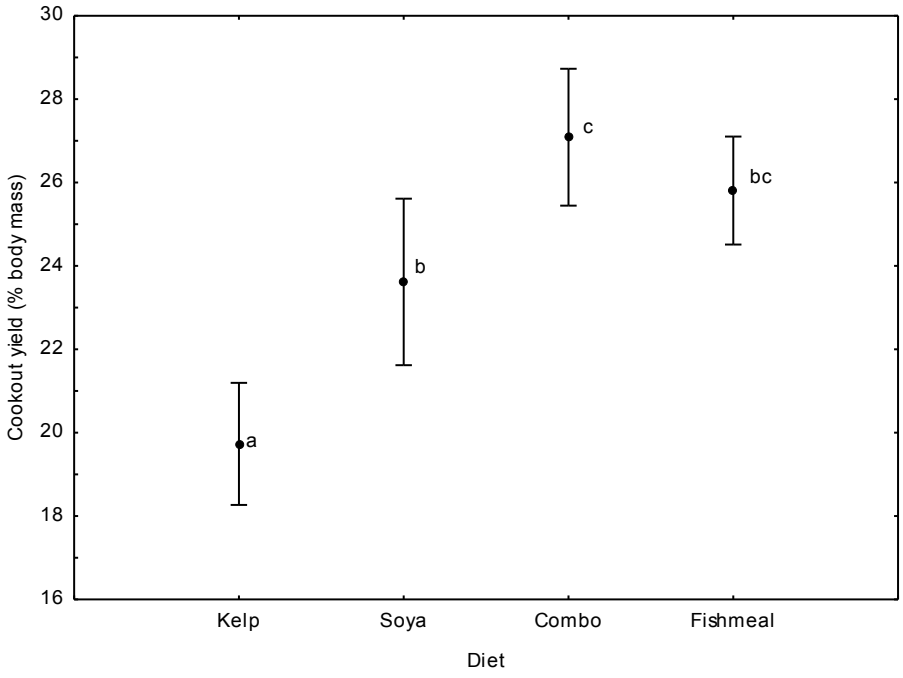

Figure 3.5: Mean cookout yield (± 95 % confidence interval) for *H. midae* fed four experimental diets for 262 days. Different letters indicate significant differences between treatment means ($F_{3, 32} = 20.9$, $p < 0.001$; Tukey's *post-hoc* test).

Gonad tissue proximate composition

There was a significant interaction between the main effects sex and diet on the gonad tissue crude protein content of farmed *H. midae* (factorial ANOVA: $F_{3, 32} = 21.4$, $p = 0.001$; Figure 3.6). Male abalone response to diet differed from that of females, in that the differences in gonad tissue crude protein content became more pronounced as both soya and fishmeal were included in the diet. As soya was included in the diet, males invested more into protein growth, while females invested less. Male abalone fed the soya diet had the highest gonad tissue crude protein content (75.94 ± 3.07 % dry mass).

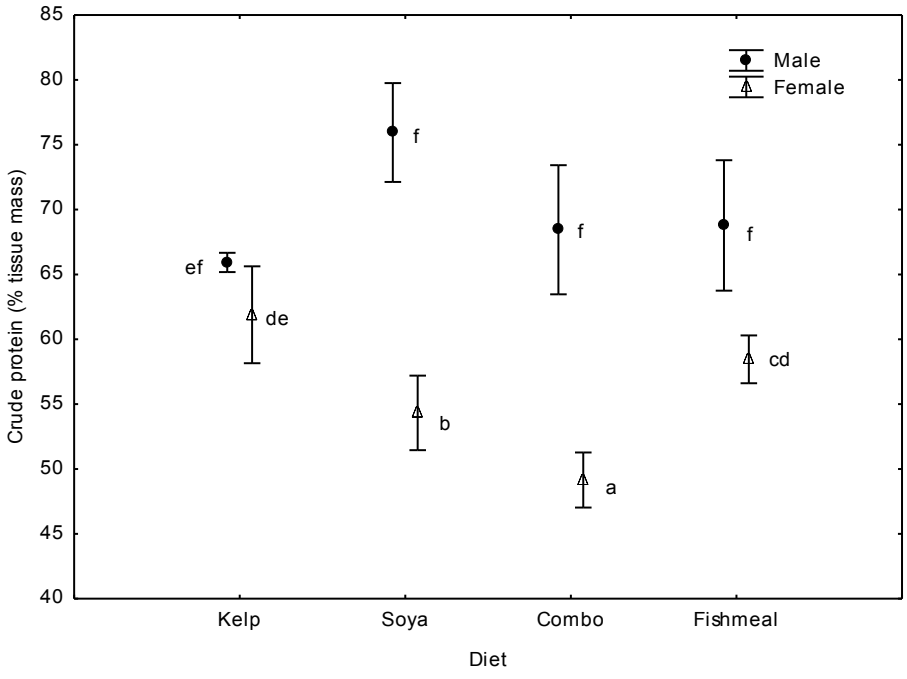

Figure 3.6: Mean gonad tissue crude protein content (± 95 % confidence interval) for male and female *H. midae* fed four experimental diets for 262 days (factorial ANOVA: $F_{3, 32} = 21.4$, $p = 0.001$; Tukey's *post-hoc* test). Different letters indicate significant differences between means ($p < 0.05$).

There was a significant interaction between the main effects sex and diet on the gonad tissue moisture content of farmed *H. midae* (factorial ANOVA: $F_{3, 32} = 6.3$, $p = 0.002$; Figure 3.7), as female response to dietary protein source differed from that of males. Female abalone fed kelp had a gonad tissue moisture content (77.64 ± 0.56 % tissue mass) which did not differ from any of the male treatments (overall mean for males: 77.63 ± 0.39 % tissue mass). In each of the dietary treatments containing fishmeal, males had higher gonad tissue moisture contents (77.71 ± 0.21 and 77.07 ± 0.39 % tissue mass, for the fishmeal and combo diets, respectively) than the females (75.09 ± 1.69 and 72.72 ± 0.36 % food mass for the fishmeal and combo diets, respectively).

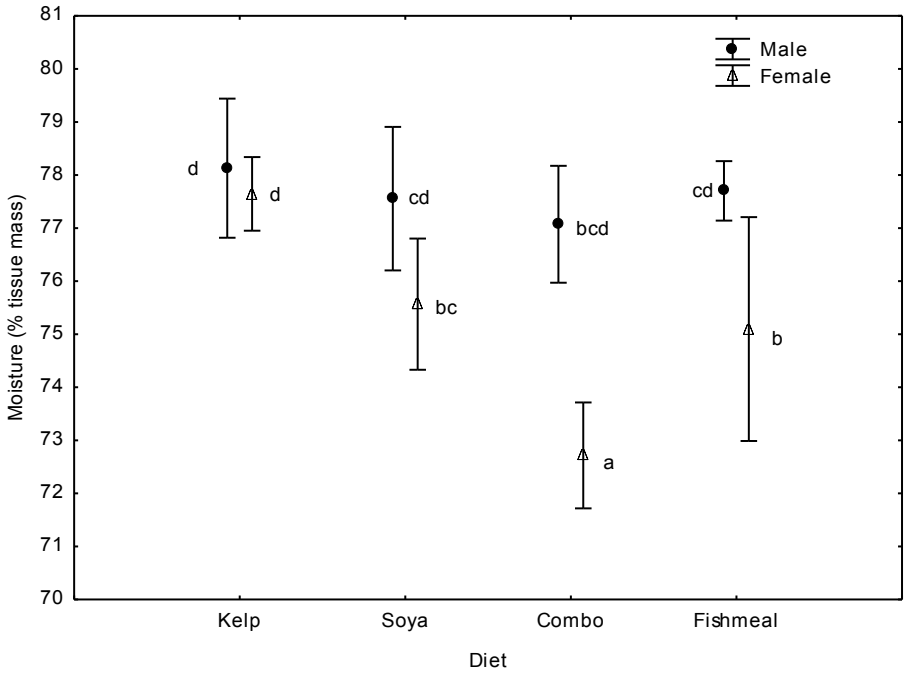

Figure 3.7: Mean gonad tissue moisture content (± 95 % confidence interval) for male and female *H. midae* fed four experimental diets for 262 days (factorial ANOVA: $F_{3, 32}$ = 6.3, p = 0.002; Tukey's *post-hoc* test). Different letters indicate significant differences between means ($p < 0.05$).

There was a significant interaction between main effects sex and diet on the gonad tissue crude lipid content of farmed *H. midae* (factorial ANOVA: $F_{3, 32}$ = 32.5, p = 0.001; Figure 3.8). The gonad tissue lipid content of females was higher than that of male abalone. In the females, abalone fed kelp had significantly lower gonad tissue crude lipid content (11.81 ± 0.81 % dry mass) than those fed the artificial diets, with the abalone fed the artificial diets that contained fishmeal having higher gonad tissue lipid content (21.56 ± 0.71 and 24.51 ± 0.49 % dry mass for the fishmeal and combo diets, respectively) than those fed the soya diet (18.51 ± 0.95 % dry mass). The same pattern was observed for the males with abalone that were fed on diets containing fishmeal

having a higher gonad tissue lipid content than those fed the soya diet. Lipid content did not differ between males fed kelp and the soya diet, or between males fed on kelp and fishmeal.

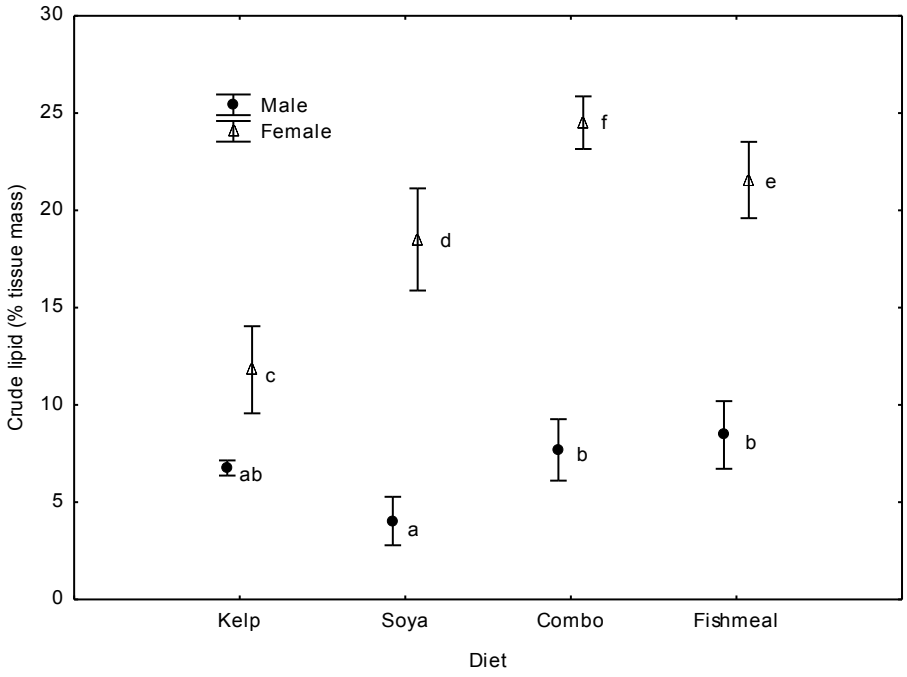

Figure 3.8: Mean gonad tissue crude lipid content (± 95 % confidence interval) for male and female *H. midae* fed four experimental diets for 262 days (factorial ANOVA: $F_{3, 32}$ = 32.5, p = 0.001; Tukey's *post-hoc* test). Different letters indicate significant differences between means ($p < 0.05$).

3.4 Discussion

Abalone growth

Abalone fed on the artificial diets grew differently from those fed on kelp. This finding is supported by previous work conducted on *H. midae,* in which abalone fed artificial feeds grew better than those fed kelp (Francis *et al.*, 2008; Hatting, 2006). Prior research conducted on a farm adjacent to the farm on which the present study was conducted showed that abalone size was lower when abalone were fed kelp compared to those fed artificial diets, during a 14 month trial (Hattingh, 2006). This has been attributed to a lack of nutrients in the natural diet when compared with artificial diets, with the relatively high nutritional content of formulated feeds probably causing enhanced growth (Dlaza *et al.*, 2008). In addition, abalone have the capacity to synthesize carbohydrates, a large component of artificial diets proteins (Durazo-Beltrán *et al.*, 2003). It is possible that the abalone fed on kelp grew differently from those fed on the artificial diets due to the lower dietary protein level present in kelp when compared with the artificial diets. In addition, it is possible that the total lipid and fatty acid composition of the diets would play an important role in influencing growth rates. Were this study to be reproduced, it may be worthwhile to determine the proximate analysis of the macro-algae at regular intervals throughout the trial period since the nutritional status and chemical composition of kelp varies seasonally.

FCR differed between treatments in which artificial feed was fed, but was the same for abalone fed on kelp and those fed the soya only diet. This suggests that kelp and soya are utilized equally as effectively as each other, but less effectively than

animal proteins. Animal-based proteins are more readily digestible than plant-based proteins (Durazo-Beltrán *et al.*, 2003). Fishmeal diets generate more muscle tissue, and abalone fed fishmeal diets have been shown to gain the most protein when compared with other diets, suggesting the high digestibility of fishmeal by abalone (Tung & Alfaro, 2012). *Haliotis midae* possess endogenous gut bacteria that secrete enzymes into the lumen of the intestine in order to facilitate the degradation of polysaccharides (Erasmus *et al.*, 1997). These bacteria have likely evolved within the abalone digestive system to assist in the efficient use of nutrients present in *E. maxima,* the natural diet of *H. midae* by using cellulase, an enzyme rarely found in animals (Erasmus *et al.*, 1997). These bacteria may assist *H. midae* in breaking down natural plant-based diets (Erasmus *et al.*, 1997). Since the metabolic activity of gut micro-flora does not provide a source of essential fatty acids in *H. fulgens*, the higher digestibility of animal proteins renders animal proteins a more efficient protein source (Durazo-Beltrán *et al.*, 2003). In the present study the abalone fed on diets containing only plant-based protein sources had an FCR which was 82 % higher, i.e., less efficient, than for abalone fed on the combo diet, which contained both fishmeal and soya meal. This suggests that *H. midae* is more efficient at utilizing fishmeal as a protein source for growth than the plant proteins.

Gonad size

The reduced reproductive growth of abalone fed kelp might be due to a grazing inhibitory response of kelp. Vascular plants produce secondary metabolites which may have a negative impact on grazer performance (Toth *et al.,* 2005). For example,

gastropods fed on previously grazed seaweed tissues showed a significantly lower percentage of viable eggs (Toth *et al.*, 2005). This suggests that kelp may display induced resistance to grazing, inhibiting reproductive growth; but this remains to be tested in future work.

Diet quality, i.e. protein level, lipid content, and fatty acid composition, may influence *H. midae* growth rates. Gonad size was the smallest after 262 days for abalone fed on kelp, when compared with those fed artificial diets. Provision of high-quality diets for juvenile *H. asinina* promoted gonad maturation, while low protein and low fat algal diets failed to promote maturation (Capinpin & Corre, 1996). In a trial conducted on the urchin *Strongylocentrotus purpuratus,* the final gonad weight and the gonad indices were lower in sea urchins fed kelp than in those fed an artificial diet (Azad *et al.,* 2011). Similarly, *H. tuberculata* fed a natural diet expended less energy on reproductive tissue than abalone fed formulated diets (Tyler, 2006).

Within the artificial diets, soya had a significant influence on abalone gonad size. Abalone fed on either of the diets containing soya meal had larger gonads than those fed on the diet which lacked soya meal, i.e. only fishmeal. Only a single study on the effects of soya on abalone gonad development has been conducted to date, in which abalone fed on soybean concentrate had higher gonad indices than those fed on fishmeal (Tung & Alfaro, 2012). It was suggested that the heavy gonads and high GSI may have been as a result of the high amounts of polyunsaturated fatty acids provided by the soybean diet (Tung & Alfaro, 2012). Though these results suggest that abalone brood stock development should focus on the use of high quantities of

soybean, there is no evidence of the influence of soya on gamete quality and this should be examined in further research. In addition, examining gonad microscopy would provide useful information. Evaluation of the influence of diet on gonad development at the microscopic level is currently being conducted by a student at the Department of Ichthyology and Fisheries Science, Rhodes University.

A second proposed reason for the effects of soya on abalone gonads is the presence of phytoestrogens. Soybean meal has been used widely as a protein source for farmed fish, and this plant-derived protein source has been shown to induce oestrogenic activity in fish (El-Sayed *et al.*, 2012). It was proposed that the major phytoestrogens present in soybeans, daidzein and genistein, may either act as oestrogens or have inhibitory effects (Monteiro *et al.*, 2000; Bennetau-Pelissero *et al.*, 2001; Green & Kelly, 2009). The larger gonad of soybean-fed abalone in the present study may be a result of oestrogenic activity caused by soybean meal, but this has yet to be confirmed and should be evaluated in future work. If so, this may have implications for (a) improved dietary formulations for abalone grow-out and (b) brood stock diet development for *H. midae*.

Although the diet formulations were standardized for protein content and energy level, the lipid quantities were not the same. The quantity and profile of fatty acids may have an influence on gonad development (Soudant *et al.*, 1996b). This is a factor which should not be overlooked, and future work should seek to evaluate the influence of fatty acid quantity and profile on gonad development and maturation in farmed haliotids.

Cookout yield

Diet influenced the proportion of meat yielded from abalone after the canning process. Abalone fed the formulated diets yielded on average 29 % more meat after processing when compared with those fed kelp. This is similar to previous work conducted on *H. midae* in which abalone fed on artificial diets yielded up to 18 % more cooked product than those fed on kelp (Hattingh, 2006; Jones & Britz, 2006). While Hattingh (2006) went further to show that there was no difference in canning yield between artificial diets tested, the present study also showed no difference in canning yield between artificial diets with the single protein sources, soya or fishmeal.

The abalone fed the combo diet with two protein sources yielded the greatest quantity of meat after processing. A greater meat yield was achieved when kelp additive was included in the artificial diet fed to *H. midae* when compared to those fed the artificial diet with no kelp additive (Winkler, 2010). It appears as though supplying abalone with an array of protein sources increases the variety of amino acids available for assimilation into muscle tissue. It is possible that *H. midae* utilize the amino acids present in the fishmeal for muscle formation and growth since the amino acid profiles of soya and brown fishmeal differ (Masumoto *et al.*, 1996). In order to increase the percentage of meat yielded after canning, it is advisable to include fishmeal in the diet since fishmeal diets generate more muscle tissue (Tung & Alfaro, 2012). The present study suggests that feed costs can still be reduced by replacing at least 30 % of the fishmeal with soya meal, without influencing cookout yield. In addition, retaining fishmeal in the diet should retain the palatability of *H. midae*, since the accumulation of dietary lipids from diets containing fishmeal in the muscle may give the distinctive ‘fishy’

flavour (Dunstan *et al.*, 1996). It is acceptable to replace a proportion of fishmeal with an alternative protein source, like soya meal, since abalone are able to convert or synthesize amino acids in order to balance their profiles according to their anabolic requirements (Tung & Alfaro, 2012).

Gonad tissue proximate composition

The gonad tissue proximate composition of *H. midae* was influenced by both diet and sex. Protein source did not influence gonad tissue protein content in males, while in the females, protein content of the gonad tissue was lower when soya meal was present in the diet. *Haliotis iris* fed diets with different protein levels showed an increase in tissue protein with an increase in dietary protein level, but it was also suggested that the difference in metabolic rates, as a result of different amino acid compositions, could account for differences in protein deposition within the soft tissue (Tung & Alfaro, 2011). This suggests that gonad tissue protein deposition is dependent on the source, and hence the amino acid profile of dietary protein.

Protein source influenced gonad tissue moisture content. Kelp-fed abalone and those fed a formulated diet with soya only as a protein source resulted in the same gonad tissue moisture content for both male and female abalone. Fishmeal as a protein source resulted in males storing more moisture in their gonad tissue than females. Within the males, protein source did not influence gonad tissue moisture content, while the females fed the combo diet containing fishmeal and soya had the lowest gonad tissue moisture content. The gonad tissue of the sea urchin *S. purpuratus* fed kelp had

more moisture in their gonad tissue than those fed artificial diets (Azad *et al*., 2011). It has been suggested that differences in moisture content could be attributed to differences in feed conversion and protein efficiency ratios (García-esquivel *et al.,* 2007). In the present study, the FCR was the lowest for abalone fed the combo diet, containing both soya and fishmeal. Thus, the findings in the present study are similar to those of García-esquivel *et al.* (2007), suggesting that moisture content of female *H. midae* is linked to FCR, while males are unaffected by protein source.

Both sex and diet influenced gonad tissue lipid deposition. Female abalone deposited more lipids in their gonad tissue than males. Cytoplasmic transfer of molecules occurs from the hepatopancreas to the gonad, and via eggs to the larvae (Nelson *et al.*, 2002). Thus, the higher lipid level in females may be providing nutrient stores for the eggs. Within the females, abalone fed kelp deposited less lipid in the gonad tissue than those fed artificial diets. Abalone fed on artificial diets had higher foot lipid contents when compared with kelp-fed abalone (Dunstan *et al.*, 1996). Lipid deposition was the greatest when abalone were fed on the combo diet containing both fishmeal and soya. Certain fatty acids in the diet play important roles in gonadogenesis (Uki *et al.*, 1986; Nelson *et al.*, 2002). Females utilized the range of fatty acids available in the combination diet to deposit a greater quantity of lipids in the gonad tissue, while fewer lipids were deposited when only a single protein source was present in the diet.

Conclusion

Administering the natural diet, *E. maxima*, is not only labour-intensive and unsustainable, but also yielded growth, which differed from abalone fed artificial diets. Abalone had to ingest greater quantities of kelp, probably to account for the reduced protein content and low digestibility of *E. maxima.* In addition, kelp-fed abalone had the smallest gonads when compared with the gonads of abalone fed the artificial diets.

Growth was not different between any of the artificial diets, suggesting that the proportion of fishmeal present in the diet can be reduced without affecting growth. However, if farms are growing abalone for canning purposes, while the same growth rates were achieved in the present study for abalone fed on any of the artificial diets, processing yield was the greatest when only fishmeal was present in the diet. This suggests that farms, which are producing canned produce, may consider a predominantly fishmeal-based diet. An only fishmeal-based diet, according to the present study, resulted in smaller gonads and a greater canning yield when compared with the other treatments.

In summary, diet can alter body composition of abalone. Increasing the quantity of soya in a brood stock diet may increase gonad size, while a higher proportion of fishmeal may improve canning yields. If whole animals are being sold, the fishmeal protein content can be substituted with soya up to 30 %.

CHAPTER 4

The influence of dietary protein and energy level on growth and gonad size

4.1 Introduction

Dietary energy is stored when the gonads are in early stages of oogenesis and this energy storage is activated during gonad growth and development (Webber, 1970). Large gonad size as a proportion of body size is characteristic of the reproductive pattern of abalone, and large amounts of metabolic energy are required to produce these large gonads (Webber, 1970). Since gonad production is such an energetically expensive process, it is possible that altering the dietary protein and energy levels could influence gonad production in abalone. This chapter evaluates the interaction of dietary protein and energy on growth, gonad size and gonad composition in *Haliotis midae.*

A specific composition of essential fatty acids is required to meet the requirements for reproduction (Soudant *et al.,* 1996a). The importance of fatty acid composition of the diet has been identified for gametogenesis and embryogenesis in *Pecten maximus* (Soudant *et al.*, 1996b). Carbohydrates are the predominant energy source for *Haliotis corrugata* and *H. midae*, and diets should be modified so that dietary lipid levels are reduced to adequately meet the essential fatty acid requirements (Britz, 1996a; Montaño-Vargas *et al.*, 2005; Green, 2009). Most organisms are unable to synthesize these polyunsaturated fatty acids (PUFAs) *de novo* (Uki & Kikuchi, 1984; Mai *et al.*, 1995b). It is therefore important to ensure that animals are provided with an adequate quantity of lipids.

It is possible that an interaction between protein and lipids may influence growth or gonad development. A high demand was placed on lipid metabolism since maturation raised the fatty acid requirements of the sea urchins *Psammechinus miliaris* and

Paracentrotus lividus (Cook *et al*., 2007). The authors suggested that the increased demand on lipid metabolism influence protein transport and enzyme activity as protein and lipid metabolism are closely linked, and that the activity of these enzymes and the transport of proteins provide sea urchins with the essential fatty acids required for gametogenesis (Cook *et al*., 2007). The metabolism of protein is connected to aspects of lipid metabolism through the production of proteins involved with enzyme activity as well as transporter proteins responsible for long-chain PUFA synthesis (Burdge *et al.*, 2002). In addition, *Haliotis tuberculata coccinea* fed high-protein *Gracilaria cornea* showed high egg numbers, as well as increased eff and cytoplasm diameter when compared with lower protein diets (Bilbao *et al.*, 2012). Thus, it was hypothesized that dietary protein and energy would interact to influence growth, gonad size or gonad composition in *H. midae.*

Despite observing an increase in growth rate with an increase in dietary protein level, Britz (1996b) suggested that the optimal dietary protein level in formulated diets for abalone could only be identified once the optimum dietary protein to energy ratio has been identified. Green (2009) showed that, if dietary energy levels were maintained at an adequate level, it was possible to reduce the dietary protein level from 34 to 20 % without having an adverse effect on growth. This study, however, investigated abalone whole body growth as a function of diet composition. The present study aims to determine the effect of the interaction between dietary protein and energy on growth and gonad size of farmed *H. midae.*

The response of the abalone to diet depended on their size, when comparing *H. midae* of two size classes (0.2 – 1.0 and 7.0 – 14.0 g abalone^{-1}; Britz and Hecht 1997). Larger *H. midae* had a greater protein requirement than smaller abalone (Britz & Hecht 1997), while Green *et al.* (2011a) suggested that there was no evidence to indicate that body size influenced protein requirement in *H. midae*.

Weight gain correlated positively with dietary protein level between 27 and 47 % for *H. midae* and between 5 and 44 % protein for *H. discus hannai* (Uki & Watanabe, 1992; Britz, 1996b). Despite proteins, carbohydrates and lipids all being nutritionally important to growth in abalone, Nelson *et al.* (2002) suggested that the lipid class and lipid content present in a diet may be vital to abalone nutrition. This is supported by a study which showed that growth rates of *H. discus hannai* were positively correlated with the level of lipid inclusion in the diet (Uki *et al.*, 1986).

Haliotis midae fed on artificial diets were significantly influenced by the dietary protein and energy levels, within a protein to energy ratio range of 1.45 – 3.2 g protein MJ^{-1} (Britz & Hecht, 1997). Weight gain was greatest at the highest protein level (44 %), while growth was lower in abalone that were fed diets with 10 % fat than in those fed diets with 2 and 6 % fat. In addition, the proximate analysis of the abalone showed that dietary protein to energy ratio significantly influenced carcass composition (Britz & Hecht, 1997). It was concluded Britz & Hecht (1997) that a dietary lipid level of 10 % is considered too high for maximal growth in *H. midae.* Although high lipid levels appear to interfere with enzyme activity, leading to reduced growth rates, high dietary lipid levels also appeared to promote gonad maturation (Green, 2009).

Haliotis rufescens fed on diets with the protein to energy ratio ranging from 1.48 - 2.52 g protein MJ^{-1} grew best above 2.39 g protein MJ^{-1}, and a reduction in both dietary protein and lipid were suggested, as this did not lead to a reduction in growth (Gómez-Montes *et al.*, 2003). The range of protein to energy ratios in the present study overlapped with the upper range of values used by Green (2009) and included the exceeded the value above which Gómez-Montes *et al.* (2003) obtained the best growth. However, it was shown that the protein requirements of *H. midae* are dependent on the quantity of dietary energy, and that the ability of abalone to use the energy present in the lipids differed depending on the protein level of the diet (Green, 2009). The Thus, it is important to evaluate the interaction between dietary protein and energy, and the resultant impact on growth and gonad size in *H. midae* as this aspect of abalone nutrition has not been addressed.

Since growth and reproduction can be influenced by dietary protein and lipid level, the aim of this study was to test in what way dietary protein and energy influence growth and gonad size in farmed *H. midae.* The objectives of this study were to determine whether dietary protein and dietary lipid levels interacted to influence somatic weight gain, length gain, cookout yield, gonad bulk index or gonad tissue proximate composition.

From these aims, the following research hypotheses were proposed:

H_{o1}: Dietary protein and energy level do not interact to cause an effect on growth and gonad size in *H. midae*.

H_{a1}: Dietary protein and energy level interact to influence growth and gonad size in *H. midae*.

H_{o2}: Dietary protein level has no effect on growth and gonad size in *H. midae*.

H_{a2}: Dietary protein level influences growth and gonad size in *H. midae*.

H_{o3}: Dietary energy level has no effect on growth and gonad size in *H. midae*.

H_{a3}: Dietary energy level influences growth and gonad size in *H. midae*.

This study compared growth rate, feed conversion ratio, gonad size, cookout yield and gonad tissue proximate composition of abalone fed diets with different protein and energy levels, within a protein to energy range of 1.41 - 2.46 g protein MJ^{-1}.

4.2 Materials and methods

Experimental system

This experiment was conducted on farm 1 concurrently with the experiment described in Chapter 3. The experimental system for the two trials was identical and was described in Chapter 2 (*Experimental system, Farm 1*). The present trial comprised of 20 baskets.

Tank 1

Inlet

Ch 3	Ch 3
Diet 1	Diet 3/ Ch 3
Ch 3	Diet 4
Ch 3	Diet 1
Diet 4	Ch 3
Diet 3/ Ch 3	Diet 2

Outlet

Tank 2

Inlet

Ch 3	Ch 3
Diet 2	Diet 3/ Ch 3
Diet 1	Diet 4
Ch 3	Ch 3
Ch 3	Diet 1
Diet 2	Diet 4

Outlet

Tank 3

Inlet

Diet 4	Ch 3
Diet 4/ Ch 3	*farm feed
Ch 3	Diet 2
Ch 3	Diet 3/ Ch 3
Diet 2	Ch 3
Diet 1	Ch 3

Outlet

Figure 4.1: Placement of treatment baskets within each of the three tanks. The diets used in the present trial are described below in the *Experimental diets and feeding regime* section. The trials for Chapter 3 and Chapter 4 were run concurrently.

Experimental animals

The same size class (48.75 ± 3.45 g abalone^{-1}) and batch of animals, as described in Chapter 3 were used for this trial. Stocking (8.92 ± 0.26 kg basket^{-1}) was done as described above (Chapter 3, *Experimental animals*) according to farm protocol.

Experimental diets and feeding regime

Twenty baskets were randomly assigned to four treatments, with five replicates per treatment. Average values for the abalone within each basket were used for analysis, making the basket the unit of measurement. All four treatment diets were pelleted feeds based on proprietary formulations (Abfeed® - S34, Marifeed Pty Ltd). They were manufactured under industrial conditions. The diets were formulated to have two protein levels (22 and 33 % of dry mass; Table 4.1) and each of these dietary protein levels had two energy levels (15.59 and 13.50 MJ kg^{-1}). A combination of fishmeal and soya meal was used as the protein source based on proprietary formulations. The abalone were fed daily as described in Chapter 2 (*Experimental animals and feeding*).

Table 4.1: Diet formulations for the four pelleted dietary treatments that were fed to the abalone during the trial.

	Diet 1	**Diet 2**	**Diet 3**	**Diet 4**

Formulation				
Protein (% dry matter)	22.00	22.00	33.00	33.00
Energy (MJ kg^{-1})	15.60	13.50	15.60	13.50
Lipid (% dry matter)	5.00	2.00	3.00	3.00
Protein to energy ratio (g MJ^{-1})	1.41	1.63	2.13	2.46

Data collection

Abalone growth

Abalone were collected, weighed and measured according to procedures described in Chapter 3 (*Data collection, Growth*) in order to compare changes in average abalone mass throughout the trial, mass gain during the trial and feed conversion ratio (FCR) between treatments. The total biomass per basket, i.e., replicate, was recorded at the start of the trial, after 147 days and at the end of the trial (262 days).

Gonad size

Five male and five female abalone per basket were collected, purged and processed according to the methods described in Chapter 3 (*Data collection, gonad size*) in order to obtain estimates of gonad size (GBI).

Cookout yield

Abalone were collected, taken to the factory and processed as described in Chapter 3 (*Data collection, cookout yield*) to determine the percentage of meat yield of abalone body mass after processing.

Gonad tissue proximate composition

Tissue samples for this trial were collected and sent to the Feed Evaluation Unit, University of KwaZulu-Natal, Pietermaritzburg, South Africa, for proximate analysis as described in Chapter 3 (*Data collection, gonad tissue proximate composition*).

Statistical analysis

The assumptions for the equality of variance were tested using Levene's test (Levene 1960) and the assumption of normality of distribution of residuals was tested using the Shapiro-Wilk test (Shapiro & Wilk 1965). The effect of time on average abalone mass was tested using a repeated measures analysis of variance (repeated measures ANOVA). The interaction of the independent variables sex, dietary protein level and dietary energy level on the dependent variables biomass gain, FCR, GBI, cookout yield and gonad tissue protein, moisture and lipid content were tested using a factorial analysis of variance (factorial ANOVA) using the data collected at the end of the study (day 262). Tukey's *post-hoc* test was used to identify significant differences between treatments. The change in dependent variables biomass gain, GBI and

cookout yield as a function of dietary protein to energy ratios were tested using regression analysis. Analyses were conducted using the Statistica 10® software package. Data presented in tables are means ± standard deviation, while figures are presented with means ± 95 % confidence intervals.

4.3 Results

Abalone growth

There was no interaction between main effects dietary protein level and dietary energy level on the average mass gained by each individual abalone over 262 days (factorial ANOVA: $F_{1,15} = 2.7$, p= 0.119). There were significant differences in mass gain between individual abalone fed on diets with two protein concentrations (ANOVA: $F_{1, 15} = 9.9$, p= 0.007; Figure 4.2) and energy levels (ANOVA: $F_{1, 15} = 14.8$, p= 0.002; Figure 4.3). The average mass gain was higher for abalone fed on diets with the high protein level (35.84 ± 1.34 g abalone^{-1}) than those fed on the low protein diets (31.61 ± 1.39 g abalone^{-1}).

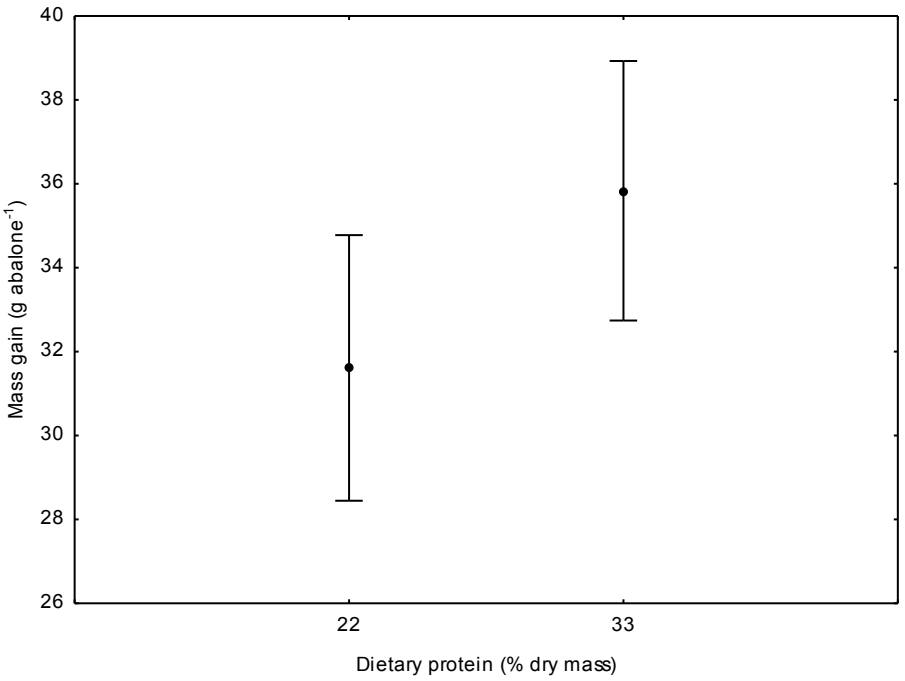

Figure 4.2: Average mass gained (± 95 % confidence intervals) by abalone fed diets with two dietary protein levels for 262 days ($F_{1, 15}$ = 9.9, p= 0.007).

The average mass gained by abalone was higher for abalone fed on the diets with the low energy level (36.38 ± 1.31 g abalone^{-1}) than those fed on the diets with the high energy level (31.12 ± 1.23 g abalone^{-1}).

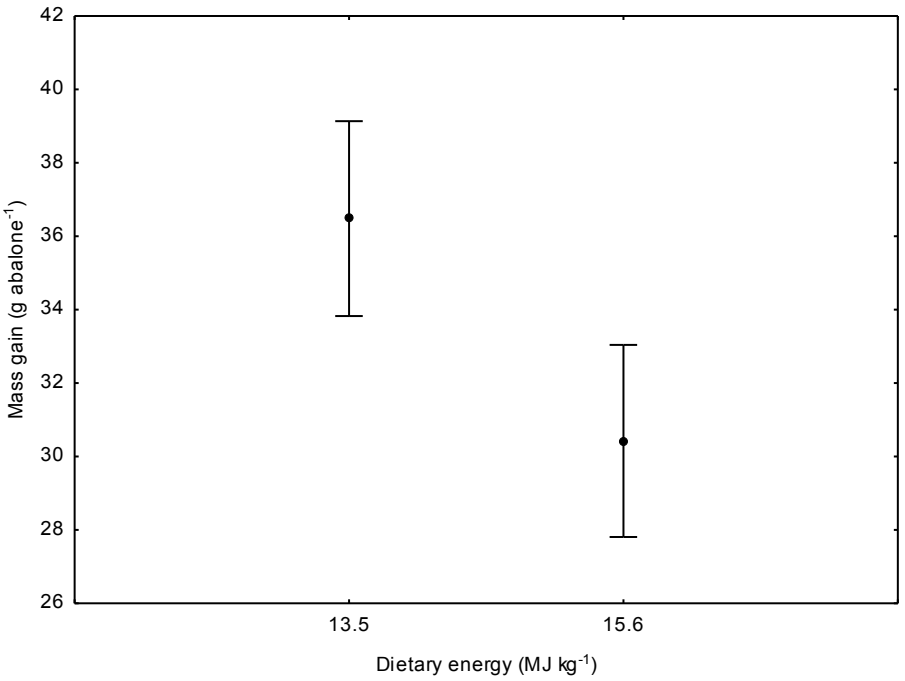

Figure 4.3: Average mass gained (± 95 % confidence intervals) by abalone fed diets with two dietary energy levels for 262 days (ANOVA: $F_{1, 15} = 14.8$, p= 0.002).

There was no significant difference between treatment means in the average starting weight of the animals (one-way ANOVA: $F_{3, 15} = 0.2$, p = 0.931), with abalone weighing on average 48.61 ± 3.48 g abalone^{-1}. There was a significant interaction between the main effects time and dietary protein level on average abalone mass (g abalone^{-1}) with abalone fed the diets with the higher protein level (33 % dry mass) growing faster than those fed the low protein (22 % dry mass) for 262 days (repeated measures ANOVA: $F_{2,30} = 4.7$, p = 0.017; Figure 4.4). Similarly, there was an interaction between the main effects time and dietary energy level on abalone mass with abalone fed the high energy diets growing faster than abalone fed the low energy diets (repeated measures ANOVA: $F_{2,30} = 8.1$, p = 0.002; Figure 4.5).

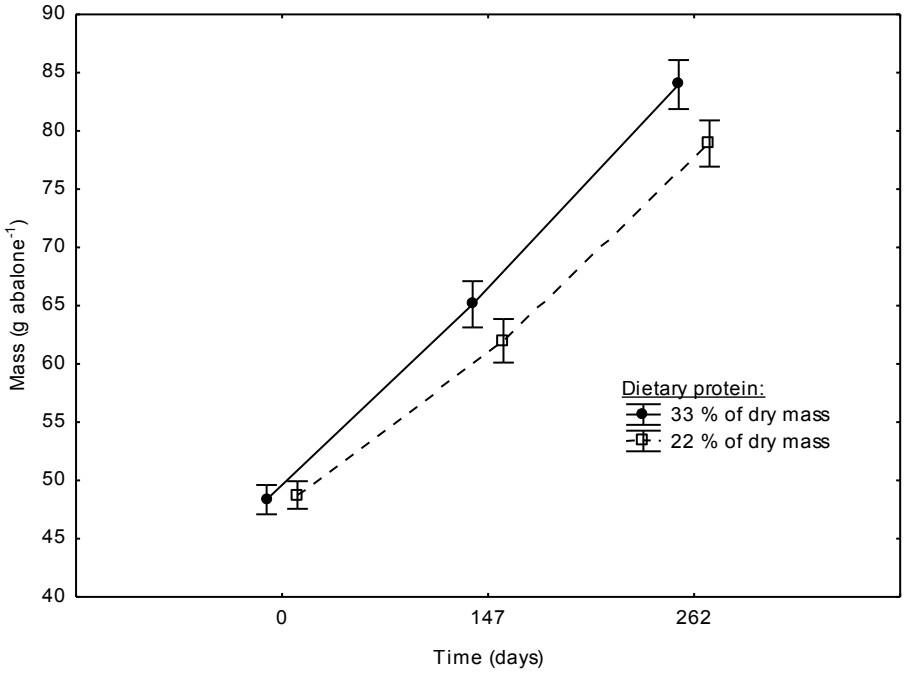

Figure 4.4: Average individual abalone mass (± 95 % confidence intervals) for abalone fed diets with two protein levels (% dry mass) for 262 days (repeated measures ANOVA: $F_{2, 30} = 4.7$, $p = 0.017$).

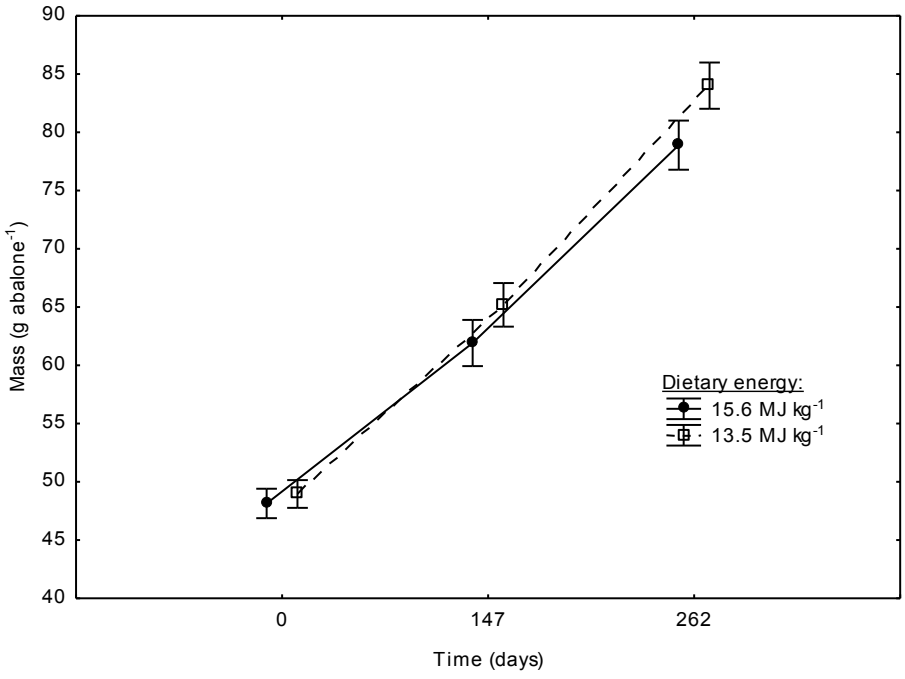

Figure 4.5: Average individual abalone mass (± 95 % confidence intervals) for abalone fed diets with two energy levels (g protein MJ^{-1}) for 262 days (repeated measures ANOVA: $F_{2, 30} = 8.1$, $p = 0.002$).

There was no interaction between the main effects dietary protein level and energy level on the average biomass of abalone gained in each basket after 262 days (factorial ANOVA: $F_{1,15} = 2.8$, $p = 0.113$). There was no significant difference in the average biomass of abalone gained between baskets of abalone fed on diets with two dietary energy levels (factorial ANOVA: $F_{1,15} = 0.1$, $p = 0.858$), with these baskets of abalone gaining a mean 5.77 ± 0.61 kg during the trial. Dietary protein level significantly affected the average abalone biomass gained ($F_{1,15} = 16.8$, $p = 0.001$; Figure 4.6), with baskets of abalone fed on the diets with the high protein level (6.47 ± 0.31 kg $basket^{-1}$)

gaining more biomass than baskets with abalone fed on the low protein diets (5.09 ± 0.15 kg basket^{-1}). No mortalities occurred during the study.

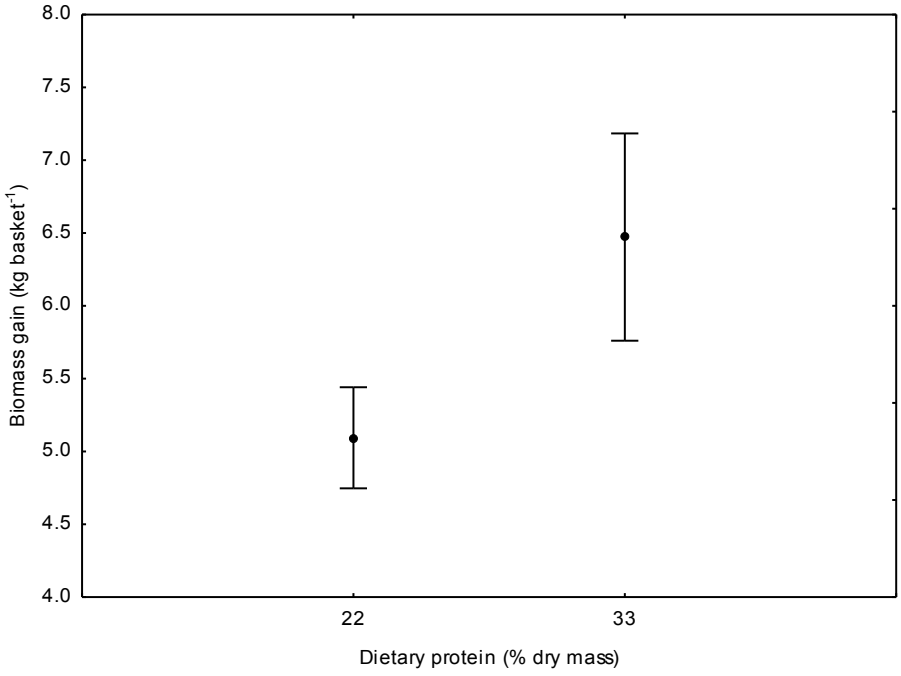

Figure 4.6: Average biomass (± 95 % confidence intervals) of abalone gained per basket after 262 days (factorial ANOVA: $F_{1, 15} = 16.8$, $p = 0.001$).

Dietary protein to energy ratio (x) had a significant influence on the average biomass of abalone gained (y) per basket ($y = 4.22\ x^{0.49}$, $r^2 = 0.48$; regression analysis: $F_{1, 17} = 15.5$, $p = 0.001$; Figure 4.7), with biomass gain increasing as a function of dietary protein to energy ratio.

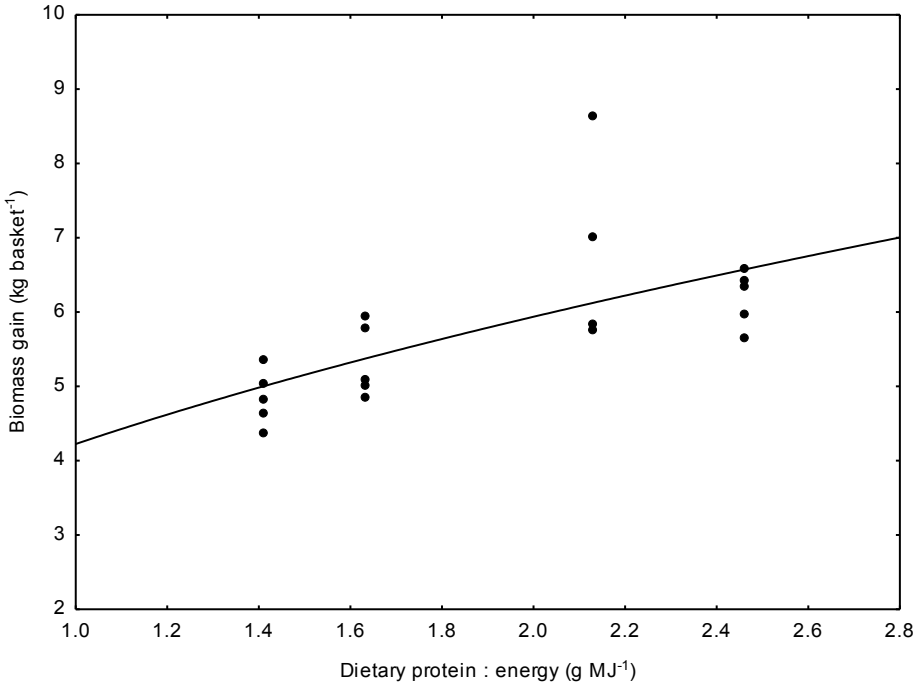

Figure 4.7: Changes in the abalone biomass gained per basket as a function of dietary protein to energy ratio ($y = 4.22\ x^{0.49}$, $r^2 = 0.48$; regression analysis: $F_{1, 17} = 15.5$, $p = 0.001$).

There was a significant interaction between the main effects dietary protein and energy level on FCR (factorial ANOVA: $F_{1, 15} = 13.8$, $p = 0.002$; Figure 4.8). There was no difference in FCR between abalone fed on the low energy level diets (average FCR: 1.73 ± 0.08). In the animals fed on the high energy diets, a higher FCR was recorded when abalone were fed the high protein diet (1.89 ± 0.08) than when fed on a low protein diet (1.56 ± 0.08).

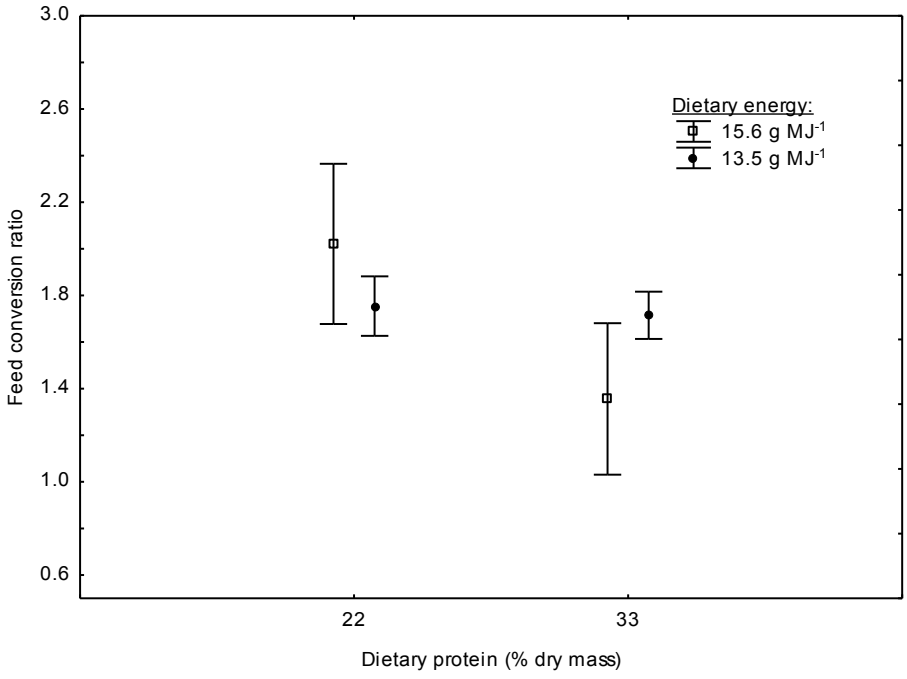

Figure 4.8: Average FCR (± 95 % confidence interval) for abalone fed diets with different protein and energy levels (factorial ANOVA: $F_{1,15} = 13.8$, $p = 0.002$).

Gonad size

There was no interaction between the main effects sex, dietary protein and energy level on GBI in farmed *H. midae* (factorial ANOVA: $F_{1, 32} = 0.4$, $p = 0.528$; Table 4.2). There was also no significant interaction between main effects sex and dietary protein level (factorial ANOVA: $F_{1, 32} = 0.5$, $p = 0.507$), sex and dietary energy level (factorial ANOVA: $F_{1, 32} = 0.7$, $p = 0.418$) or dietary protein and energy level (factorial ANOVA: $F_{1, 32} = 0.5$, $p = 0.497$) on GBI. Dietary energy level had no significant effect on GBI (one-way ANOVA: $F_{1, 32} = 1.3$, $p = 0.259$), while dietary protein level had a significant influence on GBI (one-way ANOVA: $F_{1, 32} = 7.1$, $p = 0.012$; Figure 4.9).

Abalone fed the diet with the higher protein level invested more into gonadal development (GBI: 81.92 ± 5.76) than abalone fed the diet with the lower protein level (GBI: 58.67 ± 6.16). There was no significant difference in the GBI of male and female farmed *H. midae* (one-way ANOVA: $F_{1, 32} = 0.2$, $p = 0.693$).

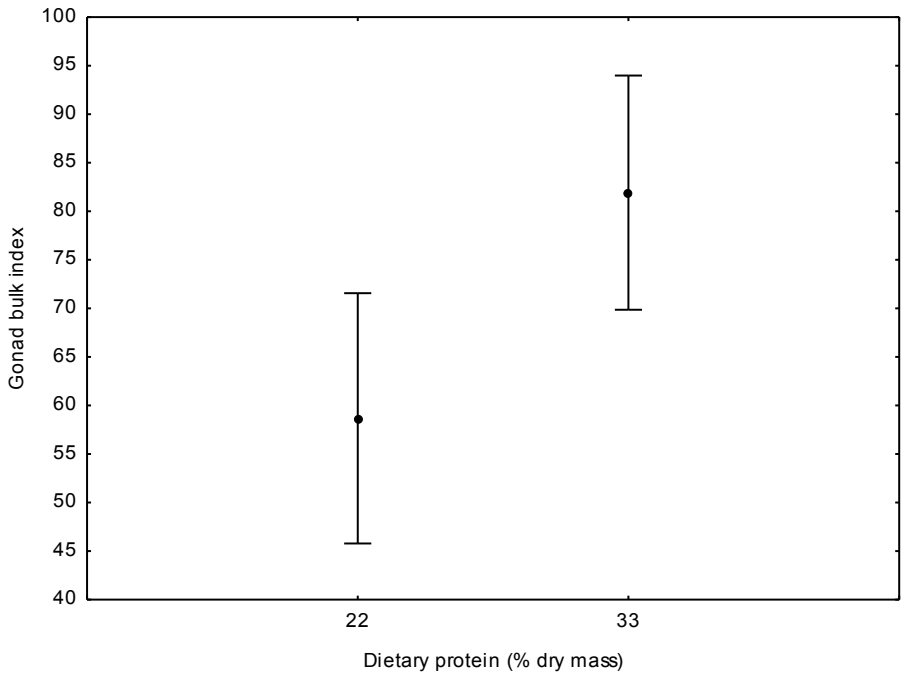

Figure 4.9: Average GBI (± 95 % confidence interval) for abalone fed diets with different dietary protein levels for 262 days (factorial ANOVA: $F_{1,32} = 7.1$, $p = 0.012$).

Table 4.2: Average male and female gonad bulk index (± standard deviation) for abalone fed diets with different protein and energy levels over 262 days.

Dietary protein (%)	**22**	**22**	**33**	**33**
Dietary energy (MJ kg^{-1})	**15.6**	**13.5**	**15.6**	**13.5**
Male				
Gonad bulk index	56.13 ± 11.25	70.55 ± 27.92	85.09 ± 28.64	76.38 ± 21.43
Female				
Gonad bulk index	45.21 ± 13.69	62.81 ± 45.56	74.73 ± 15.16	91.49 ± 37.64

Gonad bulk index (y) of both male and female abalone increased as a function of dietary protein to energy ratio ($y = 42.02\ x^{0.81}$; $r^2 = 0.19$; regression analysis: $F_{1.38} = 8.9$; $p = 0.005$; Figure 4.10). Average GBI ranged from a minimum of 50.67 ± 13.14 at the lowest protein to energy ratio (1.41 $g.MJ^{-1}$) to the highest average GBI (83.93 ± 29.96) in *H. midae* that were fed the diet with the highest ratio of protein to energy (2.46 g MJ^{-1}).

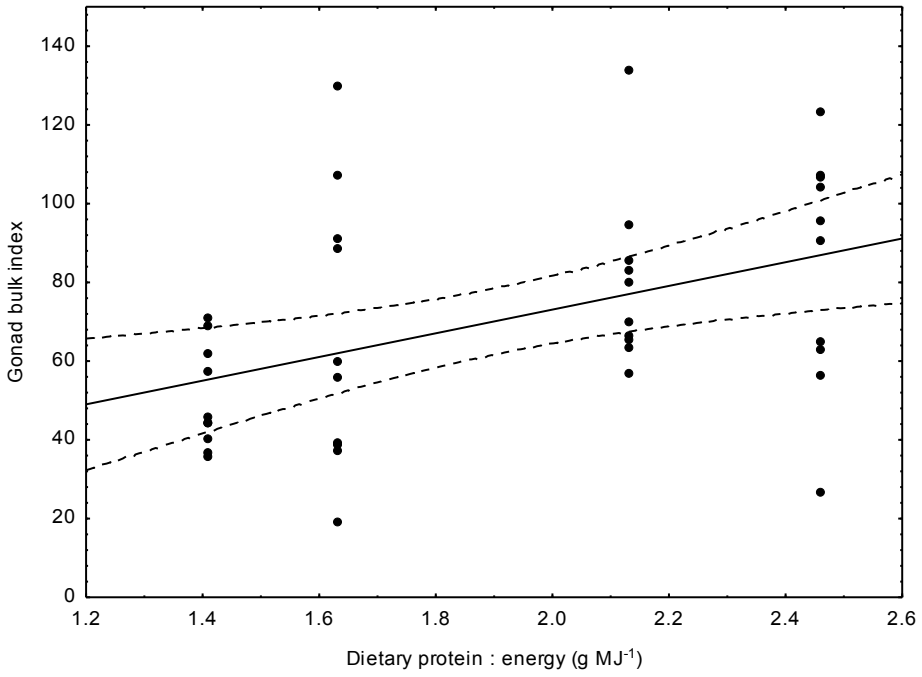

Figure 4.10: Change in mean (± 95 % confidence interval) GBI as a function of dietary protein to energy ratio ($y = 42.02\ x^{0.81}$; $r^2 = 0.19$; regression analysis: $F_{1.38} = 8.9$; $p = 0.005$).

Cookout yield

There was no interaction between the main effects sex, dietary protein and energy level on the quantity of cooked meat as a percentage of total body mass after processing (factorial ANOVA: $F_{1, 28} = 0.2$, $p = 0.641$). There was no interaction between the main effects dietary energy level and sex (factorial ANOVA: $F_{1, 28} = 0.4$, $p= 0.547$) or dietary protein and energy level (factorial ANOVA: $F_{1, 28} = 0.8$, $p= 0.373$) on cookout yield, however there was a significant interaction between the main effects dietary

protein level and sex on cookout yield (factorial ANOVA: $F_{1, 28}$ = 13.8, p= 0.001; Figure 4.11). Male abalone responded differently to dietary protein content than female abalone, with female abalone having no difference in cookout yield between the low and high protein content diets (25.85 ± 0.89 % body mass), while male abalone that were fed on the low protein diets yielded a greater proportion of cooked meat (26.67 ± 0.33 % body mass) than those fed on the high protein diet (24.71 ± 0.55 % body mass). There was a significant effect of dietary energy level on cookout yield when data for both sexes were combined (one-way ANOVA: $F_{1, 28}$ = 14.4, p= 0.001; Figure 4.12), with animals fed on the high energy diet yielding a higher proportion of cooked meat (26.24 ± 0.28 % body mass) than those fed on the low energy diet (24.96 ± 0.31 % body mass).

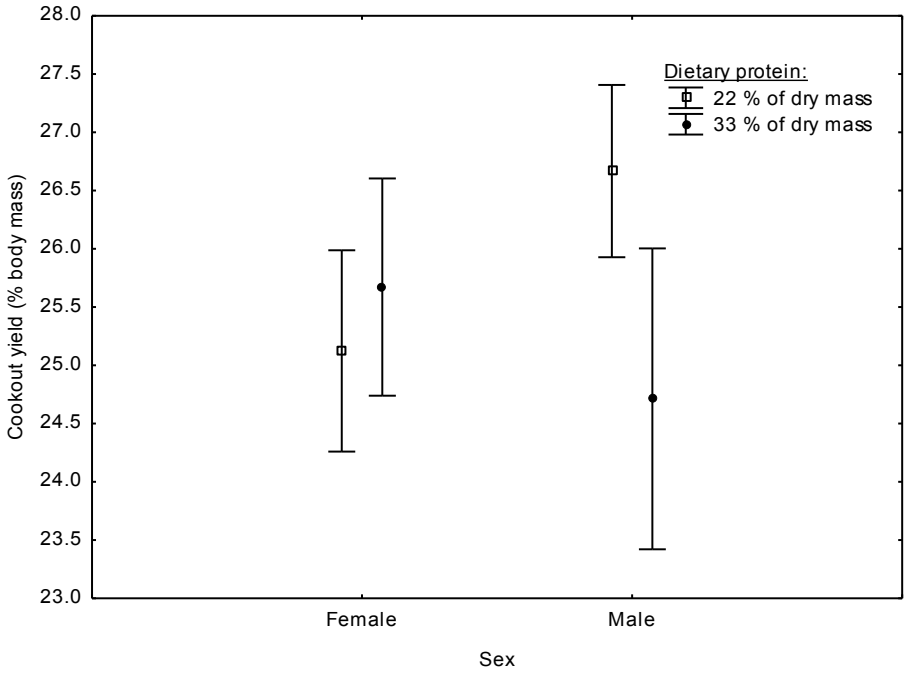

Figure 4.11: Average percentage of meat yielded after processing (± 95 % confidence interval) for male and female abalone fed diets with two protein levels (factorial ANOVA: $F_{1,28} = 13.8$, $p = 0.001$).

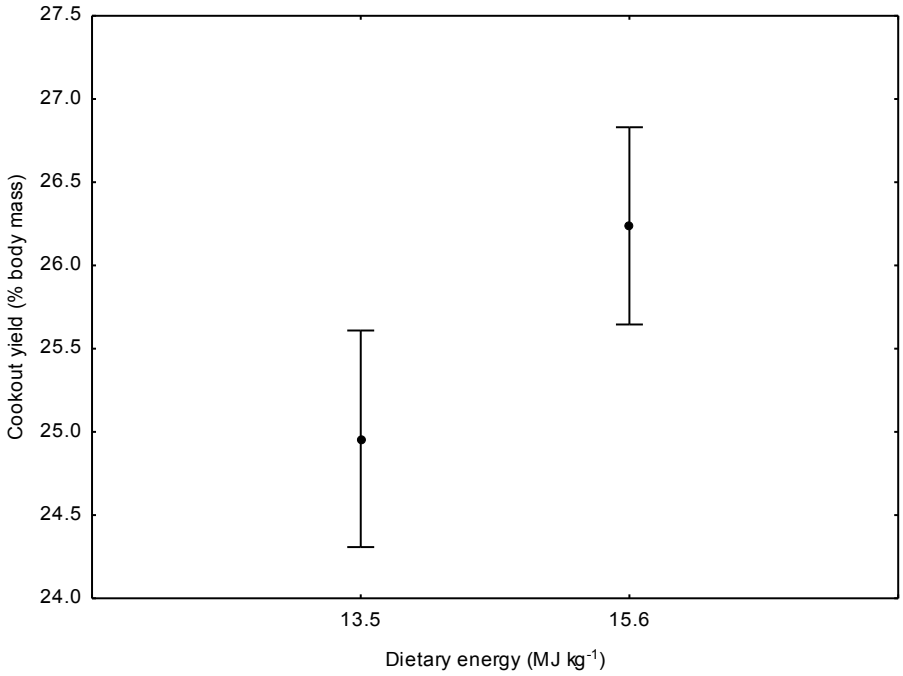

Figure 4.12: Average (± 95 % confidence interval) cookout yield between abalone fed diets with two dietary energy levels (one-way ANOVA: $F_{1,28} = 14.4$, p= 0.001).

Female *H. midae* fed diets with different ratios of protein to energy showed no difference in cookout yield (regression analysis: $F_{1, 16} = 0.1$, p = 0.976), while cookout yield for male abalone decreased as a function of dietary protein to energy ratio (y = 31.18 – 2.88x, $r^2 = 0.58$; regression analysis: $F_{1, 16} = 21.9$, p = 0.001; Figure 4.13).

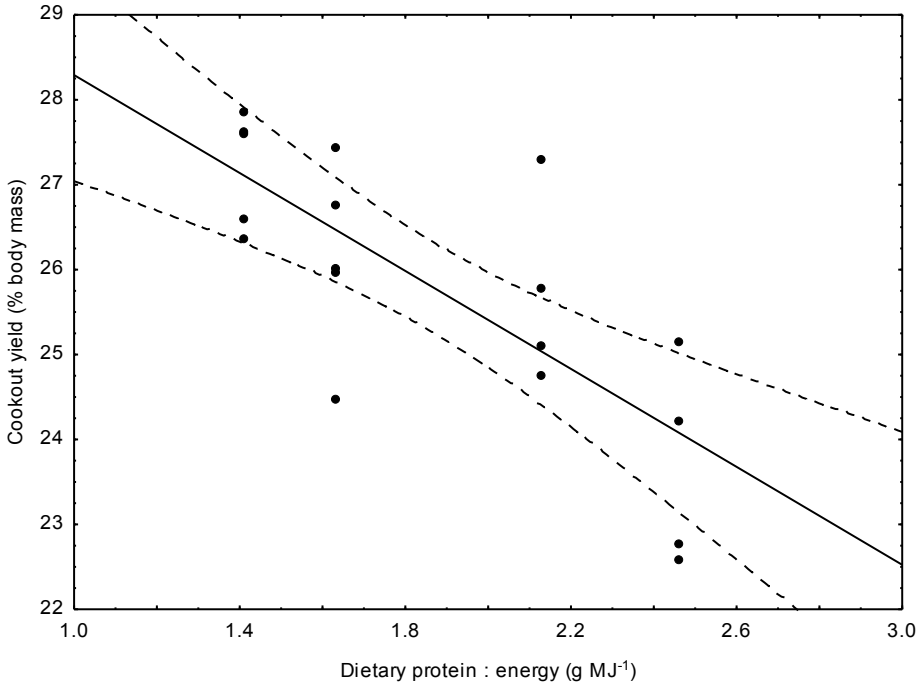

Figure 4.13: The average percentage cookout yield (± 95 % confidence interval) as a function of protein to energy ratio for male *H. midae* (y = 31.18 – 2.88x, r^2 = 0.58; regression analysis: $F_{1, 16}$ = 21.9, r^2 = 0.58, p = 0.001).

Gonad tissue proximate composition

There was no interaction between the main effects sex, dietary protein and energy level on gonad tissue protein content in farmed *H. midae* (factorial ANOVA: $F_{1, 32}$ = 1.1, p = 0.295; Table 4.3). There was no interaction between main effects sex and dietary energy level (ANOVA: $F_{1, 32}$ = 2.6, p = 0.118) or dietary protein and energy level (ANOVA: $F_{1, 32}$ = 0.1, p = 0.901) on gonad tissue crude protein content. There was a significant interaction between main effects sex and dietary protein level on gonad

tissue crude protein content (ANOVA: $F_{1, 32} = 5.9$, $p = 0.021$; Figure 4.14), with male and female abalone responding differently to the difference in dietary protein content.

Table 4.3: Gonad tissue crude protein, moisture and crude lipid content of male and female *H. midae* fed diets with different dietary protein and energy levels. Values expressed are means ± standard deviations.

Dietary protein (%)	**22**	**22**	**33**	**33**
Dietary energy (MJ kg^{-1})	**15.6**	**13.5**	**15.6**	**13.5**
Male				
Crude protein (%)	64.91 ± 2.36	68.72 ± 3.00	68.45 ± 4.01	67.95 ± 1.65
Moisture (%)	77.48 ± 0.85	77.87 ± 1.43	77.07 ± 0.89	77.19 ± 0.93
Crude fat (%)	9.88 ± 1.73	6.51 ± 0.76	7.69 ± 1.27	7.34 ± 0.64
Female				
Crude protein (%)	49.63 ± 2.24	51.80 ± 2.26	49.16 ± 1.71	50.47 ± 2.44
Moisture (%)	75.84 ± 1.57	74.36 ± 1.40	72.72 ± 0.80	73.62 ± 1.12
Crude fat (%)	21.22 ± 2.24	22.44 ± 2.14	24.51 ± 1.09	22.98 ± 1.66

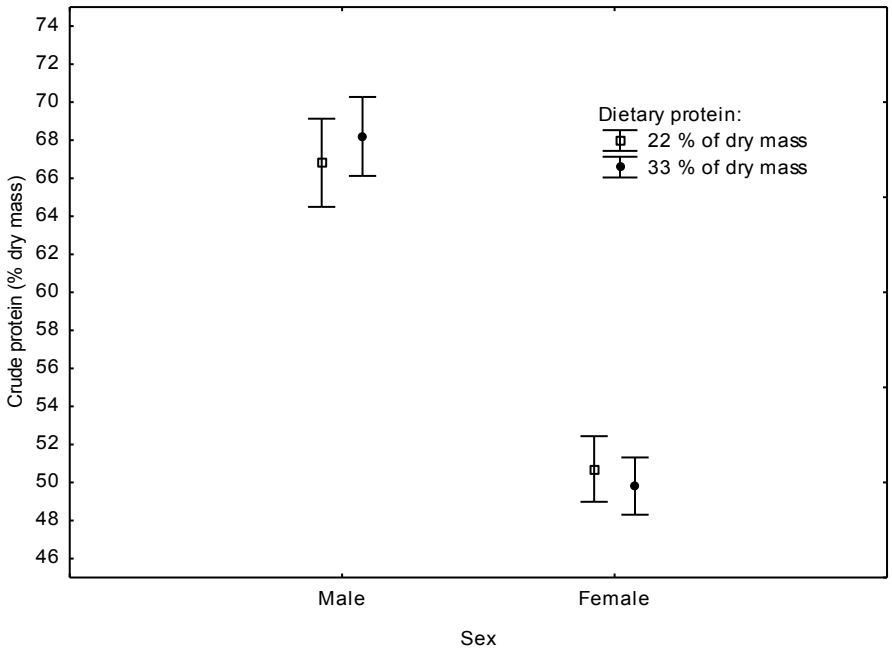

Figure 4.14: Average gonad tissue crude protein content (± 95 % confidence interval) of male and female abalone (factorial ANOVA: $F_{1, 32} = 5.9$, $p = 0.021$), at two dietary protein concentrations.

There was no interaction between the main effects sex, dietary protein and energy level on gonad tissue moisture content in farmed *H. midae* (factorial ANOVA: $F_{1, 32} = 3.4$, $p = 0.076$). There was no interaction between the main effects sex and dietary protein level (factorial ANOVA: $F_{1, 32} = 3.5$, $p = 0.071$), sex and dietary energy level (ANOVA: $F_{1, 32} = 0.5$, $p = 0.482$) or dietary protein and energy level (ANOVA: $F_{1, 32} = 2.1$, $p = 0.165$) on gonad tissue moisture content. Dietary energy level had no effect on gonad tissue moisture content in farmed *H. midae* (ANOVA: $F_{1, 32} = 0.1$, $p = 0.947$). Dietary protein level had no effect on gonad tissue moisture content (ANOVA: $F_{1, 32} =$

3.6, p = 0.064). There was a significant effect of sex on gonad tissue moisture content with male gonad tissue having a higher moisture content (77.39 ± 0.23 % dry mass) than females (74.13 ± 0.37 % tissue mass) (ANOVA: $F_{1, 32}$ = 79.1, p = 0.001; Figure 4.15).

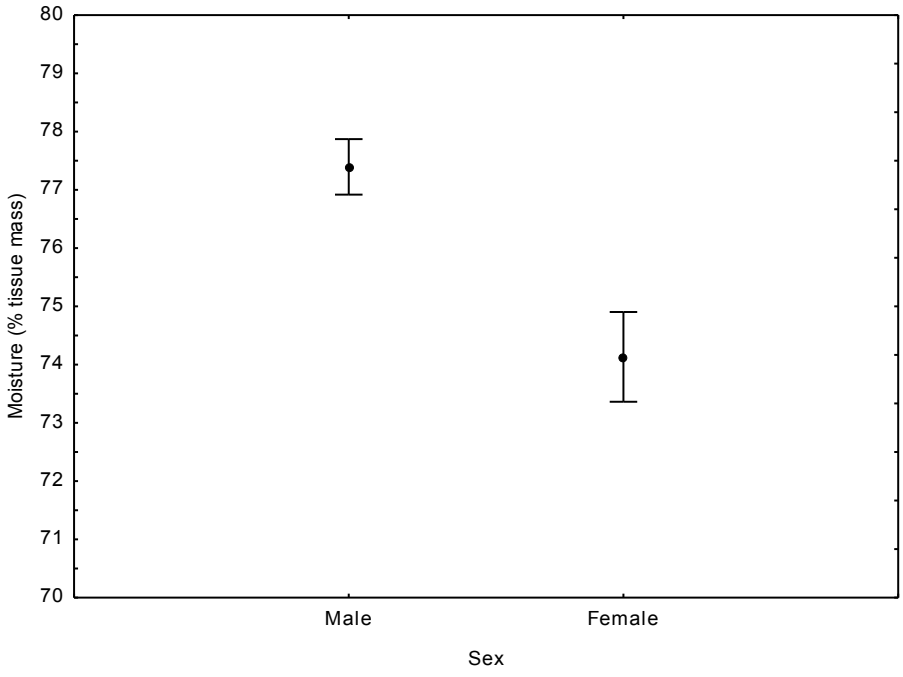

Figure 4.15: Average gonad tissue moisture content (± 95 % confidence interval) for male and female abalone (ANOVA: $F_{1, 32}$ = 79.1, p = 0.001).

Dietary protein to energy ratio had a significant influence on gonad tissue moisture content in farmed *H. midae* (regression analysis: $F_{1, 18}$ = 8.7, r^2 = 0.29, p = 0.009) with gonad tissue moisture content decreasing as a function of protein to energy

ratio ($y = 76.92\ x^{-0.06}$, where y is the gonad tissue moisture content and x is the ratio of dietary protein to energy; Figure 4.16).

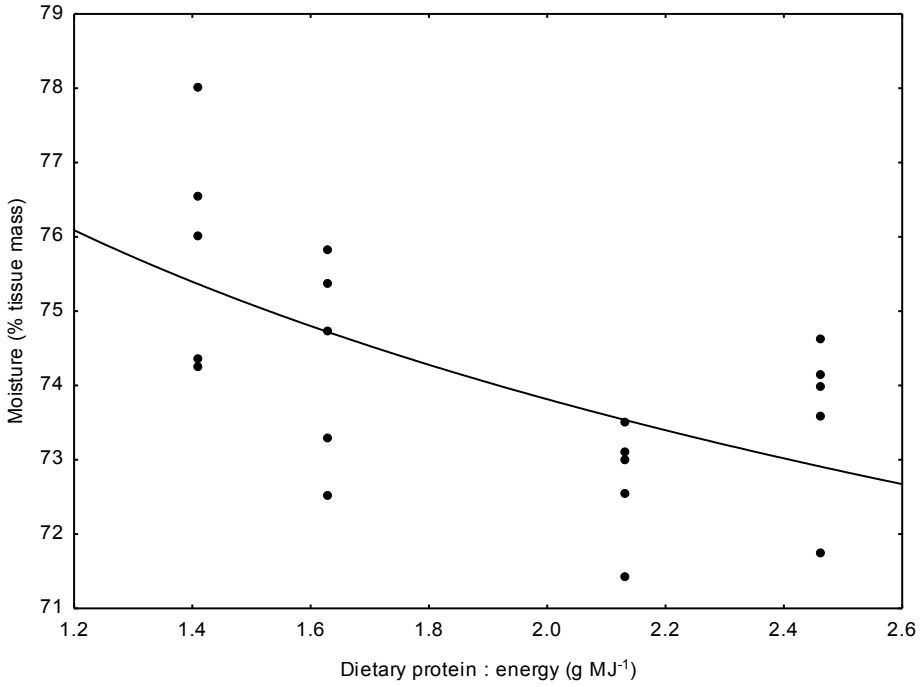

Figure 4.16: Decrease in gonad tissue moisture content (% tissue mass) as a function of dietary ratio of protein to energy ($y = 76.92\ x^{-0.06}$; $r^2 = 0.29$, $p = 0.009$).

There was a significant interaction between the main effects sex, dietary protein and energy level on gonad tissue fat content in farmed *H. midae* (factorial ANOVA: $F_{1,32}$: 7.4, $p = 0.011$; Figure 4.17). Female abalone had higher gonad tissue lipid levels (female average: 22.79 ± 1.36 % dry mass) than male abalone (male average: 7.85 ± 1.44 % dry mass) when fed on any of the dietary treatments. Males fed the low protein

(22 % of dry mass) and low energy diet had lower gonad tissue lipid content than those fed the low protein and high energy diet.

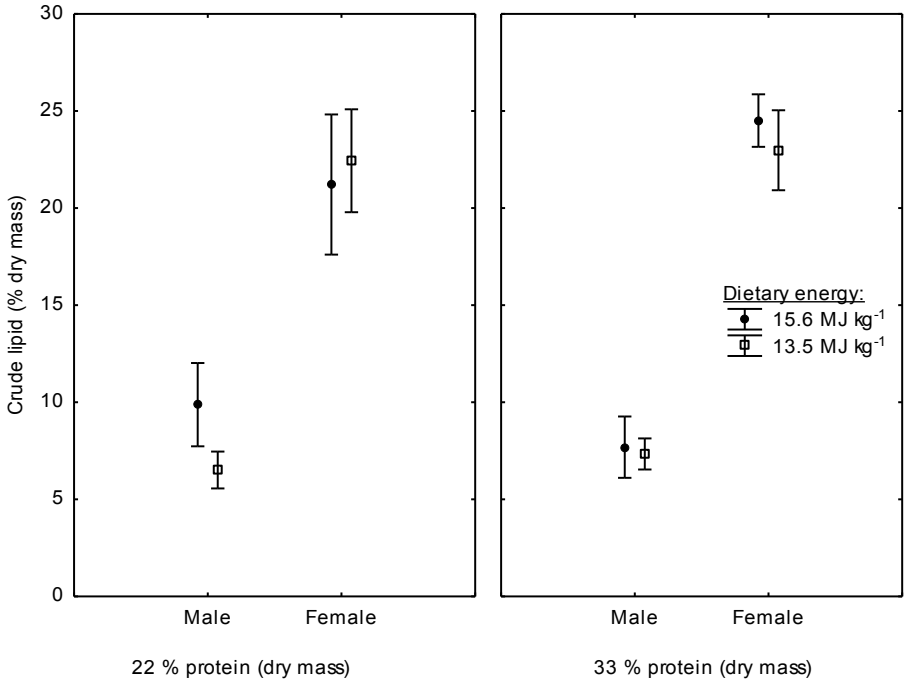

Figure 4.17: Average gonad tissue crude lipid content (± 95 % confidence interval) for male and female abalone fed on diets with two protein (22 and 33 % of dry mass) and two energy levels (13.5 and 15.6 MJ kg^{-1}) for 262 days (factorial ANOVA: $F_{1, 32}$: 7.4, p = 0.011).

4.4 Discussion

Abalone growth

Dietary protein level influenced growth in farmed *H. midae* independent of dietary energy level as Abalone weight gain increased with an increase in dietary protein level. Average abalone size was 5 % larger for the abalone fed the 33 % protein diet after 147 days, and 4.5 % larger after 262 days. Average abalone mass gained was 12.7 % higher when abalone were fed diets with the high protein concentration, while basket biomass gain was 27 % higher for baskets of abalone fed on the high protein diets. The lowest energy level used in this study was 13.5 MJ kg^{-1}, a value above which Green *et al.* (2011b) estimated that dietary protein level could be reduced from 26 % to 18 %, without reducing the growth rate of *H. midae.* Research predicted an increase in growth rate with an increase in dietary protein level (Britz, 1996b; Britz & Hecht, 1997; Mai *et al.*, 1995a; Tung & Alfaro, 2011). Another study conducted on the growth of *H. midae* suggested that optimal growth was attained at a protein inclusion level of 35.9 % of dry mass, above and below which growth was reduced (Sales & Britz, 2003). These mixed results suggest that there may be some factor besides dietary protein level (i.e. fatty acid composition), which is acting in conjunction with the protein level to influence the growth rate of farmed abalone.

Dietary energy level influenced farmed abalone growth independent of protein level. The abalone that were fed the low energy diet (13.5 MJ kg^{-1}) were on average 5.3 % larger after 147 days, and 8.3 % larger at the end of the trial (262 days). The dietary energy levels during this trial had been chosen to be within the optimal ranges

described for *H. midae* (Green *et al*., 2011b). On average, individual abalone fed the low energy diet gained 18.9 % more mass during the trial than those fed on the high energy diet, suggesting that 13.5 MJ kg^{-1} is a potentially suitable dietary energy level that provides good growth in farmed *H. midae* if used within the protein levels tested in the present study and by Green *et al.* (2011b).

The ratio of protein to energy in the diet influenced the total biomass gained within a basket, with more biomass being gained as the ratio of protein to energy increased. *Haliotis fulgens* fed diets with protein to energy ratios ranging from 1.48 - 2.52 g protein MJ^{-1} grew better when the protein to energy ratio was above 2.39 g protein MJ^{-1} (Gómez-Montes *et al.,* 2003). These findings are likely due to the increased intake of crude protein when it is available in a high-proportion diet (Britz, 1996b). If there is insufficient energy available in the diet, protein may be used as the energy reserve for maintenance (NRC, 1983).

Dietary protein and energy level interacted to influence FCR. Feed conversion was not influenced by energy level when abalone were fed the low protein (22 % dry mass) diets. However, FCR was lowest when abalone were fed the high protein and high energy diet. Green (2009) suggested that the protein requirements of *H. midae* are dependent on the quantity of dietary energy available, and *H. midae* had an improved FCR with increasing ratio of dietary protein to energy (Britz & Hecht, 1997). The present study suggests that *H. midae* are more efficient at utilizing protein for growth than dietary energy.

Gonad size

Gonad size was the same for both sexes of *H. midae.* Since haliotids are broadcast spawners, reproductive investment is energetically expensive in both sexes. (Crofts, 1929; Boolootian *et al*., 1962; Newman, 1967). Feeding male and female *H. midae* the diets used in this study may not have resulted in differential investment into gonad growth since both sexes responded the same to the diets in the present study.

It was possible to increase gonad size by increasing the level of dietary protein. Abalone fed the high protein diet had gonads which were 39.6 % larger than those fed the low protein diets. Studies have shown a positive relationship between dietary protein level and gonad production in sea urchins (de Jong-Westman *et al.*, 1995; Cook & Kelly, 2007; Cook *et al.*, 2007). When there was more protein available in the diet, abalone converted more of their diet into gonad growth.

In addition, gonad growth was increased as the proportion of dietary protein to energy increased. This differs from a study in which diets high in lipids stimulated gonad development when compared with *Haliotis tuberculata* and *Haliotis discus hannai* fed on algal diets with low lipid levels (Mercer *et al.*, 1993). Green (2009) suggested that abalone are unable to use lipids as an energy source, and suggested that high levels of dietary lipids inhibited the synthesis of proteins and carbohydrates. In addition, Green (2009) suggested that high dietary lipid levels appeared to promote gonad maturation, however the present study suggests that abalone are relying on the protein present in the diet for gonad growth. This finding was further supported by the increase in gonad size with an increase in the ratio of dietary protein to energy in the present study. This

leads to the hypothesis that essential nutrients required for reproductive development are being supplied by the protein in the diet.

Cookout yield

Male and female *H. midae* differed in the proportion of meat yielded after processing. Female abalone had the same cookout yield regardless of diet, while male abalone fed the low protein diet had a cookout yield which was 7 % higher than in those fed the high protein diet. *Haliotis midae* of 73 g abalone^{-1} had a higher cookout yield when fed a low protein (22 %) diet when compared with those fed diets with protein levels of 26 or 34 % (Winkler, 2010). This suggests that water retention may be higher when abalone are fed the higher protein diet, resulting in greater loss of water to heat during processing.

An increase in dietary energy level increased the cookout yield of abalone, independent of sex. One of the steps which are most responsible for yield loss when canned mussel meats are processed is the thermal processing which renders the canned product sterile for long-term shelf storage (Almonacid *et al.*, 2011). This thermal processing has been proven to reduce abalone meat quality and yields as a result of the loss of water, and water-soluble compounds, reducing the final product to only 60 % of the initial meat weight (Brown *et al.*, 2008). It has been suggested that this loss is as a result of the insolubility of lipids in water, with an increase in foot lipid levels limiting water loss during processing (Dunstan *et al.*, 1996).

The proportion of meat yielded after canning decreased as the ratio of protein to energy in the diet was increased. In addition to the water-insolubility of lipids (Brown *et al.*, 2008), the denaturation of proteins has been suggested as a reason for yield loss during processing (Almonacid *et al.*, 2011). Yield loss is thought to be related to the osmotic gradient between the abalone meat and brine solution in the cans, as well as temperature (Vosloo & Vosloo, 2006; Brown *et al.*, 2008). As there was more protein available in the diet to convert into foot muscle protein as the protein to energy ratio in the diet increased, there was more foot muscle protein which was likely to be degraded at high temperatures. The quantity of water-insoluble lipids would decrease with an increase in protein to energy ratio, resulting in more water in the foot muscle. Water loss and protein denaturation were most likely responsible for the decreased canning yield as the dietary protein to energy ratio increased.

Gonad tissue proximate composition

Dietary protein and energy level influenced gonad lipid deposition differently between male and female *H. midae.* Females had higher gonad tissue lipid levels than males. Diet influenced the lipid profiles of abalone (Nelson *et al.*, 2002). Lipids are regarded as a highly important food reserve for oocytes, and have been attributed to assuring the viability of bivalve larvae as well as ensuring hatchery success (Pazos *et al.*, 1997). The lipids present in embryos are derived from the mother and used for membrane formation, as well as an energy source during larval development (Soudant *et al.*, 1999). Females are depositing greater amounts of lipid in the gonad tissue to

ensure viability of their offspring, while males don't have the same lipid demands for spermatogenesis (Soudant *et al.*, 1996b). Males require a greater quantity of protein in their gonads to provide the spermatozoa with protein and to build the complex interconnecting system of fibers that support the tail movement (Buckland-Nicks *et al.*, 1982). This has implications for brood stock diet formulation, suggesting that females should be provided with a diet high in the necessary fatty acids required for gonad development, while male abalone should be provided with a high protein diet. However, this could not be concluded from data obtained in the present study and should form the basis of further research.

Abalone stored more moisture in their gonads as the proportion of dietary protein to energy in the diet increased, with females having higher moisture content in their gonad tissue than males. The biochemical composition of the reproductive tissue in the gastropod *Strongylocentrotus droebachiensis* was influenced by diet, since the gonads serve as a reservoir for the accumulation of nutrients (Liyana-Pathirana *et al.*, 2002). The gonad tissue moisture content of these sea urchins increased as the lipid content decreased. The abalone in the present study showed an inverse relationship between lipid and moisture content in the gonad tissue with males having a higher gonad tissue moisture content which corresponded to lower lipid content when compared with females. Salmonid body lipid content is both endogenously and exogenously controlled, and that the whole body moisture content is inversely related to body lipid (Shearer, 1994). The same effect was noticed in the present study where *H. midae* gonad tissue moisture content is dependent on the lipid content, and it is possible to manipulate both

the gonad tissue lipid and moisture content by altering the dietary protein to energy ratio.

Conclusion

Growth of *H. midae* was influenced by dietary protein and energy levels. Increasing the protein level improved growth rate, while high energy levels reduced growth. When sufficient energy was available in the diet, protein may have been used as the energy source for growth since abalone are more efficient at utilizing protein than energy for growth. In addition to dietary protein being the primary contributor to growth, protein appears to provide the essential nutrients for gonad growth since gonad size and protein content increased with an increase in dietary protein level. Increasing the dietary protein level may improve growth rates and animal weight for farms which intend on selling live produce, however, many farms process their animals so as to sell canned abalone.

Farms that intend on canning their abalone should consider a high lipid content diet, since the amino acids present in the dietary protein is synthesized and stored in the foot tissue, may become degraded and cooked out during thermal processing. The present study showed that as the proportion of protein to energy in the diet decreased, meat loss during processing decreased. This is likely due to the presence of water insoluble lipids, which are not lost during thermal processing.

Lastly, no published work was found that focussed on brood stock diet development for *H. midae.* The present study showed that females deposited a greater

quantity of lipids in their gonad tissue, while male abalone deposited a greater quantity of protein in their gonad tissue. This suggests that in order to provide females with the required essential fatty acids, a high lipid diet should be fed. The optimal lipid inclusion levels in the diet remains to be tested.

CHAPTER 5

Concluding discussion

This study was conducted to determine monthly and seasonal changes in the growth rate and gonad size of farmed *Haliotis midae* in Hermanus, South Africa. Growth and gonad size were correlated to determine whether investment in reproductive tissue was causing abalone to divert ingested energy from growth into reproductive development. The effects of a range of protein sources, i.e. *Ecklonia maxima*, soya meal and fishmeal or their combinations, on growth and gonad size were evaluated. Lastly, the effects of dietary protein and energy level, as well as the interaction between dietary protein and energy level on growth and gonad size were evaluated.

Studies have highlighted temperature and nutrient availability as the two most important factors in influencing seasonal growth and reproductive patterns in haliotids (Newman, 1968; Lopez & Tyler, 2006). Nutrient supply was constant throughout the year in the present study and was unlikely to have influenced gonad development. This conclusion was similar to a suggestion by Newman (1968), who showed that food availability could not explain seasonal growth patterns in wild *H. midae.* Thus, it was tested whether temperature influenced seasonal growth and reproduction. As water temperature did not reach a growth-limiting level throughout the study, this variable may not have influenced gonad development.

Haliotis midae are asynchronous spawners with only part of wild populations spawning each month (Wood & Buxton, 1996b). This variation in gonad size was visible in the range within the gonad size data, with individuals with reduced gonads and others with greatly enlarged gonads being observed in the same month. Within each month, there were individuals with large gonads, but just prior to the identified spawning periods

of wild *H. midae*, the proportion of individuals with enlarged gonads increased. Despite the presence of two peaks in gonad size, growth did not appear to be reduced during these periods. Some authors suggested that growth rates were reduced when abalone channelled energy into gonad development (Shepherd & Hearn, 1983; Capinpin & Corre, 1996). In this study it appeared that *H. midae* invested energy into growth and reproduction without the need for a trade-off. Abalone may have been fed diets that provide a sufficient quantity of nutrients and energy to sustain both processes.

In optimizing artificial diets in order to reduce the use of expensive dietary constituents to meet the minimum required quantities that yield favourable growth rates, focus has been on changing protein and energy levels in the diet. Unlike a recent study conducted on *H. midae* in which it was possible to reduce the dietary protein level without influencing growth (Green *et al.*, 2011b), protein level in the present study could not be reduced from 33 to 22 % of dry mass without influencing growth, as growth was greater in the 33% protein treatment. Growth rates in the study done by Green *et al.* (2011b) fell below average commercial farm growth rates, while those of the present study fell within the range of average commercial farm growth rates. Green *et al.* (2011b) suggest that the suppressed growth rates were probably due to a non-nutritional factor. Dietary energy level did not influence growth of *H. midae* in the present study, probably because the dietary energy levels were above the minimum required energy level of 13.5 MJ kg^{-1}, below which growth-reducing effects have been observed (Green *et al.*, 2011b).

Little research focus has been on gonad growth and development in *H midae*. Only one study to date has evaluated the reproductive response of *H. midae* to diet, which suggested that high dietary lipid levels may be promoting gonad maturation (Green *et al.*, 2011a). When compared with the low lipid diets, 65 – 70 mm *H. midae* fed the high lipid diet were found to be sexually mature at the end of the trial (Green *et al.*, 2011a). Results from the present study suggested that there was no influence of dietary energy level on gonad size; however protein level, as well as the ratio of protein to energy influenced gonad size (Chapter 4). An increase in the level and proportion of protein in the diet increased gonad size. If dietary protein is providing the essential amino acids for gonad development, it is possible that the various sources of protein are providing abalone with different amino acid profiles, which may result in varied influences on body or gonad growth. The influence of dietary protein sources on growth in *H. midae* has been studied (Britz, 1996a; Dlaza *et al.*, 2008). Plant-derived protein sources were less effective at achieving good growth than animal-derived proteins (Britz, 1996a; Bautista-Teruel et al., 2003; Dlaza *et al.* 2008). However, the use of plant-derived soya meal as a sole protein source during this study yielded interesting results as high growth rates could be sustained with the use of soya meal, an alternative to fishmeal. However, soya meal also favoured gonad growth. Thus, changing the dietary protein source influenced gonad size in farmed *H. midae*. Gonad growth was greatest when soya was present in the diet when compared with kelp and fishmeal. Soya plants contain a high concentration of isoflavones, a group of phytoestrogens (Cederroth & Nef, 2009). These plant-derived oestrogens may have endocrine-promoting effects. The present study showed that gonad growth was enhanced when soya was present in the

diet. It should be tested whether this may be as a result of the presence of phytoestrogens, or the presence of high levels of polyunsaturated fatty acids present in soybean diets. This study has provided reference data for further research into understanding the influence of soya as a protein source on gonad growth and development in abalone.

Dietary protein to energy ratio increased processing yield for female abalone, while processing yield for males decreased with an increase in protein to energy ratio. This means that males fed on the diets with the lower protein to energy ratios were able to provide more processed and marketable product per unit biomass than those fed diets with raised protein to energy ratios. Thus, with the reduced cost of such a diet, and with similar growth rates between treatments, it may be worthwhile for the industry to consider using this diet for animals that are to be canned. It may be feasible to change the diet prior to canning in order to optimize canning yield. In addition, the difference between male and female *H. midae* in response to processing yield between treatments suggests that sex-sorting abalone before sending male abalone which have been fed on a low protein to energy ratio diet may be beneficial.

Research and management implications

The present study has placed a foundation for further research into understanding abalone gonad development, as well as the influence of diet on reproduction. There was no difference in gonad size between male and female *H. midae*, a character common to broadcast spawners (Crofts, 1929; Newman, 1967).

Despite this, nutrient allocation into the gonad tissue varied, with male abalone depositing more protein in their gonad tissue, and females depositing more lipids, which are essential for gametogenesis. These findings may prove important in future brood stock diet development, in which it would appear beneficial to provide males with high protein diets, and females with high lipid diets. Since male and female brood stock are kept in separate tanks and the number of breeding individuals on the farms is relatively low, it is important to develop a high quality diet, while it is also feasible to administer a sex-specific diet.

In addition to the relative quantities of protein and energy in the diets, the source of protein influence gonad size. Soya protein enlarged the gonads when compared with kelp and fishmeal. However, the combination of fishmeal and soya meal in the diet may have provided a range of fatty acids. This suggests that female brood stock development should, in addition to the high lipid diet, replace a high proportion of fishmeal with soya meal, but still include some fishmeal.

The use of different diets also influenced the processing yields of abalone, which is an important consideration since a large proportion of produce is marketed canned. There was no difference in cookout yield between animals fed fishmeal or soya meal, however including both protein sources in the diet may have provided abalone with that composition of amino acids that influenced processing yield. Thus, it is suggested that farms marketing canned abalone reduce the amount of fishmeal present in their diets and replace it with soya meal. This reduction in the quantity of fishmeal may reduce the cost of feeds, without a loss of marketable product.

Shortcomings and improvements in experimental design

This study was conducted under commercial farming conditions. The monthly monitoring (Chapter 2) took place without manipulating the farm conditions. One of the reasons suggested for observing variations in monthly growth rates was the varied abundance and nutritional value of benthic diatoms. Measuring the nutrient content and abundance or availability of benthic diatoms at intervals throughout the trial would have added value to this study. It may have been possible to account for extra nutrients in the system than the pellets fed to the abalone. A comparison in benthic diatom abundance would also allow for a comparison between the two farms which have slightly different management strategies.

Since this study was conducted on-farm, it is important to note that there were certain parameters and conditions which could not be controlled to as great a degree as would they be during a laboratory experiment. Holding several treatment baskets within a single tank meant that tank position varied between treatments, however this was accounted for by having five replicates at various positions throughout three tanks. In addition, there was no way of determining whether adjacent treatments were influencing each other, but the random block design accounted for as much of this risk as possible. A method rectifying this error, or negating the risk, would have been to have had each replicate basket in an individual closed system with no external influences. To do this on-farm would have required a full tank of each treatment replicate, which would equate

to 4 200 kg of abalone, as opposed to the 360 kg required with the design used in this study.

The gonad bulk index used in this study has flaws. The gonad tissue surrounds the conical appendage and as a result it is not possible to simple section out the gonad tissue, weigh it and express this mass as a proportion of soft tissue mass or eviscerated mass. Microscopic analysis of the gonad tissue fed the range of experimental diets would have added significant value to this study. This would also have allowed for accurate evaluation of the influence of the experimental diets on maturation in cultured *H. midae*. This microscopic evaluation is presently being undertaken by a Master of Science candidate at the Department of Ichthyology and Fisheries Science, Rhodes University.

Lastly, an animal does not have a requirement for crude protein or energy *per se*, but rather a requirement for specific amino acids and fatty acids. The potential influence of fatty acid type and content on growth and gonad development was suggested throughout the thesis. This study served to probe the broader gaps in understanding the relationship between diet and growth or gonad development in haliotids. The foundation has been laid to further research where particular focus should be on the influence of amino acid profiles, fatty acid profiles and phytoestrogens on growth and gonad development in cultured *H. midae.*

Conclusion

The present study highlighted the possibility to manipulate gonad growth in farmed *H. midae*, a basis on which future research should focus. Both dietary protein source and level, and energy level influenced farmed abalone growth and gonad development, and it should be tested to what extent diet formulation should be based on the choice of marketable product.

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