

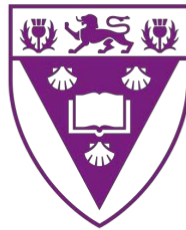
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QUANTIFYING FEED INTAKE AND FEEDING INTENSITY USING TWO  
EXPERIMENTAL CONDITIONS AND THE EFFECT OF DIFFERENT  
FEEDING STRATEGIES ON THE PRODUCTION PARAMETERS OF  
FARMED SOUTH AFRICAN ABALONE, *Haliotis midae*

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

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at



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*Where leaders learn*

By

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## Abstract

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As abalone are slow-growing animals there is a high investment of capital, running costs and labour in commercial abalone farming. Revenue needs to be maximised by achieving fast growth rates. A foremost driver of abalone growth is feed intake and feeding intensity of the abalone as well as the feeding regimen a farm utilises. While feed intake is well-documented in *H. midae*, there is a paucity of information regarding this aspect in abalone above 70 g and the relationship between feeding intensity, feed conversion rate (FCR) and daily growth rate needs to be documented. Similarly, there are a limited number of studies dealing with the effects of different pellet types of the same formulation, that differ only in shape and size, on feed intake and production in *H. midae*. This study made use of both a controlled laboratory experiment and commercial-scale farm experiment with the aim of testing whether feed intake information produced under laboratory conditions can provide good estimates of feed intake under farming conditions, which can further be implemented into feeding strategies on abalone farms. This study quantified daily feed intake (F) in 10 – 20 g, 100 – 110 g and 150 – 160 g abalone weight classes using two different pellet types and determined the relationship between the duration of feed availability and feed intake under laboratory conditions. The effect of three different pellet type strategies (fed daily: a leaf-only strategy = L d<sup>-1</sup>, a short-pellet only strategy = SP d<sup>-1</sup>, and a strategy that used both = B d<sup>-1</sup>) on abalone production parameters under farming conditions such as growth rate, feed conversion ratio (FCR), feeding intensity (FI) and weight variation of 30 – 40 g *H. midae* was also investigated. A commercial-scale farm study was designed to test the effect of four commercially relevant feeding regimens (three size-specific regimens and one commercially practiced feeding method) on the growth, FCR, feeding intensity and weight variation of these three abalone weight classes while taking into account an economic model to assess the profitability of each feeding regimen.

Daily feed intake as a percentage of body mass (% BM d<sup>-1</sup>) was a function of abalone weight class between the 10 – 20 g and two larger weight classes 100 – 110 g and 150 – 160 g ( $p < 0.0001$ ), however feed intake was not different between the 100 – 110 g and 150 – 160 g weight classes. Pellet type did not affect feed intake in all abalone weight classes ( $p = 0.15$ ). Feed intake (F) was positively linearly correlated with duration of feed availability (h) in the 10 – 20 g weight class ( $r^2 = 0.76$ ,  $p < 0.00001$ ) and logarithmically correlated in the 100 – 110 g ( $r^2 = 0.25$ ,  $p < 0.02$ ) and 150 – 160 g ( $r^2 = 0.52$ ,  $p < 0.0001$ ) weight classes (10 – 20 g abalone<sup>-1</sup>:

$F (\% \text{ BM}) = 0.02(h) + 0.1976$ , 100 – 110 g abalone<sup>-1</sup>:  $F (\% \text{ BM}) = \log_{10}(h)*0.17 + 0.17$ , 150 – 160 g abalone<sup>-1</sup>:  $F (\% \text{ BM}) = \log_{10}(h)*0.36 + 0.07$ .

There was no significant difference in monthly average abalone weight, daily growth rate (G), FCR and feeding intensity between each pellet type strategy (G:  $p = 0.60$ , FCR:  $p = 0.62$ , FI:  $p = 0.54$  ). However, abalone grew well over the 112-day growth period with average abalone weight increasing significantly between each monthly sample time (pooled pellet type strategy: Huynh-Feldt correction;  $p < 0.00001$ ). Furthermore, abalone fed the leaf-only pellet type strategy ( $L \text{ d}^{-1}$ ) fell into higher weight classes after a 112-day growth period (Z test: 50 – 70 g: 72%) which was 16.1 % higher compared to the  $SP \text{ d}^{-1}$  and  $B \text{ d}^{-1}$  strategies. Daily growth rate ( $r^2 = 0.34$ ,  $p < 0.01$ ) and FCR ( $r^2 = 0.42$ ,  $p < 0.004$ ) was negatively correlated and FCR was positively correlated with daily feeding intensity, respectively ( $G = - 2.59 \text{ (FI)} + 1.526$ ,  $\text{FCR} = 8.8082 \text{ (FI)} - 2.7108$ ). Feeding regimen affected the production parameters of three abalone weight classes. The method practiced on the farm resulted in the best growth in the 10 – 20 g abalone weight class. The farm feeding method resulted in slower yet more efficient growth rates (lowest FCR values) in the abalone weight classes, 100 – 110 g and 150 – 160 g abalone<sup>-1</sup>. However, size-specific feeding regimens resulted in the fastest growth rates but resulted in higher FCR values (less efficient). The relationship between feeding intensity, daily growth rate and FCR all showed that an increase in feeding intensity results in increased daily growth rates and FCR values. The economic model suggests that the higher FCR values associated with size-specific regimens, which have higher associated costs to producing abalone, were greatly outweighed by the growth attained by the abalone in the 100 – 110 g and 150 – 160 g weight classes. The size-specific regimens generated a higher potential monetary value of abalone after a 112-day period, which would consequently result in higher income for abalone farms. For abalone ranging from 100 – 110 and 150 – 160 grams, the economic model suggested that in a quarterly grading schedule (112 days) that abalone be fed the size specific daily rations, which is a function of body mass, at 0.35 %  $\text{BM d}^{-1}$  and 0.352 %  $\text{BM d}^{-1}$ , respectively.

The two experimental conditions used in this study produced feed intake and production parameter information that is beneficial to South African abalone farmers. The small-scale laboratory study produced information on feed intake that can be used as reference values as to what abalone in these weight classes can consume on a daily basis. The laboratory study can provide estimates of feed intake under farming conditions but should only be used as minimum

values when determining size-specific feeding regimens. To maximise abalone growth, farmers should utilise size-specific feeding regimens for abalone above 30 g. Daily growth rate and FCR can be predicted as a function of the abalone's feeding intensity. Further studies are needed to determine the effects of abalone weight class on production parameters when testing different pellet types as well as an exploration into behavioural studies focusing on diet preferences. Additionally, future studies need to take into consideration abalone above the weight of 100 g with additional focus of research on behavioural, genetic and environmental aspects on abalone feed intake.

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### List of abbreviations and definitions

<b>Abbreviation</b>	<b>Definition</b>
% BM	Percentage of body mass
% BM d <sup>-1</sup>	Percentage of body mass per day
F (% BM d <sup>-1</sup> )	Feed intake
FI (% BM d <sup>-1</sup> )	Feeding intensity
G (% BM d <sup>-1</sup> )	Daily growth rate as a percentage of body mass per day
FCR	Feed conversion ratio, shown as a ratio of kilograms of feed used to produce one kilogram of abalone

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## Ethical Clearance

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The experiments of this study were ethically approved by the Rhodes University Animal Research Ethics Committee (approval numbers: 2022-2711-6540, 2020-1279-3300).

# CHAPTER 1

## General Introduction

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### **South African haliotids**

The South African abalone, or perlemoen, *Haliotis midae* Linnaeus 1758, is one of 90 members of the molluscan family Haliotidae within the class Gastropoda. The meaning of *Haliotis* refers to the general shape of abalone and associates such shape to a “sea ear” or “ear-like” shape. There are six species of abalone found along the coast of South Africa of which two are commercially, culturally and recreationally important namely the blood-spotted abalone *Haliotis spadicea* and the South African abalone *H. midae* (Muller 1986; Oliver 2004). *H. spadicea* is not commercially attractive but it has a cultural value to the indigenous people of South Africa that inhabit the coastline (Muller 1986; Troell *et al.* 2006). The abalone family has distinguishable features that separate themselves from other molluscs (Oliver 2004). The foremost feature displayed by *H. midae* is the oval shaped yet moderately convex and spiralling shell which protects both the vital organs of the abalone and a large muscular foot. The size of the shell varies between 12 and 20 cm based on the age and mass of the animal (Oliver 2004). The most famous feature is the plethora of colours shown by the underside and occasionally the topside of the shell that gives off a silver, purple and blue hue.

*Haliotis midae* are found on rocky shorelines, between the low tide mark and approximately 10 metres deep (Barkai and Griffiths 1986). The geographical distribution stretches along the entire coastline of South Africa, spanning the Indian and Atlantic Ocean waters, ranging from the Transkei on the east coast to St Helena Bay on the west coast, respectively (Newman 1965, Barkai and Griffiths 1987). The temperature along the coast ranges from 12 – 21 °C. Along these coastlines, an abundance of food is found, in the form of micro- and macroalgae, which is eaten depending on the abundance of abalone in the corresponding area to the feed, the abalone’s life stage and the ease of access to the feed (Barkai and Griffiths 1986).

### **Feed intake in *H. midae***

Abalone are classified as opportunistic herbivores that depend on a plant-based diet that changes throughout their development (Sales and Britz 2001). Abalone possess a radula, which is an anatomical feature that is used in feeding. Abalone will grasp onto food with this radula, which leads to the ingestion of the food. Abalone diets change from planktonic diatoms as free-swimming larvae to sessile diatoms as juveniles, and then to attached seaweeds as adults (Sales and Britz 2001, Troell *et al.* 2006). *Haliotis midae* consume a wide range of algae and have shown to have a preference for kelp *Ecklonia maxima* and red algae *Plocamium spp.* as adults, but a higher preference for *Ulva spp.* is observed in juveniles in winter months (Barkai and Griffiths 1986). Once abalone become adults and focus their feed selection towards seaweeds, a nocturnal feeding pattern becomes evident (Shepherd and Turner 1985). This is possibly due to the abalone's response to predator avoidance and to minimize feeding competition (Bullon *et al.* 2022). Similarly, evidence shows that in abalone farm tank environments, their heightened nocturnal activity leads to lower dissolved oxygen levels at night compared to the daytime due to an increase in aerobic metabolism (Yearsley 2008).

Abalone tend to select their feed on the basis of the following reasons; environmental conditions, such as water quality, population density, the abalone's characteristics, such as size, species and age and the type and availability of feed such as formulated versus natural feed and the duration of feed availability (Barkai and Griffiths 1986, Barkai and Griffiths 1987, Sales and Britz 2001, Troell *et al.* 2006).

#### *Water quality*

Feed intake is a function of water temperature and abalone size (Britz *et al.* 1997), with feed intake increasing with temperature from 12 °C up to an observed threshold of 20 °C in *H. midae*. These temperatures coincide with the average sea temperature ranges where abalone are found in the wild (Barkai and Griffiths 1986). When temperatures exceed 20 °C, oxygen becomes less readily available due to a heightened metabolic response of the abalone and increased demand for oxygen (Harris *et al.* 1999). Dissolved oxygen is also influenced by temperature, consequently adding an influential effect on feed intake. With an increase in temperature, dissolved oxygen becomes less soluble in water and this requires less dissolved oxygen to reach 100% oxygen saturation (Harris *et al.* 1999, Naylor *et al.* 2011).

### *Size*

Feed intake as a percentage of body mass in *H. midae* decreases as a function of body mass. Small abalone consume food at a higher percentage of body mass. How feed intake is converted into weight gain can be explained by the interaction between growth rate and feed conversion ratio. In *H. fulgens*, abalone under 30 mm shell length of approximately 0.125 g abalone<sup>-1</sup> consumed on average 1.0 % of their body mass per day, and abalone of 30 mm shell length and over ( $\pm 0.636 - 2.257$  g abalone<sup>-1</sup>) ingested 0.5 % and 0.2% of their body mass per day of a formulated diet, respectively (Farias *et al.* 2003). In *H. midae*, abalone with an average weight of 1.7 g abalone<sup>-1</sup> consumed approximately 0.5% of their body mass per day of formulated feed (Britz 1996 a). There is a multitude of information regarding the optimisation of formulated feeds, which consequently quantify feed intake in *H. midae*, however, generally smaller juvenile abalone have been used in studies to quantify feed intake.

For instance, Britz *et al.* (1997) used abalone up to 60 mm in shell length at a weight of approximately 30 g abalone<sup>-1</sup>. The study determined the effects of water temperature and body mass on feed intake in *H. midae* and showed that at a temperature of 18 °C an abalone at this weight consumed approximately 0.20 % BM d<sup>-1</sup>. Abalone size classes that were tested to quantify feed intake, and Green *et al.* (2011) determined the effect of protein : energy ratio of formulated feed on feed intake in the 29 g weight class and found that abalone of this weight consumed approximately 0.44 – 0.54 % BM d<sup>-1</sup> depending on the treatments.

Thus, more research is needed regarding feed intake in *H. midae* above 60 mm shell length or 30 g in weight emphasizing the need to quantify feed intake in *H. midae* in larger weight classes which are larger than 30 g abalone<sup>-1</sup>.

### *Stocking density*

In land-based farms space is a limiting factor for production. Stocking density is an important variable to maximise abalone production while taking into account space limitations (Mgaya and Mercer 1995). In aquaculture environments, access to feed is an important factor to consider with population density influencing competition for food (Lloyd and Bates 2008, Buss *et al.* 2015). Stocking density directly influenced growth in two *H. tuberculata* weight classes where an increase in stocking density resulted in a decline in abalone growth and consequently feed intake (Mgaya and Mercer 1995). The study found that after a 226-day growth period, abalone of 0.50 g at lower densities (597 abalone m<sup>-2</sup>) realized a growth rate that was 39 %

faster than in abalone at higher densities (2177 abalone m<sup>-2</sup>) and in abalone of 1.91g, a 76 % faster growth rate was observed at lower densities compared to higher densities (Mgaya and Mercer 1995). The differences in the growth rates observed in the study by Mgaya and Mercer (1995) were attributed to intraspecific competition within the abalone tanks, which ultimately led to a less efficient access to feed. Similarly, Lloyd and Bates (2008) illustrated that density-dependent interactions such as population density influenced the movement of abalone, resulting in competition for available space and the ability for abalone to find food in aquaculture environments (Lloyd and Bates 2008). For example, at lower population densities, twice the number of *H. kamtschatkana* were eliciting feeding behaviour and movement, compared to higher densities (Lloyd and Bates 2008).

### *Withholding feed*

Withholding feed is sometimes necessary on abalone farms when temperatures exceed a maximum physiological threshold. For example, a significant decline in feed intake was observed in *H. midae* when water temperatures exceeded 20 °C (Britz *et al.* 1997). Similarly, with an increase in water temperatures comes an increase in nutrient leaching of formulated feeds as the pellets are less stable at higher temperatures (Britz *et al.* 1997, Sales and Britz 2001). In the events of devastating red tides, a harmful algal bloom may result in the withholding of feed as water intake from the ocean must be stopped to avoid contamination of the tank water. In some cases, abalone can survive without food for a long time. For instance, gut evacuation studies involved withholding feed after acclimation periods for up to 96 hours with no effect on abalone mortality (Shipton and Britz 2001). Similarly, *H. midae* had a gastric evacuation time of 18 – 24 hours after feed was consumed, emphasising the need for withholding feed for longer than 24 hours when conducting gut content analyses and dry-weight analyses of feed and faecal matter (Britz *et al.* 1996).

In starved molluscs, metabolic rates slow down which can lead to compensatory growth (Francis *et al.* 2008) when feeding resumes. Compensatory growth can occur when animals have been starved or restricted in terms of feed provided which results in brief growth spurts when feeding is returned to normal compared to abalone of the same size that were not starved (Fermin 2002). In this process, an increase in feed intake occurs until the animal's metabolism stabilises.

Withholding feed is also important when conducting laboratory feed intake experiments, specifically when studying gut evacuation times and gut content analyses (Britz *et al.* 1996, Jobling *et al.* 2001). This is done to ensure that there is no food present in the gut from a previous meal. Withholding feed can result in an increase in feeding response, which may lead to overestimating feed intake. Buss *et al.* (2015) illustrated that in *H. laevigata*, a restricted feeding ration resulted in a heightened level of abalone movement in search of food, even during the daytime, which contradicts the assumption that *H. laevigata* are exclusively nocturnal foragers (Buss *et al.* 2015; Currie *et al.* 2016). As this is not starvation but rather restricted feeding, the absence of feed can result in increased foraging behaviour. Therefore, determining the influence of starvation or withholding feed on feed intake is needed to establish whether methods using starvation produce an accurate representation of feed intake.

#### *Feeding duration*

It is assumed that abalone are photophobic gastropods (Barkai and Griffiths 1987, Britz *et al.* 1996), feeding nocturnally. Gut fullness and gastric evacuation have been studied in *H. midae* (Britz *et al.* 1996). The study showed that gut fullness was reached six hours after feed was given between 18:00 and 24:00 in abalone of an average of 14 g body mass. On the contrary, the duration of feed availability was also shown to impact the feeding behaviour in *H. laevigata*, where feeding a restricted feeding ration resulted in increased foraging and alert behaviour at night and during the daylight hours (Buss *et al.* 2015).

Some authors utilised abalone juveniles, as higher weight classes are of higher commercial value and are cumbersome to source. For example, Britz (1996 b) and Britz and Hecht (1997) quantified feed intake in *H. midae* of 1.46 g abalone<sup>-1</sup>, and in the 7 – 14 g abalone<sup>-1</sup> weight classes, respectively, to determine the optimal dietary requirements for *H. midae*.

Therefore, the duration of feed availability and its effect and relationship on feed intake needs to be quantified in a wide range of abalone weight classes in order to better understand the biology of the species and provide recommendations for farmers.

## **Abalone commercial production parameters**

### *Abalone growth*

In their natural habitat, *H. midae* can reach a maximum age of 30 years and attain a shell length of approximately 200 mm (Newman 1965, Sales and Britz 2001, Venter *et al.* 2018). In captivity, abalone grow approximately 30 mm per year and take about four years to attain a weight of approximately 80 g (Troell *et al.* 2006). *Haliotis midae* has an optimal physiological temperature range of 12 – 20 °C with a maximum growth rate at 20 °C (Britz *et al.* 1997). Growth rates of 0.08 – 4.5 % BM d<sup>-1</sup> were reported in the length class of 10 – 17 mm shell length in *H. midae* fed formulated feed (Britz and Hecht 1997, Sales and Britz 2001). Growth rate is an important parameter in the calculation of feeding intensity. Determining an optimised daily ration that realises efficient growth, consequently maximising profits on abalone farms, can be done using feeding intensity studies. There is a paucity of published information on the quantification and effects of feeding intensity in South African abalone aquaculture.

### *Regimens and feeding intensity*

The feeding regimen adopted by an abalone farm is the foremost driver of abalone growth rate. Feeding the correct ration size is important, for example, if abalone are overfed, there is waste of feed and possible deterioration of water quality (Britz *et al.* 1994). If abalone are underfed, a decrease in growth rate results in economic loss. However, farmers do not determine the feeding regimen based on the size of the abalone due to the absence of data on feed intake. While farms have established a feeding method that involves the feeding of abalone based on how much feed is left over from a previous feeding, there is still a need to intensify and optimise feeding regimens.

Generally, feeding intensity can be defined as the rate at which feed is consumed (per unit body mass per unit time). More specifically however, feed conversion ratio and the rate at which abalone grow are important variables to consider for economic calculations. Feeding intensity is a function of the rate of growth after a given growth period, and the efficiency at which feed is converted into growth (feed conversion ratio, FCR)  $FI (\% \text{ BM d}^{-1}) = G \times FCR$ , where G is the growth rate (% BM d<sup>-1</sup>). This equation is a modified version of one shown by Britz *et al.* (1997) who used the growth and FCR data of their study to predict daily feed consumption of *H. midae* up to 60mm shell length at different temperatures under laboratory conditions and

was described as  $C_t = FCR_t \times \text{percentage weight gain day}_t^{-1}$ , where C is feed consumption, and t is water temperature and percentage weight gain is the increase in weight gain as a percentage of body mass per day. However, the relationship between feeding intensity, FCR and growth rate is not well documented in *H. midae*.

Some studies explored feed intake, growth rate and FCR, but did not incorporate feeding intensity into their scope of research. For example, Britz (1996 a) determined the suitability of different protein sources on growth and FCR in *H. midae* of an average weight of 1.76 g, which resulted in an average growth rate of 0.9 % BM d<sup>-1</sup> for abalone fed a fishmeal based pellet at an average FCR of 0.8:1. Therefore, using the formula to calculate feeding intensity, the feeding intensity would be 0.72 % BM d<sup>-1</sup> for abalone of 1.76 g abalone<sup>-1</sup> fed a fishmeal based pellet after a 124-day growth period. Additionally, Britz (1996 a) also quantified feed intake using dry-weight analysis under laboratory conditions and determined feed intake of fishmeal-based pellets to be 0.8 % BM d<sup>-1</sup>. The difference between feed intake and feeding intensity observed in the study conducted by Britz (1996 a) shows that using dry weight analysis results in a representation of feed intake that is 11 % higher as a percentage of body mass, which emphasizes the need to explore feeding intensity further under both laboratory conditions and commercial scale farming conditions.

As abalone grow at different rates depending on their weight, the quantification of feeding intensity as a function of abalone weight class is needed. Feeding a size-specific daily ration based on the feeding intensity of a given abalone weight class will optimise growth and enhance farm efficiency. Similarly, feed intake values and feeding intensity values should be compared between abalone of different size classes. Doing this would give an improved understanding of the biology of the abalone which can lead to the implementation of either of these values into feeding regimens used to calculate daily rations on abalone farms.

#### *Feed conversion ratio*

Feed conversion ratio is an important commercial production parameter. It measures the efficiency at which feed is consumed and converted into mass gain of the abalone. It can be defined as the dry mass of feed needed (kg) to produce one kg wet weight of abalone. The higher the feed conversion ratio the less efficient the feed was converted. The optimal relationship needed on an abalone farm is a high growth rate with lower, more efficient FCR-

values. There are instances where slow growth and low FCR coincide, but this can be related to the quality or availability of the diet or water quality. For example, Bautista-Teruel *et al.* (2003) found that *H. asinina* fed a plant protein-based feed, had a lower growth rate and less efficient FCR in comparison to abalone fed an animal-plant protein combination, which had a higher growth rate at a slightly less efficient FCR. This was explained due to a lower feed intake of the plant-based protein feed which could be narrowed down to diet selection issues and feed preferences of the abalone.

The FCR-values for natural diets are higher than formulated diets (Britz 1996 a). Values for macroalgae-based diets such as *P. corallorhiza* and *E. maxima* were 2.8 and 3.4 (dry weight basis) which was higher than the formulated feeds with an FCR range of 0.7 – 1 (Britz 1996 a). This is due to formulated diets having a higher protein content than macroalgae diets, leading to faster growth and less feed required.

#### *Size variation*

Size variation of abalone in a practical farming context plays a big role in the grading process of the abalone. The effect of feeding regimen on size variation in *H. midae* has not been studied. Understanding the role of feeding intensity and its influence on size variation can lead to improvements in farm management.

#### **Water quality: optimal conditions**

As environmental factors affect feed intake and growth in abalone, it is important to understand which ranges of water quality parameters are suitable for feed intake and fast growth. These water quality parameters include water temperature, dissolved oxygen, pH, total ammonia, (TAN) and free ammonia (FAN). As formulated feeds have a higher protein content than the natural diet, which may result in heightened metabolic processes of farmed abalone, these values need to be measured.

#### *Dissolved oxygen*

Dissolved oxygen concentration is important for aerobic metabolism. In aquaculture systems, where nutrient loads are high due to wasted feed, an increase in biological oxygen demand can

reduce the availability of oxygen to the farmed species. If dissolved oxygen is a limiting factor, it reduces the capacity for aerobic metabolism (Harris *et al.* 1999). Dissolved oxygen between 7.04 – 9.12 mg L<sup>-1</sup> (89.9 % – 96.6 %) resulted in no reduction in growth of *H. midae* (Naylor *et al.* 2011). Similarly, Yearsley (2008) showed that abalone grew sufficiently well at dissolved oxygen levels of 7.0 – 7.2 mg L<sup>-1</sup>. If no reductions in growth are observed at these dissolved oxygen levels, then it is assumed that feed intake was not affected.

### *pH*

In aquatic environments, a weak carbonic acid (H<sub>2</sub>CO<sub>3</sub>) is formed during respiration of abalone, with the release of carbon dioxide (CO<sub>2</sub>). The dissociation of H<sub>2</sub>CO<sub>3</sub> results in the release of hydrogen ions (H<sup>+</sup>), which lowers the pH of water (Sanni and Forsberg 1996). An increase in metabolic processes, such as respiration can lead to a lowering of pH to a minimum threshold which can become a limiting factor for growth and feed intake. The minimum pH at which growth was reduced was 7.6 for *H. midae* (Naylor *et al.* 2011).

### *TAN and FAN*

Ammonia is a nitrogenous waste excreted by abalone (Barkai and Griffiths 1987). The concentrations of ionised and free ammonia are affected by pH, temperature and salinity and free ammonia is toxic to aquatic organisms (Thurston *et al.* 1981). The total ammonia nitrogen (TAN) is the ammonia concentration that is analytically measured in water samples. Total ammonia is known as the sum of the gaseous form of NH<sub>3</sub> and the ammonium ion NH<sub>4</sub><sup>+</sup>, while NH<sub>3</sub> (FAN) is the most toxic form for aquatic animals. Reddy-Lopata *et al.* (2006) suggested that a FAN concentration above 7 µg L<sup>-1</sup> was the level at which a 50 % reduction in growth was observed in juvenile *H. midae* with an estimation of 5 % reduction at 4 µg L<sup>-1</sup>. Therefore, FAN values below 4 µg L<sup>-1</sup> may not limit growth and feed intake.

### **The South African abalone farm industry**

Abalone farms use land-based intensive flow through aquaculture systems (Sales and Britz 2001, Robertson-Andersson *et al.* 2007). Commercial farms with these intensive systems were established in South Africa in the 1990s. Other abalone producers operate in Asia, New Zealand and the USA (Sales and Britz 2001).

Both *H. spadicea* and *H. midae* contain edible flesh that is commonly consumed by the people along the coastline of Southern Africa. However, due to a ban on fishing for abalone due to illegal fishing and overexploitation of both species, this is no longer permitted. Of the six species, *H. midae* is the largest species that is a commercially viable. There is an environmentally sustainable yet successful abalone aquaculture industry in South Africa (Troell *et al.* 2006; Venter *et al.* 2018). The species used for aquaculture, *H. midae*, is seen as a delicacy to Asian countries such as China and Japan, based on its large attainable size. These attributes drive the industry's success, primarily through export (Venter *et al.* 2018).

Being a lucrative industry, although relatively young in terms of industrial establishment, research and development has played a pivotal role in the industry's success. Research has allowed for the enhancement of the industry, improving farming practice and consequently the production of abalone. For example, researchers investigated crucial aspects of *H. midae* relating to farming practice and strategy such as spawning and seed production (Genade *et al.* 1985, Genade *et al.* 1988, Fleming 1999), the effect of environmental conditions on abalone physiology and feed intake (Hecht 1994, Britz *et al.* 1997), the development and refinement of superior formulated feeds (Britz *et al.* 1994, Fleming *et al.* 1996, Sales and Britz 2002), the effect of water quality on abalone growth and health (Yearsley 2008, Naylor *et al.* 2011) and abalone feeding behaviour (Barkai and Griffiths 1987, Knauer *et al.* 1996). Many of these aspects have been studied under different experimental environments such as smaller-scale well-controlled laboratory conditions and larger-scale commercial farming conditions.

### *Experimental conditions*

There are two experimental conditions used to produce information on the biology and farming of abalone. One of which is a well-controlled environment under laboratory conditions which is generally used to produce information at high levels of precision (Jobling *et al.* 2001). For instance, water temperature can be controlled and kept constant which is needed to determine the effect of different temperatures on feed intake and growth of *H. midae* as was conducted by Britz *et al.* (1997) who used thermostatically controlled aquarium heaters to control water temperature. Furthermore, given the influence of temperature on feed intake and growth of abalone, a well-controlled environment is needed to produce precise information with no confounding variables. Similarly, to quantify feed intake, there needs to be an environment that best simulates what occurs on abalone farms while maintaining a system that allows for

maximum precision when collecting data. For example, using dry-weight analyses to quantify feed intake needs an experimental system that allows for the least amount of sampling error, which could be due to the loss of uneaten feed (Jobling *et al.* 2001). This is not manageable under the second experimental condition, a larger-scale commercial farm environment. Larger scale experiments under farming conditions can be used to produce information on production parameters with larger sample sizes of abalone and is a direct simulation of what conditions occur on abalone farms making it more applicable to abalone farmers (Sales and Britz 2001, Troell *et al.* 2006). Comparing feed intake information and feeding intensity of *H. midae* under laboratory and farming conditions, respectively, will show whether well-controlled laboratory conditions can produce accurate information regarding abalone feed consumption on abalone farms. This has many practical implications as laboratory experiments can be used for future research and development on abalone farms. For instance, to test new formulated feeds, different water quality variables and aspects of abalone behaviour on feed intake and production parameters.

#### *Pellet types*

Testing new formulated feeds on abalone farms is an important parameter considered in the research and development departments (Troell *et al.* 2006). A multitude of studies have tested and optimised new formulations on growth and feed intake in *H. midae* but no studies have been conducted testing two pellet types of the same feed formulation but of different shape and size in *H. midae*. In this case, it is important to consider the feeding biology of *H. midae*.

When abalone find feed, they grasp onto the feed using a radula, which is mainly dependent on what the proximity is of the feed to the abalone (Barkai and Griffiths 1986). With regard to pellet sizes, smaller pellets will have a higher surface area to volume ratio interaction with the surrounding environment than larger pellets (Piedecausa *et al.* 2010), which affects the dispersion of pellets when spread into tanks and baskets containing abalone. Furthermore, due a higher surface area to volume interaction with surrounding water, leaching rates of formulated feeds are affected, with smaller pellets losing a higher percentage of mass to the water than larger pellets. For example, Piedecausa *et al.* (2009) showed that smaller pellets had a higher maximum TAN leaching rate compared to larger pellets when testing formulated feeds used for feeding farmed gilthead seabream *Sparus aurata* and seabass *Dicentrarchus labrax*. The study showed that in 15 °C water, pellets of 2mm diameter had a higher TAN value than

8-mm feed pellets after a 60-minute immersion period of 289.31  $\mu\text{g g}^{-1}$  TAN compared to 72.15  $\mu\text{g g}^{-1}$ , respectively. Thus, the size and shape of the pellet could influence leaching rates feed intake and consequently the growth of abalone and will be an important parameter to study. Hypothetically, larger pellet sizes could result in a longer duration of consumption per pellet but may take longer to detect, while smaller pellets are easily dispersed and are easier to detect but have a shorter consumption period per pellet.

Quantifying feed intake and growth of different pellet sizes could result in different feeding strategies for abalone farmers. In saying this, there have been studies conducted on the effects of different pellet types of the same formulation on production parameters in *H. asinina* (0.7 – 2.6 g abalone<sup>-1</sup>) by Lebata-Ramos *et al.* (2021). The study tested two pre-existing commercial practical diets pellet types known as “flakes” and “noodles” which differed both in shape and size with noodles measuring 1 cm long by 0.15 cm diameter and irregular-shaped thin flakes having a surface area of 2-3 cm<sup>2</sup>. Each pellet type had a refined and unrefined version. Lebata-Ramos *et al.* (2021) found that *H. asinina* fed the refined and unrefined version of flakes resulted in the better growth rates compared to the noodle treatments. Therefore, due to the results produced by Lebata-Ramos *et al.* (2021), there is a need to quantify growth using different pellet types of the same formulation in *H. midae*.

Despite the all-encompassing depth of research aimed to improve production, there is a need for research on the effect of stocking density and withholding feed on feed intake in *H. midae*. There have been numerous studies done on the refinement of formulated feeds with regard to nutrient content and digestibility, but there is a need to test the effect of different formulated pellet types of the same dietary composition on feed intake and growth of *H. midae*. Similarly, there is a shortage of research to improve the understanding the role of feeding intensity into commercial feeding regimens of *H. midae*, and the effect it has on production parameters such as growth rate, FCR and size variation. Furthermore, there is a paucity of information regarding feed intake and feeding intensity in *H. midae* above 60 – 70 g, emphasizing the necessity to quantify these variable in abalone above 70 g.

## **Aim and objectives**

The aim of this study was to quantify feed intake and the effect of feeding intensity on abalone production parameters using laboratory experiments and larger scale farm experiments.

The objectives of this study were to:

- Investigate the effect of production variables such as stocking density and withholding feed on feed intake when feeding resumes under laboratory conditions;
- Quantify the relationship between feed intake, abalone weight class and duration of feed availability under laboratory conditions;
- Investigate the influence of commercially relevant formulated feed pellet types on feed intake under laboratory conditions and abalone production parameters under farming conditions;
- Determine the effect of feeding regimens on abalone production parameters under farming conditions;
- Compare the findings of the experimental chapters with one another and with information from the literature;
- Explore whether feed intake trials under laboratory conditions can provide good estimates of feed intake when compared with feeding intensity under farming conditions;
- Provide suggestions for feeding methods and farming practices used on commercial abalone farms

## CHAPTER 2

### Feed intake in farmed South African abalone, *Haliotis midae*, Linnaeus

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#### **Introduction**

Feed intake in *H. midae* is greatly affected by water temperature and abalone size (Britz *et al.* 1997). Britz *et al.* (1997) illustrated that feed intake increased with temperature up to 20°C and decreased as water temperature exceeded 20°C. Feed intake (% of body mass per day) decreased logarithmically as a function of an increase in abalone size (Britz *et al.* 1997). Feed intake is also a function of the duration of food availability. Under laboratory conditions, gut fullness in juvenile *H. midae* was reached six hours after feed was given and a high percentage of the feed was consumed within six hours between 18:00 and 24:00 (Britz *et al.* 1996). The study showed that after six hours of feed availability, abalone consumed approximately six percent of their gut length's volume and 2 % BM of solids, and these values slowly declined throughout the night until the end of the sample period 36 hours later.

*Haliotis midae* is a nocturnal feeder, showing heightened feeding activity during the dark phase as compared to the light phase based on gut content and proximate analyses under small scale laboratory conditions (Britz and Sales 2001, Britz *et al.* 1996, Britz *et al.* 1997). Similarly, studies on feeding behaviour of cultured abalone indicated that between 60-80% abalone showed heightened levels of feeding activity at night on natural and pelletised feed until satiation was observed (Uki 1984, Knauer *et al.* 1996). Furthermore, Yearsley (2008) showed a decrease in dissolved oxygen concentrations during the night which was hypothesised to be due to the heightened levels of feeding activity in farm tank environments. Understanding peak feeding activity under laboratory conditions can provide abalone farms with feeding strategy options, with regard to the time-of-day farms should feed the abalone.

On land-based farms, where space is a limiting factor, stocking density is an important variable to maximise abalone production (Mgaya and Mercer 1995). Stocking density or population density and access to feed create an environment where competition for feed among abalone influences feed intake and growth (Lloyd and Bates 2008, Buss *et al.* 2015). Lloyd and Bates (2008) suggested that in both the wild and in hatcheries, growth and feed intake were impacted by density-dependent factors as a response to intraspecific competition for food or space. For example, an increase in stocking density resulted in a decrease in feed intake and growth in *H.*

*kamtschatkana* (25 – 45 mm shell length) with abalone at lower densities consuming 52 % more kelp (1.45 g kelp abalone<sup>-1</sup>) compared to higher densities (0.95 g kelp abalone<sup>-1</sup>) (Lloyd and Bates 2008). While the effect of stocking density on growth of farmed abalone has been studied, little is known about the effects of stocking density on feed intake in *H. midae*. It is also important for future studies involving feed intake, specifically in laboratory experiments, to understand feed intake as function of abalone density, which is expressed as percentage surface occupation. Percentage surface occupation can be defined as the number of abalone needed to occupy a given percentage of available surface area in a farm tank or specific basket design (Heath and Moss 2009).

Withholding feed is necessary when studying gut content analyses or gut evacuation times to ensure that the intestine does not contain food from a previous meal. Similarly, quantifying feed intake requires starvation for accurate results. In feed intake experiments, a pre-experimental starvation period can increase feeding response resulting in an overestimation of feed intake. Buss *et al.* (2015) observed that farmed *H. laevigata* elicited a 22% higher alert behaviour by moving more readily in daylight hours when fed restricted rations. Thus, a decrease in ration size may have an effect on behaviour and feed intake, especially since *H. laevigata* is a crepuscular and nocturnal forager that stops feeding after dawn (Buss *et al.* 2015; Currie *et al.* 2016). A period of withholding feed prior to an experiment may increase feed intake when feed becomes available. Therefore, determining the influence of starvation on feed intake is needed to establish how withholding feed influences feed intake.

Nutrient leaching loss from formulated diets due to prolonged immersion in water is a major concern in the industry (Fleming *et al.* 1996, Bansemer *et al.* 2015). Formulated pellets lost a substantial amount of content to the surrounding water, but this has since been optimised (Britz and Hecht 1997, Sales and Britz 2001). The optimised starch-bound protein rich pellets are dried but absorb water to soften, simulating the natural diet of abalone, kelp (Britz *et al.* 1994, Bansemer *et al.* 2015). In this process, a certain percentage of mass is lost to the environment. Quantifying feed intake using dry weight analysis where leaching indices are not quantified, leads to an overestimated feed intake value. Since this study utilised dry-weight analysis, quantifying the leaching rate of the feed used, is important and will be used to correct results from the feed intake studies.

Testing new formulated feeds on abalone farms is crucial to the research and development departments on abalone farms (Troell *et al.* 2006). Many studies have dealt with testing new formulations of growth and feed intake, but there is a paucity of information that pertains to testing two pellet types of the same feed formulation but of different shape and size on feed intake in *H. midae*. However, there have been studies conducted on the effects of different pellet types of the same formulation on growth in *H. asinina*. A study by Leбата-Ramos *et al.* (2021) found that abalone fed “flakes”, resulted in better growth compared to the other pellet types used. While feed intake was not quantified in their study, this ultimately shows that feed intake can potentially be influenced by pellet type of different shapes and sizes. Therefore, there is a need for quantifying feed intake of different pellet sizes which could result in different feeding strategies for abalone farmers.

Of the many studies conducted to optimise feed formulations and protein requirements for abalone, feed intake has also been quantified. However, not many studies use abalone that are close to marketable size or larger than marketable size of 70 g abalone<sup>-1</sup>. Therefore, understanding the effect that population density and withholding feed have on feed intake in *H. midae* may influence farming strategies. Similarly, a better understanding of the relationship between feed intake, weight class and duration of feed availability will aid in optimising feeding strategies in the commercial abalone industry. Therefore, this research aimed to:

- Quantify the effect of population density and withholding of feed, on feed intake when feeding resumes
- Determine a leaching index model of formulated feeds
- Quantify daily feed intake in three commercial abalone weight classes of 10 – 20 g, 100 - 110 g and 150 – 160 g abalone<sup>-1</sup>, using two pellet types of different sizes of the same formulation
- Determine the relationship between feed intake and the duration of feed availability in three abalone weight classes in order to gain an understanding of peak feeding activities under small scale experiments

## **Materials and methods**

### *Experimental system*

The experiments were conducted at HIK Abalone Farm (Pty) Ltd., in Hermanus South Africa (34°26'04.35" S; 19°13'12.51" E). The experimental system simulated a flow-through system (figure 1). It consisted of 15-L rectangular plastic tanks (36 cm x 27 cm x 16 cm), with a basket insert made of 6 mm oyster mesh (32 cm x 25 cm x 14 cm). Sea water was supplied from the hatchery, which was pumped from the sea and filtered through a drum filter (85 µm) and passed multiple times through a foam fractionator. Water temperature (°C) was controlled to 1.5 °C warmer than the sea temperature using a heat exchanger utilised in the hatchery. Water was supplied to each tank through a polyvinylchloride (PVC) water supply system. Water was gravity-fed into tanks through a 10-mm inlet and drained through a 20-mm outlet standpipe (figure 1, top) at the opposite end of the tank. Each basket contained a baffle plate on the standpipe side of the basket closest to the outlet to allow for particles to sink to the bottom (flow direction caused the feed to hit the baffle plate and sink) and allow for a negligible amount of matter to leave the experimental tank through the top of the outlet standpipe. Each basket contained a longitudinally-sectioned half of a 160-mm pipe (200 mm x 80 mm x 80 mm) to shelter the experimental animals. Each tank was covered with an outer dark casing and a dark floating plate to prevent light from entering the tank as abalone are photophobic. Aeration was supplied using aquarium tubing (5 mm diameter) installed with a 20-mm PVC airline along the water supply.



Figure 2.1: (Top): 15 L rectangular plastic tank (36 cm x 27 cm x 16 cm), with a basket insert made of 6-mm oyster mesh (30 cm x 25 cm x 14 cm) while showing outlet standpipe;

(Bottom): Eight of the 18 experimental tanks showing aeration tubing, PVC inlet, dark casing and dark floating plate.

### *Experimental animals and feed*

Abalone were obtained from the on-farm grow-out platforms, weighed to the nearest 0.1 gram using a scale (DIGI DS-530) and were randomly allocated into experimental baskets. The abalone acclimatised to the experimental system for six days, and feed was withheld 36 hours prior to the commencement of each experiment to ensure that abalone were not satiated from feeding during the acclimation period. During the acclimation period, the experimental tanks were fully cleaned every morning at 11:00 the following day. Abalone from each weight class came from the same batch numbers per weight class used in the experiments, which is the date at which they are placed from the hatchery onto the grow out platforms on a farm and were considered to be of similar age for all experiments. Abalone were fed Abfeed S32 leaves and short pellets (composition (g kg<sup>-1</sup>) for both pellet types: 320 g protein, 430 g carbohydrates, 20 – 60 g lipids). The difference between short pellets (SP) and leaves (L) was the weight and dimension per pellet. Short pellets weighed on average 0.2 ± 0.01 g pellet<sup>-1</sup> and leaves 2 g ± 0.01 g pellet<sup>-1</sup>. The dimension of short pellets was approximately 0.7 cm x 0.6 cm and leaves were 3 cm x 2 cm.. The nutrient composition of each pellet type was the same.

Table 2.1: Weight class ranges used in each feed intake experiment. Experiment 1 = Effect of population density on feed intake, 2 = effect of withholding of feed, on feed intake when feeding resumes, 3 = Effect of pellet type and weight class on feed intake and 4 = effect of duration of feed availability on feed intake in three weight classes.

Experiment	Weight class (g)	Weight range (g)
1	100 – 110	97.2 – 103.2
2	100 – 110	97.5 – 108.2
3	10 – 20	10.7 – 11.7
	100 – 110	107 – 109
4	150 – 160	156.3 – 160
	10 – 20	7.5 - 10
	100 – 110	94.7 – 106.9
	150 – 160	143.3 – 160

### *Data collection*

During sampling, uneaten feed was siphoned out and filtered through a filtration contraption comprised of a sieve bucket with a fixed 160-mm PVC socket and a lock tight insertable 150-mm PVC pipe. The contraption allowed for the entire tank volume to pass through it. At the sieve bucket a 25- $\mu$ m filter paper (Macherey-Nagel, REF: 431 015, medium-fast retention, Germany) was inserted per sample and was removed after each sample, stored and dried at the earliest convenience at 100°C using a convection oven. Each sample containing uneaten feed was then weighed multiple times using a micro-mass balance (DENVER SARTORIUS TR-104; 01-914-105, United Kingdom) throughout the drying period until the values stopped to decrease and stabilise, obtaining a final dry weight (g). Each filter paper dry weight (g) was initially weighed and included in the calculations for final dry weight consumed. Dry matter (%) of the feed was also determined before the experiment adjusting the initial dry weight (g) accordingly. This method was used in all feed intake trials related to the experimental system described. Water quality parameters such as water temperature (°C), dissolved oxygen (mg L<sup>-1</sup>), oxygen saturation (%) and pH were recorded at every sampling time in every experiment in the present study using hand-held dissolved oxygen probes and pH probes (dissolved oxygen and temperature: OxyGuard H02A2, pH: OxyGuard H04PP, Denmark).

### *Experimental design and statistical analyses*

#### *Leaching index*

The relationship between leaching index (% mass of feed lost) and duration of immersion of the formulated feed was determined using the experimental system with no experimental animals. There were six sampling times (17:00, 20:00, 23:00, 02:00, 05:00, 08:00, 11:00), each with triplicate samples. Data analysis was done using the averaged samples of the triplicates. This gave a three hourly leaching rate to be used as leaching index adjustments in all feed intake related trials by adjusting the mass of feed consumed (g) to avoid overestimation of feed intake.

Leaching index was calculated using the following formula:

$$L (\% \text{ lost}) = 100 - \left(\frac{r}{i} \times 100\right)$$

Where  $L$  is the leaching index shown as a percentage mass lost,  $r$  is the dry weight of feed recovered (g),  $i$  is the initial dry weight of feed

*Experiment 1: Population density on feed intake*

The effects of two population densities (% covered by abalone) were tested on feed intake (% body mass) and the interaction of these variables with the duration of feed availability, in one weight class (100 – 110 g abalone<sup>-1</sup>). The two population densities were 18 % and 25 % of the available surface area covered by the experimental animals in a basket. There were three sampling times: six hours, 12 hours and 18 hours post-feeding (23:00, 05:00 and 11:00). There were three replicates per treatment per sample time. This amounted to 18 experimental units. The experimental units were positioned randomly throughout the experimental waterway.

The formulae below were used to calculate the number of abalone needed per tank for a given population density  $p$  (Heath and Moss 2009).

$$\text{Area abalone}^{-1} = \pi \times \text{shell radius}^2$$

$$\text{Total number of abalone} = (\text{total available surface area} / \text{area abalone}^{-1})$$

$$\text{At population density } p: \text{Number of abalone} = p \times (\text{total number of abalone})$$

*Experiment 2: The effect of withholding feed on feed intake when feeding resumes*

The effect of a 48-hour feed withholding period on feed intake with the duration of feed availability was tested in one abalone weight class (100 – 110 g), the same animals as used in experiment one. There were two treatments, withheld and fed (pre-fed states), and three sample times (23:00, 05:00 and 11:00). There were three replicates, per treatment per sample time. This amounted to 18 experimental units. All experimental units were stocked at the same density of 18 % of total available surface area and at similar biomass values. Half the experimental units were randomly selected and not fed 48 hours prior to the commencement of the experiment (“withheld treatment”) whereas the other half were fed (“fed treatment”). All experimental units were fully cleaned with minimal disturbance to the animals before the experiment at 11:00 on the day of the experiment. The trial commenced at 17:00 later that day when all abalone were fed at 1.5 % body mass, which is in excess of what can be consumed to ensure that feed did not run out.

*Experiment 3: Effect of two pellet types and three abalone weight classes on daily feed intake*

The effect of pellet type on feed intake was tested using two formulated feed pellet types, leaves and short pellets, on three abalone weight classes over six days. The abalone weight classes used were 10 – 20, 100 – 110 and 150 – 160 g abalone<sup>-1</sup>. There were three replicates per weight class and three replicates for leaching control tanks without abalone. Abalone were fed at 1.5 % BM each day at 17:00, of each pellet type on alternating days; on day one they fed leaves in all experimental baskets, and on day two short pellets. This was repeated for six consecutive days in order to simulate on-farm feeding regimens. This resulted in three replicates per pellet type per weight class. Uneaten feed was siphoned out the following day at 17:00 (24-hour period). Each sampling period took approximately 15 minutes to siphon out all uneaten feed in each experimental basket. Therefore, abalone were fed 15 minutes later each day over the six-day experimental period.

*Experiment 4: The relationship between duration of feed availability and feed intake*

Using new abalone, feed intake was quantified as a function of the duration of feed availability over an 18-h feeding period in the same three commercially relevant weight classes (10 – 20 g, 100 – 110 g and 150 – 160 g abalone<sup>-1</sup>). Each weight class was tested separately, and data were treated as being collected in separate trials. The feeding period of 18 hours, consisted of six sampling times, 20:00, 23:00, 02:00, 05:00, 08:00 and 11:00. There were three replicates per sampling time, giving 18 experimental baskets. Abalone were fed at 1.5 % BM at 17:00 using short pellets. The sampling times consisted of two crepuscular phases, 17:00 to 20:00 and 05:00 to 08:00, a nine-hour dark phase between 20:00 to 05:00, and a three-hour light phase from 08:00 to 11:00. The leaching index model was used to obtain leaching correction values in these experiments.

*Feed intake*

Feed intake (F) was expressed in mass of feed consumed (g) which is a function of the dry matter-adjusted dry weight (g) provided minus leaching index and the dry weight of remaining feed. This can be expressed as a percentage of body mass (% BM) which is the function of mass consumed per unit biomass in a tank.

$$\text{Feed Intake (mass consumed g)} = G - (DM\% G) - (L\% R) - R$$

G was the dry weight of feed provided (g), DM was the dry mass index of the feed provided (%), L was the leaching index (% lost), R was the dry weight of the remaining feed (g)

And:

$$\text{Feed Intake (\% BM)} = \frac{\text{mass consumed (g)} \times \text{biomass (g)}}{100}$$

Percentage of feed consumed over 12 hours (3 hours crepuscular and 9 of dark phase) in each weight class was calculated using the average percentage consumption after 12 hours of feed availability and the feed intake (% BM) at a given time (t).

$$\text{Percentage consumed (\%)} = \frac{F(t)}{\text{Average consumed after 12 hours (\%)}} \times 100$$

#### *Data analysis*

The averages of feed intake were compared between each population density and pre-fed state and the interaction of these variables with duration of feed availability, using a multi-factorial analysis of variance (ANOVA). Similarly, the effect of the interaction between weight class and pellet type on average feed intake were tested using factorial ANOVA. Assumptions of equality of variance and normality of residuals were tested using Levene's test. Repeated analysis of variance (ANOVA) was used to compare the average feed intake between each pellet type within each 24-hour period (time). The assumptions for repeated measures ANOVA were checked using Mauchly's test of sphericity. If sphericity was violated, Huynh-Feldt and Greenhouse-Geisser corrections of F-statistics were used. Tukey's post-hoc test was used to compare the differences between the averages of the dependent variables against the independent variables. The relationship of feed intake as a function of weight class, duration of feed availability and water quality were determined using least-squares regression analysis. As the trials of each weight class were done separately in experiment four, a pairwise comparison of slopes analysis was performed to compare the three models of percentage consumed as a function of the duration of feed availability over the first 12 hours. As there were three models to compare using pairwise regression analysis, a Bonferroni correction value of  $p < 0.016$  was calculated ( $\alpha = 0.05/3$ ), as there were three planned pair-wise comparisons. If the p-value was below 0.016 then there was a significant difference between slopes. Analysis

of covariance (ANCOVA) was used to compare the slopes between models. Values are presented as average and standard errors (SE), unless stated otherwise.

## Results

### *Water quality*

Water temperature (°C), dissolved oxygen (mg L<sup>-1</sup>), oxygen saturation (%) and pH were recorded at every sampling time in each of the four experiments.

Table 2.2: Water quality variables across all feed intake experiments, including their minimum and maximum values. SD = standard deviation.

Experiment no.	Experiment	Water quality variable	Average	Minimum	Maximum	SD
1	Population density	pH	7.94	7.77	8.1	
		Temperature (°C)	17.16	16.5	17.56	0.42
		dissolved oxygen (mg L <sup>-1</sup> )	7.71	7.52	7.97	0.13
		Oxygen saturation (%)	99.46	97	102.8	1.67
2	Withholding feed	pH	7.95	7.8	8.07	
		Temperature (°C)	17.54	16.8	17.9	0.42
		dissolved oxygen (mg L <sup>-1</sup> )	7.57	7.43	7.69	0.08
		Oxygen saturation (%)	98.43	96.7	100	1.04
3	Pellet type, size and daily feed intake	pH	8.03	7.89	8.13	
		Temperature (°C)	18.75	18.3	19.2	0.31
		Dissolved oxygen (mg L <sup>-1</sup> )	7.72	7.43	8.17	0.17
		Oxygen saturation (%)	102.79	99.7	108.2	2.17
4	10 – 20 g abalone <sup>-1</sup>	pH	7.8	7.76	7.88	
		Temperature (°C)	16.16	15.5	17	0.46
		Dissolved oxygen (mg L <sup>-1</sup> )	7.93	7.68	8.14	0.13
		Oxygen saturation (%)	99.97	97.3	103	1.16
	100 – 110 g abalone <sup>-1</sup>	pH	7.86	7.74	8.06	
		Temperature (°C)	17.39	16.7	18.1	0.44
		Dissolved oxygen (mg L <sup>-1</sup> )	7.76	7.63	7.86	0.07
		Oxygen saturation (%)	100.22	98.6	102.2	0.96
150 – 160 g abalone <sup>-1</sup>	pH	7.79	7.7	7.88		
	Temperature (°C)	16.6	16	17.5	0.51	
	Dissolved oxygen (mg L <sup>-1</sup> )	7.89	7.72	8.06	0.1	
	Oxygen saturation (%)	100.41	98.2	102.2	0.94	

### *Leaching indices used for experiment four*

Leaching index (% mass lost) was linearly related with time of immersion (least squares regression analysis,  $r^2 = 0.90$ ,  $p < 0.001$ , figure 2.2). Average leaching index (% mass lost) after three hours was  $4.40 \pm 1.28$  % mass lost increasing linearly to  $16.70 \pm 1.01$  % after 18 hours.

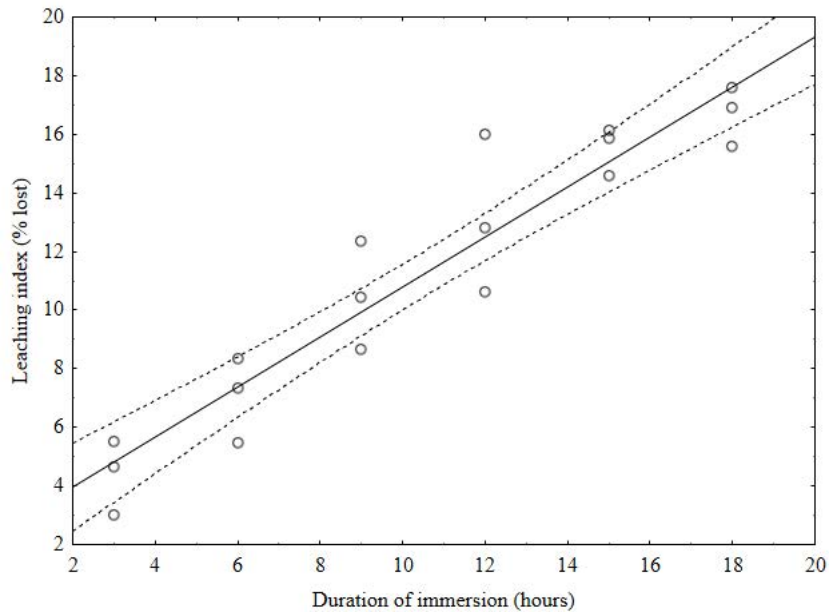


Figure 2.2: Leaching index (% lost) of short pellets as a function of duration of immersion (hours) at an average temperature of 17.7 °C (least-squares regression analysis,  $r^2 = 0.90$ ,  $p < 0.0000$ ,  $L$  (% lost) =  $0.8533x + 2.253$ ). The correction values derived from this model were used in experiments one, two and four.

### *Experiment 1: The effect of two population densities on feed intake*

There was no significant interaction between population density and the duration of feed availability for average feed intake (ANOVA;  $F_{2,12} = 0.94$ ,  $p = 0.415$ ). Feed intake was positively logarithmically related to the duration of feed availability (least-squares regression analysis;  $r^2 = 0.19$ ,  $p < 0.0363$ , figure 2.3a). Feed intake ranged from 0.27 – 0.34 % BM after the first six hours of feed availability (table 2.3). Feed intake increased by 0.07 % BM from six to 18 hour of feed availability. Feed intake ranged from 0.28 – 0.51 % BM at the end of the 18 hour period at an average of 0.37 % BM (table 2.3).

*Experiment 2: The effect of withholding feed on feed intake when feeding resumes*

There was no significant interaction between pre-fed state and the duration of feed availability for average feed intake (ANOVA;  $F_{2, 12} = 1.1542$ ,  $p = 0.348$ ). Feed intake showed a significant relationship with the duration of feed availability, with feed intake increasing logarithmically as the duration of feed availability increased (least-squares regression:  $r^2 = 0.22$ ,  $p < 0.026$ , figure 2.3 b). Feed intake ranged from 0.26 – 0.40 % BM after six hours (table 2.3). At the end of 18 hours of feed availability, feed intake ranged from 0.32 – 0.49 % BM at an average of 0.40 % BM.

Table 2.3: Average feed intake (% BM) and feed intake minimum and maximum at each sample time after the duration of feed availability (hours).  $n = 6$  at each sampling time, Min = Minimum, Max = Maximum, SE = Standard error of the average

Experiment	Duration (h)	Sample time	Feed intake	Min	Max	SE
1	6	23:00	0.3	0.27	0.34	0.01
	12	05:00	0.34	0.25	0.39	0.02
	18	11:00	0.37	0.28	0.51	0.03
2	6	23:00	0.33	0.26	0.4	0.02
	12	05:00	0.38	0.26	0.51	0.04
	18	11:00	0.4	0.32	0.49	0.03

Experiment 1 = The effect of population density on feed intake in *H. midae* and experiment 2 = The effect of withholding feed on feed intake when feeding resumes.

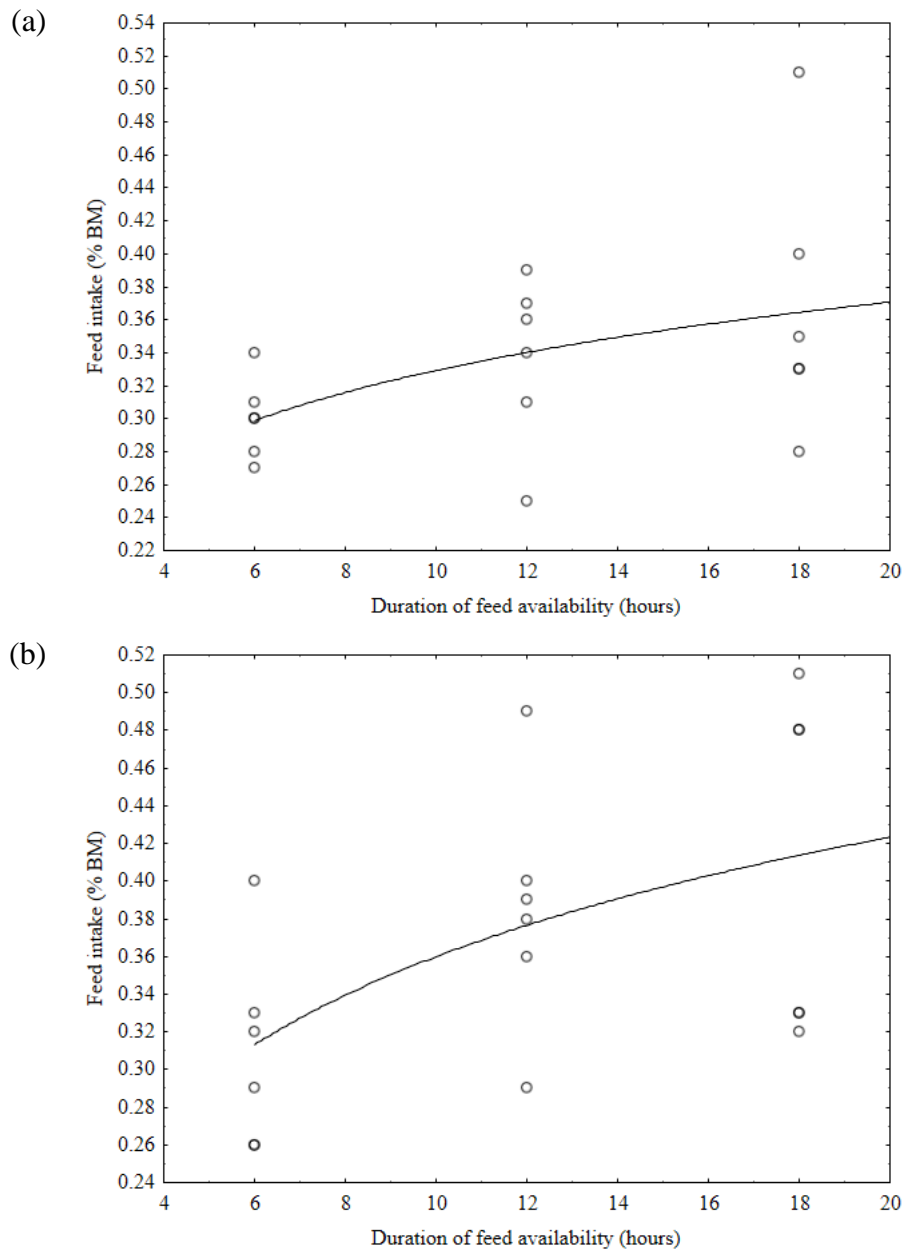


Figure 2.3: Feed intake (%BM) as a function of the duration of feed availability (hours) of 100 – 110 g abalone weight class. (a): Experiment 1: least-squares regression analysis;  $r^2 = 0.19$ ,  $p < 0.036$ ,  $F (\% \text{ BM}) = \text{Log}_{10} x (0.1378) + 0.1915$ . (b): Experiment 2: least-squares regression:  $r^2 = 0.22$ ,  $p < 0.026$ ,  $F (\% \text{ BM d}^{-1}) = \text{Log}_{10} x (0.2107) + 0.1491$ .

*Experiment 3: The effect of two pellet types and three abalone weight classes on daily feed intake*

There was no significant interaction between pellet type and weight class for feed intake (factorial ANOVA;  $F_{2,48} = 1.92$ ,  $p = 0.15$ ), but feed intake depended on weight class (ANOVA;  $F_{2,48} = 132.1$ ,  $p < 0.0001$ , figure 2.5 a). Average feed intake as % BM was highest for the smaller weight class (10 – 20 g abalone<sup>-1</sup>) at 0.55 % BM d<sup>-1</sup>, which was significantly higher than in the 100 – 110 g and 150 – 160 g weight classes. There was no significant difference in feed intake between the 100 – 110 g and 150 – 160 g weight classes at 0.303 % BM d<sup>-1</sup> and 0.301 % BM d<sup>-1</sup>, respectively (Tukey's post hoc test).

The SP pellet type leached the most at an average of 20.8 % loss after 24-hour immersion period with the L pellet type leaching 15.1 % (ANOVA;  $n = 9$  per pellet type,  $F_{1,16} = 66.1$ ,  $p < 0.0001$ , figure 2.4).

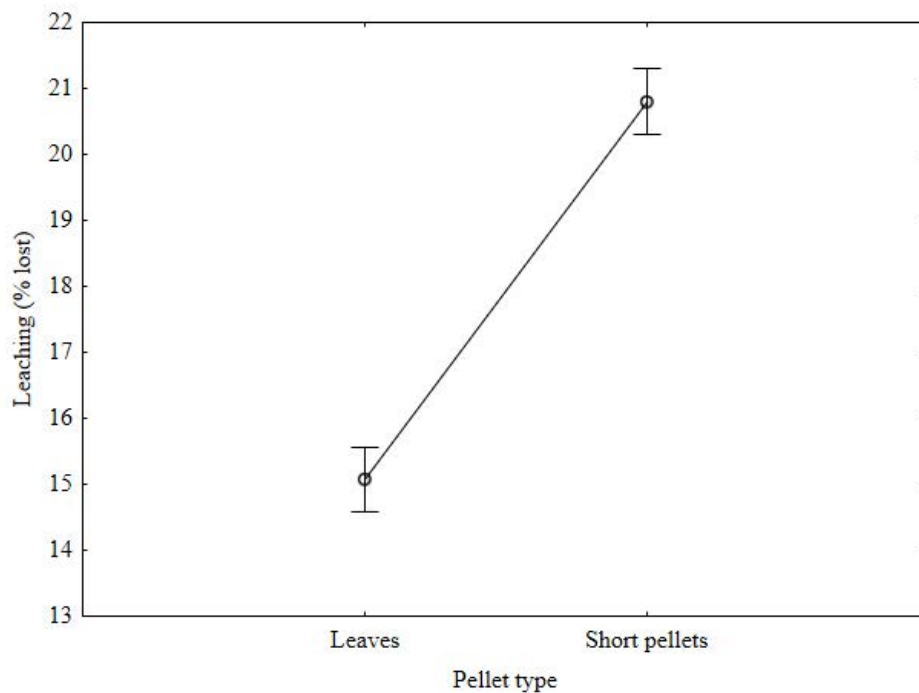


Figure 2.4: Average daily leaching index (% mass lost) of two formulated pellet types. Short pellets lost an average of 20.8% of mass and leaves lost and average of 15.1 % mass after 24 hours.

There was a significant interaction between weight class and time (repeated ANOVA;  $F_{10, 30} = 2.307$ ,  $p < 0.03$ , figure 2.5 b). Average feed intake was consistently higher in the 10 – 20 g abalone<sup>-1</sup> weight class over six consecutive days in comparison to the other weight classes. Over six consecutive days, average daily feed intake for the 10 – 20 g weight class ranged from 0.46 – 0.58 % BM d<sup>-1</sup>, 0.25 – 0.34 % BM d<sup>-1</sup> for the 100 – 110 g weight class and 0.24 – 0.36 % BM d<sup>-1</sup> for the 150 – 160 g weight class (table 2.4). The daily feed intake for each weight class did not follow the same trend over the six consecutive days. There was a drop in average daily feed intake between day two and three, SP (2) and L (3), in the 10 – 20 g weight class from 0.57 to 0.46 % BM d<sup>-1</sup> (figure 3.2 b), whereas, there was an increase in average daily feed intake between day four and five, SP (4) and L (5), in the 150-160 g weight class from 0.29 to 0.36 % BM d<sup>-1</sup> (figure 2.5 b).

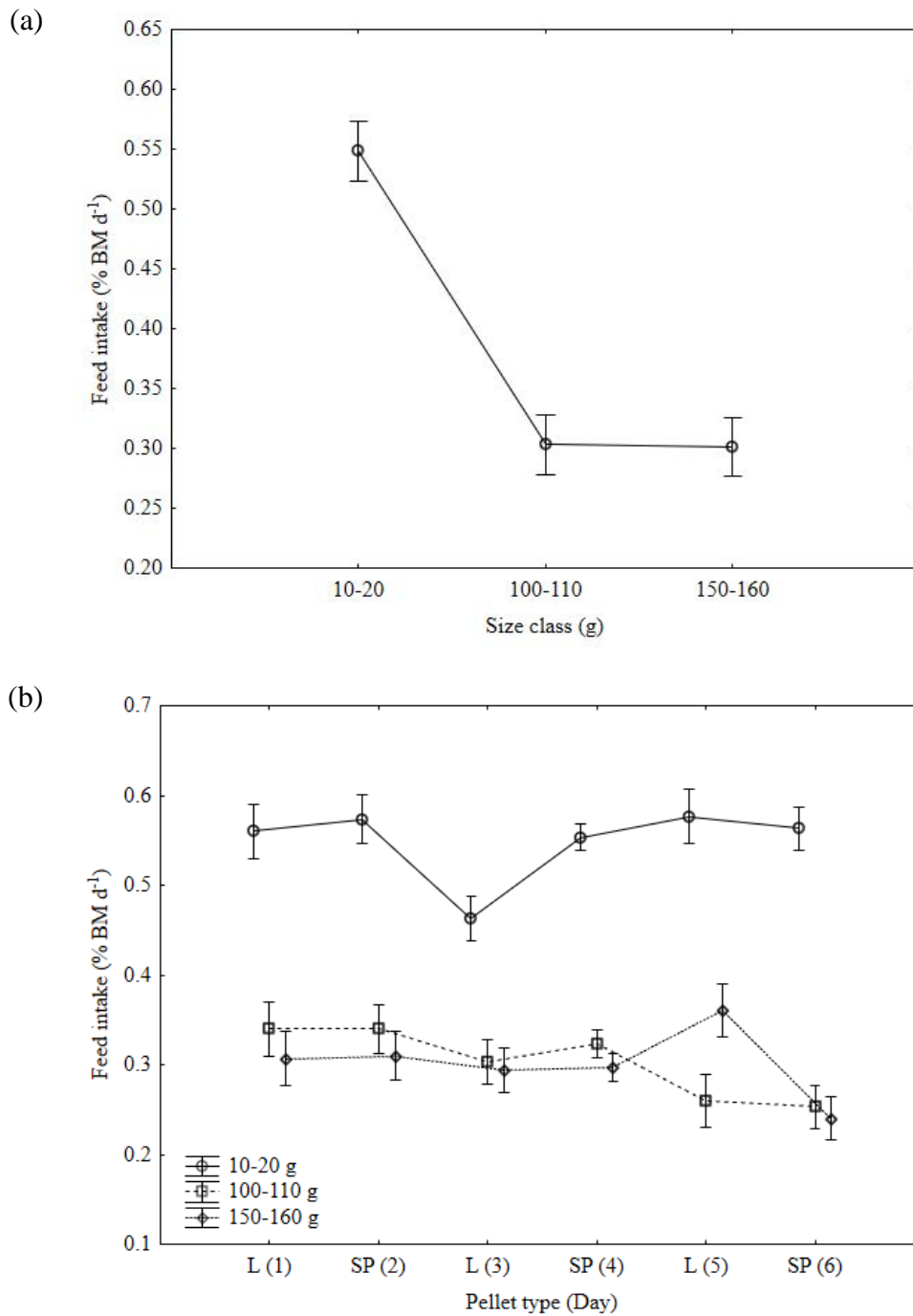


Figure 2.5 (a): Daily feed intake (%BM d<sup>-1</sup>) as a function of three abalone weight classes and (b): Daily feed intake as a function of abalone weight class over six days fed L and SP pellet type on alternating days (numbers in brackets = day). Vertical bars denote the standard error of the averages.

Table 2.4: Average daily feed intake of each weight class in experiment one, with minimum and maximum of daily average feed intake over the six-day period. SE = standard error of the

Weight class	Average	Minimum	Maximum	SE
10-20	0.548	0.46	0.58	0.04
100-110	0.304	0.25	0.34	0.04
150-160	0.3	0.24	0.36	0.04

*Experiment 4: The relationship between the duration of feed availability and feed intake*

Feed intake was a function of the duration of feed availability in all three weight classes. Feed intake in the 10 – 20 g weight class had a linear relationship with duration of feed availability (least squares regression analysis;  $r^2 = 0.76$ ,  $p < 0.00001$ , figure 2.6 a, table 2.5). Feed intake continued to increase into the early hours of the morning (05:00 to 11:00) between 12 – 18 hours of feed availability (figure 2.6 a, table 2.5). Feed intake increased logarithmically with an increase in the duration of feed availability for the 100 – 110 g and 150 – 160 g weight classes (least squares regression; 100 – 110 g:  $r^2 = 0.25$ ,  $p < 0.01$ , 150-160 g:  $r^2 = 0.52$ ,  $p < 0.0004$ , figure 2.6 b, table 2.5). Feed intake was highest after 12 hours of feed availability at an average of 0.41 % BM for the 100 – 110 g weight class which is the end of the dark phase at 05:00 (table 2.5). Feed intake after 15 hours of feed availability in the 150 – 160 g abalone weight class resulted in an average feed intake of 0.53 % BM (table 2.5), which is three hours into the light phase, i.e., 05:00 to 08:00 (table 2.5).

Percentage consumption over the first 12 hours of feed availability (3 hours of crepuscular light and 9 hours of dark phase) increased linearly as a function of duration of feed availability for all three weight classes (least squares regression; 10 – 20 g abalone<sup>-1</sup>:  $r^2 = 0.54$ ,  $p < 0.01$ , 100-110 g abalone<sup>-1</sup>:  $r^2 = 0.66$ ,  $p < 0.001$ , 150 – 160 g abalone<sup>-1</sup>:  $r^2 = 0.47$ ,  $p < 0.01$ , figure 2.7). The slopes of each model did not differ from each other (pairwise comparison of slopes; Bonferroni correction value ( $p < 0.016$ ); 10 – 20 g abalone<sup>-1</sup> with 100 – 110 g abalone<sup>-1</sup>:  $p = 0.07$ , 10-20 g with 150 – 160 g abalone<sup>-1</sup>:  $p = 0.04$ , 100 – 110 g with 150 – 160 g abalone<sup>-1</sup>:  $p = 0.53$ ). The intercepts did differ between each model (ANCOVA;  $F_{1,28} = 699.7$ ,  $p < 0.00001$ , figure 2.7). The intercept for 10 – 20 g abalone weight class was significantly higher than for the other two weight classes, thus they consumed a higher percentage of the feed provided over a 12-hour period.

There were no correlations between feed intake and water quality variables across all weight class trials (least squares regression; temperature: 10 – 20 g:  $p = 0.29$ , 100 – 110 g:  $p = 0.07$ , 150 – 160 g:  $p = 0.52$ , dissolved oxygen: 10 – 20 g:  $p = 0.42$ , 100 – 110 g:  $p = 0.96$ , 150 – 160 g:  $p = 0.34$ , pH: 10 – 20 g:  $p = 0.09$ , 100 – 110 g:  $p = 0.07$ , 150 – 160 g:  $p = 0.63$ ).

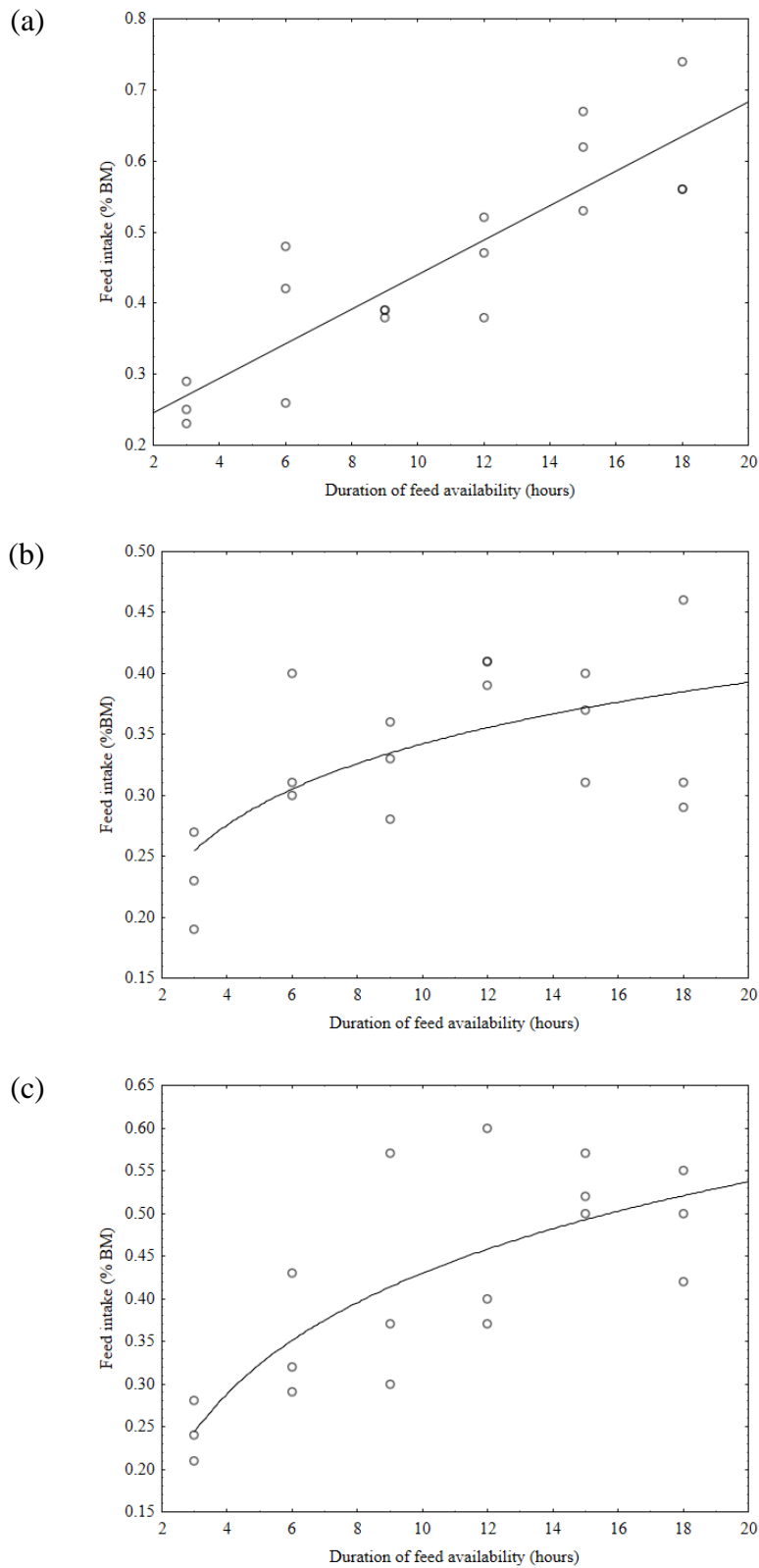


Figure 2.6: Feed intake (% BM) as a function of feed availability in 10 – 20 g (a), 100 – 110g (b) and 150 – 160 g (c) abalone weight classes. (a) Feed intake (% BM) =  $0.02x + 0.1976$ ,  $r^2 = 0.76$ ,  $p < 0.0000$ , (b) Feed intake (% BM) =  $\log_{10}(x) \cdot 0.17 + 0.17$ ,  $r^2 = 0.25$ ,  $p < 0.02$ , (c) Feed intake (% BM) =  $\log_{10}(x) \cdot 0.36 + 0.07$ ,  $r^2 = 0.52$ ,  $p < 0.0001$ .

Table 2.5: Average feed intake of each weight class at each sampling time after respective durations of feed availability (n=3 for each sample time). Light phase: C1 = crepuscular phase 1 (17:00 – 20:00), D = dark phase (20:00 – 05:00), C2 = crepuscular phase 2 (05:00 – 08:00), L = light phase (08:00 – 11:00). SE = standard error of the average.

Weight class (g)	Duration (hours)	Sampling time	Light phase	Feed intake	Min	Max	SE
10 – 20	3	20:00	C1	0.26	0.23	0.29	0.02
	6	23:00	D	0.39	0.26	0.48	0.07
	9	02:00	D	0.39	0.38	0.39	0.00
	12	05:00	C2	0.46	0.38	0.52	0.04
	15	08:00	L	0.60	0.53	0.67	0.04
	18	11:00	L	0.62	0.56	0.74	0.06
100 – 110	3	20:00	C1	0.23	0.19	0.27	0.02
	6	23:00	D	0.33	0.30	0.40	0.03
	9	02:00	D	0.32	0.28	0.36	0.02
	12	05:00	C2	0.41	0.39	0.41	0.01
	15	08:00	L	0.36	0.31	0.40	0.02
	18	11:00	L	0.35	0.29	0.46	0.05
150 – 160	3	20:00	C1	0.24	0.21	0.28	0.02
	6	23:00	D	0.35	0.29	0.43	0.04
	9	02:00	D	0.42	0.30	0.57	0.08
	12	05:00	C2	0.46	0.37	0.60	0.07
	15	08:00	L	0.53	0.50	0.57	0.02
	18	11:00	L	0.49	0.42	0.55	0.04

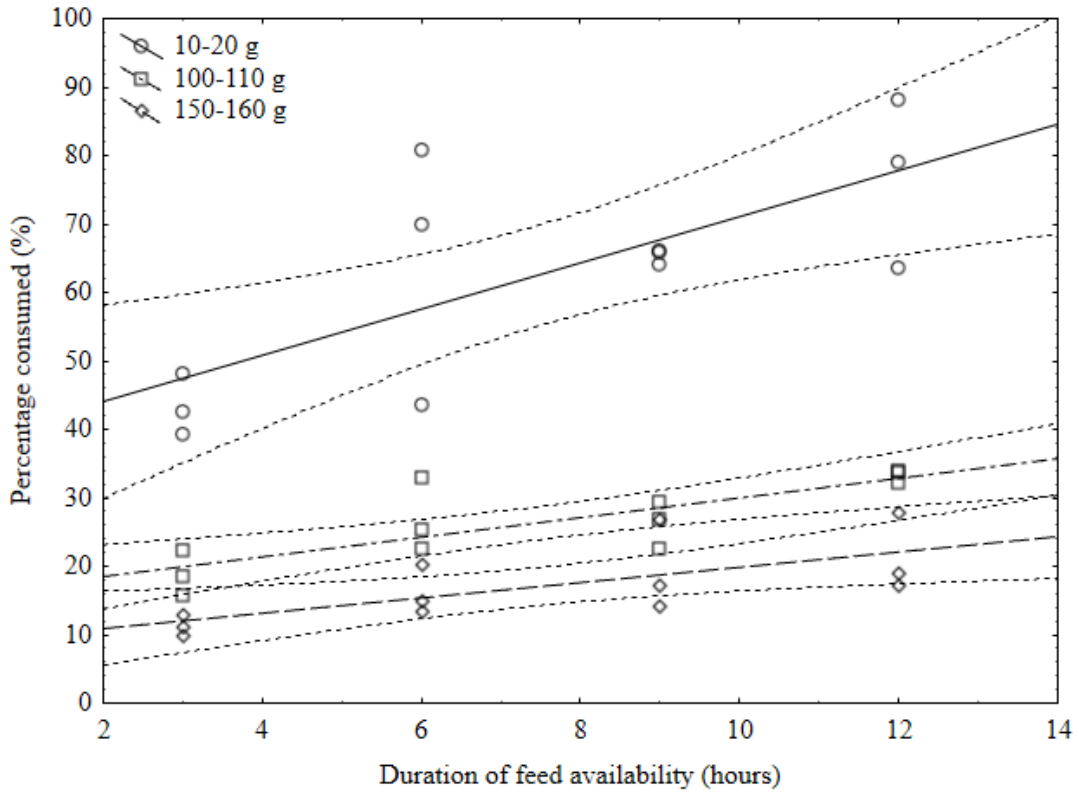


Figure 2.7: Percentage feed consumed (%) as a function of the duration of feed availability over 12 hours in three abalone weight classes. (10 – 20 g abalone<sup>-1</sup>: % consumption = 3.47x + 37.3,  $r^2 = 0.54$ ,  $p < 0.01$ , 100 – 110 g abalone<sup>-1</sup>: % consumption = 1.43x + 15.62,  $r^2 = 0.66$ ,  $p < 0.001$ , 150 – 160 g abalone<sup>-1</sup>: % consumption = 1.12x + 8.76,  $r^2 = 0.47$ ,  $p < 0.01$ ). Dashed lines indicate  $\pm 95$  % confidence intervals.

## Discussion

### *Water quality and feed intake*

Water temperature fluctuated between each sampling time by 0.42 °C at an average of 17.16 and 17.54 °C in experiment one and two, respectively, by 0.31°C between each day in experiment three and between each weight class trial by 0.46, 0.44 and 0.51 °C in the 10 – 20 g, 100 – 110 g and 150 – 160 g trials, respectively, in experiment four (table 2.2). The average water temperature over the six days of experiment three was 18.75 °C. The average water temperatures of the three trials in experiment four were 16.16, 17.39 and 16.60 °C. Feed intake in *H. midae* increased with an increase in water temperature with a high feed intake at a water temperature of 20 °C (Britz *et al.* 1997). This is due to the fact that Haliotids are thermoconforms which means that physiological processes are controlled by temperature (Prosser 1991, Garcia-Esquivel *et al.* 2007). Feed intake over 20 °C leads to a decrease in feed intake due to the breaking down of metabolic processes and physiological thresholds (Britz *et al.* 1997). Similarly, a water temperature below 12 °C resulted in a slowing of metabolism and feeding activity in *H. midae* (Britz *et al.* 1997). The water temperatures in all four experiments, are within the optimal temperature range of 12 – 20 °C for feed intake in *H. midae* and given that there were no correlations between temperature and feed intake, the narrow fluctuations in temperatures recorded within and between each experiment, are likely to have had no effect on feed intake.

Dissolved oxygen concentration from 7.04 – 9.12 mg L<sup>-1</sup> (89.9 % – 96.6 % saturation) resulted in no reduction in growth of *H. midae* in a study by Naylor *et al.* (2011). Similarly, Yearsley (2008) showed that abalone grew well at dissolved oxygen concentrations of 7.0 – 7.2 mg L<sup>-1</sup>. If dissolved oxygen is below these ranges, it becomes a limiting factor for feed intake and growth by reducing the capacity for aerobic metabolism (Harris *et al.* 1999). Due to the abalone's feeding activity, there is an increased demand for oxygen with evidence suggesting that due to the abalone's nocturnal feeding behaviour, there were lower dissolved oxygen concentrations at night compared to the day (Yearsley 2008). Therefore, it is suggested that the average dissolved oxygen concentrations in all experiments were inside the above-mentioned dissolved oxygen ranges (table 2.2). There were no relationships between dissolved oxygen and feed intake for each trial in experiment four, therefore, the dissolved oxygen concentrations likely had no effect on feed intake.

The pH of seawater is on average in the range of 8.08 to 8.33 (Marion *et al.* 2011). The average pH values in the present study across all four trials ranged from 7.79 – 8.03, which is lower than values provided by Marion *et al.* (2011) However, the ranges of pH in all experiments are well over the physiological apparent minimum of 7.6 for *H. midae*, where growth reduction was observed (Naylor 2012). Naylor (2012) indicated that pH was the first limiting water quality parameter in serial-use waterways. As pH in the current study was greater than 7.6 and as there were no correlations between feed intake and pH, pH was unlikely to have influenced feed intake.

### *Sampling method and system design*

Feed intake information in chapter two was measured using dry-weight analyses, which involved the sampling method of siphoning out uneaten feed into a custom-made filtration apparatus containing filter papers to allow an entire experiment tank content to pass through but catch uneaten feed. In this process, it is possible to weigh other solids such as faeces and suspended solids, however, before each experiment, feed was withheld for 48-hours to ensure the clearing of the abalone's gut from a previous meal in the acclimation periods. After this period, each tank was emptied and refilled, the morning of the commencement of each experiment. Given that Britz *et al.* (1996) illustrated that the solids within the gut contents of juvenile *H. midae* (14 g abalone<sup>-1</sup>) after feeding emptied to pre-feeding levels 38 hours after feed was given, the period of withholding feed of 48 hours in combination with tank cleaning methods in the present study should account for a negligible effect of other solids when sampling uneaten feed.

### *Experiment 1 and 2*

There were no effects of abalone population density and withholding feed on feed intake of *H. midae* in the present study. However, these two experiments allowed for the quantification of feed intake over 18 hours of feed availability in the 100 – 110g weight class. Feed intake after 18 hours was 0.37 and 0.4 % BM in experiments one and two, respectively. Feed intake increased by 0.07 % BM between 6 and 18 hours of feed availability. After six hour of feed availability in experiment one and two, feed intake was 0.3 and 0.33 % BM, respectively and proportionally, 81% and 83% were consumed after the first six hours of feed availability in this weight class. This is similar to a study conducted by Britz *et al.* (1996) who indicated that gut

fullness in juvenile *H. midae* occurred six hours after feed was given at the time of 24:00, indicating that the bulk of the feed was consumed within six hours, between 18:00 and 24:00.

These two experiments quantified feed intake in the 100 – 110 g abalone weight class over 18 hours, from 17:00 through to 11:00 the following day, incorporating a light and dark phase into the experiment. The influence of light and dark phases and the relationship it may have with feed intake and abalone size was quantified in experiment four.

### *Experiment 3: Daily feed intake*

Pellet type did not influence feed intake, but feed intake was a function of abalone weight class. Physiological models regarding feed intake as a function of animal weight expressed as a percentage of body mass in aquatic species is an almost unbreakable relationship for all aquatic organisms (Jobling *et al.* 1993, Britz *et al.* 1997). “The relationship between growth and feed intake with body weight can be described with a power function, and logarithmic transformation, where an increase in body weight results in a lower feed intake per body mass” (Jobling *et al.* 1993). For instance, the study conducted by Britz *et al.* (1997) described feed intake as a function of *H. midae* size of approximately 15 to 60 mm shell length at different temperatures from 12 °C to 20 °C. The study found that feed intake was highest at 20 °C, with abalone of 15 mm shell length consuming approximately 2.5 % BM d<sup>-1</sup> and abalone of 60 mm shell length consuming approximately 0.5 % BM d<sup>-1</sup>, resulting in the construction of a logarithmic relationship that shows an increased feed intake as a percentage of body mass of smaller abalone compared to a lower feed intake in larger abalone.

The present study, however, did not show a similar trend as Britz *et al.* (1997) and the above-mentioned model regarding feed intake as a percentage of body mass is not fully confirmed by the results of this study. For instance, feed intake as a function of abalone weight class was not apparent throughout all the weight classes used in the present study. While abalone in the smallest weight class of 10 – 20 g abalone<sup>-1</sup> consumed a significantly higher percentage of body mass at 0.55 % BM d<sup>-1</sup> compared to the larger abalone weight classes of 100 – 110 g and 150 – 160 g abalone<sup>-1</sup>, there was no significant difference in feed intake between the 100 – 110 g and 150 – 160 g abalone<sup>-1</sup> weight classes which were pooled at an average daily feed intake of 0.30 % BM d<sup>-1</sup>. This indicates that abalone that are 50 g heavier have the potential to

consume the same amount of food, as a percentage of body mass, compared to abalone that are 50 g lighter, which in this case is between the 100 – 110 g and 150 – 160 g weight classes.

Furthermore, Green *et al.* (2011) determined the effect of dietary protein : energy contents on growth in *H. midae* at different water temperatures, consequently quantifying feed consumption in abalone of 29 g. The results of their study showed that abalone in this weight class consumed 0.55 % BM d<sup>-1</sup> when fed a diet containing 26 % protein with a low energy content, which is similar to the daily feed intake values of the present study in the 10 – 20 g weight class which was quantified at 0.55 % BM d<sup>-1</sup>. While the study conducted by Green *et al.* (2011) used different formulated feeds, and the abalone used were marginally larger, the comparison of the feed intake results of the present study with the results of Green *et al.* (2011), shows that the information produced in the present study can be used as comparatives for existing and future studies dealing with feed intake in *H. midae*.

In addition, Gomez-Montes *et al.* (2003), tested the effect of different protein:energy formulations on feed intake in *H. fulgens* (0.20 g abalone<sup>-1</sup>). The study quantified feed intake to range across each of the treatments from 1.43 – 1.65 % BM d<sup>-1</sup>. Given that the abalone used in the study by Gomez-Montes *et al.* (2003) of an average weight of 0.2 g abalone<sup>-1</sup>, are smaller than the smallest weight class in the present study of 10 – 20 g abalone<sup>-1</sup>, the higher consumption as a function of body mass conforms with the logarithmic relationship between feed intake and body weight, with smaller abalone consuming a higher percentage of body mass than larger abalone, in this case. Furthermore, the results in the present study can be used as reference values for other abalone species and the information produced in this study should be taken into consideration when quantifying feed intake in abalone weight classes above 100g.

An increase in abalone size from 10 to 100 g abalone<sup>-1</sup> resulted in a lower feed intake as a function of an increase in abalone weight. However, the similar feed intake values (% BM) between the two larger weight classes of 100 – 110 g and 150 – 160 g abalone show the need for further studies to explore such a phenomenon. Given that there is a paucity of information regarding daily feed intake in *H. midae* above 70 g, the present study has shown that farmed *H. midae* that fall in the weight range of 100 – 160 g have the potential to consume the same amount of food on a daily basis. Therefore, with regard to feed intake of *H. midae*, the present study quantified feed intake of abalone in weight classes that have not been published for this

species. The present study has produced information regarding feed intake in *H. midae* that can be used as reference values for future studies in *H. midae* and other abalone species.

#### *Experiment 4: Duration of feed availability*

Feed intake in all weight class trials had a significant relationship with the duration of feed availability. In the 10 – 20 g weight class, feed intake continued to increase linearly throughout the duration of feed availability through the light phase. Abalone began to consume food in the first crepuscular phase (sunset) all the way through to the end of the light phase (table 2.5). Feed intake after 18 hours reached an average of 0.62 % BM ranging from 0.56 – 0.74 % BM (table 2.5). Britz (1996 a) showed that feed consumption in 10 g *H. midae* weight class, ranged from 0.5 – 0.8 % BM d<sup>-1</sup> when animals were fed casein-based and fishmeal formulated feeds. In comparison to experiment three, daily feed intake of this weight class was at an average of 0.55 % BM d<sup>-1</sup>. Given that feed intake was quantified over 18 hours in experiment four, the remaining six hours are absent, and cannot be extrapolated using the regression model, but rather compared to experiment three.

Average feed intake in the 100 – 110 g weight class after 18 hours of feed availability was 0.35% BM. However, after 12 hours of feed availability, feed intake was the highest at 0.41% BM. Abalone in this weight class, like the 10 – 20 g weight class, began to consume food in the first crepuscular phase at 0.23 % BM but did not consume more food after the dark phase between the second crepuscular phase and light phase (table 2.5). The significant relationship between feed intake and the duration of feed availability in this weight class suggests that feed intake slowed down although feed remained available. Feed intake after 18 hours can then be estimated to be in the range of 0.23 – 0.41 % BM. Comparatively with experiment three, the average daily feed intake of this weight class was 0.30 % BM d<sup>-1</sup> and it ranged from 0.25 – 0.34 % BM d<sup>-1</sup>. There is a paucity of information pertaining to feed intake in *H. midae* of this weight class using dry-weight analysis, which gives room for future validation studies.

Feed intake in the 150 – 160 g weight class increased logarithmically as the duration of feed availability continued. Like the previous two weight classes, feed intake began in the first crepuscular phase and resulted in an average feed intake of 0.24 % BM. Interestingly, feed intake in this weight class, abalone consumed a higher percentage of body mass compared to

the 100 –110 g abalone weight class of 0.49 % BM after 18 hours. Which show a similar trend with the information produced in experiment three, where larger abalone consumed a higher percentage of body mass compared to smaller abalone. Feed intake was highest after 15 hours of feed availability at 0.53 % BM, with abalone consuming feed into the second crepuscular phase between 05:00 – 08:00.

The results of the present study suggest that peak feeding activity occurs at night or under darker conditions than during the day for larger abalone, with smaller abalone of 10 – 20 g abalone weight class feeding consistently into the daylight hours. This suggests that peak feeding activities is also a function of abalone weight class meaning that smaller abalone have the potential to consume food under light conditions where larger abalone do not. Similarly, all size classes began to consume food under sunset conditions (crepuscular phase one, table 2.5), and it shows that abalone should be fed just before sunset.

#### *Feed intake and abalone aquaculture*

The utilisation of daily feed intake values can be crucial information to abalone farmers. The values obtained in the present study show what *H. midae* across all the relevant weight classes can consume on a daily basis under small-scale laboratory conditions. This study presents an opportunity to test whether quantifying feed intake under smaller scale well-controlled laboratory experiments can provide accurate feed intake representations of what occurs on abalone farms, which can further be implemented as the basis for determining daily rations. Using the values to determine a daily ration can be calculated using: Ration size (g) =  $(F \times \text{biomass})/100$ , where F is the feed intake value as a % body mass per day and biomass is the mass (g) of the animals in a given basket or tank.

However, due to the short experimental periods in the present study and the experimental system, using what abalone can consume as the basis to determine daily feeding rations could potentially lead to wrong estimates. Given that these experiments were not run in a farm tank and basket environment, the feed intake values in the present study should be compared to a longer on-farm growth trial that focuses on the influence of feeding intensity on production parameters. Feeding intensity takes into account the growth rate of the animal and feed conversion ratio of the feed, essentially taking into account the efficiency at which abalone consume food that results in growth. Feeding intensity can be calculated using;  $FI = G \times FCR$ .

This is a modified version of a formula that has been used by Britz *et al.* (1997) who tested the effect of temperature on feed consumption and growth in *H. midae* (up to 60 mm shell length). The formula used by Britz *et al.* (1997) took the form:  $C = \text{daily growth rate (\% BM d}^{-1}) \times \text{FCR}$ , where C is consumption of feed and FCR is feed conversion ratio. Britz *et al.* (1997) however quantified “feeding intensity” in abalone up to 60 mm shell length ( $\pm 20 - 30 \text{ g abalone}^{-1}$ ), which emphasizes the need to research feeding intensity in abalone of similar weight classes of the present study due to a paucity of information regarding growth, FCR and feeding intensity in larger abalone.

## **Conclusion**

The quantification of feed intake in the weight range of 10-160 g abalone<sup>-1</sup> has produced important information that can be used as comparatives in future studies regarding the biological understanding of *H. midae*. The improved understanding in this aspect can provide abalone farmers with critical information that could potentially benefit the farm. Daily feed intake as a function of body weight in abalone above 100 g did not conform with normal physiological models regarding feed intake, which show the need for future studies regarding feed intake in *H. midae* above 100 g.

The present study determined that abalone population densities of 18% and 25 % surface occupation does not effects feed intake in *H. midae*, however further studies need to determine the effect of these densities on abalone production parameters. Withholding of feed for 48 hours did not affect feed intake when feeding resumed, which is important information for future studies involving gut content analyses and proximate analyses when quantifying feed consumption of abalone. The correlation of feed intake as a function of the duration of feed availability indicates that abalone begin feeding at sunset which holds value to farmers when determine times to feed abalone. The 10 – 20 g weight class consumed feed throughout the duration of feed availability, into the light phases, which shows that abalone farmers can feed this weight class at any time of the day. This relationship should be tested in future studies that potentially incorporates behavioural aspects of *H. midae* feed intake.

## CHAPTER 3

The effect of formulated pellet type and feeding regimen on growth, feed conversion ratio, daily percentage feeding intensity and size variation in farmed *Haliotis midae*

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### **Introduction**

Feeding pelletised feed on abalone farms has become a feeding strategy around the globe (Sales and Britz 2001). A multitude of research has gone into the development of pellet nutrient composition and optimised formulations (Britz *et al.* 1994, Fleming *et al.* 1996, Sales and Britz 2001, Sales and Britz 2002). However, there are no published studies that have tested pre-existing commercial pellet types, i.e., size and shape, of the same formulation and nutrient composition in *H. midae*.

The size and shape of food items are considered to be important at each stage of ingestion (Stradmeyer *et al.* 1988, Smith *et al.* 1995). These stages are the detection of the pellet, the acceptability of the pellet and finally ingestion. Furthermore, the size and shape of a pellet may influence the detectability of the pellet and probability that an animal will find the pellet for ingestion. Detectability and probability of detection can be directly influenced by the surface area to volume interaction with the surrounding environment of the pellet. For example, as pellet size increases surface area to volume ratio decreases (Piedecausa *et al.* 2010). Therefore, it can be hypothesised that smaller pellets can result in a higher dispersion due to the higher surface area to volume ratio, however this can be influenced by the feeding regimens used by farms.

Different pellet types have been tested on production parameters in *H. asinina* of 0.7 – 2.6 g abalone<sup>-1</sup> weight class (Lebata-Ramos *et al.* 2021). The study tested the effect of different pellet shapes on abalone growth, FCR and morality. The pellet shapes were called “flakes” and “noodles” which differed mainly in shape with flakes having a more irregular thin shape with the surface area of 2 – 3 cm<sup>2</sup> and noodles having a length of 1 cm at a diameter of 1.5 mm. Abalone fed the flakes treatments resulted in a heavier final weight that was 4 % heavier and higher growth rates that were 8 % faster compared to the noodle treatments while FCR (2.2 –

2.5:1) and mortality did not differ between pellet types. This stresses the need to quantify production using different pellet types of the same nutrient composition in *H. midae*.

Additionally, size and shape of pelletized feed has been studied in salmon. Stradmeyer *et al.* (1988) tested the effect of pellet type (shape, size and texture) on feed preference in Atlantic salmon *Salmo salar*. Farmed *S. salar* preferred a pelletised feed that more accurately represented the shapes and sizes of feed particles that resemble prey in the wild. Due to *S. salar* being a predatory fish, long thin and long fat pellets were ingested more readily than round flat pellets which was hypothesised due to the pellets shape resembling small fish prey (Stradmeyer *et al.* 1988). Similarly, the long thin and long flat pellets were better suited to the mouth morphology of *S. salar*, possibly leading to a higher consumption of these pellet types compared to round pellets.

Similarly, a prerequisite for the optimal and efficient production of salmonid species, is the understanding of the size of food particles used as it affects the acceptance of the feed, and the growth of the animal (Jobling *et al.* 1993). For example, growth of Atlantic salmon (4 – 20 cm length) is most rapid when they are fed particles with a diameter that is 2.2 – 2.6 % of the fork length (Wankowski and Thorpe 1979) as was determined by Tabachek (1988), where growth of arctic charr was most rapid when fed food particles with a diameter that was 2.4 % of the fork length.

Given the anatomical features and feeding behaviour of *H. midae*, namely a radula to grasp onto feed once feed is found during the abalone's opportunistic grazing behaviour (Barkai and Griffiths 1986), the size and shape of the pellet could present an influencing factor in aquaculture. For instance, larger pellet sizes could result in a longer duration of consumption per pellet but may take longer to detect, while smaller pellets are easily dispersed and are easier to detect but have a shorter consumption period per pellet. Therefore, and hypothetically, feeding both large and small pellets to abalone could result in a combination of a high dispersion of pellets and favourable long duration of consumption per pellet once detected.

The South African abalone *Haliotis midae* is a slow growing gastropod, however, *H. midae* grows faster in the abalone land-based farming industry compared to *H. midae* in the wild (Hahn 1989; Troell *et al.* 2006). Accelerated growth rates in captivity are due to a more efficient

feed conversion ratio (FCR) with the implementation of artificial diets with optimised protein levels (Britz *et al.* 1994). Furthermore, such accelerated growth rates in captive *H. midae* are due to convenient access to the feed, where the consumption of the feed as a function of body size decreases with an increase in abalone size (Britz *et al.* 1994).

Abalone farmers are investigating feeding regimens that improve the on-farm growth rate of the abalone, while also accounting for the cost of feeding (Troell *et al.* 2006; Venter *et al.* 2018). As the grow-out phase is longer than it is in most aquaculture species, this phase contributes a major portion of the cost of abalone aquaculture production. Some of these costs include the cost of the feed, the labour associated with feeding, the water pumping costs and electricity costs for farm operations. Research is required to minimise these costs and in abalone aquaculture, there is limited information regarding this aspect (Currie *et al.* 2016). Typically, the grow out phase begins with an abalone weight of approximately 1–5 g abalone weight class, which have been reared in the hatchery. *H. midae* grow only two to three centimetres per year and take approximately four years to reach a weight of approximately 80 g abalone<sup>-1</sup> (Venter *et al.* 2018), which emphasises the investment abalone farms put into growing abalone to market size.

Similarly, testing two pellet types may yield results that are beneficial on abalone farmers. Understanding the effects of different pellet types on production parameters provide different feeding strategies that can possibly implemented into feeding regimens. Production parameters such as daily growth rate, feeding intensity and feed conversion ratio, are greatly affected by the feeding regimen used by a farm (Sales and Britz 2001). Feeding a ration size that is size- and species-specific is of vital importance as it presents the animal with the daily feed requirement for consistent growth (Chua and Teng 1982, Britz *et al.* 1994, Sales and Britz 2001). Optimising feeding regimens can allow for maximum growth at a high efficiency, and because feed intake controls growth and metabolic rates, an inefficient feeding regimen can decrease profit on farms (Goddard 1996, Kaiser *et al.* 2011). A measure of this can be calculated using feeding intensity. Feeding intensity is a function of the rate of growth and the efficiency at which feed is converted into growth after a given growth duration or period (feed conversion ratio, FCR):  $FI = G \times FCR$  (Britz *et al.* 1997). Which is a modified version of a formula used to calculate feed consumption ( $C = FCR \times \text{percentage weight gain per day}$ ) in *H. midae* using the growth and FCR data by Britz *et al.* (1997).

In aquaculture, a fundamental driver for success and generation of revenue is fast growth of the species. Research and development aim to maximise growth while maintaining efficient feed conversion ratios (FCR). Abalone farms generally utilise a feeding method where abalone of all weight classes are fed to apparent satiation using a similar ration size, as this approach is practically and logistically manageable. This can lead to certain weight classes being underfed while some are overfed, which results in wasted feed and deteriorating water quality or potential loss in growth (Britz *et al.* 1994). In order to maximise growth, the optimisation of feeding regimens and feeding intensity needs to be explored.

As feed intake increases due to a better access to the feed in abalone farms, it can be used as a means for calculating daily rations (Britz *et al.* 1994). A term that encompasses feed intake without directly measuring consumption can be defined as “feeding intensity”. Feeding intensity is calculated using the growth rate and FCR achieved to attain the growth rate after a certain growth period (G multiplied by the FCR), essentially meaning it encompasses that rate at which animals consume food that results in growth at a certain feed conversion efficiency. While feeding intensity isn’t the term that is used by studies that determine abalone feed consumption, studies that produce information on abalone growth with corresponding FCR values can estimate abalone feed intake. For instance, Britz *et al.* (1997) quantified growth and FCR of *H. midae* at different water temperatures and further predicted abalone feed consumption at the same temperatures using both the growth rate and FCR of growth trial results. Nevertheless, many studies involving abalone production parameters do not include the term “Feeding intensity” or at least the calculations for it. Additionally, the relationship between feeding intensity, growth rate and FCR is not well documented in *H. midae*.

For example, Britz (1996 a) determined the effect of different protein contents on growth and FCR in *H. midae* (1.76 g abalone<sup>-1</sup>) which resulted in an average growth rate of 0.9 % BM d<sup>-1</sup> (% body mass per day) for abalone fed a fishmeal based pellet with an average FCR of 0.8:1. Therefore, using the formula to calculate feeding intensity, the feeding intensity would be 0.72 % BM d<sup>-1</sup> for abalone of 1.76 g abalone<sup>-1</sup> fed a fishmeal based pellet after a 124-day growth period. Although feeding intensity and feed intake both involve abalone feed consumption, feeding intensity takes into account the rate at which abalone grow in relation to what they consume and can therefore be compared as follows. Britz (1996 a) found that feed intake in (%)

BM d<sup>-1</sup>) of the fishmeal pellet was 0.8 % BM d<sup>-1</sup> and comparing it to the resultant feeding intensity of the trial of 0.72 % BM d<sup>-1</sup>, the difference in percentage is 11 % i.e., using 0.8 % BM as a means for calculating a daily ration for 1.76 g abalone weight class could result in 11 % higher daily ration as a percentage of body mass compared to if 0.72 % BM d<sup>-1</sup>. This further emphasises the need to quantify feeding intensity and the possibility of using it as the basis for determining daily feeding rations.

On abalone farms, the size-grading process of abalone is important in identifying the slower growing individuals (Heath and Moss 2009). Keeping faster growing individuals together can result in a more efficient management of abalone that will reach a marketable size in a shorter time. Similarly, keeping slower growing abalone together can result in an increased growth rate due to the lack of fast-growing competitors in the New Zealand abalone *H. iris* (Heath and Moss 2009). Size-frequency distributions can be used to identify the slower and faster growing abalone in an abalone population (Leighton and Boolootian 1963). Furthermore, using the standard deviation and average of the population in a z-test, will allow for the estimation and probability that an animal will be in or above a given size range. There are no studies involving the use of weight-frequency distributions in *H. midae* as a measure of size variation with the calculation of the probabilities of abalone falling into a given size class after a growth period fed a specific feed.

Therefore, the relationship between feeding intensity with growth and FCR needs to be explored. A high feeding intensity could be due to both a high growth rate and FCR which means growth was less efficient, which would need to be explored further by conducting an economic analysis. To quantify the optimal feeding intensity would be to determine a feeding regimen that results in the highest growth rate at the lowest FCR. Utilising a feeding regimen that provides an optimal daily ration of feed could result in a feeding intensity at the lowest FCR without reducing growth.

Therefore, the aim of this study was to determine and quantify the effect of three pellet type strategies using a daily ration feeding regimen on daily growth rate, daily feeding intensity, feed conversion ratio and size variation in 30 – 40 g abalone weight class and to determine the relationship between feeding intensity, daily growth rate and FCR between pellet types. Furthermore, three commercially relevant weight classes, 10 – 20 g, 100 – 110 g and 150 – 160

g abalone<sup>-1</sup> of *H. midae* were used to test the effect of four industrially relevant feeding regimens on growth, feeding intensity, FCR and size variation.

## **Methods and materials**

The present study consisted of two experiments focusing on production parameters such as daily growth, feed conversion ratio, daily feeding intensity and size variation by testing in experiment one, the effects of different pellet types and in experiment two, the effects of feeding regimen on these parameters.

### *Experimental system*

Experiments were conducted at HIK Abalone Farm (Pty) Ltd., in Hermanus South Africa (34°26'04.35" S; 19°13'12.51" E) from August to December 2021. Abalone were kept in rectangular farm tanks (3 m x 2 m x 1.5 m) which were part of the grow-out platforms in a flow-through sea water system. Sea water was pumped and filtered through a 100- $\mu$ m micro-screen drum filter directly from the sea and then gravity-fed to each tank on the grow out platforms. Water was supplied from one end (inlet) and exited the tanks at the opposite end through a standpipe (outlet). Each tank in experiment two had twelve baskets, six along each half of the tank and six baskets in one tank (smaller tanks) in experiment one. Each basket consisted of "oyster mesh" and contained vertical plastic racks, providing surface area to the abalone in the basket (3.2 m<sup>2</sup>). On top of the vertical racks was a floating horizontal feeder plate. Feed was provided on top of the feeder plate and underneath the feeder plate between the vertical racks (figure 3.1 a). Aeration was delivered through two 20-mm polyvinylchloride (PVC) airline pipes positioned along the length of the bottom of the tank. The tanks were shaded from direct sunlight using two layers of 80 % mesh shade cloth. The feeder plate also served as a covering at the water surface.

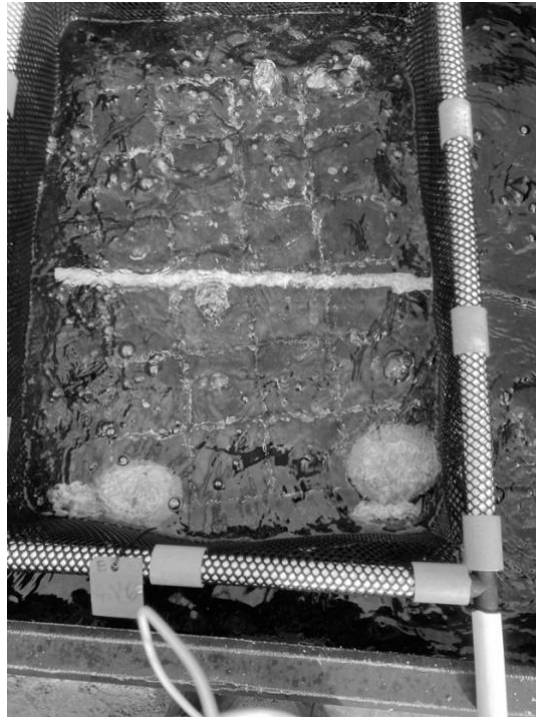


Figure 3.1 (a): Basket image made of 8 mm “oyster mesh” and top feeding plate submerged and held down by PVC pipe hook.

#### *Feeding regimen calculation*

This study used historical HIK-specific industrial feeding data as reference values. The treatment values were calculated using feeding data from routine feeding periods on the farm. The farm data consisted of the periods August to the end of November in the years 2016, 2018 and 2019 and showed the average biomass per weight class per basket, i.e., the average number of abalone per basket multiplied by the average abalone weight per basket. The historical data coincided with the time of year when the present study was conducted. The average amount of feed fed per basket of abalone per day was calculated over the period mentioned above.

The reference value FI (E) was then calculated based on what was fed daily on average across all weight classes. Therefore, the reference value FI(E) is expressed as a function of abalone body mass (%BM d<sup>-1</sup>) and was calculated using:

$$FI (E) = \frac{\text{average amount of feed fed daily (g)} \times 100}{\text{average number abalone per basket} \times \text{average weight per abalone (g)}}$$

Table 3.1: Feeding intensities (% BM d<sup>-1</sup>) used to calculate daily rations of each pellet type strategy (experiment one) and feeding regimen (experiment two) for each weight class using calculations mentioned above. FI (+10) and FI (-10) are 10% above and below FI (E) respectively.

Experiment	Weight class (g)	FI (E)	FI (+10)	FI (-10)
1	30-40	0.454	-	-
	10-20	0.600	0.66	0.540
2	100-110	0.350	0.385	0.315
	150-160	0.320	0.352	0.288

Experiment 1 = effect of pellet type on production parameters in 30-40 g abalone<sup>-1</sup> weight class, Experiment 2 = The effect of feeding regimen on production parameters using three weight classes.

### *Experimental design and statistical analysis*

#### *Experiment 1: The effect of pellet type on abalone production parameters*

The effect of a size specific daily ration feeding regimen on growth (% body mass day<sup>-1</sup> or % BM d<sup>-1</sup>), FCR and realized feeding intensity (% body mass day<sup>-1</sup> or % BM d<sup>-1</sup>) in the 30 – 40 g abalone weight class was tested using three feed pellet type strategies over four 28-day periods or 112-days. These strategies were a short pellet only ration fed daily (SP d<sup>-1</sup>), a leaf only ration fed daily (L d<sup>-1</sup>), and a strategy feeding both short pellets and leaves ration daily (B d<sup>-1</sup>). All strategies were fed at a feeding intensity of 0.454 % BM d<sup>-1</sup>, which was calculated using the before-mentioned formula (table 3.1). There were two baskets replicates per strategy across three tanks, with each tank consisting of six baskets in total i.e., 18 experimental units (figure 3.1 b). The average of the two basket replicates per tank was treated as a replicate.

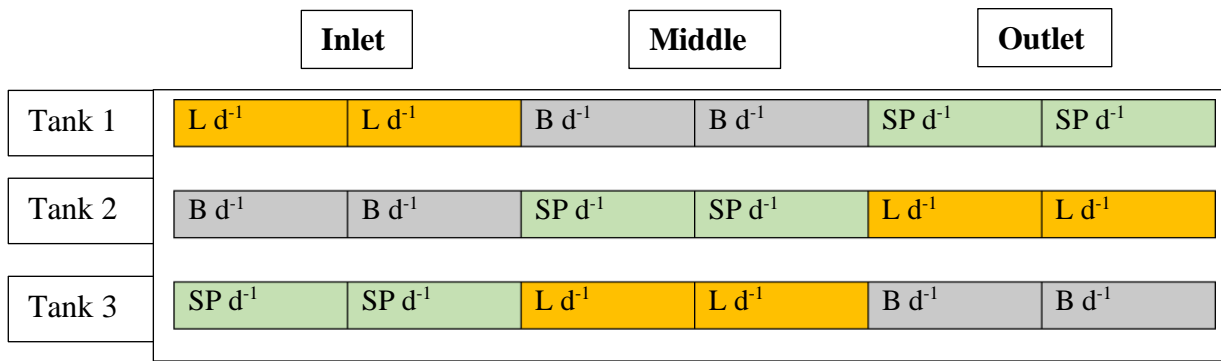


Figure 3.1 (b): Block design showing allocation of treatments across three tanks, in each basket position, for experiment one. L d<sup>-1</sup> = daily leaves strategy SP d<sup>-1</sup> = daily short pellet strategy, B d<sup>-1</sup> = both (leaves and short pellets) fed daily.

*Experiment 2: The effect of feeding regimen on abalone production parameters*

The effect of feeding intensity regimen (FI) on daily growth rate, daily feeding intensity and FCR were tested using four feeding intensity regimens for each of three abalone weight classes over four 28-day feeding periods i.e., for 112-days. The abalone weight classes used were 10 – 20, 100 – 110 and 150 – 160 g abalone<sup>1</sup>. Of the four feeding regimens, three were adjusted feeding methods FI (E), FI (+10), FI (-10) and the farm feeding method FI (FARM). The FI (E) regimens for the 10 – 20 g, 100 – 110 g and 150 – 160 g weight classes were 0.60 %BM d<sup>-1</sup>, 0.350 %BM d<sup>-1</sup> and 0.32 %BM d<sup>-1</sup> respectively, with FI (+10) and FI (-10), 10 % above and below the FI (E) reference value respectively (table 3.1). The FI (FARM) regimen was based on the feeding method used on the farm. All abalone were fed daily. There were six replicates per feeding regimen across three weight classes.

*Tank cleaning*

Tanks were cleaned every week. This involved moving the baskets from the first tank to an empty tank with clean water nearby to the same basket positions as before, after which, the empty and dirty tank were drained, scrubbed and refilled. The baskets of the next dirty tank were placed into the new clean tank in the same basket positions. The process was repeated until all tanks were cleaned. Flow rates were checked using a standard farm flow measure and set to a flow rate of approximately 120 L h<sup>-1</sup>.

### *Experimental animals*

The abalone in the 30 – 40 g weight class that were used in the study were sorted during a farm grading process. Each of the 18 baskets in experiment one was filled with an average of  $8.1 \pm 0.3$  kg abalone, which is the stocking density used for this weight class. Thirty abalone were weighed to obtain average individual weights ( $\text{g abalone}^{-1}$ ). There were no differences in average initial basket weights ( $\text{kg basket}^{-1}$ ) and individual weights across each basket position ( $F_{2, 15} = 0.54$ ,  $p = 0.59$  and  $F_{2, 15} = 0.61$ ,  $p = 0.55$ , respectively). Abalone in each weight class for experiment two were sorted during a farm grading process and placed into baskets. Thirty abalone per replicate were weighed to obtain the initial average abalone weight. The baskets with the weight classes of 10 – 20 g, 100 – 110 g and 150 – 160 g  $\text{abalone}^{-1}$  were stocked at an average of  $4.99 \pm 0.41$  kg  $\text{basket}^{-1}$ ,  $12.44 \pm 0.03$  kg  $\text{basket}^{-1}$  and  $13.9 \pm 0.13$  kg  $\text{basket}^{-1}$ , respectively, which is the stocking density used for these weight classes. Baskets were allocated randomly across the three tanks in experiment one and the six tanks in experiment two. All the tanks in each experiment were in the same row on the farm. After the baskets were randomly allocated, they were assigned a treatment using a block design, with each replicate represented in three positions (two baskets per position) along the length of each tank and throughout six tanks, to randomise any potential basket position effect. i.e., two baskets at the inlet, middle and outlet regions. Figure 3.1 b which showing the block design of experiment one can be used as the basis for the block design for experiment 2.

### *Data collection*

Abalone were grown in the three tanks for experiment one and six tanks for experiment two for 112-days. Average abalone weight ( $\text{g abalone}^{-1}$ ) was obtained by weighing abalone ( $n = 10$  from each basket) every 28-days to the nearest 1 g using a mass balance (DIGI DS-530, UK). This involved the random sampling of abalone, which were quickly dried using a sponge to eliminate excess water weight. The average change in weight of the  $n = 10$  abalone sample per basket was used to adjust the feeding ration within each treatment. The average of monthly weight gain per abalone among each feeding regimen was used to calculate the new biomass for the next 28-day period:  $\text{New biomass} = \text{average weight abalone}^{-1} \text{ at the end of a 28 day-feeding period} \times \text{number of abalone basket}^{-1}$ . Abalone numbers in each basket remained the same every month as there were no mortalities. The adjusted ration was calculated using:  $\text{Ration (g)} = (\text{FI} \times \text{biomass g})/100$ . Where FI is the feeding regimen feeding intensity value (table 3.1) and biomass is the new calculated biomass of a basket in a treatment. After the 112-

day growth period, the abalone went through a farm grading process, which is done every four months. For this, 50 abalone were weighed per basket from the 10 – 20 g weight class and 30 abalone from each basket in the 30 – 40 g, 100 – 110 g and 150 – 160 g weight classes. These data were used to construct weight frequency distributions.

Average percentage daily growth rate ( $G$ ) was calculated using the formula;  $G$  (%BM  $d^{-1}$ ) =  $((W_e/W_0)^{(1/(t-1))} - 1) \times 100$ , where  $W_0$  and  $W_e$  = average weight of abalone (g abalone $^{-1}$ ) in a basket at the beginning and end of a growth period, respectively, and  $t$  = time of the growth period (d). FCR was calculated by dividing the mass of dry food fed per abalone (g) by the total biomass gain (g) in each basket during a feeding period. Daily percentage feeding intensity was determined at the end of the growth period based on the growth rate of the abalone and the FCR for each basket ( $FI = G \times FCR$ ).

#### *Experimental feed*

Abalone were fed Abfeed S32<sup>®</sup> leaves and short pellets (composition (g kg $^{-1}$ ) for both pellet types: 320 g protein, 430 g carbohydrates, 20 – 60 g lipids) in the respective baskets once daily at 16:00. The difference between short pellets and leaves was the weight and dimension per pellet. Short pellets weighed on average  $0.2 \pm 0.01$  g pellet $^{-1}$  and leaves  $2 \text{ g} \pm 0.01$  g pellet $^{-1}$ . The dimension of short pellets was approximately 0.7 cm  $\times$  0.6 cm and leaves were 3 cm  $\times$  2 cm. For experiment one feed was weighed out weekly to the nearest gram, for each pellet type strategy, a bucket for L  $d^{-1}$ , SP  $d^{-1}$  and two buckets separating leaves and short pellets (50:50) for B  $d^{-1}$ . This is a ratio that is fed on the farm. Of these buckets a daily ration of 0.454 % BM  $d^{-1}$  for each treatment across 6 baskets were split into six daily portions using a tray designed to hold the approximate daily ration.

For experiment two, feed was weighed weekly to the nearest gram for the baskets assigned to the FI (E), FI (+10) and FI (-10) regimens per weight class. For each feeding regimen there were two buckets separating leaves and short pellets at a 50:50 ratio (a ratio that is used on the farm), as both of the pellet types were fed each day for the FI (E), FI (+10) and FI (-10) regimens. Of these buckets a daily ration (at respective % BM  $d^{-1}$ , table 3.1) for each feeding regimen per weight class across six replicates was split into six daily portions using a tray designed to hold the approximate daily ration for that feeding regimen of a certain weight class. Before feeding the baskets assigned the FI (FARM) regimen, baskets were checked for unfed

feed. If there were more than 15 pellets or leaves left over, the abalone were not fed on that day. If there were less than 15 pellets, a daily ration of the same size was fed across all weight classes using a standardised farm feeding method. Leaves and short pellets were fed on alternating days, as is done commercially. At the end of each week, the remaining amount of feed for the FI (FARM) regimen was weighed to estimate how much feed was fed daily. Daily rations were adjusted every 28 days based on the change in biomass per weight class per pellet type strategy and feeding regimen using the previously mentioned formulae ( $\text{Ration (g)} = (\text{FI} \times \text{biomass (g)})/100$ ).

#### *Water quality*

Daily sea water temperature ( $^{\circ}\text{C}$ ) was recorded. Weekly tank water quality samples were measured in all experiments using a hand-held dissolved oxygen probe and a pH probe (dissolved oxygen and temperature: OxyGuard H02A2, pH: OxyGuard H04PP). This included water temperature ( $^{\circ}\text{C}$ ), pH, dissolved oxygen ( $\text{O}_2 \text{ mg L}^{-1}$ ) and oxygen saturation of the water (% saturation). The samples were recorded from three sections in each tank, the inlet, the middle and the outlet. Due to the flow dynamics of the tank, measuring the effects of pellet type and feeding regimen on water quality within a basket would have been inaccurate due to cross contamination of water from each basket along the tank from the inlet to the outlet. Therefore, water quality within each basket position was not recorded, but rather the water that surrounded the basket position. Flow rates were set every three days using the farm protocol at a standard flow rate of approximately  $120 \text{ L h}^{-1}$ . TAN samples were taken from the outlet region of each tank after each 28-day feeding period, along with temperature, pH and dissolved oxygen. The samples were sent to an external service to be analysed (AL ABBOTT & Associates). FAN was calculated using the values for TAN, temperature, pH and salinity of the water at the time the TAN samples were recorded (Bower and Bidwell 1978).

Table 3.2: Temperature (°C), dissolved oxygen (mg L<sup>-1</sup>), oxygen saturation (%), pH, TAN (µg L<sup>-1</sup>) and FAN (µg L<sup>-1</sup>) of all three tanks over a 112-day growth period. SE = standard error. (Experiment one: n = 48 samples per water quality variable and experiment two: n = 144 except TAN and FAN, where n = 4 for both experiments).

Experiment	Water quality variable	Average	Minimum	Maximum	SE
1	Temperature (°C)	16.1	14.3	19.2	0.11
	Dissolved oxygen (mg L <sup>-1</sup> )	7.87	6.71	8.36	0.02
	Oxygen saturation (%)	99.56	91.9	105.2	0.17
	pH	7.87	7.7	8.02	-
	Total ammonia nitrogen (TAN, µg L <sup>-1</sup> )	143.41	100	210	5
	Free ammonia nitrogen (FAN, µg L <sup>-1</sup> )	3.23	1.73	5.87	0.41
	2	Temperature (°C)	16.37	14.9	19.3
Dissolved Oxygen (mg L <sup>-1</sup> )		7.73	6.91	8.86	0.03
Oxygen saturation (%)		97.82	91.3	112.3	0.29
pH		7.86	7.64	8.02	-
Total ammonia nitrogen (TAN, µg L <sup>-1</sup> )		176.6	100	260	20.02
Free ammonia nitrogen (FAN, µg L <sup>-1</sup> )		3.55	1.17	6.33	0.001

Experiment 1 = effect of pellet type on production parameters in 30-40 g abalone<sup>-1</sup> weight class, Experiment 2 = The effect of feeding regimen on production parameters using three weight classes.

#### *Preliminary feeding regimen pilot study*

To determine a suitable percentage above and below FI (E), a two-week preliminary pilot study was conducted separately from the animals used in the present study before the commencement of experiments one and two. The pilot study tested the suitability of 10% above and below FI (E) and 20% above and below FI (E) in abalone of similar weight classes. The abalone were fed based what was left over from the previous feeding. At the beginning of each week, feed for each day was weighed. Abalone were fed when there were fewer than 20 pellets left in the basket. If there were more than 20 pellets, feed was withheld until the next day. A score out of seven was determined at the end of each week which comprised the number of times the abalone were fed. The study found that feeding 20 % above FI (E) daily resulted in feeding eight daily rations worth of feed out of a total of 14 daily rations compared to the 10% above FI (E) which resulted in 13 daily rations being fed out of the total 14 daily rations daily for each weight class. The 10 % and 20 % below FI (E) treatments were both fed 14 out 14 times after the two-week trial was complete, however abalone fed 20 % below FI (E) began to exhibit stress-like behaviour by clustering together, and in some instances began to “walkout” of the farm tanks (pers. obs). Therefore, feeding 20 % above and below FI (E) would have resulted

in overfeeding and underfeeding, respectively. This justified the use of FI (E) and 10 % above and below FI (E) to be used in experiment two.

### *Data analysis*

Repeated measures analysis of variance (ANOVA) was used to compare average abalone weight between each pellet type strategy (experiment one) and feeding regimen (experiment two) within each 28-day feeding period (time). This was done separately for each of three weight classes in experiment two as farming conditions, e.g., stocking densities differed between weight classes. The assumptions for repeated measures ANOVA were checked using Mauchly's test of sphericity. If sphericity was violated, Huynh-Feldt and Greenhouse-Geisser corrections of F-statistics were used. The averages of daily growth rate, feeding intensity and FCR at the end of the 112-day growth period were compared using a one-way analysis of variance (ANOVA) using the averages of two baskets per basket position in experiment one and each single basket replicate across all experimental tanks in experiment two. Assumptions of equality of variance and normality of residuals were tested using Levene's test. If these assumptions were not met, the data were log-transformed, and the test was repeated. If there were significant differences between treatment means based on the ANOVA results, Tukey's post-hoc test was used to compare the differences between the averages of the dependent variables across each 28-day feeding period and between each pellet type strategy and feeding regimen. Least square regression analysis was used to determine the correlation between feeding intensity, daily growth rate and FCR. At the end of the 112-day growth period, weight data of were recorded to generate frequency distributions for each feeding regimen per weight class. Coefficient of variation (CV) was calculated using the standard deviation and average weight of each population of each feeding regimen per weight class, to explore the extent of variability in relation to the average weight of abalone after 112 days. Bartlett's test was used to test for equality of variance between feeding regimens and frequency histograms were used to explore weight frequency distributions. A one-sample z-test (1 - p value) was used to determine the probability that the average population size will be equal to or greater than a given weight value for each feeding regimen and abalone weight class. The probability values were then used to determine what percentage of abalone would be expected to fall between selected weight class ranges. A significance level of  $p = 0.05$  was used for all tests.

## Results

### *Experiment 1: The effect of pellet type on abalone production parameters*

There was no significant interaction between pellet type strategy and time (repeated ANOVA; Mauchly's sphericity;  $p < 0.0001$ ; Huynh-Feldt correction;  $p = 0.8$ ), but there was an effect of time on average abalone weight (pooled pellet type strategy, repeated ANOVA; Mauchly's sphericity;  $p < 0.0001$ ; Huynh-Feldt correction;  $p < 0.00001$ ), with the initial average abalone weight of 34.64 g abalone<sup>-1</sup> and the final average weight of 54.95 g abalone<sup>-1</sup> after the 112-day growth period. The final weight of the abalone was 1.5 times greater than the initial weight. Average abalone weights were significantly different between each 28-day feeding period (Tukey's post-hoc test).

Daily growth rate did not differ between each pellet type strategy (ANOVA;  $F_{2, 15} = 0.51$ ,  $p = 0.60$ ). The pooled averages between each pellet type strategy were 0.42 % BM d<sup>-1</sup> after a 112-day growth period. Daily feeding intensity was not affected by pellet type strategy (ANOVA;  $F_{2, 15} = 0.64$ ,  $p = 0.54$ ), with the pooled average daily feeding intensity at 0.43 % BM d<sup>-1</sup>. Pellet type strategy did not affect FCR (ANOVA;  $F_{2, 15} = 0.49$ ,  $p = 0.62$ ). The average FCR for the pooled pellet type strategies was 1.05:1.

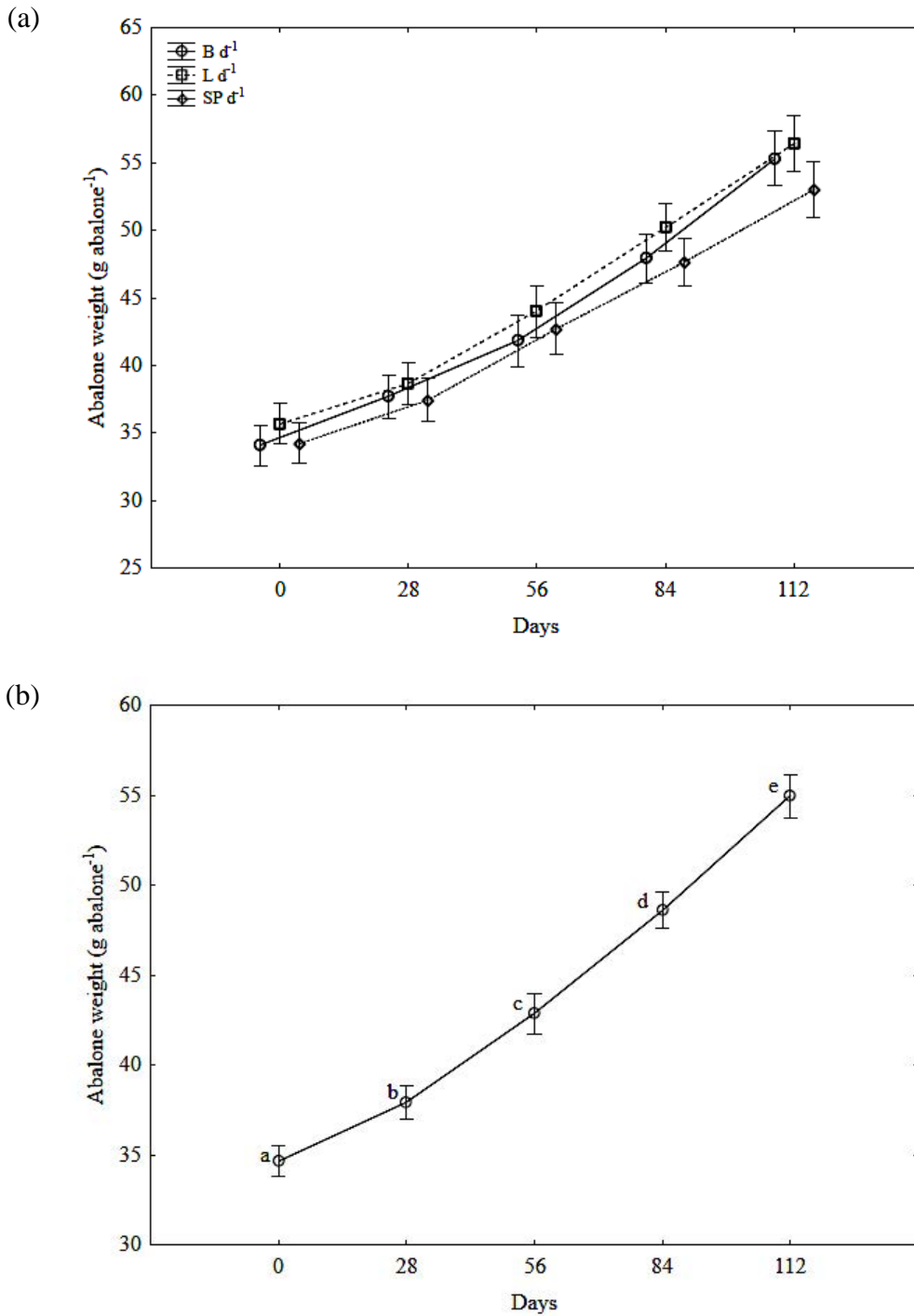


Figure 3.2 (a): The effect of pellet type on abalone weight during a 112-day growth period. (b): Pooled abalone weights of each pellet type strategy over 112-day growth period. Superscripts indicate significant differences between each 28-day period. Vertical bars denote the SE of the average. B d<sup>-1</sup> = both pellet types, L d<sup>-1</sup> = Leaves only pellet type strategy per day, SP d<sup>-1</sup> = short pellet only pellet type strategy per day.

Daily growth rate was a linear function of daily feeding intensity (least squares regression;  $r^2 = 0.34$ ,  $p < 0.01$ ), and it decreased with an increase in feeding intensity. Pooled daily growth rates of the three pellet type strategies ranged from 0.27 – 0.55 %BM d<sup>-1</sup> after a 112-day growth period (table 3.4). Feed conversion ratio was linearly related with daily feeding intensity (least squares regression;  $r^2 = 0.42$ ,  $p < 0.004$ ), and increased with an increase in feeding intensity. Daily growth rate decreased with an increase in FCR (least squares regression;  $r^2 = 0.92$ ,  $p < 0.0001$ ). FCR ranged from 0.71:1 – 1.64:1 and resulted in an average daily growth rate of 0.42 % BM d<sup>-1</sup> (table 3.4).

The variances of the weight distribution data were significantly different between each pellet type strategy (Bartlett's test;  $p < 0.01$ ). The weight distribution data for the B d<sup>-1</sup> strategy had the highest CV of 18.03% and the L d<sup>-1</sup> strategy had the lowest CV at 14.33%. The L d<sup>-1</sup> strategy had the highest probability of abalone falling in the 50 – 70 g size range (Z (55.62, 7.97); 50-70 = 72%). The SP d<sup>-1</sup> strategy had the highest probability of abalone falling in the lower size range 45 – 50 g (Z (54.67, 9.83); 45-50 = 18%).

Table 3.3: Parameters between each 28-day feeding period, showing the average mass fed daily based on the change in biomass between each 28-day feeding period.

Parameter	28	56	84	112
Initial weight (g abalone <sup>-1</sup> )	34.64	37.92	42.83	48.59
Final weight (g abalone <sup>-1</sup> )	37.92	42.83	48.59	54.95
Initial Biomass (kg basket <sup>-1</sup> )	8.06	8.82	9.96	11.33
Final Biomass (kg basket <sup>-1</sup> )	8.82	9.96	11.33	12.81
Mass fed (g basket day <sup>-1</sup> )	37	40	45	51
Total fed (kg basket month <sup>-1</sup> )	1.02	1.12	1.27	1.44

Table 3.4: The effect of three pellet type strategies on daily growth rate, feeding intensity and feed conversion ratio in *H. midae* after a 112-day growth period. G = daily growth rate (% BM d<sup>-1</sup>), FI = feeding intensity (% BM d<sup>-1</sup>), FCR = feed conversion ratio. As there were no significant effects of pellet type strategy, the data from all treatments were pooled.

Pellet type strategy	G	FI	FCR
B d <sup>-1</sup>	0.438	0.422	1.01
L d <sup>-1</sup>	0.416	0.426	1.03
SP d <sup>-1</sup>	0.397	0.432	1.12
Pooled average	0.42	0.43	1.05
Pooled ranges	0.27 – 0.55	0.38 – 0.45	0.71 – 1.64

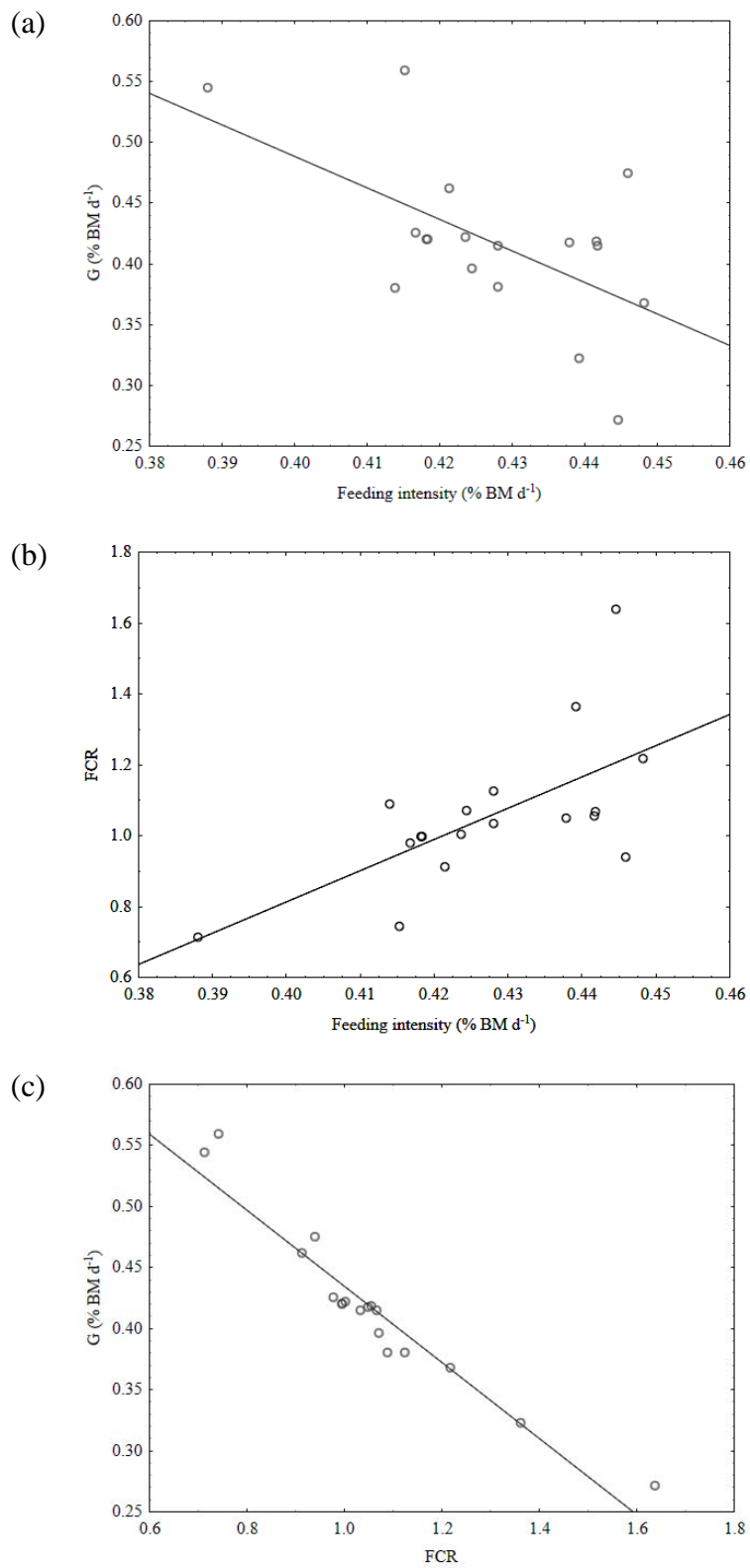
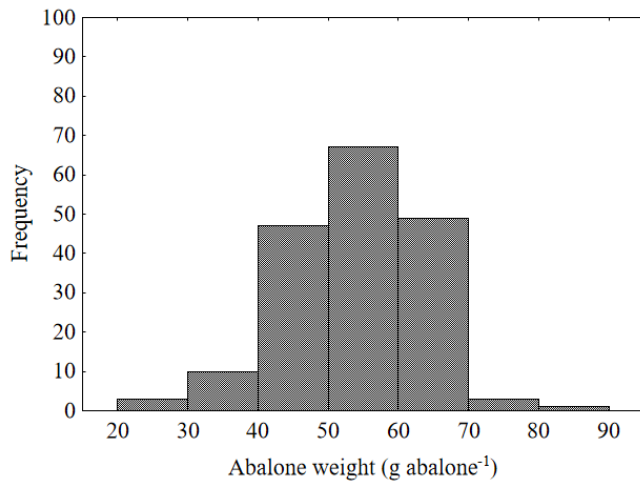


Figure 3.3 (a): Daily growth rate (G) as a function of daily feeding intensity (least squares regression;  $r^2 = 0.34$ ,  $p < 0.01$ ,  $G = -2.5952(x) + 1.526$ ), (b): Feed conversion ratio (FCR) as a function of daily feeding intensity (least squares regression;  $r^2 = 0.42$ ,  $p < 0.004$ ,  $FCR = 8.8082(x) - 2.7108$ ), (c): Daily growth rate as a function of FCR (least squares regression;  $r^2 = 0.92$ ,  $p < 0.0001$ ,  $G = 0.3122(x) - 0.7567$ ).



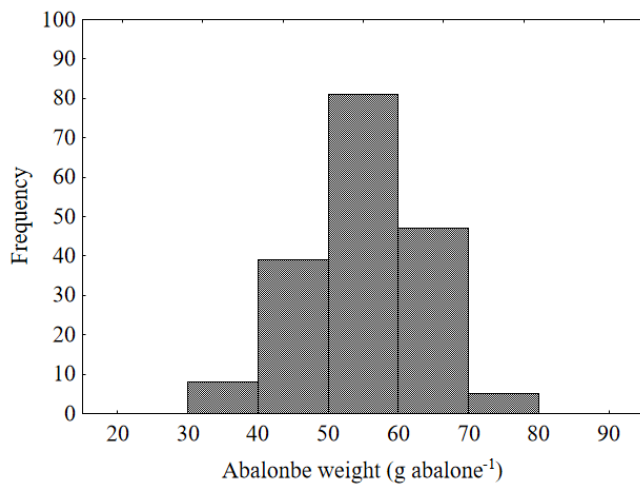
(a)

Pellet type strategy: B d<sup>-1</sup>

$x = 54.77$

$s = 9.86$

$CV = 18.03 \%$



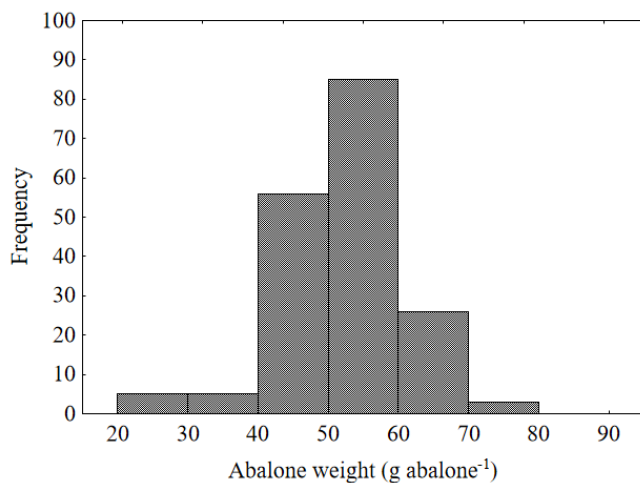
(b)

Pellet type strategy: L d<sup>-1</sup>

$x = 55.62$

$s = 7.97$

$CV = 14.33 \%$



(c)

Pellet type strategy: SP d<sup>-1</sup>

$x = 53.22$

$s = 8.59$

$CV = 16.14 \%$

Figure 3.4: Weight (g abalone<sup>-1</sup>) frequency distributions of the 30 – 40 g abalone weight class after a 112-day growth period for each pellet type strategy (n = 180 per pellet type strategy).  $x$  = average,  $s$  = standard deviation,  $CV$  = coefficient of variation.

Table 3.5: Z-test probability values using the weight frequency values,  $x$  = average,  $s$  = standard deviation to calculate the expected probability ( $p$ ) of values above  $X$  = abalone weight. To determine at which probability abalone fall into each size range,  $p$  values above  $X$  were subtracted to calculate the percentage of abalone that fall into the commercially relevant size ranges. Remaining percentages are not shown as they fall out of the commercially relevant size ranges shown in the table.

Weight class	Pellet type strategy	Z (x, s) = X	Probability (p)	Size range (g)	Percentage
30 – 40	B d <sup>-1</sup>	Z(54.67, 9.83) = 45	0.84	45-50	15%
		Z(54.67, 9.83) = 50	0.68		
		Z(54.67, 9.83) = 70	0.06	50-70	62%
		Z(54.67, 9.83) = 75	0.02		
	L d <sup>-1</sup>	Z(55.62, 7.97) = 45	0.91	45-50	15%
		Z(55.62, 7.97) = 50	0.76		
		Z(55.62, 7.97) = 70	0.04	50-70	72%
		Z(55.62, 7.97) = 75	0.01		
	SP d <sup>-1</sup>	Z(53.22, 8.59) = 45	0.83	45-50	18%
		Z(53.22, 8.59) = 50	0.65		
		Z(53.22, 8.59) = 70	0.03	50-70	62%
		Z(53.22, 8.59) = 75	0.01		

## *Experiment 2: The effects of feeding regimen on abalone production parameters*

There were no mortalities across all weight classes over the 112-day growth period. Analyses were performed to compare treatment means within each of the three weight classes.

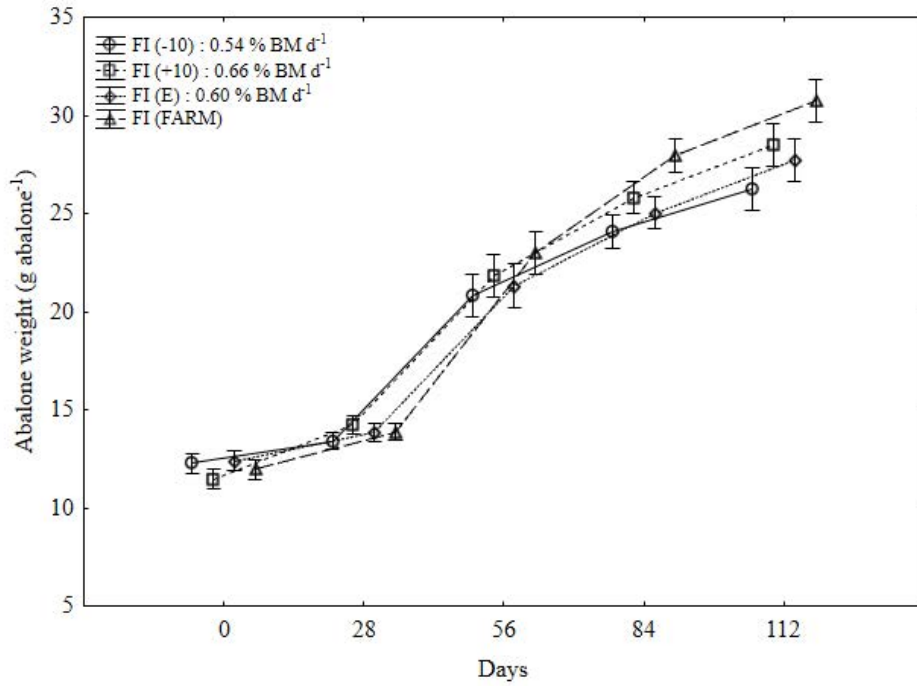
### *10 – 20 g abalone weight class*

There was no significant interaction between feeding regimen and time for average abalone weight (g abalone<sup>-1</sup>) (repeated ANOVA; Mauchley sphericity;  $p < 0.012$ , Huynh-Feldt;  $p = 0.09$ , figure 3.5 a), however, average abalone weight was significantly different between each 28-day feeding period over the 112-day growth period (repeated ANOVA; Huynh-Feldt;  $p < 0.0001$ , figure 3.5 b), i.e., with an initial average weight of 12.02 g abalone<sup>-1</sup> and a final average weight of 28.31 g abalone<sup>-1</sup> after the 112-day growth period (pooled average of all feeding regimens, figure 1b).

Daily growth rate (% BM d<sup>-1</sup>) did not differ between feeding regimens (one-way ANOVA;  $F_{3,20} = 3.11$ ,  $p = 0.05$ ), however, feeding regimen affected feeding intensity (% BM d<sup>-1</sup>) and FCR (one-way ANOVA; feeding intensity;  $F_{3,20} = 56.44$ ,  $p < 0.0001$ , FCR (log);  $F_{3,20} = 9.46$ ,  $p < 0.0004$ , table 3.6). Feeding intensity was significantly lower for the 112-day growth period in the FI (FARM) regimen at 0.42 % BM d<sup>-1</sup> compared to the other three regimens, FI (E) at 0.58 % BM d<sup>-1</sup>, FI (+10) at 0.60 % BM d<sup>-1</sup> and FI (-10) at 0.58 % BM d<sup>-1</sup> (Tukey's post-hoc). Feeding intensity in the FI (+10) regimen was 43 % higher than the FI (FARM) regimen. FCR was significantly lower for abalone fed the FI (FARM) regimen at 0.50:1 in comparison to the other feeding regimens. FCR was a function of feeding intensity and was positively correlated with feeding intensity (least square regression;  $r^2 = 0.52$ ,  $p < 0.00007$ , figure 3.6 b).

Weight frequency distribution indicated that the FI (FARM) regimen had a higher probability of abalone falling in the upper weight class (Z (25.91, 8.61); 35-45 g = 14%, table 3.7 a), after 112 days in comparison to the other feeding regimens. The FI (-10) regimen (figure 3.7 a, table 3.7 a) had the highest probability of abalone falling in the smallest size range (Z (22.9, 6.69); 15 – 25 = 50%). The FI (+10) regimen showed a 39% probability that abalone will fall in the size range 25 – 35 g abalone<sup>-1</sup>.

(a)



(b)

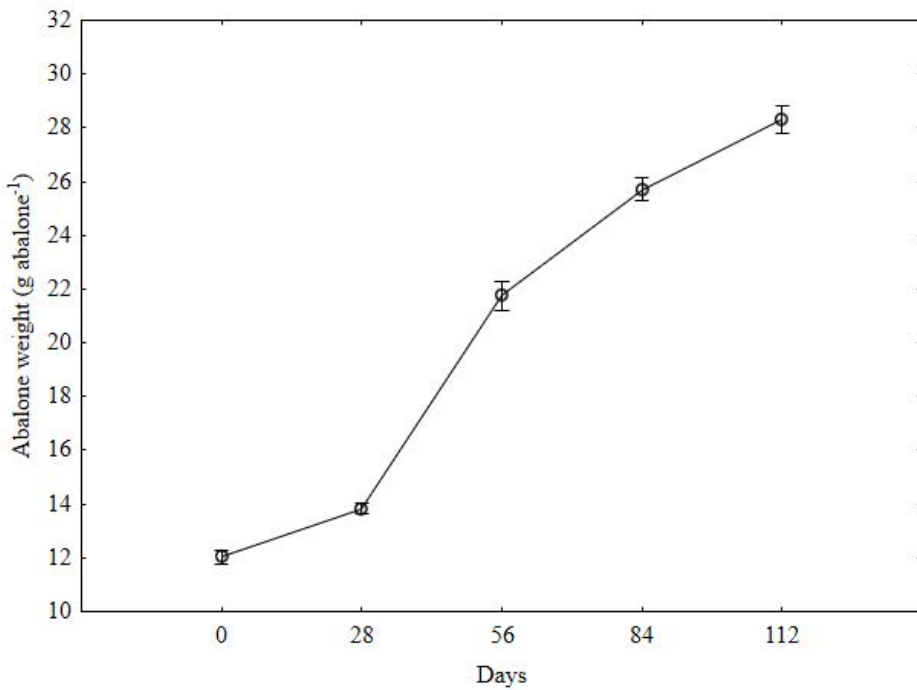


Figure 3.5 (a): Average weight (g abalone<sup>-1</sup>) of 10 – 20 g abalone weight class during a 112-day growth period fed four feeding regimens. (4.1b): Average weight (g abalone<sup>-1</sup>) of 10-20 g abalone weight class over 112-day period (pooled data). All vertical bars represent standard errors of the average. Legend in 3.5 (a) are FI values based on table 3.1.

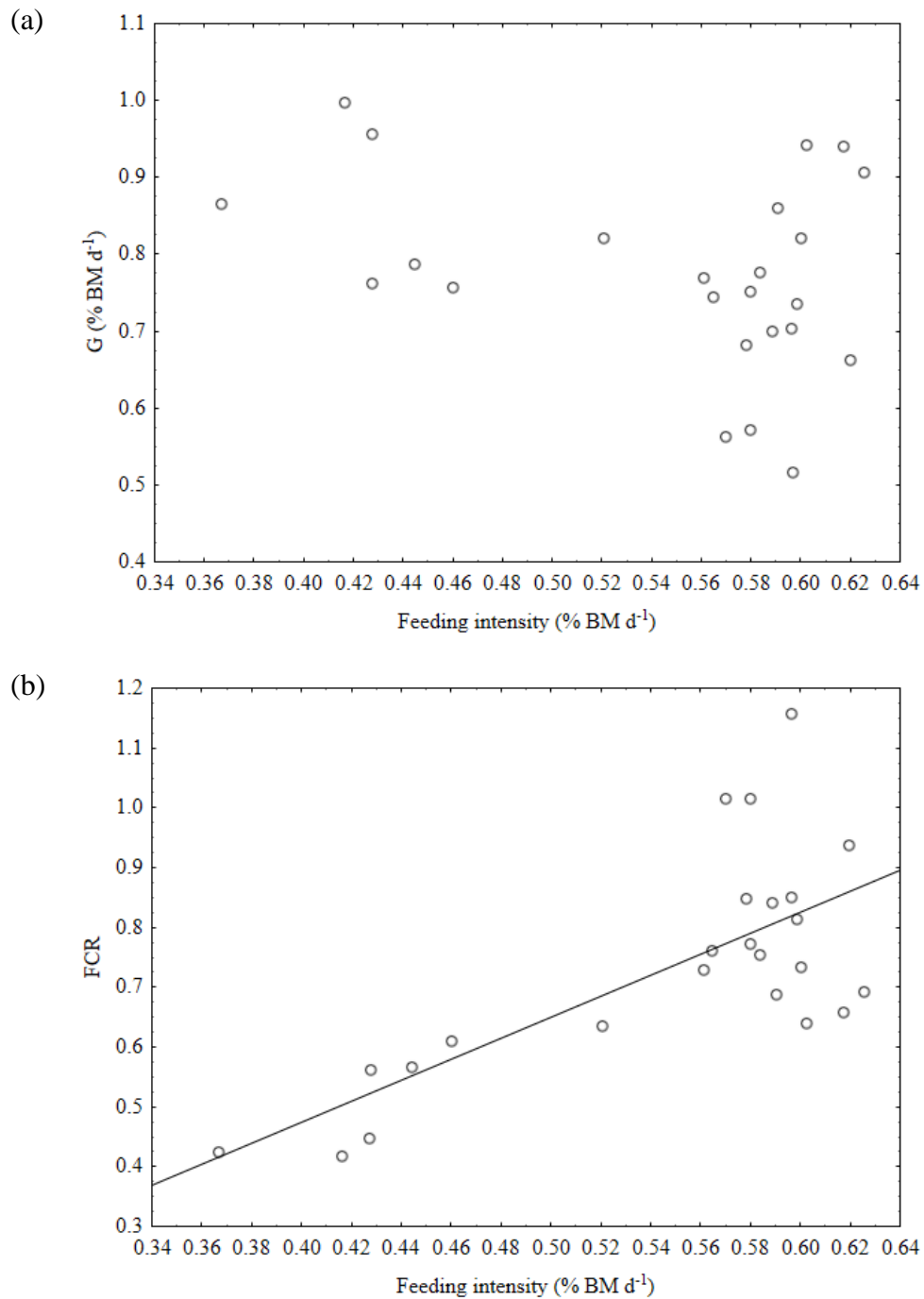
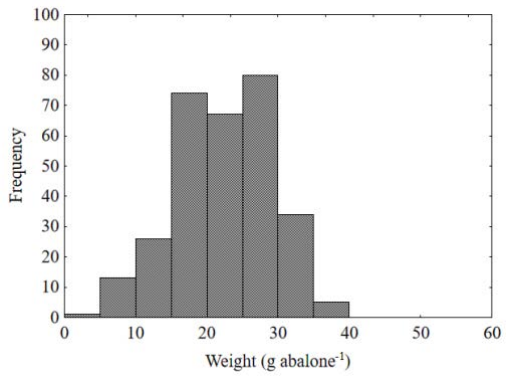


Figure 3.6 (a): The relationship between feeding intensity (% BM d<sup>-1</sup>) and daily growth rate (% BM d<sup>-1</sup>) in the 10 – 20 g abalone weight class after a 112-day growth period ( $p = 0.11$ ,  $r^2 = 0.11$ , average daily growth rate = 0.55 % BM d<sup>-1</sup>). (b): The relationship between feeding intensity (% BM d<sup>-1</sup>) and FCR in the 10-20 g abalone weight class after a 112-day growth period ( $p < 0.0001$ ,  $r^2 = 0.52$ , FCR = 1.7518 x - 0.2263).



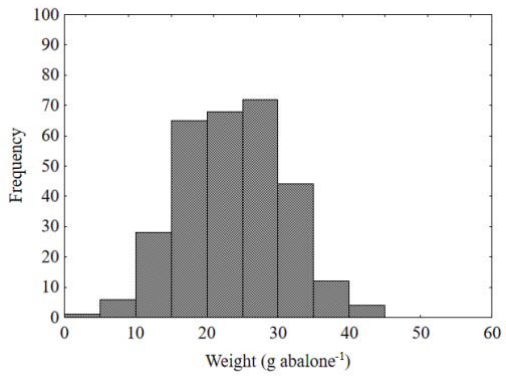
(a)

Regimen: FI (-10)

$x = 22.90$

$s = 6.69$

$CV = 29.2 \%$



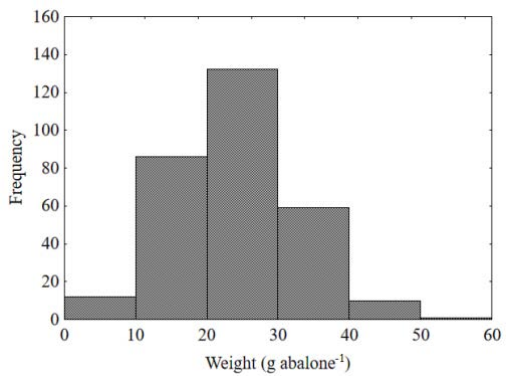
(b)

Regimen: FI (+10)

$x = 24.25$

$s = 7.22$

$CV = 29.8 \%$



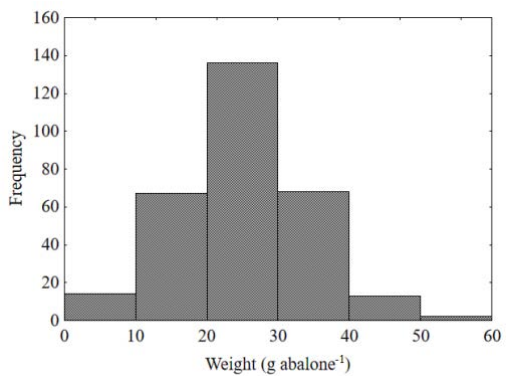
(c)

Regimen: FI (E)

$x = 24.63$

$s = 8.67$

$CV = 35.19 \%$



(d)

Regimen: FI (FARM)

$x = 25.91$

$s = 8.86$

$CV = 34.19 \%$

Figure 3.7: Weight ( $\text{g abalone}^{-1}$ ) frequency distributions of the 10 – 20 g abalone weight class after a 112-day growth period for each feeding regimen ( $n = 300$  per feeding regimen).  $x$  = average,  $s$  = standard deviation,  $CV$  = coefficient of variation (%).

### *100 – 110 g abalone weight class*

There was no interaction between feeding regimen and time for average abalone weight (repeated ANOVA;  $F_{12, 80} = 1.36$ ,  $p = 0.20$ ; figure 3.8 a), however average abalone weight differed between each 28-day feeding period with an initial weight of 103.72 g abalone<sup>-1</sup> and a final weight of 131.45 g abalone<sup>-1</sup> after the 112-day growth period (repeated ANOVA;  $F_{4, 80} = 503.77$ ,  $p < 0.00001$ , figure 3.8 b).

Daily growth rate was affected by feeding regimen over the 112-day growth period and differed between the FI (+10) and FI (FARM) regimens but not between the FI (E) and FI (-10) regimens (ANOVA;  $F_{3, 20} = 3.43$ ,  $p < 0.037$ , table 3.6). The daily growth rate was 16.6 % higher for the FI (+10) regimen at a rate of 0.24 % BM d<sup>-1</sup> than the FI (FARM) regimen at 0.20 % BM d<sup>-1</sup>. Feeding intensity differed across all feeding regimens (ANOVA;  $F_{3, 20} = 1039.3$ ,  $p < 0.0001$ , table 3.6) and was the highest for abalone fed the FI (+10) regimen and lowest for the FI (FARM) regimen at 0.38 and 0.26 % BM d<sup>-1</sup>, respectively, with the FI (E) and FI (-10) regimens at 0.36 and 0.31% BM d<sup>-1</sup>, respectively. FCR was lower for abalone fed the FI (FARM) regimen at 1.33:1 with FI (E) having the least efficient FCR of 1.67:1. FCR differed significantly between the FI (E), FI (+10) and FI (FARM) regimens and the FI (-10) regimen did not differ from the FI (FARM) regimen.

Daily growth rate positively correlated with feeding intensity (least squares regression;  $r^2 = 0.25$ ,  $p < 0.013$ , figure 3.9 a). Feed conversion ratio significantly positively correlated to feeding intensity (least squares regression;  $r^2 = 0.40$ ,  $p < 0.001$ , figure 3.9 b).

The weight distribution data of the FI (FARM) regimen had the highest probability of 25% of abalone falling in the lower size range of 110 – 120 g ( $Z(125.9, 10.58; 110-120 \text{ g} = 25\%$ , table 3.7 b). The FI (-10), FI (+10) and FI (E) feeding regimens showed a higher probability of abalone falling in the in the upper size ranges, in comparison to the FI (FARM) regimen (table 3.7 b).

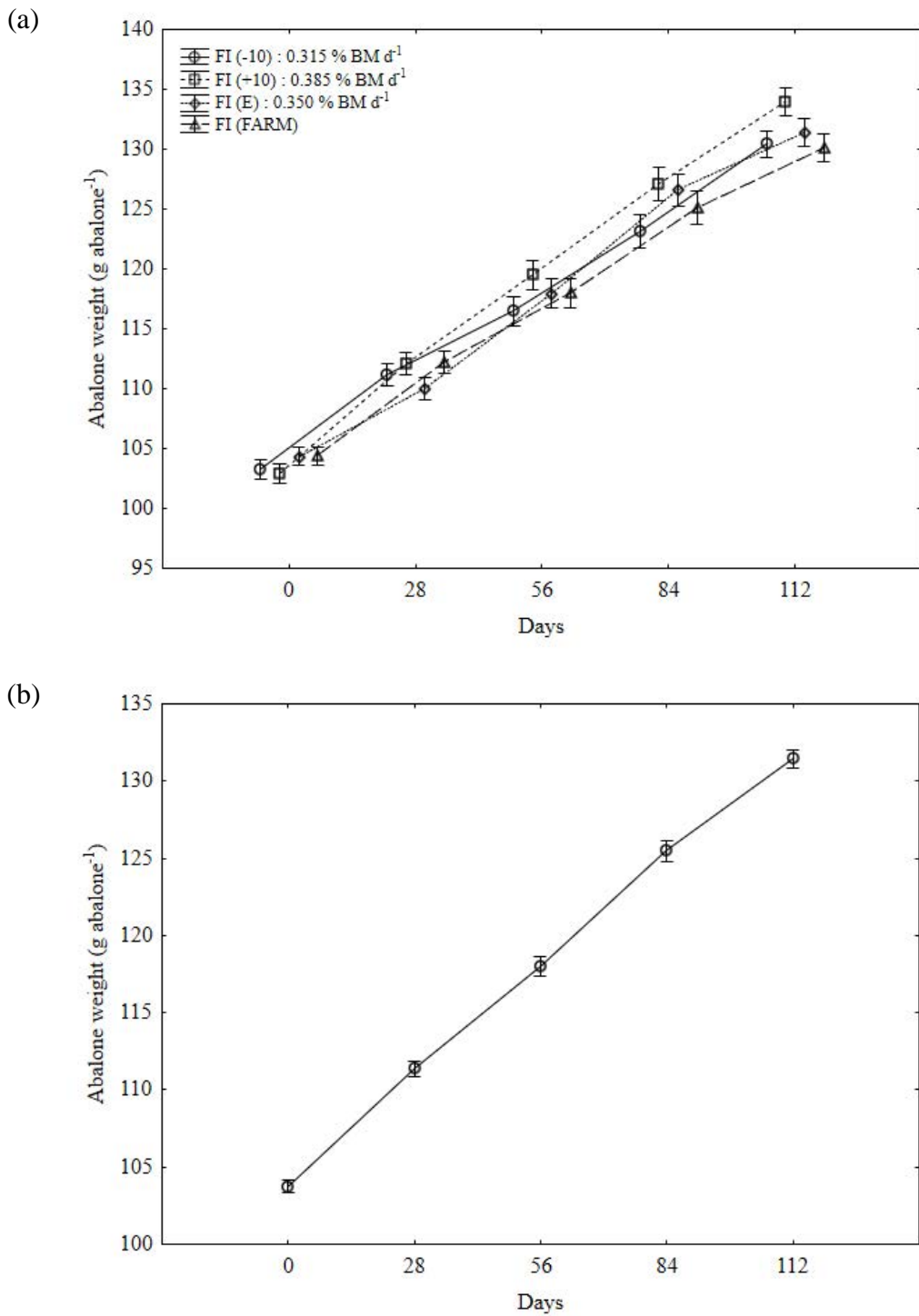


Figure 3.8 (a): Average weight (g abalone<sup>-1</sup>) of 100 – 110 g abalone weight class during a 112-day growth period fed four feeding regimens. (b): Average weight (g abalone<sup>-1</sup>) of 100 – 110 g abalone weight class over 112-day period. All vertical bars represent standard errors of the average. Legend in 3.8 (a) are FI values based on table 3.1.

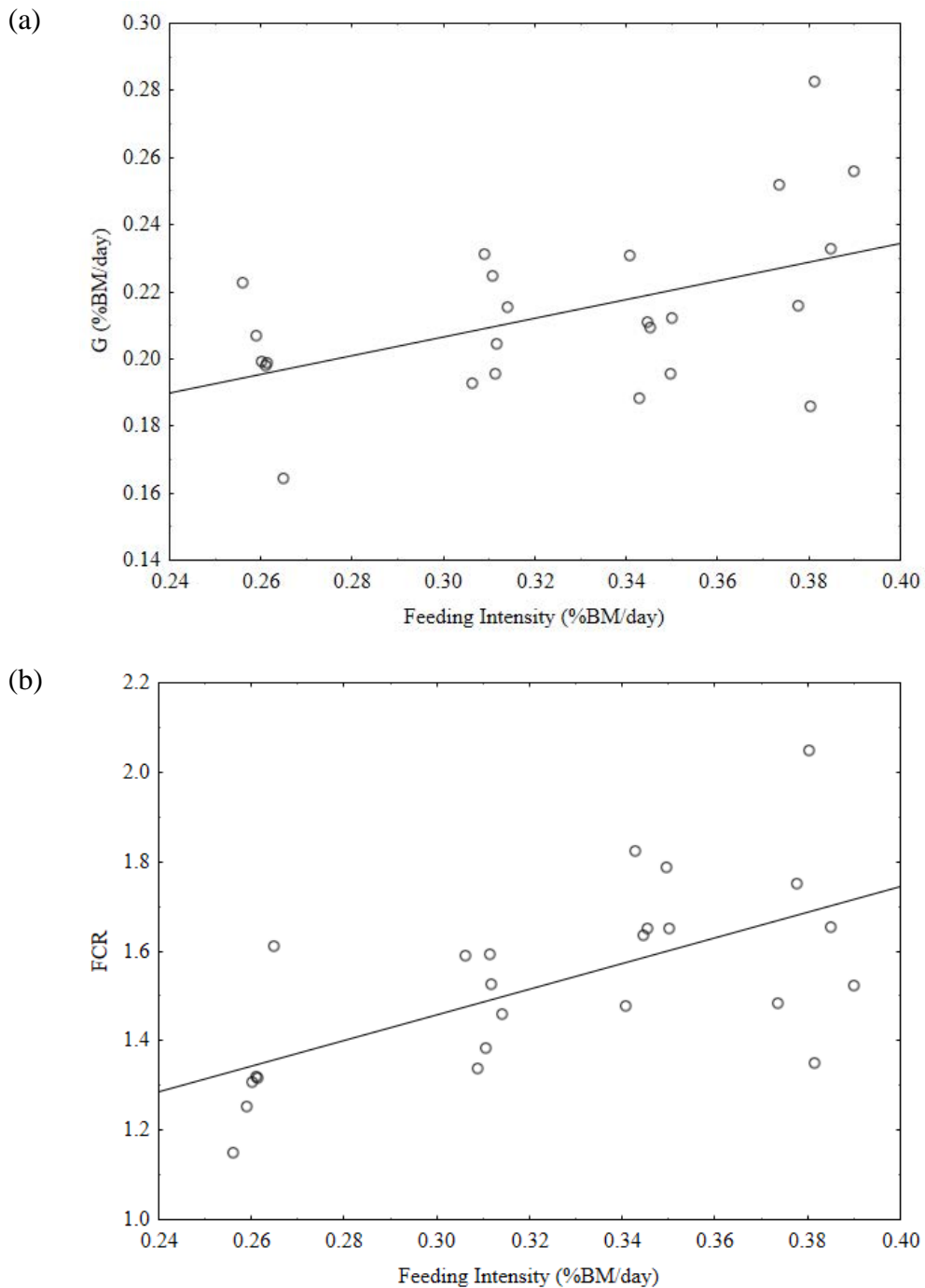
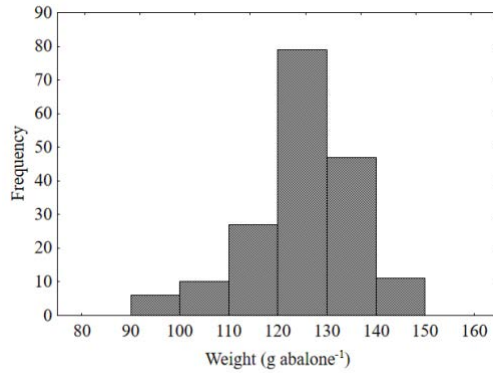
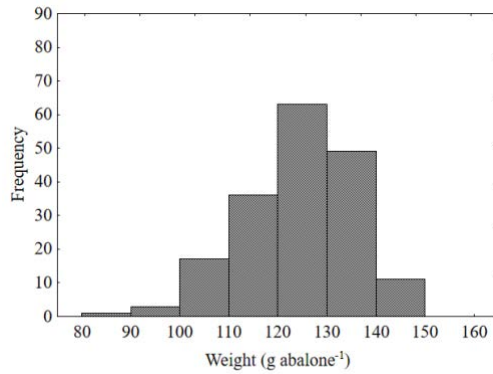


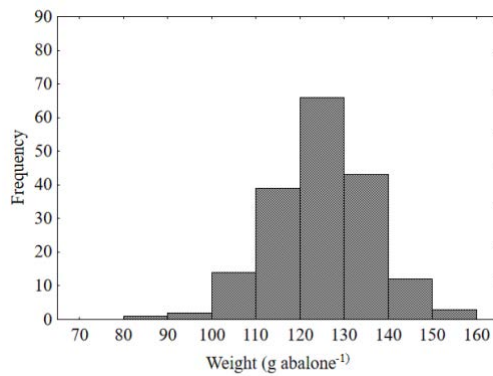
Figure 3.9 (a): The relationship between feeding intensity (% BM d<sup>-1</sup>) and daily growth rate (% BM d<sup>-1</sup>) in the 100 – 110 g abalone weight class after a 112-day growth period ( $p < 0.01$ ,  $r^2 = 0.24$ ,  $G = 0.2784x + 0.1232$ ). (b): The relationship between feeding intensity (% BM d<sup>-1</sup>) and FCR in the 100 – 110 g abalone weight class after a 112-day growth period ( $p < 0.001$ ,  $r^2 = 0.40$ ,  $FCR = 2.8715x + 0.5962$ ).



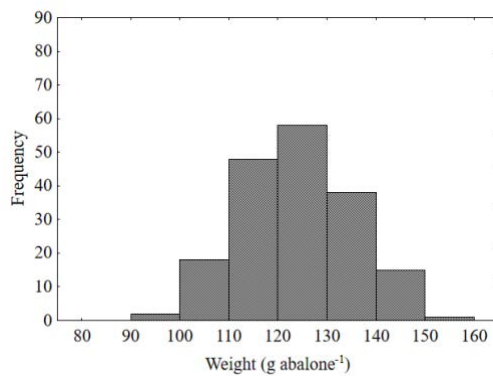
(a)  
 Regimen: FI (-10)  
 $x = 125.9$   
 $s = 10.58$   
 $CV = 8.40 \%$



(b)  
 Regimen: FI (+10)  
 $x = 125.2$   
 $s = 11.28$   
 $CV = 9.01 \%$



(c)  
 Regimen: FI (E)  
 $x = 125.6$   
 $s = 11.44$   
 $CV = 9.11 \%$



(d)  
 Regimen: FI (FARM)  
 $x = 124.6$   
 $s = 11.07$   
 $CV = 8.88 \%$

Figure 3.10: Weight (g abalone<sup>-1</sup>) frequency distributions of the 100 – 110 g abalone weight class after a 112-day growth period for each feeding regimen (n = 180 per feeding regimen).  $x$  = average,  $s$  = standard deviation,  $CV$  = coefficient of variation (%).

### *150 – 160 g abalone weight class*

Abalone fed the FI (FARM) regimen had the lowest weight gain of all the feeding regimens with FI (+10) having the highest over the 112-day growth period (repeated ANOVA; Mauchly sphericity;  $p < 0.00001$ , Huynh-Feldt;  $p < 0.01$ , figure 3.11 a). The average abalone weight in the FI (+10) and FI (FARM) regimens after 112 days were 198.88 g abalone<sup>-1</sup> and 189.58 g abalone<sup>-1</sup>, respectively. There were no significant differences between average weight up until 84-days of feeding, but there was a difference after 112-days between FI (+10) and FI (-10).

Daily growth rate was significantly different between abalone fed the FI (+10), FI (-10) and FI (FARM) regimens but not the FI (E) regimen (ANOVA;  $F_{3, 20} = 6.92$ ,  $p = 0.0022$ , table 3.6). Abalone fed the FI (+10) regimen had the highest daily growth rate of 0.22 % BM d<sup>-1</sup> that was 22.22% higher than the FI (FARM) regimen at 0.18 % BM d<sup>-1</sup>. Feeding intensity of the abalone was significantly different between each feeding regimen (ANOVA;  $F_{3, 20} = 1892.7$ ,  $p < 0.0001$ , table 3.6). The feeding intensity for abalone in the regimens FI (+10), FI (E), FI (-10) and FI (FARM) were 0.35, 0.32, 0.29 and 0.23 % BM d<sup>-1</sup>, respectively. Feed conversion ratio differed between the FI (+10), FI (E) and FI (FARM) regimens but not the FI (-10) regimen (ANOVA;  $F_{3, 20} = 4.72$ ,  $p = 0.011$ , table 3.6). The abalone in the FI (FARM) regimen had the most efficient FCR at 1.32:1 with the least efficient being the FI (E) regimen at 1.58:1. The FI (+10) regimen that had the highest daily growth rate had an FCR of 1.58:1.

Both daily growth rate and FCR were positively correlated to feeding intensity (G:  $r^2 = 0.46$ ,  $p < 0.0003$ , figure 3.11 b and FCR:  $r^2 = 0.38$ ,  $p < 0.0001$ , figure 3.11 c). This indicates that at a higher feeding intensity, abalone grew at a faster rate but converted the feed less efficiently.

Weight distribution data indicated that the FI (+10) regimen showed a higher probability of abalone falling in the upper weight class of 195 – 215 g compared to the FI (FARM) regimen at 24% compared to 15% of, respectively. The FI (FARM) regimen showed the highest probability of all regimens of abalone falling in the lower size range 160 – 180 g ( $Z(181.83, 13.12)$ ; 160-180 = 40 %, table 3.7 c).

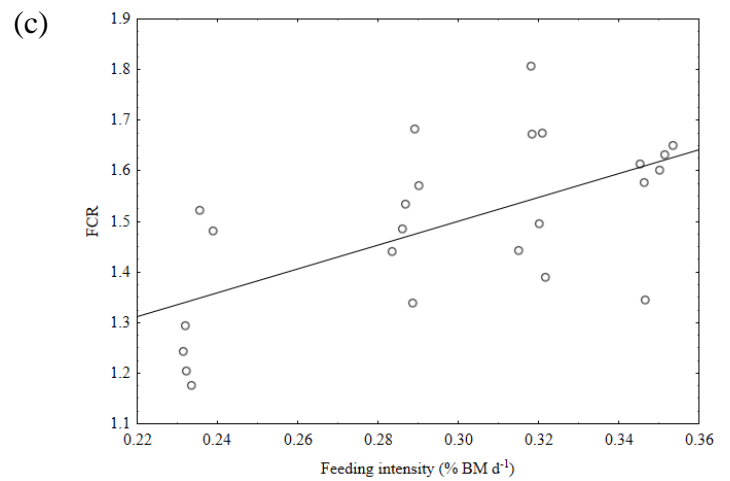
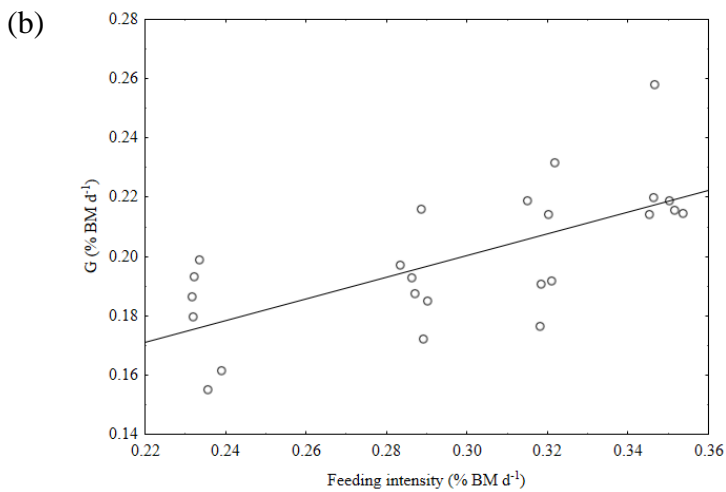
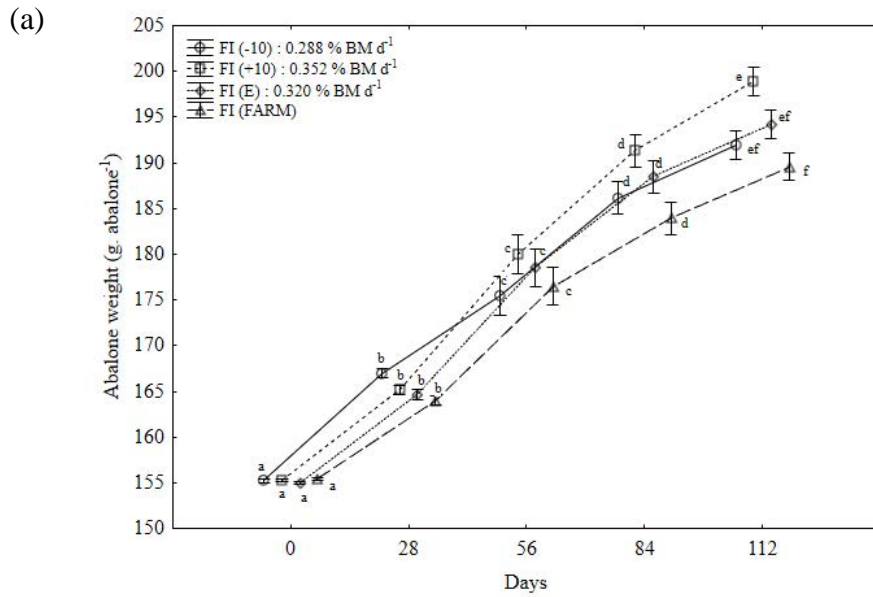
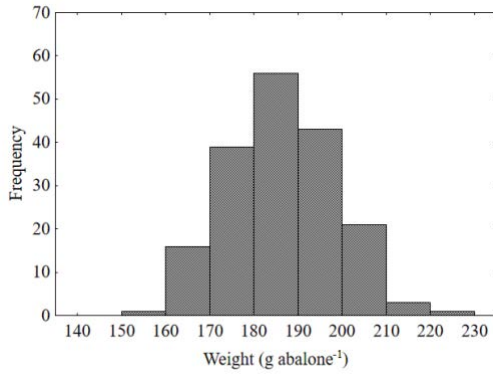
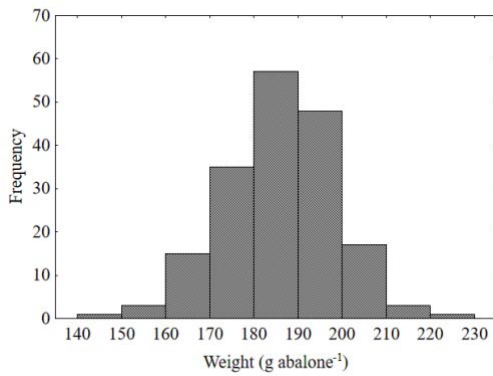


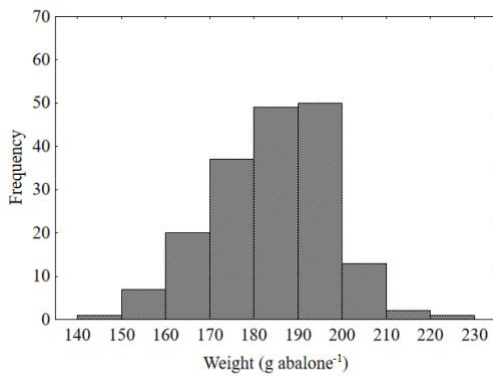
Figure 3.11 (a): Average weight ( $\text{g abalone}^{-1}$ ) of 150–160 g abalone weight class during a 112-day growth period fed four feeding regimens. (b): The relationship between feeding intensity ( $\% \text{ BM d}^{-1}$ ) and daily growth rate ( $\% \text{ BM d}^{-1}$ ) ( $p < 0.001$ ,  $r^2 = 0.46$ ,  $G = 0.3654x + 0.0908$ ). (c): The relationship between feeding intensity ( $\% \text{ BM d}^{-1}$ ) and FCR in the 150–160 g abalone weight class after a 112-day growth period ( $p < 0.001$ ,  $r^2 = 0.38$ ,  $\text{FCR} = 2.3584x + 0.7926$ ). Vertical bars are standard errors of the average. Superscript letters indicate significant differences between feeding regimens.



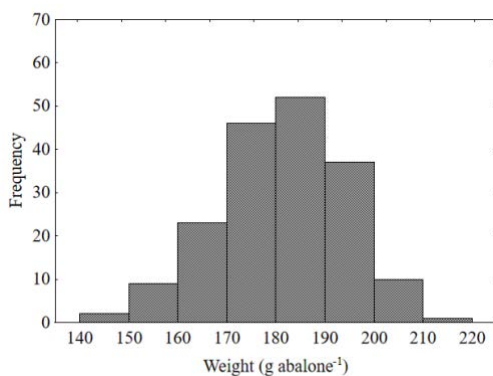
(a)  
 Regimen: FI (-10)  
 $x = 186.5$   
 $s = 12.15$   
 CV = 6.51 %



(b)  
 Regimen: FI (+10)  
 $x = 186.7$   
 $s = 12.24$   
 CV = 6.55 %



(c)  
 Regimen: FI (E)  
 $x = 184.5$   
 $s = 13.36$   
 CV = 7.24 %



(d)  
 Regimen: FI (FARM)  
 $x = 181.8$   
 $s = 13.12$   
 CV = 7.21 %

Figure 3.12: Weight (g abalone<sup>-1</sup>) frequency distributions of the 150 – 160 g abalone weight class after a 112-day growth period for each feeding regimen (n = 180 per feeding regimen).  $x$  = average,  $s$  = standard deviation, CV = coefficient of variation (%).

Table 3.6: The effect of four feeding regimens on growth, feeding intensity and FCR in *H. midae* over a 112-day growth period: W0 and We = initial average weight (g abalone<sup>-1</sup>) and final average weight (g abalone<sup>-1</sup>), respectively, over 112 days; G = growth rate (% BM d<sup>-1</sup>); FI = feeding intensity (%BM d<sup>-1</sup>); FCR = feed conversion ratio. Different superscript letters indicate significant differences between feeding regimen averages (Tukey's post-hoc test). Each weight class is treated separately.

Weight class (g)	Feeding regimen	W0	We	G	FI	FCR
10 – 20	<i>FI (-10)</i>	12.27	26.25	0.69 <sup>a</sup>	0.58 <sup>a</sup>	0.86 <sup>a</sup>
	<i>FI (+10)</i>	11.46	28.50	0.83 <sup>a</sup>	0.60 <sup>a</sup>	0.73 <sup>a</sup>
	<i>FI (E)</i>	12.39	27.73	0.73 <sup>a</sup>	0.58 <sup>a</sup>	0.84 <sup>a</sup>
	<i>FI (FARM)</i>	11.96	30.75	0.85 <sup>a</sup>	0.42 <sup>b</sup>	0.50 <sup>b</sup>
100 – 110	<i>FI (-10)</i>	103.26	130.42	0.21 <sup>a</sup>	0.31 <sup>a</sup>	1.48 <sup>ab</sup>
	<i>FI (+10)</i>	102.92	133.92	0.24 <sup>b</sup>	0.38 <sup>b</sup>	1.63 <sup>b</sup>
	<i>FI (E)</i>	104.35	131.40	0.21 <sup>a</sup>	0.35 <sup>c</sup>	1.67 <sup>b</sup>
	<i>FI (FARM)</i>	104.39	130.08	0.20 <sup>ac</sup>	0.26 <sup>d</sup>	1.33 <sup>ac</sup>
150 – 160	<i>FI (-10)</i>	155.21	191.97	0.19 <sup>a</sup>	0.29 <sup>a</sup>	1.51 <sup>a</sup>
	<i>FI (+10)</i>	155.22	198.88	0.22 <sup>b</sup>	0.35 <sup>b</sup>	1.57 <sup>a</sup>
	<i>FI (E)</i>	154.93	194.25	0.20 <sup>ab</sup>	0.32 <sup>c</sup>	1.58 <sup>a</sup>
	<i>FI (FARM)</i>	155.42	189.58	0.18 <sup>a</sup>	0.23 <sup>d</sup>	1.32 <sup>b</sup>

Table 3.7 a: Z-test probability values using the weight frequency values,  $x$  = average,  $s$  = standard deviation to calculate the expected probability ( $p$ ) of values above  $X$  = abalone weight.  $p$  values above a given value,  $X$  were subtracted from  $p$  values below a given value  $X$ , to determine the probability at which abalone fall into a range of 15-25 g, 25-35 g and 35-45 g abalone<sup>-1</sup>. Remaining percentages are not shown as they fall out of the size ranges shown in the table. (Top: 10 – 20g, middle (b): 100 – 110g, bottom (c): 150 – 160g).

(a)

Weight class (g)	Feeding regimen	Z (x, s) = X	Probability (p)	Size range (g)	Percentage
10 – 20	FI (-10)	Z(22.9, 6.69) = 15	0.88	15-25	50%
		Z(22.9, 6.69) = 25	0.38	25-35	34%
		Z(22.9, 6.69) = 35	0.04	35-45	3%
		Z(22.9, 6.69) = 45	0.00		
	FI (+10)	Z(24.25, 7.22) = 15	0.90	15-25	44%
		Z(24.25, 7.22) = 25	0.46	25-35	39%
		Z(24.25, 7.22) = 35	0.07	35-45	7%
		Z(24.25, 7.22) = 45	0.00		
	FI (E)	Z(24.63, 8.86) = 15	0.86	15-25	38%
		Z(24.63, 8.86) = 25	0.48	25-35	36%
		Z(24.63, 8.86) = 35	0.12	35-45	11%
		Z(24.63, 8.86) = 45	0.01		
	FI (FARM)	Z(25.91, 8.86) = 15	0.89	15-25	35%
		Z(25.91, 8.86) = 25	0.54	25-35	39%
		Z(25.91, 8.86) = 35	0.15	35-45	14%
		Z(25.91, 8.86) = 45	0.02		

(b)

Weight class (g)	Feeding regimen	Z (x, s) = X	Probability (p)	Size range (g)	Percentage
100 – 110	FI (-10)	Z(125.9, 10.58) = 110	0.93	110-120	22%
		Z(125.9, 10.58) = 120	0.71	120-140	62%
		Z(125.9, 10.58) = 140	0.09	140-160	9%
		Z(125.9, 10.58) = 160	0.00		
	FI (+10)	Z(125.2, 11.28) = 110	0.91	110-120	23%
		Z(125.2, 11.28) = 120	0.68	120-140	58%
		Z(125.2, 11.28) = 140	0.09	140-160	9%
		Z(125.2, 11.28) = 160	0.00		
	FI (E)	Z(125.6, 11.44) = 110	0.91	110-120	23%
		Z(125.6, 11.44) = 120	0.69	120-140	58%
		Z(125.6, 11.44) = 140	0.10	140-160	10%
		Z(125.6, 11.44) = 160	0.00		
	FI (FARM)	Z(124.6, 11.07) = 110	0.91	110-120	25%
		Z(124.6, 11.07) = 120	0.66	120-140	58%
		Z(124.6, 11.07) = 140	0.08	140-160	8%
		Z(124.6, 11.07) = 160	0.00		

(c)

Weight class (g)	Feeding regimen	Z (x,s) = X	Probability (p)	Size range (g)	Percentage
150 – 160	FI (-10)	Z(186.5,12.15) = 160	0.99	160-180	28%
		Z(186.5,12.15) = 180	0.70	180-195	46%
		Z(186.5,12.15) = 195	0.24	195-215	23%
		Z(186.5,12.15) = 215	0.01		
	FI (+10)	Z(186.7,12.24) = 160	0.99	160-180	28%
		Z(186.7,12.24) = 180	0.71	180-195	46%
		Z(186.7,12.24) = 195	0.25	195-215	24%
		Z(186.7,12.24) = 215	0.01		
	FI (E)	Z(184.5,13.36) = 160	0.97	160-180	33%
		Z(184.5,13.36) = 180	0.63	180-195	42%
		Z(184.5,13.36) = 195	0.22	195-215	20%
		Z(184.5,13.36) = 215	0.01		
	FI (FARM)	Z(181.83,13.12) = 160	0.95	160-180	40%
		Z(181.83,13.12) = 180	0.56	180-195	40%
		Z(181.83,13.12) = 195	0.16	195-215	15%
		Z(181.83,13.12) = 215	0.01		

## Discussion

### *Water quality*

Water temperature, dissolved oxygen, pH and FAN concentrations were all within acceptable ranges for abalone growth. The water temperature ranged from 14.3 – 19.3 °C across both experiments one and two at an average of 16.10 °C and 16.37 °C over the 112-day growth period in both experiment one and two, respectively, which is within the optimal physiological range (12-20 °C) determined by Britz *et al.* (1997). Water temperature did not change along the length of the tank which was also found by Yearsley (2008). Yearsley (2008) quantified that water temperature did not change in the tank's regions from the inlet to the outlet of flow through tanks. This could be due to the high flow rates that are set under intensive farming conditions which allows for water to be exchanged regularly to attempt to keep water temperature constant along the length of flow through tanks.

Similarly, average dissolved oxygen concentration of all the tanks in both experiment one and two were above a range of 7.0 – 7.2 mg L<sup>-1</sup> determined by Yearsley (2008) in flow-through systems. Sufficient growth rates in 20 – 30 g *H. midae* fed high protein formulated feeds were observed at this range by Yearsley (2008). Furthermore, Naylor *et al.* (2011) showed that in serial-use raceways, a dissolved oxygen concentration and oxygen saturation range of 7.04 – 9.12 mg L<sup>-1</sup> (89.9 % – 96.6 %), resulted in no reduction in growth after determining the first limiting water quality variable in that study. Therefore, the dissolved oxygen concentrations in the present study likely had no effect on abalone growth.

The lowering of pH in both serial-use raceways and flow through tanks is attributed to increased metabolic processes such as aerobic respiration while feeding and moving (Yearsley 2008, Naylor *et al.* 2011). In flow through systems, pH decreases from the inlet to the outlet (Yearsley 2008). This was observed in the present study's experiment one with the average pH decreasing from inlet to the outlet with values of 7.91 – 7.84, respectively. However, given the apparent minimum of 7.6 determined by Naylor *et al.* (2011) who showed that at an average pH value of 7.75 good growth was observed and that pH below 7.6 resulted in significantly lower weight gain in *H. midae*. Given that experiments one and two had an average pH of 7.87 and 7.86, respectively, which is slightly higher than the pH of 7.75 measured by Naylor *et al.* (2011), pH likely had no effect on growth.

The principle nitrogenous waste excreted by abalone is ammonia (Barkai and Griffiths 1987, Naylor *et al.* 2011). Uneaten feed can lead to increased ammonia concentrations due to the bacterial decomposition of the feed, especially with feeds containing high protein levels (Yearsley *et al.* 2009, Naylor *et al.* 2011). It has been documented that the FAN concentration that reduced growth by up to 5 % in juvenile *H. rubra* and *H. midae* was at FAN < 4 µg L<sup>-1</sup> and reduced by up to 50 % above 7 µg L<sup>-1</sup> (Huchette *et al.* 2003, Reddy-Lepata *et al.* 2006). FAN concentrations in the present study of 3.23 µg L<sup>-1</sup> and 3.55 µg L<sup>-1</sup> one experiments one and two, respectively, FAN likely had no effect on growth. However, a more in depth study is needed to quantify the effect of FAN on growth when testing the effect of feeding daily rations on feeding intensity in *H. midae*.

### *Experiment 1*

Abalone fed at 0.454 % BM d<sup>-1</sup> had an average daily growth rate of 0.42 % BM d<sup>-1</sup>. Additionally, despite the absence of significant effects between each pellet type strategy, the Z-test indicated that there was a higher probability of abalone falling in the upper weight class when fed the L d<sup>-1</sup> strategy. On average, 72 % of the abalone fell in in the size range of 50 – 70 g when fed L d<sup>-1</sup> at a ration size 0.454 % BM d<sup>-1</sup> for 112-days, which is 16.1% higher than the B d<sup>-1</sup> and SP d<sup>-1</sup> strategies.

The three models derived in the present study can be used to predict important production parameters for the 30 – 40 g abalone weight class. Figure 3.3a showed the relationship between feeding intensity and growth. The model suggests that as feeding intensity increases, daily growth rate decreases with maximum growth rate of 0.55 % BM d<sup>-1</sup> at a daily feeding intensity of 0.38 % BM d<sup>-1</sup>. Considering this, a feeding intensity range of 0.38 – 0.45 % BM d<sup>-1</sup> should be used as the basis for calculating a daily ration for *H. midae* when starting with the 30 – 40 g weight class.

Furthermore, given the relationship between feeding intensity and FCR, a daily ration of 0.38 – 0.45 % BM d<sup>-1</sup> will result in an average FCR of 1.05:1, which is lower and more efficient in comparison to a study by Green *et al.* (2011). The study compared different protein levels (18 – 26 % protein) and energy concentrations of formulated ABFEED diets on their effect on growth, FCR and feed intake of *H. midae* of 29-g weight and found that FCR was not different

between protein at FCR = 2.48:1 across all protein feeds. The higher FCR values observed by Green *et al.* (2011) could be due to the different energy contents of feed used in the study, with feeds containing lower energy resulting in higher FCR values. However, dietary protein levels did not differ and the pooled values of each treatment in the study could also account for the higher values of FCR. The present study was conducted under farming conditions whereas Green *et al.* (2011), conducted an experiment under a well-controlled laboratory environment, which could also account for the differences in FCR of *H. midae* between the two studies.

### *Experiment 2*

Feeding regimen had a significant effect on growth rate, feeding intensity and FCR. The results suggest that feeding regimens should be size specific, to attain faster growth rates while maintaining efficient FCRs. Feeding regimens also influenced size variation after a growth period of 112 days, which may have practical applications. Size-specific daily rations which were the FI (E), FI (+10) and FI (-10) regimens resulted in a higher daily growth rate, feeding intensity and less efficient FCR over the 112-day growth compared to the farm feeding method, FI (FARM) regimen in the 100 – 110 g and 150 – 160 g abalone<sup>-1</sup> weight classes however, this was not observed in the 10 – 20 g abalone<sup>-1</sup> weight class. Similarly, size specific daily rations resulted in a higher percentage of abalone falling into the higher weight classes after a 112-day growth period. The 10 – 20 g abalone<sup>-1</sup> weight class grew at the same rates after a 112-day growth period between all feeding regimens, however the FI (FARM) regimen showed a lower daily feeding intensity and FCR after the 112-day growth period, and a higher percentage of abalone falling into the upper weight classes. As revenue is produced on a per-weight-basis on abalone farms, identifying a feeding regimen that results in a higher growth rate, a greater generation of abalone biomass and a larger number of heavier abalone with higher potential value is important to abalone farmers (Troell *et al.* 2006, Francis *et al.* 2008). It is important however, to take into consideration the costs associated with feeding and abalone growth, which will be explored with the results of this experiment and economic calculations.

### *Growth*

Although daily growth rate was not influenced by feeding regimen in the 10 – 20 g abalone<sup>-1</sup> weight class in the present study, using the model of daily growth rate as a function of feeding intensity, average daily growth rate for this weight class after a 112-day growth period was 0.55 % BM d<sup>-1</sup>. Abalone grew significantly larger over a 112-day growth period with a pooled average initial weight of 12.02 g abalone<sup>-1</sup> growing to an average pooled final weight of 28.30 g abalone<sup>-1</sup> which is a final average weight of 2.3 times more than the initial average weight after 112 days. Britz and Hecht (1997) conducted a study that quantified daily growth rate of *H. midae* of a similar weight class (7 – 14 g abalone<sup>-1</sup>) under laboratory conditions, at a range of 0.1 – 0.45 % BM d<sup>-1</sup> when testing the effect of dietary protein:energy ratios on growth and FCR (24 – 44 % protein content). The general trend observed by Britz and Hecht (1997) showed that an increase in protein contents of feed resulted in higher daily growth rate and the ranges of daily growth rate achieved by abalone were largely due to the ranges of different nutrient contents used. Even though the maximum growth rate of 7 – 14 g abalone<sup>-1</sup> weight class observed by Britz and Hecht (1997) used different nutrient contents to the present study, the daily growth rate of the present study is 22 % higher keeping in mind that the present study was conducted under commercial farming conditions. This suggests that estimating growth under laboratory conditions can potentially lead to an underestimated representation of abalone growth rates of this weight class compared to the growth rates achieved under farming conditions. Furthermore, a 22 % higher daily growth rate of the present study compared to that of Britz and Hecht (1997) implies that abalone will reach larger more valuable weight classes in a shorter period of time which will have many benefits to abalone farmer.

Growth was significantly affected by feeding regimen for the 150 – 160 g abalone<sup>-1</sup> weight class. The final average weight and daily growth rate of this weight class after 112-days was significantly higher for the FI (+10) regimen compared to the FI (FARM) regimen. The present study shows that a daily ration of 0.352 % BM d<sup>-1</sup>, the FI (+10) regimen resulted in an average daily growth rate of 0.22 % BM d<sup>-1</sup>. Interestingly, there was no difference in average abalone weight up to 84-days of feeding, but after this time period, abalone attained significantly heavier weights under the FI (+10) regimen compared to the FI (FARM) regimen, which is where the significant difference was found using repeated measures ANOVA. This implies that the daily ration feeding method only began to have an effect of average abalone weight after 112-days of feeding and when the abalone were over approximately 185 grams.

There is a paucity of information regarding abalone growth for abalone above 60 grams, therefore this study contributed to quantifying growth rates as a function of abalone size, especially under farming conditions. The present study shows that the smaller abalone size class grew at a higher percentage of body mass every day in comparison to the two larger size classes. This conforms with a suggested model for aquatic organisms (Jobling *et al.* 1993), that suggests that an increase in body weight results in growth rates as a higher percentage of body mass.

Growth rates were better for abalone fed daily size-specific rations. However, due to many farms aiming to improve growth rates at the most efficient rate to maximise profits, feed conversion ratios need to be considered. Similarly, although the growth rate achieved using the FI (+10) regimen was higher than the FI (FARM) regimen, further economic analyses need to consider the FCR of each regimen to explore the economic aspects associated with higher growth rates and the FCR of the regimen.

#### *Feed conversion ratio*

Feed conversion ratios differed depending on the feeding regimen across all weight classes in the present study. The FI (FARM) regimen had the lowest FCR at 0.50:1 for the 10 – 20 g abalone<sup>-1</sup> weight class. Indicating that the previously mentioned average daily growth rate of 0.55 % BM d<sup>-1</sup> rate can be achieved at an efficient FCR. The FCR value below one could be accounted for with the calculation of FCR as it considers as dry feed fed per wet weight gain. When FCR values are below 1, it is possible that abalone were holding excess water when weighed. In addition, however, FCR values below one have been documented in *H. midae* in other studies. For example, Knauer *et al.* (1996), compared growth performance in juvenile *H. midae* (0.7 g weight), and quantified that on a pelletised diet, FCR was 0.44:1 while abalone grew well. The low FCR in the present study is comparable with Knauer *et al.* (1996), and suggests that in the present study, abalone in the 10 – 20 g weight class are efficient converters of pelletised feed.

Feed conversion ratio in the 100 – 110 g abalone weight class was less efficient for the FI (+10) regimen at an average of 1.63:1 compared to the FI (FARM) regimen which was 1.33:1 after 112 days. This suggests that abalone under the FI (FARM) regimen were fed less feed to realise

a daily growth rate that is 20 % lower than the FI (+10) regimen. However, the FI (-10) regimen which was fed a daily ration of 0.315 % BM d<sup>-1</sup>, which resulted in a lower FCR of 1.48:1 at an average daily growth rate of 0.21 % BM d<sup>-1</sup> which is a 5 % higher growth rate than the FI (FARM) regimen. Similarly, for the 150 – 160 g abalone weight class in the present study, the feeding regimens using daily rations resulted in higher, less efficient FCRs compared to the FI (FARM) regimen. The FI (+10) regimens resulted in the highest growth rate in this size class too, at an FCR of 1.57:1. Given that higher growth rates were achieved by regimens using daily rations, an investigation into the feeding intensity of each regimen for each weight class is needed. Similarly, the relationship between feeding intensity with daily growth rate and FCR has not been documented in *H. midae* which emphasises the need to understand the effect of and possible implementation into feeding strategies on farms.

#### *Feeding intensity*

Feeding intensities were different across feeding regimens across all weight classes in the present study. As there is limited information regarding feeding intensity in abalone, this study is crucial to the understanding of feeding intensity and the role it plays in abalone biology with further implications associated with abalone farming. The present study has quantified feeding intensity which has allowed for the construction of models regarding daily growth rate and FCR as a function of feeding intensity after a 112-day growth period. These models can be used to predict daily growth rate and FCR at a given feeding intensity for *H. midae* in the weight classes of the present study under commercial farming conditions rather than in a well-controlled laboratory experiment. This gives a better representation of what occurs on farms making it applicable to abalone farmers.

For efficient growth of abalone in the 10 – 20 g weight class, abalone should not be fed more than 0.42 % BM d<sup>-1</sup> in order to achieve a good FCR, as suggested by the results. As abalone fed the FI (FARM regimen) grew better, it is suggested that this method be used to grow this weight class of abalone for a period of 112-days.

Similarly, for efficient growth in the 100 – 110 g abalone weight class, abalone should not be fed more than 0.31 % BM d<sup>-1</sup>, which was the feeding intensity resultant of the FI (-10) regimen after 112-days. Furthermore, given the correlation between feeding intensity and FCR in this weight class, to achieve faster daily growth rates, abalone should not be fed more than 0.38 %

BM d<sup>-1</sup>, as this leads to higher FCR and the consequential higher costs to obtain such growth. Abalone in the 150 – 160 g weight class grew most efficiently under the FI (FARM) regimen and is suggested that abalone should not be fed more than 0.23 % BM d<sup>-1</sup>. For faster growth, yet higher FCR and associated costs, abalone should not be fed more than 0.35 % BM d<sup>-1</sup>.

Considering the range of feeding intensities realized by the abalone being fed both size-specific daily rations and farming method, a further economic analysis needs to be conducted to weigh up the costs and benefits of each feeding regimen. For instance, it should be discussed whether the benefits of faster growth of abalone outweigh the costs associated with a less efficient FCR.

#### *Size variation and z test*

Size variation is an important variable to discuss as it has many commercial implications with regard to the size-grading aspects of abalone production (Heath and Moss 2009). In the process, slower growing organisms are sorted apart from the faster growing individuals as it is hypothesised that mixing slow and fast-growing abalone can result in the faster growing abalone outcompeting the slower growing abalone in a farm tank environment (Heath and Moss 2009).

The z-test data in the present study showed that abalone fed size-specific daily rations fell into larger weight classes after 112 days in the 100 – 110 g and 150 – 160 g abalone weight classes compared to the farm method. For instance, the 150 – 160 g weight class showed a 60 % higher probability  $((24\% - 15\%) / 15\%)$  of abalone falling in the highest weight class of 195 – 215 g abalone<sup>-1</sup> fed a daily ration of 0.352 % BM d<sup>-1</sup> after 112-days in comparison to abalone fed under the farming method FI (FARM) which had a 43 % higher probability that abalone fell in the lowest weight class. Similarly, in the 100 – 110g weight class abalone fed the FI (FARM) method resulted in 13 %  $((25 - 22) / 22)$  higher probability of falling in the lowest weight class of 110 – 120 g compared to the other feeding regimens using daily rations. The smaller weight class 10 – 20 g abalone weight class shows that a 27 % higher percentage of abalone fell into the upper weight class of 35 – 45 g abalone under the farm feeding method in comparison to the second highest, FI (E).

Each weight class on an abalone farm has different potential monetary value. The larger the abalone weight class, the longer the period of monetary investment to achieve larger weights. This emphasises the need to include weight frequency data into economic calculations.

## **Conclusion**

Water quality was well within the acceptable range for *H. midae* growth. Feeding the leaves only pellet strategy may result in a higher percentage of abalone falling in a higher weight class after a 112-day growth period. Daily growth rate and FCR were significantly correlated with feeding intensity with higher feeding intensities resulting in lower daily growth rates and higher FCR values. A daily ration of 0.454 % BM d<sup>-1</sup> resulted in efficient growth of *H. midae* for all pellet type strategies. Abalone of 30 – 40 g weight class at the beginning of a growth period should be fed this daily ration and given the change in mass after 112-day, the daily ration should be adjusted as a function of the total biomass of abalone in a basket.

Feeding size specific daily rations resulted in faster growth rates than the commercial feeding method in larger abalone, however, leading to less efficient FCR values. The relationship between feeding intensity, FCR and daily growth rate has been correlated and can be used to estimate a range of FCR and daily growth rate values as a function of abalone feeding intensity. Feeding abalone daily rations, resulted in a higher probability of abalone falling into higher weight classes and lower probability of abalone falling in the lower weight class ranges, after 112 days in the larger abalone weight classes compared to the farm feeding method. Further economic analysis is needed to weigh up the benefits of faster growth at higher FCR values and the consequential costs of production versus efficient growth rates at lower FCR values and lower associated costs.

## CHAPTER 4

### General Discussion

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The aim of this study was to quantify feed intake and feeding intensity across a range of commercially relevant weight classes of *H. midae* using two experimental conditions, (1) a small-scale well controlled environment and (2) a commercial farming environment in order to produce information on feed intake and feeding intensity as a function of *H. midae* weight class and pellet type. The present study used laboratory style experiments to produce a better understanding of feed intake using abalone weight classes that are commercially important to the industry. Experimental system design under laboratory conditions were small-scale but proportionally designed to match conditions that occur commercially such as basket design, flow rates, abalone handling procedures, aeration and abalone densities using a water temperature-controlled flow through system. In addition, two different formulated pellet types that have not been studied, were used to achieve the feed intake data. Farm scale experiments produced information regarding important production parameters such as growth, feeding intensity, FCR and size variation of *H. midae* under different feeding regimens. Farm scale experiments were performed under commercial conditions with tank size, basket size and design, water flow rates, stocking densities and abalone handling methods were set to be the same for all treatments. The information produced on abalone production parameters was achieved using a commercial feeding method that was compared to feeding regimens using abalone size-specific daily rations, as well as the use of different pellet types (shape and size) of the same nutrient composition.

The information produced on feed intake and feeding intensity can be used to make feeding method suggestions for abalone farmers, as well as to ascertain whether small-scale laboratory conditions can produce good estimates of abalone feed consumption under farming conditions. Given that the experiments incorporated system designs that are closely matched to those used in the industry, the results of this thesis are applicable to South African abalone farmers.

The results (chapters 2 and 3) can be used to (a) understand the relationship between feed intake, abalone weight class and the duration of feed availability in *H. midae*, in some abalone weight classes that have not been studied, (b) quantify the effects of different formulated pellet

types on feed intake and growth of *H. midae*, (c) quantify growth, feeding intensity and FCR as a function of size-specific feeding regimens, (d) correlate production parameters such as growth and FCR to feeding intensity of *H. midae*, (e) utilize weight-frequency distributions to analyse and explore size variation of *H. midae* under different feeding regimens, (f) determine an optimal feeding method based on a combination of data from feed intake and feeding intensity experiments, (g) predict *H. midae* feeding intensity as a function of abalone weight under farming condition and (h) provide a description of water quality conditions across all experimental chapters.

For chapters 2 and 3, abalone weight classes of 10 – 20 g, 100 – 110 g and 150 – 160 g abalone<sup>-1</sup> were used. Furthermore, experiment one in chapter 3 used abalone of the 30 – 40 g weight class which is the weighted average of abalone stock at HIK abalone farm, Hermanus, South Africa (M. Naylor – HIK Abalone Farm, pers. Comm.). The wide range of abalone weight classes used to produce information of feed intake and production parameters is beneficial to the understanding of *H. midae* biology as there is a paucity of published information regarding these aspects above abalone weights of 60 g abalone<sup>-1</sup>.

### **Water quality**

Part of Chapter 1 explored literature with regard to water quality conditions needed to realize good abalone growth and what is best suited for feed intake in *H. midae*. Water quality variables such as water temperature, dissolved oxygen, pH, total ammonia nitrate (TAN) and free ammonia nitrate (FAN) were measured to show the environmental conditions represented in all experiments from Chapter 2-4. However, TAN and FAN were not measured in the feed intake experiments (chapter 2) due to the short period of abalone housed within each experiment. Rather than correlating the effects of water quality variables on abalone feed intake and growth, the conditions observed in the experiments serve as a description of a range of conditions within optimal *H. midae* physiological tolerance for feed intake and growth. The only controlled water quality variable in the present study was water temperature in chapter 2, as it directly effects feed intake in *H. midae* (Britz *et al.* 1997).

All water quality variables in the present study under both small-scale laboratory conditions and farming conditions were within acceptable ranges for abalone feed intake and production

as explored and discussed in chapters 2 and 3. However, a more in-depth study is needed to quantify the effect of FAN on growth when testing the effect of feeding daily rations on feeding intensity in *H. midae* as well as an exploration into the pH of abalone farm inlets.

The effect of feeding regimen and pellet type on water quality variables was not quantified in the present study (chapter 3). This was largely due to the system design and block design of treatments in the chapters and the flow dynamics of the farm tanks and the “oyster mesh” basket design. The farm tanks are flow through tanks with water coming in from an inlet and a unidirectional flow of water towards the outlet where water exits the tank. Measuring the specific water quality conditions within a basket within a tank is not possible due to this movement of water as the movement of water allows for possible cross contamination of water from baskets directly next to and around the basket being measured. The only way to overcome this obstacle, would be to design an experiment where cross-basket water contamination is not a confounding variable. A study would have to use tank replicates assigned a specific feeding regimen (treatment), where all baskets within a tank containing abalone are fed under the same feeding regimen (treatment). In saying this however, for example, Chapter 3 experiment one of the present studies shows that feeding between 1.02 – 1.44 kg basket<sup>-1</sup> (table 3.3) of protein rich feed across 18 baskets containing 30 – 40 g abalone resulted in average FAN concentration of 3.23 µg L<sup>-1</sup> under farming conditions over 112-days. Additionally, the abalone grew well in both experiments of chapter 3 which indicates that the daily rations fed over 112 days results in acceptable water quality environment for *H. midae* production.

With regard to future studies involving pH, there are studies have focussed on the effects of ocean acidification on pH in natural environments. Hartin *et al.* (2016) shows that the present averages of seawater pH values (approximately 8.1) can reduce by up to 0.32 units by the year 2100 due to increased carbon emissions from anthropogenic sources, such as fossil fuel emissions. This emphasizes that the lower than “normal” pH values observed in each chapter could be attributed to both the influence of ocean acidification and the release of carbon dioxide via the abalone’s metabolic activities. Future studies should focus on the historical pH values at the inlet pumps of farms to assess such a phenomenon.

### **Effect of formulated feed pellet type on feed intake and production parameters**

The pellet types used in chapters 2 and 3, are those that are used in the industry, specifically at HIK abalone farm, which makes the results of this thesis applicable to the farmers. While there were no significant effects of pellet type (size) on growth and feed intake, chapter 3 showed that abalone fed leaves under a size specific daily ration feeding regimen resulted in a higher probability of abalone falling in larger weight ranges after a 112-day growth period. This emphasizes the need for further studies exploring the diet preferences under farming conditions.

In the wild, *H. midae* change their diet depending on the life stage and availability of food (Barkai and Griffiths 1986). As juveniles they focus their feeding on microalgae, and this changes to a macroalgae based diet as adults. The two pellet types used in the present study, were of the same nutrient composition but differed in size and dimension. The smaller pellets (short) best simulate smaller pieces of macroalgae while the larger pellets (leaves) represent larger pieces of kelp. Given the changes in abalone diet throughout their life stages in the wild, further studies need to include different abalone size ranges while testing the effects of different pellet types on production parameters under farming conditions. Additionally, future studies should consider testing the effect of the different pellet type on abalone feeding behaviour and diet preference under laboratory conditions while using different abalone weight classes that range from juveniles to adults.

Abalone fed each pellet type strategy were represented in all the basket positions within a tank to account for the effects of water quality on abalone production and each basket consisted of the same design to “house” the abalone which contained the same stocking density of abalone at the beginning of the experiment. Short pellets would have dispersed easier within a basket as they have a higher volume to surface area ratio than the leaves and leaves would allow for abalone to feed for longer periods of time as they are larger. Given that there was no effect of pellet size and shape on growth, FCR and feeding intensity, the size variation data, however, shows that there were higher percentages of abalone falling in heavier weight classes after the growth period. Identifying the reasons for a lack of significant effects of pellet type on growth, FCR and feeding intensity is not conceivable however, in this case, due to the weight variation data showing a higher percentage of abalone falling into larger weight classes after 112 days

of being fed leaves only, this emphasizes the need to explore feeding behaviour studies while testing the effects of the three pellet type strategies used in the present study in future studies.

### **Abalone size, feed intake, growth, feeding intensity and identifying optimal daily rations**

Chapters 2 and 3 produced information on feed intake, and production parameters such as growth and feeding intensity using three commercially relevant abalone weight classes namely, 10 – 20, 30 – 40, 100 – 110 and 150 – 160 g abalone<sup>-1</sup>. The present study has contributed valuable information with regards to these variables as there is a paucity of information related to these variables in abalone above 60 g abalone<sup>-1</sup>. The limited information can be largely accounted for due to the logistical reasons for sourcing abalone of larger sizes as they are of high value to abalone farms. Given the use of the larger abalone weight classes in the present study and the use of abalone below 60 grams at which there is a multitude of associated research, the present study has quantified feed intake, growth and feeding intensity as a function of abalone weight or size.

Chapters 2 and 3 were conducted under different experimental conditions with chapter two being a laboratory study, which was a well-controlled environment and chapter 3 was an experiment under commercial farming conditions. These two experimental conditions need to be compared with regards to determining daily rations for farmed *H. midae* ultimately exploring whether feed intake experiments under laboratory conditions can provide accurate estimates of feed intake under farming conditions. This can then be used further as a means to determine daily rations of abalone farms.

Chapter 2 quantified daily feed intake in abalone from 10 – 160 g abalone<sup>-1</sup>. Feed intake in the 10 – 20 g weight class was quantified at 0.55 % BM d<sup>-1</sup>, but between the 100 – 110 g and 150 – 160 g weight classes, feed intake was not different. This implies that these two weight classes eat the same amount of food per body mass which does not conform with the previously mentioned assumptions of feed intake as function of body mass decreasing with weight. This has many practical applications to abalone farmers as this suggests that these two weight classes could be fed the same amount of feed as they do not eat more or less than each other. However, this was not the case for growth and feeding intensity results produced in chapter 3.

Chapter 3 had two experiments that quantified growth, FCR and feeding intensity in four abalone weight classes, which includes the same three weight classes used in chapter 2 for experiment two of chapter 3 and an additional 30 – 40 g weight class in experiment one of chapter 3. The information produced by chapter three has allowed for the construction of models that use the feeding intensity of abalone to predict daily growth rate and FCR over a 112-day growth period which is the commercial grading period at HIK abalone farm. The quantification of *H. midae* growth can be categorized into a weight class range of 10 – 160 g weight class. The resultant daily growth rates of the 10 – 20 g, 30 – 40 g, 100 – 110 g and 150 – 160 g weight classes were at 0.55, 0.42, 0.22 and 0.19 % BM d<sup>-1</sup> after 112-days of growth (pooled average across all treatments in experiment one and two, Chapter 3, table 3.4, table 3.6), which conforms with the previously mentioned assumption by Jobling *et al.* (1993) that growth as a function of body mass decreases with an increase in body mass.

Furthermore, like growth the feeding intensity information produced in chapter 3 too conforms with this model assumption as the quantification of the same weight classes, 10 – 20 g, 30 – 40 g, 100 – 110 g and 150 – 160 g and resulted in pooled averages across all treatments used in all experiments at 0.55, 0.43, 0.33 and 0.30 % BM d<sup>-1</sup>. This is shown in a logarithmic model (figure 4.1) that describes feeding intensity as a function of the above-mentioned *H. midae* weight classes. The model was constructed using least squares regression analysis showing a strong relationship between these two variables and can be used to predict abalone feeding intensity under farming conditions over a 112-day growth period in abalone weights up to 160 g. Furthermore, these feeding intensity values can be used as a basis for determining daily rations on abalone farms for abalone up to 160 g. However, these feeding intensity values show a different trend regarding feed intake as function of abalone weight in weight classes above 100 g, and therefore need to be compared with feed intake results from chapter 2 as both involve abalone feed consumption but they are calculated differently.

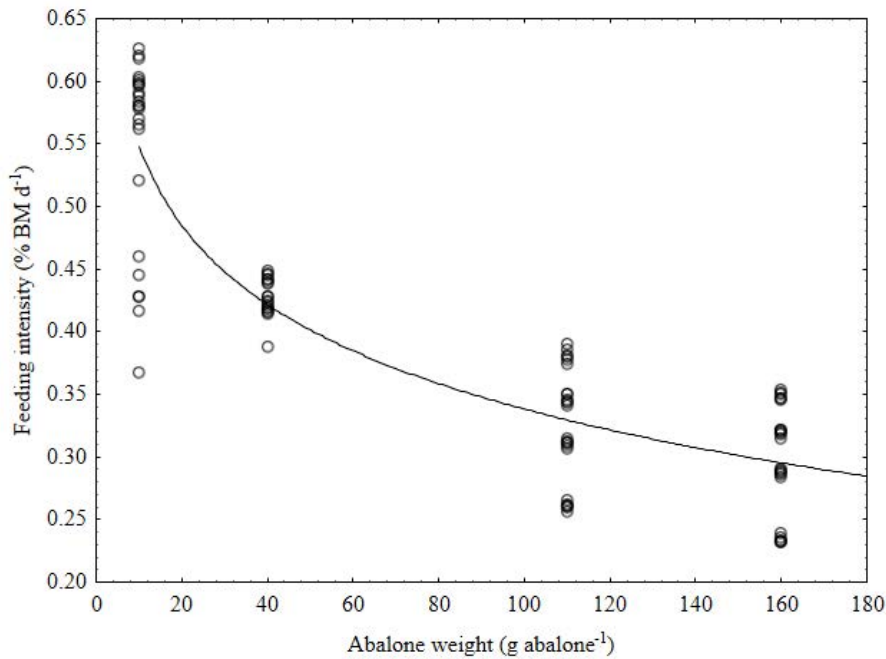


Figure 4.1: Feeding intensity as a function of abalone weight after a 112-day growth period (least squares regression analysis;  $r^2 = 0.72$ ,  $p < 0.0000$ ,  $FI (\% \text{ BM } d^{-1}) = -0.21\text{Log}_{10}(w) + 0.7583$ , where  $FI$  = feeding intensity and  $w$  = abalone weight (g)).

#### *Feed intake, feeding intensity and daily ration calculations*

When comparing feed intake to feeding intensity, it is crucial to first identify what each of these variables mean. For instance, feed intake in the present study was determined using dry weight analysis and is a direct representation of the amount of food the abalone has consumed. Whereas feeding intensity is what abalone consume as function of the rate and efficiency at which abalone grew over a given growth period, in this case 112 days (Feeding intensity = daily growth rate multiplied by the FCR).

The quantification of feed intake and daily feeding intensity values in the smallest weight class (10 – 20 g) used in the present study are shown to be 0.55 % BM  $d^{-1}$  which indicates that both a small-scale laboratory study and large-scale farm study can be used to determine daily rations for feeding this weight class. However, the results from chapter 3 indicate that the farm feeding method resulted in the best and most efficient growth in the small weight class which means that calculating daily rations for this weight class is unnecessary based on the farming methods used (based on apparent satiation, see chapter 3 “*experimental feed*”). Furthermore, the feed

intake and feeding intensity values should still be used as reference values on abalone farms as they incorporate what this weight class can “actually” consume and what they consume that results in an optimised growth performance.

The 100 – 110 g weight class was the middle abalone weight class used in the chapters 2 and 3, and information regarding feed intake and feeding intensity at this weight class contributes to a further understanding of the biology of *H. midae* given the paucity of information above 60 g abalone<sup>-1</sup>. Chapter 2 quantified daily feed intake at 0.30 % BM d<sup>-1</sup> and chapter 3 quantified feeding intensity at 0.33 % BM d<sup>-1</sup> (pooled feeding intensity for all treatments in chapter 3, experiment 2) which indicates that under farming conditions over a longer period compared to laboratory conditions in the presents study resulted in abalone potentially consuming 9.1 % higher as a percentage of body mass. However, chapter 3 shows that abalone of this weight class grew the fastest when fed a size-specific daily ration of 0.385 % BM d<sup>-1</sup>, FI (+10) regimen, which resulted in a daily feeding intensity of 0.38 % BM d<sup>-1</sup> after 112 days. In this case, abalone of this weight class consumed feed at 25.4 % higher as a percentage of body mass when compared to the feed intake value of 0.30 % BM d<sup>-1</sup> from chapter 2. This indicates that abalone in this weight class can potentially consume a much higher percentage of their body mass when exposed to farming conditions which can also be shown by higher FCR values, as more feed was consumed and converted into mass. It is therefore recommended that the feeding intensity values obtained in chapter 3 be used as the means for determining daily rations for this weight class to produce faster growth rates. In addition, the feed intake value of 0.30 % BM d<sup>-1</sup> is best represented by the daily feeding intensity of 0.31 % BM d<sup>-1</sup> from chapter 3, which is the resultant feeding intensity of the abalone fed size specific daily ration of 0.315 % BM d<sup>-1</sup>, FI (-10) regimen. This daily feeding ration resulted in slower growth than the FI (+10) regimen, however it was more efficient (lower FCR) and can be recommended to farmers that this regimen be used to maintain abalone growth with lower associated costs. However, further economic analysis is needed to determine what benefits the farmer while using feeding intensity as the principal variable in determining profitability.

Similarly, the biggest weight class used in the present study (150 – 160 g) showed a daily feed intake of 0.30 % BM d<sup>-1</sup>, which is the same feed intake result as the abalone weight class that is 50 g lighter (100 – 110 g) from chapter 2 and a resultant pooled feeding intensity of 0.30 % BM d<sup>-1</sup> across all feeding regimen treatments in chapter 3. However, abalone grew the fastest

when fed the FI (+10) regimen which was a size specific daily ration of 0.320 % BM d<sup>-1</sup> and resulted in a daily feeding intensity of 0.35 % BM d<sup>-1</sup>. This illustrates that abalone of this weight class can potentially consume food at a higher percentage of body mass (16.2 % higher) compared the feed intake value observed in chapter 2. Thus, it is recommended that the daily feeding intensity values produced in chapter 3 are to be used as a means to calculate daily rations for abalone of this weight class in order to maximise growth. In saying this, an economic model that uses the resultant feeding intensities of chapter 3, experiment two, should be considered in order to determine the profitability of each feeding regimen.

### **Economic considerations and optimal daily rations**

The generation of revenue on abalone farms depend on the farmers' ability to reduce costs of production while improving the efficiency of growth and production of biomass (Troell *et al.* 2006). Each weight class on a farm holds a value to the farm, as it has taken time, labour, production cost and hence financial investment to produce. For instance, larger abalone hold more value per kilogram than smaller abalone due to the longer period they have been on the farm, taking into consideration the longer duration of monetary investment and costs associated with attaining a larger size. A foremost driver of growth on abalone farms is the feeding regimen a farm utilises.

For the present study, abalone were grown under the same farming conditions across all experiments in chapter 3. This entails that the cost of water pumping, electricity and labour, remained constant. The variable that changed is the cost of feed, as a different amount of feed was utilised under each feeding regimen. Depending on the cost of feed per kilogram basis, farmers should consider the change in potential value of crucial abalone weight classes using the feeding intensity values realized after a 112-day growth period, which was modelled to predict daily growth rate and FCR across all weight classes under each feeding regimen, as well as the weight variation and z-test probability scores after 112-days of growth (quarterly grading period) under each feeding regimen determined in chapter 3 (table 4.3).

The assumptions of the economic model were: (1) all costs associated with water pumping, labour and electricity were constant across all feeding regimens over a quarterly grading period and where therefore not considered in the economic analysis, (2) all abalone biomass values

and stocking densities were the same at the beginning of the growth period, (3) all feeding regimens were compared against the farm-based regimen FI (FARM), (4) the potential values are based on the number of tanks and abalone used in chapter 3, experiment two, and, (5) the 10 – 20 g abalone weight class from chapter 3, experiment two, were not used in the economic model due to the low number of marketable animals.

The feeding intensity results after 112 days from chapter 3 of the 100 – 110 g weight class were used as the basis for the models derived to predict daily growth rate and FCR as a function of feeding intensity. The economic model shows that the size specific daily ration of the FI (E) regimen resulted in a higher potential value of abalone after 112-days (grading period) compared to the FI (FARM) regimen. In addition, the model shows that using 34 % more feed in comparison to the farming method, results in a 2.2 % higher potential value to the abalone farmers, which is annualised at 7.4 % more than the farming method (table 4.1).

Similarly, using the feeding intensity results of abalone under each feeding regimen and the models to predict daily growth rate and FCR as a function of daily feeding intensity, the 150-160 g abalone weight class fed size-specific daily rations generated a higher biomass using more feed (higher FCR values) compared to the farm's feeding method (FI FARM) as was found in chapter 3, experiment two. The potential value growth attained after 112-days greatly outweighs the cost of feed associated with higher FCR of the size specific daily rations (table 4.2). The FI (+10) feeding regimen resulted in a FCR of 1.62:1 which results in the use 51 % more feed used than the FI (FARM) regimen. This results in a 4 % higher potential value of abalone after a quarterly grading period and a 13.6 % higher annualised potential value compared to the farming method (FI FARM). In this situation, the relationship between feeding intensity and FCR and feeding intensity and daily growth rate (G) shows that the costs associated with higher FCR values and faster growth is beneficial to the abalone farmers.

When addressing these economic models, it is crucial to consider the fact that the number of abalone used in the calculations are a representative of the number of abalone used in experiment two of Chapter 3. If the number of abalone considered in the calculations of the model were to be adjusted to what occurs across an entire farm (of these weight classes), the potential values should change proportionally but will present much higher more applicable potential values.

Weight class (at start) - 100-110 g	Feeding regimen				
	FI (E)	FI (-10)	FI (+10)	FI (FARM)	
<b>Production parameters</b>					
Feeding intensity (% BM d <sup>-1</sup> ) – chapter 3	0.35	0.31	0.38	0.26	0.26
Daily growth rate (% BM d <sup>-1</sup> ) – chapter 3	0.221	0.210	0.229	0.196	0.196
Total growth after 112 days (% BM)	24.71	23.46	25.65	21.91	21.91
Initial average individual weight (g abalone <sup>1</sup> )	104.35	103.26	102.92	104.39	104.39
Number of abalone (6 baskets) (constant)	720	720	720	720	720
Final average individual weight (g abalone <sup>1</sup> )	130.14	127.49	129.32	127.26	127.26
Initial biomass (kg)	75.13	74.35	74.10	75.16	75.16
Final Biomass (kg)	93.70	91.79	93.11	91.63	91.63
FCR – chapter 3	1.60	1.49	1.69	1.34	1.34
mass of feed used (kg)	29.73	25.93	32.07	22.11	22.11
Cost of feed used @ R30.23/kg	R 898.71	R 783.86	R 969.44	R 668.33	668.33
<b>Percentage feed used relative to FI (FARM)</b>	<b>34%</b>	<b>17%</b>	<b>45%</b>	<b>0%</b>	<b>0%</b>
<b>Potential value weight classes</b>					
100-110 Z test probability	9%	7%	10%	9%	9%
110-120 Z test probability	23%	22%	23%	25%	25%
120-140 Z test probability	58%	62%	58%	58%	58%
140-160 Z test probability	10%	9%	9%	8%	8%
100-110 mass/weight class (kg)	8.43	6.43	9.31	8.25	8.25
110-120 mass/weight class (kg)	21.55	20.19	21.41	22.91	22.91
120-140 mass/weight class (kg)	54.35	56.91	54.00	53.14	53.14
140-160 mass/weight class (kg)	9.37	8.26	8.38	7.33	7.33
<b>Value per weight class</b>					
100-110 @ R320/kg	R 2 698.51	R 2 056.15	R 2 979.44	R 2 638.80	2 638.80
110-120 @ R340/kg	R 7 327.21	R 6 866.07	R 7 281.01	R 7 788.13	7 788.13
120-140 @ R 362/kg	R 19 672.91	R 20 601.88	R 19 548.85	R 19 237.60	19 237.60
140-160 @ R 445/kg	R 4 169.58	R 3 676.28	R 3 728.96	R 3 261.85	3 261.85
Potential value after 112-days	R 33 868.22	R 33 200.38	R 33 538.26	R 32 926.39	32 926.39
Full potential value - cost of feed (112-days)	R 32 969.51	R 32 416.52	R 32 568.82	R 32 258.06	32 258.06
<b>Difference (%) compared to FI (farm) after 112 days</b>	<b>2.2%</b>	<b>0.5%</b>	<b>1.0%</b>	<b>0.0%</b>	<b>0.0%</b>
<b>Annualised difference (%) compared to FI (farm) - compound interest</b>	<b>7.4%</b>	<b>1.6%</b>	<b>3.2%</b>	<b>0.0%</b>	<b>0.0%</b>

Table 4.1:  
Hypothetical economic model showing the comparison of potential values of abalone of the 100 – 110 g weight class under the feeding regimens used in experiment two of chapter 3 after 112 days

Weight class (at start) - 150-160 g	Feeding regimen			
	FI (E)	FI (-10)	FI (+10)	FI (FARM)
<b>Production parameters</b>				
Feeding intensity (% BM d <sup>-1</sup> ) – chapter 3	0.32	0.29	0.35	0.23
Daily growth rate (% BM d <sup>-1</sup> ) – chapter 3	0.208	0.197	0.219	0.175
Total growth after 112 days (% BM)	23.27	22.04	24.49	19.58
Initial average individual weight (g abalone <sup>1</sup> )	154.93	155.21	155.22	155.42
Number of abalone (6 baskets) (constant)	540	540	540	540
Final average individual weight (g abalone <sup>1</sup> )	190.98	189.41	193.24	185.85
Initial biomass (kg)	83.66	83.81	83.82	83.93
Final Biomass (kg)	103.13	102.28	104.35	100.36
FCR – chapter 3	1.55	1.48	1.62	1.33
mass of feed used (kg)	30.11	27.27	33.22	21.94
Cost of feed used @ R30.23/kg	R 910.37	R 824.38	R 1 004.10	R 663.23
<b>Percentage feed used relative to FI (FARM)</b>	<b>37%</b>	<b>24%</b>	<b>51%</b>	<b>0%</b>
<b>Potential value weight classes</b>				
140-160 Z test probability	5%	3%	2%	5%
160-180 Z test probability	33%	28%	28%	40%
180-195 Z test probability	42%	46%	46%	40%
195-215 Z test probability	20%	23%	24%	15%
140-160 mass/weight class (Kg)	5.16	3.07	2.09	5.02
160-180 mass/weight class (kg)	34.03	28.64	29.22	40.14
180-195 mass/weight class (kg)	43.31	47.05	48.00	40.14
195-215 mass/weight class (kg)	20.63	23.53	25.04	15.05
<b>Value per weight class @ price/kg</b>				
140-160 @ R445/kg	R 2 294.57	R 1 365.49	R 928.70	R 2 233.05
160-180 @ R 460/kg	R 15 654.63	R 13 174.18	R 13 440.12	R 18 466.53
180-195 @ R 472/kg	R 20 443.83	R 22 207.91	R 22 656.21	R 18 948.27
195-215 @ R 482/kg	R 9 941.41	R 11 339.21	R 12 071.07	R 7 256.14
Potential value after 112-days	R 48 334.43	R 48 086.79	R 49 096.10	R 46 903.99
Full potential value - cost of feed (112-days)	R 47 424.07	R 47 262.40	R 48 092.00	R 46 240.76
<b>Difference (%) compared to FI (farm) after 112 days</b>	<b>2.6%</b>	<b>2.2%</b>	<b>4.0%</b>	<b>0.0%</b>
<b>Annualised difference (%) compared to FI (farm) - compound interest</b>	<b>8.6%</b>	<b>7.4%</b>	<b>13.6%</b>	<b>0.0%</b>

Table 4.2:  
Hypothetical economic model showing the comparison of potential values of abalone of the 150 – 160 g weight class under the feeding regimens used in experiment two of chapter 3 after 112 days

Table 4.3: Hypothetical economic potential value model input values and calculations

Feeding intensity (% BM d <sup>-1</sup> )	= The resultant daily feeding intensity (FI) after 112 days of growth, which is calculated using:  FI = G*FCR (chapter 3). FI is expressed as a percentage of body mass per day
Daily growth rate (% BM d <sup>-1</sup> )	= The daily growth rate (G) of abalone calculated as a function of daily feeding intensity; using the models derived in chapter 3  100-110 g weight class (figure 3.9 a): G = 0.2784*(FI) + 0.1232  150–160 g weight class (figure 3.11 b): G = 0.3654*(FI) + 0.0908
Total growth after 112-days (% BM)	= the daily growth rate multiplied by 112 days (the growth period) resulting in the total amount of body mass (% BM) gained after 112 days
Number of abalone	Based on the average number of abalone in a basket multiplied across six baskets per feeding regimen (constant).
Initial average individual weight	= the average initial individual weight per abalone at start of 112-day growth period
Final average individual weight (g abalone)	= Initial average individual weight + (Initial average individual weight * total growth after 112 days (%BM))
Initial biomass (kg)	= number of abalone* Initial average individual weight
Final biomass (kg)	= number of abalone* final average individual weight
FCR	FCR calculated as a function of daily feeding intensity; using the models derived in chapter 3  100-110 g weight class (figure 3.9 a): FCR = 2.815*(FI) + 0.5962  150–160 g weight class (figure 3.11 c): FCR = 2.3585*(FI) + 0.7926
Mass of feed used	=(Final biomass – initial biomass )*FCR
Cost of feed used	= mass of feed used * price (price = R30.23/kg) – this is the price for ABFEED S32 (Marifeed Pty Ltd.)
Percentage feed used relative to FI (FARM)	= percentage comparison of mass feed used to FI (FARM) =
Z-test probability	= the p value scores of Z-test in chapter 3, showing percentages at which abalone fall into given weight classes after 112-days
Mass per weight class after 112 days	= z test percentage*Final biomass
Price per weight class	= the average value per kilogram of abalone in a specific weight class
Value per weight class	= mass per weight class (z test percentage) *price per weight class
Full potential – cost of feed used	= sum of value per weight class – the cost of feed used
Annualised difference	= compound interest formula = ((difference compared to FI (FARM)) <sup>1/ (112/365))</sup> )-1

## Conclusions

The two experimental conditions used in this study were well within acceptable water quality ranges for *H. midae* to produce feed intake and production parameter information. The small-scale laboratory study to determine feed intake in three important commercial abalone weight classes and showed that feed intake does not conform with widely accepted physiological models but can be used as reference values as to what abalone in these weight classes can consume on a daily basis. These can be further implemented into daily ration calculations as minimum values. These values, however, should only be implemented into daily ration calculations if there is a need to cut down on costs associated with abalone feeding. To maximise abalone growth, farmers should utilise size specific feeding regimens for abalone above 40 grams. Daily growth rate and FCR can be predicted as a function of the abalone's feeding intensity. As farms do not readily utilize the term "feeding intensity", it can be suggested to assess on-farm data in search of growth rate and FCR information which is used to determine feeding intensity ( $FI = G \times FCR$ ). The economic model suggested that size specific daily rations produce abalone that are of higher value to the farm after a quarterly grading period. For abalone ranging from 100 – 110 and 150 – 160 grams, it is recommended that in a quarterly grading schedule (112 days) that they be fed the size specific daily rations of FI (E) and FI (+10) respectively, which is 0.35 % BM d<sup>-1</sup> and 0.352 % BM d<sup>-1</sup> respectively. Further studies are needed to determine the effects of abalone weight class on production parameters when testing different pellet types as well as an exploration into behavioural studies focusing on diet preferences thereof. Additionally, future studies need to take into consideration the use of abalone above 100 g with focussed scope of research on behavioural, genetic and environmental aspects on abalone feed intake.

## CHAPTER 5

### References

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