Abalone nutrition – growth performance of *Haliotis midae* in relation to variable artificial feeds

Riaaz Mohamed*

**University of the Western Cape, Department of Biodiversity and Conservation Biology, Private, bag X 17, Bellville, 7535, South Africa.*

Email: riaazm94@gmail.com

A thesis submitted in fulfillment of the requirements for the degree of Master of Science

in the Department of Biodiversity and Conservation Biology,

University of the Western Cape

Supervisor: Prof Mark J. Gibbons

Department of Biodiversity and Conservation Biology. University of the Western Cape

July 2020

University of the Western Cape
Private Bag X17 Bellville 7535 South Africa

Telephone: [021] 959-2255/959 2762

Fax: [021]959 1268/2266

FACULTY OF NATURAL SCIENCE

PLAGIARISM DECLARATION TO BE INCLUDED IN ALL ASSIGNMENTS, THESIS PROPOSALS ETC, BE IT FOR MARKS OR NOT:

I, **Riaaz Mohamed**, having the following student number **3338412**, do hereby declare that the following assignment entitled "**Abalone nutrition – growth performance of *Haliotis midae* in relation to variable artificial feeds**" is my own work, and that all the sources I have quoted have been indicated and acknowledged by means of complete references.

Signed this day 17 of July 2020 at The University of the Western Cape

Signature: M. R. Mohamed

Dedicated to the strongest woman I know

my mother, Adela Mia

your sacrifices inspire me to be better.

Abstract

Abalone are among the most expensive and sought-after seafood subjects. In South Africa, *Haliotis midae* is the only commercially significant abalone species and it has become increasingly sold on the global market. The importance of abalone as a mariculture subject has triggered extensive research into maximizing production, with particular emphasis on optimizing growth rates. This study aimed to assess the growth performance of *Haliotis midae* relative to 1) standard pelleted feed, and 2) kelp-inclusive pelleted feed. I assessed feed stability of the dietary treatments and growth parameters associated with abalone weight gain. Temperature and time of feed submersion were found to significantly affect feed stability. Although there were no significant differences in water stability between feeds, the kelp-inclusive feed produced significantly lower total suspended solids (TSS) than the standard pelleted feed at upper time-temperature combinations. No significant difference in growth (% weight gain) between feed treatments was observed. However, the relationship between percentage weight gain and initial animal weight differed significantly between the feed treatments, with kelp-inclusive feed yielding better growth returns over all abalone size classes. In terms of feed stability, the data provided no evidence for the use of one feed type over another, but I do recommend that at 22°C, feed be removed after 16 hours of submersion, if present. Although there was no difference in percentage weight gain between feed treatments, I recommend the use of kelp-inclusive pelleted feed for consistent growth returns over all size classes, as compared to the standard pelleted feed which yielded substantially lower percentage weight gain with increasing size. I further recommend that higher inclusion levels be tested using various macroalgal treatments, as these parameters have been shown by others to significantly influence abalone growth.

Contents

1. Introduction

1.1. *Haliotis midae*

Abalone are marine gastropods that are characterized by the presence of a large muscular foot (Wood and Buxton 1996; Schalkwyk 2011; Visser-Roux 2011) and an undivided, external calcareous shell. The class name is derived from ancient Greek, directly translating to "stomach foot" (gastro: stomach, poda: foot), which reflects the appearance that gastropods use their "stomach" for locomotion. The viscera of these animals, however, is for the most part dorsally situated and covered by a shell (Schalkwyk 2011; Visser-Roux 2011; Gerber 2013).

The genus *Haliotis* is the only one in the family Haliotidae (Geiger 1999) and it comprises approximately 56 species in tropical and temperate waters (Schalkwyk 2011). *Haliotis midae* Linnaeus 1758 is one of six abalone species found in South African waters and it is the only commercially exploited species (Sales and Britz 2001; Schalkwyk 2011; Gerber 2013). Globally, abalone is an important fishery resource and animals command high prices (Britz 1995; Johansen et al. 2011; Visser-Roux 2011; The Department of Agriculture 2012). The very high global demand for abalone, especially in the Far East, has driven market prices up (Brown et al. 2008; Cook 2016). This in turn has led to major problems with overexploitation and poaching, which has pushed wild stocks to critically low levels (Chigumira 2016), and poses problems for the economy and for the viability of local communities dependent upon it. Fortunately, abalone (including *Haliotis midae*) can now be cultured quite easily, and South Africa is one of the world's top producers of farmed abalone (Vosloo and Vosloo 2006; Schalkwyk 2011; The Department of Agriculture 2012).

1.1.1. Basic anatomy

Abalone have a low and open shell structure, which spirals outward from the apex. Respiratory pores are lined along the outer edge of the shell, increasing in size from the anterior (apex-end) to posterior end, with new pores forming as the animal grows. Abalone are permanently attached to their shells, which form during the larval stage of development (Button et al. 2011; Schalkwyk 2011; Morash and Alter 2016).

Located ventrally is the large muscular foot, used for locomotion and attachment. Protruding beyond the shell edge and encircling the foot is a sensory structure known as the epipodium, which bears epipodial tentacles. When relaxed, the eipodium and its associated tentacles are clearly visible along the shell edge. These structures are drawn into the shell when the abalone clamps down onto surfaces (Schalkwyk 2011; Gerber 2013; Morash and Alter 2016).

The mouth and anus are located ventrally at the apex side of the shell. Abalone feed using a "tongue-like" radula, which is lined with transverse rows of slender teeth used to scrape diatoms and other encrusting species off rock surfaces. Sensitive oral tentacles surround the mouth, with a light sensitive eye located to the right (Button et al. 2011; Schalkwyk 2011; Gerber 2013; Morash and Alter 2016).

1.1.2. Ecology distribution, habitat, diet

Haliotis midae is distributed from the cold waters of the SE Atlantic off the west coast of South Africa (12 ˚C), to the warmer waters of the Indian Ocean along the Eastern Cape (21 ˚C) (Tarr 1995; Sales and Britz 2001; Schalkwyk 2011). *Haliotis midae* permanently reside in the high energy subtidal zone, living between rocks and kelp forests (Sales and Britz 2001; Vosloo and Vosloo 2006).

Haliotis midae is a generalist, opportunistic herbivore. They feed primarily on diatoms and other encrusting microalgae as early juveniles (Britz 1995; Wood and Buxton 1996), but larger specimens feed on drift seaweed by trapping it under the muscular foot, though occasionally they will feed on attached seaweeds (Wood and Buxton 1996). Abalone feed on a variety of algal species, with relative abundance thought to dictate dietary composition (Poore 1970; Wood and Buxton 1996; van der Merve 2009). Abalone are nocturnal grazers, feeding at night and remaining relatively inactive during the day (Wood and Buxton 1996; van der Merve 2009; Gerber 2013).

1.1.3. Reproduction

Haliotis midae are dioecious, broadcast spawners and, depending on the locality, may spawn twice a year during spring and autumn (Wood and Buxton 1996; Sales and Britz 2001). In wild populations along the South African west coast, *Haliotis midae* is thought to be sexually mature at approximately seven years of age (Tarr 1995; Schalkwyk 2011; Gerber 2013). Along the warmer east coast and under cultured conditions, however, abalone may reach sexual maturity within three years (Tarr 1995; Sales and Britz 2001; Schalkwyk 2011; Gerber 2013).

After fertilization, eggs develop into non-feeding trochophore larvae, which further develop into planktonic veliger larvae characterized by shell formation. (Leighton 1974; Visser-Roux 2011). The larval developmental stage lasts for between five to seven days (temperature dependent), after which animals settle onto rocky substrata in shallow water and further develop into shelled juveniles, completing the process of metamorphosis (Sales and Britz 2001; Schalkwyk 2011; Visser-Roux 2011; Gerber 2013). The refuge provided to juvenile *Haliotis midae* by Cape urchins (*Parenchinus angulosus*) is vitally important to the survival of juvenile abalone (Tarr et

al. 1996). Sea urchins provide protection for the juvenile *Haliotis midae* concealed below them and, like abalone, urchins feed on a diversity of macroalgae (Mayfield and Branch 2011). Rock lobster, thus, have an indirect, but major impact on juvenile abalone survival through predation on sea urchin populations (Tarr et al. 1996; Mayfield and Branch 2011). Mayfield and George (2000) showed *Haliotis midae* and sea urchin densities to be positively correlated, and sea urchin and rock lobster densities were negatively correlate in field studies at Cape Hangklip and Hermanus (South Africa).

1.2. Abalone as mariculture subjects

The aquaculture industry is regarded as the fastest growing food production system in the world (Gerber 2013; Krohn & Britz 2016; van der Merve 2009). The extensive scientific research invested in understanding the overall biology of commercially farmed aquatic species is therefore no surprise, so as to meet the continual and growing demand for human food production through better culturing practices (Troell *et al*. 2006; Gerber 2013; van der Merve 2009). The aforementioned is even more relevant to the abalone farming industry, as according to the Food and Agriculture Organization of the United Nations (FAO 2011), its contribution to international aquaculture production, together with winkles and conchs, equated to 350 000 tons and a value of 672 million US dollars in 2009. With diminishing wild stocks since the 1970s, farm production of abalone has increased by over 750% leading up to the year 2010 (Krohn *et al.* 2016). South Africa contributed 2.8% of the global abalone supply for the year 2016 (Krohn et al. 2016). Australia, Chile, Japan, Taiwan, New Zealand and US are also important producers of abalone, but all have a production capacity lower than 700 metric tons (Gerber 2013; Cook 2014; Krohn *et al.* 2016).

In South Africa, *Haliotis midae* is the only commercially significant abalone species and it has become increasingly sought after in the global market (Krohn *et al.* 2016; Gerber 2013; Schalkwyk 2011; Troell *et al*. 2006; Britz 1995). This has led to the development of a thriving local industry that has grown to become one of the largest producers of farmed abalone after China and South Korea (Troell et al. 2006; Schalkwyk 2011; Gerber 2013; Krohn et al. 2016). According to the Department of Environmental Affairs (2009), the abalone subsector contributed 94.1% to the total production of SA's mariculture industry, valued at more than ZAR 290m. The importance of this research-driven industry to the national economy should force the expansion of production boundaries and the exploration of new ways to not only optimize production, but also minimize resource investments while meeting the former (Naylor et al. 2009; Krohn et al. 2016; Morash and Alter 2016).

1.2.1. Optimizing production

The importance of abalone as a mariculture subject has triggered extensive research into maximizing production, with particular emphasis on growth rates (Mercer et al. 1993; Britz 1995; Mai et al. 1995a; Britz 1996; Durazo-beltra et al. 2003; Gordon et al. 2006; van der Merve 2009; Naylor et al. 2009; Schalkwyk 2011). This focus reflects the fact that abalone is a slow growing species requiring prolonged resource investment, which takes, on average, three years to reach a desired market size of 100 g (Britz 1995; Britz 1996). Abalone feed, together with electricity and labour, are the three main cost in abalone production, reported to make up approximately 65% (Mouton 2018). Feeds optimized for increased growth rates would reduce resource investment into the production of animals, by shortening the growth period required to achieve market size, thereby increasing turnover. Research into the dietary needs of abalone is, and continues to be, an extensive

area of research, since food availability and nutrient uptake are among the most important limiting factors to growth. The use of artificially formulated feed has become increasingly important relative to the use of natural abalone food sources (algal species) on which the industry was first started (Britz 1995; Troell *et al*. 2006; Naylor *et al*. 2009; van der Merve 2009). Artificial feed, however, has a number of advantages over natural seaweed feed: it is reliably available and easy to administer; it can be tailored to maximize food conversion ratios and digestibility; it is easy to store and transport and imposes no geographical limits to farm location (Britz 1995; van der Merve 2009). Also, the nutritional indices of artificial feed can be easily manipulated for nutrients essential for optimizing growth rates, which are lacking (or in low concentrations) in a natural seaweed diet (Naidoo et al. 2006; Francis et al. 2008).

1.2.2. Marketability of abalone: physiochemical properties

1.2.2.1.Taste

The characteristic taste of abalone has been attributed to the ratio and amounts of free amino acids (FAA) and nucleotides (Brown et al. 2008). Omission tests for taste-active components in abalone confirm that Glutamic acid (Glu), Glycine (Gly), Betaine and Adenosine 5'-monophosphate (AMP) are responsible for abalone taste (Fuke and Konosu 1991; Brown et al. 2008). Glycogen is also believed to improve abalone taste (Brown et al. 2008). These taste-active components have been shown to vary seasonally, and highest concentrations have been observed in summer and early autumn for wild *Haliotis discus* (Watanabe et al. 1992). For farmed *Haliotis diversicolor*, however, the concentrations of taste active components were highest in winter and early spring,

suggesting that the seasonal variation in concentrations of taste-active components may be species dependent (Chiou et al. 2001).

1.2.2.2.Texture

Abalone meat has a tough texture, a market characteristic considered to be as important as taste (Brown et al. 2008). Positive correlations exist between collagen content and entire animal weight (Olaechea et al. 2009); smaller abalone are, thus, more tender than larger specimens. Changes in collagen content and subsequent abalone toughness may vary seasonally, as shown for Haliotis *discus*, with abalone being most tender and having the lowest collagen content in summer, while the opposite is true for winter (Olaechea et al. 1993).

1.2.2.3.Visual appearance

The colour of abalone is an important marketability trait and is often used as an indication of quality (Brown et al. 2008). Abalone species that have a lighter pigmentation demand higher prices, compared to darker pigmented species which require additional trimming, washing and/or bleaching before sale (Oakes and Ponte 2003). Abalone considered to be of good quality should be creamy yellow in colour, with no surface discoloration.

1.2.2.4. Size

The premium size for live cultured abalone is 80 – 100 mm (Oakes and Ponte 1996), though in future the demand-size is predicted to increase to 120 mm and above (Gordon and Cook 2004). Size preference, however, varies with product form; abalone exported live are

often smaller than those exported in canned form (Oakes and Ponte 2003). For individually quick frozen (IQF) meat of varying size, *Haliotis laevigata* demands highest prices at 1–2 pieces/lb product, compared to *Haliotis rubra*, for which highest prices are obtained at 3–5 pieces/lb product (Brown et al. 1993). Size preference, thus, is also dependent upon the species being farmed.

1.2.3. Practical application of dietary research

Wild abalone display a distinct dietary shift from encrusting algal species and diatom films on rock surfaces as juveniles, to larger macroalgal species such as *Ecklonia maxima* as they get larger (Wood and Buxton 1996). This knowledge has been important in the formulation of different macro- and micro- foods to be administered at different phases of growth on abalone production farms (Wood and Buxton 1996; Gordon *et al.* 2006; Wootton 2015; van der Merve 2009): each food type being tailored to maximize growth. Feeding methods can also be adjusted (Britz et al. 1997; Naidoo et al. 2006; Naylor et al. 2009; Green et al. 2011). A number of studies have been conducted on the effects of diet on *Haliotis midae* under culture and all have confirmed enhanced growth rates with a variable, rather than single, feed type (Troell *et al*. 2006; Naidoo *et al*. 2006; Daume *et al*. 2007). Soft-body lipid content has been found to be positively correlated with dietary lipid levels (Mai et al. 1995b; Dunstan et al. 1996). For *Haliotis diversicolor*, taste preferences were significantly higher for abalone fed an artificial diet than those fed a natural *Gracilaria* diet (Chiou and Lai 2002). The meat of *Haliotis diversicolor* fed an artificial diet also had higher levels of taste-active compounds than that of abalone fed the *Gracilaria* diet (Chiou and Lai 2002). *Haliotis iris* fed natural algal diets had

distinct darkening of the foot relative to those fed artificial diets (Allen et al. 2006). The accumulation of Dimethylesulfide (DMS), a break down product of Dimethylesulfoniopropionate (DMSP) found in some macroalgal feeds of *Haliotis midae*, and subsequently, abalone tissue, was found to cause both an undesirable taste and odour in canned abalone (Smith et. al. 2007). The food provided to abalone has been found to affect DMSP accumulation levels in abalone tissue, with *Ulva* found to cause higher accumulation levels than either artificial feeds or *Ecklonia maxima (*Smith et. al. 2007).

As an abalone's diet may influence the quality parameters of the flesh at harvest, growth rates need to be balanced against product quality. This is important with regard to feeding strategies, as various diets have been shown to yield differences in the physiochemical composition of abalone species (Britz 1995; Mai *et al*. 1995a; Mai *et al*. 1995b; Gerber 2013), from which inferences on overall body condition can be drawn.

1.2.4. Future challenges for abalone farming

Lastly, in a study by Morash & Alter (2016), considering the global crisis of climate change, they highlighted various potential challenges and adverse effects that current and future climate change are predicted to have on abalone farming. The fact that many, if not all South African abalone farms are subject to changes in environmental variables (temperature, oxygen, carbon dioxide, etc.), puts abalone production at particular risk. Current climate change models predict increased weather extremes, which will likely exacerbate fluctuations in the abovementioned variables, resulting in detrimental impacts on farms where these variables are largely uncontrolled (Morash and Alter 2016). Morash & Alter (2016) predicted lower future

abalone growth rates, resulting from increased investment in stress alleviation instead of growth. This then calls for further and continual studies to improve diet, which may not only be important for current optimization of growth rates, but also have significant importance in rehabilitation of abalone in the face of environmental stressors, and subsequent rehabilitation of farms subjected to future production challenges.

1.3. Factors affecting growth

1.3.1. Water quality

Water quality may be defined as the physiochemical and biological factors influencing the well-being of cultured aquatic organisms (van der Merve 2009). The water quality parameters that are important for optimizing growth and survival include salinity (Jarayabhand et al. 2010), organic waste concentration (Ackefors and Enell 1994; Harris et al. 1998; Wassnig et al. 2010), nitrogenous waste concentration (Ackefors and Enell 1994; Harris et al. 1998; Reddy-lopata et al. 2006; Naylor et al. 2011), dissolved gasses (Harris et al. 1999; Neori et al. 2000; Naylor et al. 2011; Kim et al. 2013), alkalinity and hardness (Harris et al. 1999; Reddy-lopata et al. 2006; Wassnig et al. 2010; Kim et al. 2013), as well as the presence of disease causing microorganisms (Karunasagar et al.; van der Merve 2009; Mills et al. 2010; Johansen et al. 2011; Salama and Murray 2011).

Good quality coastal water along the west coast of South Africa has facilitated the rapid growth of the abalone industry there (Troell et al. 2006). Abalone farms in South Africa are

situated in areas with relatively good water quality, free of industrial and/or domestic waste (Troell et al. 2006; van der Merve 2009). Large kelp beds along the west coast are supported by these water conditions, which are the primary dietary component of *Haliotis midae* (Troell et al. 2006; van der Merve 2009). Abalone farms along the South African coastline typically make use of flow-through systems, which pumps water into/through the farm directly from the ocean and back out into the ocean upon exiting the farms (Fourie 2014). This open flow-through system allows farm managers to better maintain water quality parameters within acceptable limits, by exposing them to naturally occurring conditions (Fourie 2014).

1.3.2. Temperature

As ectotherms, temperature influences various aspects of growth and maturity in *Haliotis midae*. Temperature controls the rate at which biological reactions occur, underpinning major physiological processes (Britz et al. 1997; Gilroy and Edwards 1998; Green et al. 2011). As aerobic metabolism provides most of the energy required for these metabolic processes, there is a positive correlation between environmental temperature and oxygen consumption/requirements (Morash and Alter 2016). This is, of course, a finite relationship that exists only between the upper and lower critical temperatures, above or below which animals can no longer execute biological functions (Britz et al. 1997; Morash and Alter 2016). Physiological responses will therefore yield temporal differences in growth that reflect seasonal fluctuations in temperature, which affects dissolved oxygen concentrations and oxygen requirements (Harris et al. 1999).

The developmental time of the non-feeding trophophore larva stage of *Haliotis midae* is generally temperature dependent. At 20˚C, the larval period lasts for 5 days, compared to seven days at 17.5 ˚C (Sales and Britz 2001; Schalkwyk 2011). Tarr (1993) showed that specimens along the warmer east coast mature at 33-40 mm shell width, while on the cold west coast maturity is reached at about 80 mm shell width. A temperature range between 12 and 20˚C are physiologically optimal for *Haliotis midae,* and this has direct consequences for the rate of metabolic processes and, thus, growth (Sales and Britz 2001; van der Merve 2009). Green *et. al.* (2011) found the growth of *Haliotis midae* to be temperature dependent and negatively correlated with temperature from 18 to 24˚C (also see Gilroy and Edwards (1998)). These results agreed with those obtained by Britz *et. al.* (1997), who found a temperature range between 12 and 20˚C to be physiologically optimal for *Haliotis midae.*

1.3.3. Natural vs artificial diet

Various abalone diets have been shown to produce significantly different growth rates (Britz 1995; Britz 1996; Troell et al. 2006). Being generalist herbivores of the subtidal, abalone accept a wide variety of algal species (Troell et al. 2006; van der Merve 2009; Schalkwyk 2011). Juveniles feed mainly on diatoms and other microalgal species off rock surfaces, while post-juvenile *Haliotis midae* switch to a macroalagal diet as they grow. *Haliotis midae* appear to favour specific macroalgae including *Ecklonia maxima, Laminaria pallida, Ulva* spp., and *Gracilaria* spp. (Britz 1995; Troell *et al*. 2006; Naylor *et al*. 2009; van der Merve 2009).

The growth rates of abalone feeding on natural algal diets have been shown to be slow and heterogeneous over time relative to the use of artificial diets, which yield better growth rates

over time (Britz 1995; van der Merve 2009; Naylor *et al*. 2009). This is due to the availability and balance of various nutrients required for optimum growth in formulated feeds, which are often lacking in a monotypic natural-feed diet, with protein being a primary nutritional focus for the optimization of muscle and tissue growth relative to overall abalone growth and body composition (shell growth, fat gain, etc.) (Britz 1995; Mai *et al*. 1995; Britz 1996; van der Merve 2009).

Our understanding of abalone nutrition and subsequent feeding practices have changed since the start of the culturing industry, whereby abalone were fed on a strict natural macroalgal diet (Troell et. al. 2006). In response to the growing abalone industry and the difficulties of meeting the nutritional requirement for optimized growth and production, Britz et. al. (1994) initiated the production of a simple artificial diet composed of 32% protein, 44% carbohydrates and 5% fats. This artificial feed elicited a faster feeding response than the macroalgae species, *Plocamium corallorhiza*, and when presented with a choice between the two feed treatments, always selected the artificial feed (Britz et. al. 1994). Better growth performance with the use of artificial feeds as opposed to natural algal diets have, for the most part, been attributed to the higher protein content in in artificial feeds (Britz 1995; Mai et al. 1995; Britz 1996; van der Merve 2009). Green et. al. (2011), however, showed that for *Haliotis midae*, shell growth and weight gain were independent of protein levels (22% and 26 %), provided that the dietary digestible energy was not lower than 13.5 MJ kg^{-1}. Feed consumption of *Haliotis midae* is linked to energy requirement, as abalone that have been fed diets with identical protein levels consumed significantly more feed at lower digestible energy levels (11.6 vs 16.2 MJ kg^{-1}) (Green et. al. 2011). Recent trends in abalone feed formulation have also promoted a higher inclusion levels of macroalgae, in an attempt to harness the nutrient benefits of both a formulated and natural

seaweeds diets, without the logistical difficulties of dealing with fresh macroalgae (Bansemer et. al. 2016; Nel et. al. 2017). Superior growth rates and feed conversion ratios were achieved for Haliotis *laevigata* fed diets with 5% and 10 % macroalgae inclusions, compared to artificial feeds with no macroalgal inclusion (Bansemer et. al. 2016). Similar results were achieved for *Haliotis midae,* with kelp (*E. maxima*) inclusion levels of 0.44-3.54 % (Nel et. al. 2017). Significant increases in growth have also been achieved with the use of nutrient enriched, farm-grown macroalgae, as compared to the use of wild caught macroalgae for abalone feed (Naidoo et. al. 2006; Robertson-Andersson et. al. 2011).

With the abovementioned in mind, I wish to determine whether similar enhanced growth rates can be achieved for *Haliotis midae,* as observed by Bansemer et. al. (2016) and Nel et. al. (2017), with a kelp inclusive artificial feed relative to the standard artificial feed currently used on Abagold ™ Growout farms.

1.4. Aims and objectives

The aim of this study is to investigate the effects of two commercially available artificial abalone feeds on growth performance of *Haliotis midae,* each representing a monotypic dietary administration.

Abalone fed each dietary type will be compared in terms of percentage weight gain, body condition factor, and specific growth rate after a trial period. Feed stability will be assessed based on water stability (dry matter loss) of feeds and total suspended solids after being suspended in water for different time temperature combinations.

2. Materials and Methods

2.1. Feed Stability Trials

2.1.1 Water stability Trials

The water stability of artificial dietary pellets was tested at 14 and 22˚C, resembling the lower and upper limits of the physiologically optimal temperature range for *Haliotis midae*. Three replicates of each artificial diet were assessed after 4, 8, 16 and 24 hours of submersion in filtered seawater at each of the abovementioned temperatures. For each feed type, the dry weight of three pellets was measured after being oven-dried at 100 – 105˚C for 16 hours. Pellets were then placed in 1000 ml of water (in 1000 ml glass beakers), aerated at 0.33 liters/minute. After submergence in the above mentioned water temperatures and time intervals, the artificial pellets were retrieved and oven dried at 100 - 105˚C for 16 hours. Thereafter, final dry weight was measured and water stability expressed as recovery rate of dry feed: percentage of final dry weight to initial dry weight after time *t*. For each feed type, three replicates were carried out for each temperature-time combination.

2.1.2. Total suspended solids

For each of the above trials and after the removal of pellets, the water was filtered using 1.23 µm glass microfiber filters (GF/C grade) of known weight. Thereafter, filters were rinsed with deionized water and dried in the oven at 105˚C for 17 hours. Total suspended solids (TSS) were expressed as a percentage of initial dry weight of feed pellets, using the difference in the weight of the filter to determine the amount (in grams) of TSS.

2.2. Growth response in relation to artificial feeds

2.2.1. Rearing system

Feeding trials were conducted at one of the grow-out farms of Abagold Ltd, in Hermanus. Abagold Ltd has land-based abalone production farms, utilizing a flow through system which pumps water into/through the farm directly from the ocean and back out into the ocean upon exiting the farms.

Abalone were reared in 474 x 88 x 783 cm tanks, with each tank supporting up to 16 mesh baskets (62 x 53 x 74 cm, mesh size = 6mm), in which abalone are kept. Two rows of eight baskets ran along the length of the tank. Fresh seawater entered at one end of the tank and left at the opposite end. All tanks were exposed to the same ambient temperatures and were characterized by similar dissolved oxygen (DO) concentrations and pH. Water quality parameters and inorganic nutrient levels were checked on a daily basis to ensure good growing conditions for abalone.

The stocking density per basket for each dietary administration did not exceed 18 Kg. Staff from Abagold Ltd graded the baskets seven months after the start of the trial, upon which the trial was terminated. Three grading events were initially planned for two sales weeks over a one-year period, however, due to logistical issues and unforeseen circumstances experienced by the farm, this was not possible.

2.2.2. Diet administration

Specific brood stock groups are spawned at ten-week intervals and all subsequent offspring are assigned a “sales week” at which they are to leave the farm for the market. For a

sales week, an equal number of baskets were assigned to each dietary administration: 1) SAF3000-kelp feed (1% kelp inclusion) and 2) Standard SAF3000 feed. Each basket received two handfuls (approximately 200 g – based on information provided by Abagold™ management) of its assigned feed type in the afternoon and were fed by the same individual farm workers for consistency. Animals were fed *ad libitum*, with workers assessing baskets on a daily basis for feed condition and availability.

A commercial-sized sales week (SW) with an estimated biomass of 1 450 kg comprised of 40 482 animals with an average individual weight of 35.80 g was used. The sales week comprised of 127 baskets, providing 63 replicates for SAF3000-kelp feed (1% kelp inclusion) and 64 replicates for Standard SAF3000 feed. Baskets from experimental tanks were randomly assigned a feed type, with half of the baskets assigned to each feed treatment. The average individual start weight (g) of the abalone fed kelp-inclusion feed was 58.396 ± 5.149 SE (N = 17 673) and the average individual start weight of the abalone fed Standard SAF3000 was 59.469 ± 5.361 SE (N = 17 998). There was no significant difference in initial start weight of animals between feed treatments (t = -0.28843, df = 124.87, P-$value$ = 0.7735).

2.2.3. Growth (weight) and Body Condition

For each treatment, average weight gain for abalone per basket and per animal were calculated (as a percentage of initial weight) for each feed treatment. Basket weight was used to determine average weight per animal in said basket. Number of animals per basket were counted before and at the end of the growth trial. Mortalities throughout the growth period were excluded

from the count at the end of the trial, with initial and final basket weight averages compared. Specific growth rate (*SGR*) (adapted from Britz 1996) was calculated as:

$$SGR = 100.\frac{\ln(W_f) - \ln(W_i)}{t} \qquad \textbf{Equation 1}$$

where *SGR* is the specific growth rate (% body weight gain per day), ln(*Wf*) is the natural log of the mean final weight of abalone, ln(*Wi*) is the natural log of the mean initial weight of abalone, and *t* is time, in months.

Average condition factor (length-weight relationship) was compared between dietary treatments using the equation from Britz (1996):

$$CF = 5575.\frac{W}{L^{2.99}} \qquad \textbf{Equation 2}$$

where *CF* is condition factor, *W* is weight (g) and *L* is length (mm). Length was determined from a length-weight curve using average basket weight (values provided by Abagold™).

2.4. Statistical analyses

Feed stability over discrete time intervals and between temperatures was compared using a Kruskal-Wallis *H* test, when data did not conform to assumptions of normality and homogeneity, or with ANOVA when they did. Pairwise *t*-test comparisons were computed to assess the significance of differences between treatments.

Aspects of the growth performance of abalone between the two dietary treatments were compared using either a Mann-Whitney *U*-test (in the case where assumptions of normality were not met) or Student's *t*-test. Linear models for growth performance vs animal size were generated for each feed treatment and the slopes of the models compared for significance.

All statistical analyses were conducted in the program "R", considering a statistical significance level of 95% and assuming equality.

3. Results

3.1. Feed Stability Trials

3.1.1. Water stability of feeds at various temperature-time combinations

SAF1 – kelp inclusive feed

The ANOVAs conducted for water stability over time were significant at both 14 (*F-stat* = 10.21. $P < 0.01$) and 22°C (*F-stat* = 32.14, $P < 0.01$). For SAF1, at both 14 and 22°C, water stability only significantly decreased after 16 hours of submersion. No significant changes in water stability were observed from 4 to 16 hours for either temperature treatments (Table 1.1). Comparison of water stability between temperature treatments from 4 to 16 hours revealed no significant differences, and only differed significantly after being submerged for 24 hours (Figure 1.1). Overall, submersion at 22°C yielded lower water stability than at 14°C (Figure 1.1).

SAF2 – Standard feed (no kelp)

The ANOVAs conducted for water stability over time were significant at both 14 (*F-stat* = 28.06, $P < 0.01$) and 22°C (*F-stat* = 32.39, $P < 0.01$). Water stability for SAF2 at 14°C differed significantly between all time trials, except for between 16 and 24 hours (Table 1.2). At 22°C, water stability only significantly decreased after being submerged for 24 hours. No significant changes in water stability were observed from 4 to 16 hours at 22°C. Overall, submersion at 22°C yielded lower water stability than at 14°C (Figure 1.1).

SAF1 vs SAF2

At both 14 and 22°C, SAF1 and SAF2 only differed significantly in water stability after being submerged for 24 hours. In cases when water stability differed significantly between feeds, SAF2 had higher water stability than SAF1 (Figure 1.2). At both temperatures, from 4 to 16 hours, despite no significant differences in water stability, SAF2 appears to have better water stability than SAF1 (Figure 1.2).

Table 1.1: Pairwise comparisons using *t* tests for water stability of SAF1 at 14°C and 22°C at different times.

	14°C				22°C			
Hours	4	8	16	24	4	8	16	24
4	-				-			
8	1	-			0.87	-		
16	1	1	-		0.29	1	-	
24	0.073	0.262	0.301	-	***	***	***	-

1. *** : *P* < 0.05; ** : *P* < 0.01; *** : *P* < 0.001**

Table 1.2: Pairwise comparisons using *t* tests for water stability of SAF2 at 14°C and 22°C at different times.

	14°C				22°C			
Hours	4	8	16	24	4	8	16	24
4	-				-			
8	***	-			1	-		
16	***	*	-		0.84	1	-	
24	***	**	1	-	***	***	***	-

1. *** : *P* < 0.05; ** : *P* < 0.01; *** : *P* < 0.001**

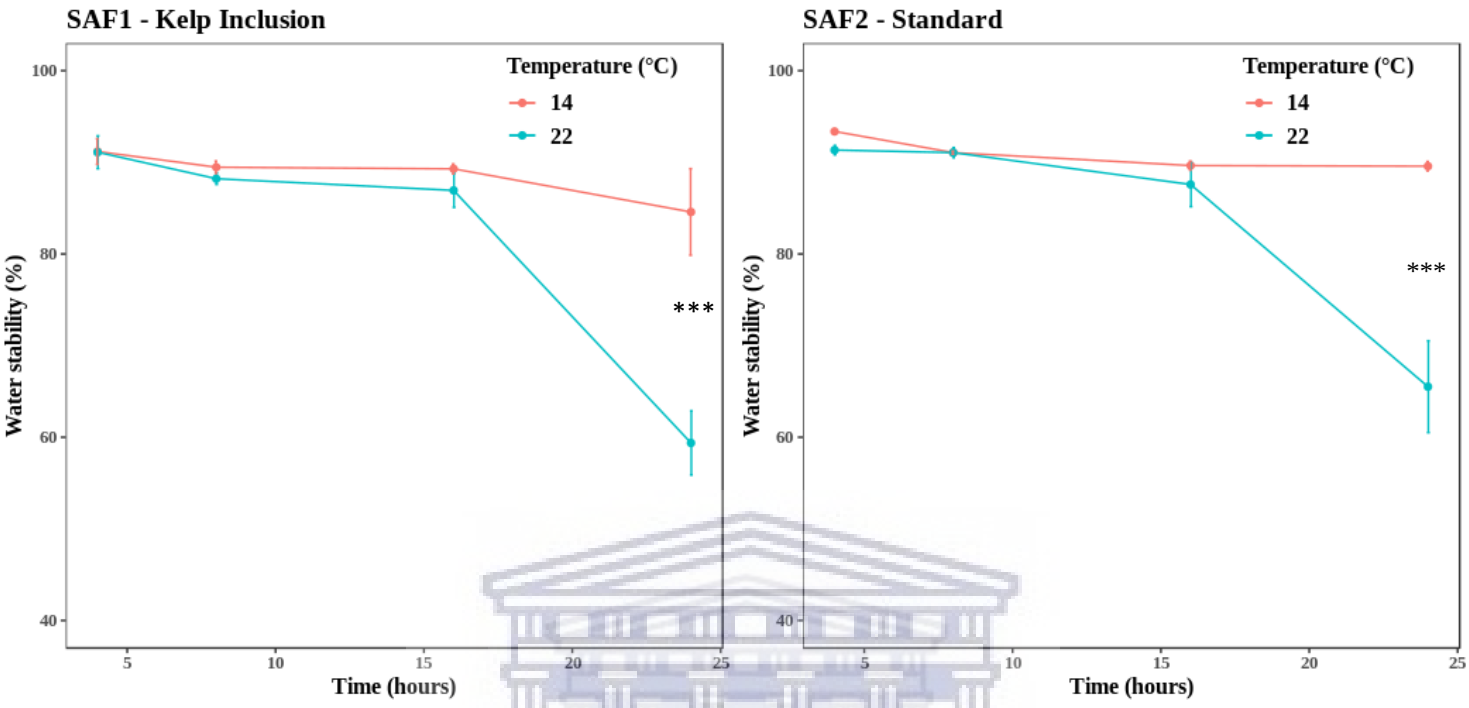

Figure 1.1: Water Stability of artificial abalone feeds after being subjected to water temperatures of 14 and 22°C over a 24 hour period. Error bars represent standard deviation around the mean. Significant differences in water stability between the two temperature treatments at each time interval are represented by: *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

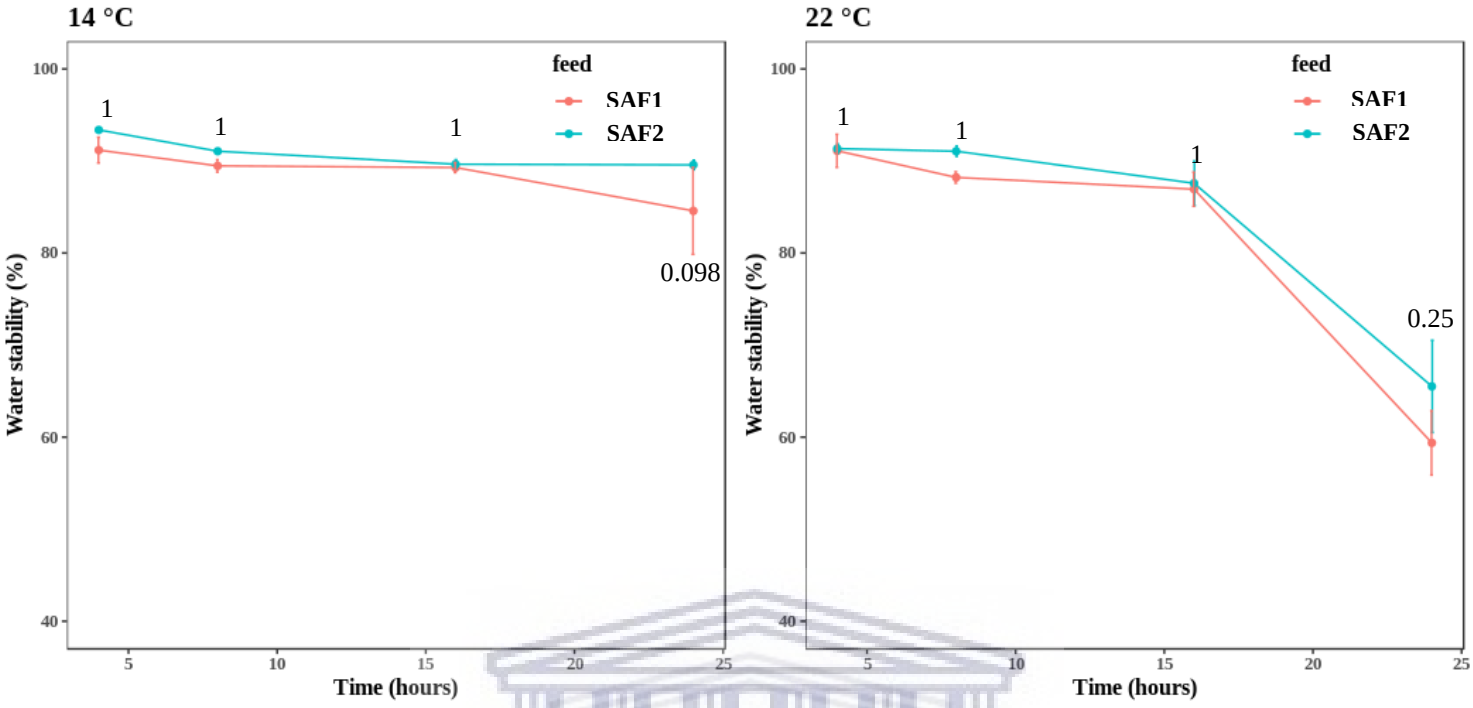

Figure 1.2: Comparison of water stability between artificial feeds after being subjected to water temperatures of 14 and 22°C over a 24 hour period. Error bars represent standard deviation around the mean. Numbers represent *P* values comparing water stability between the two feeds at each time interval. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

3.1.2. Total suspended solids (TSS) of feeds at various temperature-time combinations

SAF 1

The ANOVAs conducted for TSS over time were significant at both 14°C (*F-stat* = 5.238, $P = 0.045$) and 22°C (*F-stat* = 17.65, $P < 0.01$). For SAF1, at both 14 and 22°C, TSS only significantly increased after 16 hours (Table 2.1): there was no difference in TSS between temperature treatments over the period 4 to 16 hours (Figure 2.1), but after that a much higher

TSS% was achieved at 22°C than at 14°C.

SAF2

The ANOVAs conducted for water stability over time were significant at both 14 (*F-stat* = 6.365, *P* = 0.030) and 22°C (*F-stat* = 27.17, *P* < 0.01). For SAF2, at both 14 and 22°C, TSS only significantly increased after 16 hours (Table 2.2): there was no difference in feed performance between temperature treatments over the period 4 to 16 hours (Figure 2.1), but after that a much higher TSS% was achieved at 22°C than at 14°C.

SAF1 VS SAF2

SAF1 and SAF2 did not differ significantly in TSS for all time trials at 14°C. At 22°C, TSS between SAF1 and SAF2 only differed significantly at 24 hours, with SAF2 yielding a higher TSS% (Figure 2.2).

Table 2.1: Pairwise comparisons using t tests for total suspended solids of SAF1 submerged at 14°C and 22°C at different time intervals.

	14°C				22°C			
Hours	4	8	16	24	4	8	16	24
4	-				-			
8	1	-			1	-		
16	1	1	-		1	1	-	
24	*	*	**	-	***	***	***	-

1. *** : *P* < 0.05; ** : *P* < 0.01; *** : *P* < 0.001**

Table 2.2: Pairwise comparisons using t tests for water stability of SAF2 submerged at 14°C and 22°C at different time intervals.

	14°C				22°C			
Hours	4	8	16	24	4	8	16	24
4	-				-			
8	1	-			1	-		
16	1	1	-		1	1	-	
24	0.21	0.18	0.24	-	***	***	***	-

1. *** : *P* < 0.05; ** : *P* < 0.01; *** : *P* < 0.001**

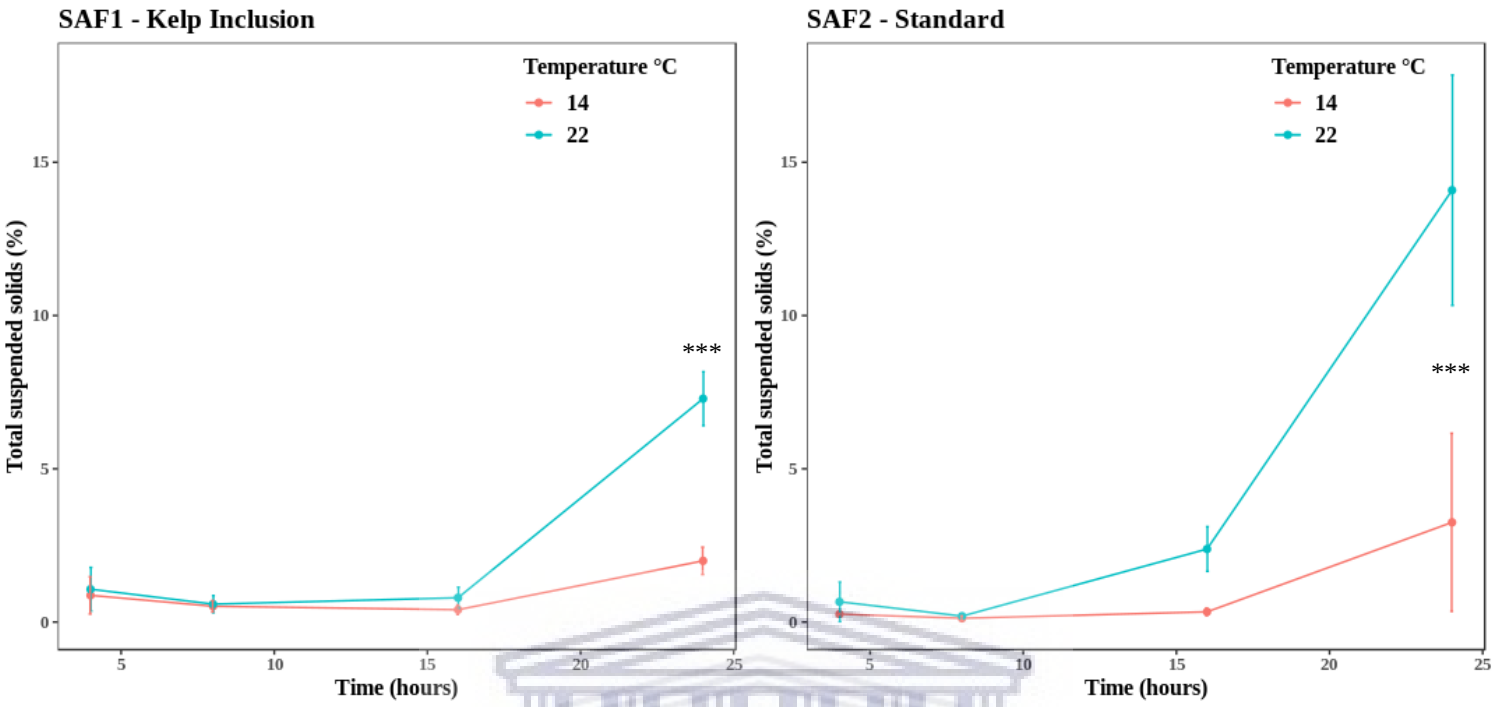

Figure 2.1: Total suspended solids (> 1.23 µm) released from artificial abalone feeds after being subjected to water temperatures of 14 and 22 °C over a 24 hour period. Error bars represent standard deviation around the mean. Significant differences in TSS between the two temperature treatments at each time interval are represented by: *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

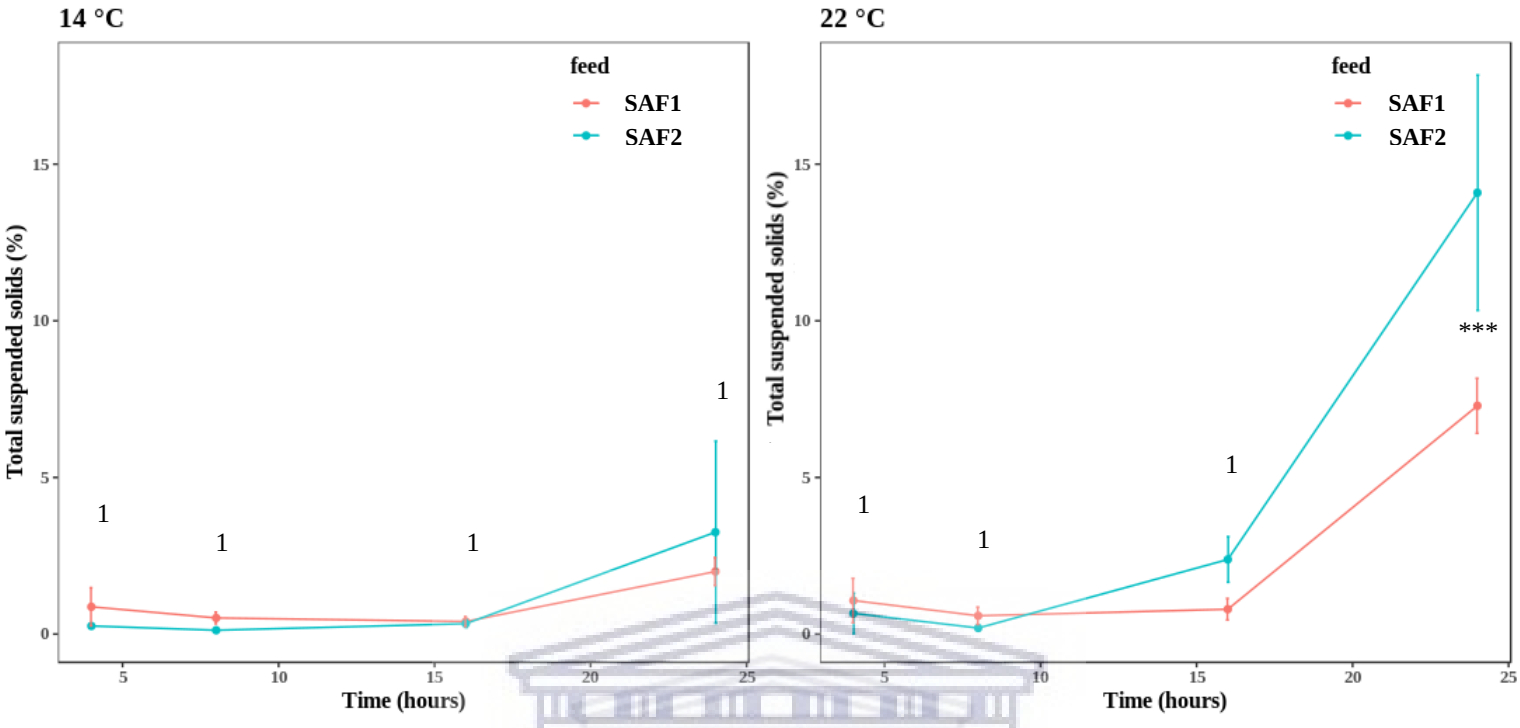

Figure 2.2: Comparison of total suspended solids (> 1.23 µm) released by artificial feeds after being subjected to water temperatures of 14 and 22 °C over a 24 hour period. Error bars represent standard deviation around the mean. Numbers represent *P* values comparing water stability between the two feeds at each time interval. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

3.2 Growth performance between diets

3.2.1. Percentage weight gain between feed treatments

The data for percentage weight gain per basket and per animal for both feed trials were not normally distributed (Figure 3.1a and 3.1b), though the SAF3000-Standard feed treatment had a wider distribution spread than SAF3000-Kelp. A Wilcox rank sum test revealed no significant difference between feed treatments when comparing percentage basket weight gain (W = 2275, P = 0.213) and percentage weight gain per animal (W = 2253, P = 0.254).

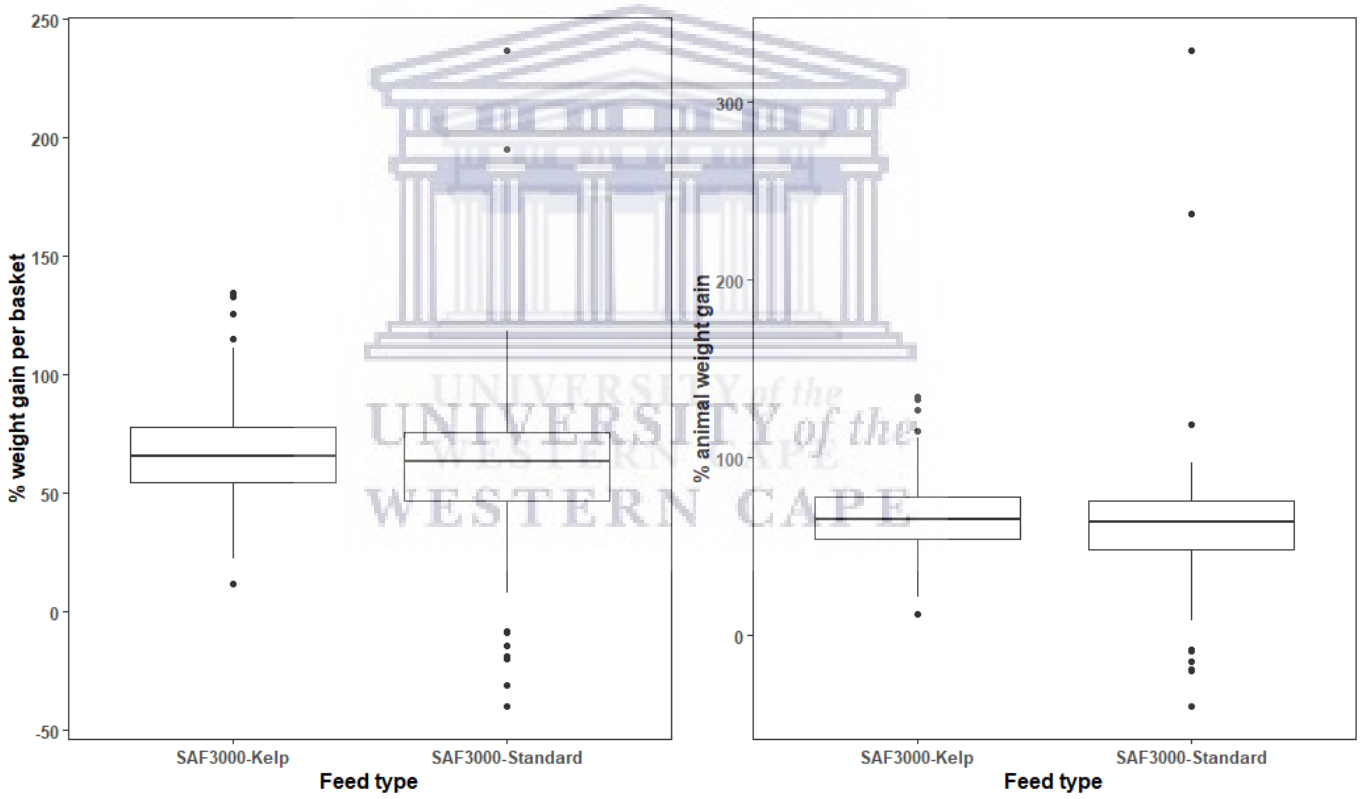

Figure 3.1: Percentage weight gain **a)** per basket and **b)** per animal relative to feed treatments after 7 months on a grow-out farm in Hermanus, South Africa. n = 63 and 64 baskets for SAF3000-Kelp and SAF3000-Standard, respectively.

There was no significant difference (W = 2275, P = 0.213) in % basket weight gain between feed treatments. Average % animal weight gain decreased with increasing animal size (Figure 3.2 and 3.3). When comparing the slopes of the linear models for the relationship between% animal weight gain and initial animal weight, the slope for animals fed SAF3000-Kelp (y = -0.334x + 87.435) was significantly smaller (t = 2.344, dof = 123, P < 0.021) than animals fed SAF3000-Standard (y = -1.048x + 124.562) (Figure 3.3).

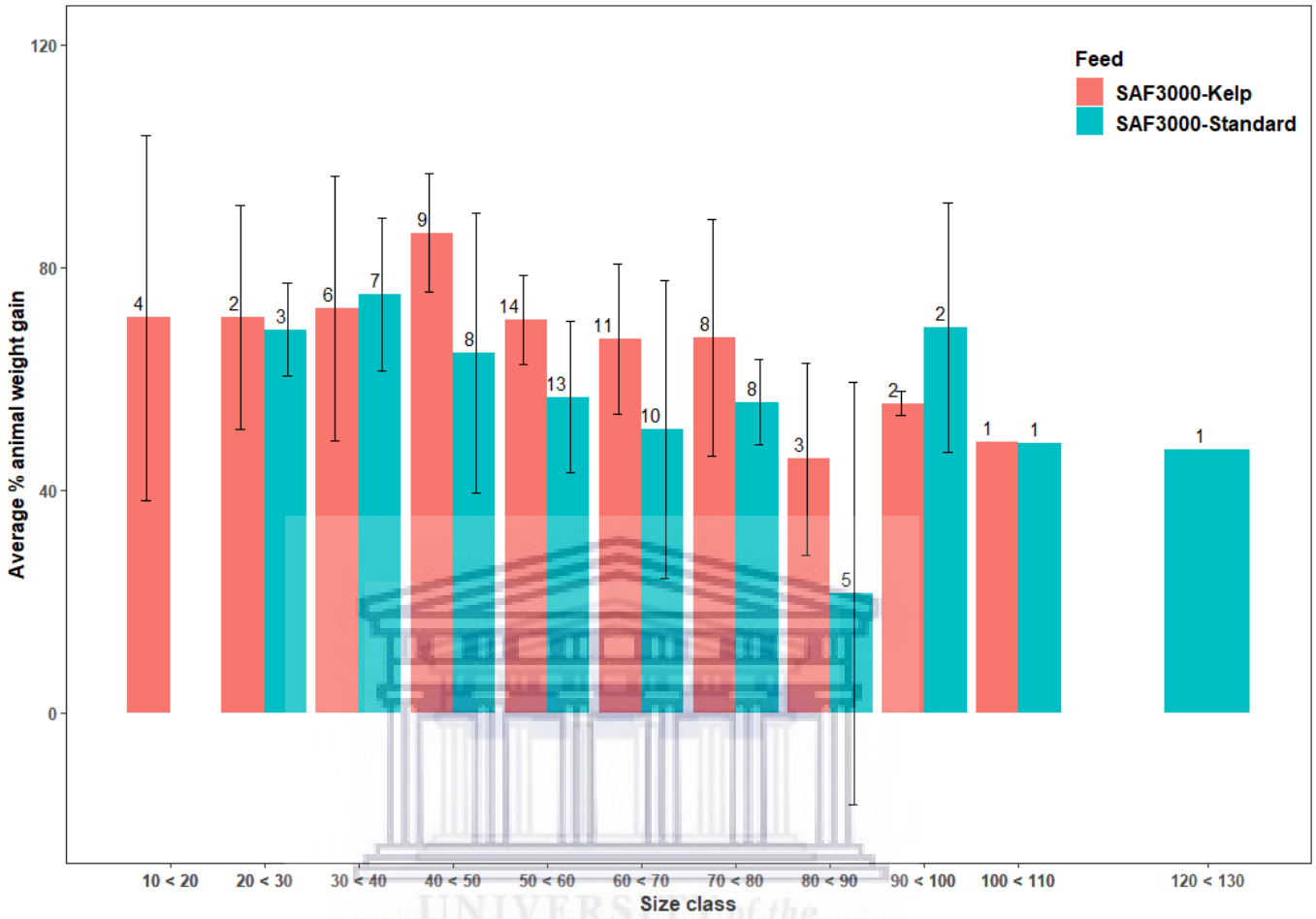

Figure 3.2: Average percent weight gain per animal per size class for feed treatments over a 7 month period. Error bars represent 95% confidence intervals around the mean. Numbers represent sample size (number of baskets) per size class for feed treatments. **Missing bars indicate no size class available for that feed treatment.**

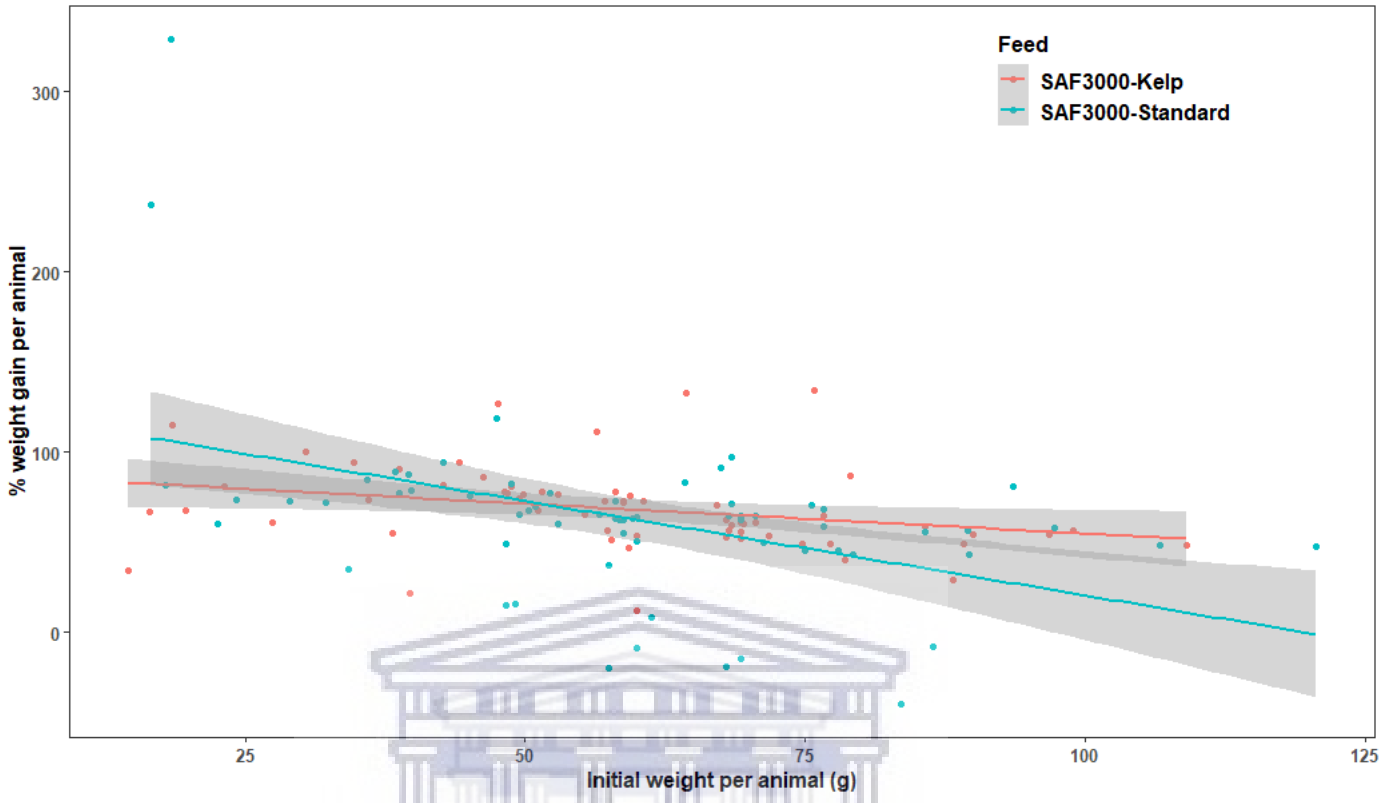

Figure 3.3: Linear relationship between% weight gain per animal as a function of initial animal weight (g) for each feed treatment over a 7-month period. Grey bandwidths represent 95% confidence intervals around linear model predictions. Regression slopes between feed treatments differ significantly (t = 2.344, dof = 123, $P < 0.05$).

3.2.2. Body condition factor between feed treatments

A Wilcox rank sum test revealed no significant difference (W = 1786, P = 0.268) in body condition factor change between feed treatments (Figure 3.4). Change in body condition factor was positively correlated with animal size for both feed treatments (Figure 3.5 and 3.6). There was no significant difference (t = -1.939, dof = 123, P < 0.055) in the slopes of the linear models for the relationship between change in body condition and initial animal weight between the feed treatments (Figure 3.6). However, the industry often considers implementing changes at a P-value < 0.2, and thus, the results could still be of use for the farm (Prof N. Vine, UFH, personal communication).

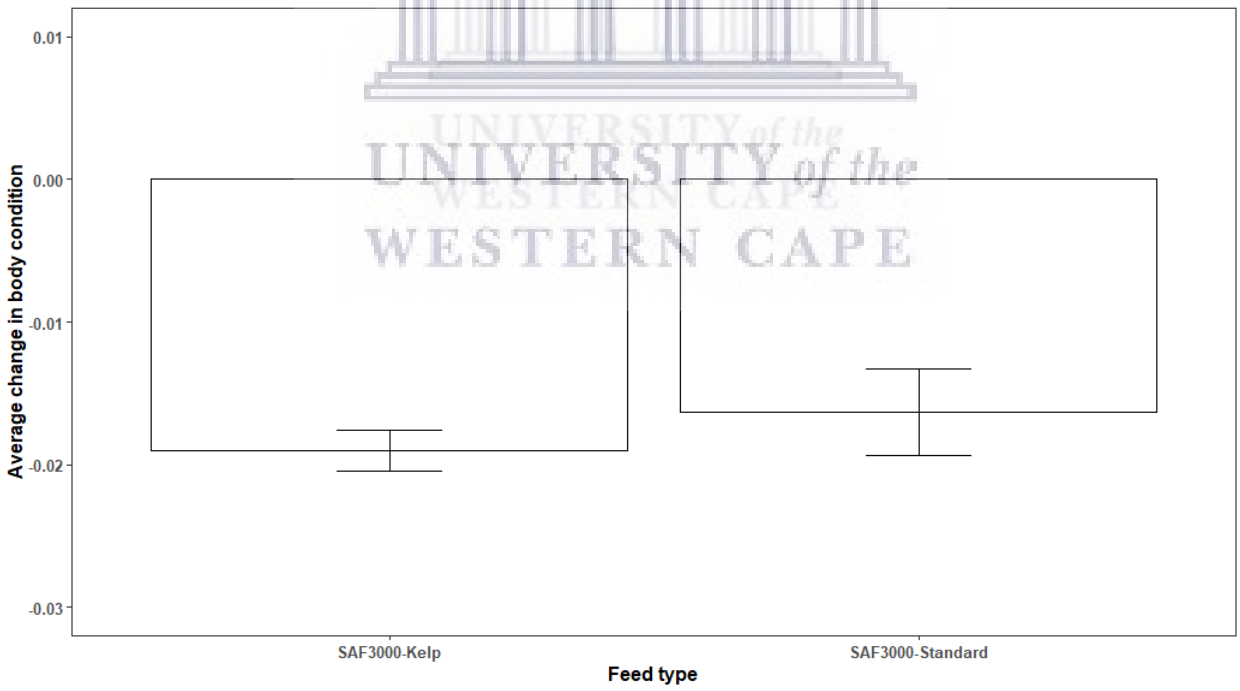

Figure 3.4: Average change in body condition for *Haliotis midae* between feed treatments. Error bars represent confidence intervals around the mean.

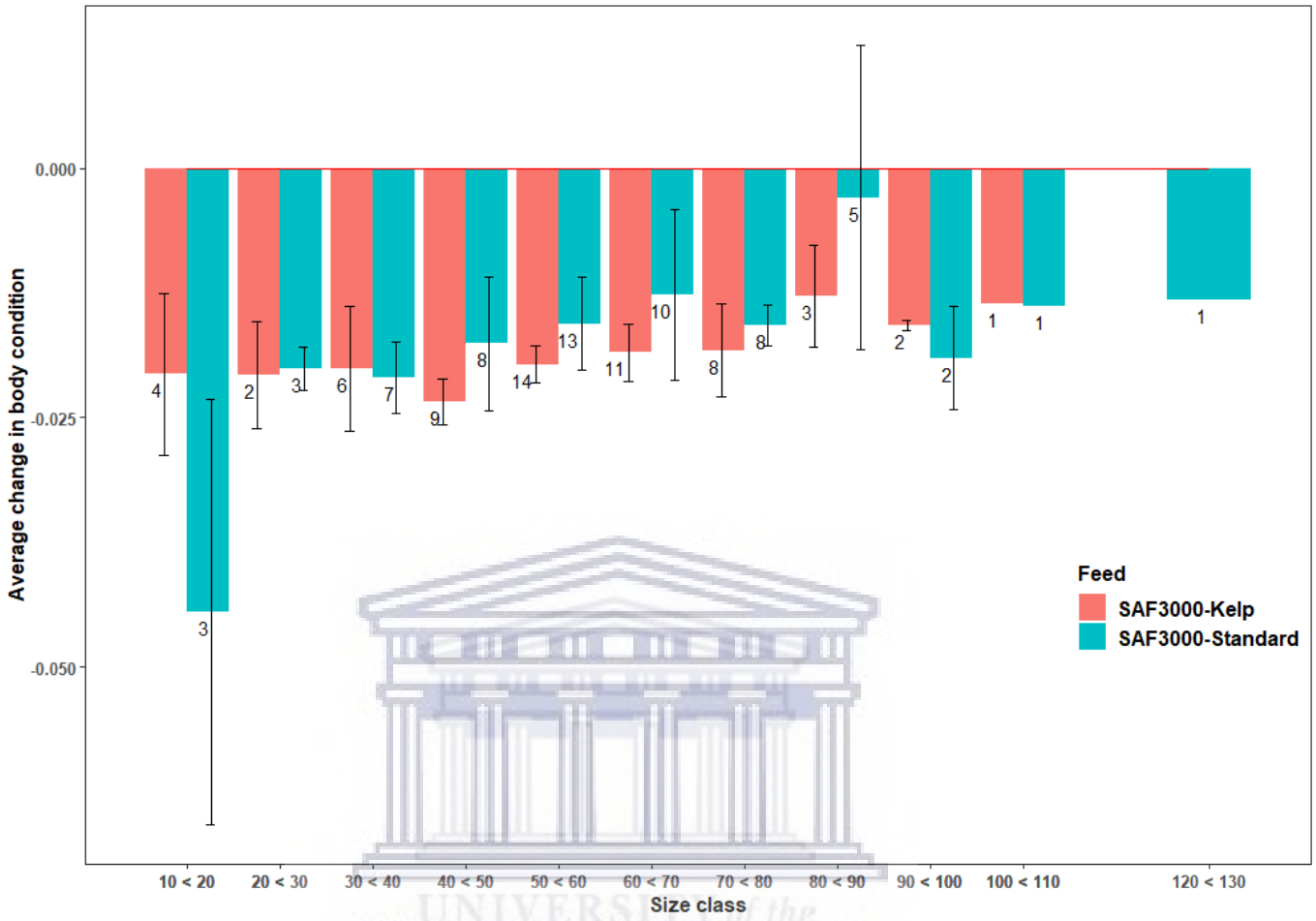

Figure 3.5: Average change in body condition for animal size classes between feed treatments over a 7 month period. Error bars represent confidence intervals around the mean. Numbers represent sample size (number of baskets) per size class for feed treatments.

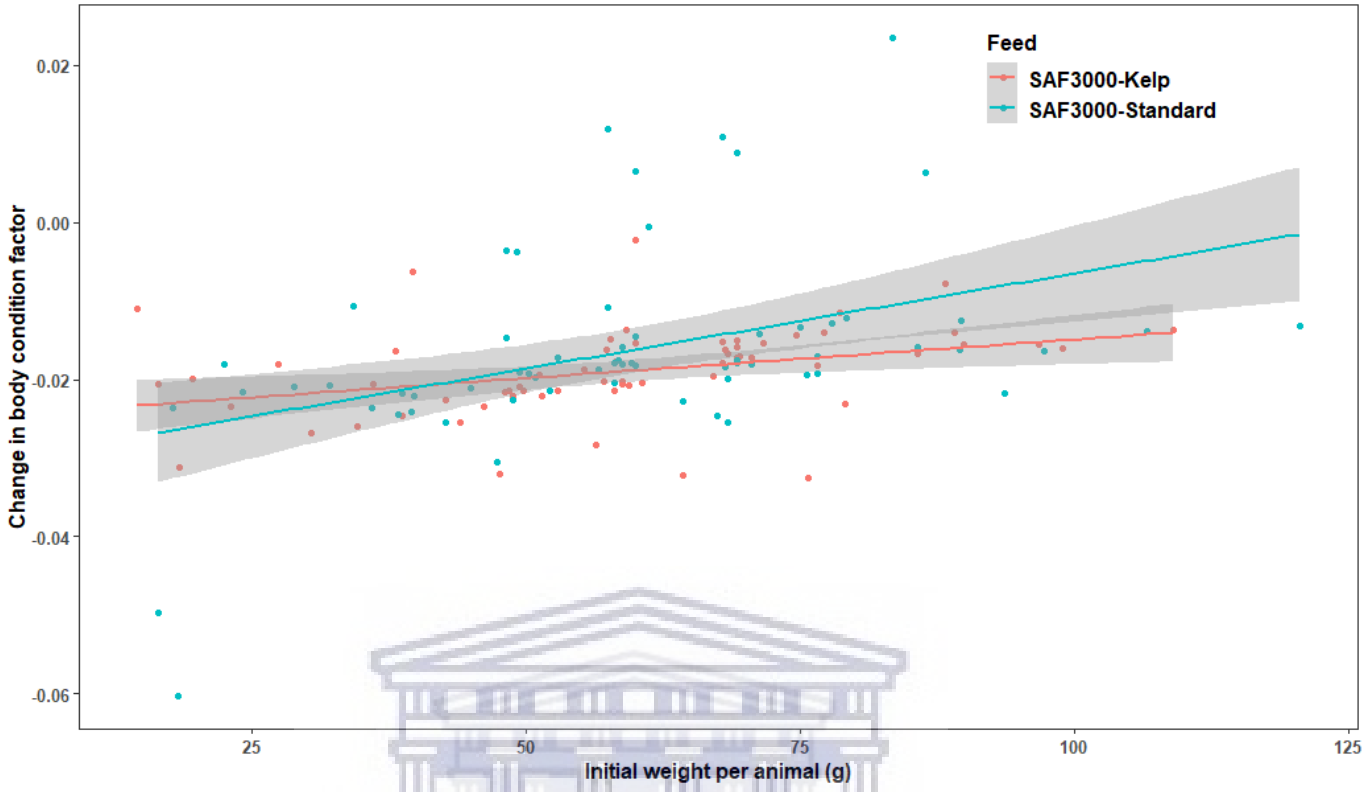

Figure 3.6: Linear relationship between change in body condition and initial animal weight (g) for each feed treatment over a 7 month period. Grey bandwidths represent 95% confidence intervals around linear model predictions. Regression slopes between feed treatments are at the threshold of significance (*t-stat* = -1.939, *dof* = 123, *P* = 0.055).

3.2.3. Specific growth rate between feeds treatments

A Wilcox rank sum test revealed no significant difference (W = 2294.5, P = 0.180) in daily *SGR* between feed treatments (Figure 3.7). Daily *SGR* was negatively correlated with animal size for both feed treatments (Figure 3.8 and 3.9). There was no significant difference (t = 1.948, dof = 123, P < 0.054) in the slopes of the linear models for the relationship between daily *SGR* and initial animal weight between the feed treatments (Figure 3.9).

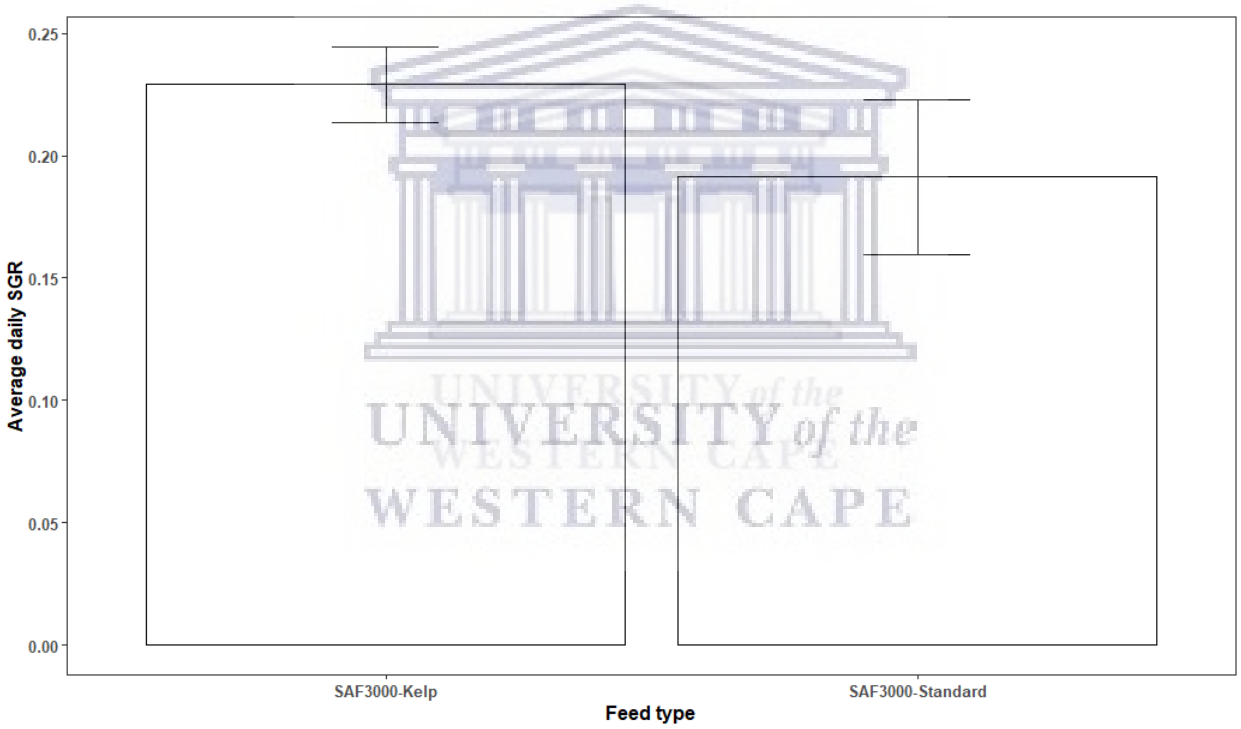

Figure 3.7: Average daily specific growth rate (SGR) per basket between feed treatments. Error bars represent 95% confidence intervals around the mean.

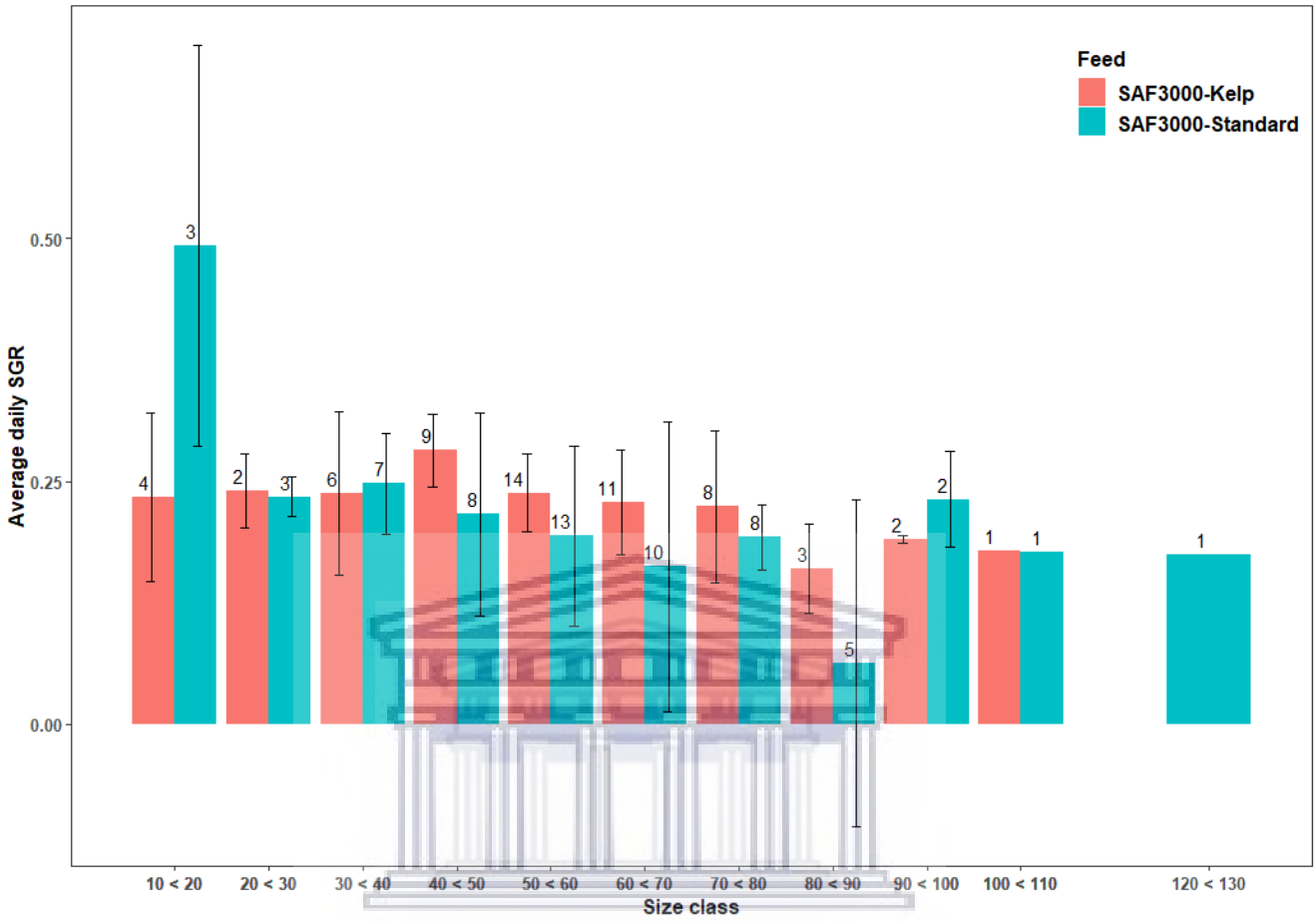

Figure 3.8: Average daily specific growth rate (SGR) for animal size classes between feed treatments over a 7 month period. Error bars represent 95% confidence intervals around the mean. Numbers represent sample size (number of baskets) per size class for feed treatments.

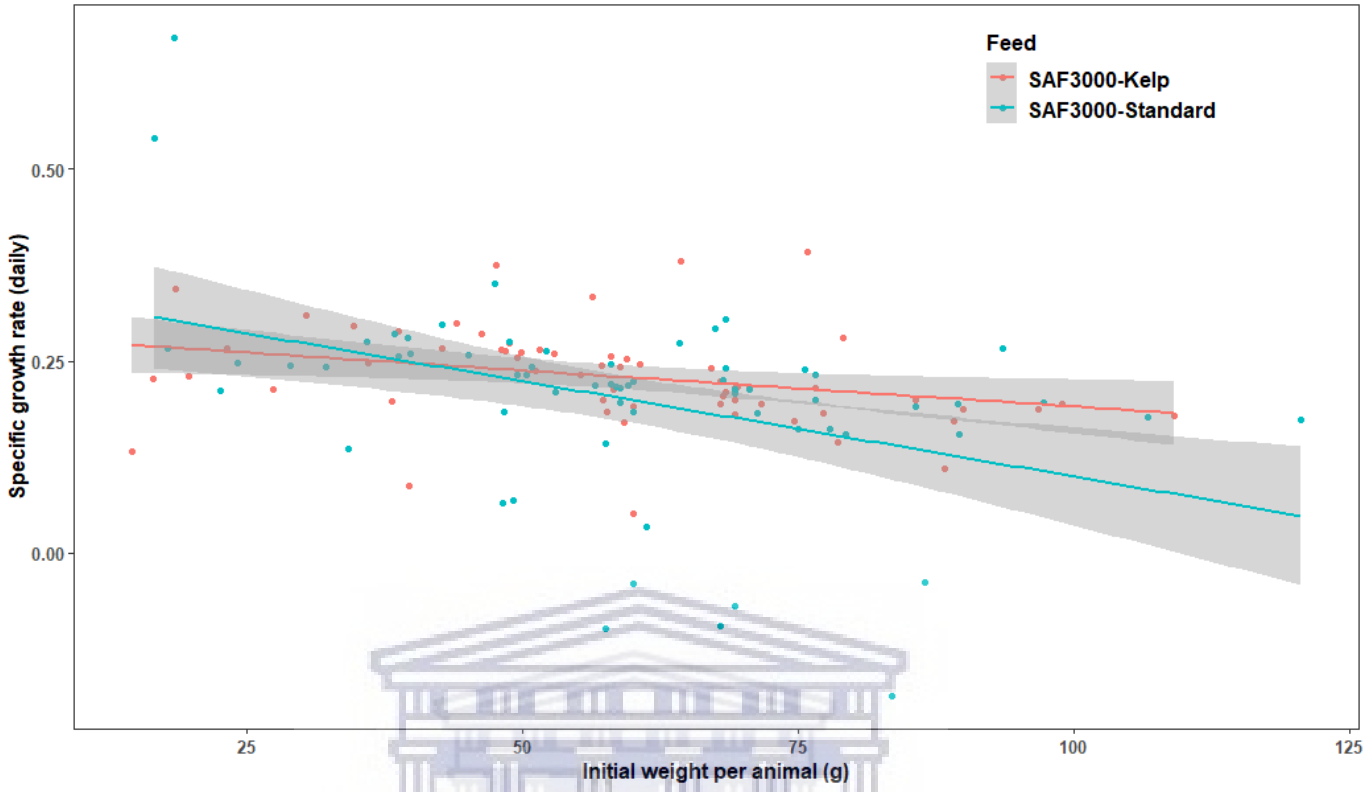

Figure 3.9: Linear relationship between daily specific growth rate and initial animal weight (g) for each feed treatment over a 7 month period. Grey bandwidths represent 95% confidence intervals around linear model predictions. Regression slopes between feed treatments are at the threshold of significance (*t-stat* = 1.948, *dof* = 123, *P* = 0.054).

4. Discussion

4.1. Feed stability

Haliotis midae experiences an average water temperature of 12 and 21°C along the west and south coast of South Africa, respectively (Tarr 1995). Artificial abalone feeds therefore need to be designed for maximum water stability within this temperature range in order to ensure that abalone have sufficient access to feed, and the nutrients therein, before disintegration (Jussila and Evans 1998; Troell et al. 2006; Bansemer et al. 2016). Moreover, abalone are slow nocturnal feeders (Wood and Buxton 1996; García-Esquivel et al. 2007), which when combined with farm operations during daylight hours, calls for the use of a feed that can maintain its structural integrity long enough for abalone to commence and complete feeding (Britz et al. 1994).

Increased precautionary measures are needed in the implementation of a feeding regime at higher temperatures (Britz et al. 1997). Based on the results here, at a water temperature of 14°C, feed can safely be left in the tanks for 24 hours without any meaningful loss in water stability for both kelp-inclusive (SAF1) and standard (SAF2) feed, with a percentage loss of 6.6 and 3.8, respectively, from 4 to 24 hours. At 22°C, however, I suggest feed to be taken out after 16 hours, as water stability significantly decreases thereafter, or feeding to commence at a different time. The decrease in water stability from 4 to 24 hours at 22°C was 31.7% and 25.8% for SAF1 and SAF2, respectively, which is significantly lower than the 4.2% and 3.8% decrease from 4 to 16 hours for SAF1 and SAF2, respectively. Temperature has also been shown to significantly influence feed consumption of *Haliotis midae*, increasing with an increase in temperature between 12 and 20 °C, and decreasing sharply from 20 to 24 °C (Britz et. al. 1997). Thus, formulated feed capable of withstanding temperature fluctuation will be highly advantageous to accommodate for

the resultant change in feeding response (Jussila and Evans 1998; García-Esquivel et al. 2007).

Feed stability has the potential to significantly influence growth performance of cultured species. *Cherax tenuimanus* (hairy marron), though a crustacean, had significantly better percentage weight gain and specific growth rate when fed a stable-form diet as compared to the unstable pelleted form (Jussila and Evans 1998). Nel (2016) indicated a significant increase in leaching of pelleted feed with increasing kelp-inclusion levels. The inclusion of different macroalgal species in the formulation of pelleted abalone feed has been shown to significantly influence feed stability (Bansemer et al. 2016). The chemical composition of some green macroalgae is thought to inhibit pellet binding, while agar or carrageenan (in red algae), and alginate (in brown algae) may improve pellet binding, thereby improving feed stability (Bansemer et al. 2016). However, when comparing the stability of feed containing *Ulva clathrate*, the kelp *Macrocystis pyrifera* and the fucoid *Ascophyllum nodosum*, it was noted that *U. clathrate* had significantly lower dry matter loss than either brown alga, and also yielded better growth (Cruz-SuÁrez et al. 2009).

As expected from the trends observed in water stability, TSS significantly increased only after more than 16 hours of feed submersion at 22°C. Though SAF2 had a higher water stability than SAF1, SAF2 had significantly higher (14.088 ± 3.757) TSS than SAF1 (7.287 ± 0.878) after 24 hours of feed submersion, which may be attributed to differences in the ability of nutrients to dissolve in water between the two feeds (Cruz-SuÁrez et al. 2009; Bansemer et al. 2016).

There are two main sources contributing to nutrient build-up in aquaculture systems: excretion from farmed animals themselves, and that resulting from the degradation of feed (Ackefors and Enell 1994). Ammonia build-up in aquaculture systems has been shown to

negatively affect both growth and survival of *Haliotis midae* (Huchette and Day 2003; Reddy-lopata et al. 2006), with sensitivity to ammonia decreasing with increasing abalone size (age). Similar results were observed for greenlip abalone, *Haliotis laevigata* (Harris et al. 1998). Feeds that have a higher water stability are, of course, preferred when seeking to lower the risk of toxic ammonia levels in aquaculture systems. Feed with a higher water stability prevents the potential for excessive nutrient leaching and subsequent ammonia build-up (Ackefors and Enell 1994). With reference to the current study, there was no statistically significant difference in water stability between the two feeds used. Ammonia toxicity, when considering water stability and leaching appears to be of concern only at 22°C after more than 16 hours of submersion, regardless of the type of feed used. There is, thus, no need for further consideration in an attempt to select a more water stable feed as they perform similarly in this regard. I did, however, find that at 22°C after 24 hours of feed submersion, SAF3000 – Standard had significantly higher TSS% than SAF3000 – kelp inclusion, indicating a higher potential for leached particulate feed to dissolve in water for the kelp – inclusion feed. The aforementioned difference in TSS% may be attributed to the use of dried kelp in the kelp-inclusion feed, which has a greater potential to dissolve in water.

4.2. Growth performance relative to artificial feeds

The use of dried kelp in the formulation of artificial feed is becoming increasingly popular, as macroalgae are the primary constituent of wild abalone diets and are thought to be beneficial for abalone growth performance (Mercer et al. 1993; Naidoo et al. 2006; Bansemer et al. 2016). The inclusion of dried macroalgae in formulated feed provides an opportunity to explore the benefits

of formulated feeds without the complete abandonment of macroalgae (Naidoo et al. 2006; Kemp et al. 2015; Bansemer et al. 2016; Nel 2016). This study, however, indicates that there was no major difference in growth performance between SAF3000-Standard and SAF3000-kelp inclusion (1%). These results contrast with those of Nel et al. (2017), in which formulated feed with kelp inclusion had a positive effect on mass gain by *Haliotis midae*. All kelp (*Ecklonia maxima*) supplemented diets (ranging from 0.44 to 3.54% inclusion) yielded significantly higher mass gain than control diets (Nel et al. 2017). Statistically significant improvements in *FCR* and *PER* were observed with increasing kelp inclusion levels (Nel et al. 2017). Greenlip abalone, Haliotis *laevigata*, fed a diet of macroalgae-inclusive feeds grew better than those on a feed without any macroalgal inclusion (Bansemer et al. 2016). The latter authors noted that a feed with 5% *Ulva* sp inclusion yielded significantly higher growth than 0% inclusion, but beyond 10% inclusion, growth was similar to 0 and 5% inclusion. In contrast, *Haliotis tubervulata coccinea* fed a formulated diet with a 43% inclusion had significantly poorer growth than fresh macroalgae when fed in excess (Bansemer et al. 2016).

Therefore, when considering macroalgal inclusions in feed, one must consider both the percent inclusion and the species to be used, as abalone growth may respond differently according to the interaction between the aforementioned factors (Mercer et al. 1993). Also, the inclusion of dried macroalgae in formulated feed may have benefits beyond only dietary requirements as they may be used as a chemosensory stimulus to evoke a feeding response in abalone. *Haliotis rufescens* had improved consumption and digestibility on formulated feed with dried inclusion of giant kelp (*Macrocystis pyrifera*) (Garcia-Esquivel and Felbeck 2009). Australian abalone, *Haliotis rubra*, have also been shown to have distinct chemosensory preferences among macroalgal species (Fleming 1995), with Bansemer et al (2006) confirming a significant effect of dietary macroalgal

inclusion on growth performance of the greenlip abalone. The perceived benefits from macroalgal inclusion have led to the South African feed production company, Marifeed^{TM}, producing a 5% kelp (*Ecklonia maxima*) inclusion feed as an attractant for *Haliotis midae* (Bansemer et al. 2016).

The use of formulated feed has proven to yield better growth rates than natural macroalgal diets (Britz 1995; Makhande 2008; Garcia-Esquivel and Felbeck 2009; van der Merve 2009; Naylor et al. 2009). This is largely due to the higher nutritional value of artificial feeds and the presence of essential nutrients required for optimum growth, which are often lacking or "insufficient" in natural seaweeds diets (Britz 1995; Mai *et al*. 1995; Britz 1996; van der Merve 2009). Despite the widely accepted use of formulated feed as a superior option for both nutritional and logistical reasons (Britz 1995; van der Merve 2009; Naylor *et al*. 2009), Naidoo et al (2006) achieved superior growth rates form a natural seaweeds diet relative to formulated feed. In fact, all natural seaweeds diets outperformed the use of formulated feed alone (Naidoo et al. 2006). Similar results were achieved for hybrid Australian abalone (*Haliotis rubra x Haliotis laevigata*), with six mixed macroalgal diets (all using different ratios of *Grateloupia turuturu*, *Ulva australis* and *U. laetevirens*) outperforming two commercially available formulated feeds in both weight and length growth rates, despite having almost 33% less protein content (Mulvaney et al. 2013). These results highlight the fact that natural seaweed diet, despite having lower protein content, have higher digestible protein and production energy value, with a more suitable amino acid profile than the commercial feed used (Mulvaney et al. 2013).

Growth rates on natural seaweeds diets have been enhanced by the protein enrichment of macroalgal species, through on farm production in abalone/fish effluent (Naidoo et al. 2006; Mulvaney et al. 2013). Juvenile *Haliotis midae* grow significantly better when fed farm-grown, protein enriched *Ulva lactuca* compared to wild *U. lactuca* stocks (Robertson-Andersson et al.

2011). This has promoted the implementation of on-farm macroalgal production through bioremediation and integrated multi-trophic aquaculture practices (Naidoo et al. 2006; Mulvaney et al. 2013). The use of artificial feed, however, remains a superior option over natural seaweeds diets (Britz 1995; van der Merve 2009; Naylor *et al*. 2009).

The use of a mixed or rotational dietary strategy has been shown to yield superior growth rates compared to a monotypic dietary strategy (Stuart and Brown 1994; Sales and Britz 2001; Naidoo et al. 2006). These results are supported by the analysis of wild *Haliotis midae*, which have a variety of macroalgae in their gut (Barkai and Griffiths 1986). Juvenile *Haliotis midae* have been shown to grow significantly better on a mixed diet of kelp (*Ecklonia maxima*) and *Ulva lactuca*, compared to monotypic administration of either macroalgal species (Robertson-Andersson et al. 2011). The same can be said for a mixed diet incorporating both pelleted and natural seaweeds diets, as *Haliotis midae* has been shown to grow significantly better on a mixed diet of pelleted and natural seaweed (*U. lactuca*), compared to the monotypic administration of either feed type (Makhande 2008). Superior growth performance from mixed/rotational diets is thought to reflect a more balanced nutrient and amino acid dietary composition, as essential amino acids become limiting in a monotypic macroalgal diet (Bansemer et al. 2016). The fact that I found no significant difference in growth (%) herein between the kelp-inclusion feed and the control feed may be attributed to the form in which the feed was administered (Naidoo et al. 2006). This study made use of dried, milled kelp for artificial feed inclusion, which has been shown to yield the poorest growth rates relative to fresh macroalgae and artificial feeds (Naidoo et al. 2006; Makhande 2008). This may be as a consequence of consuming bacteria associated with fresh kelp, which improve digestibility and assimilation of nutrients, as well as the fact that the drying process likely significantly decreases nutrient availability to abalone (Troell et al. 2006). Kelp-supplemented

diets result in a more balanced gut bacterial composition relative to non-kelp formulated feeds, which have greater variability in the composition of dominant bacterial species (Nel et al. 2017). Artificial feed used in combination with fresh kelp, however, produced significantly better growth rates than the use of artificial feed alone (Naidoo et al. 2006), which may be a better feeding strategy compared to the incorporation of dried kelp in feed formulation. I do, however, suggest that higher kelp-inclusion levels be tested, which will allow for comparability with the use of fresh kelp on a dry weight basis. For red abalone (*Haliotis rufescens*), a macroalgal inclusion level of 31% was too low to significantly increase growth rates relative to control pelleted feeds, but significantly increased at a 75% inclusion level. The major macroalgal dietary constituents (e.g. *Ulva spp*, *Plocamium spp*, etc.) of wild *Haliotis midae* should also be tested for inclusion in formulated feeds, as growth performance varies with species and abalone size (Mercer et al. 1993; Stuart and Brown 1994; Sales and Britz 2001; Naidoo et al. 2006).

Inspection of the relationship between percent animal weight gain and initial animal weight between the two feed treatments revealed that the kelp inclusion feed had more consistent weight gain across all size classes, compared to the standard formulated feed which had a significantly steeper decline in percent weight gain with increasing size. Size-dependent growth potential was also found in farmed *Haliotis rufescens* (red abalone) (Steinarsson and Imsland 2003) and *Haliotis discus hannai* (Pacific abalone) (Wu et al. 2009). Steinarsson and Imsland (2003) showed that red abalone ranging from 21 to 66 mm average shell length had similar maximum growth rates, but that this decreased significantly for animals larger than 80 mm. Pacific abalone have higher specific growth rates with increasing size, however, these abalone were grown *in situ* (Wu et al. 2009). Regardless, the aim of dietary formulation is to achieve maximum, consistent growth

performance across all size classes. The observations presented here are consistent with those reported by Troell et. el. (2006), confirming relatively good growth rates for *Haliotis midae* until at least 50 mm shell length, after which farmers tend to include fresh kelp into the diet for improved growth. In support of this decreased growth potential with increased abalone size, *Haliotis fulgens* (green abalone) has been shown to exhibit decreased feed consumption (% feed consumed as a function of body weight) with increased size, thereby also limiting growth potential (García-Esquivel et al. 2007). Differences in the linear models for percent weight gain across size classes between the feed treatments may, therefore, be attributed to the inclusion of dried kelp, which yielded more consistent weight gain across all size classes compared to those achieved with standard SAF3000 (Troell et al. 2006). Being composed of 25% ash on a dry weight basis, the inclusion of dried kelp in artificial feed is beneficial for increased shell growth, which is often limited in larger abalone fed only formulate feed (Troell et al. 2006). The high ash content of dried kelp may, therefore, be used to meet essential mineral requirements of *Haliotis midae*, providing a natural replacement for artificial ash inclusion.

5. Conclusion

For both feed treatments, temperature significantly influenced water stability only after 24 hours submersion, with greater dry matter loss at 22°C compared to 14°C. Water stability between the feed treatments did not differ significantly at any temperature-time combinations. Both feed treatments differed significantly in *TSS* only after 24 hours submersion for 14 vs 22°C treatments. Similarly, feed treatments differed significantly in *TSS* only at 22°C, after 24 hours submersion, with kelp-inclusive feed having lower *TSS*. I therefore have no recommended feed treatment in terms of feed stability between the feeds tested herein, but recommend that at higher temperatures of at least 22°C, feed be removed from tanks after 16 hours of submersion. At 14°C, feed can comfortably be left in tanks for at least 24 hours with no meaningful dry matter loss. For more extensive studies comparing stability of various feeds, the chemical leaching of feeds should be assessed to determine the extent of macronutrient after being submerged in water for various time-temperature combinations.

There was no significant difference in growth (% weight gain) between feed treatments. However, the relationship between percentage weight gain and initial animal weight differed significantly between the feed treatments, with kelp-inclusive feed yielding better (and more consistent) growth returns over all abalone size classes compared to standard SAF3000. I therefore recommend the use of kelp-inclusive SAF3000 for more consistent growth returns over all size classes of *Haliotis midae*. Based on the available literature, I suggest that higher inclusion levels be tested using various macroalgae specimens (which form a natural part of *Haliotis midae* diet), as adjustment to these facors have been shown to significantly influence abalone growth performance.

Due to the lack of sufficient growth data, results herein must be interpreted with caution and provide for only a preliminary assessment. Only one sales week was used with two data points for animals from each feed treatment: initial and final animal weights, over a seven-month period. For future studies aimed at answering similar questions to those herein, I suggest the use of multiple sales weeks, more grading events, and extended trial periods to increase the reliability of observed growth trends and add power to the statistical tests used.

Acknowledgements

I would like to thank Abagold (Ltd) for providing the facilities and animals need to conduct the growth trials, and Specialised Aquatic Feeds (Co.) for providing the formulated diets used for feed stability and growth trials. Many thanks to the National Research Foundation (NRF) and project Phuhlisa at the South African Institute for Aquatic Biodiversity (SAIAB), for their financial support throughout the study. I would also like to thank Tim Ponton (UWC), for his assistance with sample collection and logistics. To my supervisor, Prof MJ Gibbons, Thank you for your continued support both academically and personally.

References

Ackefors H, Enell M. 1994. The release of nutrients and organic matter from aquaculture systems in Nordic countries. *Journal of Applied Ichthyology* 10:225–241.

Allen VJ, Marsden ID, Ragg NLC, Gieseg S. 2006. The effects of tactile stimulants on feeding, growth, behaviour, and meat quality of cultured Blackfoot abalone, *Haliotis iris*. *Aquaculture* 257(1–4):294–308.

Bansemer Matthew S., Qin JG, Harris JO, Duong DN, Currie KL, Howarth GS, Stone DAJ. 2016. Dietary inclusions of dried macroalgae meal in formulated diets improve the growth of greenlip abalone (Haliotis laevigata). *Journal of Applied Phycology* 28(6):3645–3658.

Bansemer Matthew S, Qin JG, Harris JO, Howart GS, Stone DA. 2016. Nutritional requirements and use of macroalgae as ingredients in abalone feed. *Reviews in Aquaculture*. 8(2):121–135.

Barkai R, Griffiths CL. 1986. Diet of the South African abalone Haliotis midae. *South African Journal of Marine Science*. 4(1):37–44. doi:10.2989/025776186784461891.

Britz PJ. 1995. The nutritional requirements of haliotis midae and development of a practical diet for abalone aquaculture [PhD thesis]. South Africa: Rhodes University. 150 p.

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

Britz PJ, Hecht T, Knauer J, Dixon MG. 1994. The Development of an Artificial Feed for

Abalone Farming. *South African Journal of Science* 90:7–8.

Britz PJ, Hecht T, Mangold S. 1997. Effect of temperature on growth, feed consumption and nutritional indices of Haliotis midae fed a formulated diet. *Aquaculture* 152(1–4):191–203.

Brown MR, Sikes AL, Elliott NG, Tume RK, Tume RONK. 2008. Physicochemical Factors of Abalone Quality : A Review. *BioOne* 27(4):835–842.

Button C, Hubbard KE, Kashiwada J V, Moore JD, Rogers-bennett L, Lampson K, Stein D, Taniguchi IK. 2011. Status of the Fisheries Report: Abalones, Haliotidae.

Chigumira G. 2016. POLICY BRIEF: 4 / 2016 - Maximising niche markets: South African abalone. South Africa: Trade and Industrial Policy Strategies. 7 p.

Chiou TK, Lai MM. 2002. Comparison of taste components in cooked meats of small abalone fed different diets. *Fisheries Science* 68(2):388–394.

Chiou TK, Lai MM, Shiau CY. 2001. Seasonal variations of chemical constituents in the muscle and viscera of small abalone fed different diets. *Fisheries Science* 67(1):146–156.

Cook PA. 2014. The Worldwide Abalone Industry. Scientific Research 5:1181–1186.

Cook PA. 2016. Recent Trends in Worldwide Abalone Production. *Journal of Shellfish Research* 35(3):581–583.

Cruz-SuÁrez LE, Tapia-Salazar M, Nieto-LÓpez MG, Guajardo-Barbosa C, Ricque-Marie D. 2009. Comparison of Ulva clathrata and the kelps Macrocystis pyrifera and Ascophyllum nodosum as ingredients in shrimp feeds. *Aquaculture Nutrition* 15(4):421–430.

Department of Trade and Industry [DTI]. 2016 April. Maximising niche markets: South African

abalone. South Africa: Department of Trade and Industry. 7 p.Dunstan GA, Baillie HJ, Barrett SM, Volkman JK. 1996. Effect of diet on the lipid composition of wild and cultured abalone. *Aquaculture* 140:115–127.

Durazo-beltra E, Toro-vazquez JF, Vasquez-pela C, Viana T. 2003. Effect of triacylglycerols in formulated diets on growth and fatty acid composition in tissue of green abalone (Haliotis fulgens). *Aquaculture* 224:257–270.

Fleming AE. 1995. Growth , intake , feed conversion efficiency and chemosensory preference of the Australian abalone, *Haliotis rubra*. *Aquaculture* 132:297–311.

Fourie L. 2014. The evaluation of biological, mechanical and chemical methods to contain South African abalone species (Haliotis midae) [dissertation]. South Africa: Stellenbosch University. 104 p.

Francis TL, Maneveldt GW, Venter J. 2008. Growth of market-size abalone (Haliotis midae) fed kelp (Ecklonia maxima) versus a low-protein commercial feed. African Journal of Aquatic Science. 33(3):279–282.

Fuke S, Konosu S. 1991. Taste-active components in some foods: A review of Japanese research. *Physiology and Behaviour* 49(5):863–868.

Garcia-Esquivel Z, Felbeck Haliotis 2009. Comparative performance of juvenile red abalone, Haliotis rufescens, reared in laboratory with fresh kelp and balanced diets. *Aquaculture Nutrition* 15(2):209–217.

García-Esquivel Z, Montes-Magallón S, González-Gómez MA. 2007. Effect of temperature and photoperiod on the growth, feed consumption, and biochemical content of juvenile green

abalone, Haliotis fulgens, fed on a balanced diet. *Aquaculture* 262(1):129–141.

Gerber ME. 2013. Quantitative assessment of yield traits between family groups of the cultured abalone, Haliotis midae, during the process of canning [PhD]. South Africa: University of Stellenbosch. 74 p

Gilroy A, Edwards SJ. 1998. Optimum temperature for growth of Australian abalone : preferred temperature and critical thermal maximum for blacklip abalone, Haliotis rubra (Leach), and greenlip abalone, Haliotis laevigata (Leach). *Aquaculture Research* (1994):481–485.

Gordon HR, Cook PA. 2004. World abalone fisheries and aquaculture. *Journal of Shellfish Research* 23(4):935–939.

Gordon N, Neori A, Shpigel M, Lee J, Harpaz S. 2006. Effect of diatom diets on growth and survival of the abalone Haliotis discus hannai postlarvae. *Aquaculture* 252:225–233.

Green AJ, Jones CLW, Britz PJ. 2011. The protein and energy requirements of farmed South African abalone Haliotis midae L . cultured at optimal and elevated water temperatures. *Aquaculture Research* 42:1653–1663.

Harris JO, Maguire GB, Edwards S, Hindrum SM. 1998. Effect of ammonia on the growth rate and oxygen consumption of juvenile greenlip abalone , Haliotis laevigata Donovan. *Aquaculture* 160:259–272.

Harris JO, Maguire GB, Edwards SJ, Johns D. 1999. Low dissolved oxygen reduces growth rate and oxygen consumption rate of juvenile greenlip abalone , Haliotis laevigata Donovan. *Aquaculture* 174:265–278.

Hooper C, Day R, Slocombe R, Benkendorff K, Handlinger J. 2011. Effect of movement stress

on immune function in farmed Australian abalone (hybrid Haliotis laevigata and Haliotis rubra). *Aquaculture* 315:348–354.

Huchette SMH, Koh CS, Day RW. 2003. The effects of density on the behaviour and growth of juvenile blacklip abalone (Haliotis rubra). *Aquaculture International* 11:411–428.

Huchette SMH, Koh CS, Day RW. 2003. Growth of juvenile blacklip abalone (Haliotis rubra) in aquaculture tanks : effects of density and ammonia. *Aquaculture* 219:457–470.

Jarayabhand P, Kruiroongroj W, Chaisanit C. 2010. Effects of Stocking Density on Growth Performance of Thai Abalone , Haliotis asinina , Linnaeus 1758 , Reared Under a Semiclosed Recirculating Land-Based System. *BioOne* 29(3):593–597.

Johansen L, Jensen I, Mikkelsen H, Bjørn P, Jansen PA, Bergh Ø. 2011. Disease interaction and pathogens exchange between wild and farmed fi sh populations with special reference to Norway. *Aquaculture* 315:167–186.

Jussila J, Evans LH. 1998. Growth and condition of marron Cherax tenuimanus fed pelleted diets of different stability. *Aquaculture Nutrition*. 4(3):143–149.

Karunasagar Indrani, Vinod MG, Kennedy BOB, Vijay A, Deepanjali A, Umesh KR, Karunasagar Iddya. Biocontrol of Bacterial Pathogens in Aquaculture with Emphasis on Phage Therapy. *Diseases in Asian Aquaculture* 5:535–542.

Kemp JOG, Britz PJ, Toledo Agüero PH. 2015. The effect of macroalgal, formulated and combination diets on growth, survival and feed utilisation in the red abalone Haliotis rufescens. *Aquaculture* 448:306–314.

Kim TW, Barry JP, Micheli F. 2013. The effects of intermittent exposure to low-pH and low-

oxygen conditions on survival and growth of juvenile red abalone. *Biogeosciences* 10:7255–7262.

Leighton DL. 1974. The influence of temperature on larval and juvenile growth in three species of southern California abalones. *Fishery Bulletin* 72(4):1137–1145.

Mai K, Mercer JP, Donlon J. 1995a. Comparative studies on the nutrition of two species of abalone, Haliotis tuberculata L. and Haliotis discus hannai Ino. IV. Optimum dietary protein level for growth. *Aquaculture* 136(1–2):165–180.

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

Makhande ED. 2008. Growth of the South African Abalone (Haliotis midae) on Three Diets, Under Commercial Conditions [MSc]. South Africa: Nelson Mandela Metropolitan University. 52 p.

Mayfield S, Branch GM. 2011. Interrelations among rock lobsters, sea urchins, and juvenile abalone: implications for community management. *Canadian Journal of Fisheries and Aquatic Sciences* 57: 2175–2185.

Mercer J, Mai K, Donlon J. 1993. Comparative studies on the nutrition of two species of abalone, Haliotis tuberculata Linnaeus and Haliotis discus hannai Ino I. Effects of algal diets on growth and biochemical composition. *Invertebrate Reproduction and Development* 23:75–88.

van der Merve E. 2009. Toward best management practices for the growth of the abalone Haliotis midae Linnaeus on a commercial South African abalone farm [MSc]. South Africa:

University of the Western Cape. 115 p.

Morash AJ, Alter K. 2016. Effects of environmental and farm stress on abalone physiology : perspectives for abalone aquaculture in the face of global climate change. *Review Aquaculture* 8:342–368.

Mouton A. (2018, Feb 21). South Africa's abalone farmers are turning to renewables to power the growth of their industry - which is set to double production levels in the next five years. From: https://writing.colostate.edu/guides/page.cfm?pageid=206&guideid=13.

Mulvaney WJ, Winberg PC, Adams L. 2013. Comparison of macroalgal (Ulva and Grateloupia spp.) and formulated terrestrial feed on the growth and condition of juvenile abalone. *Journal of Applied Phycology* 25(3):815–824.

Naidoo K, Maneveldt G, Ruck K, Bolton JJ. 2006. A comparison of various seaweed-based diets and formulated feed on growth rate of abalone in a land-based aquaculture system. *Journal of Applied Phycology* 18:437–443.

Naylor MA, Kaiser H, Jones CLW. 2011. Water quality in a serial-use raceway and its effect on the growth of South African abalone, Haliotis midae. *Aquaculture Research* 42:918–930.

Naylor RL, Hardy RW, Bureau DP, Chiu A, Elliott M, Farrell AP, Forster I, Gatlin DM, Goldburg RJ, Hua K, et al. 2009. Feeding aquaculture in an era of finite resources. *Proceedings of the National Academy of Sciences* 106:15103–15110.

Nel A. 2016. The effect of kelp supplementation in formulated feed on the production performance and gut microbiota of south african abalone (haliotis midae) [PhD]. South Africa: Rhodes University. 151 p.

Nel A, Pletschke BI, Jones CLW, Kemp J, Robinson G, Britz PJ. 2017. Effects of kelp Ecklonia maxima inclusion in formulated feed on the growth, feed utilisation and gut microbiota of South African abalone Haliotis midae. *African Journal of Marine Science* 39(2):183–192.

Neori A, Shpigel M, Ben-ezra D. 2000. A sustainable integrated system for culture of fish, seaweed and abalone. *Aquaculture* 186:279–291.

Oakes FR, Ponte RD. 1996. The abalone market: Opportunities for cultured abalone. *Aquaculture* 140:187–195.

Olaechea RP, Ushio H, Watabe S, Takada K, Hatae K. 2009. Toughness and Collagen Content of Abalone Muscles. *Bioscience, Biotechnology, and Biochemistry* 57(1):6–11.

Poore GCB. 1970. Ecology of New Zealand abalones , haliotis species (Mollusca : Gastropoda). New Zealand Journal of Marine and Freshwater Research. 6:11–22.

Reddy-lopata K, Auerswald L, Cook P. 2006. Ammonia toxicity and its effect on the growth of the South African abalone Haliotis midae Linnaeus. *Aquaculture* 261:678–687.

Robertson-Andersson D V., Maneveldt GW, Naidoo K. 2011. Effects of wild and farm-grown macroalgae on the growth of juvenile South African abalone Haliotis midae Linnaeus. *African Journal of Aquatic Science* 36:331–337.

Salama NKG, Murray AG. 2011. Farm size as a factor in hydrodynamic transmission of pathogens in aquaculture fish production. *Aquaculture environment interactions* 2:61–74.

Sales J, Britz PJ. 2001. Research on abalone (Haliotis midae L .) cultivation in South Africa. *Aquaculture Research* 32: 863-874.

Schalkwyk HJ Van. 2011a. Assessment of yield traits between family groups of the cultured abalone (Haliotis midae) in South Africa [MSc]. South Africa: Stellenbosch University. 81 p.

Smit AJ, Robertson-Andersson DV, Peall S, Bolton JJ. 2007. Dimethylsulfoniopropionate (DMSP) accumulation in abalone Haliotis midae (Mollusca: Prosobranchia) after consumption of various diets, and consequences for aquaculture. Aquaculture 269: 377–389.

Steinarsson A, Imsland AK. 2003. Size dependent variation in optimum growth temperature of red abalone (Haliotis rufescens). *Aquaculture* 224(1–4):353–362.

Stuart MD, Brown MT. 1994. Growth and diet of cultivated black-footed abalone, Haliotis iris (Martyn). *Aquaculture* 127(4):329–337.

Tarr RJQ. 1995. Growth and movement of the South African abalone *Haliotis midae*: A reassessment. *Marine and Freshwater Research.* 46(3):583–590.

Tarr RJQ, Williams PVG, MacKenzie AJ. 1996. Abalone, sea urchins and rock lobster: A possible ecological shift that may affect traditional fisheries. *South African Journal of Marine Science* 17:319–323.

The Department of Agriculture Forestry and Fisheries. 2012. Aquaculture annual report 2011 south africa. South Africa: Department of Agriculture Forestry and Fisheries. 30 p.Troell M, Robertson-andersson D, Anderson RJ, Bolton JJ. 2006. Abalone farming in South Africa : An overview with perspectives on kelp resources , abalone feed , potential for on-farm seaweed production and socio-economic importance. *Aquaculture* 257:266–281.

Vilchis LI, Tegner MJ, Moore JD, Friedman CS, Riser L, Robbins TT, Dayton PK, Robbins TT,

Dayton PK. 2005. Ocean Warming Effects on Growth , Reproduction , and Survivorship of Southern California Abalone. *Ecological Applications* 15:469–480.

Visser-Roux a. 2011. Reproduction of the South African abalone (Haliotis midae) [PhD]. South Africa: University of Stellenbosch. 88 p.

Vosloo A, Vosloo D. 2006. Routes of water loss in South African abalone (Haliotis midae) during aerial exposure. *Aquaculture* 261:670–677.

Wassnig M, Roberts RD, Krsinich A, Day RW. 2010. Effects of water flow rate on growth rate , mortality and biomass return of abalone in slab tanks. *Aquaculture Research* 41:839–846.

Watanabe H, Hideaki Y, Hiroshi Y. 1992. Seasonal variations of extractive components in the muscle of disk abalone. Nippon Suisan Gakkai 58: 921 - 925.

Wood A, Buxton C. 1996. Aspects of the biology of the abalone Haliotis midae (Linne, 1758) on the east coast of South Africa. 1. Feeding biology. South African Journal of Marine Science. 17:61–68.

Wood AD, Buxton CD. 1996. Aspects of the biology of the abalone *Haliotis midae* (Linne, 1758) on the east coast of South Africa. 2. Reproduction. South African Journal of Marine Science. 17:69–78.

Wu F, Liu X, Zhang G, Wang C. 2009. Effects of the initial size, stocking density and sorting on the growth of juvenile pacific abalone, haliotis discus hannai ino. *Aquaculture Research* 40:1103–1110.