Testing the suitability of local seaweeds and formulated feed as a food source for abalone (*Haliotis midae* Linnaeus) in an Integrated Land-based Aquaculture System

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I declare that

"*Testing the suitability of local seaweeds and formulated feed as a food source for abalone (*Haliotis midae *Linnaeus) in an Integrated Land-based Aquaculture System*"

is my own work, that it has not been submitted for any degree or examination in any other University, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

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1.1 Introduction to Aquaculture

Aquaculture is officially defined by the United Nations Food and Agriculture Organization (FAO) as the farming of aquatic organisms (FAO, 1990). Farming implies some sort of intervention in the rearing process to enhance production, such as regulating stocking, feeding, protection from predators, etc, and also implies individual or corporate ownership of the stock being cultivated (FAO, 1997). Aquatic organisms are farmed either in freshwater, ponds/lakes, in tanks on land, or in the ocean (Ryther, 1981; Tacon & De Silva, 1997; Troell et al., 1999b). Farming of marine organisms is referred to as mariculture (Kautsky et al., 1997; Sadovy & Vincent, 2002; Neori et al., 2007).

Aquaculture dates back 4 000 years to the culture of carp in China, and before that to Egypt where early pictorial depictions dating to 2500 BCE show tilapia being fished out of a tank (Bardach, 1972; Swann, 1992). The earliest known written record of fish culture techniques is attributed to Fan Li from China, who in 475 BCE described propagation methods, pond construction and growth characteristics of common carp (Beveridge & Little, 2002). Aquaculture has progressed from early freshwater farming of species like carp and tilapia, to marine farming of high value species like salmon, shrimp to mass culture of seaweeds (Lee, 1998; Beveridge & Little, 2002; Buschmann et al., 1995; Edding & Tala, 2003).

Aquaculture has rapidly expanded world-wide primarily due to the rising demands for seafood (FAO, 2006), the collapse in global capture fisheries (Naylor et al., 2000; Pauly et al., 2002; Troell et al., 2004), the decrease in arable land resources (Holmes, 1996; Naylor et al., 2000) and the exhaustion of natural freshwater resources (Delgado et al., 2003; De Silva, 2007). For these reasons, aquaculture has progressed and evolved in societies pressurized by

the inability of natural resources to provide enough high quality protein foods for their increasing populations (Tacon & De Silva, 1997; Costa-Pierce, 2003). In the last century alone there has been a surge in agricultural practices (Falcon, 1970; Michaels, 1982; Parks, 2001), resulting in what had been referred to as the Green Revolution[1]. More recently, the Blue Revolution[2] of aquaculture is becoming an industrial mode of food production (Stonich & De La Torre, 2002; Troell et al., 2004; White et al., 2004; Volpe, 2005; Neori et al., 2007). World aquaculture of fish, crustaceans, molluscs and aquatic plants has grown significantly from a production of below 1 million tonnes in the early 1950s, to a production of 62.9 million tonnes in 2005 (FishStat Plus, 2006). This figure is growing rapidly with an average annual global growth rate of 8.8% (FAO, 2006).

With most wild stocks already heavily depleted, over fished or fully exploited, there is increasing scientific evidence pointing to the dramatic declines in global catches (Cook et al., 1997; Pauly et al., 1998, 2002; Caddy & Garibaldi, 2000; Watson & Pauly, 2001; Meyers & Worm, 2003; De Silva, 2007; FAO 2006, 2007). Sustaining fish supplies from capture fisheries will therefore not be able to meet the growing global demand for aquatic food (FAO, 2006). The shortfall in food fish supplies to meet the demands of a growing global population as well as the increasing levels of seafood consumption, will consequently have to be met through aquaculture (Rawlinson & Forster, 2000; Delgado et al., 2003; Muir, 2005; De Silva, 2007). Fish and shellfish produced from aquaculture already accounts for nearly 43% of all seafood consumed by humans (FAO, 2007).

[1] Green Revolution refers to the worldwide transformation of agriculture that had led to significant increases in the production of cereal crops (due to the introduction of pesticides, improved seed strains, fertilizers and irrigation systems) during the 1960's and 70's (Falcon, 1970; Michaels, 1982; Parks, 2001).

[2] Blue Revolution refers to the more recent increase in aquaculture production of a diverse array of aquatic species in order to meet the global demand for protein and to curb the decline in capture fisheries (Stonich, 2002; Stonich & De La Torre, 2002).

Aquaculture is currently recognized as the fastest growing food production sector globally and the vast majority of aquaculture takes place in Asia (Gordon & Cook, 2001, 2004; Muir, 2005; De Silva, 2007). In 2004, countries in the Asian and Pacific regions accounted for 91.5% and 80.5% of aquaculture production and value respectively (FAO, 2004). More recent statistics now show that China alone accounts for 69.6% of the production and 51.2% of the total value of global aquaculture (FAO, 2006).

1.2 Aquaculture Practices

1.2.1 Aquaculture systems and their technology

Aquaculture may be defined in terms of the extensive and intensive farming production yields, based on the size of area used (Bardach, 1987; Tacon & De Silva, 1997; Troell et al., 1999b; Muir, 2005). Extensive or traditional aquaculture systems are closest to natural fisheries (Kautsky & Folke, 1989; Troell et al., 1999b, 2004; Naylor et al., 2000; Muir, 2005), requiring minimal inputs (Stickney, 1994; Tacon, 1995; Tacon & De Silva, 1997) with low stocking densities that offer relatively low yields (Muir, 1995, 2005; Stonich, 2002; Troell et al., 2004). Extensive systems typically do not involve feeding of the cultured organism because food is obtained directly from the natural resources of the system (FAO, 1995; Tacon & De Silva, 1997). Such systems exclude predators and so ensure control of competitors (Naylor et al., 2000). Intensive systems on the other hand require a large amount of input (Troell et al., 1997; Folke et al., 1998; Tacon, 2005; Tacon et al., 2006) with high stocking densities that offer relatively high yields (Brzeski & Newkirk, 1997; Stonich, 2002; Stonich & De La Torre, 2002; Troell et al., 2004; Muir, 2005). In such systems all the nutritional requirements of the cultured organism are provided artificially (FAO, 1995; Naylor et al., 2000). There is thus complete independence of the availability of natural food

and complete dependence on the use of commercial feeds (Tacon & De Silva, 1997; Naylor et al., 1998; Kautsky et al., 2001; FAO, 2006).

Between the extremes of extensive and intensive aquaculture lies varying degrees of semi-intensive aquaculture. Here there is partial feeding with commercial feeds (Tacon De Silva, 1997; Troell et al., 1999b; Naylor et al., 2000; Muir, 2005) and possible fertilization of the natural food supply (Muir, 1995; Naylor et al., 2000; Kautsky et al., 2001). Semi-intensive systems require less input and generate lower yields than intensive systems (Stonich & De La Torre, 2002; Muir, 2005).

Cultured organisms are usually marketed at the stage of their life cycle that is equivalent to the life cycle stage at which the wild stock of that particular organism is harvested/fished (Muir, 1995, 2005). For example, pelagic species are harvested at the adult/mature stage, at the point where the greatest concentrations of wild stock of that species would occur (Muir, 2005). While some aquaculture operators choose to focus on particular stages of the life cycle of the cultured organism (FAO, 1997; Kautsky et al., 2000; Troell et al., 2004), other operators choose to control the entire life cycle (Naylor et al., 2000). The latter is achieved by having hatchery systems together with grow-out systems on the same farm (Sales & Britz, 2000; Troell et al., 2006).

In terms of systems technology, the main containment units for aquaculture species are ponds (Pagand et al., 2000; Neori et al., 2003; Xu et al., 2008), cages (Troell et al., 1997; Karakassis et al., 2000; Lojen et al., 2005), net pens (Blouin et al., 2007; Wang et al., 2007), tanks (Bourne et al., 2006; Macbeth et al., 2007), raceways (Martin et al., 1987; Christiansen et al., 2007; Faulk et al., 2007) and long-line ropes (Chaitanawisuti & Menasveta, 1987; Duckworth

& Battershill, 2003; Brehmer et al., 2006). The type of system employed is largely dependent on the cultured organism. For example, shrimps are usually cultured in ponds (Funge-Smith & Briggs, 1998) whereas mussels are usually cultured on long-line ropes (Chaitanawisuti & Menasveta, 1987). Concerns for water conservation and waste discharges have, however, prompted the increased use of closed land-based recirculating aquaculture systems (Rawlinson & Forster, 2000; Faulk et al., 2007). This has allowed producers to have a degree of control over environmental factors (such as temperature, salinity, oxygen, predators, diseases, etc.) in order to prevent the aquaculture species from having a negative impact upon the environment (Rawlinson & Forster, 2000; Chen et al., 2002; Stickney, 2002; Troell et al., 2006).

1.2.2 Forms of Aquaculture

There are many different forms of aquaculture namely monoculture, co-culture and polyculture. The most common form of aquaculture is monoculture and typically involves the cultivation of just one species (Tacon & De Silva, 1997; Neori et al., 2004). Many examples of this type of aquaculture exist such as oysters (Bishop & Petersen, 2005; Wells & Jernakoff, 2006), fish (Wassef et al., 2001; Hong & Zhang, 2003) and seaweeds (Buschmann et al., 1995; Edding & Tala, 2003).

The cultivation of two or more different non-competitive species within the same farming system is referred to as polyculture (Tacon & De Silva, 1997; Stickney, 2000; Langdon et al., 2004; Neori et al., 2007). Traditional, freshwater polyculture (notably of rice and fish) started around 220 AD (Li, 1987; Tian et al., 1987, 1993; Liu & Cai, 1998; Beveridge & Little, 2002; Fernando, 2002). In this early polyculture system, water containing the animal by-products or wastes was directed to the terrestrial component as fertilizer in order to

enhance productivity (Liu & Cai, 1998; Frei & Becker, 2005; Maike et al., 2007). Polyculture is the forerunner of the modern day integrated aquaculture (Kautsky et al., 1997; Troell et al., 2003; Neori et al., 2004). Although the former can include terrestrial as well as aquatic species that may or may not benefit the system, the latter integrates different aquatic species within one system, with each organism benefitting the system in some way (Neori & Shpigel, 1999; Troell et al., 1999a; Chopin et al., 2004, 2006; Neori et al., 2004, 2007).

Co-culture is a modern term that is used to describe a form of polyculture in which two or more complementary organisms are grown together in the same culture medium (Edwards, 2004; Langdon et al., 2004; Pierri et al., 2006). Co-culture with species of the same trophic level is generally not beneficial to the system (Brzeski & Newkirk, 1997). However, integrating species of different trophic levels can be greatly beneficial to all facets of the system (Krom & Neori, 1989; Neori et al., 1989, 2001, 2004; Neori & Shpigel, 1999; Troell et al., 1999a; Chopin et al., 2004; Haya et al., 2004; Lander et al., 2004; Ridler et al., 2006). This form of aquaculture has recently been described as Integrated Multi-Trophic Aquaculture (IMTA) (Neori et al., 2004, 2007; Pierri et al., 2006; Chopin et al., 2006; Blouin et al., 2007; Ridler et al., 2007; Neori, 2007).

An example of IMTA is the integration of fish or shrimp culture with the culture of seaweed (referred to as the inorganic extractive) and/or shellfish (referred to as the organic extractive) (Shpigel & Neori, 1996; Neori et al., 1998, 2000, 2007; Chopin et al., 2001; Jones et al., 2001; 2002; Schuenhoff et al., 2003). In such a system, wastes (inorganic ammonia, phosphorous and organic solids) from one organism are used as inputs (fertilizers, food) for another. This results in the optimal use of resources and reduces the risk of eutrophication (Naylor et al., 2000; Chopin et al., 2001; Mazzola & Sarà, 2001; Tian et al., 2001; Chen et

al., 2002; Jones et al., 2002; Troell et al., 2003; Edwards, 2004; Lander et al., 2004; Neori et al., 2004; Pierri et al., 2006). One of the main aims of IMTA is that all co-cultured species become harvestable crops with some commercial value (Chopin et al., 2001; Fei, 2004; Neori et al., 2004, 2007; Chopin et al., 2006; Neori, 2007). IMTA therefore creates balanced systems for environmental sustainability and product diversification (Chopin et al., 2001; Pauly et al., 2002; New, 2003; Shumway et al., 2003; Troell et al., 2003; Neori et al., 2004, 2007; Tournay, 2006; Neori, 2007).

1.3 The costs, impacts and sustainability of Aquaculture

Expansion and intensification of aquaculture has been on the rise (Chopin et al., 2001; D'Abramo et al., 2002; Delgado et al., 2003) and increasing the stocking density of individual organisms is now the norm (Stonich & De La Torre, 2002). Consequently, aquaculture systems require greater management of inputs, which in turn lead to a greater generation of waste products and an increased potential for the spread of disease (Brzeski & Newkirk, 1997; Funge-Smith & Briggs, 1998; Naylor et al., 2000; Heuch, 2001). Intensive monoculture of shrimp and salmon for example, extract all the resources from the system (Naylor et al., 1998; Tian et al., 2001; Stonich, 2002; Marra, 2005) and consequently have negative environmental impacts as the effluent from these systems are often released directly into the surrounding waterways. This causes pollution problems (stemming from fertilizers, undigested feed and biological waste) and contributes to coastal eutrophication (Gowen & Bradbury 1987; Folke & Kautsky, 1989; Shpigel et al., 1993; Beveridge et al., 1994; Folke et al., 1994; Troell & Berg, 1997; Funge-Smith & Briggs, 1998; Naylor et al., 1998; 2000; Chopin et al., 1999; Troell et al., 1999b; Angel et al., 2000; Chopin et al., 2001; Stonich &

De La Torre, 2002; Delgado et al., 2003; Read & Fernandes, 2003; Marra, 2005) that may even lead to harmful algal blooms (Huang & Li, 2002; Pitcher et al., 2002).

In addition, many aquaculture practices such as shrimp and carnivorous fish ponds have large ecological footprints[3] (Kautsky et al., 1997; Folke et al., 1998; Wolowicz, 2005). Shrimp and fish ponds require very large ecosystem areas to sustain production and consequently occupy large coastal areas in developing countries (Troell et al., 1999b; Stonich & Bailey, 2000). These aquaculture practices often cause degradation of the coastal marine and freshwater ecosystems (e.g. mangroves) and are usually abandoned when the ecosystem becomes degraded and can no longer sustain production (Naylor et al., 2000; Stonich & De La Torre, 2002).

The demand for fishmeal and fish-oil (from wild-caught 'lower-value' fish such as hake, tilapia and milkfish) as feed for aquaculture is on the increase (Tacon & De Silva, 1997; Naylor et al., 1998; D'Abramo et al., 2002; Delgado et al., 2003; Tacon, 2005; Neori et al., 2007) because of the high quality animal protein, essential fatty acids and trace minerals they contain (Hertrampf & Piedad-Pascual, 2000; Tacon, 2004). The aquaculture sector is currently heavily dependent upon the use of fishmeal and fish oil within compound aquaculture feeds (Folke & Kautsky, 1989; 1992; Asche & Tveteras, 2004; Tacon, 2004; Pike, 2005). Naylor et al. (2000) argues that although aquaculture can significantly boost world fish production, there is a sizable environmental trade-off by the increased demand in wild-caught fish turned into fishmeal and fish-oil for 'higher-value' species such as salmon and sea-bass (Naylor et al., 1998; Tacon, 2004; Marra, 2005). Currently, 12kg of wild caught fish are required to produce 1kg of fish oil (used within commercial feeds) and 4kg of wild-

[3] Ecological footprint is a tool for gauging the sustainability of global fisheries, where resource inputs are compared to the production outputs (Kautsky et al., 1997; Folke et al., 1998; Wolowicz, 2005)

caught fish (in the form of fishmeal) are required to produce 1kg of farmed salmon (Tuominen & Esmark, 2003; Tacon, 2005; Kaushik, 2006). There are, however, many new research attempts to identify alternative sources of oil (especially of polyunsaturated fatty acids) and protein to counter diminishing supplies of fishmeal for use in commercial feeds (Chopin et al., 2001; Powell, 2003; Tacon, 2005).

According to Neori et al. (2007) intensive fishmeal-fed monocultures are ecologically imbalanced, unsustainable, and therefore cannot meet the rising demand for seafood. This viewpoint had been previously expressed by a number of other authors (e.g. Brzeski & Newkirk, 1997; Stickney, 2002; Stonich & De La Torre, 2002; Chopin & Reinertsen, 2003; Shumway et al., 2003; Neori et al., 2004). Due to the increasing limited nature of fishmeal and soy meal, using seaweeds as aquaculture feeds (Buschmann et al., 2001; 2005; Stirk & van Staden, 2004), and more recently as biofilters (Neori et al., 1991; 1996; Angel & Gordin, 1996; Neori & Shpigel 1999; Troell et al., 1999a, b; Jones et al., 2002; Neori et al., 2003; Schuenhoff et al., 2003; Demetropoulos & Langdon, 2004a,b; Xu et al., 2008), is a direction in which the blue revolution mariculture industry has shifted focus (Diamond, 2002; Troell et al., 2003, 2004; Neori et al., 2007; Neori, 2007).

The new mariculture focus is being referred to as the greening of the blue revolution, where there is a shift from intensive monoculture of shrimp and carnivorous fish to the cultivation of seaweeds, herbivores and omnivorous organisms (e.g. sea cucumbers, sea urchins, and grey mullet) in integrated systems (Diamond, 2002; Neori et al., 2007). There has also been a shift from harvesting seaweeds in the wild to the farming of seaweeds (seaweed mariculture) (Buschmann et al., 1996, 2005; Lüning & Pang, 2003; Gutierrez et al., 2006; Troell et al., 2006) as a result of scientific research into the life cycles and growth characteristics of

seaweeds with economic potential (Chopin et al., 2001; Wikfors & Ohno, 2001; Neori et al., 2007).

1.4 The use of seaweeds in Mariculture

Seaweed mariculture is increasingly becoming one of the more popular sectors of world aquaculture (FAO, 2000; Muller-Feuga, 2000; Troell et al., 2003). This is so because seaweeds are low in the food chain and extract their nourishment directly from the sea (Fei, 2004; Neori et al., 2004). Seaweed mariculture thus involves relatively low inputs (Wikfors & Ohno, 2001; Buschmann et al., 2005). Currently, the largest sector of the seaweed mariculture industry is concentrated in Asia and is represented by seaweed production for human consumption (Chopin et al., 2001; Wikfors & Ohno, 2001; Neori, 2007). Besides food for human consumption (Craigie et al., 1999; Davis & Kris-Etherton, 2003; Fei, 2004; Sahoo & Yarish, 2005), seaweeds are cultivated for a number of other reasons, including high value biochemicals (such as antibiotics, cosmetics and nutritional additives - Bhadury & Wright, 2004; Smit, 2004), phycocolloids (agar, carrageenans, and alginates) (Zemke-White & Ohno, 1999; Buschmann et al., 2005), fertilizers (Stirk & van Staden, 2004) and food for cultivated marine animals (Shpigel et al., 1999; Buschmann et al., 2005). Research into using seaweeds as full or partial replacements for fishmeal in order to supply carnivorous fish diets with balanced proteins and oils, is already underway (Mai et al., 1996; Wahbeh, 1997; Chopin et al., 2001; Powell, 2003).

Seaweed mariculture has been around for centuries. For example, the culture of *Porphyra* spp. was established in Japan some 400 years ago (Chopin et al., 2001) and still continues today (Buschmann et al., 2005; Blouin et al., 2007; He & Yarish, 2007). Modern seaweed

mariculture includes, for example, *Laminaria* spp. (Buck & Buchholz, 2005; Pang et al., 2007), *Undaria* spp. (Uchida & Murata, 2002), *Ulva* spp. (Shpigel et al., 1999; Schuenoff et al., 2003), and *Gracilaria* spp. (Buschmann et al., 1994, 1995, 2001; Anderson et al., 1998; Morinho-Soriano et al., 2002; Smit et al., 2003) to name but a few. Specific taxa are selected for integrated culture with shrimp, fish and/or shellfish because of their capacity for nutrient uptake as biofilters and for rapid growth (Chopin et al., 1999, 2001; Troell et al., 1999b, 2003; Neori et al., 2003). Such taxa include *Gracilaria chilensis* Bird, McLachlan et Oliveira (Bushmann et al., 1996, 2005; Troell et al., 1997), *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine et Farnham (Njobeni, 2006), *Gracilaria lichenoides* Greville (Xu et al., 2008), *Gracilaria parvispora* Abbott (Nelson et al. 2001), *Gracilaria edulis* (Gmelin) Silva (Jones et al., 2001), *Palmaria* spp. (Demetropolous & Langdon, 2004a,b), *Porphyra* spp. (Chopin et al., 1999), *Ulva lactuca* Linnaeus (Vandermeulen & Gordin, 1990; Cohen & Neori, 1991; Neori et al., 1991, 2003; Shpigel & Neori, 1996; Goldberg et al., 1998; Shpigel et al., 1999; Robertson-Andersson, 2003, 2006; Robertson-Andersson et al., 2007; Schuenhoff et al., 2003) and *Ulva rigida* C. Agardh (Jimenez del Río et al., 1996; Boarder & Shpigel, 2001).

Seaweeds used in integrated aquaculture systems are referred to as nutrient-scrubbers because they soak up nutrients like a sponge (Chopin et al., 1999; Troell et al., 2003; Neori et al., 2004, 2007; Neori, 2007). Seaweeds can assimilate as much as 90% of the ammonium produced by intensive fish culture (Cohen & Neori 1991; Neori et al., 1991, 1996, 2000; Jimenez del Río et al., 1996; Buschmann et al., 1996; Shpigel & Neori, 1996; Neori & Shpigel, 1999). This characteristic is what is required in intensive fish aquaculture where most of the nitrogen is released as ammonium and not nitrate (the nutrient usually taken up by seaweeds after bacterial oxidation of ammonia) (Neori, 1996; Troell et al., 1999b; Carmona et al., 2001; Lojen et al., 2005).

Integrating seaweeds into shrimp, fish and shellfish aquaculture not only counterbalances nutrient inputs, but also other metabolic aspects such as dissolved oxygen, carbon-dioxide and acidity levels (Harris et al., 1999; Xu et al., 1999, 2008; Neori et al., 2003; Schuenhoff et al., 2003). Seaweeds, unlike mechanical biofilters that contain bacteria (Rawlinson & Forster, 2000), do not compete with the shrimp, fish or shellfish for dissolved oxygen and so complex devices are not needed when seaweeds are integrated into the system (Chopin et al., 2001; Neori et al., 2007). Furthermore, night-time oxygen consumption by seaweeds is much lower than their daytime oxygen production (Neori et al., 2004); seaweeds clearly therefore produce much more oxygen than they use up. In an integrated system this is beneficial to the system and to the cultured animal as the seaweeds generally increase the oxygen levels in the water (Schuenhoff et al., 2003; Neori et al., 2004).

1.5 Abalone Mariculture World-wide

1.5.1 Taxonomy and Biogeography

Abalone are commercially important marine gastropods (Oakes & Ponte 1996; Kawamura et al., 1998; Sales & Britz, 2000, 2001) belonging to the Phylum Mollusca (Archeogastropoda: Haliotidae). Geiger (1998) evaluating all of the 200 taxa known to have been ascribed to some 18 genera within the Haliotidae, found the use of generic names other than *Haliotis*, to be unjustified. Geiger (1998, 2000) does, however, not rule out the possibility of other valid genera outside of *Haliotis*, if there is adequate evidence to substantiate this claim. While most authors (Hahn, 1989; Fallu, 1991; Simpson, 1994; Lyon, 1995; Elliot, 2000) believe that there are approximately one hundred (100) *Haliotis* species globally, Geiger (1998, 2000) is of the opinion that there are only some 56 extant species and 10 extant subspecies. Despite these conclusions, many authors (e.g. Suzuki & Imai, 1998; Nakamura & Archdale, 2001;

Weber & Vinogradov, 2001; Baine & Side, 2003; Yuasa & Suzuki, 2005) still report on genera other than *Haliotis* and yet other authors (e.g. Sales & Britz, 2001; Bester et al., 2004; Sales & Janssens, 2004) recently reported that there are currently about 90 *Haliotis* species worldwide.

Distributed world-wide, the Haliotidae are found in coastal temperate and tropical waters and are native to most coastal waters except those around South America and the Atlantic coast of North America (Najmudeen & Victor, 2004; Degnan et al., 2006). Abalone inhabit rocky substrates, reefs and crevices in the intertidal zone (Hecht, 1994; Geiger, 2000; Degnan et al., 2006) at temperatures ranging from 2°C to 30°C (Leighton, 2000) and occur at depths usually down to 30m. They can, however, be found at depths down to 100m, but maximum population densities occur at depths between 3-10m where there is an abundant natural seaweed food supply (Leighton 2000; Ebert, 2001).

A geographic analysis of maximum shell length in living abalone conducted by Estes et al. (2005) revealed that small-bodied species (i.e., shell lengths <150mm), while most common in the Tropics, have a cosmopolitan distribution. Large-bodied species (i.e., shell lengths >150mm) on the other hand occur exclusively in cold-water ecosystems that are dominated by kelps and other large seaweeds (Estes et al., 2005). The largest species, *H. rufescens* Swainson (red abalone) from North America, for example, reaches 31 cm in shell length and almost 5kg in weight (Leighton, 2000). Larger species of abalone are mainly distributed in the temperate zones (coasts of Africa, Australia, Japan, New Zealand, southeast Asia, and western North America - Degnan et al. 2006) possibly because these regions are rich in nutrients and oxygen, and food is abundant (Jarayabhand & Paphavasit, 1996; Leighton,

2000; Somero, 2002). The greatest abalone species diversity notably occurs in the southwestern Pacific and Indian Oceans (Leighton, 2000; Degnan et al., 2006).

1.5.2 Life History

Abalone are dioecious (individuals of separate sexes) and broadcast spawners releasing their gametes into the water column for fertilization (Hobday et al., 2000). Abalone life history can be broadly categorized into five stages: embryo, larvae, postlarvae, juvenile and adult (McShane, 1992; Kawamura et al., 1998). A female can produce as many as 10 million eggs (high fecundity) with a mortality rate of 35-90% (McShane, 1992; Rogers-Bennett et al., 2004b). Eggs usually hatch within 24 hours of fertilization (Kawamura et al., 1998). The embryonic stage typically lasts a number of hours and concludes with the hatching of the pelagic, actively swimming, lecithotrophic (non-feeding), ciliated trochophore (Jaeckle & Manahan, 1989; McShane, 1992). The trochophore carries a yolk supply derived from the egg (which is its only source of energy - Kawamura et al., 1998; Moran & Manahan, 2003) and remains in the water column for 2-15 days (Kawamura et al., 1998; Leighton, 2000; Sawatpeera et al., 2001).

In natural habitats, larval abalone settle largely on crustose coralline algae in the presence of certain chemical cues (e.g. Gamma aminobutyric acid and amino acids - McShane, 1992, 1995; Roberts & Nicholson, 1997; Daume et al., 1999; Day & Branch, 2000; Roberts et al., 2004), diatom layers (Takami et al., 1997a; Daume et al., 1999; Gordon et al., 2004), abalone mucous trails (Searcy-Bernal et al., 1992, 1996; Takami et al., 1997b) and bacterial films (Roberts, 2001). Larvae then begin feeding mainly on benthic microflora, predominantly diatoms such as *Amphora* spp., *Cocconeis* spp., *Navicula* spp. and *Nitzschia* spp. (Kawamura & Takami 1995; Kawamura et al., 1998, 1999; Daume et al., 1997, 1999, 2000; Roberts,

2001; Searcy-Bernal et al., 2001; Correa-Reyes, 2002; Simental et al., 2004; Onitsuka et al., 2007). At this stage they are known as postlarvae, a stage that lasts for about two months, during which time the animal grows to 1.5-2.5mm (Shepherd & Daume, 1996; Leighton, 2000).

The end of the postlarval stage is signified by the appearance of the first respiratory pore (Kawamura et al., 1998; Najmudeen & Victor, 2004). At this point there is an increase in feed intake and a shift in the feeding habit from microalgal to macroalgal feeding (Simental et al., 2004; Onitsuka et al., 2007). This usually occurs around 5-10mm shell length but can begin as early as 2-4mm shell length such as the case with *H. discus hannai* Ino (Kawamura et al., 1998). This new feeding habit is often due to an increase in mouth size (Fleming et al., 1995b), changes in the morphology of the radula (Kawamura et al., 2001; Onitsuka et al., 2004), and/or changes in the digestive capabilities (Roberts et al., 1999; Kawamura et al., 2001; Johnston et al., 2005).

1.5.3 Use and Mariculture

Abalone have long been utilized as a food source by humans (Leighton, 1989; Bravo et al., 2001; Newsome et al., 2004). They are a highly valuable seafood, prized for their delicate meat that is in prime demand in Asia where abalone products form part of traditional cuisine and ceremony (Sales & Britz, 2000; Najmudeen & Victor, 2004). The ornamental shells of abalone with their inner iridescent layer are also valuable and are used to make jewelry and crafts (Sales & Britz, 2000; Caballero-Alegria et al., 2004).

Abalone are particularly susceptible to overfishing because of their high value (Huchette & Clavier, 2004; Uchino et al., 2004). The continuous unsustainable levels of harvesting in

many countries, habitat loss, elevated seawater temperatures (due to El Niño), disease and poaching, has depleted wild stocks and caused the decline in global abalone fisheries (Daniels & Floren, 1998; Harris et al., 1998; Shepherd et al., 1998; Bautista-Teruel & Millamena, 1999; Hobday et al., 2000; Shepherd, 2000; Tegner et al., 2001; Shepherd & Rodda, 2001; Gordon & Cook, 2004; Ponce-Diaz et al., 2004). Abalone are also vulnerable to overfishing because they are slow-growing, easy to capture, and because they display unpredictable recruitment (Tegner & Butler, 1989; Rogers-Bennett et al., 2004a). For these reasons, it has been necessary to cultivate abalone to meet the growing demand for abalone products world-wide (Gordon & Cook, 2001; Troell et al., 2006). Although overfishing has resulted in a rapid decline in wild abalone fisheries, there has been a dramatic increase in the global production of cultured abalone (Gordon & Cook 2001, 2004).

Research on the culture of abalone began in Japan and China in the early 1900's, and significant developments with regards to hatchery systems came about in the 1960's and 1970's (Uki & Kikuchi 1984; Leighton, 2000). The development of abalone cultivation has since grown rapidly and is now widespread in many countries including Australia, Chile, China, Iceland, Ireland, Japan, Mexico, New Zealand, South Africa, Taiwan and the USA, with more than 15 species now being commercially harvested and cultivated (Gordon & Cook, 2004; Sales & Janssens, 2004).

Globally, the most important commercial species are: the red abalone *H. rufescens* Swainson, the green abalone *H. fulgens* Philippi and the pink abalone *H. corrugata* Gray (McBride, 1998; Leighton, 2000; Viana, 2002; Flores-Aguilar, 2003; Rogers-Bennett et al., 2004) in North America; the blacklip abalone *H. rubra* Leach and the greenlip abalone *H. laevigata* Donovan (Coote et al., 2000; Daume & Ryan, 2004a; Dunstan et al., 2007) in Australia; the

black-footed paua *H. iris* Gmelin (Tong & Moss, 1992; Roberts et al., 2001, Allen et al., 2006) in New Zealand; and the disc abalone *H. discus hannai* Ino and the small abalone *H. diversicolor supertexta* Reeve (Yoo, 1989; Nie, 1992; Nie & Wang, 2004; Zhang et al., 2004a) in Asia. The current demand for abalone world-wide exceeds the supply by about 3000 tonnes and is expected to exceed supply by more than 7000 tonnes by the end of 2007 (Fishtech, 2007). The largest commercial abalone producer and consumer in the world is China with over 300 farms and a total production of approximately 4500 metric tonnes recorded in 2003 (Gordon & Cook, 2004; Zhang et al., 2004a), which is expected to reach 5000 metric tonnes by the end of 2007 (Fishtech, 2007). Abalone production currently holds the top position in terms of commercial value among farmed molluscan products in China (Zhang et al., 2004a).

Abalone can be cultured extensively by releasing seed on the sea bed using long line rafts or by being suspended in barrels or cages in the water column of sheltered bays or coastlines (Cai & Huang, 2000; Nie & Wang, 2004). Alternatively they can be cultured intensively on land in concrete seawater tanks (Gordon & Cook, 2001, 2004; Zhang et al., 2004a). Synchronized spawning is induced artificially using hydrogen peroxide, seawater treated with ultraviolet light, or temperature alteration (Ebert, 2001; Leighton, 2000; Nie & Wang, 2004). For fertilization success, farms usually maintain a high density of wild broodstock (McShane, 1991; Babcock & Keesing, 1999) or conditioned broodstock (individuals that are induced to spawn year-around or at regular and predictable times) (Freeman et al., 2006; Graham et al., 2006) ensuring that there is sufficient seed supply (Zhang et al., 2004a).

The induction of larval settlement is important in determining the survival rate of early postlarval growth stages (Daume et al., 1999; Roberts et al., 1999; Takami et al., 2002) and

diatom layers are frequently used for recruitment of larvae in culture (Daume et al., 1999; Roberts, 2001; Daume & Ryan, 2004a; Gordon et al., 2004). More recently, *Ulvella lens* Crouan, a green seaweed that has been used successfully in Japan, is currently on trial in many other countries as a cue for larval settlement (Daume et al., 2000, 2001, 2003; Daume & Ryan, 2004a; Daume, 2006). Alternative methods such as the Stott's Abalone Postlarvae Production System (SAPPS - Stott et al., 2003a,b; 2004a,b) (in which plastic plates are sprayed with a mixture containing formulated microparticle diets, agar, and juvenile mucus trails) are currently also being considered.

Growth of abalone is exponential during the postlarval and juvenile stages, but slows down once they reach sexual maturity (Hahn, 1989; Lee, 2004). In postlarval abalone, growth is generally influenced by several factors including type of feed (Kawamura et al., 1998, 1999; Roberts et al., 1999; Siquieros-Beltrones & Voltolina, 2000; Strain et al., 2006; Onitsuka et al., 2007), quantity of food (e.g. diatom density - Day et al., 2004; Daume, 2006), quality of food (Daume et al., 2000; Carbajal-Miranda et al., 2005), digestibility and nutritional composition of food (Kawamura & Takami 1995; Roberts et al., 1999; Kawamura et al., 1998; Daume et al., 2000, 2003; Searcy-Bernal et al., 2001; Day et al., 2004; Carbajal-Miranda et al., 2005), water temperature (Leighton, 2000; Lu et al., 2004), feeding regime (i.e.starvation period - Roberts et al., 2001; Takami et al., 2000; Du & Mai, 2004), photoperiod (Garcia-Esquivel et al., 2007) and light intensity (Searcy-Bernal et al., 2003; Gorrostieta-Hurtado & Searcy-Bernal, 2004). In older abalone, growth is influenced by type of feed (Seviere-Zaragoza et al., 1998; Krautachue et al., 2004; Lee, 2004; Daume, 2006; Strain et al., 2006), nutritional composition of food (Britz 1996a,b; Shipton & Britz, 2001a; Sales & Britz, 2003; Sales et al., 2003a,b; Lee, 2004; Viera et al., 2005), feed supplementation (Dlaza et al., 2008), water temperature (Lopez et al., 1998, Grubert & Ritar,

2003; Steinarsson & Imsland, 2003; Searle et al., 2006), feeding regime (i.e. starvation period – Francis et al., 2007), salinity (Edwards, 2003; Cheng et al., 2004), photoperiod (Garcia-Esquivel et al., 2007), rearing system (Chen & Lee, 1999), culture basket design (Dlaza, 2006), stocking density (Mgaya & Mercer, 1995; Capinpin et al., 1999), disease (Simon et al., 2004, 2005; Hansen et al., 2006; Troell et al., 2006), oxygen consumption (Xu et al., 1999; Cheng et al., 2004) and levels of ammonia in the water (Harris et al., 1997; Neori et al., 1998; Hindrum et al., 2001; Reddy-Lopata et al., 2006). Each of these above factors are species-dependent, influencing some species more than others.

1.5.4 Seaweeds as abalone feed

Abalone have different seaweed preferences and their growth in the wild is often determined by seaweed abundance and availability (Barkai & Griffiths 1987; Seviere-Zaragoza et al., 2001). Seaweed selectivity by abalone has been studied by analysis of gut contents (Leighton & Boolition, 1963; Barkai & Griffiths, 1986, 1987; Sheperd & Steinberg, 1992; Seviere-Zaragoza et al., 1998; Tahil & Juinio-Menez, 1999) and by feed experiments (Simpson & Cook, 1998; Seviere-Zaragoza, 2001; Alcantara & Noro, 2005, 2006; Viera et al., 2005; Strain et al., 2006). This knowledge is invaluable in countries like China for example, where abalone are fed primarily on seaweeds during the grow-out period (Nie & Wang, 2004; Zhang et al., 2004a).

Several characteristics of seaweed can affect the rate at which they are consumed and consequently the growth of abalone (Barkai & Griffiths 1986; Fleming et al., 1995a,b; Wood & Buxton, 1996; Alcantara & Noro, 2005). Abalone feeding rates are linked to the toughness (Chen, 1989; Shepherd & Steinberg, 1992; McShane et al., 1994; Corazani & Illanes, 1998), nutritional value (Fleming, 1995a; Fleurence et al., 1999; Rosen et al., 2000; Wong &

Cheung, 2001), and to some extent, the chemical defenses of seaweeds (Steinberg, 1985; Fleming, 1995a; Winter & Estes, 1992; Stepto & Cook, 1996). Alcantara & Noro (2005) found that *H. diversicolor supertexta* had higher consumption rates on brown leathery seaweeds such as *Sargassum* spp., red corticated seaweeds such as *Gracilaria* spp., and green foliose seaweeds such as *Ulva* spp. and suggested that this could be because of the texture (mucilaginous) of these seaweeds that suited this abalone. Fleming (1995a) found that *H. rubra* had higher consumption rates on red seaweeds such as *Jeannerettia lobata* [J. D. Hooker] Harvey and *Laurencia botryoides* [C. Agardh] Gaillon and suggested that this could be because of the high digestible nitrogen content of these seaweeds. Stepto & Cook (1996) on the other hand, found that *H. midae* Linnaeus had higher consumption rates on the red seaweed *Porphyra capensis* Kützing and suggested that this could be because of the low polyphenolic content and thus weaker chemical defenses of this seaweed.

The biochemical composition of seaweeds varies among species (Fleurence et al., 1999; Rosen et al., 2000; Wong & Cheung, 2001) and results in heterogeneous growth rates. For example, the biochemical composition of *Macrocystis pyrifera*, a brown seaweed, is poor, ranging from 5-12% protein, 0.5-1% lipids, and 46-50% carbohydrates (Simental et al., 2004). Red seaweeds have been shown to have a high protein content recorded at up to 47% for *Porphyra* spp. and up to 35% for *Palmaria* spp. (Fleurence et al., 1999; Rosen et al., 2000). Green seaweeds such as *Ulva* spp. have been shown to have a protein content ranging from 5-26% (Nisizawa et al., 1987; Fleurence et al., 1999; Shpigel et al., 1999; Wong & Cheung, 2001; Robertson-Andersson, 2003; Robertson-Andersson et al., 2006, 2007). Although wild seaweeds have a low protein content, abalone in the wild have adapted to diets that are relatively low in protein (Coote et al., 2000). Seaweeds that are grown in farm effluent, however, as opposed to their wild counterparts, have been shown to have a

significantly higher protein content (Neori et al., 1998, 2003; Neori & Shpigel, 1999; Shpigel et al., 1999; Robertson-Andersson, 2003; Robertson-Andersson et al., 2006, 2007). This characteristic has rendered them suitable for use as abalone feed (Neori & Shpigel, 1999; Shpigel et al., 1999; Boarder & Shpigel, 2001; Demetropolous & Langdon, 2004a,b).

Food preferences vary between abalone species. For example, *H. rufescens*, *H. corrugata* and *H. fulgens* prefer the kelps *Macrocystis pyrifera* Linnaeus and *Laminaria farlowii* Setch (Tutshulte & Connell, 1988a,b; Buschmann et al., 1995, 2005). Red seaweeds on the other hand such as *Gracilaria* spp. are the food of choice of *H. iris* (Marsden & Williams, 1996; Allen et al., 2006). *H. laevigata* and *H. rubra* also prefer red seaweeds such as *Pterocladia* spp., but do feed on brown seaweeds such as *Macrocystis pyrifera* in the absence of red seaweeds (Shepherd & Steinberg, 1992; Tahil & Juinio-Menez, 1999).

Many farms use mainly wild seaweed for the grow-out of abalone because of their availability and low cost (Buchal et al., 1998; Kawamura et al., 1998; McBride, 1998; Simental et al., 2004; Buschmann et al., 2005; Anderson et al., 2006; Troell et al., 2006). Cultured seaweed such as *Laminaria japonica* Areschoug, *Undaria pinnatifida* Harvey, *Sargassum* spp. *Gracilaria* spp., *Porphyra* spp. and *Ulva* spp. are increasingly being utilized as food (Chen, 1989; Mai et al., 1996; Nie & Wang, 2004; Zhang et al., 2004a). Additionally, salted or cold-stored *Laminaria* spp. serve as the substitute for fresh kelp on many abalone farms (Nie & Wang, 2004; Zhang et al., 2004a).

1.5.5 Formulated feeds as abalone feed

The availability and cost of storing seaweeds, as well as the uncertain sustainability of harvesting wild seaweed for abalone feed, has led to the development and testing of various formulated feeds (Uki & Watanabe, 1992; Viana et al., 1993; Britz et al., 1994; Mgaya & Mercer, 1995; Britz, 1996a,b; Capinpin & Corre, 1996; Fleming et al., 1996; Knauer et al., 1996; Corazani & Illanes, 1998; Lopez et al., 1998; Capinpin et al., 1999; Chen & Lee, 1999; Kruatrachue et al., 2000; Serviere-Zaragoza et al., 2001; Shipton & Britz, 2001a; Fariás et al., 2003; Sales & Britz, 2003; Sales et al., 2003a; Lee, 2004; Lee et al., 2004; Montano-Vargas et al., 2005; García-Esquivel & Felbeck, 2006). Formulated feeds are usually composed of a mixture of animal and plant products, and contain high levels of protein with different fatty acid profiles to those of seaweeds (Britz 1996a,b; Knauer et al., 1996; Sales & Britz, 2000, 2001; Sales et al., 2003a; Daume & Ryan, 2004b). Although formulated feeds are costly, they have been shown to produce comparatively better growth than seaweeds and are therefore increasingly being used by farmers as an alternative to seaweeds (Fleming et al., 1996; Troell et al., 2006).

Nutritional research on formulated feeds for abalone has identified suitable dietary sources of carbohydrates (Lee et al., 1998b), protein (Mai et al., 1995a; Lee et al., 1998a, 1999; Coote et al., 2000; Gomez-Montes et al., 2003; Sales & Britz, 2003; Sales et al., 2003a; Montano-Vargas et al., 2005), lipids and essential fatty acids (Mai et al., 1995b, 1996; Lee & Park, 1998; Durazo-Beltrán et al., 2003; Xu et al., 2004; Montano-Vargas et al., 2005), and vitamins and minerals (Tan & Mai, 2001; Kangsen et al., 2003; Zhang et al., 2004b, 2007; Zhu et al., 2004). The major ingredients of formulated diets for postlarvae are dried kelp powder and fish meat powder (microparticulate diets) (Stott et al., 2003a,b; Zhang et al., 2004). The ingredients in formulated feed for grow-out abalone usually include fishmeal,

casein, soybean meal, cottonseed meal and wheatgerm meal (Fleming et al., 1996; Shipton & Britz, 2001a; Sales & Britz, 2003; Sales et al., 2003a; Kruatrachue et al., 2004; Lee, 2004).

Although abalone satisfy their energy requirements primarily from carbohydrate (Hahn, 1989; Lee et al., 1998b), proteins are vital for tissue deposition (Wilson, 1994; Britz et al., 1996a; Lee, 2004), while dietary lipids play important roles in providing essential fatty acids (EFA) and fat-soluble nutrients for the normal growth of the animal (Durazo-Beltrán et al., 2003; Lee, 2004; Xu et al., 2004). In addition, proteins and lipids found in abalone eggs fuel the development and metamorphosis of the larvae (Litaay et al., 2001; Moran & Manahan, 2003; Fukazawa et al., 2005) and there is evidence that specific dietary lipids play a crucial role in gonadogenesis (Uki & Watanabe 1992; Nelson et al., 2002). The type of diet therefore affects the composition of the eggs and ultimately larval performance (Litaay et al., 2001; Daume & Ryan 2004b).

Carbohydrates form a large part of formulated feeds because they are the primary energy source that drives all metabolic processes (Hahn, 1989; Fleming et al., 1996; Lee et al., 1998b). The natural diet of abalone consists primarily of carbohydrate (up to 50%) (Fleming et al., 1996; Sales & Britz, 2001) and the abalone digestive system is therefore naturally adapted to feed on high levels of carbohydrates (Fleming et al., 1996; Garcia-Esquivel & Felbeck, 2006). This fact is supported by the high amounts of complex carbohydrases found in the abalone gut (Garcia-Esquivel & Felbeck, 2006). The carbohydrate requirement of abalone is therefore high and formulated feeds usually consist of between 30% and 60% of carbohydrates in the form of wheat flour, maize flour, sodium alginate, dextrin, starch and bran (Fleming et al., 1996; Lee, 2004).

Proteins are vital for tissue deposition (Wilson, 1994; Britz et al., 1996a; Lee, 2004) and so sufficient amounts of protein are essential for optimal growth. Britz & Hecht (1997) showed, for example, that the growth rate and nutritional composition of abalone were significantly affected by the level of proteins in their diets. Fallu (1991) suggested that the protein content of formulated diets should comprise at least 30% of the feed. Fleming et al. (1996) found that the protein composition in formulated feeds generally ranged from 20% to just over 50% of the feed.

There is evidence to show that the high protein content found in formulated feeds can be broken down by the proteases that are found throughout the abalone's gut (Knauer et al., 1996; Erasmus et al., 1997; Seviere-Zaragoza et al., 1997; Picos-Garcia et al., 2000). Protease activity is a key determinant of the digestibility and assimilation efficiency of ingested proteins (Edwards & Condon, 2001; Garcia-Carreno et al., 2003; Saitongdee et al., 2004; Garcia-Esquivel & Felbeck, 2006). It has been found that abalone can modulate their enzyme levels to optimize their utilization of dietary substrates (Edwards & Condon, 2001; Garcia-Carreno et al., 2003; Garcia-Esquivel & Felbeck, 2006). Abalone are therefore able to digest diets high in protein content and consequently are able to adapt to consuming formulated feeds. Taylor (1992) identified the optimal protein level in formulated diets as the level at which growth efficiency (i.e. weight gain relative to food intake) and protein utilization (i.e protein retention relative to intake) are both maximized. The optimum dietary protein requirement for *H. kamtschatkana* Jonas, for example, was found to be 30% of the feed (Taylor, 1992), that of *H. laevigata* was found to be 27% (Coote et al., 2000) and that of *H. discus hannai* and *H. tuberculata* Linnaeus to be between 24-36% (Mai et al., (1995a). If these protein levels are exceeded, however, the excess proteins are simply metabolized as

energy or excreted and the most expensive dietary component will not be effectively used (Wilson, 1994; Mai et al., 1995a; Coote et al., 2000; Lee, 2004).

Abalone cannot synthesize all the fatty acids required for normal cellular function and growth, and so they rely heavily on dietary sources for these essential fatty acids (EFA) (Lee; 2004, Xu et al., 2004). It has been shown that reduced feed rations or provision of feeds low in EFA, result in slower growth of abalone (Floreto et al., 1996; Mai et al., 1996; Dunstan et al., 2000). However, since abalone have low levels of lipases (enzymes that break down lipids) in their guts (Gomez-Pinchetti & Garcia-Reina, 1993; Britz et al., 1994), they cannot use excess lipids effectively as an energy source (Lee et al., 1998b; Lee, 2004). Although Mai et al. (1995b) reported an optimum lipid requirement in a formulated diet for *H. discus hannai* to be about 3-7%, Van Barneveld et al. (1998) showed that dietary lipid content of more than 3% in the form of either fish oil or vegetable oil, or a combination of both, had a negative influence on amino acid digestibility in *H. laevigata*.

There are, however, a number of drawbacks to using formulated feeds. These include the leaching of nutrients from the formulated feeds (Marchetti et al., 1999; Shipton et al., 2002; Sales et al., 2003b), feed instability in the water system (Fleming et al., 1996; Guzman & Viana, 1998), the costs of the protein sources (fishmeal and casein, for example, are costly) (Fleming et al., 1996, Krautachue et al., 2004) and feed availability (in some countries such as China, formulated feeds are not readily available - Zhang et al., 2004a). Furthermore, with the decline in fisheries, there is concern relating to the future supply and demand of fishmeal for abalone feed (Troell et al., 2006), leading to efforts to reduce its use as the major protein source in commercial feed formulations (Lee et al., 1998, 2004). Also, because the exact nutritional requirements of abalone have not been fully demonstrated (digestible energy has

recently been advocated to also be of importance – Britz. *pers. com*.), it is not possible to balance dietary essentials to suit all the nutritional requirements of the abalone worldwide (Zhang et al., 2004a; Montano-Vargas et al., 2005).

Disease and infestation is another factor that affects abalone growth and survival when formulated feeds are used and is a serious threat to abalone culture with many farms suffering heavy losses due to bacterial and viral infections (Mai et al., 1996; Li et al., 1998; Liu et al., 2000, 2001; Wang et al., 2000; Lee et al., 2001; Fang et al., 2002; Nicolas et al., 2002; Shuhong et al., 2004; Cai et al., 2006). Culture-environment problems such as Withering Syndrome (Friedman et al., 2003; Braid et al., 2005; Balseiro et al., 2006), the infiltration of protozoan flagellates in the blood (Chen et al., 2004) and the infestation of polychaetes (such as sabellid worms) that live on the surface of the inside of the abalone shell (Oakes & Fields, 1996; Ruck & Cook, 1998; Chen et al., 2001; Caballero-Alegria et al., 2004; Culver & Kuris, 2004; Vargas et al., 2005), all cause serious losses. Furthermore, the shells of abalone can be as expensive as the meat itself and although some epibionts (organisms that live on the abalone shell) do not affect the quality of the meat, they can perforate the shell, destroying the mother-of-pearl (Clavier, 1992; Kuris & Culver, 1999).

The sustainability of abalone fisheries is becoming a strong focus (Sales & Britz, 2000, 2001; Troell et al., 2006). In Japan, for example, the focus is on wild abalone fisheries enhancement. To achieve this, the Japanese have for decades now been seeding and out-planting hatchery-produced seed and hatchery-reared juveniles into the sea in order to enhance their wild stocks (Masuda & Tsukamoto, 1998; Simizu & Uchino, 2004). For this purpose, nearly 30 million seed are released annually in Japan (Kawamura, 2003). Other countries such as Mexico have also followed suit (Preece et al., 1997; Guzman del Proo et al.,

2004) and research into the possibilities and effectiveness of seeding is also ongoing for countries such as Australia (Shepherd, 2000; Molony et al,. 2003; Heasman, 2006) and pioneering work has been undertaken on the West Coast of South Africa (Hauck & Sweijd, 1999).

1.6 Mariculture of *Haliotis midae* Linnaeus in South Africa

1.6.1 Biogeography of wild *Haliotis midae*

There are six haliotid species indigenous to Southern African waters, namely *H. parvum* Linnaeus, *H. spadicea* Donovan, *H. queketti* Smith, *H. speciosa* Reeve, *H. pustulata* Reeve and the largest, *H. midae* Linnaeus (Tarr, 1992; Cook, 1998). *Haliotis midae* is the only species of commercial importance (Hecht & Britz, 1992; Hecht, 1994; Hauck & Sweijd, 1999) reaching a maximum size of about 200mm shell length after about 30 years (Tarr, 1992; Sales & Britz, 2000, 2001). Sexual maturity in *H. midae* occurs around 7 years of age (Tarr, 1995) and fecundity is high, with individual females producing several millions of eggs (Barkai & Griffiths, 1988; Sales & Britz, 2001).

Haliotis midae consumes a wide range of seaweeds in the wild (Barkai & Griffiths 1986; Stepto & Cook, 1996). The preferred food item of juveniles is *Ulva* spp., probably because it is tender as well as more abundant in shallower water (Sales & Britz, 2000). As abalone age, their preferences change, with larger abalone consuming more red seaweeds. Kelp is, however, also largely consumed by older individuals, probably because of its abundance and availability within the abalone habitat (Barkai & Griffiths, 1986; Stepto & Cook, 1996).

In the wild, *H. midae* has a relatively wide temperature range and it has been suggested that temperature is probably the primary determinant for its distribution (Britz et al., 1997). Older individuals are generally found within a temperature range of 12-20ºC, while juveniles prefer an optimal temperature of around 24ºC (Britz et al., 1997; Sales & Britz, 2000). *Haliotis midae* is, however, able to survive acute exposure to temperature extremes, from 8 – 25ºC (Hecht, 1994).

1.6.2 The South African abalone industry

Abalone cultivation in South Africa began because of the drastic decline of natural stocks due to commercial exploitation and poaching (Hauck & Sweijd, 1999; Sales & Britz, 2000; Troell et al,. 2006). It was shown that the life cycle of abalone could be closed (Genade et al. 1988), and that the feed conversion ratio (FCR) was such that there would be sufficient kelp available as feed with growth rates that were higher than those obtained in the wild (Troell et al., 2006). Despite the local fishery restrictions on the size of abalone caught (114mm shell breadth) (Tarr, 1992), natural stocks continued to decrease because of poaching (Hauck & Sweijd, 1999). Although the total allowable catch (TAC) for 2004/2005 was 237 tonnes, the estimated poached tonnage was 1185 tons (du Plessis, 2006).

First attempts at cultivation of *H. midae* were made in 1981 when wild abalone were captured and successfully spawned to produce spat and juveniles (Genade et al., 1988). Unlike most species, however, *H. midae* does not usually spawn if collected ripe from the wild. Hatcheries have consequently learned to condition their broodstock to spawn at regular intervals (Sales & Britz, 2000, 2001). Access to relatively cheap labour, together with favourable coastal water quality and infrastructure, has facilitated the rapid growth of the South African abalone industry (Troell et al., 2006). Most farms employ high density pump-

ashore flow-through systems (Cook, 1998; Sales & Britz, 2001), where seawater is pumped into land-based tanks and also re-circulated, with most farms having both hatchery and on-growing facilities (Cook, 1998; Sales & Britz, 2000; Troell et al., 2006). It takes about 4 to 5 years to grow an abalone from seed to market size (100mm, approx. 80 g) (Sales & Britz, 2001; Troell et al,. 2006).

South Africa is currently the largest producer of abalone outside of Asia (FAO, 2004). The destination for most of the abalone produced in South Africa (over 95%) is the Far East, mainly China, Hong Kong, Japan, Malaysia, The Philippines, the Republic of Korea, Singapore and Taiwan (CITES, 2007). Abalone are sold either live, canned, frozen or dried (Gordon & Cook, 2001, 2004), and fetch high prices of $34 - $38 per kg when live, and $600 - $850 per case when canned (du Plessis, 2006).

There are currently 13 abalone farms in operation in South Africa with a production of more than 850 tons per annum (Robertson-Andersson et al., 2007). In 2007, production of the South African farmed abalone industry reached 1000 tonnes (Belemani Semoli, Deputy Director, Marine Aquaculture Management unit, Department of Environmental Affairs and Tourism) and the projected figure for the year 2010 is 1200 tonnes (du Plessis, 2006). The expansion of the industry is expected to continue (Troell et al., 2006), although concerns of over-supply internationally to the Asian markets (du Plessis, 2006) and the strengthening Rand against the US Dollar up until 2007 discouraged investment into new ventures. However, current weakening of the Rand offers renewed hope of investment.

1.6.3 Factors affecting growth of *H. midae* culture

In culture it is important to maintain the proper controls to ensure healthy abalone. Britz et al. (1997) found that in culture a temperature of 12-20 ºC is physiologically optimal for grow-out *Haliotis midae*. In contrast, temperatures above 20ºC resulted in a decline in growth rate and a deterioration of the feed conversion ratio (FCR) and protein efficiency ratio (PER). In addition, Britz et al. (1997) found that at temperatures in excess of 20ºC mortality increased significantly, suggesting a gradual breakdown in physiological processes. Temperature has also been found to influence ammonia excretion and oxygen consumption rates (Lyon, 1995) as well as feeding and respiration rates (Barkai & Griffiths, 1987, 1988) as all of these increase with increasing temperature (Barkai & Griffiths, 1987, 1988; Lyon, 1995; Britz et al., 1997).

Ammonia (a nitrogenous compound) is derived from protein degradation and bacterial activity (Hindrum et al., 2001; Reddy-Lopata et al., 2006). High levels of ammonia impact negatively on dissolved oxygen (DO) and consequently also on abalone growth and survival because the more ammonia present in the water system, the less oxygen is consumed (Harris et al., 1997; Basuyaux & Mathieu, 1999; Xu et al., 1999; Hindrum et al., 2001; Huchette et al., 2003; Cheng et al., 2004). Reddy-Lopata et al. (2006) showed that ammonia at sub-lethal levels can be highly toxic to *H. midae*, causing a substantial reduction in growth (by more than 50% of standard growth rates) that can be costly to the farmer. This is because the abalone become stressed when there is an insufficient oxygen supply (Sales & Britz, 2001).

An ideal culture system promotes the even distribution of animals, ready access to feed, and minimum contact of animals and feed with faecal wastes (Fleming & Hone, 1996). Stocking density therefore is an important factor that influences abalone growth. It has been found that the growth of individual abalone decreases as stocking density increases (Mgaya & Mercer,

1995; Capinpin et al, 1999). This suggests density-dependent competition for food or space (Mgaya & Mercer, 1995; Capinpin et al., 1999). Furthermore, in South Africa, the success of the sabellid polychaete *Terebrasabella heterouncinata* Fitzhugh is attributed to the high stocking densities of abalone in intensive abalone farming systems that have reduced water movement in comparison to wild habitats (Chalmers, 2002; Simon et al., 2004, 2005). When access to feed is limited, abalone growth is invariably negatively affected. While not much is known in this regard, Dlaza (2006) found that the interior design of some culture baskets greatly affect feed accessibility and subsequent growth in *H. midae*.

1.6.4 Feed

1.6.4.1.Natural diets

Feed is an important and costly component of aquaculture that determines growth, and ultimately the size and quality of marketable abalone. In culture, newly metamorphosed *H. midae* are reared on diatoms such as *Cocconeis sublittoralis* Hendey and *Amphora proteoides* Hustedt (Sales & Britz, 2001). Postlarval abalone are weaned onto fresh seaweed or formulated feed at 4-6mm shell length (Sales & Britz, 2000). Grow-out abalone, on the other hand are fed harvested kelp (*Ecklonia maxima* [Osbeck] Papenfuss, *Laminaria pallida* Greville), formulated feeds, red seaweed (e.g. cultured *Gracilaria gracilis*) or green seaweed (e.g. *Ulva lactuca* Linnaeus) (Robertson-Andersson, 2003; Njobeni, 2006; Troell et al., 2006). Combinations of these feeds are often also fed, together with the red seaweeds *Porphyra* spp., *Aeodes* spp., *Plocamium* spp., *Gelidium* spp., and *Gracilariopsis* spp. (Simpson & Cook, 1998, Sales & Britz, 2000). These seaweeds are added as mixtures or in a rotation diet to improve growth rates (Simpson & Cook, 1998; Sales & Britz, 2000). *E. maxima* together with its three epiphtyes (*Gelidium vittatum* [Linnaeus] Kuetzing,

Polysiphonia virgata [C. Agardh] Sprengel and *Carpoblepharis flaccida* [C. Agardh] Kütz, are also fed to *H. midae* (Anderson et al., 2006).

Seaweed preference and consumption by *H. midae* is greatly affected by the nutritional value and the characteristics (e.g. shape, toughness, presence or absence of plant chemical compounds, % ash content, digestible nitrogen content, etc) of the seaweed (Fleming, 1995b; Stepto & Cook, 1996; Simpson & Cook, 1998; Alcantara & Noro, 2005). The nutritional value of the seaweed is primarily determined by its protein content can affect the rate at which it is consumed (Fleming et al., 1996; Simpson & Cook, 1998). Fleming (1995b) suggested that the low availability of digestible nitrogen in brown and green seaweeds causes a lower preference for these seaweeds than for red seaweeds. Stepto & Cook (1996) argued that *H. midae* prefers red seaweed because of its higher nitrogen content. The higher nutritional content of red and brown seaweeds as compared to green seaweeds (Fleurence et al., 1999; Rosen et al., 2000) is considered a function of the phycocolloids that make their cells mucilaginous and thus absorbent to minerals and other nutrients (Hashim & Chu, 2004; Lodeiro et al., 2005). In addition to these features, the shape (Watson & Norton, 1985; Alcantara & Noro, 2005), toughness (Padilla, 1985; Alcantara & Noro, 2005) as well as the presence of polyphenolic compounds (Stepto & Cook, 1996; Alcantara & Noro, 2005), may influence the palatability of seaweeds.

Certain compounds such as dimethylsulfoniopropionate (DMSP) that are produced by seaweeds such as *E. maxima*, *G. gracilis* and *U. lactuca*, are found to influence the taste characteristics of abalone that feed on them, and possibly also their growth rates (Van Alstyne et al., 2003, 2007; Smit et al., 2007). The levels to which abalone accumulate DMSP depends on the concentration of DMSP in their food and Smit et al. (2007) found that *U.*

lactuca contained higher concentrations of DMSP than either *E. maxima* or *G. gracilis*. Cultivated abalone have consistently been found to have higher levels of DMSP than wild abalone, particularly when feeds are high in DMSP (Troell et al., 2006; Smit et al., 2007). The accumulation of this compound in cultivated abalone often results in a bad taste and odour (Stefels, 2000) that is exacerbated by the canning process, possibly because heating during canning converts DMSP to DMS (a sulphur compound) (Smit et al., 2007).

The exact function of DMSP is not known. However, acrylic acid, a breakdown product of DMSP is known to deter feeding and may serve as a defense mechanism (Van Alstyne et al., 2003, 2007). DMS levels can be reduced through depuration (placing abalone on a diet that has low DMSP levels) before canning and shipping (Smit et al., 2007). However, low levels of DMSP also affect the taste of the abalone meat as DMS yielded during cooking contributes to the characteristic abalone taste (Robertson-Andersson et al., 2007). Abalone fed manufactured feeds that contain low levels of DMSP generally lack the characteristic taste and texture quality of wild abalone (Smit et al., 2007) and this too is a concern for commercial abalone farming. Generating a balance in taste is therefore of critical importance.

The growing demand for fresh *E. maxima*, which is the major feed for farmed abalone in South Africa (Troell et al., 2006; Francis et al., 2007) has greatly increased harvesting of this kelp (Anderson et al., 2006; Rothman et al., 2006). Anderson et al. (2006) showed that while *Ecklonia maxima* recovered from harvesting after 2.5 years, its three obligate epiphytes (*Gelidium vittatum*, *Polysiphonia virgata* and *Carpohlepharis flaccida*) that play an important role in the ecosystem, take more than 3.5 years (up to 4.5 years) to recover. The longer recovery time for these kelp epiphytes could influence farming operations as the

epiphytes add to the bulk of the kelp and also contribute to better growth rates because of the mixture of seaweeds of higher protein value (Owen et al., 1984; Cook & Claydon, 1991; Day & Fleming, 1992; Stuart & Brown, 1994; Fleming, 1995b; Simpson & Cook, 1998; Shpigel et al., 1999; Nelson et al., 2002).

1.6.4.2. Formulated feeds and supplementation

With sustainable harvesting of kelp being of concern, formulated feeds provide an alternative to limits placed on the harvesting of kelp, offering convenience and cost benefits to farm operators and they are increasingly being used on South African farms (Sales & Britz, 2001). It has been shown that the digestive physiology of *H. midae* can readily adapt to formulated diets (Knauer et al. 1996). In addition to using formulated feeds on grow-out abalone, formulated diets are now also being used to wean abalone off diatoms on many South African farms (Sales & Britz, 2001). Formulated diets with varying ingredients (such as carbohydrates [wheat-flour, maize flour, alginate, dextrin, sucrose, starch and bran – Fleming et al., 1996; Lee, 2004], animal proteins such as casein, fishmeal, *Spirulina* spp. [Britz, 1996a,b; Knauer et al., 1996; Britz & Hecht, 1997], plant proteins [such as soybean meal, sunflower oil and corn gluten – Shipton & Britz, 2001a, 2002; Sales & Britz, 2003; Sales et al., 2003a], lipids [Britz 1996a,b; Britz & Hecht, 1997; Britz et al., 1997], amino acids [Shipton et al., 2002; Sales & Britz, 2003], vitamins and minerals [Sales & Britz, 2001; Sales et al., 2003b]) have been tested on *H. midae*, and results have shown that formulated diets produce good growth with good Feed Conversion Ratios.

Carbohydrates are the cheaper component of formulated feeds compared to the generally expensive protein component (Fleming et al., 1996; Lee, 2004) and *H. midae*, like all other abalone, metabolise carbohydrates for energy (Fleming et al., 1996). The dietary crude

carbohydrate requirement for good growth in *H. midae* was found to range between of 43-48% (Sales & Britz, 2001; Sales & Janssens, 2004). Natural diets of *H. midae* usually consists of up to 50% carbohydrates while that of formulated diets consist of 30-60% carbohydrates (Fleming et al., 1996; Sales & Britz, 2001). Britz et al. (1996b) found that poor growth rates were generally obtained when *H. midae* was fed kelp. These authors suggested that while kelp may have satisfied the carbohydrate energy requirements of *H. midae*, there was insufficient crude protein available for tissue deposition. More recently, digestible energy is proving to be an additional limiting factor (over and above carbohydrate energy and crude protein concentrations) (Britz *pers. com*.).

Britz (1996b) suggested that the optimum dietary crude protein requirement of *Haliotis midae* was 470gkg^{-1} (47%). However, Sales et al. (2003a) more recently argued that it was only 358.7gkg^{-1} (36%). Britz & Hecht (1997) found that larger abalone tended to have higher protein requirements than smaller abalone and that the higher the protein in the diet, the higher the protein deposition in larger abalone. Shipton & Britz (2001b) confirmed these findings showing that growth rate was positively correlated with animal size when formulated diets of similar protein levels were fed.

It is known that abalone have a limited ability to utilize high levels of dietary lipid (Uki & Watanabe, 1992; Lee et al., 1998b; Lee, 2004). This too has been shown for *H. midae* that has a low level of lipase activity in its gut suggesting that it also has a limited ability to digest fat (Britz et al., 1996; Knauer et al., 1996). This could explain why diets containing high levels of lipid (6-10%) produced significantly lower growth rates in *H. midae* (Britz & Hecht, 1997). A lipid content of 10% in formulated diets was therefore suggested to be too high for *H. midae* to maintain a maximum growth rate (Britz & Hecht, 1997). Britz & Hecht (1997)

did, however, find that the tissue lipid content of abalone increased with dietary lipid content, and that smaller abalone contained significantly higher levels of lipids than larger abalone.

In addition to carbohydrates, crude proteins and lipids, *H. midae* also requires certain essential (supplied as part of the diet) and non-essential amino acids (synthesized by the abalone); when these are supplied in the correct ratios with the appropriate digestibility, growth is optimized (Sales & Britz, 2003; Sales et al., 2003a). Feeds are generally formulated according to tissue amino acid patterns and there is currently insufficient knowledge of growth limiting amino acids, or the rate of turnover of individual amino acids (Fleming et al., 1996; Sales & Britz, 2001, 2003). Mean apparent amino acid digestibility of different ingredients (such as fishmeal, casein, corn gluten meal, soybean meal, cottonseed meal, sunflower meal, canola meal, peanut meal, lupins and faba beans) in formulated feeds has been determined and casein, soybean meal and lupins have been found to produce the highest values (Shipton and Britz 2002, Bautista-Teruel et al., 2003; Gómez-Montes et al., 2003; Sales & Britz, 2003; Sales et al., 2003a; Lee, 2004). Shipton et al. (2002) attempted to identify growth responses in juvenile *H. midae* fed on graded levels of synthetic amino acids, but despite effective encapsulation techniques to prevent leaching of amino acids into the water, their study was unsuccessful. As far as we know, no other attempts have been made at identifying feed responses in *H. midae* fed on graded levels of synthetic amino acids.

In addition to the leaching of amino acids, leaching of other nutrients such as vitamins (e.g. water-soluble vitamin C and Pantothenic Acid - Zhu et al., 2004; fat-soluble vitamins A, D - Zhang et al., 2004, 2007; vitamin K - Tan & Mai, 2001) and minerals (e.g. phosphorous - Sales et al., 2003b; calcium - Tan & Mai, 2001; zinc - Mai et al., 2003) also occur. Leaching of nutrients into the water system often leads to poor water quality, as well as reducing the amount of essential nutrients that the abalone consumes (Shipton & Britz, 2002; Sales et al.,

2003b). Fortification of formulated feeds with essential vitamins and minerals ensures that farmed abalone consume an adequate and balanced supply of these nutrients for optimal performance (Marchetti et al., 1999; Sales et al., 2003b).

The major feed on South African farms is kelp (Anderson et al., 2006; du Plessis, 2006; Francis et al., 2007) which is low in protein (ca. 5-15%), has an unbalanced amino acid profile, a high ash content (up to 25%) and a high Feed Conversion Ratio compared to Abfeed®-S34 (53% carbohydrates, 33-35% protein, 1.2% fat, 0.9% crude fibre, 5% ash and approximately 10% moisture - Marifeed Pty Ltd, 2007) (Troell et al., 2006). Abfeed®-S34 is a local formulated feed containing mainly fishmeal, soybean meal, starch, vitamins and minerals, and younger abalone gain relatively more weight on a combination of these ingredients than on seaweeds alone (Britz, 1994, 1996b; Knauer et al., 1996; Sales & Britz, 2001). Although kelp produces better growth in shell length compared to Abfeed®-S34, it is weight gain that is more important in commercially farmed abalone (Britz, 1996b; Simpson & Cook, 1998). Abalone farmers, however, prefer using kelp or a combination of kelp and Abfeed®-S34 for grow-out abalone because abalone fed Abfeed®-S34 alone produce nutrient-rich faeces that promote sabellid polychaete infestation (Chalmers, 2002; Potgieter, 2005; Simon et al., 2004, 2005). In addition, Dlaza et al. (2008) showed that formulated feeds supplemented with fresh wild seaweed produced even better growth in abalone than those based on formulated diets alone.

Food supplementation is increasingly proving to be vital in commercial abalone farming. Macey and Coyne (2005) showed, for example, that supplementing feed with selected probiotics (bacteria that enhance digestion) improved growth rates by up to 8% in smaller sized *H. midae*, and up to 33% in larger individuals. This was because probiotics improve

protein digestion and absorption in the intestinal region of the digestive tract. Macey and Coyne (2005) also suggested that bloating, which is caused by a proliferation of bacteria (*Vibrio anguillarum* Bergeman) in the gut of abalone cultured under high temperatures, could possibly be prevented by using probiotics. Probiotic bacteria can hydrolyze a variety of complex polysaccharides found in seaweeds (Britz & Hecht, 1997; Erasmus et al., 1997) and for this reason their use is becoming increasingly important in commercial abalone farming.

In addition to feeds supplementation, some abalone farms have developed novel approaches to the culture of their abalone. Some farms, for example, co-culture seaweeds such as *U. lactuca* and *G. gracilis* along with *H. midae* in integrated mariculture systems where the seaweeds serve as biofilters (Robertson-Andersson, 2003; Njobeni, 2006; Robertson-Andersson et al., 2006, 2007). Seaweed growth in abalone effluent is better than that in natural seawater, and by removing dissolved nitrogen from the abalone effluent, improves the water quality, while increasing their phosphorous and protein content, thereby making them a better feed for abalone (Sales & Britz, 2000; Steyn, 2000; Robertson-Andersson, 2003; Njobeni, 2006; Robertson-Andersson et al., 2006, 2007; Troell et al., 2006). In addition to using formulated feeds there is now an increasing trend in culturing seaweeds on South African farms for such benefits and to counter drawbacks associated with high levels of dissolved nitrogen that can lead to ammonia toxicity (Robertson-Andersson, 2003; Njobeni, 2006; Robertson-Andersson et al., 2006, 2007).

1.6.5 Sustainability of our abalone resource

Poaching and over-fishing of wild abalone stocks are increasing. Proudfoot et al. (2006) showed that even the most remote locations are now being targeted and will soon be over-fished to a point where sustainability and recovery of natural populations are unlikely. It

appears now that global demand can be met only through the cultivation and rehabilitation of wild stocks in a process where hatchery produced seed are stocked into local kelp beds for both wild stock enhancement and commercial harvesting (de Waal & Cook, 2001a,b; de Waal et al., 2003).

The success of the abalone industry in South Africa thus far has been due largely to a high degree of co-operation between the private sector and government-backed institutions. In addition, the formation of stakeholder bodies such as the Abalone Farmer's Association of South Africa (AFASA) has greatly facilitated communication between competing investors. The continued future success, however, will rely heavily on research investment into a number of new fields including disease prevention, integrated systems design, studies on nutrient cycling, feed technologies and optimisation, probiotic research, reseeding, and even genetics.

2.1 Aims of Study

Most research on natural seaweed diets as feed for abalone in culture has focused on single-species diets (Viana et al., 1993; Mai et al., 1995b; Britz et al., 1996b; Capinpin & Corre, 1996; Seviere-Zaragoza et al., 1998, 2001; Bautista-Teruel & Millamena, 1999; Tahil & Juinio-Menez, 1999; Rosen et al., 2000; Bautista-Teruel et al., 2002; Simental et al., 2004; Alcantara & Noro, 2005; Viera et al., 2005; Daume, 2006; Strain et al., 2006). Single-species diets of red seaweeds generally produce better growth than either brown or green seaweeds because of their higher protein content (Simpson & Cook, 1998; Fleurence et al., 1999; Shpigel et al., 1999; Rosen et al., 2000; Boarder & Shpigel, 2001; Kruatachue et al., 2004; Najmudeen & Victor, 2004; Alcantara & Noro, 2005; Viera et al., 2005). Research (e.g. Stepto & Cook, 1996; Simpson & Cook, 1998) has shown that *Haliotis midae* prefers red seaweeds to brown or green seaweeds possibly because of their higher protein content.

Wild *H. midae* have been found to have a variety of seaweeds in their guts (Newman, 1968; Barkai & Griffiths, 1986, 1987), suggesting that they are naturally selecting a mixture of seaweeds. Mixed diets (Owen et al., 1984; Cook & Claydon, 1991; Day & Fleming, 1992; Fleming, 1995a; Simpson & Cook, 1998, Nelson et al., 2002) and rotational diets of different single-species (Simpson & Cook, 1998) of fresh wild seaweeds, have long been known to produce better growth in *H. midae* than single-species diets fed over a long period of time. Since the biochemical composition of seaweeds vary among species (Fleurence et al., 1999; Rosen et al., 2000; Wong & Cheung, 2001), it has been suggested (see Day & Fleming, 1992; Stuart & Brown, 1994; Simpson & Cook, 1998) that single-species diets are only of higher nutritional value when they form part of a mixed diet as they may provide essential nutrients that might otherwise be lacking. In addition, growth on single-species diets tends to decrease

over time whereas mixed diets tend to sustain higher growth (Day & Fleming, 1992; Stuart & Brown, 1994; Simpson & Cook, 1998; Bautista-Teruel & Millamena, 1999; Shpigel et al., 1999).

Despite mixed diets of wild seaweeds producing relatively good growth, formulated feeds have consistently been shown to outperform both single-species and mixed wild seaweed diets (see Hahn, 1989; Viana et al., 1993; Britz, 1996b; Fleming et al., 1996; Knauer et al., 1996; Bautista-Teruel & Millemena, 1999; Bautista-Teruel et al., 2002; Kruatachue et al., 2004; Lee, 2004). This is largely due to the fact that formulated feeds generally have high nutritional values (particularly crude protein) compared to wild seaweed. The protein requirement of *H. midae* for example is high, currently accepted to be about 36% (Sales et al., 2003). This high protein requirement was met using the formulated feed Abfeed®-S34 and *H. midae* grown on Abfeed®-S34 have been shown to achieve maximal growth rates (Britz et al., 1996; Sales & Britz, 2001; Shipton et al., 2001b; Sales et al., 2003).

Research (e.g. Shpigel & Neori, 1996; Neori & Shpigel, 1999; Shpigel et al., 1999; Robertson-Andersson, 2003; Robertson-Andersson et al., 2006, 2007) has shown that farm-grown seaweeds grown in abalone and fish effluent have substantially higher protein contents than their wild counterparts. It has even been shown that some seaweeds have a high nutrient uptake capacity and can assimilate as much as 90% of the ammonium released into farming systems (Buschmann et al., 1996, Shpigel & Neori, 1996; Neori & Shpigel, 1999; Neori et al., 2000). This characteristic not only makes them excellent biofilters (Shpigel & Neori, 1996; Shpigel et al., 1999; Troell et al., 1999a; 2003; Chopin et al., 2001; Jones et al., 2002; Neori et al., 2003; 2004; Schuenoff et al., 2003; Xu et al., 2008), but considerably increases their protein content rendering them suitable for use as abalone feed (Shpigel & Neori, 1996;

Neori et al., 1998, 2000; Neori & Shpigel, 1999; Shpigel et al. 1999, Boarder & Shpigel, 2001; Demetropoulos & Langdon, 2004a,b). Subsequently, these protein-enriched seaweeds have been shown to produce good growth as single-species diets and in mixed diets (Neori et al., 1998; Shpigel et al., 1999; Boarder & Shpigel, 2001; Neori et al., 2004; Demetropolous & Langdon, 2004).

Some South African abalone farms currently grow seaweed together with abalone in integrated aquaculture systems for use as abalone feed (du Plessis, 2006; Troell et al., 2006; Robertson-Andersson et al., 2007). *Ulva lactuca* is presently the most widely used seaweed and there are a number of reasons why this seaweed is preferred. Firstly, *U. lactuca* is a morphologically simple seaweed that lacks chemical defenses (Stuart & Brown; Fleming, 1995a; Shpigel et al., 1999). It is therefore a safe, non-toxic seaweed that has even been used for human consumption (Neori et al., 2004). Secondly, the growth of *U. lactuca* and its culture within farming systems has been well studied and documented (see Neori et al., 1998, 2004; Shpigel et al., 1999; Robertson-Andersson, 2003; Robertson-Andersson et al., 2007). In particular, this seaweed has a nutrient uptake capacity that is one of the highest known among seaweeds (Shpigel et al., 1999; Neori et al., 2004). *Ulva lactuca* consequently has a high growth rate because of its high surface to volume ratio (Neori et al., 2004), which allows it to sustain a rapid production of biomass (Neori et al., 1996; Shpigel et al., 1999; Robertson-Andersson et al., 2007). Thirdly, as it grows largely vegetatively (and does not become reproductive in the system), its life cycle is easy to control, making it an easy species to cultivate (Shpigel et al., 1999; Robertson-Andersson, 2003; Neori et al., 2004).

On farms growing *U. lactuca* as feed for their abalone, the seaweed is also being used as a biofilter, essentially improving the culture environment of these farms by removing excess

nutrients from their systems. These farm-grown *U. lactuca* have been shown to have a higher protein content of 36 – 44% as opposed to their wild counterparts that have a protein content of only 3-24% (Neori & Shpigel, 1999; Shpigel et al., 1999; Robertson-Andersson, 2003; Robertson-Andersson et al., 2007). Wild, naturally low protein *U. lactuca* produces poor growth rates in abalone such as *H. tuberculata* (Shpigel et al., 1999), *H. discus hannai* (Mercer et al., 1993), *H. iris* (Stuart & Brown, 1994) and in particular, *H. midae* (Simpson & Cook, 1998). The protein content recommended for maximum growth of *H. midae* has been most recently shown to be 36% (Sales et al., 2003). This suggests that protein-enriched *U. lactuca* has the potential to be a successful feed on South African abalone farms. Farm-grown, protein-enriched *U. lactuca* has already been shown to significantly improve the growth rates in abalone species such as *H. tuberculata* (Neori et al., 1998; Shpigel et al., 1999) and *H. discus hannai* (Shpigel & Neori 1996; Neori & Shpigel, 1999; Shpigel et al., 1999).

No study thus far has documented the effects of farm-grown, protein-enriched *U. lactuca* on the growth of *H. midae* in a mixed diet. Similarly, no research has yet been done comparing farm-grown *U. lactuca* against Abfeed®-S34 (formulated feed) and kelp (most commonly used natural feed) on the growth of *H. midae*. The growth of *H. midae* on farm-grown *U. lactuca* as compared to those grown on wild *U. lactuca* has also not been attempted. The aims of this study were therefore: 1) to test the suitability of various seaweed-based diets against that of the formulated feed Abfeed®-S34 on the growth of the abalone *H. midae*; and 2) to compare the growth of *H. midae* fed protein-enriched *U. lactuca* as opposed to those fed wild, naturally low protein *U. lactuca* in both single-species and mixed feeds.

A comparison of various seaweed-based diets and formulated feed on the growth rate of the abalone *Haliotis midae* Linnaeus in a land-based aquaculture system

3.1 Abstract

The effect of different diets on the growth of the cultured South African abalone, *Haliotis midae*, was investigated. Growth of juvenile *Haliotis midae* was monitored on a commercial abalone farm over a period of 9-months in an experiment consisting of 9 treatments with 4 replicates (n = 250 individuals per replicate). The treatments were: fresh kelp (*Ecklonia maxima*) blades (seaweed control); Abfeed®-S34 (formulated feed control); kelp + Abfeed®-S34; dried kelp pellets; dried kelp blades; dried kelp stipes; fresh kelp with the epiphyte *Carpoblepharis flaccida*; a mixed diet (*Gracilaria gracilis*, *Ulva lactuca*, and kelp) and a rotational diet (abalone were fed 1 of the 9 treatments for the first week and then kelp for the next 3 weeks). Results show that abalone grow well on all fresh seaweed combinations, but do best on a mixed diet. The likely reason for the success of the mixed diet is that the red and green seaweeds were farm grown, with an increased protein content. Dried kelp in any form produced poor growth. Abalone fed on the mixed diet grew at 0.066 mm day^{-1} shell length and 0.074 g day^{-1} body weight; this corresponds to 24.09 mm shell length and 27.01 g body weight increase per annum. Abalone fed on dried kelp grew at only 0.029 mm day^{-1} shell length and of 0.021 g day^{-1} body weight. Abalone grown on Abfeed®-S34 grew at 0.049 mm day^{-1} shell length and 0.046 g day^{-1} body weight which corresponds to 17.88 mm and 16.79 g increase per annum; this is better than the dried seaweed feeds, but poorer than the fresh seaweed combinations. This study shows that seaweed diets, particularly if the diets include seaweeds grown in farm effluent, are good substitutes for formulated feeds.

Key words: abalone, Abfeed®-S34, diet, growth, seaweed, *Ecklonia maxima*, *Haliotis midae*.

3.2 Introduction

The South African abalone, *Haliotis midae* Linnaeus, is a highly sought after delicacy in the Far East, which is also the destination of 95% of the product from the local fishery (CITES, 2007). Of 6 abalone species in South Africa, only *H. midae* is presently fished commercially. Although the South African abalone fishery has existed since 1949, the first attempts at cultivating *H. midae* commercially were only made in 1981 when captured specimens were successfully spawned to produce spat and juvenile abalone (Genade et al., 1988). As of 2001, twelve abalone farms, with an estimated investment of US$12 million, had been established on the South African coast (Sales & Britz, 2001). By 2003, this value had increased to 18 farms, with a projected production of 527 and 700 tons per annum for 2003 and 2004 respectively (Gerber, 2004). Currently, however, there are only 13 farms in operation with a production of more than 850 tonnes per annum (Robertson-Andersson et al., 2007). In 2007 production of farmed abalone in South Africa reached 1000 tonnes (Belemani Semoli, Deputy Director, Marine Aquaculture Management unit, Department of Environmental Affairs and Tourism) and the projected figure for the year 2010 is 1200 tonnes (du Plessis, 2006).

The proper nutrition and the resulting growth of cultured abalone are critical factors in the successful culture of this animal. While *H. midae* can reach a maximum size of about 200 mm shell length at an age of over 30 years in the wild, farm production is aimed towards an average size of only 100 mm, which is currently achieved after 5 years (Sales & Britz, 2001). Abalone growth is extremely slow and quite often varies with size and age. Diet is therefore very important and it has been shown that different diets produce different growth rates

(Leighton, 1974; Britz, 1996a; Guzmán & Viana, 1998; Shpigel et al., 1999; Boarder & Shpigel, 2001; Bautista-Teruel et al., 2003).

Abalone begin to feed immediately after larval settlement, initially consuming benthic diatoms (Tutschulte & Connell, 1988). As they grow, they begin feeding on seaweeds and may change from one species of seaweed to another as they mature (Stepto & Cook, 1993). Preferences exist, with red seaweeds being favoured by a number of different abalone species (Tutschulte & Connell, 1988; Shepherd & Steinberg, 1992; Stepto & Cook, 1993; Fleming, 1995). Juveniles begin to eat seaweeds at about 10mm shell length and will eat from 10-30% of their body weight in seaweeds each day. These high feeding rates are due to the high water content and relatively low protein content of seaweeds (Hahn, 1989).

Research conducted thus far has dealt mainly with the natural diet of wild abalone, single-species diets in culture, and more recently, the production of formulated diets (Simpson & Cook, 1998; Sales & Janssens, 2004). Wild abalone generally feed on a broad selection of seaweeds, normally with at least two species being found in the gut at any one time (Barkai & Griffiths, 1986). This implies that abalone typically select more than just a single species and preferentially choose a mixture of algae. In this study we test the effects of various diets on the growth of juvenile abalone in commercial aquaculture systems, including a formulated feed, dry and fresh kelp, and a mixture of kelp, kelp epiphytes, and farm grown seaweeds.

3.3 Materials and methods

3.3.1 Experimental animals

Abalone of a specific age class often vary in size because of their differential feeding rates. For this reason, juvenile abalone of the same age and similar size were chosen as test animals. Hatchery-reared animals (from the Jacobsbaai Sea Products farm - 17º 53' 12.5" E, 32º 58' 2.5" S, Western Cape, South Africa), spawned in September 2000, approximately 22 months old, 34.7 ± 5.8 mm in shell length, and 7.8 ± 3.8 g in body weight, were used to test the growth response of juvenile abalone fed on 9 different diets. Flow through seawater (700 ± 100 Lh^{-1}), moderately aerated, was supplied at a temperature of 15.5 ± 2.5 ºC in the holding tanks. Abalone were grown in culture baskets, with a stocking density of 5kg (± 500 individuals) per basket. Each basket was subdivided, using mesh, to produce 2 replicates (a stocking density of ± 250 individuals per replicate) and two baskets were used for each treatment, i.e. n = 4 replicates. Growth was monitored over a 9-month period.

3.3.2 Diets

The 9 diets consisted of: fresh kelp (*Ecklonia maxima* [Osbeck] Papenfuss) blades (seaweed control); Abfeed®-S34 (formulated feed control); kelp + Abfeed®-S34; dried kelp pellets; dried kelp blades; dried kelp stipes, kelp with a red algal epiphyte (*Carpoblepharis flaccida* [C.Agardh] Kützing.); a mixed diet (*Gracilaria gracilis* [Stackhouse] Steentoft, Irvine et Farnham, *Ulva lactuca* Linnaeus and kelp); and a rotation diet (where the abalone were fed 1 of the 9 treatments for the first week, and then kelp for the following 3 weeks). Abfeed®-S34 (Marifeed Pty Ltd, South Africa) is an abalone formulated feed containing fishmeal (55 %), starch, *Spirulina* spp. (10 %), vitamins and minerals (Fleming et al., 1996). The approximate analysis of Abfeed®-S34 is 34.6 % protein, 43.3 % carbohydrates, 5.3 % fat, 1.2

% Crude fibre, 5.7 % ash and ~10 % moisture (Marifeed Pty Ltd, South Africa). All kelp was harvested locally. Kelp was chosen as a seaweed control because it is this seaweed that is most commonly used as fresh abalone feed in South Africa. In addition, prior to the start of the experiment, kelp was fed to all experimental animals. Abfeed®-S34 was used as a formulated control feed because at the time it was the most common artificial food pellet used on commercial abalone farms in South Africa. *Ulva lactuca* and *G. gracilis* comprising the mixed diet were obtained on the farm from a cultured stock grown in abalone and fish (turbot) effluent. These seaweeds grown in abalone and turbot effluent have considerably increased nitrogen content over that of seaweed collected from local seashores (Robertson-Andersson, 2003; Robertson-Andersson et al., 2006, 2007). *Ulva lactuca* grown in these systems has an average protein content of 33.4 % when grown in abalone waste, and 36.6 % when grown in turbot waste as opposed to 3.7-19.9 % in wild *U. lactuca* (Robertson-Andersson, 2003).

Representative animals were selected from each treatment (n = 30 at 0-2 months, n = 40 at 3-8 months and n = 50 at 9 months to compensate for differential growth). Abalone shell length and body weight were measured once a month for 9 months.

Daily growth rates in terms of body weight (DGBW) and shell length (DGSL) (see Simpson & Cook, 1998) were calculated as follows:

$$DGBW = (W_1 - W_0)/t$$

$$DGSL = (L_1 - L_0)/t$$

W_0 = mean initial weight, W_1 = mean final weight, L_0 = mean initial length, L_1 = mean final length, and t = time in days.

3.3.3 Body Weight/Shell Length Ratio

The body weight-to-shell length ratio (BW/SL) was calculated for all 9 diet treatments. The BW/SL ratio (Mean final weight / Mean final length) gives an indication of the flesh volume per unit shell length growth for each of the 9 diet treatments. BW/SL rations are important in that they indicate the mass of abalone per unit shell length. Thus at marketable size (80 mm-100 mm), the value of an abalone priced by weight will be dependent on the BW/SL ratio. Certain diets will therefore produce more valuable abalone.

3.3.4 Condition factor

Similar to the BW/SL ratio, the condition factor is a concept that was developed to account for the relationship between the weight of abalone gained per unit shell ratio (see Britz 1996 b).

$CF = [BW\ (g) / SL\ (mm)^{2.99}] \times 5575$

Where CF = the condition factor, BW = the mean body weight, SL = the mean shell length and 2.99 & 5575 are Britz (1996b) constants.

3.3.5 Statistical analysis

All data are expressed as means ± se. A two-way analysis of variance (ANOVA: Zar, 1984) was used to compare and analyze the effect of the various treatments on shell length and body weight over time. Differences among treatment means were considered significant at $P < 0.05$.

3.4 Results

3.4.1 Diets

Dried kelp in any form (blades, stipes, and pellets) produced poor growth in both weight ($P < 0.05$) and shell length ($P < 0.05$) when compared against the fresh seaweed treatments and Abfeed®-S34 (Figures 1 & 2). In comparing the remaining treatments, shell length produced no meaningful comparison, as there was no significant difference between any of the fresh seaweed treatments and the Abfeed®-S34 ($P = 0.37$). Body weight, however, produced more meaningful differences.

There were significant differences in body weight between treatments after only 5 months ($P < 0.05$). The mixed diet (0.074 g day^{-1}) produced the best growth followed by the rotation diet and epiphyte treatments (0.059 g day^{-1}), and the fresh kelp diet (0.056 g day^{-1}). A surge in growth can be seen after month 7 (Figure 2). This is due to a thinning of the sample size (n = 150 per replicate) as growth of the sample had become density dependent by month 7. Generally those diets that contained more than one seaweed and those that included Abfeed®-S34, produced good growth rates (Tables 1 & 2; Figures 1 & 2). The mixed diet, ranked 1, produced the best growth rates for both shell length (0.066 mm day^{-1}) and body weight (0.074g day^{-1}). Abfeed®-S34 (0.049 mm day^{-1} shell length & 0.046 g day^{-1} body weight), ranked 6, did not perform as well as the combination feeds, but still performed better than the dried seaweed treatments. Dried kelp (pellets and blades) produced the lowest growth (Tables 1 & 2; Figures 1 & 2). .

3.4.2 BW/SL ratios and Condition factor

The mixed diet produced the highest BW/SL ratio (0.528 g mm^{-1}) and the dried blade treatment the lowest (0.306 g mm^{-1}) (Table 2). Again the BW/SL ratios were generally higher for those diet treatments that contained a combination of fresh feeds i.e., the mixed diet (0.528 g mm^{-1}, ranked 1), the rotation diet (0.469 g mm^{-1}, ranked 2), the epiphyte diet (0.464 g mm^{-1}, ranked 3), and the kelp + Abfeed®-S34 diet (0.463 g mm^{-1}, ranked 5). The kelp only diet produced a comparatively good BW/SL ratio (ranked 4 at 0.463 g mm^{-1}). Similarly, while all animals showed positive (i.e. >1; relatively "fat" individuals, see Britz 1996b) condition factors at the start of the experiment, only those treatments (except for the kelp + epiphyte treatment) that comprised largely combinations of fresh seaweed gained weight relative to shell length (see Table 2). Here again, the mixed diet performed very well producing relatively "fatter" individuals than most of the other treatments.

3.5 Discussion

Good growth rates are important in ensuring that cultured animals reach a marketable size and condition within a time that is economically viable. Previous studies on *Haliotis* sp. have shown that "mixed" diets produce better growth rates than single-species diets (Owen et al., 1984; Day & Fleming, 1992; Fleming, 1995, Simpson & Cook, 1998). Wild *H. midae*, in particular, are found to have a variety of algae in their guts, with *E. maxima* forming the larger percentage of the gut contents, followed by red or green algae (Newman, 1968; Barkai & Griffiths, 1986, 1987). This suggests that abalone are naturally selecting a mixture of seaweeds.

Our data are consistent with previously published works in that abalone grown on a combination of different seaweeds perform better than those grown on a single species. Single-species diets, and in particular dried feeds, produce poor growth in abalone. Duncan & Klekowski (1975), for example, stated that essential nutrients might become limiting in experiments where animals are fed single-species diets, which could result in poor growth rates. Various single-species algal diets have been tested in an attempt to identify those diets that maximize the growth rate of abalone in culture. Day & Fleming (1992) demonstrated that on some single-species algal diets *H. rubra* initially grew at a steady rate then failed to grow for the remainder of the trial, suggesting that nutrients were lacking in the diet. Stuart and Brown (1994) suggested that a variety of algae are better able to meet the preferences and nutritional requirements of cultured abalone over extended periods of time.

Kelp (*E. maxima*) is the most abundant algal species along the southwest coast of southern Africa, and is the most likely food source for abalone farms developing along this area of the coast (although in the northern west coast of South Africa and in Namibia it is largely replaced by *Laminaria pallida* (Greville *Ex* J. Agardh) (Stegenga et al., 1997). However, Stepto & Cook (1993) found that *E. maxima* was the least preferred of three algae fed to *H. midae*. They suggested that this might be due to high phenolic levels. Simpson & Cook (1998) also stated that in a mixed diet it is likely that *Ecklonia* spp. would be avoided, which would negate the purpose of feeding *Ecklonia* spp. as part of a diet. Additionally, kelp is low in protein content (5 %) and high in water content (68-91 %) (Hahn, 1989; Robertson-Andersson, 2004; Smith, 2007). All of this probably accounts for the relatively low growth obtained by the kelp only diet when compared against the other fresh seaweed combinations. In this study fresh kelp, however, was able to produce better growth than all dried feed including the formulated feed, Abfeed®-S34. The better performance of kelp over Abfeed®-S34 is contrary to the industry norm of Abfeed outperforming kelp (see Hahn, 1989; Britz, 1996b; Bautista-Teruel et al., 2003). As kelp was the diet prior to the start of the experiment, this may explain the relatively poor performance of Abfeed®-S34, as abalone in this size-range become habituated to kelp and are slow to change to Abfeed®-S34 (Peter Britz, pers. comm.). Other contributing factors which may explain the poorer performance on Abfeed®-S34 may be poorer water quality as Abfeed tanks require a greater water flow rate than was reported here, and access to the feed pellets as baskets without "feeder plates" may restrict access to the feed.

What then determines the feeding preference? Fleming (1995) suggested that preference for certain algae might be due to the presence of essential nutrients not available in other algae. A number of authors (Day & Fleming, 1992; Stuart & Brown, 1994; Fleming, 1995; Simpson

& Cook, 1998) showed that algae like *Ulva* and *Gracilaria* species constitute a poor diet when supplied singly, but they may be of great value when supplied as part of a mixed diet, thereby supplying essential nutrients to the diet. These studies, however, referred to wild stocks and not protein-enriched *Ulva* and *Gracilaria* species as were used in this study. This probably accounts for the higher abalone growth on our mixed diet.

A number of previous studies have come to similar conclusions. Neori et al. (1998), for example, have shown that a number of *Ulva* spp. are able to remove up to 90 % of dissolved nitrogen from aquaculture effluent. The culture of *Ulva* spp. in nutrient rich waters increases their protein content roughly 3 to 10 fold (Shpigel et al., 1999; Boarder & Shpigel, 2001; Robertson-Andersson, 2003; Robertson-Andersson et al., 2007). This enriched *Ulva* has subsequently been shown to improve growth in, for example *H. tuberculata* (Neori et al., 1998; Shpigel et al. 1999), *H. discus hannai* (Shpigel et al., 1999), and *H. roei* (Boarder & Shpigel, 2001).

The BW/SL ratio and the Condition Factor (CF) is are important determinants of the economic viability of different diets. The mixed diet had the greater ability to increase the ratio of body weight to shell length and our results have shown that although shell length growth rates of abalone fed on combination diets remained relatively constant over time, the body weight growth rates showed a significant increase over time. This is important for the commercial farmer, in that growth is dependent on the feed used for abalone aquaculture, largely because abalone are sold on a per weight basis. The BW/SL ratio and the CF thus give an indication of what feeds are more likely to produce “fatter”, heavier animals. Similar to our findings, Dlaza et al. (2008) found that abalone fed formulated feeds supplemented with fresh wild seaweed, produced higher CF values than those fed formulated feeds only. It

is clear therefore from these studies that seaweeds can improve the growth and condition of abalone cultured in integrated aquaculture systems.

In conclusion, natural diets of fresh seaweed, either as kelp alone or combinations of kelp and other seaweeds, produced the best growth in abalone, with the mixed diet performing best. Dried seaweed in any form produced the lowest growth. Although not as good as the fresh feeds, the formulated diet Abfeed®-S34 as a single feed performs better than the dried seaweed feeds.

3.6 Tables

Table 1. Mean shell length and growth rates of juvenile abalone fed over the 9-month growth period (270 days).

Diet Treatment	Mean Initial Length (mm ± se)	Mean Final Length (mm ± se)	Growth Rate ($mm.day^{-1}$)	Growth Rate ($mm.year^{-1}$)
Mixed diet	34.74 ± 0.20	52.60 ± 0.24^{a}	0.066^{a}	24.09^{a}
Fresh kelp + Epiphyte	33.81 ± 0.18	49.94 ± 0.25^{b}	0.060^{b}	21.90^{b}
Rotation	34.76 ± 0.20	50.35 ± 0.23^{b}	0.058^{b}	21.17^{b}
Fresh kelp	34.45 ± 0.21	49.24 ± 0.24^{c}	0.055^{c}	20.07^{c}
Fresh kelp + Abfeed®-S34	34.75 ± 0.18	49.09 ± 0.25^{c}	0.053^{c}	19.34^{c}
Abfeed®-S34	34.04 ± 0.18	47.27 ± 0.25^{d}	0.049^{d}	17.88^{d}
Dried kelp pellets	34.34 ± 0.19	43.53 ± 0.19^{e}	0.034^{e}	12.41^{e}
Dried kelp stipe	34.31 ± 0.18	42.82 ± 0.21^{f}	0.032^{f}	11.31^{f}
Dried kelp blade	34.58 ± 0.20	42.36 ± 0.18^{f}	0.029^{f}	10.58^{f}

Table 2. Mean wet weight, growth rates, BW/SL ratios and Condition factor (CF) of juvenile abalone fed over the 9-month growth period (270 days) by increasing order of dietary rank based on relative weight gain.

Diet treatment	Mean Initial Weight (g±se)	Mean Final Weight (g±se)	Growth Rate (g.day^{-1})	Growth Rate (g.year^{-1})	BW/SL (g.mm^{-1})	Initial CF	Final CF	CF difference	Rank Order
Mixed diet	7.83 ± 0.13	27.78 ± 0.36^{a}	0.074^{a}	27.01^{a}	0.528^{a}	1.079	1.107	0.028^{a}	1
Rotation	7.72 ± 0.12	23.61 ± 0.36^{bc}	0.059^{bc}	21.54^{bc}	0.469^{b}	1.062	1.072	0.011^{b}	2
Fresh kelp + Epiphyte	7.22 ± 0.13	23.15 ± 0.32^{cd}	0.059^{cd}	21.54^{cd}	0.464^{b}	1.079	1.078	-0.001^{c}	3
Fresh kelp	7.61 ± 0.11	22.81 ± 0.26^{d}	0.056^{d}	20.44^{d}	0.463^{b}	1.075	1.107	0.032^{a}	4
Fresh kelp + Abfeed®-S34	7.92 ± 0.12	22.75 ± 0.28^{cd}	0.055^{cd}	20.08^{cd}	0.463^{b}	1.090	1.115	0.024^{a}	5
Abfeed®-S34	7.45 ± 0.11	19.85 ± 0.27^{e}	0.046^{e}	16.79^{e}	0.420^{c}	1.091	1.089	-0.002^{c}	6
Dried kelp pellets	7.66 ± 0.12	14.31 ± 0.18^{f}	0.025^{f}	9.13^{f}	0.334^{d}	1.095	1.055	-0.040^{d}	7
Dried kelp stipe	7.65 ± 0.11	13.39 ± 0.16^{g}	0.021^{g}	7.67^{g}	0.316^{d}	1.069	1.020	-0.049^{e}	8
Dried kelp blade	7.61 ± 0.11	13.32 ± 0.17^{g}	0.021^{g}	7.67^{g}	0.306^{d}	1.085	0.935	-0.150^{e}	9

3.7 Figure captions

Fig. 1. Increase in abalone shell length for all diet treatments over the 9-month period.

Fig. 2. Increase in abalone body weight for all diet treatments over the 9-month period.

3.8 Figures

Figure 1.

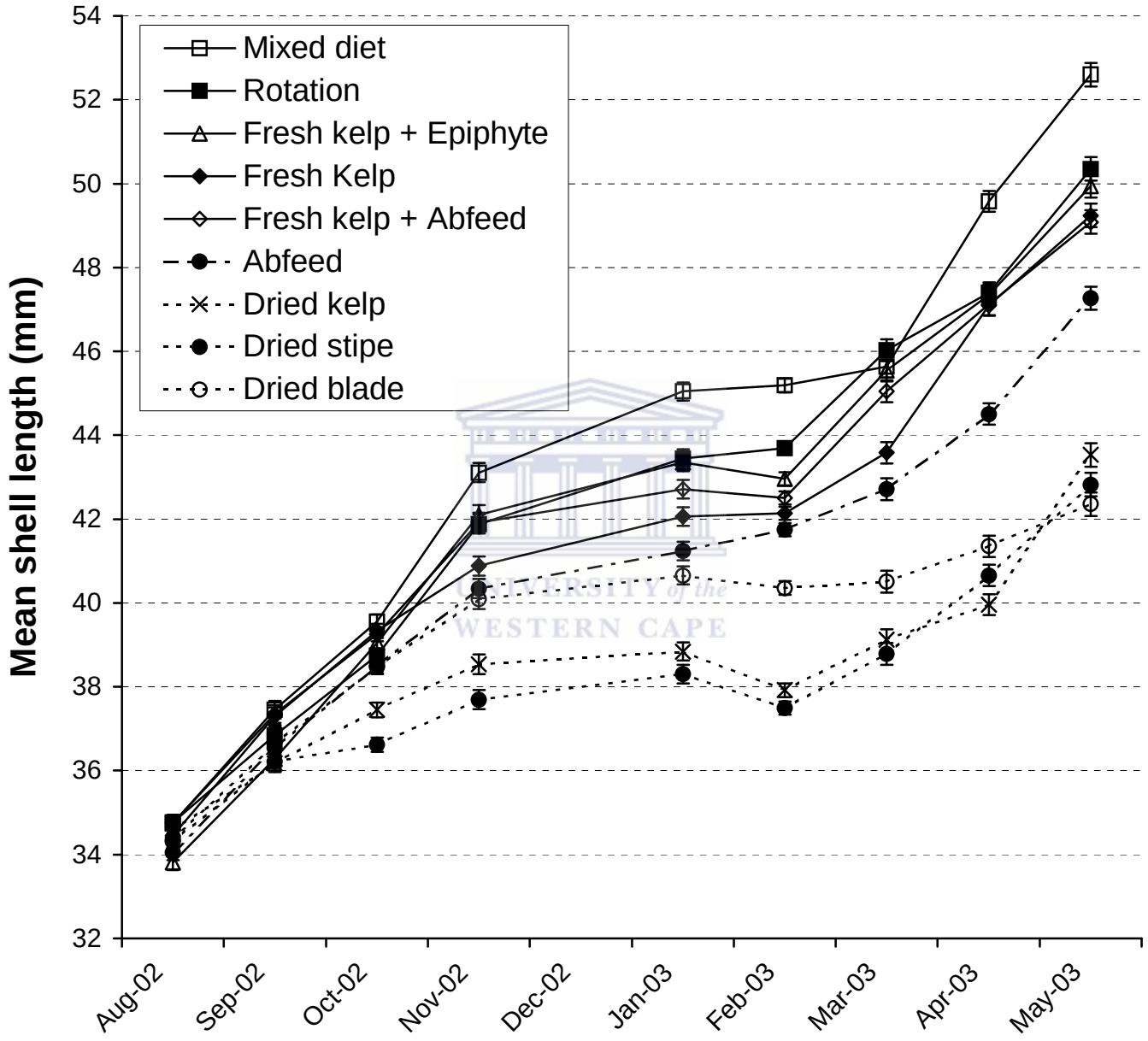
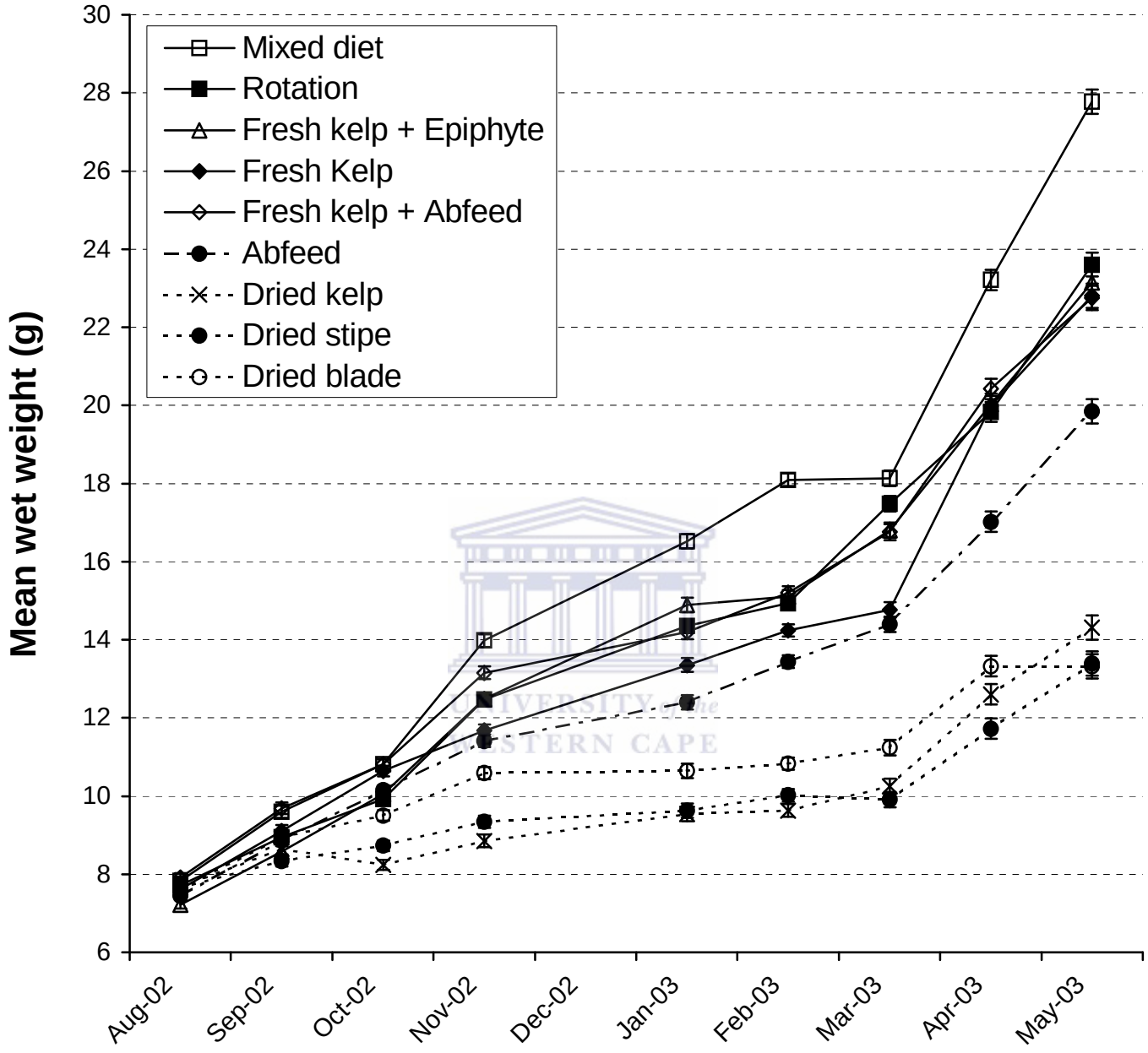


Figure 2.


Mean wet weight (g)
30
28
26
24
22
20
18
16
14
12
10
8
6
Mixed diet
Rotation
Fresh kelp + Epiphyte
Fresh Kelp
Fresh kelp + Abfeed
Abfeed
Dried kelp
Dried stipe
Dried blade
Aug-02
Sep-02
Oct-02
Nov-02
Dec-02
Jan-03
Feb-03
Mar-03
Apr-03
May-03

A comparison of feeds incorporating wild and farm-grown *Ulva lactuca*

on the growth of juvenile *Haliotis midae* Linnaeus in a land-based aquaculture system

4.1 Abstract

The effect of different diets on growth of juvenile South African abalone, *Haliotis midae*, was investigated on a commercial abalone farm over a period of 8-months in an experiment consisting of 6 treatments with 4 replicates (n = 250 individuals per replicate). The treatments comprised: fresh kelp (*Ecklonia maxima*) blades (seaweed control), Abfeed®-S34 (formulated feed control), kelp + Abfeed®-S34, kelp + farmed *Ulva lactuca*, farmed *U. lactuca*, and wild *U. lactuca*. Farmed *U. lactuca* for the experiment was grown in aquaculture effluent. Results show that abalone grew best on a combination (mixed) diet that included farmed, protein-enriched seaweeds. Abalone fed kelp + farmed *U. lactuca* (0.431 ± 0.02% weight.day^{-1} SGR; 61.530 ± 0.02 µm.day^{-1} DISL; 1.093 final CF) performed the best of all diets tested. Abalone fed wild *U. lactuca* (-0.095 ± 0.01% weight.day^{-1} SGR; 2.414 ± 0.02 µm.day^{-1} DISL; 0.820 final CF) produced the poorest growth that decreased over time. Farmed, protein-enriched *U. lactuca* (0.255 ± 0.02% weight.day^{-1} SGR; 38.777 ± 0.03 µm.day^{-1} DISL; 1.013 final CF) on its own performed considerably better than wild *U. lactuca*. Abalone grown on Abfeed®-S34 (0.330 ± 0.02% weight.day^{-1} SGR; 48.993 ± 0.02 µm.day^{-1} DISL; 1.061 CF) produced better growth than both farmed and wild *U. lactuca* but did not perform as well as the mixed diet. Abfeed®-S34, however, had the highest FCE value (0.147). This study showed that seaweeds grown in aquaculture effluent produce better growth in abalone than their wild counterparts and such protein-enriched seaweeds produce even better growth in abalone in combination with other seaweeds as mixed diets. In addition, seaweeds grown in aquaculture effluent can produce better growth in abalone than formulated feed when fed as a mixed diet, providing a good substitute for the costly formulated feeds.

Key words: abalone, farm-grown, growth, *Haliotis midae,* mixed diet, protein content, *Ulva lactuca*

4.2 Introduction

Kelp (*Ecklonia maxima* [Osbeck] Papenfuss) is the major fresh feed for farmed abalone in South Africa (Troell et al., 2006; Francis et al., 2007). With the expansion of the abalone industry, the demand for fresh kelp has been growing (Anderson et al., 2006; Rothman et al., 2006). In 2003, more than 7000 tons of fresh kelp fronds were harvested and this figure is expected to increase (Robertson-Andersson et al., 2007). This has already caused many kelp beds to reach limits of sustainable harvesting particularly in kelp concession areas with a high number of abalone farms (Troell et al., 2006).

Kelp is cheaper than formulated feeds (although labour costs associated with feeding kelp are higher than with feeding formulated feed, kelp is still cheaper with respect to its price per tonne) but its future availability as the primary abalone fresh feed is uncertain (Troell et al., 2006). As an alternative to kelp, some farms grow seaweeds for use as abalone feed in their land-based aquaculture systems, with two of these farms depending solely on farm-grown seaweeds to meet the bulk of their feed requirements (du Plessis, 2006; Troell et al., 2006; Robertson-Anderson et al, 2007). These latter farms culture the seaweed *Ulva lactuca* Linnaeus with *Haliotis midae* Linnaeus in an integrated mariculture system where the seaweeds serve not only as biofilters, but also as feed for abalone (Robertson-Andersson, 2003; Troell et al., 2006; Robertson-Andersson et al., 2007).

Farm-grown seaweeds such as *U. lactuca* have higher protein contents than their wild counterparts (Neori & Shpigel et al., 1999; Shpigel et al., 1999; Neori et al., 2003, 2004; Robertson-Andersson, 2003; Robertson-Andersson et al., 2006, 2007), growing better in abalone effluent than in natural seawater (Shpigel et al., 1999; Neori et al., 2004). The

seaweed removes dissolved nitrogen from the abalone effluent and this results in a considerable increase in both its phosphorous and protein content (Sales & Britz, 2000; Steyn, 2000; Robertson-Andersson, 2003; Robertson-Andersson et al., 2006, 2007). *Ulva lactuca* is therefore a valuable biofilter with a high nutrient absorption capacity (Vandermeulen & Gordin, 1990; Cohen & Neori, 1991; Neori et al., 1991, 2003, 2004; Jimenez del Río et al., 1996; Shpigel & Neori, 1996; Goldberg et al., 1998; Shpigel et al., 1999; Robertson-Andersson, 2003; Robertson-Andersson et al., 2006, 2007).

The trend of cultivating seaweeds such as *U. lactuca* on South African farms is increasing (du Plessis, 2006; Troell et al., 2006; Robertson-Andersson et al., 2007). *Ulva lactuca* has already been shown to be a good choice of seaweed for cultivation on abalone farms (see e.g. Shpigel et al., 1996; Neori & Shpigel et al., 1999; Shipgel et al., 1999; Neori et al., 2003, 2004) and its value as a biofilter and protein-enriched feed for *H. midae* is being investigated on South African abalone farms. Information on how farm-grown, protein-enriched seaweeds may benefit the growth of *H. midae* is, however, still lacking. This study, was therefore undertaken to compare the growth of *H. midae* on both farm-grown and wild *U. lactuca* and to determine how it compares as a feed to both kelp and the high protein formulated feed Abfeed®-S34.

4.3. Materials and methods

4.3.1 Experimental system

All research was conducted on the Jacobsbaai Sea Products (JSP - 17º 53' 12.5" E, 32º 58' 2.5" S, Western Cape, South Africa) abalone farm. Moderately aerated seawater with a flow-through seawater of 700 ± 100 $L.h^{-1}$ was supplied at 15.5 ± 2.5°C in concrete holding tanks (5500 x 1300 x 550mm; length, width, depth). The flow direction within each tank was alternated weekly to compensate for end effects. Abalone were grown in culture baskets (800 x 570 x 250mm; length, width and depth respectively) that were subdivided with vertically orientated feeding plates to increase the surface area. A horizontal feeder plate (600 x 380mm) was centrally positioned above the vertical plates. This design provided optimum access to feed with no visible feed wastage.

4.3.2 Experimental animals

Grow-out (abalone with a shell length > 20mm) juvenile abalone from the same broodstock and of the same age and similar size were chosen as test animals. Hatchery-reared animals from the JSP abalone farm, spawned in August 2001, approximately 22 months old and measuring 35.94mm ± 0.16 in shell length and 8.71g ± 0.12 in body weight, were used in this study. Abalone were grown in culture baskets with a stocking density of 5kg (± 500 individuals) per basket. Each basket was subdivided, using mesh, to produce 2 replicates (a stocking density of ± 250 individuals per replicate) and two baskets were used for each treatment (diets), i.e. $n = 4$. After 5 months, each replicate was thinned down to 150 individuals to reduce intraspecific competition.

4.3.3 Diets

The six diets consisted of: fresh kelp (*E. maxima*) blades (seaweed control); Abfeed®-S34 (formulated feed control); kelp + Abfeed®-S34; kelp + farmed *U. lactuca*; farmed *U. lactuca* on its own; and wild *U. lactuca*. The approximate analysis of the formulated feed, Abfeed®-34 is 34.6% protein, 43.3% carbohydrates, 5.3% fat, 1.2% Crude fibre, 5.7% ash and ~10% moisture (Marifeed Pty Ltd, South Africa). Kelp blades were chosen as a seaweed control because it is this seaweed that is most commonly used as fresh abalone feed in South Africa. Abfeed®-34 was used as a formulated feed control because at the time it was the most common artificial food pellet used on commercial abalone farms in South Africa. *Ulva lactuca* was obtained from the JSP farm from a cultured stock grown in abalone and fish (turbot) effluent. *Ulva lactuca* grown in these systems has an average protein content of 33.4% and 36.6% when grown in abalone and turbot waste respectively as opposed to 3.7-19.9% in wild *U. lactuca* (Robertson-Andersson, 2003).

4.3.4 Sampling and data collection

The experiment was conducted over 8 months. For shell length and body weight measurements, representative animals were selected monthly from each treatment (n = 30 at 0-2 months, n = 40 at 3-7 months and n = 50 at 8 months to compensate for differential growth). Before all weight measurements, abalone were blotted dry to remove excess water. Weight was recorded to 0.01g while shell length was measured along the longest axis to the nearest 0.01mm.

Daily increment in shell length (DISL) was calculated according to Zhu et al. (2002):

$$DISL\ (\mu m.day^{-1}) = [(SLt - SLi)/t] \times 1000$$

Where SLt = final mean shell length, SLi = initial mean shell length, and t = the feeding trial period in days.

Abalone specific growth rate (SGR) was calculated according to Britz (1996b):

$$SGR\ (\%\ weight.day^{-1}) = \{[In(Wf)-In(Wi)]/t\}\ X\ 100$$

Where In(Wf) = the natural log of the final mean weight, In(Wi) = the natural log of the initial mean weight, and t = the feeding trial period in days.

4.3.5 Condition factor (CF) and feed conversion efficiency (FCE)

The condition factor which is an index that was developed to account for the relationship between the weight of the abalone gained per unit shell length, was calculated using the formula of Britz (1996b).

$$CF = [BW\ (g) / SL\ (mm)^{2.99}] \times 5575$$

Where CF = the condition factor, BW = the mean body weight, SL = the mean shell length and 2.99 & 5575 are Britz (1996b) constants.

The feed conversion efficiency was calculated according to Simpson & Cook (1998):

$$FCE = [growth / ration] \times 100$$

Where FCE = feed conversion efficiency, growth = the wet body weight (g) gain per day, and ration = the wet feed (g) intake per day in grams.

4.3.6 Statistical analysis

All data are expressed as means ± se. Data for experimental replicates were pooled as no significant differences were found between them. A two-way analysis of variance (ANOVA: Zar, 1984) was used to compare and analyze the effect of the various treatments on shell

length and body weight over time. Differences among treatment means were considered statistically significant at $P < 0.05$.

4.4 Results

4.4.1 Growth

Ulva lactuca grown in aquaculture effluent produced better growth in *H. midae* than their wild counterparts. In particular, protein-enriched *U. lactuca* performed even better in combination with kelp as a mixed diet. However, protein-enriched *U. lactuca* on its own was not a good substitute for the formulated feed Abfeed®-S34.

The combination (or mixed) diet that contained kelp and farmed, protein-enriched *U. lactuca*, produced significantly better growth than any of the other diets, ranking highest in all growth categories (SGR = 0.431 ± 0.02% weight.day^{-1}, DISL = 61.530 ± 0.02µm.day^{-1}) ($P < 0.001$) (Table 1; Figs 1 & 2). Of the single feed diets, kelp (SGR = 0.357 ± 0.02% weight.day^{-1}, DISL = 52.242 ± 0.02µm.day^{-1}), ranked highest, performing better than even Abfeed®-S34, results that are atypical of the norm (see Britz, 1996b). Farmed, protein-enriched *U. lactuca* (SGR = 0.255 ± 0.02% weight.day^{-1}, DISL = 38.777 ± 0.03µm.day^{-1}) on its own perfomed considerably better than wild *U. lactuca* (SGR = -0.095 ± 0.01% weight.day^{-1}, DISL = 2.414 ± 0.02µm.day^{-1}) ($P < 0.001$). Of particular interest is the surge in growth that was observed after 5 (weight) – 6 (length) months, due to the thinning of the sample size.

4.4.2 Condition factor and FCE

All feeds produced negative CF responses (Table 1) suggesting that the abalone in this study gained more length relative to weight i.e. all diets produced relatively "thin" animals (see Britz, 1996b). The overall CF trends and ranking followed very closely those of the SGR and DISL values. Feed conversion efficiency was highest for Abfeed®-S34 (0.147), substantially

greater than that of the farmed, protein-enriched *U. lactuca* (0.011) diet and even more so than wild *U. lactuca* (-0.014) ($P < 0.001$).

4.5 Discussion

Farm-grown, protein-enriched seaweeds, whether as a single diet or as part of a combination or mixed diet, produce good growth in abalone. When fed as part of a mixed diet these seaweeds can perform better than formulated feeds. The farm-grown *U. lactuca* used in this study had a protein content averaging 33.4% - 36.6%. Sales et al. (2003) concluded that the optimum protein content for the growth of *H. midae* is 36%. The protein content of our farmed *U. lactuca* was within this range and so this no doubt explained the relatively good growth obtained with this seaweed.

Shpigel et al. (1999) suggested that it was necessary to feed other seaweed species together with protein enriched *U. lactuca* as part of a mixed diet in order to achieve commercially acceptable growth rates. This was suggested because it was argued that mixed diets essentially compensated for nutrients and attractants generally lacking in single-species diets. As part of a combination feed with kelp, farm-grown *U. lactuca* produced the best growth even better than the Abfeed®-S34 only diet, an artificial feed that was specifically formulated for *H. midae*. Farm-grown *U. lactuca* on its own, however, was not a suitable substitute for Abfeed®-S34. As suggested by previous research (e.g. Stuart & Brown, 1994; Fleming, 1995b; Shpigel et al., 1999), while their (i.e. farm-grown *U. lactuca* and Abfeed®-S34) protein contents were similar, the differences in their performance was probably due to the nutrients and attractants lacking in *U. lactuca* by those present in Abfeed®-S34.

The primary results of this study are consistent with many previous studies. Farm-grown, protein-enriched *U. lactuca* had previously been shown to produce good growth in abalone species such as *H. tuberculata* Linnaeus (Neori et al., 1998; Shpigel et al., 1999) and *H.*

discus hannai Ino (Shpigel & Neori 1996; Neori & Shpigel, 1999; Shpigel et al., 1999). In this study, farm-grown, protein-enriched *U. lactuca* as a single feed sustained the growth of *H. midae* over the period of the experiment. Wild *U. lactuca*, on the other hand, performed poorly as a single-species diet, producing the poorest growth in *H. midae*. Wild seaweeds are known to have a low protein content (Mercer et al., 1993; Fleming et al., 1996; Fleurence et al., 1999; Neori & Shpigel, 1999; Shpigel et al., 1999; Tahil & Juinio-Menez, 1999; Rosen et al., 2000; Wong & Cheung, 2001; Bautista-Teruel et al., 2002; Robertson-Andersson, 2003; Demetropoulos & Langdon, 2004; Robertson-Andersson et al., 2006, 2007) that results in relatively poor growth in abalone (Fleming, 1995b; Fleming et al., 1996; Neori & Shpigel, 1999; Shpigel et al., 1999; Rosen et al., 2000; Kruatachue et al., 2004; Lee, 2004; Neori et al., 2004).

Previous growth studies on *H. midae* have used only wild, presumably low-protein seaweeds (Cook & Claydon, 1991; Owen et al., 1994; Stepto & Cook, 1996; Simpson & Cook, 1998). While wild seaweeds as part of a mixed diet were shown to produce relatively good growth in *H. midae*, wild single-species seaweed diets were shown to produce comparatively poor growth (Owen et al., 1994; Stepto & Cook, 1996; Simpson & Cook, 1998, Nelson et al., 2002) probably due to a lack of a mixture of nutrients (Stuart & Brown, 1994; Fleming, 1995b). Wild, low-protein seaweeds, whether as single-species or in combination as mixed diets, however, have never been able to produce growth comparable to formulated feeds that are high in crude protein (Viana et al., 1993; Fleming, 1995b; Fleming et al., 1996; Bautista-Teruel & Millemena, 1999; Bautista-Teruel et al., 2002; Kruatachue et al., 2004; Lee, 2004).

While the primary results of this study are consistent with many previous studies, there is one noticeable exception. Of the single feed diets in this study, kelp performed better than the

formulated feed Abfeed®-S34, results that are contradictory to previously published work (see Britz, 1996b). While this study did achieve a substantially higher FCE value with the use of Abfeed®-S34, these values are considerably lower than those obtained in other studies (see e.g. Britz, 1996b; Britz et al., 1997; Shipton & Britz, 2001b). The lower FCE values obtained suggest that the design of the culture baskets was responsible for some wastage (although not noticeable) of the Abfeed®-S34 feed.

Ulva lactuca has long been known to be a good candidate for aquaculture. This species has a high nutrient uptake capacity (Neori et al., 1998, 2004; Neori & Shpigel, 1999; Shpigel et al., 1999), a rapid growth rate (Neori et al., 1996; Shpigel et al., 1999; Robertson-Andersson et al., 2006), and a relatively simple life cycle that is easy to control (Shpigel et al., 1999; Robertson-Andersson, 2003; Neori et al., 2004; Robertson-Andersson et al., 2006). Seaweeds such as *U. lactuca* that are co-cultured together with *H. midae* are of benefit, firstly because they serve as biofilters and secondly as potential high-protein feed (Shpigel et al., 1999; Neori et al., 2003, 2004; Troell et al., 2003, 2006, Robertson-Andersson et al., 2006; 2007).

In conclusion, this study is the first to document the effect of farm-grown, protein-enriched *U. lactuca* on the growth of *H. midae*. Seaweeds grown in aquaculture effluent perform better than their wild counterparts and such protein-enriched seaweeds perform even better in combination with other seaweeds as mixed diets. While protein-enriched *U. lactuca* on its own was not a good substitute for the formulated feed Abfeed®-S34, in combination with other fresh seaweeds as a mixed diet, it does, however, perform better than the formulated feed. Co-culturing *U. lactuca* with *H. midae* clearly has many benefits for the South African abalone farmer.

4.6 Table

Table 1. Growth parameters (SGR, DISL, CF and FCE) of juvenile abalone fed the six diet treatments by decreasing order of dietary rank. Comparative values with the same letter are not statistically different.

Diet treatment	Mean Final Weight (g)	Mean Final Length (mm)	SGR (%weight.day^{-1})	DISL (µm.day^{-1})	Initial CF	Final CF	CF Diff.	FCE	Rank		
									SGR	DISL	CF
Kelp + farmed *Ulva lactuca*	24.308 ± 0.28^{a}	50.521 ± 0.21^{a}	0.431 ± 0.02^{a}	61.530 ± 0.02^{a}	1.106	1.093	-0.013^{a}	N/A	1	1	1
Kelp + Abfeed®-S34	21.878 ± 0.24^{b}	50.185 ± 0.18^{b}	0.368 ± 0.02^{b}	54.901 ± 0.01^{b}	1.041	1.003	-0.038^{b}	N/A	2	2	2
Kelp	21.194 ± 0.28^{c}	49.503 ± 0.23^{c}	0.357 ± 0.02^{c}	52.242 ± 0.02^{c}	1.040	1.013	-0.027^{c}	0.020^{b}	3	3	2
Abfeed®-S34	19.680 ± 0.29^{d}	47.539 ± 0.23^{d}	0.330 ± 0.02^{d}	48.993 ± 0.02^{d}	1.136	1.061	-0.075^{d}	0.147^{a}	4	4	4
Ulva lactuca (farmed)	17.568 ± 0.26^{e}	46.486 ± 0.22^{e}	0.255 ± 0.02^{e}	38.777 ± 0.03^{e}	1.080	1.013	-0.067^{e}	0.011^{c}	5	5	4
Ulva lactuca (wild)	7.164 ± 0.09^{f}	36.950 ± 0.15^{f}	-0.095 ± 0.01^{f}	2.414 ± 0.02^{f}	1.096	0.820	-0.276^{f}	-0.014^{d}	6	6	6

4.7 Figure captions

Fig. 1. Increase in abalone shell length for all diet treatments.

Fig. 2. Increase in abalone body weight for all diet treatments.

4.8 Figures

Figure 1.

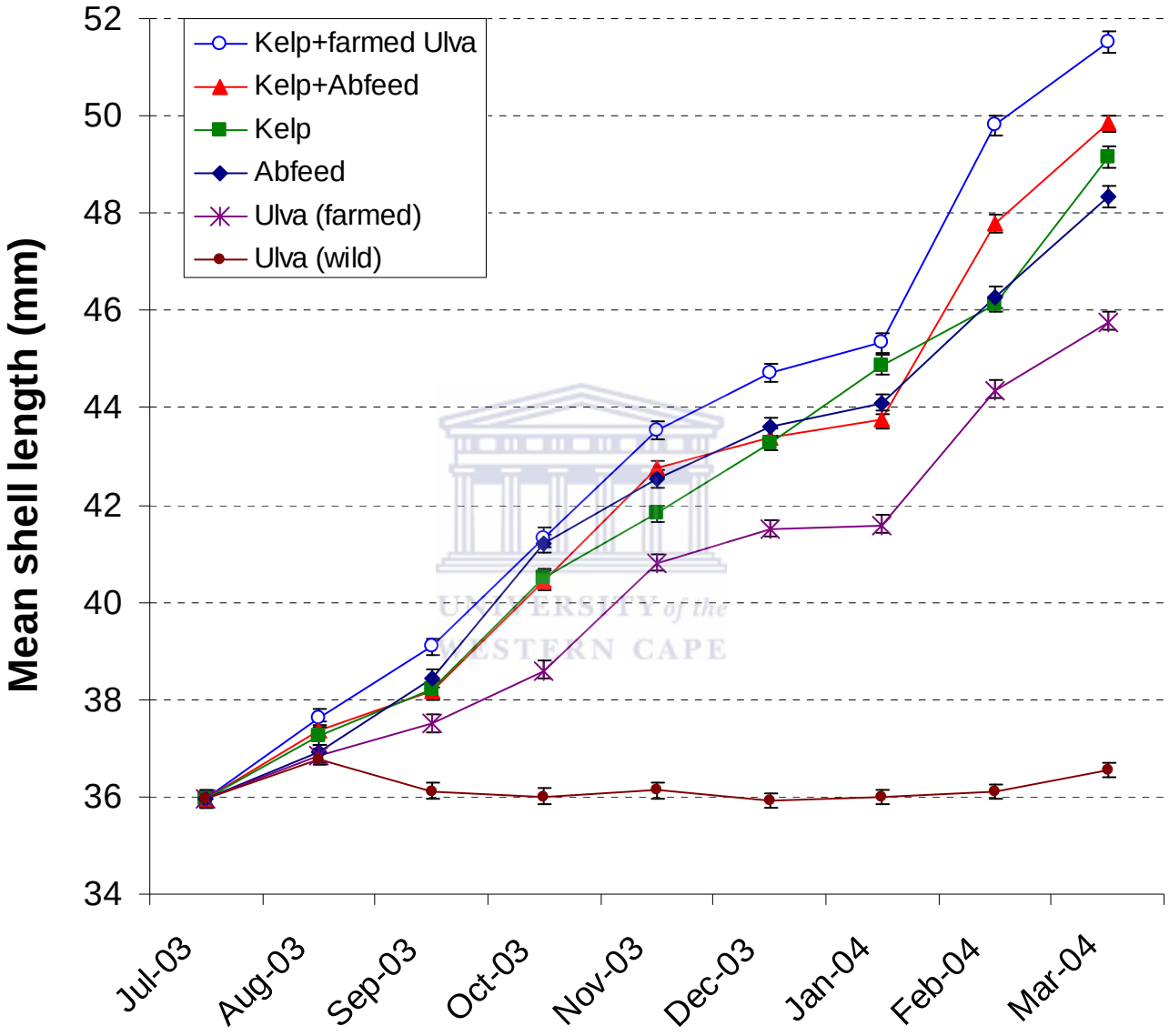
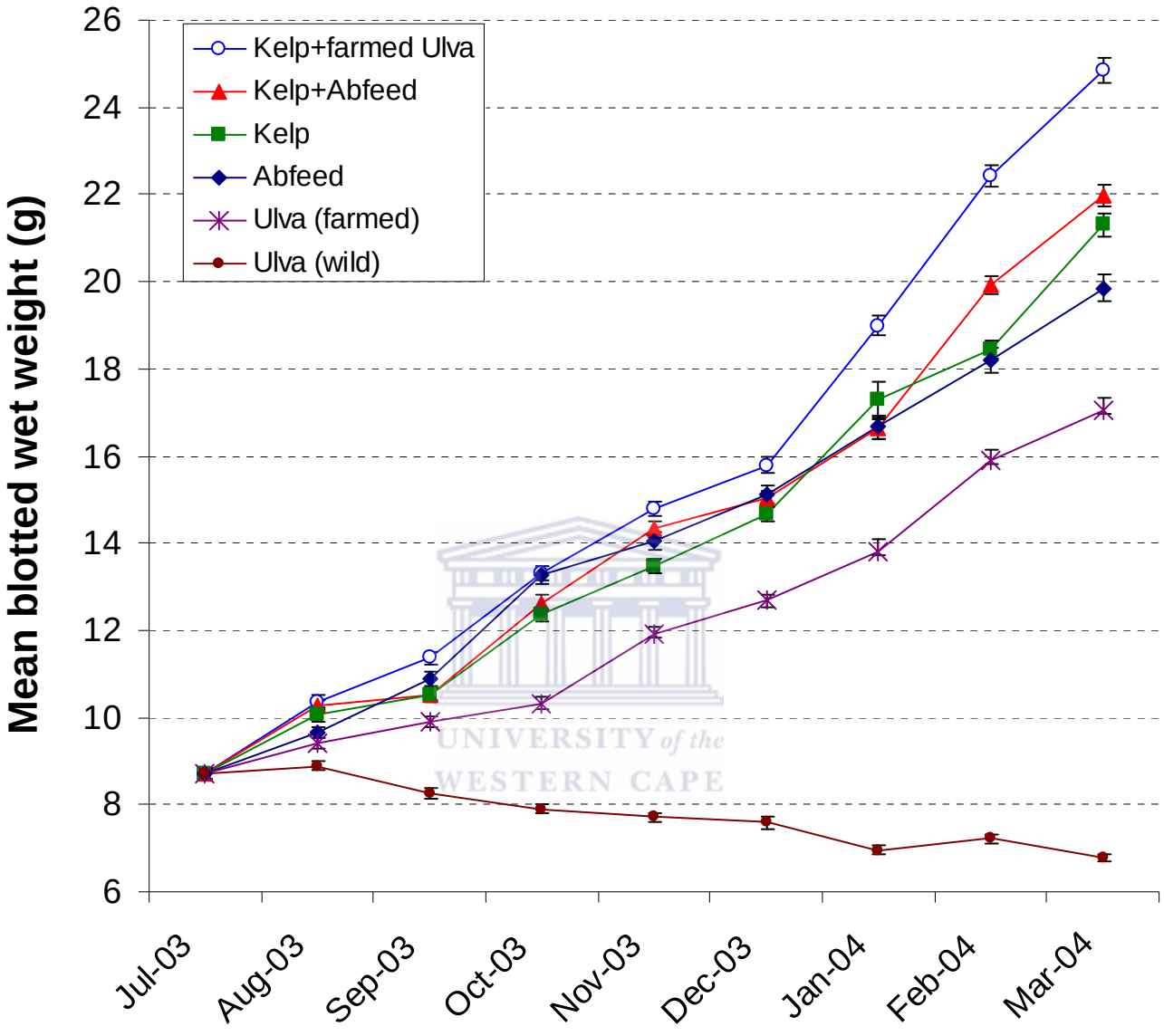


Figure 2.



5.1 General Discussion

The findings of this research have shown that diet strongly influences the growth response of cultured abalone. Of particular importance is the nutritional content and value of the diet, and the efficiency of its conversion to body weight. Although many studies (see e.g. Nie et al., 1986, 2000; Viana et al., 1993; Britz et al., 1996b; Fleming et al., 1996; Knauer et al., 1996; Lee, 1998a, 2004; Bautista-Teruel & Millemena, 1999; Sales & Britz, 2001; Serviere-Zaragoza et al., 2001; Bautista-Teruel et al., 2002; Gómez-Montes et al., 2003; Daume & Ryan, 2004a; Krautachue et al., 2004; García-Esquivel & Felbeck, 2006; Strain et al., 2006) have shown that formulated feeds produce better growth in cultured abalone than fresh seaweeds, this research has shown that fresh, protein enriched seaweeds, can outperform formulated feeds.

The major constituent of most formulated feeds for abalone is fishmeal, which makes up to 60% of the feed (Fleming et al., 1996). There is, however, a need to find alternative protein sources for formulated feeds, and alternative protein-rich feeds to formulated feeds (Chopin et al., 2001; Powell, 2003; Tacon, 2005). There are several reasons for this. Firstly, there have been dramatic declines in global catches due to wild fish stocks being heavily depleted, over-fished or fully exploited (Cook et al., 1997; Pauly et al., 1998, 2002; Caddy & Garibaldi, 2000; Watson & Pauly, 2001; Meyers & Worm, 2003). This has lead to diminishing supplies of fishmeal for use in commercial feeds (Naylor et al., 2000; Chopin et al., 2001; Powell, 2003; Tacon, 2005). Alternative sources of protein are therefore imperative as the demand for fishmeal (Tacon & De Silva, 1997; Naylor et al., 1998; D'Abramo et al., 2002; Delgado et al., 2003; Tacon, 2005; Neori et al., 2007) and aquaculture products (Naylor et al., 2000; Pauly et al., 2002; Troell et al., 2004; FAO, 2006) has

increased. Secondly, the Asian market increasingly prefers abalone that have been grown on natural feeds as opposed to those grown on formulated feeds, arguing that the abalone meat tastes better when grown on natural feeds (Kevin Ruck *pers. com.*) Since the South African abalone industry exports 95% of their product to the overseas market (mostly the Far East) (CITES, 2007), the use of natural feeds is becoming increasingly important. In addition, many seaweeds contain compounds such as dimethylsulfoniopropionate (DMSP) that contributes to the unique flavour and taste of cooked abalone meat (Van Alstyne et al., 2003; Smit et al., 2007, Van Alstyne et al., 2007). Formulated feeds contain low levels of DMSP and therefore abalone fed on these feeds, lack the characteristic taste and texture of wild abalone (Smit et al., 2007). Still other reasons for finding alternative protein and feed sources include the fact that formulated feeds are costly (Fleming et al., 1996, Krautachue et al., 2004), they often become unstable in the cultivation system (Fleming et al., 1996; Guzman & Viana, 1998), many leach nutrients (Marchetti et al., 1999; Shipton et al., 2002; Sales et al., 2003b), and most are not readily available in some countries (such as China - Zhang et al., 2004a). In addition, because the exact nutritional requirements of all commercially cultivated abalone have not been fully demonstrated, it is not possible to balance dietary essentials to suit all the nutritional requirements of abalone worldwide (Zhang et al., 2004a; Montano-Vargas et al., 2005).

This research has shown that protein enriched seaweeds cultured in aquaculture effluent, are a good substitute for the fishmeal-based protein found in formulated feeds. Grown in sufficient quantities, the culture of seaweeds as feed for abalone can therefore counter the diminishing fishmeal supplies (Buschmann et al., 2001, 2005; Diamond, 2002; Troell et al., 2003, 2004; Stirk & van Staden, 2004; Neori et al., 2007; Neori, 2007). In addition to this, culturing seaweeds with abalone in an integrated mariculture system also has other added benefits such

as serving as biofilters (Neori et al., 1991, 1996, 2003, 2004, 2007; Angel & Gordin, 1996; Neori & Shpigel 1999; Troell et al., 1999a, b; Boarder & Shpigel 2001; Jones et al., 2002; Robertson-Andersson, 2003; Schuenhoff et al., 2003; Demetropoulos & Langdon, 2004a,b; Njobeni, 2006; Robertson-Andersson et al., 2006, 2007; Neori, 2007; Xu et al., 2008). In such systems, the aquaculture effluent serves as a substitute for fertilizers that are generally used for growing seaweeds, thereby reducing production costs (Muir, 1995; 2005; Neori et al., 2004, 2007)

In integrated mariculture systems, seaweeds grow better in the aquaculture effluent than in the natural seawater, removing dissolved nitrogen from aquaculture effluent (Neori et al., 1996, 2004; Shpigel & Neori, 1996; Neori & Shpigel 1999; Shpigel et al., 1999; Robertson-Andersson, 2003; Robertson-Andersson et al., 2006, 2007). In this way, they improve the water quality of the system, by not only counterbalancing nutrient inputs (Neori et al., 2003, 2004; Troell et al., 2003), but also low dissolved oxygen (Harris et al., 1999; Xu et al., 1999), high ammonia (Reddy-lopata et al, 2006), high or low carbon-dioxide (Xu et al., 1999, 2008; Neori et al., 2004) and acidity levels (Xu et al., 1999, 2008). Through this nutrient and chemical recycling these seaweeds increase their protein and phosphorous content, which make them a good source of high protein fodder for abalone (Sales & Britz, 2000; Steyn, 2000; Robertson-Andersson, 2003; Njobeni, 2006; Robertson-Andersson et al., 2006, 2007; Troell et al., 2006). In addition, seaweeds typically also produce much more oxygen than they use up (their night-time oxygen consumption is lower than their daytime oxygen production) and in integrated systems this is beneficial to the system and to the cultured animals (Schuenhoff et al., 2003; Neori et al., 2004).

Wild seaweeds generally have a low protein content (Mercer et al., 1993; Fleming et al., 1996; Fleurence et al., 1999; Neori & Shpigel, 1999; Shpigel et al., 1999; Tahil & Juinio-Menez, 1999; Rosen et al., 2000; Wong & Cheung, 2001; Bautista-Teruel et al., 2002; Robertson-Andersson, 2003; Demetropoulos & Langdon, 2004; Robertson-Andersson et al., 2007). Feeding one species of wild seaweed as a single-species feed for prolonged periods of time often results in poor growth (Hahn, 1989; Day & Fleming, 1992; Mercer et al., 1993; Stuart & Brown, 1994; Fleming, 1995b; Fleming et al., 1996; Shpigel & Neori, 1996; Simpson & Cook, 1998; Bautista-Teruel & Millamena, 1999; Neori & Shpigel, 1999; Shpigel et al., 1999, Rosen et al., 2000; Fermin, 2002; Nelson et al., 2002; Kruatachue et al., 2004; Lee, 2004) and our research has supported this finding. However, mixed diets of fresh wild seaweeds have been shown to produce better growth in abalone than single-species diets (Owen et al., 1984; Cook & Claydon, 1991; Day & Fleming, 1992; Fleming, 1995a; Simpson & Cook, 1998, Nelson et al., 2002). This finding too has been supported by the current research. Day & Fleming (1992) suggested that single-species diets of wild seaweeds are of great value when they form part of a mixed diet as they provide essential nutrients to the diet that might otherwise be lacking. This was supported by Stuart & Brown (1994) who argued that a variety of seaweeds is better able to meet the preferences and nutritional requirements of cultured abalone over extended periods of time.

The culture of seaweeds in nutrient rich waters increases their protein content roughly 3 to 10 fold (Shpigel et al., 1999; Boarder & Shpigel, 2001; Robertson-Andersson, 2003; Robertson-Andersson et al., 2007). These protein enriched seaweeds have been shown to produce good growth as single species diets (Shpigel et al., 1996, 1999; Neori et al., 1998, 2004; Boarder & Shpigel, 2001; Demetropolous & Langdon, 2004). Neori & Shpigel (1999) found that feeding farm-grown, protein enriched seaweed tripled their abalone production when

compared to feeding wild seaweed. In addition, farm-grown, protein enriched seaweeds have been shown to produce even better growth as part of a mixed diet with other wild seaweeds (Neori & Shpigel, 1999; Shpigel et al., 1999). The current research supports these statements and has shown that farm-grown, protein enriched seaweeds produce relatively good growth when fed as a single-species diet, and even better growth when fed as part of a mixed diet producing better growth in abalone than those grown on formulated feed.

Seaweeds that are chosen for culture with abalone in integrated systems have certain beneficial characteristics that make them suitable for use as abalone feed and beneficial to the system. They generally lack chemical defenses (that usually deter grazing in abalone in the wild) (Stuart & Brown, 1994; Fleming, 1995a) and are morphologically simple (Shpigel et al., 1999; Neori et al., 2004). These two characteristics usually make them attractive and palatable to abalone (Stuart & Brown, 1994; Shpigel et al., 1999; Neori et al., 2004; Alcantara & Noro, 2005). Furthermore, such seaweeds have high growth rates defined by their high surface to volume ratios (Shpigel et al., 1999; Neori et al., 2004), which sustain a rapid production of biomass (Neori et al., 1996; Shpigel et al., 1999; Robertson-Andersson et al., 2007). They also have life cycles that are easy to control and are therefore easy to cultivate (Neori et al., 2004) as they are vegetative and do not become reproductive in the system (Shpigel et al., 1999; Robertson-Andersson, 2003). In addition to these characteristics, such seaweeds have a global distribution, are easily available and occur in abundance in their geographic localities (Shpigel et al., 1999; Robertson- Andersson, 2003).

Most South African abalone farmers use kelp as the primary feed for their abalone (Anderson et al., 2006; Francis et al., 2007), often in combination with formulated feeds (du Plessis, 2006). One of the primary reasons is that kelp is still a cheaper option to formulated feed

(Troell et al., 2006). Abalone are known to be inefficient feeders with approximately 63% of the energy from feed consumed being lost in faeces (Sales & Britz, 2001). This fact can pose a problem because abalone that consume protein-rich formulated feeds, excrete nutrient-rich faeces that in turn promote sabellid polychaete infestations (Chalmers, 2002; Simon et al., 2004, 2005; Potgieter, 2005). In combination, uneaten formulated feed and protein-rich faeces, often cause poor water quality in aquaculture systems (Chopin et al., 1999; Troell et al., 2003; Neori et al., 2004, 2007; Neori, 2007). These latter factors can lead to an accumulation of ammonia that can be toxic to abalone when present in high levels (Reddy-Lopata et al., 2006). By culturing seaweeds with abalone in integrated systems, all of this can be avoided.

In South Africa, kelp harvests are reaching their limits of sustainability, particularly in kelp concession areas with a high number of abalone farms (Troell et al., 2006). Culturing seaweeds for use as abalone feed thus promotes the sustainable use of kelp resources. In addition, culturing seaweeds also promotes a healthy coastal ecosystem since kelp harvesting can have a negative effect on the kelp bed ecosystem because it supports a range of epiphytic fauna and flora (Anderson et al., 2006).

As a feed, kelp produces higher shell growth rates (because of its high ash content), but lower growth in body weight compared to formulated feeds (Britz, 1996b; Simpson & Cook, 1998; Gómez-Montes et al., 2003; García-Esquivel & Felbeck, 2006). This suggests that abalone fed kelp typically invest more nutrients into shell growth than into body weight. In the current study, however, kelp was shown to produce better growth in body weight than the formulated feed tested. This finding is not supported in the published literature (see e.g. Britz, 1996b; Knauer et al., 1996; Seviere-Zaragoza et al., 2001; Gómez-Montes et al., 2003;

García-Esquivel & Felbeck, 2006). Although we did expect to produce better growth in shell length when fed kelp compared to Abfeed®-S34, we do not expect this for growth in weight. While the reason for this eludes us, the answer(s) may lie in the culture system environment (e.g. temperature, basket design, water quality, etc.) that perhaps was not conducive to the application of the formulated feed. None-the-less, to the abalone farmer, growth in body weight is more important as cultured abalone are sold by weight. Abalone specific growth rates (SGR), condition factors (CF) and feed conversion efficiencies (FCE) are therefore important in determining whether a feed is able to produce valuable, heavier individuals within the shortest possible time, and with the least amount of available feed. This study has demonstrated that farm-grown, protein enriched seaweeds, when incorporated into combination with other seaweeds (mixed diets), can produce relatively heavier abalone with higher specific growth rates in a shorter time than formulated feed.

In conclusion, feeding abalone a diet of fresh, protein enriched, cultured seaweeds provides a number of economical benefits to the abalone farmer. Firstly, it saves farming costs because the seaweeds are cultured in aquaculture effluent, thus negating the use of fertilizers. Secondly, this provides an effective means of nutrient recycling, waste removal and aeration of the aquaculture system. Thirdly, such seaweeds incorporated into combination or mixed diets, result in substantially higher abalone growth rates compared to those abalone fed only single-species diets or formulated feeds. Ultimately, if grown in sufficient quantities, this could reduce the dependency and use of expensive formulated feeds. Besides these obvious cost benefits, integrating abalone and seaweed aquaculture alleviates the pressures that we are placing on our natural seaweed resources and the environment.

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