The Effects of Differences in Feeding Regime and of Export Simulations on the Growth of the abalone *Haliotis midae* Linnaeus

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I declare that

"***The Effects of Differences in Feeding Regime and of Export Simulation on the Growth of the abalone <u>Haliotis midae</u> Linnaeus***"

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 Aquaculture

The term aquaculture can be defined as the farming or culture of aquatic organisms, including fish, crustaceans, molluscs and aquatic plants (Kautsky et al. 2001, Furey and Pitman 2003). From a commercial perspective, the objective of aquaculture is to maximize production of species in demand and to achieve maximum economic benefits (Troell et al. 2004). Aquaculture is a highly diverse activity and species choice is important as different species are favoured by different culture environments (Reay 1979, Folke and Kautsky 1992, Troell et al. 2004).

Species may be farmed by means of monoculture, co-culture or polyculture. Modern-day operations are usually monoculture systems employing high stocking densities (Kautsky and Folke 1989, Naylor et al. 2000, Kautsky et al. 2001, Langdon et al. 2004, Neori et al. 2004, Troell et al. 2004). Monoculture can be seen as a common practice in which only one species is farmed (Kautsky and Folke 1989, Folke et al. 1997). Large monocultures often severely change the ecosystem and often cause negative effects on the environment (i.e. causing changes in the ecosystem) as well as the culture organism itself by making the surrounding water less suitable or unusable (Kautsky and Folke 1989, Kautsky et al. 2001). Co-culture occurs when two or more species are cultured together in the same environment. This system depends on the selection of complementary organisms (e.g. abalone and seaweed) resulting in processes that are mutually beneficial for both cultured organisms (Langdon et al. 2004). Co-culture may promote nutrient cycling since one organisms waste is used by another to enhance the overall production of the system (Kautsky et al. 2001, Langdon et al. 2004, Neori et al. 2004). Polyculture is a common integrated farming practice using techniques of mix fed species (e.g. finfish, shrimp), herbivorous species and extractive species (e.g. shellfish, seaweeds) and aims to increase crop diversity within the farm area (Naylor et al. 2000, Neori

et al. 2004). These systems usually make use of inputs (e.g. photoautotrophic plants counteracting environmental effects of heterotrophic fed fish and shrimp through the restoration of water quality) and generate less waste (Kautsky et al. 2001, Yokoyama et al. 2002, Neori et al. 2004).

1.1.1 Aquaculture intensity

The nature of the aquaculture system can be categorized into extensive, intensive and semi-intensive. Extensive aquaculture generally occurs in simple systems such as salty dams, ponds or lakes. With extensive culture, little or no food is added to the aquaculture system and organisms feed on food that occurs naturally in the system (Gowen et al. 1990, Beveridge and Little 2002, Furey and Pitman 2003). If extensive cultures are managed well, they may be less expensive than other systems, but the disadvantage is the relatively low productivity resulting from the relatively low stocking capacity (Furey and Pitman 2003). Extensive culture has been shown to bring about ecological change by influencing the structure of food webs and the impoverishment of aquatic environments (Gowen et al. 1990, Folke and Kautsky 1992, Naylor et al. 1998, Troell et al. 2004, Hari et al. 2006).

Intensive aquaculture makes use of recirculation or flow-through water systems or a combination of both systems, but may also include ponds and open water cages (Ackefors 1999, Furey and Pitman 2003, Troell et al. 2004). Intensive aquaculture requires artificial feed and aeration, with water quality being managed on a regular basis. An advantage to intensive culture is that higher productivity is achieved compared to extensive aquaculture, but there is the disadvantage of extra expenses with feeding-labour, water pumping and capital outlays (Kautsky et al. 2001, Furey and Pitman 2003, Troell et al. 2004). Intensive aquaculture has been shown to bring about the enrichment of ecosystems by the release of

metabolic waste products and uneaten feed into the aquatic system (Folke and Kautsky 1989, Gowen et al. 1990, Beveridge et al. 1994). This system requires high levels of technical and biological expertise (Ackefors 1999, Furey and Pitman 2003, Cohen et al. 2005).

Semi-intensive aquaculture is a mixture between extensive and intensive systems. Generally, extensive cultures become semi-intensive once artificial feeds are added to the system and/or once there is some degree of water quality management through aeration or waste management treatment (Midlen and Redding 1998, Beveridge and Little 2002, Furey and Pitman 2003). Advantages of using semi-intensive cultures is the higher stocking densities thus higher production than extensive culture, lower capital costs and lower operating cost than intensive cultures, and low to medium management levels (Furey and Pitman 2003).

1.1.2 Aquaculture facilities

Commercial aquaculture enterprises may consist of various aquatic production facilities. The use of these facilities depends on the size of the farm and the type of organism(s) being farmed, and are usually of four types: ponds, cages, raceways and recirculating systems (Swann 1992, Beem 1998). Further distinctions can be made within each type of facility depending on the level of culture intensity employed by the producer.

The earthen pond (that ranges from a small farm pond to one specifically built for aquaculture) is the most common production system in use today (Swann 1992, Beem 1998). In the United States for example, the commercially important channel catfish is farmed in earthen ponds (Sealey et al. 1999, Hargreaves and Tucker 2003). Physical-chemical techniques for the intensification of pond culture have included in-pond cages and raceways, water blending and shading by the algal community, and the direct flocculation and removal

of algal and bacterial biomass from ponds (Busch and Goodman 1983, Lorio 1994, Brune et al. 2003). Microbial processes such as nitrification or denitrification, photosynthesis and heterotrophic bacterial regrowth, are used to help reduce ammonia levels in a conventional pond (Brune et al. 2003, Schnieder et al. 2005) since high levels of ammonia are known to occur at toxic levels in fish ponds (Swann 1992).

Cage culture is an alternative to pond culture where dykes or levees are not available (Swann 1992). Cage culture uses an existing water resource but encloses the species in a cage or basket, which allows water to flow freely between the species and the existing water source. This type of culture has been successfully employed in the Amazon basin (Gomes et al. 2006), Australia (Rowland et al. 2004), the Philippines (Capinpin et al. 1999) and Taiwan (Liao et al. 2004).

Flow-through or raceway systems are usually enclosed channel systems (Masser and Lazur 1997, Beem 1998) that require large volumes of high quality water that is obtained from a source tank (Swann 1992, Summerfelt et al. 2004, Terlizzi et al. 2004). Production values for flow-through systems are greater than that of ponds or cages because the continual exchange of fresh water helps remove waste products from the system and supplies adequate amounts of oxygen to the cultured organisms (Swann 1992, Troell et al. 1999, Furey and Pitman 2003). Advantages of flow-through or raceway systems are improved water quality, reduced manpower, and higher stocking densities. Disadvantages of this system are however: a rapid spread of disease; less reaction time when problems occur; and larger volumes of effluent containing waste from cultured species (Yoo et al. 1995, Masser and Lazur 1997, MacMillian et al. 2003, True et al. 2004).

A closed, recirculating system refers to an aquatic facility that recirculates the water rather than passing it through only once; here water is purified and used continually (Swann 1992, Stickney 1996). Re-use systems use a percentage of their water several times before discharging it (Summerfelt et al. 2004, Terlizzi et al. 2004). These systems are generally expensive and require that the producer has advanced technical skills available to the farmer (Terlizzi et al. 2004). The system generally consists of filter tanks that remove waste products and fed particles, and biological filters that convert toxic ammonia to nitrate (which is considered harmless) (Swann 1992, Furey and Pitman 2003).

1.2 Abalone aquaculture

The decreasing commercial catch and high market demand for abalone (Haliotidae, Gastropoda) in both export and domestic markets have promoted interest in the culture of this species (Hahn 1989, Gordon and Cook 2001, Bautista-Teruel et al. 2003). To meet the increasing demand of the Asian market, the culture of abalone is increasing in many countries such as Australia, Chile, China, Iceland, Ireland, Japan, Mexico, New Zealand, South Africa, Taiwan and the USA (Gordon and Cook 2001, Sales and Britz 2001, Huchette et al. 2003, Sales and Janssens 2004). Global production of abalone reached 22,600 metric tonnes (this including poaching of 3,700 metric tonnes) in 2002 (Gordon and Cook 2004). Of this total, 8,600 metric tonnes was farmed abalone, which had a production value of approximately US$ 0.8 billion (Gordon and Cook 2004). For 2007, world abalone production and consumption has been predicted to be 21,460 tonnes (19,468 metric tonnes) in which 12,060 tonnes (10,941 metric tonnes) are farmed abalone (Wayne Barnes, Abalone Farmers Association of South Africa, pers. comm.).

1.2.1 Factors affecting the growth of abalone

The international literature abounds with experimental data that investigates the factors affecting the growth of abalone. Such factors include water temperature, stocking density, water quality (e.g. dissolved oxygen, ammonia) and nutrition (feeds). These factors will be discussed in greater detail below.

1.2.1.1 The physical-chemical environment

Abalone have been found to have conservative thermal responses and show little tendency of adapting to altered thermal environments; they also have the low ability to withstand acute thermal shock (Reynolds and Casterlin 1979, Gilroy and Edwards 1998, Díaz et al. 2000). Different abalone species have different preferred temperature optima and it is assumed that this phenomenon separates temperate from tropical species. Preferred temperature optima refers to a choice of temperature in which motile organisms tend to gravitate towards a relatively narrow range of temperatures (Hecht 1994). The preferred temperature of the Australian blacklip abalone, *Haliotis rubra* Leach (16.9°C) and greenlip abalone, *H. laevigata* Donovan (18.9 °C), for example, were found to differ only slightly; the blacklip abalone had lower temperature tolerances and preferences as expected from its habitat distribution. Their 50% critical thermal maxima (the maximum temperature at which mortality is 50%), however, was 26.9 and 27.5°C respectively while their optimum temperatures were averaged out at 17.0 and 18.3°C respectively (Gilroy and Edwards 1998). Díaz et al. (2000) also showed that the red abalone, *Haliotis rufescens* Swainson from Mexico, had a preferred temperature of 18.8 ± 2.1°C, a 50% critical maxima of 27.5°C, and an optimum growth temperature of 18.4°C. Such examples highlight the variable thermal responses of abalone world-wide.

Stocking density is another important variable in aquaculture because it directly influences survival, growth, health, water quality, feeding and production (Mgaya and Mercer 1995, Hengsawat et al. 1997, Huchette et al. 2003, Rowland et al. 2006). Many studies (e.g. Mgaya and Mercer 1995, Hengsawat et al. 1997, Capinpin et al. 1999, Huchette et al. 2003) have shown an inverse relationship between abalone growth and stocking density. In aquaculture, density may affect growth directly through competition for food or space, or indirectly through the accumulation of excretory products. This has been shown with the negative relationship between growth and density with gastropods which suggests density-dependent intraspecific competition for space and food (Stimson 1970, Jarayabhand and Newkirk 1989, Parsons and Dadswell 1992, Huchette et al. 2003). In recirculating systems in particular, high stocking densities become a problem as ammonia (the main end product of nitrogen metabolism) may reach toxic levels (Basuyaux and Mathieu 1999, Huchette et al. 2003, Björnsson and Ólafsdóttir 2006).

Low Dissolved Oxygen (DO) concentration has been classified as a limiting factor for growth in aquaculture systems (it does not act directly as many toxins do) because it limits aerobic metabolism (Boyd and Watten 1989, Harris et al. 1999). Oxygen concentrations vary especially during algal blooms (Elston 1983, Lee and Arega 1999, Lee et al. 2005) and in systems that are subjected to high biological oxygen demand in which uneaten food and decaying wastes are only removed intermittently (Hindrum et al. 1996, Harris et al. 1999). Combined temperature and oxygen saturation often affects the survival of abalone species differently. Harris et al. (2005) for example, showed that juvenile *Haliotis rubra* held at 16.9°C and 97% oxygen saturation, grew faster in shell length than those maintained at 17.5°C and 111% oxygen saturation. Furthermore, *H. rubra* held at 19°C had lower survival rates for both 96% and 120% oxygen saturation compared with those maintained at either

110% oxygen saturation and 19°C or for any 17°C treatment (Harris et al. 2005). Abalone appear to require DO levels greater than 3-4 mg/l; lower levels cause disturbance in the acid-base balance as well as anaerobic metabolism (Fallu 1991, Cheng et al. 2004a). For example, DO at 5 mg/l or greater is considered optimal for the growth of *H. diversicolor supertexta* Lischke (Yang and Ting 1989).

Gastropod excretion (composed mostly of nitrogenous compounds) consists largely of ammonia (Spotte 1979). Ammonia is a toxic metabolite and stressor in abalone culture (Harris et al. 1998, Basuyaux and Mathieu 1999, Cheng et al. 2004b, Reddy-Lopata et al. 2006). Two forms of ammonia occur in aquaculture systems, namely the ionized (NH_4^+) and the un-ionized (NH_3) forms. The un-ionized or gaseous form of ammonia is the most harmful to aquaculture species because of its readiness to diffuse across fish gill membranes which are less permeable to the ionized form (Armstrong et al.1978, Thurston et al. 1981). Both forms grouped together are termed the Total Ammonia Nitrogen (TAN) (Swann 1992). Through normal biological oxidation processes (i.e. nitrification), toxic ammonia can be degraded to harmless nitrates in which ammonia (NH_3) is converted to nitrite (NO_2^-) and then to nitrate (NO_3^-) in the nitrogen cycle (Spotte 1979). For molluscs and crustaceans, nitrite (NO_2^-) becomes toxic with regards to their haemocyanin (Colt and Armstrong 1981). Nitrate (NO_3^-), however, is considered of low toxicity for most species (Epifano and Srna 1975, Muir et al. 1991). Basuyaux and Mathieu (1999) for example, found that the safe level of ammonia, nitrite and nitrate exposure for *H. tuberculata* (Linnaeus) was 1 mg $N\text{-}NH_{3\text{-}4}\ l^{-1}$, > 5 mg $N\text{-}NO_2\ l^{-1}$ and within the range of 100-250 mg $N\ l^{-1}$ respectively. With increasing stocking densities, ammonia levels become increasingly toxic and subsequently affect the growth of cultured species (Basuyaux and Mathieu 1999, Huchette et al. 2003, Björnsson and

Ólafsdóttir 2006). Ammonia levels in semi-enclosed aquaculture systems for example are generally regulated by the water exchange rate (Ford and Langdon 2000).

1.2.1.2 Nutrition

Abalone farms rely on the harvesting of macroalgae as abalone feed (Zemke-White et al. 1999, Sales and Britz 2001, Levitt et al. 2002, Anderson et al. 2003), but recently the need for nutritionally complete feeds has become more critical due to the limited supply of algae (Fallu 1991, Sales and Britz 2001, Bautista-Teruel et al. 2003, Sales and Janssens 2004, Troell et al. 2006). In recent years there has been a rapid increase in the number of research groups developing artificial (formulated) diets for abalone aquaculture. Many farmers, however, require more from a feed than nutritional quality and cost effectiveness. Abalone farmers are interested in other aspects of the feed, for example, the feed's availability, the influence of the tank system with a particular feed, the problems associated with waste removal, and aeration strategies associated with different types of artificial feeds (Rumsey 1993, Fleming et al. 1996, Bautista-Tereul and Millamena 1999, Shipton and Britz 2001, Troell et al. 2006).

Formulated feeds produced to date are similar in their proximate composition and contain a high protein and carbohydrate content, and a low lipid and fibre content (Fallu 1991, Fleming et al. 1996, Troell et al. 2006). The energy source in commercial artificial feeds is supplied primarily in the form of carbohydrates (wheat flour, maize flour, sodium algenate, dextrin, starch and bran) (Sales and Janssens 2004). Protein is the most expensive component in prepared formulated diets and is essential for soft tissue growth (Fleming et al. 1996, Guzmán and Viana 1998). Protein sources used for artificial feeds include fishmeal, defatted soybean meal, casein, soya oil cake, *Spirulina* spp., torula yeast, and even abalone viscera silage (Britz

1996a, Fleming et al. 1996, Guzmán and Viana 1998, Sales and Janssens 2004). It has been found that fishmeal is the only protein source that can support growth performance without combination; all other protein sources must be combined in order to improve growth (Fleming et al. 1996, Guzmán and Viana 1998).

1.3 Mariculture[1] of *Haliotis midae* Linnaeus

The South African abalone fishery has existed since 1949 (Steinberg 2005), but the first attempt to cultivate abalone in South Africa was in 1981 (Genade et al. 1988). Currently, the South African abalone aquaculture industry is based solely on *H. midae* despite the fact that there are five other haliotid species (*H. parvum* Linnaeus, *H. spadicea* Donovan, *H. queketti* Smith, *H. speciosa* Reeve and *H. pustulata* Reeve) that occur along the South African coast (Cook 1998, Sales and Britz 2001, Evans et al. 2004). *Haliotis midae* occurs from St Helena to the Eastern Cape Province, *H. parvum* from False Bay to East London, *H. spadicea* from Partridge Point (False Bay), Cape Peninsula to northern Kwa-Zulu Natal, *H. queketti* from the Eastern Cape Province to southern Mozambique, *H. speciosa* from Port Alfred to the Eastern Cape Province, and *H. pustulata* from northern South Africa to the Persian Gulf (Geiger 2000).

South Africa has become the largest abalone producer outside Asia (FAO 2004) with production steadily increasing (FAO 2006), and over-exploitation of wild stocks by poaching and high market prices have been the main drivers for its cultivation. There are currently approximately 22 abalone farms operating in South Africa (a few managers operate more than one farm in an area) distributed between Port Nolloth on the Atlantic coast to East

[1] The term 'mariculture' has often been interchanged with aquaculture (Reay 1979) and refers specifically to the cultivation of marine species (De Silva 1998, Lincoln et al. 1998, Troell et al. 2004).

London on the Indian Ocean (Sales and Britz 2001, Troell et al. 2006). Since *Haliotis* species are relatively slow growing (Britz 1996a), farming is both expensive and time consuming (Preece and Mladenov 1999, Sales and Britz 2001). While wild *Haliotis midae* reaches a maximum size of approximately 200 mm in shell length at an age of about 30 years (Newman 1968, Tarr 1995), farm production is concentrating on an average size of only 100mm after five years (Sales and Britz 2001). For the year 2006, the total farmed abalone production in South Africa was estimated at 890 tonnes with a turnover of US$ 22 million (Wayne Barnes, Abalone Farmers Association of South Africa, pers. comm.).

The same factors affecting the cultivation of abalone worldwide also affect the growth of local *H. midae*. Temperature in particular has been shown to be very critical. *Haliotis midae* is more abundant on the colder South West Cape coast (minimum of 12-13°C) than on the warmer east coast (maximum of 21°C) (Schumann et al. 1995, Britz et al. 1997). Studies by Hecht (1994) have shown that the preferred temperature for juvenile *H. midae* (30-45 mm) is between 24.1-24.5°C; the 50% critical thermal maximum being 27.9°C, which is substantially higher than the ambient environmental temperature. Research by Britz et al. (1997), however, have shown that a temperature range of 12-20°C is physiologically optimal for juvenile and larger *H. midae* as growth, feed consumption, protein efficiency ratio and feed conversion ratio starts to deteriorate at temperatures above 20°C.

Since the South African abalone industry only really started in the late 1980's, technologies regarding densities have generally been adopted from established industries from overseas (Sales and Britz 2001). Commercial abalone farms in South Africa thus employ an intensive system in which abalone are reared at high stocking densities in shore-based culture systems (Sales and Britz 2001). There is a lack of published or otherwise informal information with

regards to optimal stocking densities for South African abalone farms. Generally, stocking densities vary with each farm site as farming conditions usually differ.

A number of studies (e.g. Harris et al. 1998; Basuyaux and Mathieu 1999, Hindrum et al. 2001, Huchette et al. 2003) have concentrated on the influence of water quality and more specifically ammonia on the survival and growth of abalone. Most of these studies have, however, concentrated on the influence of ammonia on the growth of juvenile Australian abalone, and standard toxicity tests (determinations of lethal concentrations of ammonia in abalone) have not been fully presented. Information on ammonia toxicity such as lethal and sub-lethal concentrations and information regarding increased survival by adaptation to sub-lethal levels are largely lacking for South African abalone farms (Reddy-Lopata et al. 2006). Studies by Reddy-Lopata et al. (2006) on *Haliotis midae* have shown that tolerance to ammonia (at pH 7.8 and ambient temperature of 15°C) increases with body size.

Haliotis midae is a herbivorous species with a nocturnal feeding pattern and tends to remain largely inactive by day (Barkai and Griffiths 1987). Research into the natural diet of South African abalone have shown that these herbivores feed on a broad selection of algae with at least two species found in their gut at any one time (Barkai and Griffiths 1986). On abalone farms, abalone have traditionally been fed a diet of kelp (*Ecklonia maxima* [Osbeck] Papenfuss and sometimes *Laminaria pallida* Greville), red algae (*Gracillaria* spp. Greville, *Gracilariopsis* spp. Dawson, *Gelidium* spp. Lamouroux, *Plocamium corallorhiza* [Turner] J.D. Hooker & Harvey) and green algae (e.g. *Ulva* spp. Linnaeus) (Cook 1998, Troell et al. 2006). Kelp, however, presently constitutes the major feed for farmed abalone on the South west coast of South Africa (Cook 1998, Rotmann 1999) but this resource is approaching limits of sustainable harvesting in kelp concession areas with high abalone farm

concentrations (Anderson et al. 2003, 2006, Troell et al. 2006). For this reason (and to increase production), formulated feeds are increasingly featuring more prominently in abalone diets, and abalone feed is increasingly making up a major proportion of the production costs on South African abalone farms (Sales and Britz 2001, Bautista-Teruel et al. 2003, Sales and Janssens 2004, Troell et al. 2006).

Stepto and Cook (1996) showed that while food value, plant defences and prior diet history interacted to determine the food selectivity of juvenile *H. midae*, food value was of greatest importance. There has, however, been much debate around the natural versus artificial (formulated) feed in this regard and it has been shown that different abalone diets produce different growth rates (Britz 1996b, Stepto and Cook 1996, Guzmán and Viana 1998, Simpson and Cook 1998, Naidoo et al. 2006). While many studies (e.g. Britz 1996a, Guzmán and Viana 1998, Bautista-Teruel and Millamena 1999, Bautista-Teruel et al. 2003, Gómez-Montez et al. 2003) have shown that artificial formulated feeds produce better growth rates, other studies have shown that abalone grow equally well (if not better) on combinations of feed. Naidoo et al. (2006) for example, have shown that juvenile *H. midae* grew better when fed fresh protein-enriched algal combinations compared to when fed an artificial feed.

The use of kelp and other seaweeds versus artificial feed on abalone farms has had conflicting reports particularly as far as price of feed, availability and accessibility of fresh seaweed, food conversion ratio (FCR), cost of handling and storage, final quality and production levels of abalone, and the culture environment are concerned (Troell et al. 2006). In general, abalone grow faster on the formulated feed Abfeed®-S34 until they reach about 50 mm in shell length. Thereafter farmers tend to prefer feeding kelp or a combination of kelp and Abfeed®-S34. This is so because once abalone reach 50 mm in shell length, the

Abfeed®-S34 promotes higher incidence of sabellid infestation because the worms feed on the nutrient-rich faeces (Simon et al. 2004, Troell et al. 2006). Also, shell growth rates tend to be higher on kelp after the abalone have reached about 50 mm shell length (Jones and Britz 2006, Troell et al. 2006).

Besides the factors mentioned so far, general abalone health is also critical for "good" abalone culture. The first serious health issue to affect *Haliotis midae* was infestation in 1994 by the parasitic sabellid polychaete, *Terebrasabella heterouncinata* (Fitzhugh and Rouse) (Ruck and Cook 1998). Infected abalone were found to have severely reduced growth rates. Surveys of the South African coastline revealed that this sabellid was endemic to South Africa (Ruck and Cook 1998, Sales and Britz 2001) and therefore posed a serious threat to the local economy. Sabellids are simultaneous hermaphrodites, producing both eggs and sperm at the same time. Once larvae are viable, they crawl over the surface of the host abalone shell and locate themselves between the mantle and shell, secreting a mucous or proteinaceous tube around them (Ruck and Cook 1998). It was found that the sabellid becomes a problem on South African farms particularly when abalone are kept at high stocking densities and/or when poor hygiene and water quality conditions occur (Cook 1998).

Intensive cultivation tends to alter the composition of the indigenous protective gut flora of cultured organisms leading to increased susceptibility to disease and/or a reduction in the ability to efficiently utilize feed (Macy and Coyne 2005). There is, however, increasing evidence to suggest that both health and survival of organisms in intensive rearing systems is improved by manipulating the gut microflora with probiotic[2] microorganisms (Robertson et al. 2000, Olafsen 2001). The use of probiotics in South African *H. midae* for disease

[2] Probiotics are beneficial microbial cells such as bacteria fed to live stock to improve digestion and health (Gatesoupe 1999).

prevention and improved nutrition is becoming very popular due to an increasing demand for environment-friendly aquaculture. Studies by Macey and Coyne (2005) have shown that *Haliotis midae* fed a probiotic-supplemented diet have an improved survival and better growth rate compared to those abalone not fed the probiotics. More recent work done by Macey and Coyne (2006) has shown that three probiotic strains are able to survive and colonize in the digestive track of *H. midae*. This is the first study to report probiotic colonization of the digestive tract of *H. midae*.

Toxic algal blooms are common worldwide and pose a serious health problem to aquaculture and fishing industries. Toxic blooms by the dinoflagellate, *Karenia cristata* Botes, Sym et Pitcher for example, were shown to be responsible for mass mortalities of wild and farmed *H. midae* in 1988 (Botes et al. 2003). Studies by Botes et al. (2003) suggested ozonation as an effective mitigation measure for HABs (ozone has the ability to kill dinoflagellates) but ongoing research is still required as to its economic viability on abalone farms. There is generally a lack of published information regarding the effects of Harmful Algal Blooms on the South African mariculture industry.

Direct manhandling and continuous over-handling has also been suggested to be a threat to the health of abalone (Jonathan Venter, Jacobsbaai Sea Products, pers. comm.). Abalone are usually removed from their culture baskets with the use of a spatula that is quickly pushed under the foot (Fleming and Hone 1998). Abalone are renowned for their ability to pull their shell down rapidly and tightly onto the substratum, complicating their removal and handling on abalone farms (White et al. 1996). The continuous mechanical removal of abalone often results in injury or even death because of their slow healing rate and increased probability of bacterial infection with stress (Genade et al. 1988). Abalone also lack blood clotting agents

and any cut, which has a high probability of occurring during farm grading (handling), is potentially lethal (Tong et al. 1992, Fleming and Hone1998).

1.4 Research requirements for the cultivation of *Haliotis midae*

The Marine and Coastal Management branch of the Department of Environmental Affairs and Tourism developed in 2005 a Frontier Programme that hopes to deal with research and development lacking for the South African mariculture industry (see Pitcher 2005). The research being funded broadly falls into the following categories.

1. Nutritional requirements for abalone culture.
2. Determination of the seasonal variation in the nutritional quality of harvested kelp.
3. Key water parameters.
4. Determination of the effect of handling/grading and tank maintenance for abalone survival and growth.
5. Development of live export protocol to decrease transport mortality and weight loss in abalone.
6. Investigation of the biology of polydorid polychaetes infesting cultured abalone.
7. Identification of phytoplankton blooms resulting in abalone spat mortalities.

1.4.1 Aims of the current study

Aspects encompassed within points 1, 4 and 5 above forms part of this research and are discussed in greater detail below.

1.4.1.1 Feeding Regimes

During winter months, beach-cast kelp is plentiful when storms cause kelp to wash ashore. In summer, however, kelp has to be harvested at sea and delivered to the abalone farms.

Because of this, and many other reasons, the amount of kelp delivered to the farms is not always consistent. Often, no kelp is available for days at a time (Jonathan Venter, Jacobsbaai Sea Products, pers. comm.). Abalone are, thus often starved of kelp for short periods of time. Thus far, no assessment has been made of periodic starvation on the growth of abalone on commercial farms. The first aim of the current research was to examine the effects of different feeding regimes (using kelp as a feed) so as to assess the influence of periodic starvation on the growth of farmed *H. midae*.

1.4.1.2 Kelp vs Abfeed®-K26

Abfeed® is a formulated feed containing mostly fishmeal, soya bean meal, starch, vitamins and minerals (Marifeed Pty Ltd, South Africa). In general, abalone grow well on Abfeed®-S34 at least until they reach approximately 50 mm in shell length; thereafter they are fed kelp or a combination of kelp and Abfeed® (Jones and Britz 2006, Troell et al. 2006). Since older abalone grow better on kelp and/or the combination of kelp and Abfeed®-S34, a low protein version (Abfeed®-K26) has been developed (Jones and Britz 2006, Troell et al. 2006) for reasons already mentioned in section 3. Abfeed®-K26 contains only 26 % protein and also contains kelp which acts as a food attractant. Since it is low in protein, ammonia levels are decreased, thus improving water quality (Marifeed Pty, South Africa). Currently, little information exists relating to alternative formulated feeds for cultured abalone with a shell length of 40-70 mm cultured in various systems. Thus, the second aim of this research was to compare the growth of grow-out abalone fed kelp versus those fed the new low protein Abfeed®-K26 formulated feed in both a flow-through and a recirculation system.

1.4.1.3 Effects of different export protocols

Upon export, abalone are transported alive in polystyrene containers on ice in plastic bags containing 100% oxygen humidified with seawater (Sales and Britz 2001, O'Omolo et al. 2003, Vosloo and Vosloo 2006) Through this process market sized abalone lose between 4 and 15 % of their body mass due to evaporation and pedal mucous production (Vosloo and Vosloo 2006). Since commercially farmed abalone are sold by weight, there is a decrease in the foreign revenue as exporters are paid the landed mass (Vosloo and Vosloo 2006). Another concern is the added weight loss experienced by animals returned for various reasons to farms and then placed back into their original growing environment. Little research information exists on the effects of handling and transport of live abalone, and the development of live export protocols to decrease transport mortalities and weight loss in abalone is therefore needed. The third aim of this research was to run an export simulation, and then to determine the best growth environment to allow rapid weight gain in abalone returned from the export simulation. Ultimately, the over-arching goal of the current research was to improve our understanding of the abalone aquaculture environment so as to make appropriate recommendations for best on-farm practice.

Determining the most appropriate feeding regime for the South African abalone *Haliotis midae* Linnaeus grown on kelp

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2.1 Abstract

Beach-cast kelp (the most widely used feed for commercially grown South African abalone) is plentiful during winter months when periodic storms cause kelp to wash ashore. During summer, however, this resource is not always readily available and farmed abalone are often starved for short periods. The aim of this research was to assess how periodic kelp starvation influences growth of the commercially grown abalone, *Haliotis midae* Linnaeus. Growth of grow-out abalone was monitored on a commercial abalone farm over a period of six months and consisted of 3 treatments with 2 replicates (n = ±250 abalone per replicate). The treatments were: Control (abalone given more kelp than typically needed); Treatment 1 (abalone fed their weekly ration once a week); Treatment 2 (abalone fed half their weekly ration every 3 and then 4 days respectively). While the data at first suggest that the control animals outperform the treatment animals, after undergoing an initial adjustment period to the new feeding regime, the treatment animals perform better. Feed conversion efficiencies ($P < 0.05$) show that overall the treatment animals performed better than the control animals . The control animals generally required much more feed to produce comparable increases in both length and weight compared to the treatment animals. This study has shown that periodic bouts of starvation are beneficial to *Haliotis midae*, allowing variable growth spurts when returned to full feed rations.

Keywords: abalone, compensatory growth, feed conversion efficiency, growth, *Haliotis midae*, kelp, starvation

2.2 Introduction

Abalone (*Haliotis* spp.) are in considerable demand in the Far East where they are treated as part of traditional cuisine and ceremony (Chen 1989, Sales 1999, Sales and Britz 2001). Consequently, they have been exploited for centuries because of their food value (Barkai and Griffiths 1986). Presently there is an increasing demand for small, cocktail size abalone of 40-70mm shell length in the international market (Jarayabhand and Paphavasit 1996, Najmudeen and Victor 2004).

Worldwide there are approximately 90 species of abalone, of which 15 are harvested commercially (Sales and Janssens 2004). In South Africa only six Haliotid species occur, and of these, *Haliotis midae* (Linnaeus) is the only one that is commercially exploited (Cook 1998, Sales and Britz 2001, Evans et al. 2004). Although the South African abalone fishery has existed since 1949, the first attempt to cultivate *H. midae* was in 1981 (Genade et al. 1988, Sales and Britz 2001, Steinberg 2005). Since then, South Africa has become the largest abalone producer outside Asia (FAO 2004) with production steadily increasing (FAO 2006). This emerging market has largely been driven by over-exploitation of the wild abalone stocks by poaching and by high market prices (Cook 1998, Troell et al. 2006).

The growing South African abalone industry depends largely on a steady supply of feed resources, particularly fresh kelp (Troell et al. 2006). Currently more than 7000 tons of fresh kelp fronds are harvested annually in South Africa to feed cultured abalone and this figure is expected to increase as the growing demand for kelp by local abalone farmers also increases (Anderson et al. 2006). During the winter months, beach-cast kelp is plentiful as storms cause kelp to wash ashore. In summer, however, kelp has to be harvested at sea and delivered to the abalone farms (Anderson et al. 2006, Rothman et al. 2006). The amount of kelp delivered to

an abalone farm varies from day to day and often there are days where no kelp may be available. This then means that abalone are often starved of food for short periods. Thus far, no research has focused on the effects of periodic kelp starvation on the growth of farmed *H. midae*. The aim of this research was therefore to determine the most appropriate feeding regime for the South African abalone, *H. midae* and to assess how periodic starvation influences its growth.

2.3 Materials and Methods

2.3.1 Experimental system

The research was conducted at the Jacobsbaai Sea Products (17° 53' 12.5" E, 32° 58' 2.5" S, Western Cape, South Africa) abalone farm. Flow-through seawater with a flow rate of 850-1300 $L.h^{-1}$ and moderately aerated, was supplied at a temperature of 13.8±0.76°C in the holding tanks. The abalone were grown in culture baskets (80 x 57 x 25 cm; length, width and depth respectively) subdivided with feeding plates to increase the surface area.

2.3.2 Experimental animals

Grow-out abalone (abalone with a shell length > 20mm) were supplied by the Jacobsbaai Sea Products abalone farm. Since growth of abalone is variable, individuals of similar size and of the same gene pool (spawned May 2002) were used. These animals were subdivided into two replicate baskets of approximately 12.5 kg (± 250 individuals). The initial weight and shell length of the abalone were measured at 46.47g ± 0.25 and 64.31mm ± 0.75 respectively. Both body weight and shell length were measured monthly.

2.3.3 Daily feed consumption

A pre-investigation feeding trial was initiated two weeks prior to commencement of the study to ascertain the daily feed consumption of grow-out abalone of the size used in the experiment. It was found that ±12.5 kg of abalone of the weight and length above consumed roughly 500g of kelp per day. This data was used as the basis for the various treatments that followed and a base weight of 550g per day (realized feed consumption) was established to compensate for variable daily growth between baskets and between individuals within baskets. The pre-investigation feeding trial ran concurrently with the experiment in order to

monitor the on-going consumption as abalone grew throughout the experiment; the feed values were then adjusted accordingly.

2.3.4 Treatments

Three treatments (each with two replicates) were tested.

Control: Considered the ideal conditions in which kelp was always available, i.e. always more than the daily "realised feed consumption" (initially 550g + 50g) of kelp in the basket. Monitored daily, deteriorating kelp was always removed and fresh kelp topped up with as much as needed to always have 600g in the baskets.

Treatment 1: Initially fed 3,85kg (550g x 7) of kelp on day 1 and then again 3,85kg of kelp every 7 days later i.e. bulk feeding at 7 day intervals. This assumed that if the abalone consumed more than the daily "realised feed consumption" of 550g, they would at one stage or another have to starve until day eight when the next bulk feeding occurred.

Treatment 2: This treatment was included to compensate for any feeding pattern that might arise from treatment 1. Abalone were initially fed 1.925kg [(550g x 7)/2] on day 1 and then again 1.925kg, 3 and then 4 days later for the duration of the experiment. The 3-, 4-day cycle was maintained throughout this treatment. As half (1.925kg) of the weekly "realised feed consumption" amount of kelp was given over 2 different cycles, it was assumed that there would be both periods of sufficient kelp available (i.e. 3-day cycle), as well as periods where the abalone would be slightly starved of kelp (i.e. 4-day cycle).

By month 2 the "realised feed consumption" was increased by 50g to 600g of kelp as dictated by the separate, on-going pre-investigation experiment. This new value substituted the previous 550g values in all the treatments above and quantities were adjusted accordingly. Hereafter, the "realized feed consumption" was kept constant at 600g for both treatments 1 and 2 to build in greater starvation periods into the experiment for the latter four months, while that of the control was increased by 50g every second month.

2.3.5 Sampling and data collection

The experiment was conducted over six months. Representative animals were randomly selected from each treatment for sampling (N = 30 per replicate at 0-3 months; N = 40 per replicate at 4-6 months to compensate for later differential growth). Before all measurements, abalone were blotted dry to remove excess water. Body weight was recorded to the nearest 0.01g using an electronic balance. Shell length was measured along the longest axis of the abalone shell to the nearest 0.1mm with a vernier callipers.

Daily increment in shell length (DISL) was calculated using the formula of Zhu et al. (2002):

$$DISL\ (\mu m/day) = [(SL_t - SL_i)/ t] \times 1000$$

Where SL_t = the final mean shell length (mm), SL_i = the initial mean shell length (mm), and t = the feeding trial period in days.

Specific growth rate (SGR in % body weight.day^{-1}) was calculated using the formula of Britz (1996a):

$$SGR = [(\ln(W_f) - \ln(W_i)) / t] \times 100$$

Where $\ln(W_f)$ = the natural log of the final mean weight of abalone, $\ln(W_i)$ = the natural log of the initial mean weight of abalone, and t = the feeding trial period in days.

The Feed Conversion Efficiency (FCE) was calculated for each treatment using the formula of Simpson and Cook (1998):

FCE = (growth/ration) x 100

Where growth = the blotted wet weight (g) gained per day and ration = the blotted wet feed (g) intake per day.

2.3.6 Statistical analyses

Unless otherwise stated, all graphed data are expressed as means ± se. Data for all experimental replicates were pooled as no significant differences were found between them. As no adjustment period was allowed before the start of the experiment, the first two months were considered to be the time necessary for the treatment animals to adjust to the new feeding regimes. For this reason, and to monitor the ongoing change over time, the observations were combined as a split plot analysis of variance with treatments (control, treatments 1 and 2) as main plot factors and months as split plot factors (see Little and Hills 1972). For each treatment and basket combination, linear regression functions were fitted on mean abalone weight and length change over the periods 0-2 and 2-6 months. To do this, mean abalone weight and length were adjusted to be equal at the start of each analysis. Results from the linear regression analyses were then subjected to a one-way analysis of variance to compare treatment regression parameters. Differences among treatments were considered statistically significant at $P< 0.05$.

2.4 Results

Abalone fed a diet of kelp will typically invest more feed into length, quite often at the expense of weight (Troell et al. 2006). Growth trends in length are therefore often considered more reliable when determining farm management procedures for abalone fed kelp. This considered, the data indicate that the control animals outperform the treatment animals (Fig. 1) with a DISL of 38.123 μm/day (Table 1). No significant differences (P = 0.0975) were found between treatments 1 and 2 with regards to mean shell length gain for the entire experimental period (Fig. 1, Table 1). The performance in length in the control animals, however, comes at the expense of weight (Fig. 2) and the control is not the better performing treatment in terms of specific weight gain (SGR values in Table 1).

Upon closer examination of the actual rates of growth (visible by the slopes of the graphs) during the latter months (2-6) of the experiment, it is evident that something quite different is happening (see Figs. 3 & 4, Table 1). The data for the latter 2-6 months show that there are still no significant differences between treatments 1 and 2 for both mean shell length (P = 0.6046) and weight (P = 0.2063); both treatments, however, perform significantly better than the control (Figs. 3 & 4; see Table 1 for statistical significance). This is clearly evident from the DISL and SGR values obtained for the period 2-6 months (Table 1). Also, the treatment animals have higher feed conversion efficiencies (FCE) despite the control animals receiving more feed overall.

DISL, SGR and FCE values often mean little to the abalone farmer. When the data are converted into values that the abalone farmer can better understand, we see clearly that the treatment animals perform better than the control animals (Table 2). Despite the control animals gaining more length over the entire experimental period, much more kelp was

required to produce an equivalent amount of length in the control animals compared to the treatment animals. In terms of weight gain and the amount of kelp required to produce a net gain in weight, both sets of treatment animals perform better than the control animals. Overall, the treatment animals have higher feed conversion efficiencies that are even more striking for the latter four months (2-6) of the experiment.

2.5 Discussion

While abalone characteristically invest more feed into shell length than into body weight when fed kelp (because of the high ash and low protein contents – Troell et al. 2006), to the abalone farmer weight gain is more important. This is because revenue is generated on a per-weight-basis. Also, it is generally assumed that providing abalone with more kelp feed than they typically require, will produce optimum growth. This study has, however, shown that periodic bouts of starvation actually benefit the abalone and consequently the abalone farmer as well.

Starvation or restricted feeding is not unusual to marine invertebrates as food periodically becomes scarce or unavailable to them (see e.g. Durazo-Beltrán et al. 2004). It has been documented (e.g. Carefoot et al. 1993, Takami et al. 1995) that abalone can withstand long periods of starvation before body reserves are depleted. This is usually achieved by first metabolising carbohydrates and lipid stores, and then later body proteins (Roberts et al. 2001). Segawa (1991) suggested that carbohydrates and lipids were utilized during normal feeding, and that after about two weeks' starvation, proteins were metabolised as the main energy source. This, however, did not apply to the present study as abalone were never starved for periods longer than 1 to 2 days.

In starved molluscs, metabolic rates tend to decrease as starvation progresses (Gaty and Wilson 1986, Carefoot 1987). Many studies (e.g. Quinton and Blake 1990, Jobling and Koskela 1996) have shown that like dietary composition, reproductive state, and unfavourable environments, food restriction or starvation often causes an animal to display compensatory growth. Compensatory growth may be defined as the ability to display a rapid growth spurt when returned to full rations following brief periods of food restriction or

starvation (Weatherly and Gill 1981, Miglavs and Jobling 1989, Rueda et al. 1998). Compensatory growth is usually accompanied by hyperphagia (i.e. an increase in food intake – Gurney 2004) and displays itself in improved feed conversion efficiency values (Greef et al. 1986, Miglavs and Jobling 1989). This is clearly evident in the high FCE values obtained by the treatment animals.

In conclusion, once the "starved" animals (T1 & T2) overcame the adjustment period, their presumed slowed metabolism resulted in rapid weight gain through compensatory growth. Periodic kelp starvation is thus not necessarily detrimental to the South African abalone *H. midae* and may indeed be beneficial as this study has shown, no doubt because of the positive effects of compensatory growth. It should, however, be stressed that were the growth rates not examined so closely, one could so easily have missed the growth spurts evident in the latter months of the experiment. If the experiment were, however, run for a longer period, this trend would have been evident for the full data set. In addition to the main outcome of this research, this study has highlighted the importance of relatively long-term experimentation on relatively slow-growing organisms.

2.6 Acknowledgements

I would like to thank my supervisors Dr. Gavin Maneveldt and Mr. Jonathan Venter for their support and guidance of this research project. Their comments, advice and constructive criticisms were valuable and lead to the success of this research project. Thank you to the Department of Biodiversity and Conservation Biology at the University of the Western Cape and the Jacobsbaai Sea Products farm for providing research facilities and technical support. I would like to acknowledge the South African National Research Foundation (NRF) and the Frontier Programme, Marine and Coastal Management (M&CM) of the South African Department of Environmental Affairs and Tourism (DEAT) for additional research funding. Thank you to Dr. Deborah Robertson-Anderson (University of Cape Town) and Dr. Cliff Jones (Rhodes University) for providing valuable discussion and comments. I would also like to thank my family and friends and the staff of the Department of Biodiversity and Conservation Biology for their moral support and inspiration throughout this research period.

2.7 Tables

Table 1. Growth parameters for abalone from the three treatments. Daily increment increase in shell length (DISL – μm/day), specific growth rate (SGR – % body weight.day^{-1}) and feed conversion efficiency (FCE) are provided for the periods 0-6 months (0-6) and 2-6 months (2-6). Means with the same letter are not statistically different.

Treatment	Control	Treatment 1	Treatment 2
DISL (0-6)	38.123^{b}	33.102^{a}	30.478^{a}
DISL (2-6)	22.124^{a}	25.725^{ab}	27.896^{b}
SGR (0-6)	0.222^{a}	0.235^{b}	0.207^{a}
SGR (2-6)	0.112^{a}	0.136^{b}	0.130^{ab}
FCE (0-6)	0.020^{a}	0.025^{b}	0.022^{ab}
FCE (2-6)	0.010^{a}	0.015^{b}	0.014^{b}

Table 2. Length and weight gained by the animals from the three treatments and the amount of kelp required to produce 1mm of shell length and 1g of body weight. All data are provided for the periods 0-6 months (0-6) and 2-6 months (2-6). Means with the same letter are not statistically different.

Treatment	Control	Treatment 1	Treatment 2
Mean shell length (mm) gain (0-6)	7.015^{b}	6.091^{a}	5.608^{a}
Mean shell length (mm) gain (2-6)	2.721^{a}	3.164^{b}	3.431^{b}
Mean weight (g) gain (0-6)	22.968^{a}	25.441^{b}	22.101^{a}
Mean weight (g) gain (2-6)	8.775^{a}	11.179^{b}	10.293^{b}
Mean kelp consumed (g) by each individual abalone (0-6)	499.214^{b}	443.8^{a}	443.8^{a}
Mean kelp consumed (g) by each individual abalone (2-6)	376.614^{b}	331.8^{a}	331.8^{a}
Amount of kelp (g) required to produce 1mm shell length (0-6)	71.168^{a}	72.864^{a}	79.138^{b}
Amount of kelp (g) required to produce 1mm shell length (2-6)	138.397^{b}	104.862^{a}	96.699^{a}
Amount of kelp (g) required to produce 1g of abalone (0-6)	21.735^{b}	17.444^{a}	20.081^{b}
Amount of kelp (g) required to produce 1g of abalone (2-6)	42.919^{b}	29.680^{a}	32.235^{a}

2.8 Figure captions

Figure 1. Increase in abalone shell length.

Figure 2. Increase in abalone body weight.

Figure 3. Increase in abalone shell length with linear regression functions fitted for the periods 0-2 and 2-6 months. Cont = Control; Trt 1 = Treatment 1; Trt 2 = Treatment 2; B1 = mean for basket 1; B2 = mean for basket 2; Predicted = the statistically determined treatment mean determined by the split plot function.

Figure 4. Increase in abalone body weight with linear regression functions fitted for the periods 0-2 and 2-6 months. Cont = Control; Trt 1 = Treatment 1; Trt 2 = Treatment 2; B1 = mean for basket 1; B2 = mean for basket 2; Predicted = the statistically determined treatment mean determined by the split plot function.

2.9 Figures

Figure 1

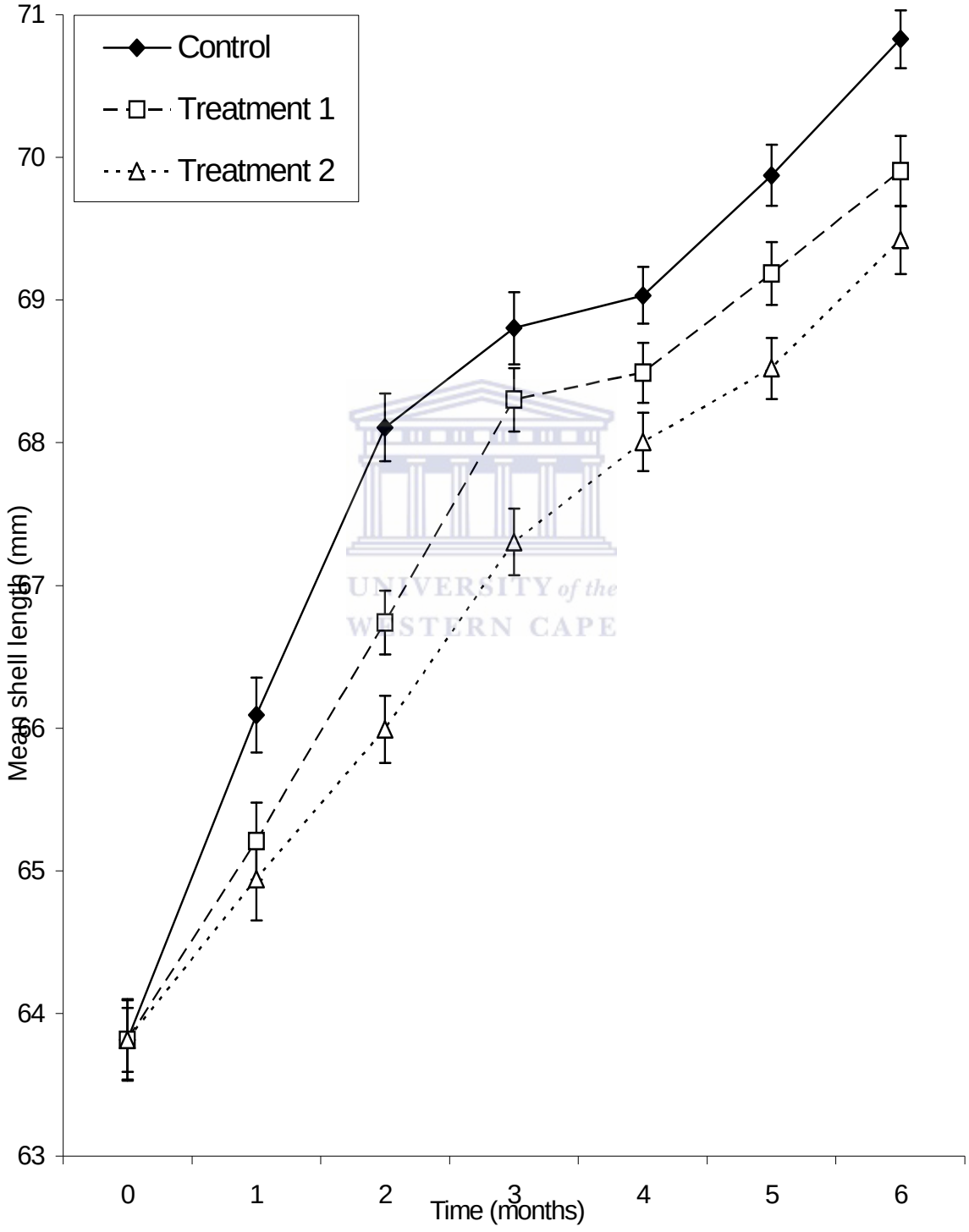


Figure 2

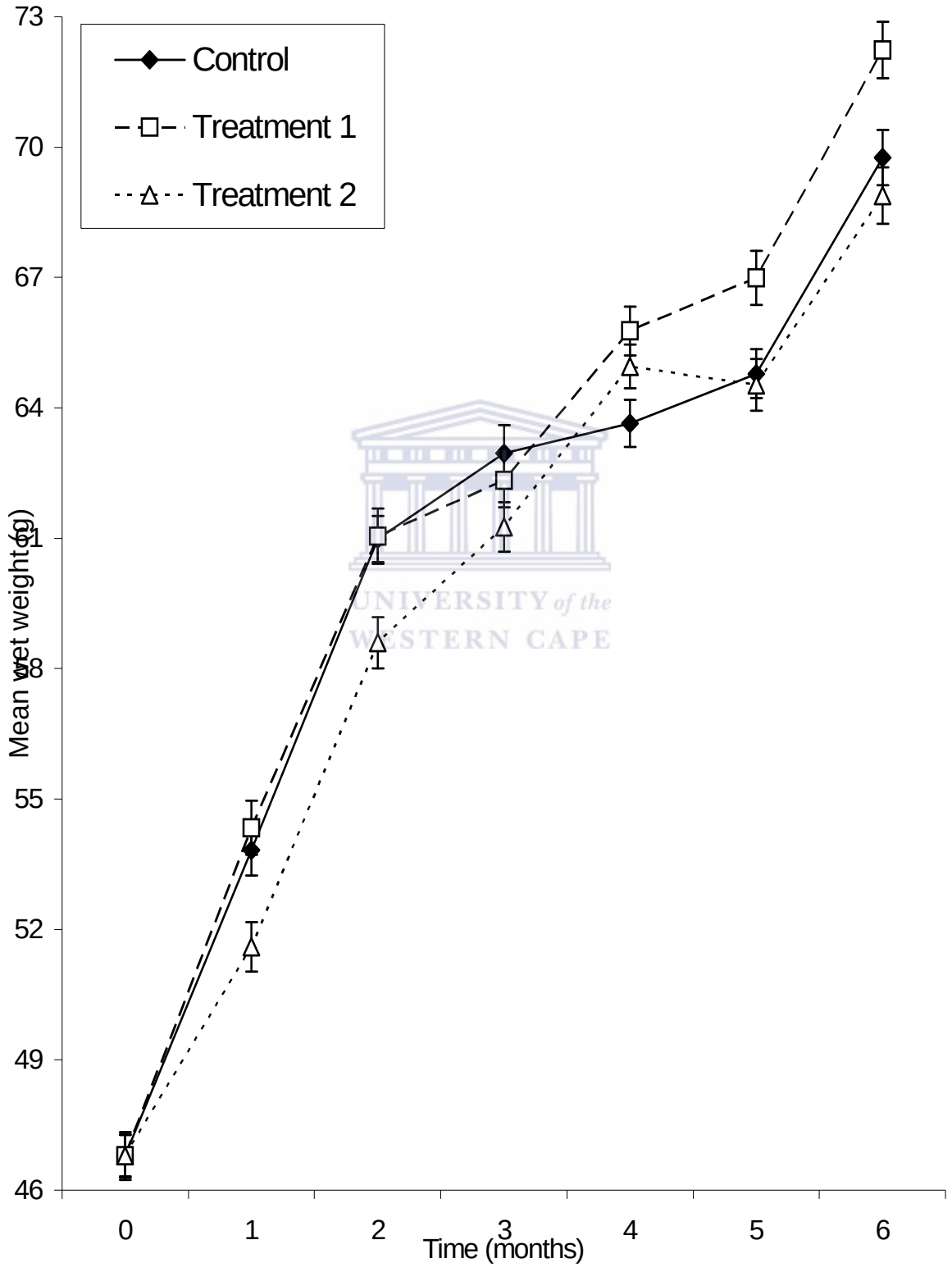


Figure 3

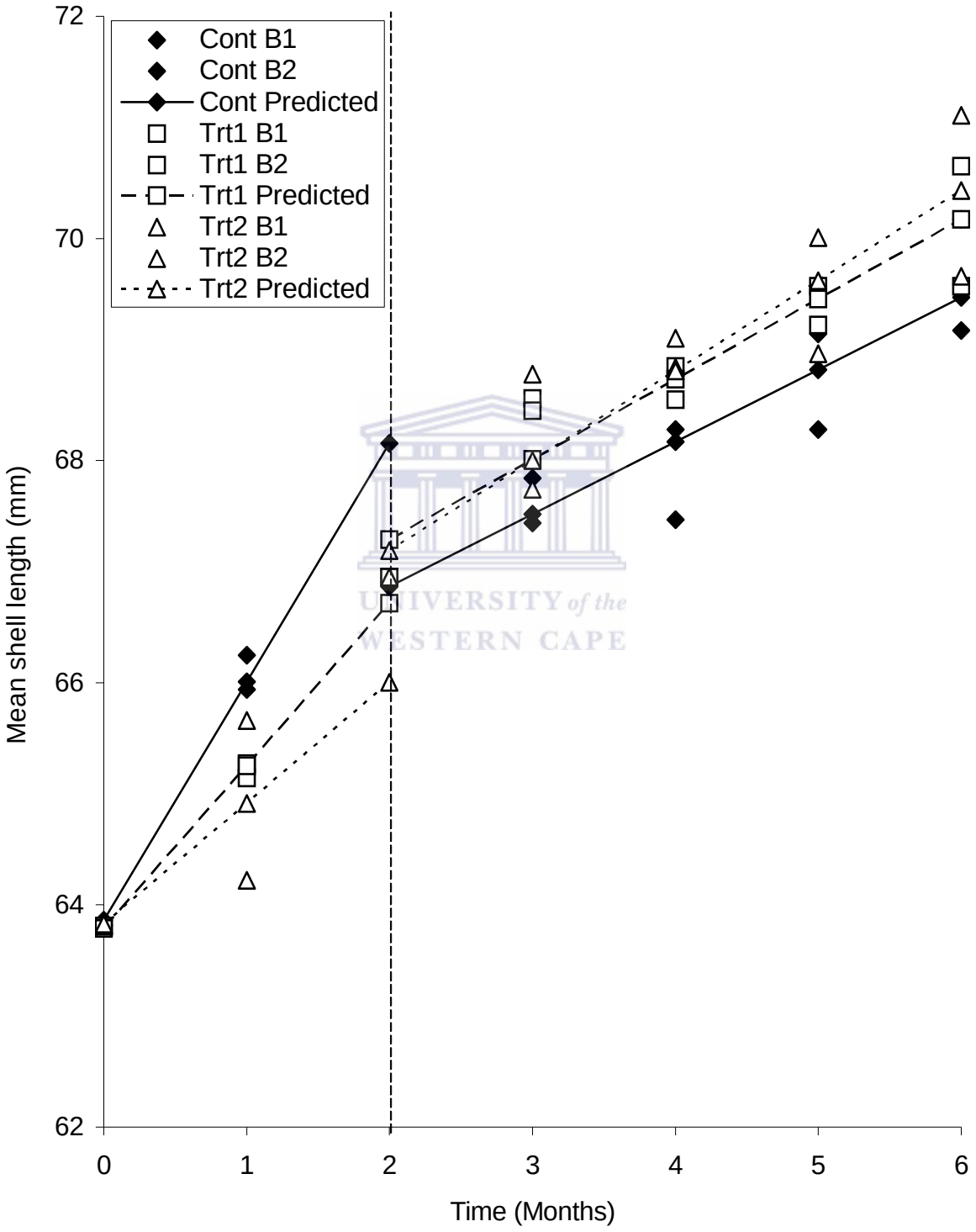
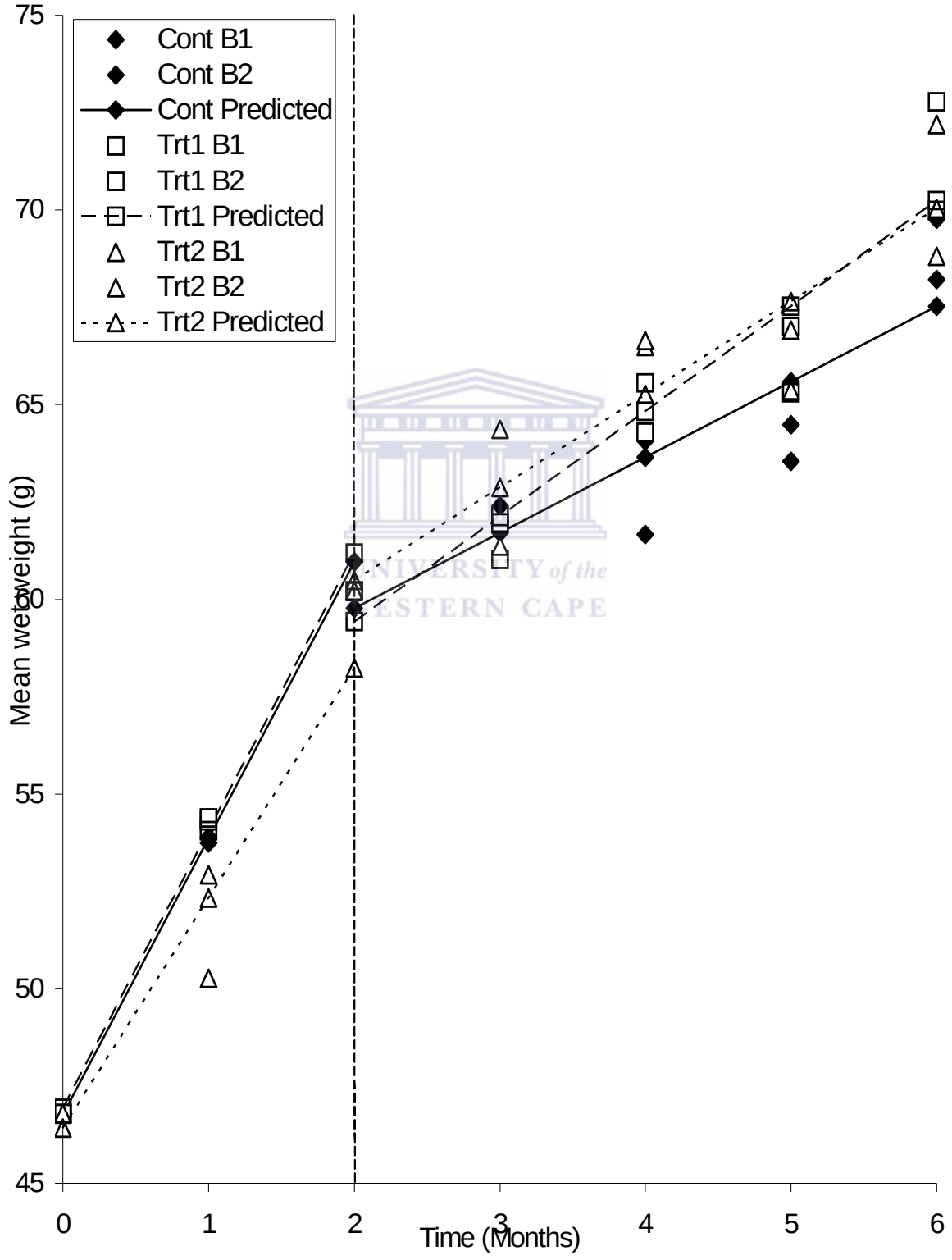


Figure 4



Comparing the growth of market-size abalone fed kelp versus the new low protein, commercially available Abfeed®-K26

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3.1 Abstract

Kelp (*Ecklonia maxima*) constitutes the major feed for farmed abalone, *Haliotis midae* Linnaeus, on the West and western South coast of South Africa. However, kelp is relatively low in protein content and is approaching limits of sustainable harvesting in kelp concession areas with high abalone farm concentrations. This has largely been the motivation for the development of a nutritionally complete, high protein feed, Abfeed®-S34 which contains 34% protein. Two of the negative effects of using Abfeed®-S34 is the higher incidence of sabellid infestation as the worms feed on the nutrient-rich faeces produced by the abalone, and the potentially negative impacts on water quality. This is particularly prevalent in culture environments with abalone of shell lengths >50mm and at relatively high water temperatures, and has prompted the development of a new low protein Abfeed®-K26 (26% protein) which does not induce these effects. The aim of our research was to compare the growth of grow-out abalone (abalone with a shell length >20mm) fed kelp with those fed the new Abfeed®-K26 in both a flow-through and a recirculation system on a local west coast commercial abalone farm. Such research has not been attempted on a commercial farm before. Results show that both feeds generally produce similar growth in abalone. No significant differences were found in shell length growth for either the recirculation ($P = 0.235$) or the flow-through ($P = 0.469$) systems for either feeds. While growth in body weight showed no significant differences in the recirculation system ($P = 0.522$), Abfeed®-K26 outperformed kelp in the flow-through system ($P = 0.014$). Abfeed®-K26 is doing exactly what it was designed to do and may no doubt prove to be of tremendous benefit to the abalone aquaculture industry as a kelp and Abfeed®-S34 substitute because it has most of the benefits of the high protein Abfeed®-S34, and none of its apparent disadvantages. However, in terms of purchasing costs, kelp is still the cheaper alternative for the JSP abalone farm.

Keywords: abalone, Abfeed®-K26, Abfeed®-S34, feed costs, formulated feed, growth, *Haliotis midae*, kelp.

3.2 Introduction

The growing South African abalone industry depends largely on a steady supply of feed resources (Troell et al. 2006). Kelp [*Ecklonia maxima* (Osbeck) Papenfuss] constitutes by far the major feed for farmed abalone on the West and western South coast of South Africa (Anderson et al. 2003, 2006, Troell et al. 2006). However, kelp is low in protein content (ca 5-15%) (Hahn 1989, Robertson-Anderson 2004, Troell et al. 2006). Furthermore, this resource is approaching limits of sustainable harvesting in kelp concession areas with high abalone farm concentrations (Anderson et al. 2003, 2006). These factors have been the motivation for the development of more nutritionally complete, high protein formulated feeds (Sales and Britz 2001, Bautista-Teruel et al. 2003, Sales and Janssens 2004, Troell et al. 2006).

The commercially available formulated feed, Abfeed® is currently the most widely used commercial abalone feed in South Africa (Troell et al. 2006) with a high protein version (Abfeed®-S34) being the first to be tested. In general, commercially grown abalone have been found to grow best on Abfeed®-S34, at least until they reach 50mm in shell length, with most farmers using it in the early stages of development (Troell et al. 2006). Once abalone reach 50mm in shell length, abalone farmers prefer to feed them kelp or a combination of kelp and Abfeed®-S34 for a number of reasons. Firstly, although kelp has a higher food conversion ratio (FCR) (Hahn 1989, Britz 1996a) and thus lower feed conversion efficiency (FCE), it is cheaper than Abfeed®-S34. Secondly, once abalone reach 50mm in shell length, the Abfeed®-S34 promotes a higher incidence of sabellid infestation (particularly on farms with poor water quality systems) since the worms feed on the nutrient-rich faeces produced (Simon et al. 2004, Troell et al. 2006). Third, the negative impacts on water quality of Abfeed®-S34 are greater than kelp, particularly at higher temperatures (Jones and Britz

2006). Forth, kelp is relatively high in ash (25% on a dry-weight basis) and thus rich in minerals (Troell et al. 2006) and often results in higher shell growth rate compared to Abfeed®-S34. Most, if not all of these factors have been the reason for the development of the new low protein, Abfeed®-K26. Now, the "old" Abfeed®-S34 is used for juvenile abalone less than 50mm in shell length, and the new low protein Abfeed®-K26 is used for abalone larger than 50mm in shell length.

A lack of information exists for abalone farmers, concerning alternative formulated feeds for grow-out abalone (abalone with a shell length of >20mm) cultured in various systems. While unpublished data exists (e.g. Jones and Britz 2006), no published accounts exist to compare results obtained with the new Abfeed®-K26. The aim of this research was therefore to compare the growth of abalone fed kelp versus those fed the new Abfeed®-K26 in a flow-through and a recirculation system on a commercial abalone farm situated on the South African west coast.

3.3 Materials and Methods

3.3.1 Experimental system

The research was conducted at the Jacobsbaai Sea Products (JSP - 17° 53' 12.5" E, 32° 58' 2.5" S, Western Cape, South Africa) abalone farm. Abalone were grown in a recirculation (200-300 $L.h^{-1}$; 60-90 hours exchange rate) and a flow-through (850-1300 $L.h^{-1}$) system. Water was supplied at a temperature of 16.05±0.48°C, and 13.8±0.76°C in the recirculation and flow-through systems respectively.

3.3.2 Experimental animals

Grow-out abalone (abalone with a shell length > 20mm) were supplied by the Jacobsbaai Sea Products abalone farm. Since growth of abalone is variable, individuals of similar size and of the same gene pool (spawned in May 2002) were used. The abalone were subdivided into two replicate baskets of approximately 12.5kg (± 250 individuals). Initial body weight and shell length of the abalone were measured at 45.65g ± 0.26 and 63.13mm ± 0.14 respectively. Both body weight and shell length were measured monthly.

3.3.3 Treatments

Two diet treatments (each with two replicates) were tested in each of the recirculation and flow-through systems.

Treatment 1: fresh kelp [*Ecklonia maxima* (Osbeck) Papenfuss] with a protein content of ca 5-15% was supplied *ad libitum*. Maintained daily, deteriorating kelp was always removed and fresh kelp was topped up.

Treatment 2: Abfeed®-K26

Abfeed®-K26 (Marifeed Pty Ltd, South Africa) is a commercially available abalone formulated feed containing kelp, formalin-free fishmeal, binders, vitamins, minerals and soya. The approximate composition of Abfeed®-K26 is given in Table 1. Animals were fed as per the manufacturer's prescription per mean body weight.

3.3.4 Sample and data collection

The experiment was conducted over six months. Representative animals were randomly selected from each basket (n = 30 per replicate at 0-3 months; n = 40 per replicate at 4-6 months to compensate for later differential growth). Before all measurements, animals were blotted dry to remove excess water. Body weight was recorded to the nearest 0.01g using an electronic balance. Shell length was measured along the longest axis of the abalone shell to the nearest 0.1mm with a vernier callipers.

Daily increment in shell length (DISL) was calculated using the formula of Mai et al. (2001) and Zhu et al. (2002):

$$DISL\ (\mu m/day) = [(SL_t - SL_i)/\ t] \times 1000$$

Where SL_t = the final mean shell length (mm), SL_i = the initial mean shell length (mm), and t = the feeding period in days.

Specific growth rate (SGR in % body weight.day^{-1}) was calculated using the formula of Britz (1996a):

$$SGR = [(\ln(W_f) - \ln(W_i)) / t] \times 100$$

Where $\ln(W_f)$ = the natural log of the final mean weight of abalone, $\ln(W_i)$ = the natural log of the initial mean weight of abalone, and t = the feeding trial period in days.

The Feed Conversion Efficiency (FCE) was calculated for each treatment, using the formula of Simpson and Cook (1998):

$$FCE = (growth/ration) \times 100$$

Where growth = the blotted wet weight (g) gained per day and ration = the blotted wet feed (g) intake per day.

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

$$CF\ (g.mm^{-1}) = [BW\ (g) / SL\ (mm)^{2.99}] \times 5575$$

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

3.3.5 Statistical analyses

All data were expressed as means ± se. Data for all experimental replicates were pooled as no significant differences were found between them. To compare the differences between the final means of the two diet treatments over time in both systems, a two-factor t-test was used. To test for correlation, the body weight and shell length of abalone from each diet treatment were compared by means of a linear regression test. Differences amongst treatments were considered statistically significant at $P < 0.05$

3.4 Results

Both feeds (kelp and Abfeed®-K26) reflect a positive correlation between body weight gain and growth in shell length (Table 2) for both systems. The data show no significant differences in the growth in shell length for either the recirculation (P = 0.235) or the flow-through systems (P = 0.469) for both kelp and Abfeed®-K26 (see Figs. 1 & 3). This is supported by the daily increment in shell length (DISL) data obtained (Table 2). While increase in body weight showed no significant difference in the recirculation system (P = 0.522) (Fig. 2), differences were found between the two feeds within the flow-through system (P = 0.014) (Fig. 4). Again this is supported by the specific growth rate (SGR) data (Table 2). However, FCE values were substantially higher for animals fed Abfeed®-K26 compared to those fed kelp (Table 2). While all animals showed positive (i.e. >1; relatively "fat" individuals, see Britz 1996a) condition factors at the start of the experiment, this trend was maintained throughout. The animals cultured in the recirculation system, however, despite having lower initial CF values, produced relatively "fatter" individuals (see Table 2). Similarly, although not comparable, the recirculation system did produce relatively higher growth values (Table 2).

DISL, SGR and FCE values often mean little to the abalone farmer. When the data are converted into values that the abalone farmer can better understand, we see clearly that those animals fed Abfeed®-K26 require much less feed to produce comparable growth than those fed kelp (see Table 3). Roughly eight (shell length) to nine (body weight) times more kelp than Abfeed®-K26 was required to produce an equivalent increase in both shell length and body weight. This result comes through strongly in the higher FCE values obtained in both systems by those abalone grown on Abfeed®-K26 (see Table 2). However, when one considers the actual purchasing costs of the feeds (see proportionate cost to produce 1mm

shell length and 1g of abalone – Table 4) it will cost the JSP abalone farmer twice as much to produce a comparable amount of growth using Abfeed®-K26.

3.5 Discussion

The results of this study are consistent with previously unpublished research. Work by Jones and Britz (Clifford Jones, *pers. comm.*) have shown that kelp could be included in artificial diets (i.e. Abfeed®-K26) and that reducing the protein level in diets of abalone larger than 50mm could be done without compromising growth. In addition, their studies have shown that the new Abfeed®-K26 produced growth in large (>50mm) abalone that was comparable to that of abalone fed the Abfeed®-S34 feed. Our data show similar trends in that the growth of abalone fed kelp and Abfeed®-K26, are comparable. What was striking in our study was that substantially less Abfeed®-K26 (11.92-12.97% - shell length; 11.29-12.53 – body weight) relative to kelp was required to produce this comparable growth (see Table 3). Also, it was suggested that temperatures of grow-out culture systems should be above 16ºC to yield optimum growth rates with Abfeed®-K26 (Marifeed Pty Ltd, South Africa). Our data show that even at lower temperatures (13.8±0.76°C), abalone fed Abfeed®-K26 cultured in a flow-through system, perform better than those fed kelp, supporting the use of the new low protein Abfeed®-K26.

Protein is the most expensive component in artificial feeds (Fleming et al. 1996). Although the production of nutritionally balanced diets have been identified as crucial to the success of the South African abalone aquaculture industry, many farmers require more from a feed than nutritional quality. Cost-effectiveness is proving to be equally important. Despite eight to nine times more kelp than Abfeed®-K26 being required to produce comparable growth, the absolute cost of feeding kelp on the JSP farm, is still substantially lower than Abfeed®-K26 (up to 2X greater) because the purchasing cost of kelp is so much cheaper than that of Abfeed®-K26. However, it must be emphasized that this cost does not take into account other expenses (e.g. transport, labour, time, etc) that potentially would affect the cost of

feeding either kelp or Abfeed®-K26. In addition, it has been reported that a number of South African abalone farms are achieving substantially better growth than the JSP farm when feeding Abfeed®-K26 (Peter Britz, *pers. comm*.). On such farms, no doubt different cost ratios will be achieved.

In conclusion, using Abfeed®-K26 could be seen as an alternative feed source for future abalone aquaculture since kelp is not only low in protein content, but also becoming increasingly limited in availability because it is approaching limits of sustainable harvesting in kelp concession areas with high abalone farm concentrations. In addition, Abfeed®-K26 has all the benefits of both kelp (high ash content for production of shell growth) and Abfeed®-S34 (relatively higher protein content for producing meat weight gain), but none of the disadvantages of both kelp (increasingly limited availability and low protein content) and Abfeed®-S34 (higher incidence of sabellid infestation and pollution problems). All in all, the new Abfeed®-K26 is doing exactly what it was designed to do, and may no doubt prove to be of tremendous benefit to the abalone aquaculture industry, particularly to those farms located substantial distances from natural kelp.

3.6 Acknowledgements

I would like to acknowledge all the people and institutions that contributed to the completion of this thesis. Thank you to my supervisors Dr. Gavin Maneveldt and Mr. Jonathan Venter for their guidance, assistance and support. I would like to thank the Department of Biodiversity and Conservation Biology at the University of the Western Cape and the Jacobsbaai Sea Products farm for providing research facilities and technical support. Thank you to the South African National Research Foundation (NRF) and the Frontier Programme, Marine and Coastal Management (M&CM) of the Department of Environmental Affairs and Tourism (DEAT) for research funding. Thank you to Dr. Cliff Jones (Rhodes University), Prof. Peter Britz (Rhodes University) and Dr. Deborah Robertson-Anderson (University of Cape Town) for their valuable comments, advice and discussion. Thank you to my family and friends and the staff of the Department of Biodiversity and Conservation Biology for supporting and inspiring me throughout the duration of my research project.

3.7 Tables

Table 1. Abfeed®-S34 and Abfeed®-K26 analysis. Both feeds were sent to an independent laboratory (Animal Production Laboratory, Institute for Animal Production, Department of Agriculture: Western Cape, Elsenberg) for compositional analysis.

Feed	Moisture (%)	Ash (%)	Protein (%)	Fibre (%)	Fat (%)	Carbohydrate (%)	KJ/100g
Abfeed®-S34	~ 10	5.65	34.68	0.9	2.37	57.3	1890.2
Abfeed®-K26	~ 10	4.6	26.18	1.2	1.12	68.1	1677.1

Table 2. Growth parameters of abalone fed the low protein Abfeed®-K26 and kelp cultured in both a recirculation and a flow-through system. Specific growth rate (SGR-% body weight.day^{-1}), daily increment increase in shell length (DISL-µm/day), feed conversion efficiency (FCE), regression factor (r, r²) and Condition factor (CF), are provided for each treatment.

System	Feed	Final weight (g)	Final length (mm)	SGR	DISL	FCE	r	r²	Initial CF	Final CF
Recirculation	**Abfeed®-K26**	81.059±0.772^{a}	72.745±0.278^{a}	0.309^{a}	51.037^{a}	0.121^{b}	0.972	0.945	1.045	1.223
	Kelp	81.587±0.610^{a}	73.214±0.235^{a}	0.319^{a}	53.585^{a}	0.031^{a}	0.995	0.989	1.040	1.212
Flow-through	**Abfeed®-K26**	75.204±0.725^{b}	71.516±0.215^{a}	0.266^{b}	46.839^{a}	0.101^{b}	0.985	0.971	1.071	1.193
	Kelp	72.673±0.716^{a}	71.218±0.244^{a}	0.257^{c}	45.220^{a}	0.023^{a}	0.992	0.984	1.062	1.173

Table 3. Length and weight gained by the animals fed the low protein Abfeed®-K26 and kelp cultured in both a recirculation and a flow-through system and the amount of feed (wet weight) required to produce 1mm of shell length and 1g of body weight. Values in brackets are the corresponding dry weights and dry weight comparisons for Abfeed®-K26. Means with the same letter are not statistically different.

System	**Recirculation**		**Flow-through**	
Feed	**Abfeed®-K26**	**Kelp**	**Abfeed®-K26**	**Kelp**
Mean shell length (mm) gain	9.391^{ab}	9.860^{a}	8.618^{b}	8.320^{bc}
Mean weight (g) gain	35.437^{a}	35.964^{a}	29.519^{b}	26.987^{c}
Mean amount of feed consumed (g) by each individual abalone	158.088^{b} (75.84^{a})	614.252^{c}	158.088^{b} (75.84^{a})	614.252^{c}
Amount of feed (g) required to produce 1mm shell length	16.834^{b} (8.08^{a})	62.300^{c}	18.343^{b} (8.80^{a})	73.825^{d}
Amount of feed (g) required to produce 1g of abalone	4.461^{c} (2.14^{a})	17.080^{e}	5.356^{d} (2.57^{b})	22.761^{f}
Ratio of feed required to produce 1mm shell length	1	3.701 (7.71)	1	4.025 (8.39)
% feed proportionately required to produce 1mm shell length	27.02 (12.97)	100	24.84 (11.92)	100
Ratio of feed required to produce 1g of abalone	1	3.829 (7.98)	1	4.250 (8.856)
% feed proportionately required to produce 1g of abalone	26.12 (12.53)	100	23.53 (11.29)	100

Table 4. Purchasing cost comparison of using kelp (wet weight) versus Abfeed®-K26 (dry weight). Note that other expenses (e.g. transport, labour, time, etc) are not included in this table.

	Recirculation		Flow-through	
	Abfeed®-K26	**Kelp**	**Abfeed®-K26**	**Kelp**
Purchasing cost per ton (ZAR)	13,500	800-1,100	13,500	800-1,100
Purchasing cost per g (ZAR)	0.0135	0.0008-0.0011	0.0135	0.0008-0.0011
Proportional cost	12.27- 16.88	1	12.27- 16.88	1
Amount of feed (g) required to produce 1mm shell length	8.08[a]	62.300[b]	8.80[a]	73.825[c]
Ratio of feed required to produce 1mm shell length	1	7.71	1	8.39
Cost to produce 1mm shell length (ZAR)	0.109	0.050-0.069	0.119	0.059-0.081
Proportionate cost to produce 1mm shell length	1.58-2.18	1	1.47-2.02	1
Amount of feed (g) required to produce 1g of abalone	2.14[a]	17.080[c]	2.57[b]	22.761[d]
Ratio of feed required to produce 1g of abalone	1	7.98	1	8.856
Cost to produce 1g of abalone (ZAR)	0.029	0.014-0.019	0.035	0.018-0.025
Proportionate cost to produce 1g of abalone	1.53-2.07	1	1.40-1.94	1

3.8 Figure Captions

Figure 1. Increase in shell length in the recirculation system using Abfeed®-K26 and kelp.

Figure 2. Increase in body weight in the recirculation system using Abfeed®-K26 and kelp.

Figure 3. Increase in shell length in the flow-through system using Abfeed®-K26 and kelp.

Figure 4. Increase in body weight in the flow-through system using Abfeed®-K26 and kelp.

3.9 Figures

Figure 1

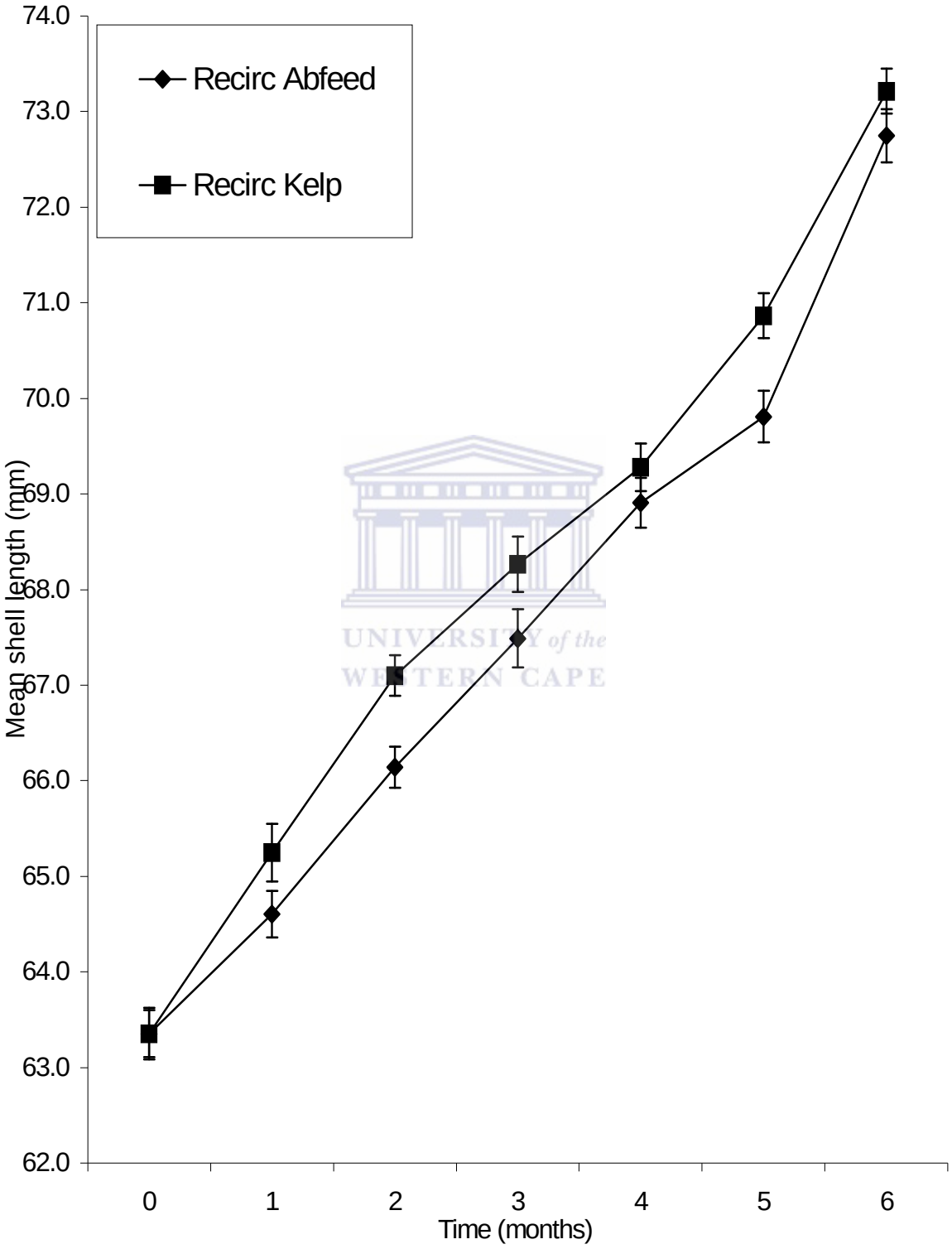


Figure 2

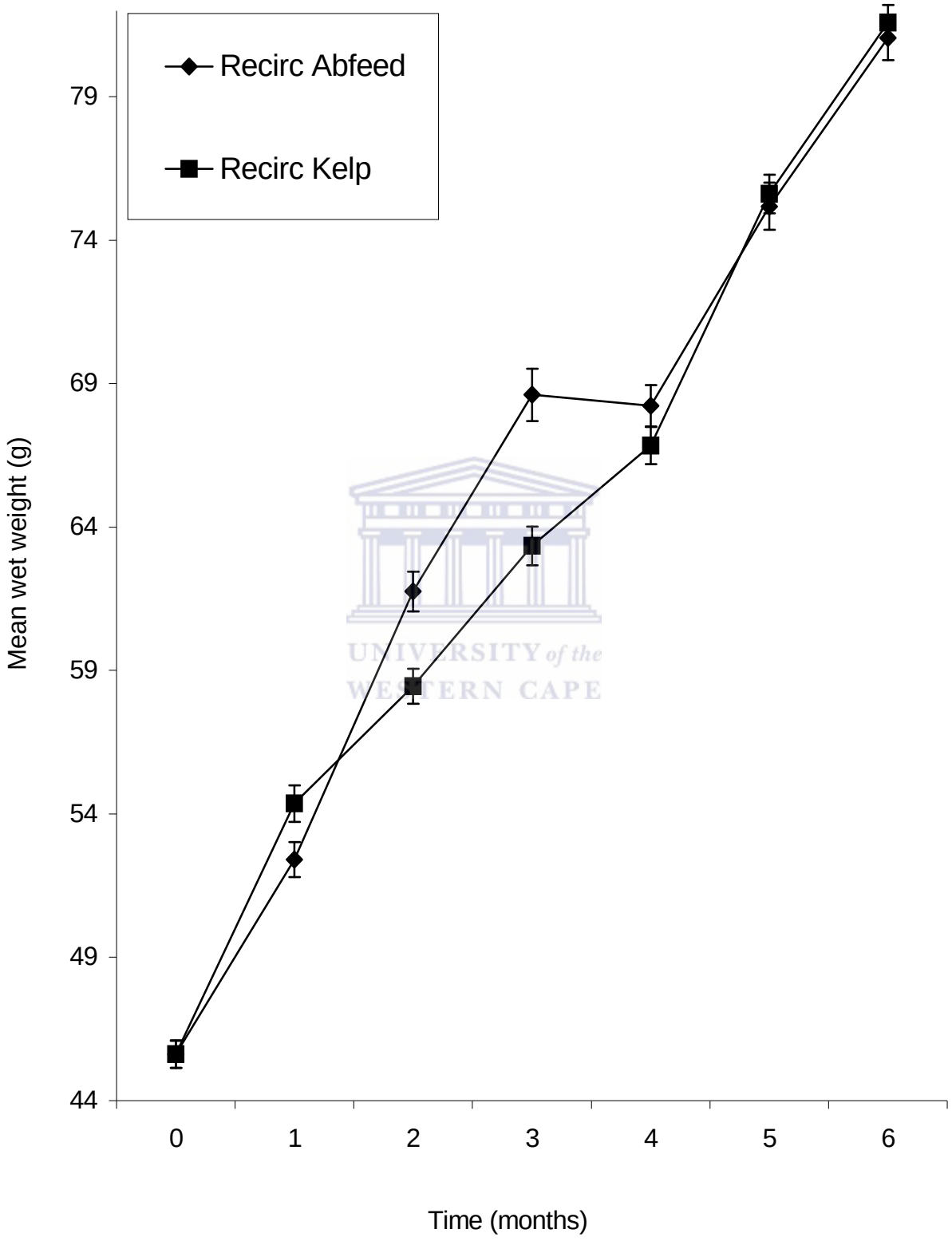


Figure 3

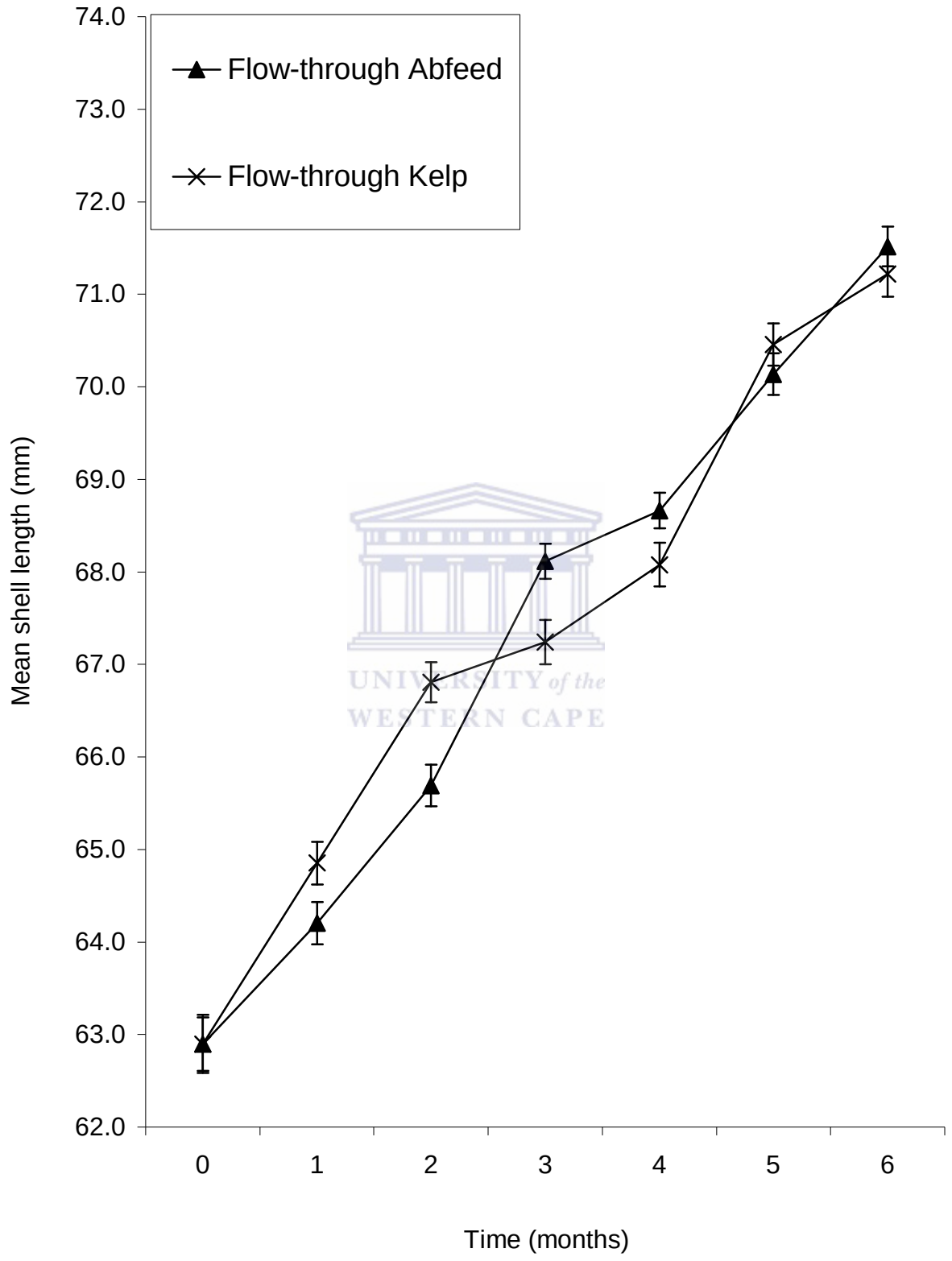
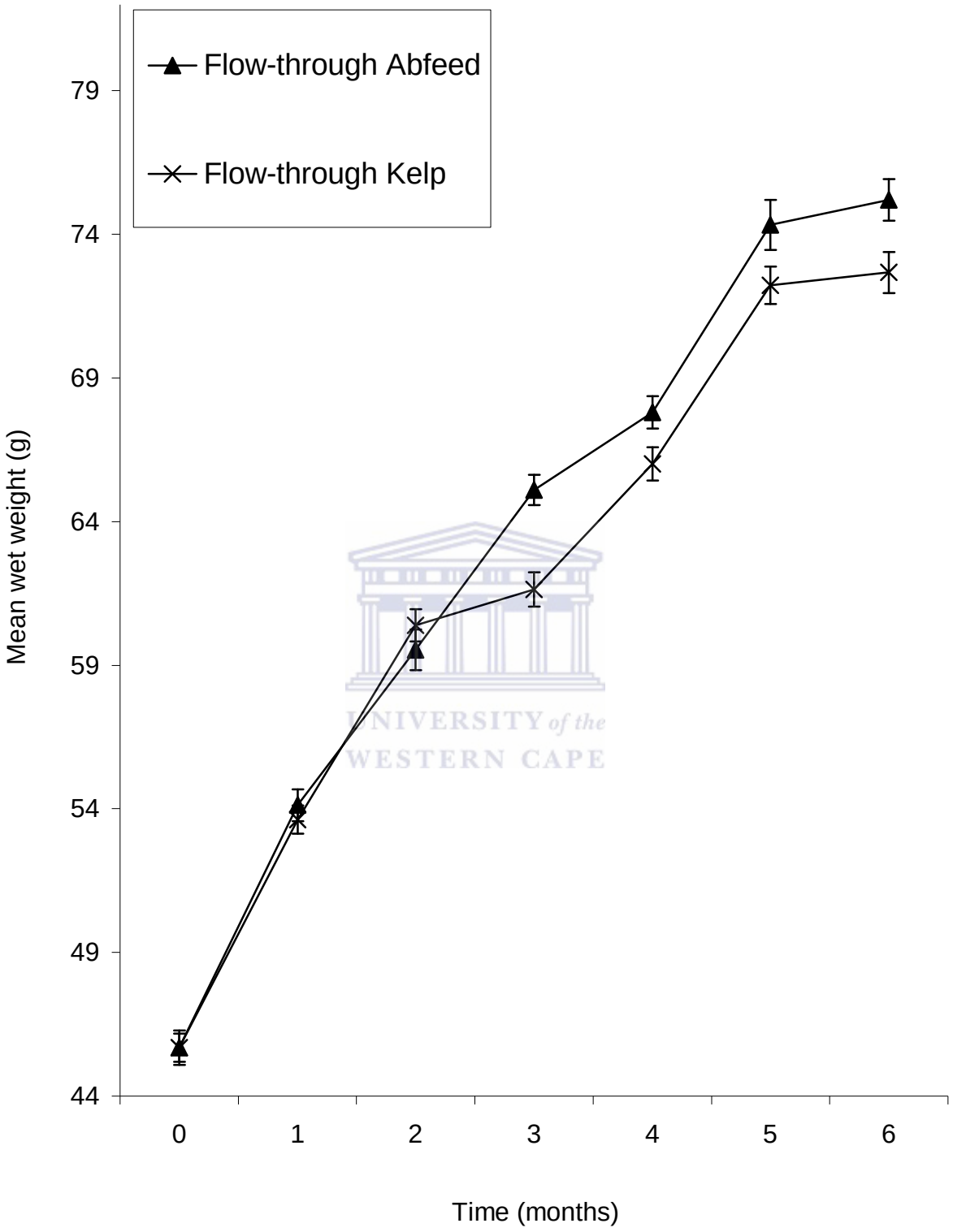


Figure 4



Effects of cultivation treatments, feed and export protocols on the recovery response of commercially farmed *Haliotis midae* Linnaeus

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4.1 Abstract

Live abalone are usually exported in polystyrene containers on ice in plastic bags containing 100% oxygen humidified with seawater for 30 to 42 hrs. Through this process, they tend to lose 4-15% of their body mass due to evaporation and pedal mucous production. Little information exists regarding live export protocols to decrease transport mortalities and weight loss during the exportation of live abalone. The aim of this research was to assess various export protocols and then to determine the best growth environment to allow rapid weight gain in the abalone, *Haliotis midae* Linnaeus returned from the export simulation. Grow-out abalone were cultured in both a flow-through and a recirculation system. Abalone were fed one of two feeds (the formulated feed Abfeed®-K26 and kelp) and subdivided into replicate baskets of ±250 abalone per replicate. Abalone were grown in a series of culture treatments to determine the effect that cultivation history may have on the responses to an export simulation. Prior to the export simulation, abalone were purged of their gut contents. An export simulation was then run for 36 hours. Our data show that prior culture history (system and feed) affects the recovery response in exported abalone. Firstly, prior cultivation history appears to determine how abalone respond to the purging process. Thereafter, the type of feed that had been provided, determines their recovery response. The data also suggests that over-handling affects the recovery response in abalone returned from an export simulation. Four of the eight treatments in which abalone were fed Abfeed®-K26 regained their post-purging weights after the export simulation, while none of those fed kelp, regained their post-purging weights.

Keywords: Abalone, Abfeed®-K26, cultivation history, export protocol, flow-through, growth, *Haliotis midae*, kelp, recirculation,

4.2 Introduction

Of the six species of abalone found in South Africa, only *Haliotis midae* Linnaeus is of commercial importance (Cook 1998, Sales and Britz 2001, Evans et al. 2004). Abalone farming is a relatively new activity in South Africa having only started in the late 1980's when Genade et al. (1988) demonstrated that it was possible to spawn *H. midae* in captivity. Since then, the development of South African abalone culture technology has been based on a combination of technology transfer and on local innovations by the industry in partnership with various research institutions (Sales and Britz 2001). South Africa has become the largest abalone producer outside Asia (FAO 2004) with production steadily increasing (FAO 2006), and over-exploitation of wild stocks by poaching and high market prices have largely been the main drivers for its cultivation (Troell et al. 2006). Consequently aquaculture of *H. midae* is rapidly becoming an economically important industry in terms of job creation and through exportation generated foreign income (Macey and Coyne 2005, Troell et al. 2006).

Farm grown *H. midae* are currently destined for freezing, canning or live export to the Far East (Gordon and Cook 2001, Vosloo and Vosloo 2006). These abalone are generally acclimatized before transportation (export) to reduce stress. This is usually done by reducing the water temperature just prior to exportation to slow down the metabolism of the abalone so that less oxygen is used (Cook and Ruck 1991). During live exportation, abalone are generally transported in polystyrene containers on ice in plastic bags containing 100% oxygen humidified with seawater (Sales and Britz 2001, O'Omolo et al. 2003, Vosloo and Vosloo 2006). Containers are sealed and are only opened once they arrive at their destination, which is usually between 30 and 42 hours later (Sales and Britz 2001). Despite these precautions, abalone still generally lose 4-15% of their body mass due to evaporation and pedal mucous production (Vosloo and Vosloo 2006). As abalone are sold by weight, weight

loss is critical for exporters and so speed of delivery is important. For this reason, all exportation is done exclusively by air (O'Omolo et al. 2003).

Exporters are paid on landed mass and since there is generally a loss of weight during exportation, foreign revenue decreases (Vosloo and Vosloo 2006). The development of live export protocols to minimize transport mortalities and weight loss in abalone are thus needed. The aims of this research were: 1) to determine the effects of cultivation treatments and feed on the recovery of commercially farmed *H. midae* from an export simulation; and 2) to determine the best growth environment to allow rapid weight gain in abalone returned from the export simulation. This research is intended to contribute to recommendations toward best on-farm practice in the culture of the South African abalone, *H. midae*.

4.3 Materials and Methods

4.3.1 Experimental system

The research was conducted at the Jacobsbaai Sea Products (17° 53' 12.5" E, 32° 58' 2.5" S, Western Cape, South Africa) abalone farm. Abalone were cultured in a flow-through system with a seawater flow rate of 850-1300 $L.h^{-1}$ and a recirculation system with a flow rate of 200-300 $L.h^{-1}$ and an exchange rate of every 60-90 hours. Water was supplied at a temperature of 13.8±0.76°C and 16.05±0.48°C in the flow-through and recirculation systems respectively.

4.3.2 Experimental animals

Grow-out abalone (abalone with a shell length > 20mm) were supplied by the Jacobsbaai Sea Products abalone farm. Since growth of abalone is variable, individuals of a similar size and of the same gene pool (spawned in May 2002) were used. The abalone were subdivided into replicate baskets of 12.5 kg abalone (± 250 individuals). Initial body weight and shell length measured 45.65g ± 0.26 and 63.13mm ± 0.14 respectively.

4.3.3 Culture history

To determine the possible effects of the past culture history and the recovery of abalone from the exportation process, a series of growth history scenarios was established and maintained over 6-months. At the start of the experiment, abalone were cultured in both the flow-through (Ft) and the recirculation systems (R). This was run for three months (see Fig. 1). Thereafter, each basket was split and subdivided into yet another two replicate baskets containing ± 125 individuals. One half of the original animals were kept in their original system (e.g. R-R),

while the other half was placed into the other system (e.g. R-Ft) (see Fig. 1). The abalone were cultured for another three months.

4.3.4 Export simulation

Prior to the export simulation, abalone were again split and subdivided into yet another two replicate baskets, this time containing only 50 individuals per basket. In keeping with the exportation process, abalone were purged of their gut contents for approximately two days (48 hrs) in purging tanks with a flow rate of approximately 1700 $L.h^{-1}$ at a temperature of 13.5±1.0°C. Thereafter an export simulation was run for approximately 36 hours. This entailed packing the animals in polystyrene containers on ice in plastic bags in a cold storage room at a temperature of 12-14°C. After the export simulation, abalone were placed into culture systems with 50 of the original animals again remaining in their original systems (e.g. R-R-R) while 50 were transferred to the other system (e.g. R-Ft-R) (see Fig. 1). This system of splitting and transfer across to the opposite culture system was employed to determine whether indeed past growth-history plays a role in the recovery process following an export simulation. This was planned because often when animals meant for export are returned to the farms (for whatever reason) the farmer typically places the recovering animals in whatever system is available to them at the time. Hereafter, abalone were bulk weighed (to reduce the added stress of individual handling) every 24 hours to monitor their rate of weight gain following the export simulation.

4.3.5 Sampling and data collection

To avoid added stress to the abalone, only bulk weights were determined for the initial (pre-purged), post-purged and post-export simulation periods. Prior to weighing, animals were drip dried to remove excess water and bulk weights recorded using an electronic balance.

Specific growth rate (SGR in % body weight.day^{-1}) was calculated for the post-purged and post export simulation periods using the formula of Britz (1996a):

$$SGR = [(\ln(W_f) - \ln(W_i)) / t] \times 100$$

Where $\ln(W_f)$ = the natural log of the final mean weight of abalone; $\ln(W_i)$ = the natural log of the initial mean weight of abalone; and t = the feeding trial period in days.

The Feed Conversion Efficiency (FCE) was calculated for only some of the system histories (i.e. Ft-Ft-Ft, Ft-Ft-R, Ft-R-Ft and R-Ft-R) using the formula of Simpson and Cook (1998):

$$FCE = (growth/ration) \times 100$$

Where growth = the drip dried wet weight gained per day; ration = the blotted wet feed intake per day.

4.3.6 Treatments

To determine the possible effects that feed may have on the post export simulation recovery of abalone, two diet treatments (each with two replicates) were tested in both the recirculation and flow-through systems.

Treatment 1: Fresh kelp [*Ecklonia maxima* (Osbeck) Papenfuss] with a protein content of ca 5-15% was supplied *ad libitum*. Maintained daily, deteriorating kelp was always removed and fresh kelp topped up.

Treatment 2: Abfeed®-K26

Abfeed®-K26 (Marifeed Pty Ltd, South Africa) is an abalone formulated feed containing kelp, formalin-free fishmeal, binders, vitamins, minerals and soya. The appropriate analysis of Abfeed®-K26 is: 9.29% moisture; 4.6% ash; 26.18% protein; 1.2% fibre; 1.12% fat and 68.1% carbohydrates (Animal Production Laboratory,

Institute for Animal Production, Department of Agriculture: Western Cape, Elsenbery). Animals were fed as per the manufacturer's prescription per mean body weight.

4.3.7 Statistical analysis

Since only bulk weights and not individual measurements were used to obtain data during the experiment, comparative statistics could not be employed to test for variability amongst the individual treatments. Descriptive statistics were thus employed and data are expressed as means only.

4.4 Results

Animals that were grown in the recirculation tanks (R-R-R, Ft-R-R, Ft-R-Ft, R-R-Ft) prior to the export simulation, all lost weight during the purging process irrespective of which feed they had been provided (see Figs. 2 & 3). In contrast, those animals that were grown in the flow-through system (Ft-Ft-R, R-Ft-R, Ft-Ft-Ft, R-Ft-Ft) all gained weight following their purge. These responses are also reflected in the SGR and Mean Wet Weight Gain (MWWG) values obtained (Table 1). Prior cultivation history appears thus to be of primary importance in affecting the response that abalone have to the purging procedure.

Animals fed kelp and that were grown in the recirculation system just prior to the export simulation, had the lowest post-purge weights. Conversely, the animals fed kelp, and that were grown in the flow-through system just prior to the export simulation, had the highest post-purge weights (Figs. 2 & 3, Table 1). Because of the different responses to the purging process due to prior cultivation history, it was necessary to adjust the data around a common post-purging mean (-36 hrs) in order to counter the effects of this initial response. The erratic growth responses suggest that the constant weighing of abalone must have had a negative impact on recovery (Figs. 4 & 5). This pattern is evident in the improbable FCE values obtained (see Table 2). The weight gained was more than likely due to re-absorption of moisture lost during the export simulation period, rather than due to actual feed consumed.

Although one of the kelp-fed treatments (R-R-R) regained their post-purging weight after 24 hrs, subsequent handling no doubt negatively impacted their responses. None of the abalone fed kelp regained their post-purging weight after 144 hrs (Fig. 4 & Table 1). Four of the eight treatments (i.e. Ft-R-Ft, Ft-R-R, R-R-Ft, R-Ft-R) fed Abfeed®-K26, however, recovered from the export simulation, regaining their post-purging weight (Fig. 5). It is

interesting to note that 3 of these 4 treatments were of animals that had been grown in the recirculation system prior to the export simulation. This is also evident from the SGR and MWWG values obtained for these system histories, with abalone fed Abfeed®-K26 generally recovering better from the export simulation than those fed kelp.

4.5 Discussion

Our data has shown that prior culture history (system and feed) affects the recovery response in exported abalone. Firstly, prior cultivation treatments (e.g. flow-through or recirculation) appeared to determine how well abalone responded to the purging process. Secondly, the type of feed that had been provided seemed to determine and affect the recovery response in abalone that underwent export procedures.

Abalone that had been grown in the recirculation system all lost weight while those that were grown in the flow-through system, all gained weight during the purging process. This result was obtained irrespective of the type of feed that had been provided. The loss of weight in animals coming from the recirculation system was probably due to both the loss of gut contents during purging as well as physiological stress experienced by the animals when taken from the warmer recirculation system and then placed into the colder purging system. This response may have been due to a temperature shock. It has been suggested (see Wood 1983, Schmidt-Nielsen 1997) that such weight loss often occurs because of an increased metabolic rate for temperature compensation in which body reserves are used. The weight gain in animals grown in the flow-through system is not generally seen as a “normal” physiological response. It is, however, possible to explain this phenomenon when we bear in mind that the purging system and the flow-through system have similar temperature regimes; only flow rates differ. Animals transferred from the flow-through system (not experiencing a temperature shock) probably kept on growing because of feed still present in their guts and because of their generally slower metabolic rates.

The irregular weight gain, loss and regain trends displayed by animals returned from the export simulation were no doubt due to stress. In order to monitor weight “gain” during the

recovery process, abalone were weighed every 24 hrs. This continuous handling was probably the stress factor and has been shown to be a threat to the health of abalone (see Genade et al. 1988, Sales and Britz 2001). Despite the constant handling, however, half of the animals fed Abfeed®-K26 did indeed regain their post-purging weights, suggesting that feed type may contribute to successful recovery. Abfeed®-K26 has specifically been designed for abalone with a shell length >50mm (see Jones and Britz 2006). Furthermore, it was interesting to note that 3 of the 4 treatments (i.e. Ft-R-Ft, R-R-Ft, Ft-R-R) that had recovered their post-purging weight, all came from the recirculation system. This suggests that the increased temperature associated with the recirculation system was to the advantage of those animals that had been fed Abfeed®-K26.

Weight regained after the export simulation was clearly not because of feed consumed. The erratic FCE values suggested that abalone were consuming kelp, when in fact those animals fed kelp, had negative mean growth values. In the case of abalone fed Abfeed®-K26, little or no feed was consumed (with low FCE values) yet weight gain occurred. The weight gain experienced by the abalone in this study was in all likelihood due to moisture absorption and not feed consumed.

In conclusion, our results have shown that prior cultivation history (system and feed) affected the recovery responses in exported abalone. Bearing this in mind, a few recommendations could be made.

1. To improve stress-resistance in abalone, Abfeed®-K26 is a better feed alternative to kelp.
2. Purging should best be performed in the same cultivation environment to minimise or even prevent weight loss due to possible temperature shock.

3. Too much handling after animals have been returned from an export procedure should be avoided.

4.6 Acknowledgements

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4.7 Tables

Table 1. Specific Growth Rate (SGR in % body weight.day^{-1}) and Mean Wet Weight Gain (g) calculated for three growth periods for abalone grown in the various systems histories when fed kelp and Abfeed®-K26. Animals are ranked according to their response to the purge and to how well they had recovered from the export simulation. The underscore in the systems column indicates the culture system just prior to the export simulation.

Feed	System	Specific Growth Rate			Mean Wet Weight Gain (g)			Rank	
		During purge (-84 to -36 hrs)	Total (-84 to 144 hrs)	Simulation and recovery (-36 to 144 hrs)	During purge (-84 to –36 hrs)	Total (-84 to 144 hrs)	Simulation and recovery (-36 to 144 hrs)	After purge	Post-recovery
Abfeed®-K26	Ft-R-Ft	-2.0030	0.2152	1.3445	-2.9	1.525	4.425	13	1
	R-R-Ft	-1.0228	0.2144	0.9072	-1.55	1.575	3.125	9	2
	R-Ft-R	1.2057	0.4881	0.4945	1.8	3.5	1.7	7	3
	Ft-R-R	-1.4916	-0.1668	0.3108	-2.15	-1.15	1	11	4
	R-Ft-Ft	1.2376	0.2112	-0.1042	1.83	1.48	-0.35	6	5
	R-R-R	-1.2804	-0.3598	-0.1904	-1.9	-2.525	-0.625	10	6
	Ft-Ft-Ft	1.1127	-0.01482	-0.5258	1.6	-0.1	-1.7	8	9
	Ft-Ft-R	1.3150	0.01107	-0.5611	1.9	0.075	-1.825	5	11
Kelp	R-R-R	-3.6222	-0.9141	-0.3200	-5.65	-6.725	-1.075	16	7
	Ft-R-R	-1.9795	-0.6057	-0.3990	-2.95	-4.25	-1.3	14	8
	R-R-Ft	-2.0141	-0.6599	-0.4979	-3.2	-4.925	-1.725	15	10
	Ft-Ft-R	1.8631	0.1050	-0.6063	2.65	0.7	-1.95	3	12
	Ft-Ft-Ft	1.9777	-0.09905	-1.0881	2.8	-0.65	-3.45	2	13
	Ft-R-Ft	-1.4717	-0.8637	-1.1692	-2.2	-5.975	-3.775	12	14
	R-Ft-Ft	1.6916	-0.2355	-1.2489	2.45	-1.575	-4.025	4	15
	R-Ft-R	2.1483	-0.2787	-1.5432	3.15	-1.875	-5.025	1	16

Table 2. The improbable Feed Conversion Efficiencies (FCE) obtained for selected abalone fed kelp and Abfeed®-K26

Feed	Cultivation treatments	Feed consumed (g)	Mean growth (g)	FCE
Kelp	Ft-Ft-Ft	20	0.335	1.673
	Ft-R-Ft	26.88	-0.263	-0.977
	Ft-Ft-R	11.25	0.8	7.111
	R-Ft-R	25	-0.338	-1.35
Abfeed®-K26	Ft-Ft-Ft	8.506	1.125	13.226
	Ft-R-Ft	0	2.352	0
	Ft-Ft-R	17.012	-0.188	-1.102
	R-Ft-R	6.962	1.4	20.11

4.8 Figure captions

Figure 1. Diagrammatic representation of the experimental design. The dotted lines represent the points of split and transfer, and of the export simulation. The underscore indicates the current culture system.

Figure 2. Mean weight gain in abalone fed kelp from the various system growth histories. The pre-purged time (-84 hrs) represents the starting point of this data set. -36 hrs represents the post-purge period and 0hrs represents the post export simulation period. Hereafter, weight measurements were calculated every 24 hrs following the export simulation. A = the purging period; B = the export simulation period. The solid horizontal line represents the starting mean (74.49g) for this recovery trial.

Figure 3. Mean weight gain in abalone fed Abfeed®-K26 from the various system growth histories. The pre-purged time (-84 hrs) represents the starting point of this data set. -36 hrs represents the post-purge period and 0hrs represents the post export simulation period. Hereafter, weight measurements were calculated every 24 hrs following the export simulation. A = the purging period; B = the export simulation period. The solid horizontal line represents the starting mean (73.48g) for this recovery trial.

Figure 4. Recovery and mean weight gain in abalone fed kelp from the various system growth histories following the export simulation. The data have been adjusted around a common post-purging mean (-36 hrs) to counter the effects that the purge has had on the growth responses. 0 hrs represents the post-export simulation period. B = the export simulation period. The solid horizontal line represents the starting mean (74.12g) for this recovery trial.

Figure 5. Recovery and mean weight gain in abalone fed Abfeed®-K26 from the various system growth histories following the export simulation. The data have been adjusted around a common post-purging mean (-36 hrs) to counter the effects that the purge has had on the growth responses. 0 hrs represents the post-export simulation period. B = the export simulation period. The solid horizontal line represents the starting mean (73.31g) for this recovery trial.

4.9 Figures

Figure 1

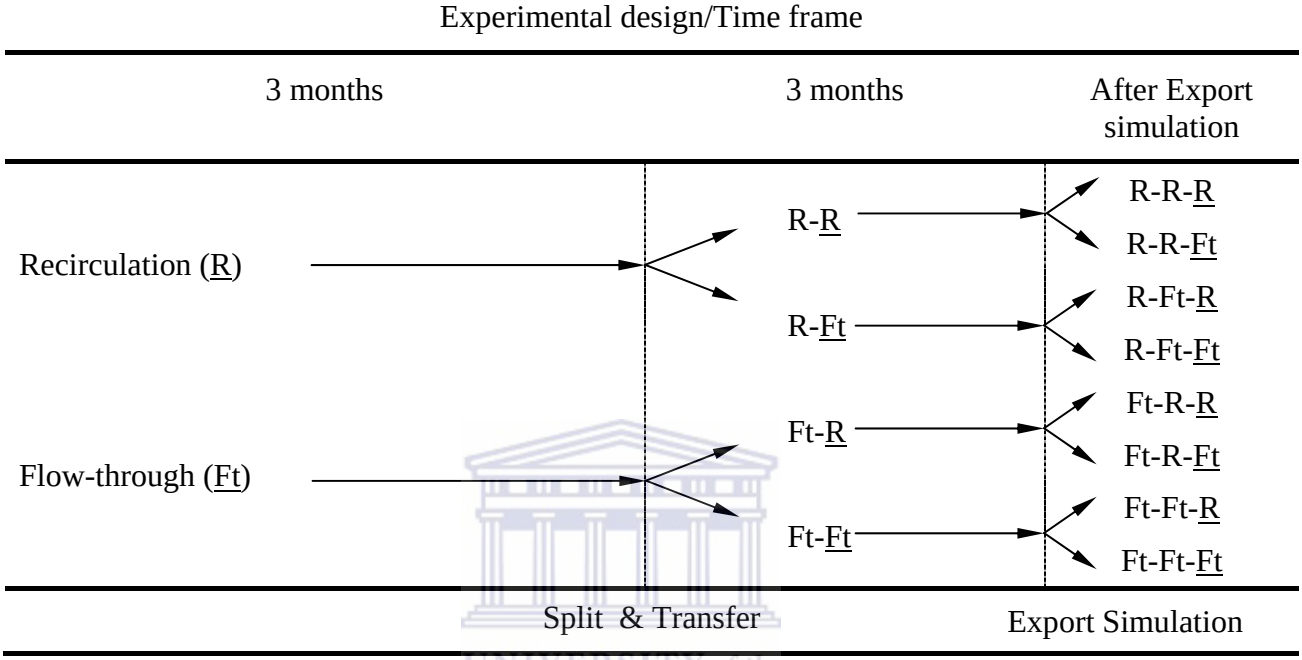


Figure 2

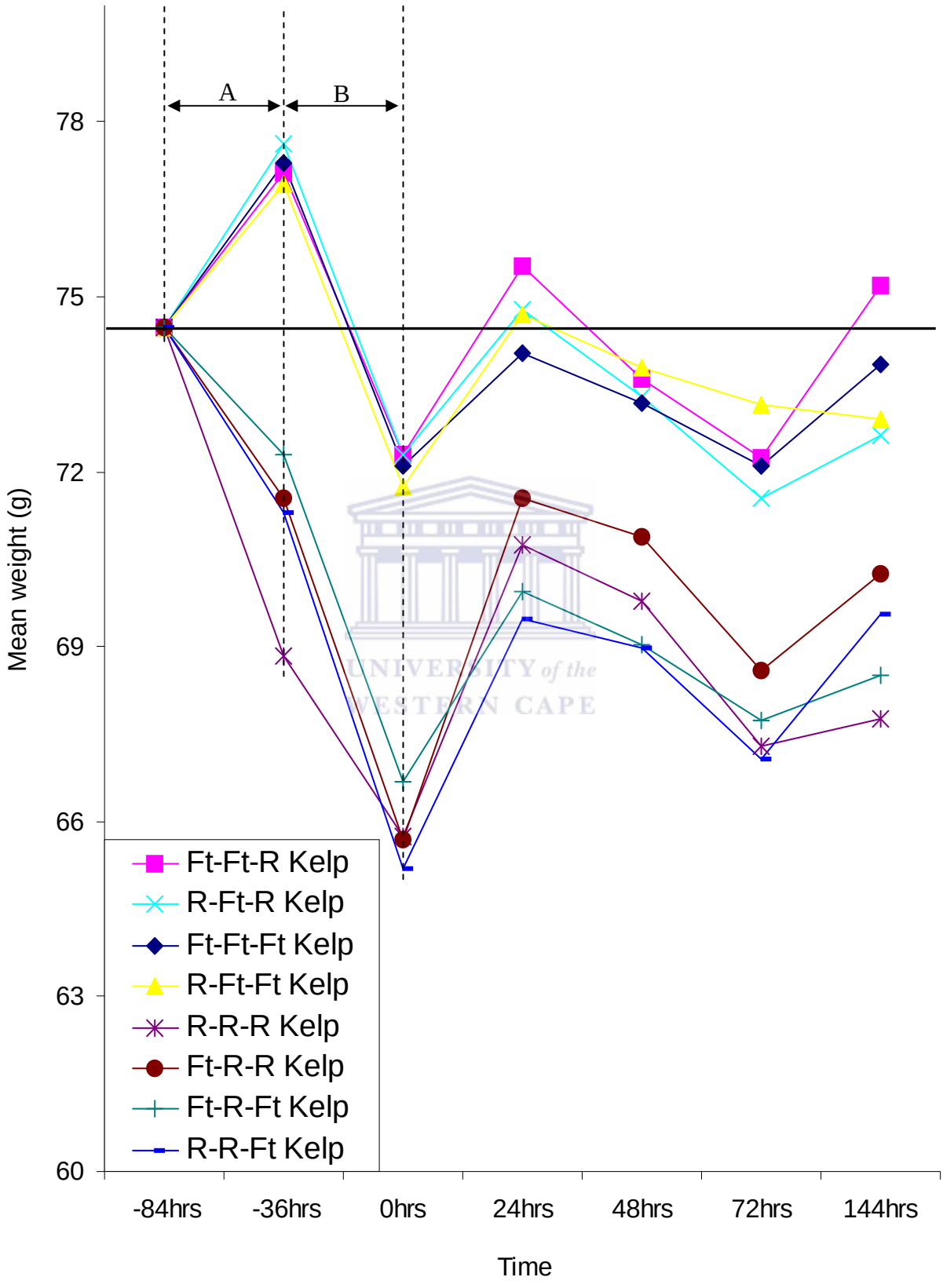
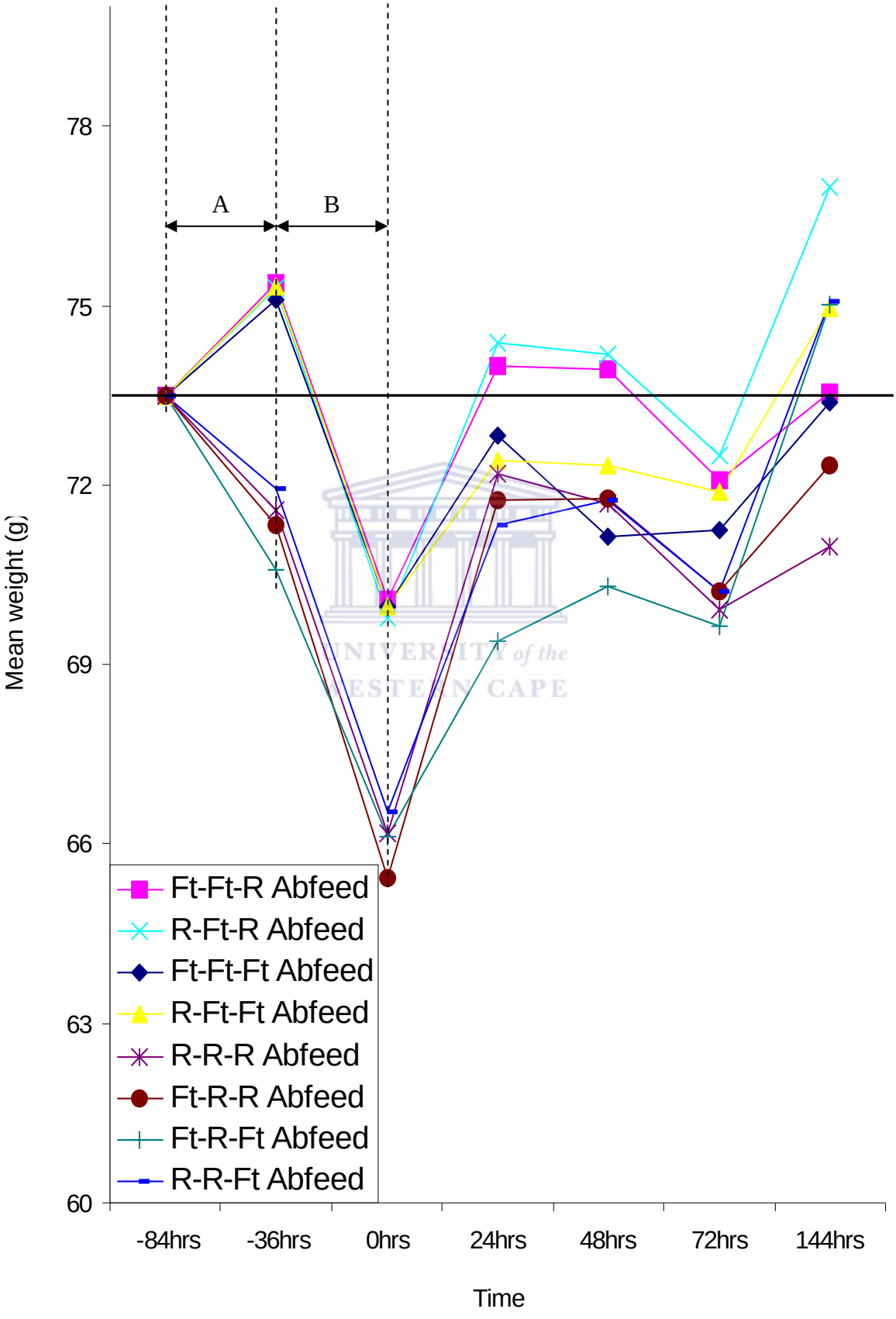


Figure 3


78
75
72
69
66
63
60
A
B
Mean weight (g)
Ft-Ft-R Abfeed
R-Ft-R Abfeed
Ft-Ft-Ft Abfeed
R-Ft-Ft Abfeed
R-R-R Abfeed
Ft-R-R Abfeed
Ft-R-Ft Abfeed
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24hrs
48hrs
72hrs
144hrs
Time

Figure 4

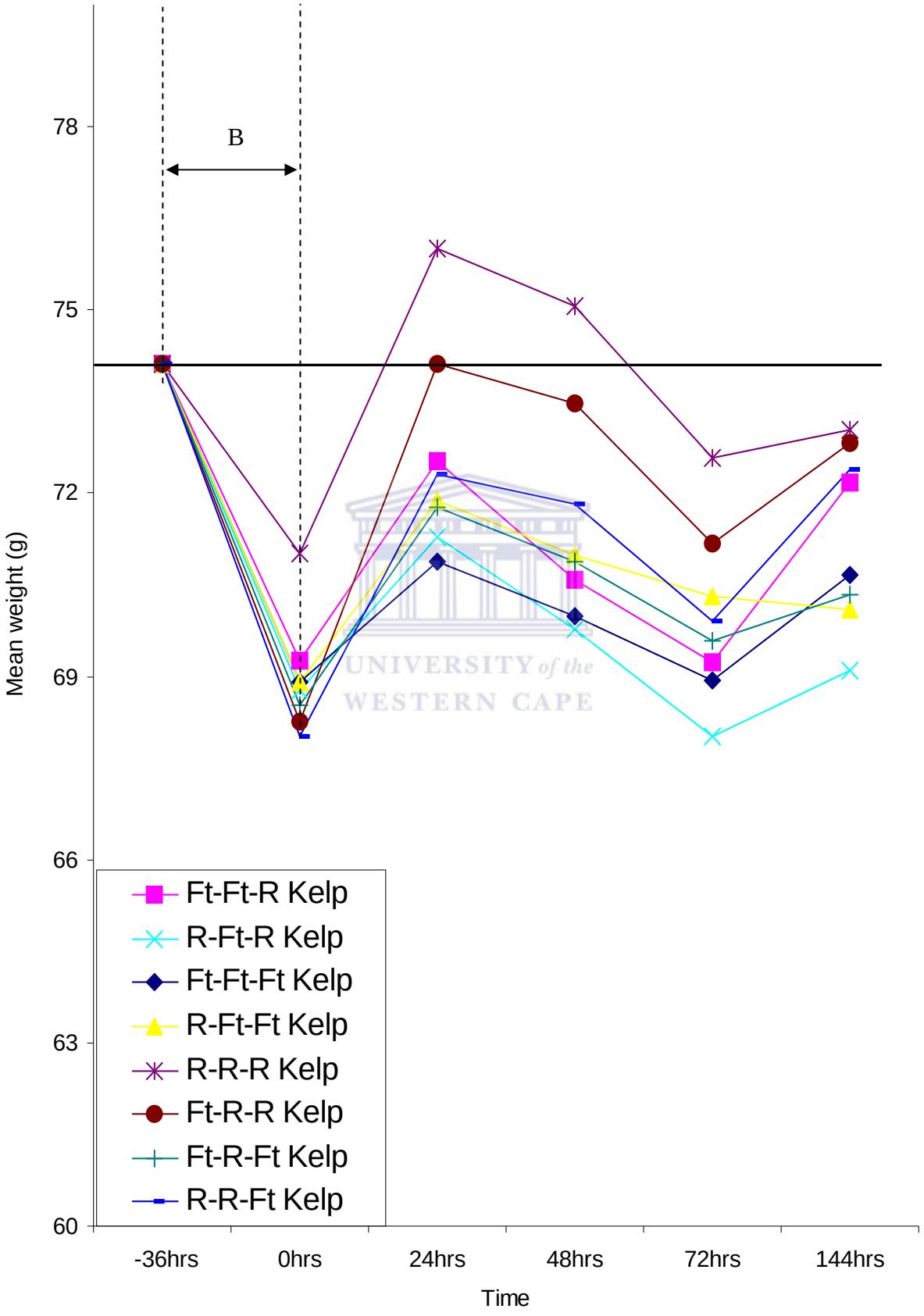
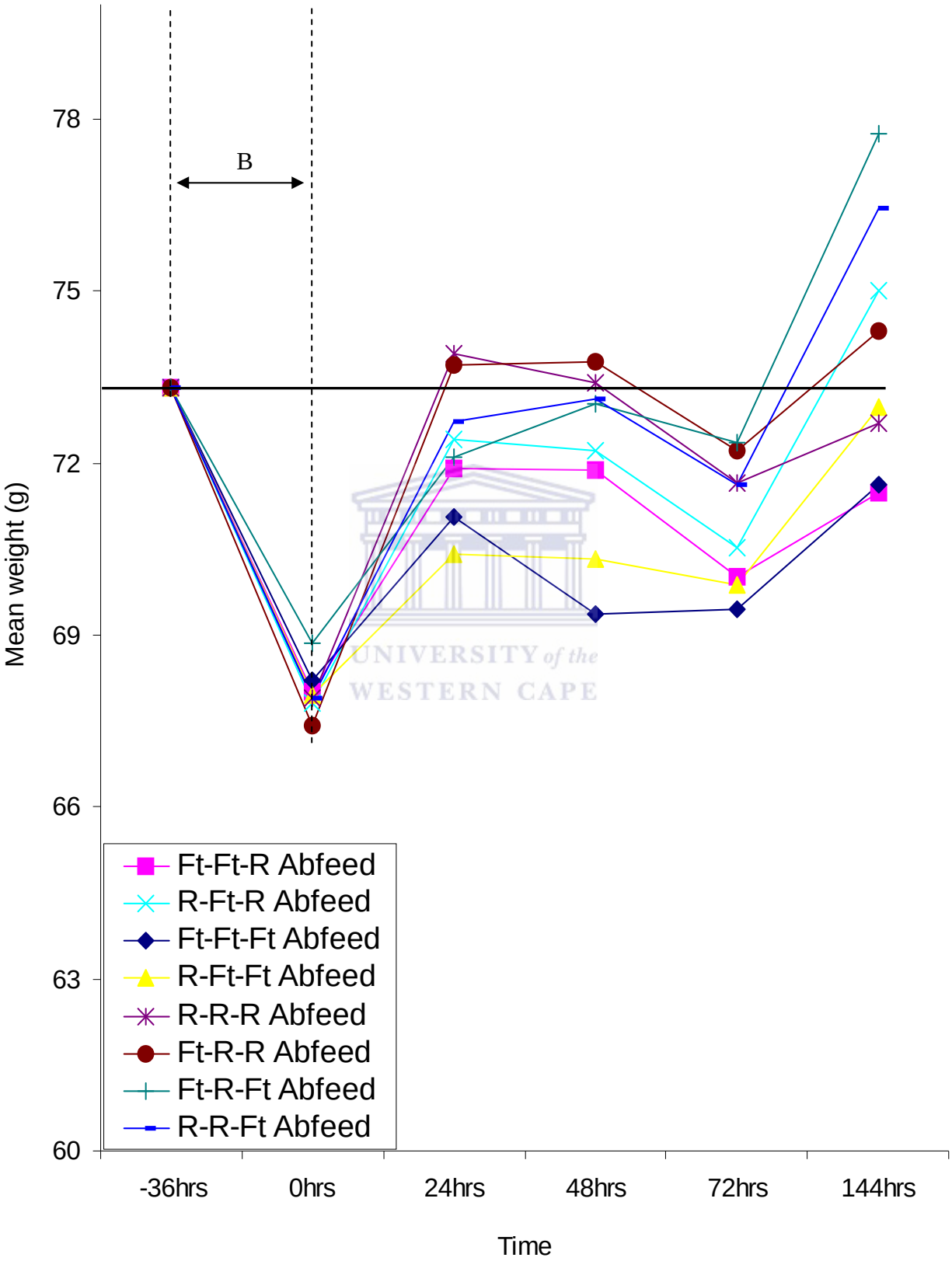


Figure 5



5. Summary and Recommendations

In 2005, the Marine and Coastal Management branch of the South African Department of Environmental Affairs and Tourism developed a Frontier Programme that was to address key research questions of relevance to the mariculture sector (see Pitcher 2005). The research and development specified in the Frontier document represents a consensus of priority by key stakeholders in the mariculture industry. Research was divided into key performance areas that addressed culture technology development, and the interactions between mariculture and the environment. The current research has attempted to address some of these questions, with an aim to promote knowledge sharing and transfer to the local abalone industry as well as to the global mariculture industry. With reference to the Frontier Programme document (see Section 4 of Chapter 1), the following key areas have been addressed.

5.1 Key Research Area 1 - Nutritional requirements for abalone culture.

5.1.1 Subsection Title 1: Determining the most appropriate feeding regime for the South African abalone *Haliotis midae* Linnaeus grown on kelp.

Background/Findings: The molluscan shellfish industry is dependent to a large extent on natural feeds such as micro- and macroalgae (Pitcher 2005). The amount of kelp in particular, delivered to abalone farms is sometimes inconsistent and abalone are consequently starved for short periods of time. Until now, little information has existed with regard to feeding regimes and the effect of periodic starvation on the growth of *H. midae* on commercial abalone farms.

This research has found that periodic kelp starvation can be beneficial to the South African abalone *H. midae*, possibly because of the positive effects of compensatory growth. Irregular bouts of starvation in particular have proven to be even more beneficial. It should, however, be stressed that were the growth rates not examined so closely, one could so easily have missed the growth spurts evident in the latter months of the experiment. Allowing for feed adjustment periods are therefore critical to fully understand the effects of differences in feeding regimes particularly when no prior pre-treatment is applied.

Farm Recommendations:

Based on the conclusions above, the following recommendations can be made:

- When changing the feeding routine, allow an adjustment period (of possibly no fewer than two months);
- If periods of starvation are foreseen, subsequent feeding should occur at irregular, rather than regular intervals.

It is not unusual for marine invertebrates to experience starvation or restricted feeding as food periodically becomes unavailable to them (see e.g. Durazo-Beltrán et al. 2004). As such, abalone have been documented (e.g. Carefoot et al. 1993, Takami et al. 1995) to withstand long periods of starvation before body reserves are depleted. Research (e.g. Quinton and Blake 1990, Jobling and Koskela 1996) has shown that like dietary composition, reproductive state, and unfavourable environments, food restriction or starvation often causes an animal to display compensatory growth. Periodic kelp starvation in the South African *H. midae* has no doubt been beneficial, possibly because of the positive effects of compensatory growth. While the international literature abounds with the positive effects of starvation in abalone (e.g. Roberts et al. 2001, Fermin 2002, Durazo-Beltrán et al. 2004), the current research may

be one of the first experiments documenting the positive effects of periodic kelp starvation in South African abalone.

5.1.2 Subsection Title 2: Comparing the growth of market-size abalone fed kelp versus the new low protein, commercially available Abfeed®-K26.

Background/Findings: Kelp constitutes the major feed for South African abalone (Anderson et al. 2003, 2006, Troell et al. 2006), but is low in protein content (ca 5-15%) (Hahn 1989, Robertson-Anderson 2004, Troell et al. 2006). Developing more nutritionally complete, high protein feeds has become important in the abalone farming industry (Sales and Britz 2001, Bautista-Teruel et al. 2003, Sales and Janssens 2004, Troell et al. 2006). It has been found that abalone with a shell length <50mm grow best on the high protein Abfeed®-S34 (34% protein). Abalone with shell lengths >50mm, however, grow equally well on kelp or a combination of Abfeed®-S34 and kelp (Britz 1996a, Jones and Britz 2006). The use of Abfeed®-S34 for larger animals has, however, impacted negatively on water quality so Abfeed®-K26 (protein content of 26%) was developed to help decrease ammonia levels and improve water quality in culture systems (Jones and Britz 2006). As no such research had been attempted before, the aim of this experiment was to compare the growth of abalone fed kelp versus the new low protein Abfeed®-K26 in both a flow-through and a recirculation system.

This research has found that growth of abalone fed kelp versus the new low protein Abfeed®-K26 was generally similar. However, despite the fact that substantially less Abfeed®-K26 than kelp was required to produce this comparable growth, it is still cheaper for the JSP commercial abalone farm to use kelp as its feed alternative. In addition, the

suggested temperature for the use of Abfeed®-K26 is 16°C (Marifeed Pty Ltd, South Africa). Our data show that Abfeed®-K26 can be used in culture systems at lower temperatures to still produce relatively similar or better growth than those animals fed kelp.

Farm Recommendations:

Based on the above conclusions, the following recommendations can be made:

- For the JSP commercial abalone farm, kelp is still the cheaper feed alternative when only purchasing costs are considered.
- With time and depending on other costs (e.g. transport, labour, time, etc) Abfeed®-K26 could prove to be the preferred choice over kelp.
- The above said though, it would be beneficial feeding abalone Abfeed®-K26 to produce "fatter" animals.
- Despite the higher purchasing costs, overall Abfeed®-K26 is an appropriate feed substitute because it has all the benefits of both kelp and Abfeed®-S34 and none of their disadvantages.

Artificial feeds have been used in Japan and China for many years already and are under development in countries such as Australia, Canada, Chile, Iceland, Korea, Mexico, New Zealand, North America, South Africa, Thailand and The Philippines (Britz 1996a, Gordon and Cook 2001, Sales and Janssens 2004). Most studies documenting the effect of artificial feeds on the growth of abalone have concentrated on high protein feeds and on their effects on juveniles (see e.g. Britz 1996a, Guzmán and Viana 1998, Bautista-Teruel and Millamena 1999, Bautista-Teruel et al. 2003, Gómez-Montes et al. 2003, Dlaza 2005, Naidoo et al. 2006). With the development of the new low protein Abfeed®-K26, unpublished laboratory work by Jones and Britz (Clifford Jones, pers. comm.) have shown that kelp could be

included in artificial diets and that reducing the protein level in the artificial feeds could be done without compromising growth. This was shown with the new Abfeed®-K26 which produced growth in large (>50mm) abalone that was comparable to that of abalone fed the high protein Abfeed®-S34 (34% protein content) (Jones and Britz 2006). We have obtained similar results showing that larger abalone (>50mm) fed a low protein formulated feed, can achieve similar growth to those fed kelp, and in some instances, grow even better. These benefits considered, the abalone farmer needs to consider the full spectrum of costs to determine the cost effectivity of the two feeds (Abfeed®-K26 and kelp).

5.2 Key Research Area 2 - Development of live export protocol to decrease transport mortality and weight loss in abalone.

5.2.1 Section Title: Effects of cultivation treatments, feed and export protocols on the recovery response of commercially farmed *Haliotis midae* Linnaeus

Background/Findings: During the exportation (transportation) of live animals, 4-15% of abalone body mass is lost through evaporation and pedal mucus production (Vosloo and Vosloo 2006). This said, there is a lack of published information regarding the effects of purging and transportation on farmed abalone. The third aim of this research was to run an export simulation and then to determine the best growth environment to allow rapid weight gain in abalone returned from the export simulation.

This research has found that prior culture and feed history affects the recovery responses of exported abalone. Firstly, prior cultivation treatment determines how well animals respond to the purging procedure. Thereafter, the type of feed provided appears to affect and determine the recovery response in exported abalone. It is suspected that the continuous handling of

animals returned from the export simulation caused unnecessary stress that certainly may have added to the irregular recovery in exported animals.

Farm Recommendations:

Based on the above conclusions, the following recommendations can be made:

- Use Abfeed®-K26 as a feed alternative to kelp as it appears to improve stress-resistance in abalone.
- Purging should be performed in as near the same culture environment as possible to minimize or prevent weight loss due to potential temperature shock.
- If returned from an export procedure, over-handling of animals should be avoided as this may affect recovery. Instead, feed consumption should be monitored as an indication of recovery.

While some studies (see e.g. Wells and Baldwin 1995, O'Omolo et al. 2003, Vosloo and Vosloo 2006) have looked at handling, metabolic stress and rates of water loss during aerial exposure and exportation, much research is still sorely lacking. Increasingly the development of live export protocols to decrease transport mortalities and weight loss in abalone is becoming important. As far as we know, this study is one of the few done in South Africa that has investigated the effects of exportation on abalone health and recovery and could serve as a guide to abalone farmers for best on-farm practice. In this regard, it is hoped that this research would pave the way for future studies as limited information exists.

5.3 Conclusion

The data obtained from these three studies will no doubt add value to the South African abalone industry. Since kelp availability is already becoming a serious problem in South Africa, our research has shown that periodic kelp starvation is beneficial to abalone farmers. As a consequence, the development of nutritionally complete formulated feeds, both low and high in protein content, is becoming increasingly important as feed alternatives to kelp. Also, while we have attempted to provide some answers to the effects of export protocols on the recovery response of abalone, much still needs to be achieved in this regard. In conclusion, this research has attempted to answer and contribute to some of the important key research areas identified in the Frontier Programme (see Pitcher 2005), and so besides contributing important information from a research perspective, the information presented in these chapters will no doubt be of benefit to the abalone commercial industry as well.

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