BIOLOGICAL AND ECONOMICAL FEASIBILITY STUDIES OF USING SEAWEEDS *ULVA LACTUCA* (CHLOROPHYTA) IN RECIRCULATION SYSTEMS IN ABALONE FARMING

By

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Thesis Presented for the Degree of

DOCTOR OF PHILOSOPHY

in the

Faculty of Science

In the Department of Botany

UNIVERSITY OF CAPE TOWN

August 2006

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DECLARATION

I declare that this thesis is my own, unaided work and has not been submitted in this or any form to another university. Where use has been made of the research of others, it has been duly acknowledged in the text.

Experimental work discussed in this thesis was carried out under the supervision of Prof. J. J. Bolton of the Department of Botany, University of Cape Town; Dr. R. J. Anderson of the seaweed unit, Marine and Coastal Management; Dr. T. Probyn Marine and Coastal Management; Dr A.J. Smit, University of Kwazulu Natal, Associate Prof. M. Troell and Dr. C. Halling of the University of Stockholm.

Chapter 2 formed the basis for a paper published in Aquaculture (Troell *et al.* 2006). Questionnaires were designed with the assistance of Dr R. Andersson and Associate Prof. M. Troell.

Chapter 3a, b were supervised by Dr. A. J. Smit and are the basis for papers in press in Aquaculture (Smit *et al.* In Press) as well as one submitted to Aquaculture (Robertson-Andersson *et al*, Submitted) and one submitted to Talanta (Smit *et* al, Submitted). Samples were analysed for DMSP and DMS by S. Peal in the Hershaw and Kinnes analytical laboratory at UCT. The taste test questionnaire was designed with the assistance of Dr. A. J. Smit. Abalone were prepared for taste testing by SPP caning in Hermanus. All abalone for DMSP and DMS canned tests were prepared and canned by SPP canning in Hermanus. Abalone were cooked and plated by staff from Viva caterers for the taste trial.

All abalone histology and heath examinations were conducted by Dr. A. Mouton.

Some sediment, bacteria, mobile macro fauna and water quality data in Chapters 5 and 6 were obtained from several small scale studies performed in the integrated abalone and seaweed system described in Chapter 6 and the feed experiment described in Chapter 5. These studies were performed by an Applied Marine Masters student at UCT and several students from the University of Stockholm doing Minor Field Studies. In each of these studies I assisted in the logistics and some of the experimental design as well as the

data collection. These studies were done to present a complete picture of the operation of an integrated system and the total effects of diet on animal cultivation. Where the data from these studies were used it was duly acknowledged in the text. Chapter six also forms the basis for a paper submitted to the Journal of Applied Phycology (Robertson-Andersson *et al.* In Press)

All research was conducted under permits obtained from the UCT research ethics committee, the UCT Science ethics committee, Department of Environmental Affairs and Tourism (RSA); Table Mountain National Parks and the SABS. For a full list of permits and the reference numbers and the indemnity form from the taste test please see appendix A.

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August 2006

We learn more by looking for the answer to a question and not finding it.
Than we do from learning the answer itself.
Lloyd Alexander

To my family
Thanks for all your love support and encouragement, without which,
this would have been impossible.

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(ii) Deborah V. Robertson-Andersson, John J. Bolton, Max Troell, Robert J. Anderson, Gavin Maneveldt, Christina Halling, A. J. Smit, Trevor Probyn and Sue Peall. 2007. The evolution of integrated seaweed aquaculture in Temperate Southern Africa. SANCOR forum meeting, Ushaka marine world/ ORI, Durban,

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ABSTRACT

Significant effort has been put into the development of cost-effective abalone (*Haliotis midae*; Gastropoda) cultivation systems in South Africa, but the limited availability of suitable seaweed for abalone food is an obstacle to future development. The aim of this study was to investigate whether a land-based recirculating seaweed-abalone integrated aquaculture system using *Ulva lactuca* was feasible as well as to test the differences between a commercial gravel bed recirculation system to an existing flow through system. These studies were carried out at two abalone farms: Danger Point (I & J) (140 km east of Cape Town) and at Jacobs Bay (JSP) (120 km north of Cape Town, South Africa). In both studies no significant difference in terms of water quality, abalone growth rates and abalone health were found. It was found that a seaweed /abalone recirculating system at the designed water exchange rates (25 %) was nitrogen limited and that the system as designed could be run at 75 % recirculation rate and remove a significant proportion of the dissolved nutrients (ammonium, phosphorus, nitrate and nitrite). It was concluded that seaweed functions well, as a biofilter. The system, both at 25 % and at 75% re-circulation, was capable of reducing the effluent concentrations and maintain, or sometimes even improve, water quality as compared to control. Total particle loading did not increase with higher re-circulation, nor does the load of fractions smaller than 35 µm or the carbon content of the particle load. Since sabellids prefer organic particles smaller than 35 µm as feed, these results indicate that, at least from a feed perspective, kelp fed re-circulating systems did not favor sabellids or other mobile macro fauna, this was echoed in the health examinations of the animals. In addition, mobile macrofauna diversity and density were similar in a recirculating system compared to a flow through system. Although dissolved oxygen production in the seaweed part of the system was 33 % higher than the flow through system, the oxygen was not being transferred to the abalone tanks. This meant that over the experimental period dissolved oxygen in the integrated system was 5 % lower than the flow through system. Temperature in the flow through system was 1 % lower than the recirculation system. Seaweed production was positively or negatively affected by external environmental effects (e.g. warm water intrusions over the

western Agulhas bank result in a 7 kg per tank decrease in seaweed production). The risk of spreading disease or rising bacterial levels in the grow-out through integrated seaweed /abalone aquaculture, was considered low.

Abalone farms want to supplement the abalone feed with cultivated *Ulva* and investigate the potential of integrated abalone seaweed systems. The *Ulva* used in this study was simple to cultivate as it grows vegetatively and was collected from free floating populations in Simons Town Harbor. It has a further benefit in its capacity to absorb nutrients and improve water quality of the aquaculture effluent.

However, macroalgae, as feeds for aquacultured abalone produce dimethylsulfoniopropionate (DMSP). DMSP levels are high in *Ulva lactuca* up to 6977 ± 1161 $\mu g.g^{-1}$ w.wt. DMSP, while *Gracilaria gracilis* and the kelp *Ecklonia maxima* contain between 0.8 ± 0.3 and 26.8 ± 20.6 $\mu g.g^{-1}$ w.wt. DMSP. DMSP levels increase in abalone tissue after they feed on these algae. A volatile breakdown product of DMSP, dimethylsulfide (DMS), is formed in abalone during canning, causing repellent tastes and odours in some batches of canned meat. The abalone adductor muscle (the part which is more commonly eaten) displays high DMSP concentrations compared to other tissues. Further, the feeding regime determines to what extent DMSP accumulates. When *U. lactuca* is fed to cultivated abalone in isolation, DMSP accumulates in the abalone to a concentration of up to 23×10^3 $\mu g.g^{-1}$w.wt. DMSP, a value of about 1.4 % of the fresh mass of the animal. A depuration phase of 3 – 6 months (depending on water temperature) prior to processing allows for the reduction of tissue DMSP levels to those seen in wild abalone, thereby ameliorating the negative effect on taste and odor. Taste tests showed that Asian people preferred abalone in its raw state with high DMSP contents while this preference changed when the abalone was cooked. Knowing the DMSP levels in feeds and its behavior in abalone tissue will lead to the development of new strategies for controlling abalone taste characteristics.

Feed not only affects taste but also affects the cultivation environment. Abalone are wasteful feeders with more than 60 % of their feed intake being converted to waste. This waste is in the form of particulates and dissolved organic nutrients. The sediments have different particle sizes and nutrient values depending on the primary feed source. The higher the nutritional value of the sediments (e.g.

a compound pellet feed), the greater mobile fauna, sabellids, *vivo* bacteria, nitrogen and carbon content the sediments will have. A mixed seaweed diet produced significantly higher phosphate concentrations of the three diets tested, while and Abfeed® diet produced significantly higher total ammonium nitrogen. The abalone farms have had a significant socio-economic impact in the coastal communities in which they are situated. They employ 840 people countrywide but if the feed, canning and seaweed industry figures are included this increases to 1200. Of this 61 % are unskilled workers, which is the highest percentage of people in these communities. The industry has expanded by 500 % in the last 10 years and is predicted to expand by another 100 % in the next 5 years. This should see South Africa retain its position as the leading aquaculture abalone country outside of Asia at least until 2010.

CHAPTER 1

INTRODUCTION

INTRODUCTION

According to the Food and Agriculture Organization (FAO) of the United Nations, most of the worlds fishing areas have reached their maximum potential for fisheries production (FAO, 2001; 2006a). This stagnation has also been accompanied by a gradual shift from the capture of large, carnivorous species of fish to smaller, less valuable species (Pauly *et al.* 1998, 2002; Naylor *et al.* 2000, Troell *et al.* 1999a,b, 2003; Halling 2004). Meanwhile production from aquaculture is increasing steadily, having doubled in the last decade. Seafood from aquaculture now supplies one third of the total consumed world wide (FAO, 2001; 2006a; Troell *et al.* 2004). The annual growth of aquaculture has averaged close to 11% for nearly two decades (Pauly *et al.* 1998, 2002; Naylor *et al.* 2000, FAO, 2001; 2006a Kautsky *et al.* 2001; Troell *et al.* 1999a,b; 2003; Halling, 2004).

Although salmon and shrimp receive the most publicity in the western world, they comprise less than 10% of global aquaculture production by weight, compared with 50% for carps and tilapias which contribute most to the domestic food supply in some developing countries (Naylor *et al.* 2000). Aquatic plant cultivation has largely been ignored when aquaculture figures are produced (Bolton, 2006), this is largely due to the fact that even though their production is high (46 % in 2004 (FAO, 2006)) their value is low (24 % in 2004 (FAO, 2006)).

Aquaculture production is also skewed geographically, with Asia producing over 90% of the global total, dwarfing Africa and Latin America with less than 0.5% and 2%, respectively (FAO, 2001). In China, with 67% of global aquaculture production, inland aquaculture production has increased at least fivefold in the past decade. It has only doubled in the rest of the world, implying large potential in other developing countries, if constraints to its expansion were removed (FAO, 2001).

To meet the future demands for food fish and proteins, as well as decreasing the pressure on wild fish stocks, aquaculture must continue to expand and increase, supplying at least 50 % of the world's seafood demand by the year 2030 (Tidwell & Allen, 2001). Increased aquaculture production has resulted from intensification and technology development (Colwell, 2002). Western

aquaculture has tended to focus on high value and high production monoculture (Chopin *et al.* 2001). This is particularly true for marine species (shrimp (Rönnbäck 2001) and fish (Chopin *et al.* 2001). A major challenge for aquaculture is to become sustainable and be based on a balanced ecosystem approach, largely due to past intensification of aquaculture resulting in environmental degradation (Folke *et al.* 1994; Naylor *et al.* 2000; Chopin *et al.* 2001). One may think that on a world scale the two types of aquaculture fed (fish and shrimp) and extractive (plants & shellfish) would at least on an environmental scale balance each other out. However, as most aquaculture is based on monoculture systems and these types of production are often geographically separate both on a local and regional scale, this environmental balancing does not occur (Chopin *et al.* 2007).

Integrated aquaculture (See Box 1.1) has therefore been suggested to increase production and sustainability (Folke & Kautsky, 1989; Naylor *et al.* 2000; Chopin *et al.* 2001; Troell *et al.* 1999a,b, 2003). Integrated aquaculture as a concept is not new. Asian countries have been practicing it through trial and error and experimentation for centuries (Li, 1987; Liao, 1992). China in particular has an ancient practice of integrated farming. This has become more refined as a consequence of governmental policies (Chopin *et al.* 2001). Western countries have only recently been "rediscovering" integrated aquaculture (Ryther *et al.* 1975, 1979; Indergaard & Jensen, 1983; Kautsky *et al.* 1996; Chopin *et al.* 1999a-d, 2001). Integrated aquaculture has the potential to reduce the dependency on external ecosystems, for food and energy, as well as minimizing the negative environmental impacts from waste release i.e. reducing the ecological footprint of an aquaculture facility (Folke *et al.* 1998; Chopin *et al.* 2001; Kautsky *et al.* 2001).

This increasing recognition, that aquaculture development should be sustainable is reflected in the literature (Foy, 1990; Farshad & Zinck, 1993; Levin, 1993; Chopin *et al.* 2001; Kautsky *et al.* 2001; Folke *et al.* 2002) (See Box 1.1). Sustainability in development has been defined as "... development that meets the needs of the present without compromising the ability of future

generations to meet their own needs..."(WCED, 1987) or "...sustainable development improves people's quality of life within the context of the Earth's carrying capacity..." (Girardet, 1992). These definitions contain two key concepts: meeting the present and future needs of the world's poor; and accepting the limitations of the environment to provide resources and to receive wastes for the present and for the future. In this thesis I will use a definition for sustainable development by Thabo Mbeki (President of the Republic of South Africa) "'...Sustainable Development' is about improving the quality of human life whilst living within the capacity of supporting ecosystems..." (Evett, 2006). Sustainable in this context means "long lasting" or "enduring", it does not means "stationary" or "status quo". Thus it focuses attention on the time frame of decision making and implies prudence, long term thinking (a longer time frame than immediate interests might dictate) and care (Evett, 2006). Development is defined as a process of advancement, growth or maturation and is about fostering human needs (Evett, 2006). Aquaculture, and particularly integrated aquaculture, needs to be assessed in terms of their ability to contribute to sustainable development (Edwards, 1998).

Box 1.1 lists current definitions surrounding aquaculture. These definitions are many and varied and often have subtleties that require intimate knowledge. An example of this is the difference between organic and sustainable aquaculture. Both follow the same definition (Box 1.1). However the central goal of certified organic production is to "...verify and communicate to consumers that production systems are in place that will promote biodiversity, biological cycles, and biological activity by managing the production system as an integrated whole" (IFOAM, 2006). This is achieved by restricting the introduction of harmful substances and practices through strict guidelines and codes of practise. Such systems strive to create and maintain a sustainable production system that works with, rather than against, the environment in which it is embedded (IFOAM, 2006). To this end the *International Federation of Organic Agriculture Movements* (IFOAM) has drafted an overlying statement of principles that organic operators must abide by as far as possible in their interactions with human populations, domestic and wild animals, and the

environment (IFOAM, 2006). Produce from this type of aquaculture may be labelled as organic.

Contrast this to sustainable aquaculture, which is long term aquaculture with the principle objective to sustainably produce and keep pace with society's food requirements without eroding natural capital (Edwards, 1998). The terminology "eroding natural capital" is a simplified way of stating that the production systems will promote biodiversity, and natural biological activity by managing the production system as an integrated whole with the environment. This is very similar to the principles of IFOAM although sustainable aquaculture does not adhere to the principles of IFOAM, and produce is also not apparently labelled as being organic.

BOX 1.1: Definitions of terms used

Aquaculture may be defined simply as the growing of aquatic flora and fauna in marine, brackish or fresh water (Swift, 1985). The term mariculture has often been interchanged with marine aquaculture (Reay, 1979). Mariculture refers specifically to the farming of marine organisms including fish, molluscs, crustaceans and plants, in marine or brackish water, with some form of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc. (Lincoln *et al.* 1998). Farming also implies individual or corporate ownership of the stock being cultivated (DEAT, 2006).

Commercial aquaculture tended to concentrate on one species with little or no crop rotation is defined as monoculture. This results in environmental impacts such as eutrophication as well as decreasing system resilience (Folke *et al.* 1997; Kautsky & Folke, 1989).

The terms polyculture and co-culture have often been interchanged. Polyculture, the cultivation of two species of the same order, aims at increasing the crop diversity within a farming system (Folke *et al.* 1997; Kautsky & Folke, 1989). In such a system there does not have to be a mutually beneficial process between the cultured species, as is with the case of co-culture (Langdon *et al.* 2004).

Integrated aquaculture or "horizontally integrated aquaculture" or "co-culture" pertains to "an output from one subsystem in an integrated farming system which otherwise may have been wasted, becoming an input to another subsystem resulting in a greater efficiency of output of desired products from the land/water area under a farmer's control" (Edwards *et al.* 1988; Langdon *et al.* 2004), thus reducing the concentration of pollutants and potentially conferring benefits to the operator, environment and stakeholder groups. Examples include the culture of seaweed and shellfish in the wastewater from shrimp ponds and marine cage facilities, and the use of constructed wetlands planted with reeds or mangroves to treat the wastewater from land based freshwater or marine/brackish aquaculture, respectively (Ajisaka & Chiang, 1993; Shpigel *et al.* 1993 and Neori & Shpigel, 1999). Traditionally, land-based systems are commonly integrated with agriculture by stocking fish in rice fields and ponds referred to as integrated agri-

aquaculture, while water-based systems involve stocking fish directly in enclosures or attaching them to substrates in water bodies such as rivers, lakes, reservoirs or bays. Integrated multi trophic aquaculture is a fed aquaculture species linked with both an organic (e.g. mussels) and inorganic (e.g. seaweed) extractive species (Chopin *et al.* 2003).

Aquaculture is a diverse activity where the degree of intensity, through manipulation and intervention in the cultured organism's life cycle is determined by the scale at which organisms are cultured (Kautsky *et al.* 2001; Naylor *et al.* 2000; Troell *et al.* 2004). Integrated agri-aquaculture or extensive aquaculture is low-cost production using extensive and semi-intensive technologies usually for small scale subsistence households (Kautsky & Folke, 1989; Edwards & Demaine, 1997). It exploits natural food chains and water recycling processes and although being environmentally friendly, it cannot sustain future global aquatic production and land management requirements (Edwards, 1998). Fertilizer and feed may be derived from on-farm by-products, although formulated and pelleted feed from agro-industry are increasingly used (Semi intensive technology) (Furey *et al.* 2003). By contrast, intensive aquaculture systems invariably depend on relatively high-cost, nutritionally complete diets, chemicals and increasing the organism density in each of the culture tanks (Naylor *et al.* 1998, 2000). Intensive aquaculture, which uses formulated feeds (Kautsky *et al.* 2001) of which only 25 – 30 % is consumed with the remainder being discharged to the environment (Kautsky & Folke, 1989; Folke & Kautsky, 1989) has many more effects on the local natural environment than either extensive or semi-intensive aquaculture.

"Sustainable aquaculture is a dynamic process, incorporating research, learning and reassessment of methods, to practice and retain natural equilibrium in aquatic ecosystems. The objective is to sustainably produce and keep pace with society's food requirements without eroding natural capital" (Edwards, 1998).

Organic aquaculture occurs in controlled aquatic vegetation zones, and aquatic species are cultured at reasonable stocking densities and maintained with natural food products and compounds. Water quality is conserved by eco-technological methods and where applicable the need to utilize organic compounds and competent materials that are sensitive to the environment without interference in natural ecosystems (IFOAM, 2006)

A system approach to sustainable integrated aquaculture

The scientific approach to understanding aquaculture, has commonly been reductionist i.e., phenomena are divided into smaller and smaller entities or variables which are studied independently. It is being recognised that this approach cannot deal adequately with complex phenomena comprising numerous interrelated variables that occur in integrated aquaculture (Folke & Kautsky, 1989; Naylor *et al.* 2000; Chopin *et al.* 2001; Troell *et al.* 1999a,b, 2003). A system approach is expansionist and holistic as it recognizes that complex phenomena comprise systems of interrelated factors (Edwards, 1994). Thus, knowledge of agriculture, ecology, economics, engineering,

environmental science and sociology may be needed to adequately appreciate certain types of integrated aquaculture systems.

Sustainability may be expressed in terms of three interrelated aspects (Figure 1.1) (Edwards, 1994): production technology, social and economic aspects, and environmental aspects. Social and economic aspects of aquaculture have received relatively little attention compared to production aspects and are major constraints to development (Ruddle, 1991, 1993). Environmental aspects are beginning to receive attention to prevent humans from exceeding the global carrying capacity.

Production technology

Production technology can be subdivided into three main aspects: cultured species, culture facility and husbandry. The choice of species cultured influences the culture facility and together these determine the type of husbandry needed for the various stages of production (hatchery, weaning and grow-out). More than 200 species are currently farmed in aquaculture (FAO, 2001) in culture facilities as diverse as rice fields, static or running water ponds, cages and pens. Husbandry may involve various methods of stock management (monoculture or polyculture; single or multiple, stocking and harvesting strategies), use of different feeds (natural, supplementary or complete feed), management of substrate and water quality, breeding programmes to produce better strains of some species, disease prevention and therapy (Edwards, 1994).

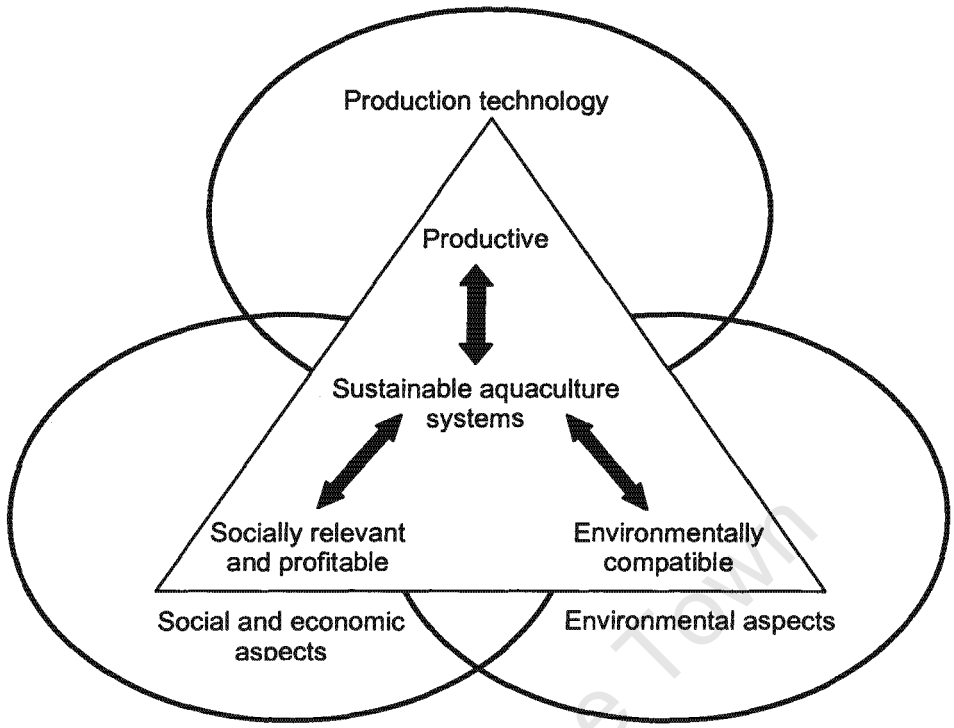

FIGURE 1.1: The three interrelated aspects of the sustainability of an aquaculture system: production technology, social and economic aspects, and environmental aspects (from Edwards, 1994).

Social and economic aspects

Social and economic factors influencing sustainable aquaculture may be considered at the macro-level (international, national and regional aspects) and the micro-level (community and farm household). Macro-level issues include world trade, national development goals, government policy, and social characteristics such as cultural attitudes and input supply and marketing. Micro-level issues are mainly alternative uses of resources (Edwards, 1994). In the South African context the economic component requires that "growth is pursued in a manner that brings economic benefits to the society at large and does not endanger its man-made and natural capital stocks" (President Mbeki in Evett, 2006). The social dimension "...is built on the premise that equity and an understanding of the human community's interdependence are basic requirements of an acceptable quality of life..." where wealth, resources and opportunity, are shared so that all involved have access to minimum standards of security, human rights and social benefits (President Mbeki in Evett, 2006).

Environmental aspects

The environment is external to the aquaculture system and includes the natural resources used for aquaculture development (e.g. land, water, nutrients and biological diversity), and also the two-way interactions between itself and the aquaculture system. The natural environment (climate, geomorphology, hydrology and soils) and its human transformation (agro-ecology, urbanization, industrialization), exert major influences on aquaculture, which may be either positive or negative. A positive interaction between aquaculture and the environment is a pond dug on a small-scale farm which functions as a nutrient trap while providing water for irrigation of rice seedlings and vegetables in addition to providing fish. Fish ponds may also be used to treat human sewage, manure from feedlot livestock as well as effluents from intensive aquaculture. Negative interactions between aquaculture and the environment are the adverse effects of pollution on aquaculture and adverse effects of aquaculture on the environment (e.g. eutrophication, misuse of chemicals, reduction of biodiversity and mangrove destruction) (Kautsky *et al.* 2001). The internal environment of the culture system is considered as part of the husbandry (Edwards, 1994).

An aquaculture farming system needs to be sufficiently productive to make it an attractive option to alternative or competing uses of resources, i.e. land and water, capital, labor, and farm by-products. They also need to mimic as much as possible the way natural ecosystems function to be sustainable (Folke & Kautsky, 1992). A systems approach is likely to be more successful in promoting aquaculture in developing countries. To do this, a thorough understanding of the resource-base of the farm, and the farmers' needs, as they perceive them is required. This must involve active participation of farmers and emphasize local resources as much as possible (Gliessman *et al.* 1981; Altieri & Anderson, 1986).

Sustainable aquaculture will be accomplished by the integration of new technology, combined with effective traditional practices, with the long term aim, to produce high quality products for local and international markets. A positive attribute of this strategy is less expense, reduced environmental damage caused by evasive packaging, refrigeration, transport, energy and waste associated with traditional intensive aquaculture.

At present both salmon and trout are being farmed organically and sustainably in countries like New Zealand, Wales, Scotland, Chile and Ireland (IFOAM, 2006) and information regarding these facilities and organic certification is freely available on the Internet.

The South African context

In South Africa, mariculture is still in its infancy, with the predominant product in the temperate regions being the South African abalone *Haliotis midae* Donovan. Of the 12 farms that comprise the Abalone Farmers Association of Southern Africa (AFASA), 4 use kelp exclusively as fodder (Bennett, 2002; Loubser, 2005). The development of compound feed (Abfeed®) for abalone has resulted in many of the seven AFASA member farms on the west coast feeding the abalone a mixture of kelp and compound feed (Bennett, 2002; Loubser, 2005). The drawback of compound feed, is that it gives the abalone a very light coloured shell and meat, which is undesirable in the Far Eastern markets and thus reduces the price of the abalone considerably (Robertson-Andersson, 2003; Gerber, 2004). A mixed diet of algae (consisting of mix of brown, red or green seaweeds) however, results in the meat having a red to brown colour, which is the ideal condition for export (Gerber, 2004).

In South Africa there are only two integrated (animal and seaweed) mariculture farms, namely: Marine Growers (Pty.) Ltd. in Port Elizabeth and Wild Coast Abalone in Haga Haga near East London (Bennett, 2002; Loubser, 2005) both on the southwest coast. Here *Gracilaria gracilis* [Stackhouse] Steentoft, Irvine et Farnham, and *Ulva rigida* C. Agardh are cultivated in tanks alongside abalone *H. midae* (Fourie, 1994; Hampson, 1998; Steyn, 2000, Robertson-Andersson, 2003; Njobeni, 2006). These farms do not have access to the large kelp beds (mostly *Ecklonia maxima* (Osbeck) Papenfuss) that the farms on the west and south-west coasts have. With the water being warmer on the south-east coast due to the influence of the warm Agulhas current, the average temperatures in the grow-out tanks on these farms are warmer than those on the west and south-west coasts. While this can lead to an increase in the growth rate of the abalone, water temperatures higher than 18° C result in the

compound feed fermenting in the stomachs of the abalone, causing high abalone mortalities (Steyn, 2000). The cultivated seaweeds therefore provide the abalone with a source of nutrition, while simultaneously acting as a biofilter for abalone effluent water (Fourie, 1994; Hampson, 1998). By integrating seaweeds with abalone culture, a number of features increase the ecological sustainability of the aquaculture system;

- The use of the same water for the seaweeds and the abalone cultures reduces seawater requirements by half (when compared to two separate systems) which will in turn decrease pumping costs;
- Biofiltration and recycling of the abalone nutrient excretions by seaweeds reduces both the nutrient input requirements for seaweed growth and the overall impact of the aquaculture operation (Vandermeulen & Gordin, 1990);
- The use of biofilter-grown seaweeds reduces the need for the destructive harvesting of natural seaweed beds and encourages "good farm management" (having an alternative food source if natural seaweed stocks are compromised);
- The chemical composition of the cultured seaweeds and hence their nutritional value to the algivores is controllable (Robertson-Andersson, 2003; Robertson-Andersson & Wilson, 2005);
- Recent studies by (Simpson & Cook, 1998; Naidoo *et al.* 2006) indicate that growth rates of the South African abalone *H. midae* are significantly higher when cultivated with mixed algal diets than when cultivated with single species diets. Thus, *Ulva* could serve to supplement the current, predominantly kelp based diet of the abalone; and
- The west coast of South Africa is often subject to toxic algal blooms (Pitcher, 1998). The threat of shellfish poisoning caused by these blooms is considerably reduced with increased water residence times, particularly if the farm can be isolated from an external seawater source for the period in which the bloom is toxic. The biofiltering function of seaweeds during recirculation could be crucial.

Objectives of this thesis

The use of integrated aquaculture is being implemented in abalone mariculture operations around South Africa. However, each farm is unique according to its environment and operational management procedures. In order to optimize production, a comprehensive understanding of the immediate interacting physical and biological variables occurring on the farms is essential. This thesis investigates the possibilities to use and develop integrated seaweed aquaculture systems for increased production and sustainability in local abalone aquaculture. This study takes place *in situ* on a commercial farm and is therefore subjected to practices which occur at a working, large scale, commercial facility, which may not necessarily have occurred had the research been laboratory based on in a more controlled research environment. This is both an advantage and a disadvantage and this will be discussed in Chapter 7.

A principal objective of this study was a review of abalone farming within an environmental and socio-economic context. Subsequent aims were to investigate the performance (in terms of health of abalone, growth rates of seaweed and abalone and water quality) of an integrated aquaculture system on a commercial scale using *Ulva* sp. cultivated in an existing abalone *H. midae* culture system on an abalone farm (Irvin and Johnson – Abalone culture division, Danger Point) on the cape south-west coast (Cape Point to Cape Agulhas), with the traditional flow-through system. This will be contrasted with the performance of a traditional flow-through system with a commercial gravel bed biofilter recirculating system, on a west coast (west of Cape Point) mariculture farm (Jacobs Bay Sea Products). The two farms were chosen to identify any differences in growth rates when grown under two differing environmental and operational conditions (south-west coast and west coast).

The thesis consists of 7 chapters. Chapter 2 reviews the abalone industry and how it interacts and affects other related industries and the environment. Chapter 3 investigates the effects of feeding cultivated algae high in dimethylsulfoniopropionate (DMSP) to cultivated abalone. Chapter 4 explores the differences between a commercial gravel bed biofilter recirculating system and a flow through system. Chapter 5 looks at how water quality is influenced

by the diets fed to abalone and how this in turn affects abalone health and growth rates. This Chapter includes several smaller studies performed by other students within this experimental system and data from these studies will be incorporated into this Chapter. Chapter 6 looks at the performance of a 25 percent integrated seaweed-abalone recirculating system for an 18 month period and a 50 % seaweed-abalone recirculating system for a 6 month period. This Chapter also includes several smaller studies performed by other students within this experimental system and data from these studies will be incorporated into this Chapter.

Chapter 7 concludes this thesis and tries to quantify potential savings from designing and operating an integrated aquaculture system. .

INTRODUCTION

According to the Food and Agriculture Organization (FAO) of the United Nations, most of the worlds fishing areas have reached their maximum potential for fisheries production (FAO, 2001; 2006a). This stagnation has also been accompanied by a gradual shift from the capture of large, carnivorous species of fish to smaller, less valuable species (Pauly *et al.* 1998, 2002; Naylor *et al.* 2000, Troell *et al.* 1999a,b, 2003; Halling 2004). Meanwhile production from aquaculture is increasing steadily, having doubled in the last decade. Seafood from aquaculture now supplies one third of the total consumed world wide (FAO, 2001; 2006a; Troell *et al.* 2004). The annual growth of aquaculture has averaged close to 11% for nearly two decades (Pauly *et al.* 1998, 2002; Naylor *et al.* 2000, FAO, 2001; 2006a Kautsky *et al.* 2001; Troell *et al.* 1999a,b; 2003; Halling, 2004).

Although salmon and shrimp receive the most publicity in the western world, they comprise less than 10% of global aquaculture production by weight, compared with 50% for carps and tilapias which contribute most to the domestic food supply in some developing countries (Naylor *et al.* 2000). Aquatic plant cultivation has largely been ignored when aquaculture figures are produced (Bolton, 2006), this is largely due to the fact that even though their production is high (46 % in 2004 (FAO, 2006)) their value is low (24 % in 2004 (FAO, 2006)).

Aquaculture production is also skewed geographically, with Asia producing over 90% of the global total, dwarfing Africa and Latin America with less than 0.5% and 2%, respectively (FAO, 2001). In China, with 67% of global aquaculture production, inland aquaculture production has increased at least fivefold in the past decade. It has only doubled in the rest of the world, implying large potential in other developing countries, if constraints to its expansion were removed (FAO, 2001).

To meet the future demands for food fish and proteins, as well as decreasing the pressure on wild fish stocks, aquaculture must continue to expand and increase, supplying at least 50 % of the world's seafood demand by the year 2030 (Tidwell & Allen, 2001). Increased aquaculture production has resulted from intensification and technology development (Colwell, 2002). Western

aquaculture has tended to focus on high value and high production monoculture (Chopin *et al.* 2001). This is particularly true for marine species (shrimp (Rönnbäck 2001) and fish (Chopin *et al.* 2001). A major challenge for aquaculture is to become sustainable and be based on a balanced ecosystem approach, largely due to past intensification of aquaculture resulting in environmental degradation (Folke *et al.* 1994; Naylor *et al.* 2000; Chopin *et al.* 2001). One may think that on a world scale the two types of aquaculture fed (fish and shrimp) and extractive (plants & shellfish) would at least on an environmental scale balance each other out. However, as most aquaculture is based on monoculture systems and these types of production are often geographically separate both on a local and regional scale, this environmental balancing does not occur (Chopin *et al.* 2007).

Integrated aquaculture (See Box 1.1) has therefore been suggested to increase production and sustainability (Folke & Kautsky, 1989; Naylor *et al.* 2000; Chopin *et al.* 2001; Troell *et al.* 1999a,b, 2003). Integrated aquaculture as a concept is not new. Asian countries have been practicing it through trial and error and experimentation for centuries (Li, 1987; Liao, 1992). China in particular has an ancient practice of integrated farming. This has become more refined as a consequence of governmental policies (Chopin *et al.* 2001). Western countries have only recently been "rediscovering" integrated aquaculture (Ryther *et al.* 1975, 1979; Indergaard & Jensen, 1983; Kautsky *et al.* 1996; Chopin *et al.* 1999a-d, 2001). Integrated aquaculture has the potential to reduce the dependency on external ecosystems, for food and energy, as well as minimizing the negative environmental impacts from waste release i.e. reducing the ecological footprint of an aquaculture facility (Folke *et al.* 1998; Chopin *et al.* 2001; Kautsky *et al.* 2001).

This increasing recognition, that aquaculture development should be sustainable is reflected in the literature (Foy, 1990; Farshad & Zinck, 1993; Levin, 1993; Chopin *et al.* 2001; Kautsky *et al.* 2001; Folke *et al.* 2002) (See Box 1.1). Sustainability in development has been defined as "... development that meets the needs of the present without compromising the ability of future

generations to meet their own needs..."(WCED, 1987) or "...sustainable development improves people's quality of life within the context of the Earth's carrying capacity..." (Girardet, 1992). These definitions contain two key concepts: meeting the present and future needs of the world's poor; and accepting the limitations of the environment to provide resources and to receive wastes for the present and for the future. In this thesis I will use a definition for sustainable development by Thabo Mbeki (President of the Republic of South Africa) "'...Sustainable Development' is about improving the quality of human life whilst living within the capacity of supporting ecosystems..." (Evett, 2006). Sustainable in this context means "long lasting" or "enduring", it does not means "stationary" or "status quo". Thus it focuses attention on the time frame of decision making and implies prudence, long term thinking (a longer time frame than immediate interests might dictate) and care (Evett, 2006). Development is defined as a process of advancement, growth or maturation and is about fostering human needs (Evett, 2006). Aquaculture, and particularly integrated aquaculture, needs to be assessed in terms of their ability to contribute to sustainable development (Edwards, 1998).

Box 1.1 lists current definitions surrounding aquaculture. These definitions are many and varied and often have subtleties that require intimate knowledge. An example of this is the difference between organic and sustainable aquaculture. Both follow the same definition (Box 1.1). However the central goal of certified organic production is to "...verify and communicate to consumers that production systems are in place that will promote biodiversity, biological cycles, and biological activity by managing the production system as an integrated whole" (IFOAM, 2006). This is achieved by restricting the introduction of harmful substances and practices through strict guidelines and codes of practise. Such systems strive to create and maintain a sustainable production system that works with, rather than against, the environment in which it is embedded (IFOAM, 2006). To this end the *International Federation of Organic Agriculture Movements* (IFOAM) has drafted an overlying statement of principles that organic operators must abide by as far as possible in their interactions with human populations, domestic and wild animals, and the

environment (IFOAM, 2006). Produce from this type of aquaculture may be labelled as organic.

Contrast this to sustainable aquaculture, which is long term aquaculture with the principle objective to sustainably produce and keep pace with society's food requirements without eroding natural capital (Edwards, 1998). The terminology "eroding natural capital" is a simplified way of stating that the production systems will promote biodiversity, and natural biological activity by managing the production system as an integrated whole with the environment. This is very similar to the principles of IFOAM although sustainable aquaculture does not adhere to the principles of IFOAM, and produce is also not apparently labelled as being organic.

BOX 1.1: Definitions of terms used

Aquaculture may be defined simply as the growing of aquatic flora and fauna in marine, brackish or fresh water (Swift, 1985). The term mariculture has often been interchanged with marine aquaculture (Reay, 1979). Mariculture refers specifically to the farming of marine organisms including fish, molluscs, crustaceans and plants, in marine or brackish water, with some form of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc. (Lincoln *et al.* 1998). Farming also implies individual or corporate ownership of the stock being cultivated (DEAT, 2006).

Commercial aquaculture tended to concentrate on one species with little or no crop rotation is defined as monoculture. This results in environmental impacts such as eutrophication as well as decreasing system resilience (Folke *et al.* 1997; Kautsky & Folke, 1989).

The terms polyculture and co-culture have often been interchanged. Polyculture, the cultivation of two species of the same order, aims at increasing the crop diversity within a farming system (Folke *et al.* 1997; Kautsky & Folke, 1989). In such a system there does not have to be a mutually beneficial process between the cultured species, as is with the case of co-culture (Langdon *et al.* 2004).

Integrated aquaculture or "horizontally integrated aquaculture" or "co-culture" pertains to "an output from one subsystem in an integrated farming system which otherwise may have been wasted, becoming an input to another subsystem resulting in a greater efficiency of output of desired products from the land/water area under a farmer's control" (Edwards *et al.* 1988; Langdon *et al.* 2004), thus reducing the concentration of pollutants and potentially conferring benefits to the operator, environment and stakeholder groups. Examples include the culture of seaweed and shellfish in the wastewater from shrimp ponds and marine cage facilities, and the use of constructed wetlands planted with reeds or mangroves to treat the wastewater from land based freshwater or marine/brackish aquaculture, respectively (Ajisaka & Chiang, 1993; Shpigel *et al.* 1993 and Neori & Shpigel, 1999). Traditionally, land-based systems are commonly integrated with agriculture by stocking fish in rice fields and ponds referred to as integrated agri-

aquaculture, while water-based systems involve stocking fish directly in enclosures or attaching them to substrates in water bodies such as rivers, lakes, reservoirs or bays. Integrated multi trophic aquaculture is a fed aquaculture species linked with both an organic (e.g. mussels) and inorganic (e.g. seaweed) extractive species (Chopin *et al.* 2003).

Aquaculture is a diverse activity where the degree of intensity, through manipulation and intervention in the cultured organism's life cycle is determined by the scale at which organisms are cultured (Kautsky *et al.* 2001; Naylor *et al.* 2000; Troell *et al.* 2004). Integrated agri-aquaculture or extensive aquaculture is low-cost production using extensive and semi-intensive technologies usually for small scale subsistence households (Kautsky & Folke, 1989; Edwards & Demaine, 1997). It exploits natural food chains and water recycling processes and although being environmentally friendly, it cannot sustain future global aquatic production and land management requirements (Edwards, 1998). Fertilizer and feed may be derived from on-farm by-products, although formulated and pelleted feed from agro-industry are increasingly used (Semi intensive technology) (Furey *et al.* 2003). By contrast, intensive aquaculture systems invariably depend on relatively high-cost, nutritionally complete diets, chemicals and increasing the organism density in each of the culture tanks (Naylor *et al.* 1998, 2000). Intensive aquaculture, which uses formulated feeds (Kautsky *et al.* 2001) of which only 25 – 30 % is consumed with the remainder being discharged to the environment (Kautsky & Folke, 1989; Folke & Kautsky, 1989) has many more effects on the local natural environment than either extensive or semi-intensive aquaculture.

"Sustainable aquaculture is a dynamic process, incorporating research, learning and reassessment of methods, to practice and retain natural equilibrium in aquatic ecosystems. The objective is to sustainably produce and keep pace with society's food requirements without eroding natural capital" (Edwards, 1998).

Organic aquaculture occurs in controlled aquatic vegetation zones, and aquatic species are cultured at reasonable stocking densities and maintained with natural food products and compounds. Water quality is conserved by eco-technological methods and where applicable the need to utilize organic compounds and competent materials that are sensitive to the environment without interference in natural ecosystems (IFOAM, 2006)

A system approach to sustainable integrated aquaculture

The scientific approach to understanding aquaculture, has commonly been reductionist i.e., phenomena are divided into smaller and smaller entities or variables which are studied independently. It is being recognised that this approach cannot deal adequately with complex phenomena comprising numerous interrelated variables that occur in integrated aquaculture (Folke & Kautsky, 1989; Naylor *et al.* 2000; Chopin *et al.* 2001; Troell *et al.* 1999a,b, 2003). A system approach is expansionist and holistic as it recognizes that complex phenomena comprise systems of interrelated factors (Edwards, 1994). Thus, knowledge of agriculture, ecology, economics, engineering,

environmental science and sociology may be needed to adequately appreciate certain types of integrated aquaculture systems.

Sustainability may be expressed in terms of three interrelated aspects (Figure 1.1) (Edwards, 1994): production technology, social and economic aspects, and environmental aspects. Social and economic aspects of aquaculture have received relatively little attention compared to production aspects and are major constraints to development (Ruddle, 1991, 1993). Environmental aspects are beginning to receive attention to prevent humans from exceeding the global carrying capacity.

Production technology

Production technology can be subdivided into three main aspects: cultured species, culture facility and husbandry. The choice of species cultured influences the culture facility and together these determine the type of husbandry needed for the various stages of production (hatchery, weaning and grow-out). More than 200 species are currently farmed in aquaculture (FAO, 2001) in culture facilities as diverse as rice fields, static or running water ponds, cages and pens. Husbandry may involve various methods of stock management (monoculture or polyculture; single or multiple, stocking and harvesting strategies), use of different feeds (natural, supplementary or complete feed), management of substrate and water quality, breeding programmes to produce better strains of some species, disease prevention and therapy (Edwards, 1994).

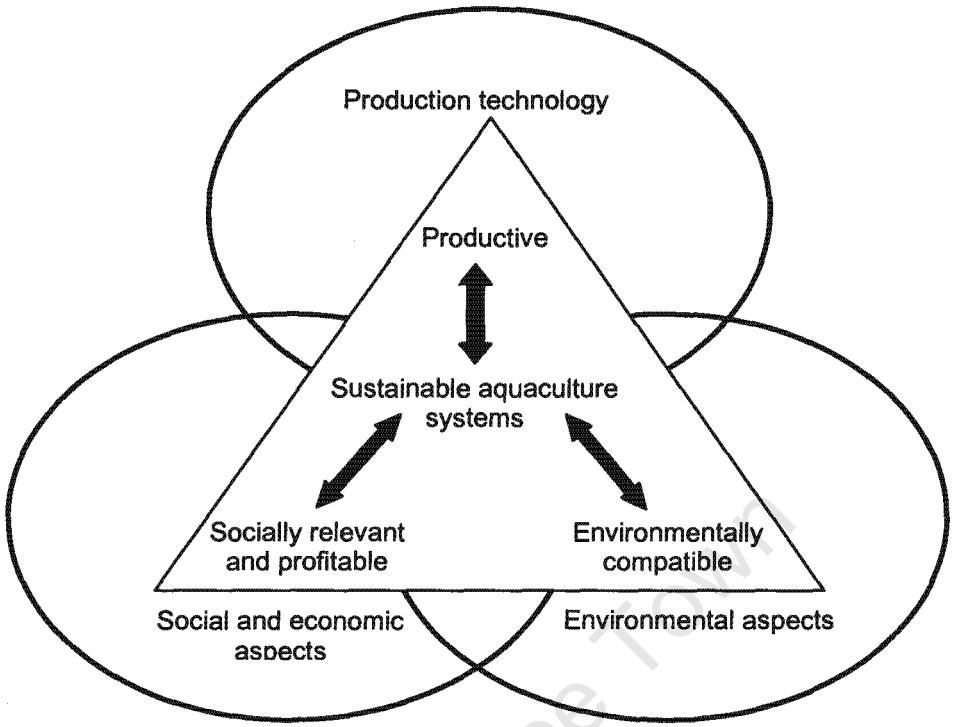

FIGURE 1.1: The three interrelated aspects of the sustainability of an aquaculture system: production technology, social and economic aspects, and environmental aspects (from Edwards, 1994).

Social and economic aspects

Social and economic factors influencing sustainable aquaculture may be considered at the macro-level (international, national and regional aspects) and the micro-level (community and farm household). Macro-level issues include world trade, national development goals, government policy, and social characteristics such as cultural attitudes and input supply and marketing. Micro-level issues are mainly alternative uses of resources (Edwards, 1994). In the South African context the economic component requires that "growth is pursued in a manner that brings economic benefits to the society at large and does not endanger its man-made and natural capital stocks" (President Mbeki in Evett, 2006). The social dimension "...is built on the premise that equity and an understanding of the human community's interdependence are basic requirements of an acceptable quality of life..." where wealth, resources and opportunity, are shared so that all involved have access to minimum standards of security, human rights and social benefits (President Mbeki in Evett, 2006).

Environmental aspects

The environment is external to the aquaculture system and includes the natural resources used for aquaculture development (e.g. land, water, nutrients and biological diversity), and also the two-way interactions between itself and the aquaculture system. The natural environment (climate, geomorphology, hydrology and soils) and its human transformation (agro-ecology, urbanization, industrialization), exert major influences on aquaculture, which may be either positive or negative. A positive interaction between aquaculture and the environment is a pond dug on a small-scale farm which functions as a nutrient trap while providing water for irrigation of rice seedlings and vegetables in addition to providing fish. Fish ponds may also be used to treat human sewage, manure from feedlot livestock as well as effluents from intensive aquaculture. Negative interactions between aquaculture and the environment are the adverse effects of pollution on aquaculture and adverse effects of aquaculture on the environment (e.g. eutrophication, misuse of chemicals, reduction of biodiversity and mangrove destruction) (Kautsky *et al.* 2001). The internal environment of the culture system is considered as part of the husbandry (Edwards, 1994).

An aquaculture farming system needs to be sufficiently productive to make it an attractive option to alternative or competing uses of resources, i.e. land and water, capital, labor, and farm by-products. They also need to mimic as much as possible the way natural ecosystems function to be sustainable (Folke & Kautsky, 1992). A systems approach is likely to be more successful in promoting aquaculture in developing countries. To do this, a thorough understanding of the resource-base of the farm, and the farmers' needs, as they perceive them is required. This must involve active participation of farmers and emphasize local resources as much as possible (Gliessman *et al.* 1981; Altieri & Anderson, 1986).

Sustainable aquaculture will be accomplished by the integration of new technology, combined with effective traditional practices, with the long term aim, to produce high quality products for local and international markets. A positive attribute of this strategy is less expense, reduced environmental damage caused by evasive packaging, refrigeration, transport, energy and waste associated with traditional intensive aquaculture.

At present both salmon and trout are being farmed organically and sustainably in countries like New Zealand, Wales, Scotland, Chile and Ireland (IFOAM, 2006) and information regarding these facilities and organic certification is freely available on the Internet.

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CHAPTER 2

ABALONE FARMING IN SOUTH AFRICA: AN OVERVIEW

INTRODUCTION

The South African abalone cultivation industry has developed rapidly and is now the largest producer outside Asia (Viana 2002; Gordon & Cook 2004; FAO 2004; Troell *et al.* 2006). Kelp (*E. maxima*) constitutes the major fresh feed for farmed abalone in SA, with 5447 tons being harvested for this purpose during 2003 (Anderson, 2003). This resource is now approaching limits of sustainable harvesting and the annual Maximum Sustainable Yield (MSY- 6-10% of total standing crop) has been reached in kelp Concession Areas with high abalone farm concentrations (Anderson, 2003).

Through its intensive nature and location the abalone cultivation industry has resulted in significant benefits to coastal communities through economic multiplier effects, but also through its inter-linkages with other industries e.g. the seaweed and abalone canning industries. With increased demand for fresh kelp (or kelp based feed) from the abalone industry, the seaweed industry has been positively affected. This has secured employment, predominantly unskilled labour. However, with the seaweed resource utilisation approaching MSY, there is a limit to its expansion. In addition abalone poaching has forced abalone fishery quotas down, resulting in decreased supply of harvested abalone to the abalone canning industry (Tarr, 2006). Abalone farming can be, and to a large extent already is, an alternative to the decrease in wild abalone fisheries. With these links and benefits in mind, the industry is reviewed from its own socio-economic perspectives as well as intermediaries with other industries e.g. the seaweed, canning and feed industries.

METHODS

The overview was based on three different questionnaires that were sent out to all SA abalone farms, all seaweed concession holders and all abalone canning factories (for details of the questionnaires see Appendix D). Information was also obtained from industry reports, from results of ongoing research programmes on integrated abalone and seaweed culture, and on coastal resource statistics obtained from coastal management authorities (Marine and Coastal Management) and census data from "Census 05 – Count Us In" by The

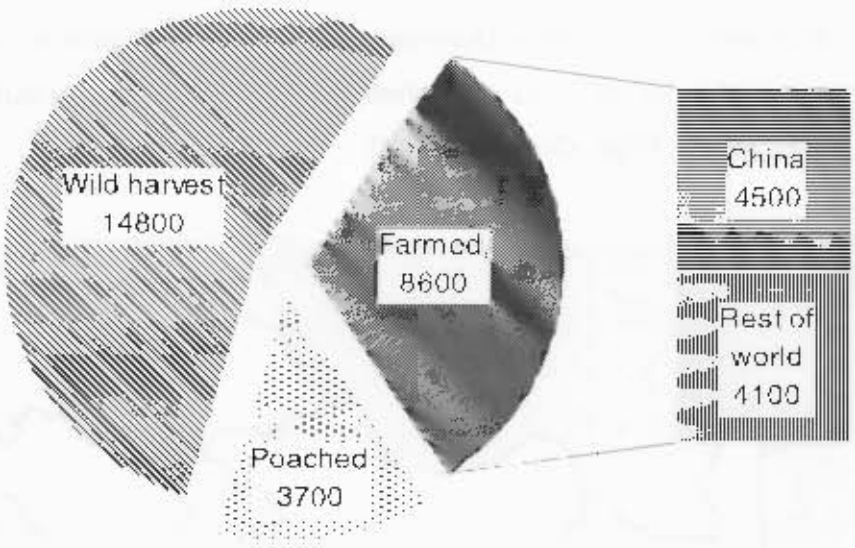

Figure 2.1: Distribution of world abalone supply (tons) for 2002 (Gordon and Cook 2004; FAO 2004)

Abalone cultivation first started in Japan more than 50 years ago, mainly directed toward ocean ranching of *H. discus hannai* for natural stock enhancement (Fujino, 1992; Viana, 2002). China started cultivation in the 1980's, and is the largest producer of cultivated abalone with over 300 farms and a total production of 4500 tons in 2003 all of which was consumed internally (Cook 2002, Viana, 2002; Gordon & Cook 2004) (Figure 2.1). Of the more than 90 existing species of abalone only fifteen are cultivated (Mahoney, 2002).

A rapid development of abalone cultivation took place in 1990s in the following countries: USA, Mexico, South Africa, Australia, New Zealand, Japan, China, Taiwan, Ireland, Iceland, and others (Hahn 1989; Gordon & Cook 2001).

Drivers for this development include: high prices being paid for abalone, and the worldwide decline in fisheries production, through poaching and over fishing.

South African abalone industry development

In South Africa, the name abalone is usually associated with one species: *Haliotis midae* Donovan, (often known by its Afrikaans name, "perlemoen" – "Dutch - mother of pearl"). However, the term abalone is internationally recognized as describing all species of the genus *Haliotis*. There are 6 abalone species endemic to South Africa (See Figure 2.2 for distribution), three of which

(*H. speciosa* Reeve, *H. quecketti* Smith and *H. pustulata* Reeve) are extremely rare. The other two, *H. spadicea* Donovan and *H. parva* Donovan, are small species not exceeding 80 and 45 mm shell length, respectively (Muller 1986, Tarr 1992, 2000; Hecht 1994; Greiger, 2004).

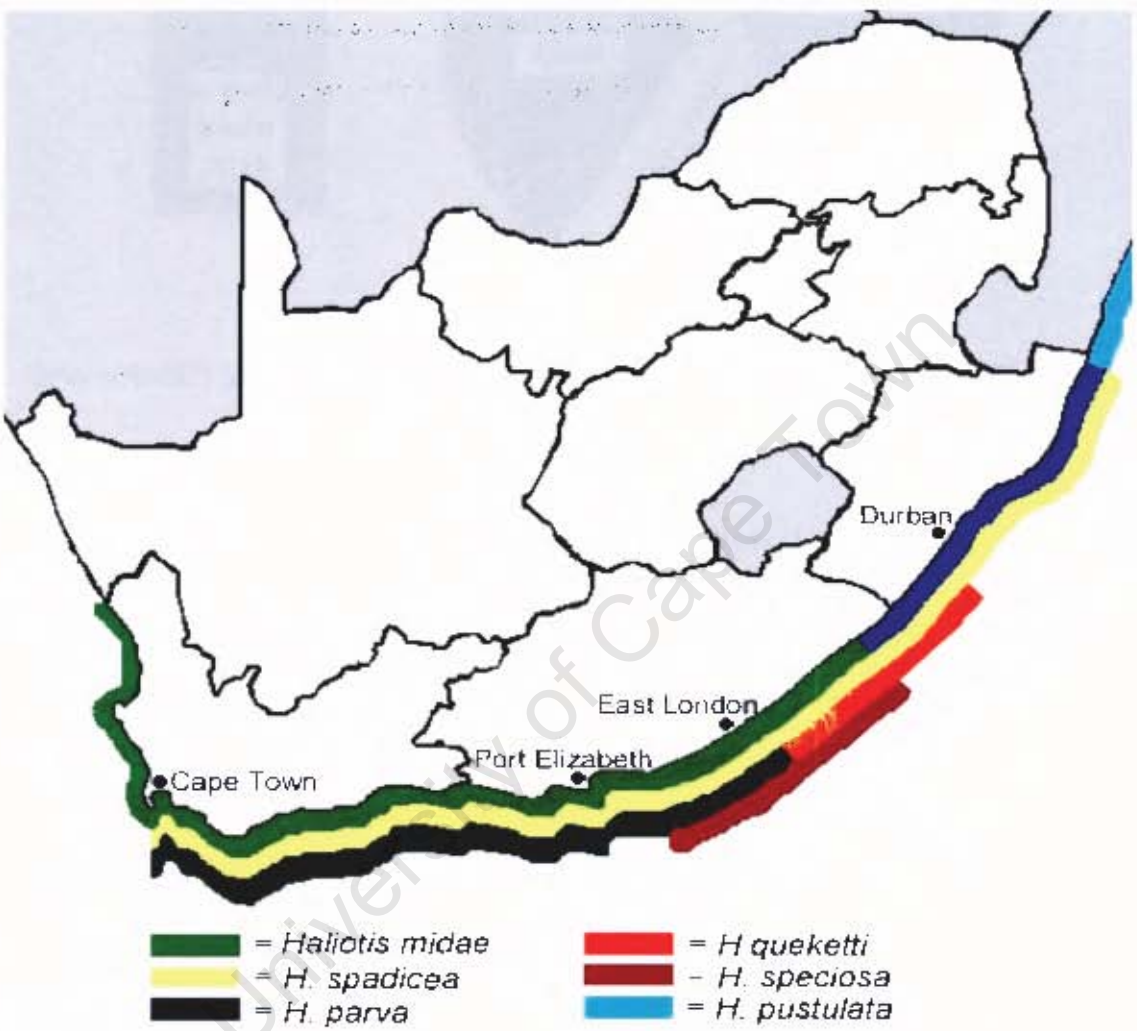

Figure 2.2: Distribution of the 6 Southern African *Haliotis* species (from Day. 1969: Tarr, 1992. Branch *et al.* 2000).

The present abalone fishery, which began in 1949, is based on subtidal stocks of *H. midae*, one of the larger haliotids (Tarr, 1992). No other abalone species of any commercial value exists in the Southern African sub-continent and *H. midae* is accordingly the only abalone species targeted in this region (Willock *et al.* 2004). Abalone attain a shell length of up to 230 mm (Hecht, 1994) and approximately 24% of their total weight is meat (Tarr, 1989). Sexual maturity may be reached at different sizes, depending on water temperature (Tarr,

1995). Abalone are dioecious (having the two sexes in separate individuals) and use external fertilization. Successful breeding depends on high densities of individuals. They can live in excess of 30 years (Tarr, 1989). This species is distributed from Cape Columbine in the north-west, to Port St Johns in the Eastern Cape Province (see Figure 2.2).

The fishery was reviewed by Newman (1964) and Tarr (1992). The traditional commercial fishery is based on about 580 km of coastline between Cape Columbine and Quoin Point (Tarr 1992, 2000; Maharaj *et al.* 2005; 2006). Not all of this is fished, however, due to unsuitable sandy areas, Marine Protected Areas, or closures of zones to the commercial fishery.

A recreational fishery existed from 1988 (See Figure 2.3 – Recreational landed), as open access with the only limitation being the daily bag limit of 5 abalone per person (reduced to 4 abalone per person in 1991) and the size limit of 114 mm shell breadth (138 mm maximum shell length, MSL). A permit system was introduced in 1983, and two years later a 3-month closed season was introduced. The recreational fishery peaked at over 750 tons in 1993 - 1994, which amounted to 122 % of the commercial TAC (ESS, 2000). Thereafter, the recreational take fluctuated around 630 tons (89 % of the commercial TAC) caught by some 34 000 permit holders, prompting the implementation of management measures to curtail further expansion of this sector (ESS, 2000). In 1997 - 1998, when one third of the season had elapsed, the Minister stopped the sale of further recreational permits and only 64 % of the permit numbers of the previous season were sold. This resulted in a drop in estimated recreational landings during the 1997/ 98 season to 302 tons. For the 1998-1999 season, the Minister again changed the season to weekends only and reduced the season length to four months. This reduced the recreational take to 123 tons, and in terms of the MLRA, the "saving" of tonnage was reallocated to other commercial sectors of the fishery. Due to the pressure of reductions in the commercial sector the Fisheries minister could no longer support a recreational fishery and the fishery was suspended indefinitely from the 2002/ 2003 season.

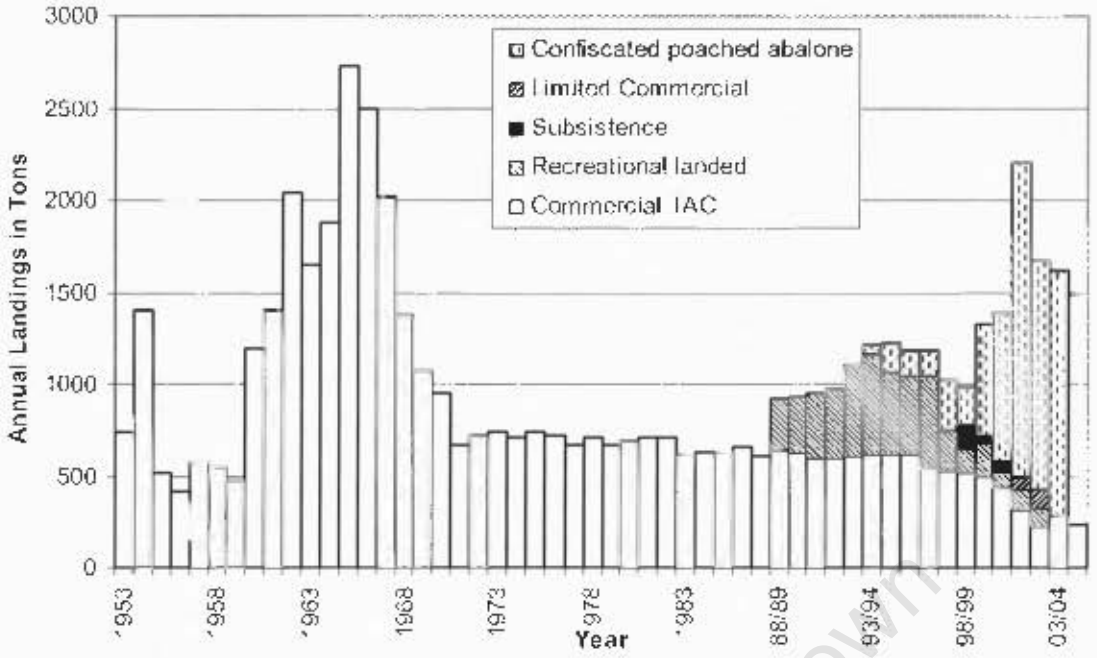

FIGURE 2.3: Abalone landings from the period 1952 to 2004 (Tarr, 1992; Maharaj *et al.* 2005, 2006). No data available at start of fisheries in 1949 to 1952. Confiscated Poached Abalone is estimated to be 10 % of total abalone poached (Gordon & Cook, 2001, 2004; Maharaj *et al.* 2005, 2006).

A new sector, subsistence fishing, was introduced in 1998 (See Figure 2.3 – Subsistence). These permits were issued to *bona fide* fishermen who could catch and sell the daily recreational limit of 4 abalone. Subsistence fisheries in the abalone and rock lobster sectors were replaced with limited (or small scale) commercial fisheries in 2001. This occurred in the high value fisheries, as a study commissioned in the late 1990's by Marine and Coastal Management concluded that it was not sustainable to maintain subsistence fisheries in high value fisheries, which are fished solely for subsistence. As subsistence fishers were not ordinarily allowed to sell their fish, many subsistence fishers sell illegally as they need finances for general purposes (clothing, food, shelter, etc.) (Kashorte, 2003; Maharaj *et al.* 2005, 2006).

An Eastern Cape Fishery existed based on small scale fishing rights, using Territorial User Rights Fisheries (TURF) regulations from 2001 to 2004 (See Figure 2.3 – Limited commercial) (Woods, 2003; Godfrey & Britz, 2005; Maharaj *et al.* 2005, 2006; Raemaekers *et al.* 2005). Harvesters were allowed 3 abalone

per week day of a MLS 100 – 114 mm, a total of 80 tons of abalone was removed during this time (Godfrey *et al.* 2005; Maharaj *et al.* 2005, 2006; Raemaekers *et al.* 2005). This fishery zone has now been closed as the area is also subject to heavy poaching (Godfrey *et al.* 2005; Godfrey & Britz, 2005; Maharaj *et al.* 2005, 2006; Raemaekers *et al.* 2005).

The commercial fishery was first regulated by permits in 1953 and since 1986 the fishery has been regulated by a minimum legal size of 138 mm shell length and 114 mm shell breadth, a restricted fishing season and a strict quota system (See Figure 2.3 – Commercial TAC) (Newman, 1964; Tarr, 1992). In 1983, the quota was changed from a production quota to a whole mass quota (total weight of the animal, including the shell also referred to as the "unshucked" weight) to address irregularities in the system (Tarr, 1992). In the 1998/ 99 season the allocation of TAC was expanded to cater for all participants in the fishery. Whereas, the TAC was previously applicable only to the commercial sector, the subsistence and recreational sectors were now also included. The TAC for 1998/99 totalled 820 tons, which was calculated as the sum of a "traditional" commercial TAC of 515 tons, a recreational take of 220 tons, and a subsistence allocation of 85 tons (ESS, 2000). However, despite this, natural stocks, and consequently commercial quotas, have been steadily decreasing over the last decade (Figure 2.3). This is a result of severe poaching in all areas (estimated at 850 tons for 2002 and 1023 tons for 2003, Gordon & Cook, 2004), higher than expected recreational catches (89 % of the commercial catch) (Griffiths *et al.* 2004; Tarr, 2006), but also a massive reduction in abalone recruitment in one particular area (Cape Hangklip to Hermanus) that used to provide the bulk of the commercial yield. This recruitment failure was caused by a unprecedented ingress of large rock lobsters (*Jasus lalandii* M. Edw.), which ate most of the large benthic invertebrates, including sea urchins (*Parechinus angulosus* Leske), turbinid snails, and juvenile abalone. These effects on abalone recruitment are compounded by the fact that the very young abalone (smaller than 18 mm diameter) live under urchins as a refuge from predators (Tarr *et al.* 1996; Day & Branch, 2000; Maharaj *et al.* 2005, 2006). The quota for the whole commercial fishery was thus limited to 237 tons in 2004 and 231 tons in 2006 (Maharaj *et al.* 2005, 2006) and decreased further in 2007

to 125 tons with closure of 3 fishery zones (Cape Times, 2006). The Department of Environmental Affairs and Tourism (DEAT) has now implemented a "focused management approach" with medium and long term (10 year) rights. This tactic is explained in DEAT 2003 policy for the allocation of commercial fishing (DEAT, 2004) and only allocates commercial fishery rights to divers, legal entities and abalone processing plants.

Poaching or the illegal harvesting of abalone is the single biggest threat to the South African abalone resource. Poaching refers to any activity, which contravenes industry regulations (outlined in the Marine Living Resources Act of 1998). Poaching is as old as the quota itself, but levels of poaching remained negligible – or at the very least containable – for the first two decades after the Commercial TAC quota's introduction (Hauck, 1997; Steinberg, 2005). All of this changed dramatically during South Africa's transition to democracy. Following the establishment of a new government in 1994 and greater emphasis on individual constitutional rights, expectations were raised among the residents of previously disadvantaged coastal communities who demanded formalized access to the abalone resource previously denied to them. Transformation of the country's fisheries was, however, considered too slow by many members of coastal communities. Illegal harvesting and trade increased (Willock *et al.* 2004). Other factors contributing to this increase include the declining value of the South African rand against major foreign currencies (Steinberg, 2005), budget cuts for many relevant government departments, including Marine Coastal Management and the South African Police Services, and continued unemployment and poverty (Willock *et. al.* 2004). Poaching began to escalate in the early 1990s (Steinberg, 2005). By the late 1990s abalone poaching had become a highly organised, multi-million dollar illicit industry, controlled by street gangs on the shoreline and by trans-national criminal enterprises on the trade routes to East Asia (Hauck & Sweijd, 1999; Steinberg, 2005). Despite increasing investments in shoreline patrolling and enforcement, the initiation of several large and well-resourced organised crime investigative projects (e.g. Project Neptune (Hauck & Hector, 2000)), and countless plans to reorganise the control of South Africa's borders, it appears that the illegal industry has been able to harvest and export South African

abalone at will (SAPS, 2001). By 2002, more abalone was being confiscated by the enforcement authorities per year than were harvested by the commercial fishery (See Figure 2.3 – Confiscated poached abalone). The illegally harvested catch has escalated annually since then, with an estimated 55% of the illegal catch being below the minimum legal size (ESS, 2000). Once the shellfish have been processed and packaged they will change hands numerous times before reaching their final destination. A major problem in halting the illegal international trade is that South Africa's abalone are not officially listed as an endangered species (Willock *et. al.* 2004). This means that if couriers can manage to smuggle the shellfish by road or chartered plane into neighbouring countries e.g. Swaziland, Botswana, Lesotho or Zimbabwe, landlocked countries with no maritime laws, they can be legally exported to the East. The Census and Statistic Department of Hong Kong shows that 200 000 kg of frozen, shucked abalone and over 100 000 kg of dried abalone were imported from Mozambique, Namibia, Tanzania, Swaziland and Zimbabwe to Hong Kong between the beginning of January 2002 and the end of June 2003 (Willock *et. al.* 2004). Abalone is not endemic to any of these countries (although it is currently being farmed in Namibia - + 5 tons exported in 2003) thus it is almost certain that all this abalone was illegally harvested in South Africa, smuggled into the other African countries, and then re-exported to Hong Kong (Willock *et. al.* 2004). When dried, abalone shrinks to one-tenth of its original size (Steinberg, 2005). A hundred tons of dried product is thus equivalent to 1 000 tons of fresh abalone. This means that, over a period of two seasons at most, considerably more South African abalone was entering Hong Kong from Southern African ports than the entire legally harvested quota. It is likely that unless poaching can be controlled, the wild abalone will be fished to commercial extinction within 5 years (Duvenhage, 2002).

A distinction needs to be made between wild abalone and cultivated abalone. The legally obtained wild product has limited market availability, in that it is restricted by a minimum harvest size. In contrast the cultivated abalone can be sold in any size. However the market for this product is largely for individuals in the size range 50 - 100 mm known as "cocktail abalone" (Hone & Flemming, 1998).

With the high prices being paid for abalone (R200-250 (US$ 30 - 40) /kg (live); Chinese black market: and up to R1 200 /kg (frozen & out of shell) & between R3 000 - R6 000 /kg (dried)) (Maharaj *et al.* 2005; 2006; Willock *et. al.* 2004) and the worldwide decline in fisheries production, it is not surprising that conditions have now combined to ensure the economic viability of abalone farming in South Africa. Initially there was reluctance to developing abalone farming due to a number of factors.

Work by Newman (1968) and Tarr (1989) suggested that growth rates of abalone in the wild were slow, taking 8 years to reach maturity. The first attempts to cultivate *H. midae* in South Africa were only made in 1981 when captured specimens were successfully spawned to produce spat and juvenile abalone (Genade *et al.* 1985, 1988). A concerted research and development effort to establish commercial abalone farming began in 1990 following awareness of initiatives in New Zealand and California, and Genade's initial work. Programs were initiated by the University of Cape Town, the Council for Scientific and Industrial Research and Rhodes University in partnerships with three fishing companies (Sales & Britz, 2001). Initial research indicated that growth rates in captivity were much faster than in the wild, and that food conversion efficiency was such that sufficient quantities of kelp would be available to feed the farm stock (Hahn 1989, Cook & Clayden 1990). In addition, access to relatively cheap labour, together with favourable coastal water quality and infrastructure has facilitated rapid growth (Figure 2.5). In addition, *H. midae* is of a very high standard and fetches a premium price on the market (Gerber, 2004). The prime demand is in eastern countries particularly: China, Hong Kong, Taiwan, Japan and Singapore, where abalone is considered one of the four "sea treasures" (abalone – representing health; sea cucumber – wisdom, Shark's fin – power and blow fish bladder – the ability to survive) (Gosse, 2000). Most farms export live (approximately three times a week) in response to overseas orders that are generally received only two to three days prior to export. The expansion of the cultivation industry is, therefore, likely to continue (Bennett, 2002; Gerber, 2004).

Most of the farms currently operating (Figure 2.4) are located in the Western Cape Province, but others exist as far north as Port Nolloth on the west coast, and East London in the Eastern Cape (where there are no kelp beds). All farms

pump seawater ashore, into land-based tanks in which the abalone are held in flow-through systems. Twelve farms have both hatchery and on-growing facilities whilst others rely on purchasing juveniles from other hatcheries. It takes between two and five years to grow an abalone from seed to market size (AFASA and SA farmers, personal communications; Viana, 2002, Loubser, 2005). The abalone cultivation industry is showing exponential growth (Figure 2.5) with 16 farms in existence (13 of which are in production) and a further 5 scheduled for development (Bennett, 2002; Loubser, 2005). In addition several farms are in the process of expanding their production.

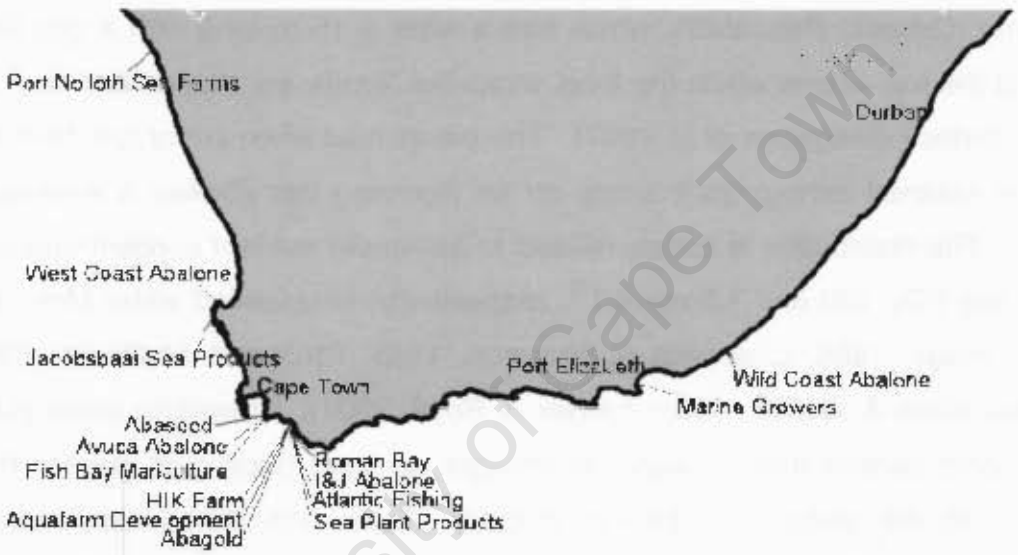

FIGURE 2.4: Location of abalone farms in South Africa.

An interesting offshoot of the farming industry has been the development of abalone ranching and abalone stock enhancement. "Ranching" activities on the west coast, are where seed are stocked into kelp beds north of the current natural distribution of *H.midae* (Sweijd *et al.* 1998; De Waal & Cook, 2001; De Waal *et al.* 2003), while "stock enhancement" experiments on the east coast at Cape Recife, are where hatchery-produced seed are re-introduced into the natural environment in the presence of wild abalone (Godfrey & Britz, 2005). The current objective of ranching operations is the commercial harvesting of the animals once they have grown to market size, while "stock-enhancement" is being used to rehabilitate over-fished stocks. It is possible therefore, that the

commercial technology that was developed to ensure profitable farms could also be used to the benefit of the natural fishery.

South African kelp resources

Kelp beds

Kelp is found along rocky coasts from just east of Cape Agulhas (34°49.8'S; 20°01.0'E), around the south west coast and up the west coast as far as Cape Frio (18°26.1'S; 12°00'E) in Northern Namibia (Levitt *et al.* 1992) (Figure 2.6). From Cape Agulhas to Cape Columbine, the dominant inshore kelp is *Ecklonia maxima* (Osbeck) Papenfuss, which has a stipe ± 15 m long with a gas-filled bulb at the top, above which the long, strap-like fronds are suspended near the water surface (Stegenga *et al.* 1997). The plants float when cut or torn free and can be washed ashore, particularly on an incoming tide (Bolton & Anderson, 1994). The distribution is largely related to maximum nutrient concentrations of NO_3^- and PO_4^+ (20 and 1,5 ug-at.l^{-1}, respectively) in upwelled water (Andrews & Hutchings, 1980; Chapman & Shannon, 1985; Probyn & McQuaid, 1985; Mitchell-Innes & Walker, 1991; Largier & Boyd, 2001). Upwelling takes place when cold central Atlantic water is brought up from depths of greater than 200 m to the surface by Ekman forcing. Even with these high nutrient concentrations, the nutrient uptake of *E. maxima* is still not saturated (Probyn & McQuaid, 1985). The kelps form dense stands between the sub-littoral fringe (at mean low water level) down to depths of ± 20 m. The upper temperature range for gametophyte survival is 26 °C, but gametophytes have been found to be fertile over a wide range of temperatures. Thus, growth of the haploid phase does not limit the distribution of the species. *E. maxima* is only found where mean monthly temperature range is between 11 - 20 °C (Bolton & Anderson, 1994).

Growth is very fast in the first year or two. The secondary fronds grow from their own secondary meristems near the base of the secondary fronds where they meet the primary frond (Bolton & Anderson, 1994). Growth in the primary frond is from a meristem at the base of the frond (Bolton & Anderson, 1994).

The primary blade can be up to 1 m long in juvenile specimens (Bolton & Anderson, 1994).

Laminaria pallida Greville ex. J. Agardh, the other dominant kelp, has a stipe up to ± 10 m long, no hollow bulb, and a digitate frond (Stegenga *et al.* 1997). It occurs almost entirely as a sub-canopy in the Agulhas to Columbine area, and seldom reaches the surface, but it may form extensive beds in deeper water. However, north of Cape Columbine and into Namibia, *L. pallida* develops a hollow stipe, and gradually replaces *E. maxima* as the dominant inshore kelp (Stegenga *et al.* 1997). Therefore, south of Cape Columbine, most of the harvestable kelp resource comprises *E. maxima*, while in the north, the bulk of the resource comprises *L. pallida*. This has implications for the supply of abalone feed, because the former species is generally considered to be a better feed for abalone. Although there is no scientific evidence for this, farms with similar cultivation temperatures have vastly differing Food Conversion Ratios (FCR) and they have also noted a difference in FCR between west and south west coast *Ecklonia*) (Robertson-Andersson, 2003 and farmer's pers. comm.).

Kelp production in the inshore ecosystem has been studied in detail in South Africa (Field *et al.* 1977, 1980). Kelp forests form diverse communities, provide various "ecosystem services" (e.g. shelter, shade, and a substratum for attachment) and interact with the hundreds of other species in the kelp bed in complex ways that are far from understood (McLean 1962, Reed & Foster 1984; Bolton & Anderson, 1997; Leliaert *et al.* 2000). They provide three major habitat zones (holdfast, mid-water, and canopy) for a multitude of organisms (Field *et al.* 1977; Velimirov & Griffiths, 1979; Allen & Griffiths, 1980; Anderson *et al.* 2006). The kelp plants themselves form a microhabitat, the stiff and upright stipe provides a substrate for many under-story seaweeds and sessile animals (Bolton & Anderson, 1997). There are a number of epiphytic algae on *E. maxima*. Three species make up the bulk of the biomass namely, *Gelidium vittatund* (L.) J. Agardh and *Polysiphonia virgata* (C. Agardh) Sprengel which occur on the stipe, and *Carpoblepharis flaccida* (C. Agardh) Kuetzing which occurs on the secondary fronds and can account for up to 5 % of frond mass in old plants (Allen & Griffiths, 1980; Stegenga *et al.* 1997; Bolton & Anderson,

1997; Anderson *et al.* 2006). Wide varieties of motile grazers are present, the majority of which do not remove entire kelp plants but rather graze on the tissue and other associated algae (Allen & Griffiths, 1980). In a study which investigated the stomach contents of the commercially important fish, Hottentot (*Pachymetopon blochii* Valenciennes) the canopy epiphytic alga, *Carpoblepharis flaccid*, was the largest component in the diet (Nepgen, 1977; Allen & Griffiths, 1980). Clinid fish which feed predominantly on meiofauna found in the canopy are in turn fed upon by seals (Penrith, 1965). Canopy fauna can exceed benthic fauna by a factor of 0.6 – 2.2 by weight (Allen & Griffiths, 1980).

Energy flow models indicate that the herbivores in kelp forests comprise a relatively small trophic group (Anderson *et al.* 1997; Field *et al.* 1977). The keystone predator in South African kelp beds is the rock lobster *J. lalandii* (Field *et al.* 1977). On the west coast and south-west coast (up to Hermanus) where rock lobster populations are high, sea urchin and juvenile abalone populations, (which shelter under the urchin) are low as urchins are a favored prey (Day, 1998). This situation is reversed on the south-west coast near Danger Point where lobster abundance is low and urchin and turbinid populations are high (Levitt *et al.* 2002). This difference in grazer abundance has an impact of the density of young kelp sporophytes. Where grazers are numerous (e.g. Danger Point) there is disproportionately high recruitment of young kelp sporophytes on the holdfasts of mature kelps, compared to on the rocks between the holdfasts (Velimirov *et al.* 1977; Fricke, 1978; Anderson *et al.* 1997; Levitt *et al.* 2002).

Kelp provides three distinct habitats for the use of birds: the kelp forest of attached plants, drift kelp on the open ocean and detached kelp that is washed up on beaches (Velimirov *et al.* 1977). Kelp forests also provide a potential source of invertebrate and fish prey as well as a refuge from storms. Kelp plants that are washed ashore on sandy beaches are consumed by large populations of the intertidal amphipod *Talorchestia capensis* (Dana), while on rocky shores it is consumed by the isopod *Ligia dilatata* (Brandt), both of which are important components of the diets of many seabirds (Velimirov *et al.* 1977).

Kelp beds are amongst the most productive biological communities. Productivity of *E. maxima* has been found to be around 4.11 – 7.76 kg.dry.wt.m^{-2}.y^{-1} and typical values of standing stock are 5.5 – 12.75 kg.m^{-2} (Bolton & Anderson, 1994). Kelp behaves like moving belts of tissue, growing at the base of the fronds and eroding at the tips (Mann *et al.* 1979). These eroding tips can contribute approximately 900 g C m^{-2}.yr^{-1} in the form of particulate matter (Dieckmann, 1978) and the bulk of this organic material is ingested as particulate matter by filter feeders (Field *et al.* 1997; Newell *et al.* 1982). Another 30 – 78 % of the material eroded at frond tips may be given off as dissolved organic carbon (300 - 700 g C m^{-2}. yr^{-1}) (Mazure & Field, 1980). This is then utilized by heterotrophic micro-organisms, via bacterial pathways (Newell *et al.* 1988), which in turn become food for suspension or deposit feeding animals, which comprise 80 % of the animal biomass in kelp beds (Field *et al.* 1997; Velimirov *et al.* 1977; Mazure & Field, 1980). Fifteen percent of the total kelp biomass is lost through kelp mortality. If this kelp is washed ashore between 60 - 80 % is assimilated by beach meiofauna (Griffiths & Stenton-Dozey, 1981). While the recovery of the kelps from harvesting may be easily measured, more subtle effects of long-term or excessive harvesting are difficult to measure, so that management has to be conservative (Anderson *et al.* 2006).

The regrowth of kelp after harvesting is therefore not only of importance to the harvesting industry but also important in an ecological context because the dynamics of recovery in the kelp forest ecosystem depend on the recovery of the kelp as keystone species. Recolonization of a harvested system will not only depend on the recovery time of the kelp but will also depend on how the associated flora and fauna are able to recolonize and stabilize the system.

The effects of commercial harvesting of *E. maxima* has been investigated since the late 1970's (Mann *et al.* 1979; Anderson *et al.* 1989; Levitt *et al.* 2002, Anderson *et al.* 2006), and kelp biomass has been shown to recover within 2.5 to 3 years of harvesting of whole sporophytes. Harvesting of *E. maxima* did not induce any measurable changes in understorey communities over a 3-year period (Levitt *et al.* 2002; Rothman *et al.* 2006). After initial harvests, understory flora was detrimentally affected but recovered as the juvenile *E. maxima* sporophytes grew and gradually reduced the light (Levitt *et al.*1992,

2000). However, populations of three obligate red algal epiphytes that grow on *E maxima* took at least two years longer to recover than the kelp itself (Anderson *et al.* 2006).

The South African seaweed industry:

Seaweed harvesting in South Africa started during the Second World War and focused initially only on red seaweeds (i.e. *Gracilaria* and *Gelidium*) from which agar was extracted (Anderson *et al.* 1989, 2003; Levitt *et al.* 1992). Collection of dried beach-cast kelp (*E. maxima* and *L. pallida*) started in 1953, with most of the material exported for alginate extraction. Although an average of about 1 000-1 500 t dw has been collected annually, from 1972 to 1977 collections reached record amounts of more then 4000 t per year with a maximum of about 5000 t DW exported in 1977 (Anderson *et al.* 1989; 2003) (Figure 2.5). There have been periodic slumps in export, usually lasting 2-3 years, when international market prices fell.

Twelve seaweed species are currently being exploited: *Ecklonia maxima*, *Laminaria pallida* (including *Laminaria pallida* var. *schinzii* Foslie), *Gracilaria gracilis* (Stackhouse) Greville from Saldanha Bay (includes *Gracilariopsis longissima* in St Helena Bay), *Gelidium abbottiorum* R. E. Norris, *G. pteridifolium* Norris, Hommersand & Fredericq, *G. pristoides* (Turner) Kuetzing, *G. capense* (S. G. Gmelin) P. C. Silva and *Plocamium corallorhiza* (Turner) Harvey. *Ulva* and *Porphyra* species are collected for a health food industry on the west coast (ESS, 2005)

Harvesting of *Ecklonia* began in the late 1970's, for the production of a commercial plant-growth stimulant (Kelpak®), and this operation continues today (Anderson *et al.* 1989; Robertson-Andersson *et al.* 2006). After the first abalone farms were built in the early 1990's, the demand for fresh kelp fronds as feed for abalone increased to nearly 6 000 t WW in 2000 (Figure 2.5). The harvest appears to have plateaued, but is likely to increase with expansion in the abalone industry.

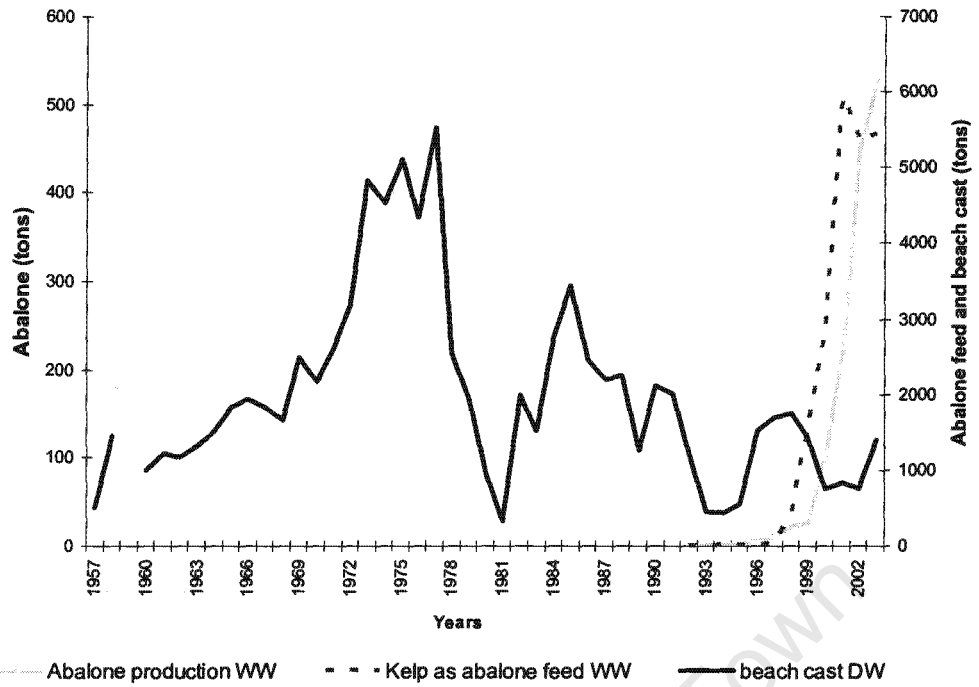

FIGURE 2.5: Abalone production from 1993 to 2004 (data from Hoffman *et al.* 2000; Cook, 1998a,b; Bennett, 2002; Gerber 2004; Troell *et al.* 2006), harvest kelp supply to abalone industry, total dried kelp production from 1957 to 2004 (data from Anderson *et al.* 1989; Troell *et al.* 2006 and MCM).

Seaweed resources in SA are promulgated under the Marine Living Resources Act (Act 18 of 1998), and are managed on an area basis by Marine and Coastal Management (MCM), with 23 rights areas ("seaweed concession areas" or SCA's) between the Namibian border and the eastern border of the Eastern Cape Province (Anderson *et al.*, 1989, 2003; GPR, 2005) (Figure 2.6). The seaweed sector is managed in terms of both a Total Applied Effort (TAE) and a Total Allowable Catch (TAC) (GPR, 2005). A single company is granted the right to harvest or collect a particular seaweed species or group of species in a SCA (e.g. either "kelp" including *E. maxima* and *L. pallida*; or "*Gelidium*" including all commercially useful species of this genus) (Anderson *et al.* 1989). The yield reported from the different concession-holders during 1986 - 2003 is shown in Table 2, which clearly shows a reduction in beach cast collections since about 1999, and increased production of harvested kelp for abalone feed and Kelpak®. In some Concession Areas (e.g. 5, 8, and 11) almost no beach-cast is now collected for export. This is because some operators no longer

consider it worthwhile, as collecting and harvesting fresh kelp fronds for sale to local abalone farms earn larger and more immediate returns. At least three of the Concession Areas, that before the advent of abalone farms produced substantial amounts of dried kelp, now produce none.

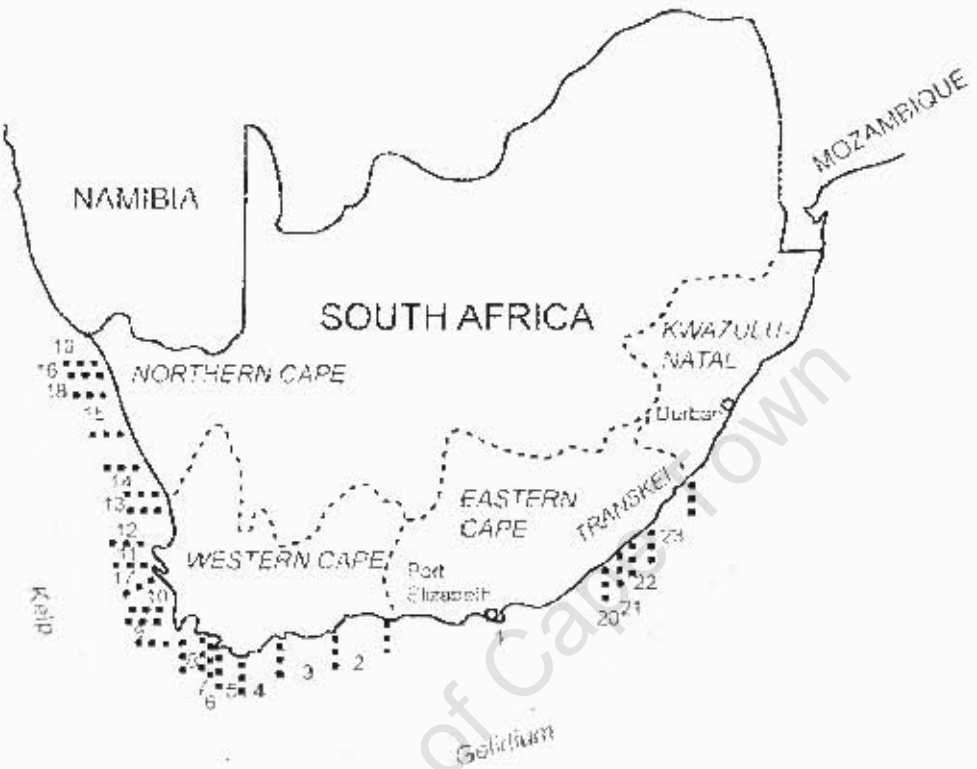

FIGURE 2.6: Map of the South African coast showing Seaweed Concession Areas (after Anderson *et al.* 2003; Troell *et al.* 2006). *Gelidium* is currently collected from area 1 and 20 – 23. In 2004 there was no collection of seaweed in areas 2, 3, 4, 10, 18, 19, 20, 22, and 23. Kelp can be collected in areas 5 – 19, excluding 17 (in which *Gracilaria* is collected). In 2005 after long term rights allocation all SCA have right holders for seaweed collection with some areas having more than 1 rights holder (GPR, 2005).

As with the abalone rights this fishery has also gone through a process of medium and long term rights allocation. This system (single right-holder per area) is aimed at preventing competitive over-exploitation of these static resources. For kelp, an annual Maximum Sustainable Yield (MSY) is set for each concession area, based on estimates of the biomass of kelp (Anderson *et al.* 1989) (See Table 2.1). In effect the MSY is set at about 6-10% of the biomass (a value similar to the estimated annual natural mortality of the kelp

(see Simons & Jarman, 1981). Furthermore, about 10% of each area is set aside as a "non-harvest zone" or "reserve" for the protection of old kelp plants and of kelp epiphytes, which occur mainly on old plants, and are an important food for certain invertebrates and line-fish (McBride, 1998; Anderson *et al.* 2006).

The potential supply of fresh kelp fronds for abalone feed is complicated by two main factors: a) distribution of kelp resources and b) distribution of kelp species and kelp bed biomass.

a) Distribution of kelp and abalone farms

Abalone farms are concentrated in two main areas, placing localised pressure on the kelp in those areas (Figure 2.4). There are 11 farms in the Kleinmond - Hermanus – Gansbaai area. Potential annual MSY (See Table 2.1) along this stretch of coast (SCA's 5 - 8) is 6475 tons fresh kelp, and in 2003, over 65 % of this was harvested. More important, in the westernmost SCA 8, close to a concentration of farms in Hermanus, 99 % of the MSY was harvested (951 out of 956 tons). The second node is in the Cape Columbine area, where there are 3 farms at present. Here an MSY of 1 550 t is available, and in 2003, 75 % of this was harvested.

Table 2.1: Kelp: Maximum Sustainable Yields (MSY) of kelp, harvests, and beach-cast amounts of fronds supplied as abalone feed, for the year 2003, by concession area (data from Marine & Coastal Management; Troell *et al.* 2006).

	a	b	c	d
Concession Area	MSY (t f wt)	Harvest (t f wt)	Harvest as % of MSY	Beach cast (t f wt)
5	1165	696	60	0
6	2680	2541	95	362
7	644/1287**	348	54/27**	192
8	956	951	99	0
9	1030/2060**	957	92/46**	0
10	143			
11	1550	1158	75	9
12	15	0	0	29
13	32	0	0	126
14	478	0	0	177
15	784	0	0	129
16	564	0	0	77
18*	137	0	0	0
19*	364	0	0	0
Totals	**10399**	**6651**	**54/39**	**1101**

*diamond areas – no collecting or harvesting at present

**Two values, First indicates frond only harvest, second is whole plant harvest.

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which are under maximal harvest pressure in terms of biomass. Thus effects or lack of significant effects to the kelp bed communities due to harvesting cannot be extrapolated to all kelp beds around the country. West coast beds tend to be less dense than South west coast beds (See Table 2.3) and thus the effect of additional light reaching the subsurface canopy may have a greater effect than has been previously documented.

Table 2.2: Commercial seaweed yields in South Africa for 2003, as reported by rights-holders. a – dried, mainly exported for alginate; b – wet beach-cast fronds supplied to abalone farms; c - harvested, all but area 9 (used for production of Kelpak®, not for abalone feed) supplied to abalone farms; d - total supplied to abalone farms. (data from MCM; Troell *et al.* 2006).

	a	b	c	d
Concession Areas	**Kelp – beach cast (tons dry wt)**	**Kelp – beach cast (tons wet wt)**	**Kelp – harvest (tons wet wt)**	**Kelp – Abalone feed (tons wet wt)**
5	0	354	696	1050
6	362	878	897	1775
7	192	523	348	871
8	0	0	951	951
9	0	0	957	0
11	9	112	1158	1270
12	29	0	0	0
13	126	0	0	0
14	177	0	0	0
15	0	0	0	0
16	0	0	0	0
Total	**1101**	**1867**	**5007**	**5917**

Table 2.3: Kelp bed density by area, from the literature from west to east.

Kelp bed	Density ($kg.m^2$)	SCA	Reference
Dangerpoint S	5.71 - 16.45	6	Levitt, 2002
Dangerpoint N	6.14 - 13.01	6	Levitt, 2002
Bettys Bay	4.9	8	Field *et al.* 1980
Millers Point	0.623	9	Frieke, 1978
Olifants Bos	5.95	9	Field *et al.* 1980
Kommetjie	7.01 - 7.9	9	Field *et al.* 1980; Simons & Jarman, 1981
Soetwater	3.8 – 13.4	9	Levitt *et al.* 1992
Oudekraal	8.39		Field, 1977
Seapoint	1.68		Field *et al.* 1980
Kreeftebaai/Sal	0.8	10	Field *et al.* 1980
Melkbosstrand	2.84	10	Field *et al.* 1980
Port Nolloth	3.29	16	Levitt, 1992

c) Harvest techniques

Harvesting of kelp for abalone feed essentially involves cutting the fronds during low tide. Almost all of the harvesting is done from boats, by workers who lean overboard and remove the fronds and primary blade by cutting through the base of the primary blade (See Figure 2.7 A). The whole "head" of kelp is then pulled aboard, leaving the stipe and holdfast to die and rot off the rock. Recovery of the kelp biomass then requires the growth of replacement sporophytes. Ashore, the fronds are then cut off and the primary blade discarded.

A non-lethal harvesting method is being tested commercially in one concession area. This method is based on the experiments of Levitt *et al.* (2002), who showed that by excising only the distal parts of the secondary blades, and leaving at least 20-30 cm of their bases (with the basal meristems) attached to the primary blade, the fronds could be repeatedly re-harvested (See Figure 2.7 C). The primary blade and stipe are left undamaged, and the yield of frond material over time was reported to be about 5 times higher than if the sporophytes were killed (Levitt *et al.* 2002). Although this allows a higher yield, there is evidence that frond re-growth rates decline as the sporophytes age, and it may prove necessary to carry out periodic lethal harvests to allow replacement plants to enter the population (Rothman *et al.* 2006). The third method of kelp harvesting is used by divers in SCA 10 only, where plants greater than 50 cm are cut (See Figure 2.7 B) in 10 m wide lanes. The plants then float to the surface and are collected when washed ashore by waves. The lanes are rotationally cut every 3 years (Simons & Jarman, 1981).

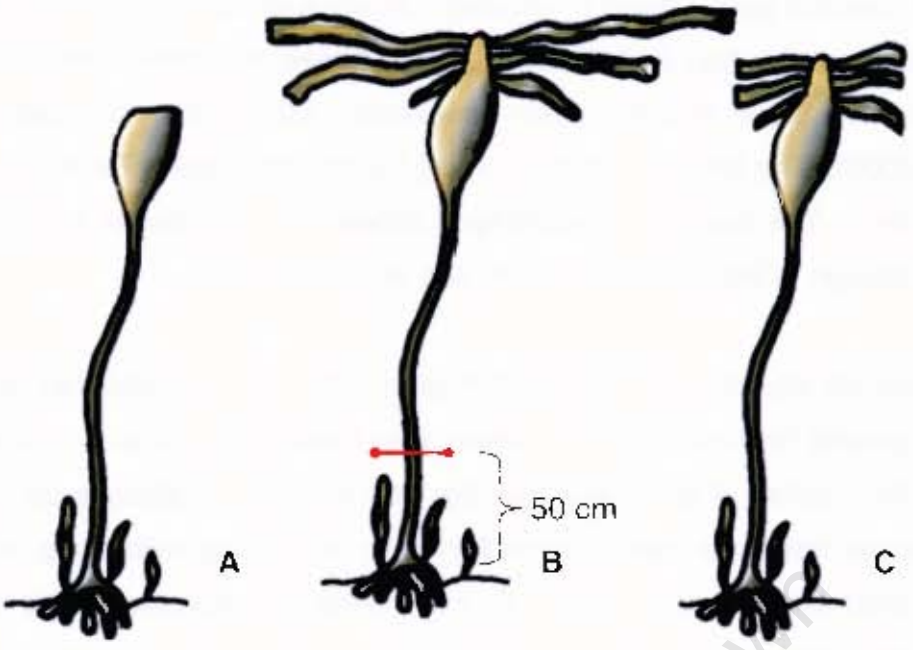


FIGURE 2.7: A is lethal harvest through primary blade; B is mature kelp plant greater than 50 cm height; C is non lethal harvest through secondary blades, 20 – 30 cm from primary blade (from Rothman *et al.* 2006).

d) History of kelp use per concession area

The use of fresh kelp as abalone feed began with the establishment of the first abalone farms in the Research and Development (R & D) stage in 1985. For most farms a period of 8 years of R & D development took place, Premier/ Atlantic Fishing in Gansbaai was the first farm to begin exporting abalone in 1991. Since then the number of farms have increased to 13 producing abalone farms. Of these, 13 farms most use a combination of kelp and compound feed, two farms use kelp only, and four use a mixed algal diet (Loubser 2005).

The only commercially available all-seaweed based feed in SA is a formulated dried feed called "Midae Meal MM-1c" being manufactured by Taurus Products (Pty) Ltd. The ingredients are mainly *Laminaria pallida* and *Ecklonia maxima* (stipes and fronds) but it contains also *Graciliaria* spp., *Gelidium* spp., *Porphyra capensis* Kuetzing and "agar agar". The wet seaweed: dry pellets ratio is between 6 - 7:1. Protein content is around 18 % which gives the abalone meat a natural taste, colour and smell. Sabellid infections have apparently not been observed with this feed and the shell colour is apparently also positively

affected (farmers pers. comm.). Although widely exported to the Far East, this feed has, however, had limited success in a series of growth trials, producing poor growth rates in abalone when compared against other formulated feeds (Dlaza, 2006). The feed is, however, being further developed (Taurus Products, pers comm.). The use of compound feed Abfeed® has increased in the last two years, although in the past, most farms fed kelp exclusively.

The farms are situated in nodes (See Figure 2.4), which means that there is a higher demand for fresh kelp on certain kelp beds (See Table 2.1) based not only on the number of farms but also the total tonnage of abalone produced in the particular nodes i.e. two forty ton farms do not use as much kelp as a one, one hundred and twenty ton farm. By correlating historical records of kelp use for abalone feed by SCA and abalone exports by SCA, we can predict the maximum amount of abalone production a kelp bed will support. In addition trends in kelp usage from the beds such as switching to a previously under utilised bed or other diets can also be detected (see Table 2.4). SCA 6 is a highly exploited concession area, which is being harvested to 95 % of its MSY and it is supporting 39 % more abalone than can theoretically be fed solely by the kelp available in that SCA (See Tables 2.1 and 2.4). The reason for this is that one farm in that SCA feeds exclusively on Abfeed®. Another SCA that is being maximally exploited is SCA 8, which is being harvested to 99 % of its MSY. Although this SCA has lower numbers of abalone, most of the harvest goes to farms situated in SCA 7 (See Tables 2.1 and 2.4).

Table 2.4: Present and maximum tons of abalone each SCA is able to support based on a kelp only diet for all farmed abalone (based on past trends of kelp use, 1991 to 2003).

SCA	Present tons of Abalone	Max tons*	% of total	equation	r^2
5	80.6	113	71.3	y = 0.823x + 7.0436	0.9775
6	145	104	139.4	y = 1.1306x - 4.6647	0.952
7	211	238	88.6	y = 0.4109x + 2.0562	0.975
8	17	37	45.9	y = 1.5642x + 41.845	0.7049
11	69	119	57.9	y = 0.8728x - 3.6982	0.8646

* Maximum tons a SCA can theoretically support if only fresh harvest and no beach cast or additional food source used.

Socio-economics

It has been said that political change in South Africa has been just short of revolutionary (Fakir, 2003). However, present socio-economic conditions have been determined by the country's apartheid past. While South Africa is classified as a middle income country, its Gini Co-efficient (the measurement of inequality) is 0.58, which is the second highest in the world after Brazil (Fakir, 2003). South Africa also has one of the worst records in terms of social indicators (health, education, safe water, fertility) (Fakir, 2003). This means that so-called previously disadvantaged individuals are in fact still disadvantaged. This can be seen when one looks at a breakdown of income categories:

By race and gender: The top 10 % income earners by races and gender: 6% of African females vs. 40 % for White females and 12 % of Black males vs. 73 % for White males (STATS SA, 1995).

By urban and rural: eight percent of rural people have an income in the top 10 % vs 34 % of people in urban areas (STATS SA, 1995).

Education: Twenty four percent of Africans have received no education, compared with 1 % of Whites. 40 % of Whites have received education up to standard 10 compared with 12 % of Africans (STATS SA, 1995).

The South African Constitution creates a set of socio-economic rights (Act 108 of 1996; Devenish, 1998). These rights include: the right to have access to adequate housing; the right to health care services; the right to sufficient food and water; and the right to social security, are entrenched in the South African Bill of Rights. The Constitution sanctions the state to "*...take reasonable legislative and other measures to achieve the progressive realisation...*" of these rights. Thus, the central purpose of the Constitution and the system of democratic, parliamentary governance that is establishes, is to "*deliver a social and economic transformation*" (Devenish, 1998; Fakir, 2003). These are reflected in the critical current goals of fisheries management (van Sitterta *et al.*

The SA abalone industry generates direct permanent employment and, although it may be small compared to other employment generating SA enterprises, it brings important benefits to previously disadvantaged coastal communities, distant from major constituencies. Because coastal resources that could be utilised by poor people are limited, these people are left with few alternatives for their livelihoods. The abalone industry, however, not only includes direct employment at the farm level, it also indirectly supports interlinked businesses such as the seaweed and abalone processing industries. Further, "supporting industries" (those which support the final assemblage, through the supply of parts (Yamazaki, 2004) and short-term contract workers from local firms are needed during build up and expansion phases. In order to quantify and evaluate the overall socio-economic benefits from the abalone industry, we need to look at the employment provided, identify worker groups and any additional benefits the workers receive from their employment (i.e. education/training).

The 13 producing farms in South Africa are all privately financed. 90 % of these farms have their own hatcheries. Investment, growth and employment in the industry are increasing yearly (See Table 2.5). The high fixed capital investment needed for abalone farming ranges between R1.6 million and R30 million for a 15 and 120 tons farm, respectively, preventing many prospective entrepreneurs from starting a farm. The gross industry investment is around R 340 million (See table 2.6) (Loubser, 2005) about R130 million of this is in infrastructure alone (Laweson-Smith, 2003). For 2004 the Gross Industry Turnover was R 193 million (Loubser, 2005). The average FOB (free on board - describes a price which includes goods plus the services of loading those goods onto some vehicle or vessel at a named location.) is around 26.8 $.kg and has remained at this level for the last three years (Loubser, 2005).

Table 2.5: Investment, employment and increase in the abalone industry from 2004 and projected forwards to 2006 (Data from Gerber 2004, Loubser 2005 and questionnaires).

Year	# of producing farms	Investment (R millions)	Tons.a^{-1}	Annual % increase	# of employees	% increase in employees
2004	13		576		556	
2005	13	197	745	27	776	28
2006	13	182	890	21	840	7.6

A. Direct employment

Abalone Industry

Cultivating abalone is labour intensive and as most of the jobs offered at a farm do not require educational or apprenticeship credentials they are open for people from poorer communities. An average farm employs 60 people and the total industry about 840 people, of which 61 % are unskilled (Table 2.6 & 2.9). The number of employees per ton of abalone exported range between 0.6 (this is low as a result of a farm employing recirculation technology resulting in decreased cleaning and daily maintenance) and 1.62, with larger farms having fewer employees per output due to "economies of scale" (See Table 2.6). Expansion of existing farms is seen as a means to compete with low exchange rates as it is possible to substantially increase output without significantly increasing running costs (Gerber 2004). Production lags behind employment for 5 years due to the slow growth of the abalone. It is possible that farms greater than 150 tons will have a lower figure of about 0.46 employee per ton once production is mature (based on farms estimated staff numbers after expansions are completed).

Wages constitute the largest expenditure (Table 2.7), and the percentage that abalone farms spend could be higher if it were not due to the local abundance of unemployed labourers (Gerber 2004). The average wage for a skilled worker

in 2004 was R5 442 p/m (US $ 833 – R: $ - 6.16:1, April 2006), while an unskilled worker received R1 813 p/m (US $ 294 April 2006).

Table 2.6: Abalone farming statistics for 2005.

	Min	Max	Average	Total
Investment per farm (millions)	R 1.6	R 30	R 15.75	R 346.5
Workers per farm	17	110	63	776
Employees per ton of exported abalone	0.6	1.62	1.1	

The average worker turnover rate for farms is reported to be less than 1 worker per year, barring two strikes on two farms where the entire workforce was replaced. This is an exceptionally low turnover and illustrates the stability of the abalone workforce (Gerber 2004).

TABLE 2.7: Distribution of running costs in the abalone farming industry as a percentage of the total running cost (n = 10) from Gerber 2004:

Running costs	%	range
Salaries and wages	31.27	22 – 40
Cost of sales*	21.5	10 – 34
Kelp	10.63	1 – 20
Repairs and maintenance	7.22	3.2 – 12
Electricity	6.77	2.7 – 9
Compound feed	5.63	0 – 10
Technology	2.08	0 – 5
R & D	3.2	0 – 5.5
Security	2.5	1 – 5

* Cost of sales includes all costs related to the selling of the product e.g. freight costs, freight insurance, health certification, fees, commission, bank charges etc.

Employment distribution within the abalone industry:

In terms of full and part time employment there is a distinction that needs to be made between farms that are in the build up phase and farms that have reached the mature phase. A farm that is in the build up phase requires more part time workers (Table 2.9). The ratio of unskilled to skilled jobs also differs between the build up and mature phase, with more skilled workers required during the build up phase (Table 2.9). Unskilled employees are mainly maintenance workers and those involved in harvesting, processing and security. Personnel working with engineering, finances, research and management are usually semi- skilled or skilled.

There is a difference in the distribution of gender during the development of the industry (Table 2.9), with an immature non-producing (i.e. not yet exporting) farm having almost 100 % male workers while a mature farm has approximately 78 % males. The reason for this change is that lifting and transporting of abalone and kelp in baskets is heavy manual labour and is therefore carried out by males. Grading and sorting occur on mature farms, allowing more females to move into the industry. With hatcheries becoming a new area on farms this also increases employment of female workers.

Race distribution within the abalone industry:

All of the unskilled employees are labourers. The Coloured and Black employees who are skilled are distributed into the following sections: management, administration, leaders/supervisors and technical staff (See Table 2.8).

The majority of the workforce resides within 0 – 30 km of the farms. Black and Coloured un-skilled and semi-skilled labour dominate on the farms, and the majority have completed the highest grade at high school

TABLE 2.8: Percentage race and skill distribution in the abalone work force

Industry		Black	Coloured	White
Abalone work force	Race distribution	45	38	17
	Unskilled	41	20	0.5
	Semi skilled	10	7	0.5
	Skilled	4	3	18
Permanent kelp harvesting work force	Race distribution	63	29	8
	Unskilled	54	5	0
	Semi skilled	8	20	1
	Skilled	1	4	7
Canning work force	Race distribution	67	24	9
	Unskilled	0	0	0
	Semi skilled	67	20	0
	Skilled	0	4	9
Abfeed® workforce	Race distribution	10	80	10
	Unskilled	10	40	0
	Semi skilled	0	30	0
	Skilled	0	10	10

B. Indirect employment

Seaweed industry

The seaweed industry was established before the abalone farming industry and has been a small but important source of employment for poorer coastal communities. The industry peaked during the 1970's, but recent changes in the global seaweed market, with increased competition and lower prices, negatively affected the industry (see figure 2.5). It is likely that the introduction of a new market, for abalone feed, has enabled a number of seaweed companies to continue operating. This change has increased profitability, as more is paid for fresh kelp as abalone feed than dry kelp for overseas alginate production (e.g. R 900 – R 1 200 per ton fresh harvested kelp vs. R 2 142 (US $ 350 – 400, April, 2006) per ton dried kelp FOB, remembering that for one ton of dried kelp, 5 tons of fresh kelp is required (Wet: Dry = 5:1 Anderson *et al.* 1989).

Start up costs for a seaweed harvesting operation range from R 250 000 to R 10 million. The total investment into the industry in 2004 was R 255.8 million. The average rand value of turnover per SCA allocated in 2004 was approximately R 2.3 million (GPR, 2005). Expenditure into research and development ranged from R 60 000 to R 600 000. The gross industry turnover for 2004 was R 12 500 000 (GPR, 2005). Six medium term rights holders made corporate social investments.

The seaweed industry at present employs 388 people who are directly and permanently employed with kelp collection of which most are unskilled labourers (See Table 2.9), the industry also employees between 600 – 1 456 part time or occasional workers, mainly for *Gelidium* harvesting. The average monthly salary for an unskilled worker is R 1 484 (US $ 240 – April 2006), while for a semi skilled worker it is R 3 208 (US $ 520 – April 2006). Unlike the abalone industry, the work force in the seaweed industry is predominantly female (GPR, 2005), but the permanent employees and levels above labourer are male dominated. Black and Coloured workers make up almost the entire labour force, but employment above the labourers are White dominated. There is a change in the race distribution with location, with companies that occur on the west coast having a higher proportion of coloureds in their work force, this changes to being Black dominated as one moves towards the east. This trend follows the community demographics. Occasional workers are mostly black females.

Future potential for increased employment in seaweed harvesting and collection may prove difficult. Apart from reaching the limits for fresh kelp harvest in some of the Concession Areas, most companies have restricted access to the resource in their Concession Areas. This is due to: poor infrastructure; privately owned land (farmland) bordering the coastline; permits for beach going vehicles; public opposition and restricted access due to marine reserves and nature reserves (ESS, 2003).

Table 2.9: Differences in percentages in labour between those employed: between an immature and mature abalone farm, in kelp harvesting vs. the entire industry, the canning industry and the Abfeed® industry.

	Build Up Farm	Mature farm	Kelp only	Entire seaweed	Canning	Abfeed®
Unskilled	50	73	59	91	0	50
Semi-skilled	40	17	30	7	87	40
Skilled	10	10	12	3	13	10
Full time	75	98 - 100	60	15	24	100
Part time	25	0 - 2	40	85	76	0
Male	98 - 100	78	51	34	83	90
Female	0 - 2	22	49	66	17	10

Most seaweed concessionaires agree that very little has been achieved towards stabilizing the kelp, *Gracilaria* and *Gelidium* industry in this country via the development of value added end products (with a few exceptions, such as the successful production of the agricultural growth stimulant Kelpak®) (ESS, 2003). As a result South Africa still competes as a raw material supplier with all the other main seaweed-producing countries (especially Indonesia and China), which limits volume stability and price. Seaweed collection for the alginate industry requires large volumes to support the market demand, and this particular market is extremely volatile. Any product arising out of new technology requires extensive research to enable product registration and sale. Markets need to be developed for new products and product nicheing is difficult.

Abalone Feed Industry

Even though the bulk of the production of compound feeds for abalone takes place abroad, some national producers exist. One local company, Marifeed PTY. LTD. Who produces Abfeed®, is the main supplier of compound feeds to the abalone farming industry. There is R & D occurring in another company on "Midae Meal MM-1c – a totally seaweed based feed. As this has not gone into production only statistics for Abfeed® were considered in this section.

Abfeed® is a formulated feed containing fishmeal (55 %), starch, *Spirulina* spp. (10 %), vitamins and minerals (Fleming *et al.* 1996). A cheaper, low protein (26 %) form of Abfeed® is currently also in production (Marifeed pers comm.).

At present 10 people work in abalone feed manufacturing in SA, a number that most probably will increase concurrently with the growth of the abalone industry and as more farms increase the use of Abfeed® in their diets. The average monthly salary was R 2 464 (US $ 400; April, 2006) in 2005, with only one female being employed in the industry. To date a total investment of around R 750 000 has been made in the industry, although this will increase as the company is planning to double its production capacity of 312 tons to 620 tons of feed in 2006/7. The company is doing R & D into probiotics and feed development with the Universities of Cape Town and Rhodes, which may increase its demand in the abalone industry further as the product becomes more economically viable.

Abalone canning industry

The first abalone factories were built in the 1960's to can wild caught abalone. Due to the fact that abalone is a high value food, the jobs created by this industry are of a more permanent nature with the staff being well trained and skilled. There is also a direct link between the abalone processing industry and the farming industry. Increased abalone poaching has resulted in decreased quotas for wild fisheries and this has made the link even stronger (See Table 2.10 and Figure 2.3). The commercial extinction of wild abalone is therefore a very serious threat to the livelihood of these companies and the communities sustained by them, but in this case, the decrease in the wild quota has been met by the increase in abalone farming.

The canning factory interviewed employs 57 people in processing but also 4 contract divers for wild harvesting. Each diver employs a crew of approximately four assistants with him on the boat. The average monthly salary earned in 2005 was R 2 464 (US $ 400, April, 2006). The work force is generally male dominated, due to the heavy lifting required (See Table 2.9). The canning industry has an investment value of around R 30 million. Besides employment generation, some of the factories also contribute towards corporate social Investment by donating funds for education (e.g. school renovations and

69 % of all employment. There is not a great gender imbalance in the unemployment statistics (Table 2.11)

TABLE 2.11: Comparison of unemployment figures between genders (Data from CSS 2005).

	Male	Female
Vrede-Sal-Jacob-StHel-Swartriet	7783	11074
Hemanus-Zwelihle	1470	2010
Kleinmond - Protea	1189	1311
Danger point-Gansbaai	1300	1833
Haga Haga	27	30

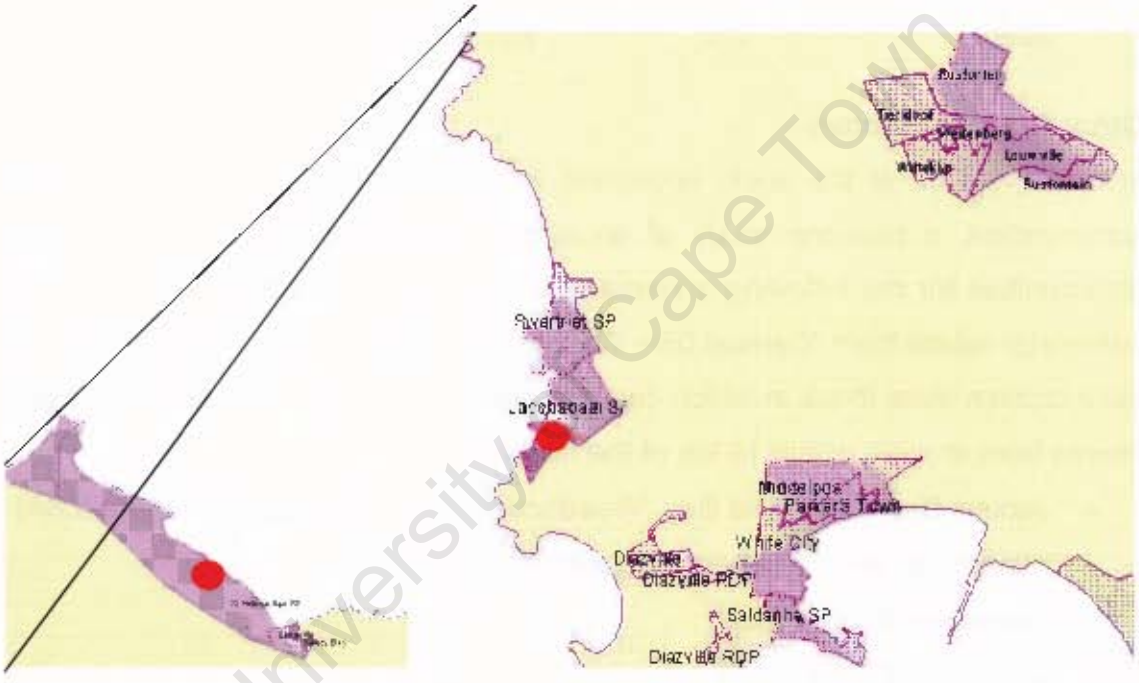


Figure 2.8A: The two farms Jacobs Bay Sea Products (in Jacobs Bay) and West Coast abalone (in St Helena Bay) (red dots) and the numerator areas used in the analysis (Purple shading). Wards from left to right: St Helena Bay, Sandy Bay, Langvlla, Rustenfontein, Tier Kloof, Witte Klip, Vredenberg, Louwville, Swartriet, Jacobs Bay, Middlepos, Parker Town, Diazville, White City, Saldanha

bursaries) (Lawson-Smith, 2003). A second canning factory was built by the Abagold Abalone Farm at an initial investment of R 8 million in 2007 and employs 16 people and cans farmed abalone exclusively.

TABLE 2.10: Data on the South African abalone canning industry.

	Canned Abalone		No of contract divers
	Wild (tons)	Farmed (tons)	
1998	95	0	50
2000	54	4	15
2002	36	30	6
2004	23	123	3
2006	7.5	200 estimated	2

Other Industry sectors

In order to look at the socio economic impact the farms have on the local communities, a baseline study of socio-economic indicators for the coastal communities for the following communities was compiled, based on data from numerator wards from "Census 05 – Count Us In" (CSS, 2005). The wards that were chosen were those in which communities existed that the farms employed people from or were within 15 km of the farms:

- Jacobs Bay, St Helena Bay, Vreedendal and Saldanha Bay (Figure 2.8A)
- Kleinmond and Protea (a small informal community)
- Hermanus (Figure 2.8B)
- Gansbaai (Figure 2.8C)
- Haga Haga and Komga (Figure 2.8D)

The areas where the farms are situated are characterised by high unemployment:

Vrede-Sal-Jacob-StHel-Swartriet	83.54 %
Kleinmond - Protea	57.64 %
Hemanus - Zwelihle	77.12 %
Danger point-Gansbaai	85.07 %
Haga Haga - Komga	45.24 %

Employment in all areas is heavily dependant on primary industries such as fishing, forestry and quarrying and secondary industries such as wholesale, retail and construction. These four sectors are responsible for between 32 and

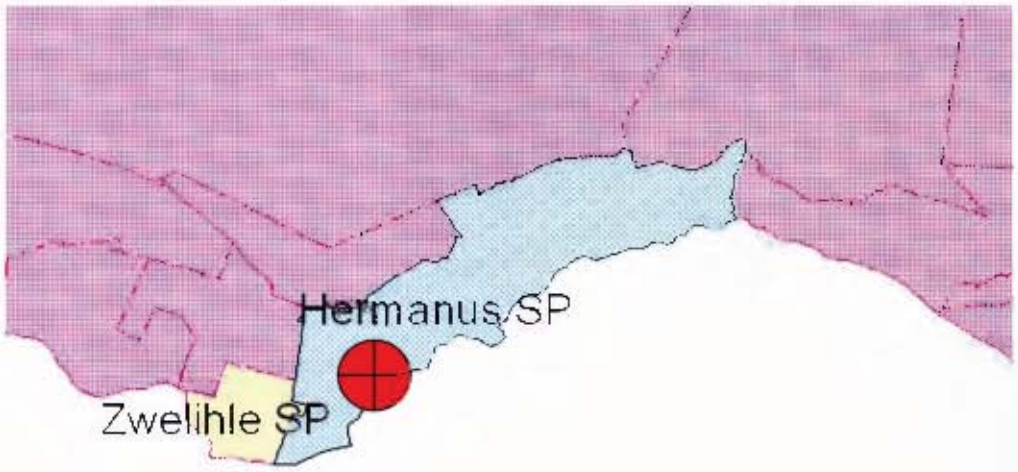


Figure 2.8B: The 5 farms HIK, Aquafarm, Bersig, Abagold. Abaseed in Hermanus New Harbour (red dot) and all the numerator areas (Blue and yellow shading, respectively). Wards: Hermanus and Zwelihle.

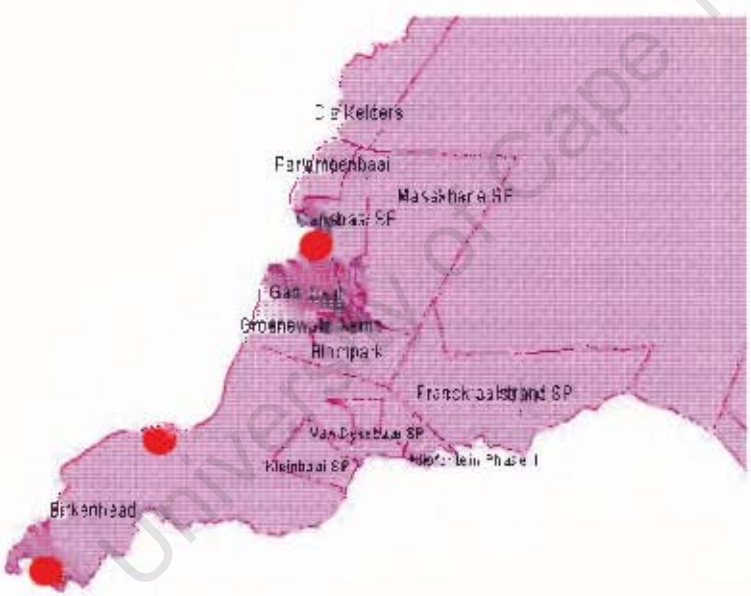


Figure 2.8C: The 3 farms I & J, Roman Bay and Atlantic Fishing (red dots) and all the numerator areas used in the analysis (Purple shading).
Wards from to left to right: Kleinbaai Birkenhead, Van Dryers Baai, Die Kelders, Klipfontein, Blompark, Franskraalstrand, Perlemoen Baai, Groeneweldskerma, Gaansbaai, Masakhane.

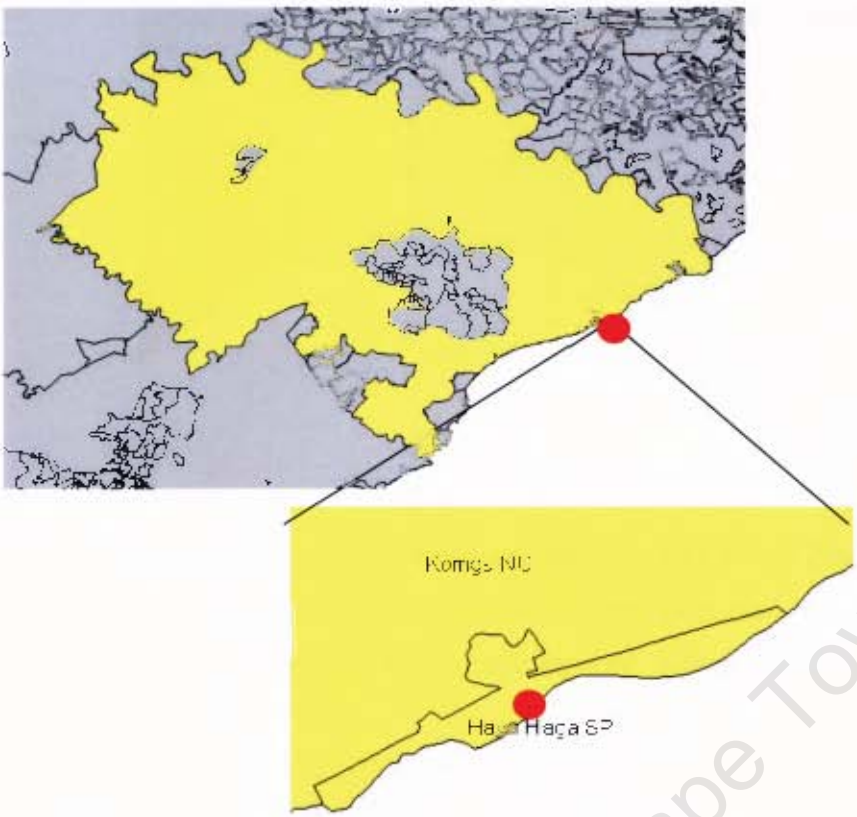


Figure 2.8D: The Wild Coast Abalone Farm (red dot) and all the numerator areas (yellow shading). Wards: Komga and Haga Haga.

TABLE 2.12: Males and females employed on the farms by area and the total number of employed people in the areas as well as the percentage of the total community employed in abalone farming.

	Employed on farms		Community Total		Community employed		% of employed community employed	
	Males	Females	Males	Females	Males	Females	Males	Females
Vrede-Sal-Jacob-St:Hel-Swartriet	74	11	19343	19218	11560	8144	0.6	0.1
Hemanus - Zwelihle	186	76	3389	3382	1919	1372	9.8	5.5
Kleinmond - Protea	20	5	2222	2115	1033	804	2.5	0.6
Danger point-Gansbaai	154	28	2657	2782	1557	949	9.9	3.0
Haga Haga - Komga	64	2	54	72	27	42	237.0	4.8

Tables 2.12 and 2.13 illustrate the numbers of male and females in each of the communities where farming occurs. By breaking farm employment statistics into each of the 3 population groups (Black, Coloured and White) it becomes apparent that abalone farms employ a large number of people in the areas

where they are situated. In the Haga-Haga area especially, one farm is responsible for employing all the Black males in that district according to the census data. If one looks at the area covered in the census data (Figure 2.8D) the values from Census 01 may be misleading. The Komga ward is very large and thus the number could reflect under-sampling in the census. The census for this ward was completed by one individual. It is a rural area with little or no infrastructure (in the form of roads for access). Given the limited time available (24 hours) in the census it is entirely likely that various dwellings were not counted. Notwithstanding, Table 2.12 illustrates how important abalone farming is to this coastal community. A trend which is evident from Table 2.13 is that employment by population group within the races changes from being predominately Coloured on the west coast (82.4 %) to being co-dominated by Black and Coloured on the south west coast (47.9 – 56.8 % Black: 27.7 – 32 % Coloured), to predominately Black (87.9 %) on the south east coast as with the seaweed industry. The White population employed by farms ranges from 12.1 – 22.4 % over the whole country and is in the minority at all farms.

TABLE 2.13: Population and gender groups in the districts and employed in abalone farming.

	Black African		Coloured		White	
	District	Farms	District	Farms	District	Farms
Hemanus-Zwelihle						
Male	3496	109	96	52	1467	27
Female	3240	41	191	21	1839	14
Total	6735	150	287	73	3306	41
Kleinmond - Protea						
Male	988	12	993	4	1238	4
Female	699	0	1021	4	1444	1
Total	1688	12	2015	8	2682	5
Danger Point-Gansbaai						
Male	1264	79	1230	46	1600	29
Female	1016	13	1274	11	1705	14
Total	2280	92	2505	57	3304	43
Haga Haga - Komga						
Male	54	57	0	0	30	7
Female	57	1	3	0	42	1
Total	111	58	3	0	72	8
Vrede-Sal-Jacob						
Male	5324	2	14780	63	4166	9
Female	4108	0	15869	7	4277	4
Total	9432	2	30650	70	8443	13

Other sectors of employment, both locally and nationally, have been stimulated by the abalone industry, these include:

Electrical Industry: Electrical costs which range between 5 – 15 % of a farms running costs. If this compared to the area of Gansbaai, the total usage of the three farms in Gansbaai is 13% of the total usage in that area (Lawson-Smith, 2003). In addition the development of the farms in the Hermanus/ Gansbaai area have allowed massive opportunities for the training and exposure of apprentices in the rural areas.

Security Industry: A huge investment in the form of outsourced security is maintained (2 – 5 % of a farms running costs). Security is an issue as one farm had its entire brood stock stolen by an armed gunman. This resulted in a loss of spawning production for more than 18 months (Simpson, pers comm.). The abalone farms all have galvanized electrified fences or a perimeter wall and an electric fence.

Engineering, Construction/ Civil, Industries: Local engineering companies are employed to complete certain tasks. The building industry is extensively used on all expansions and developing projects. Expansion of farms creates more opportunities for employment.

Scientific Equipment, Generating of Research Projects: About 3.2 % of running costs is spent on R & D, (for example from 2001 – 2006, at the University of Cape Town, the Stockholm University and the University of the Western Cape 2 Honours, 15 MSc. students (8 South African and 7 Swedish) and 1 PhD student have obtained their bursaries doing research on 2 projects in collaboration with two farms). Gerber (2004), pointed out 12 research institutes that have been or currently are involved with research into aspects of abalone and seaweed physiology and cultivation.

Other industries positively affected are Exporters, Equipment Manufacturing/ Piping/ Fittings (5 – 12 % of running costs), Flights / Airfreight, Laundry, Plastics, Transport, Insurance, HACCP as well as shell fish monitoring, veterinary services, foreign exchange and Sub contractors. Even though these are not addressed here they should be included in any overall estimation of employment.

Training and education

The Skills Development Act of 2001 is government's bid to provide education and training for all sectors of the workplace, including the unemployed. The aim of this act is to build the capacity of the work force, through the development of Sector Education and Training Authorities (SETAS) - which help bolster skills development though learnership - for each sector of the economy (Evett, 2006). This is funded by a skills levy on the pay roll, as well as government approved funding for employees who provide skills development plans and report on their implementation. The SETAS have discretionary funds - drawn from the levy income - which can be used for projects designed to assist in the achievement of specific sector priorities (Evett, 2006).

The seaweed industry has an approved work place skills plan as well as training. In 2004, R2.6 million was paid to SARS – South African Revenue Service - in skills development levies by the seaweed industry (GPR, 2005). An additional R 780 000 was spent on training Black seaweed employees (GPR, 2005). On average 3.46 % of all seaweed employees received learnerships in 2004 through SETAS (GPR, 2005).

At present a training programme for the entire abalone industry is under development (Loubser, 2005). Besides promoting very specific learning and skills for farming, it also includes basic life skills education (including reading, writing, computer literacy, financial and health issues, e.g. AIDS and Tuberculosis). Previously most farms applied their own training programmes that were farm cultivation system specific and this created problems of knowledge transferral.

Market drivers and constraints to development

In South Africa, abalone farms may only sell their abalone to final consumers. In addition, cultivated abalone are not available for sale in South Africa. In 1984, in response to complaints that abalone were not available on the local market, and in an effort to reduce the black-market trade of abalone within South Africa, it was made compulsory to market 10 % of wild production within the country. This regulation no longer applies, and 100 % of the wild product is now exported. The abalone fetches the highest unit prices of any South African fishery resource on the export market (ESS, 2000). Prior to 2003 local

restaurants depended solely on abalone supplied by recreational fishers. In 2005, two top restaurants in Cape Town applied for exemption permits to sell cultivated abalone. In 2006, the ban on the sale of cultivated abalone was lifted and the control is via a restaurant permit and book keeping system.

In general the prohibition of sales on the local market for cultured abalone has rendered the home market completely unknown. In addition the decrease in the commercial fishery and the prohibition of the recreational fishery are likely to have a positive effect for the demand for cultivated abalone in the local market. The prohibition on the local market has lead to vulnerability for South African cultivators, when prices are unfavourable in the overseas market. A local market may also allow the abalone farms space and price stability when there are problems in the overseas market. More than 60 % of the cultivated abalone is sold live at first point of sale (See Table 2.14). Since 2002 some product diversification has been introduced into the canning market, although frozen and semi-cooked exports have decreased (See Table 2.14). Ex-farm price of processed abalone is lower than ex-farm price of live abalone (Loubser, 2005).

Table 2.14: Value added to the product (From Gerber 2004 and Loubser, 2005).

	2002	**2005**
live at first point of sale	72 %	62 %
Canned	16.5 %	33 %
Frozen	10.5 %	4 %
semi-cooked exports	0.9 %	1 %

Seventy five percent of the farms sell their products under their own brand names. With more cooperation between the farms, the South African abalone could be marketed in such a way as to compete with brands such as Calmex® (a brand from California and Mexico). This would then result in an increased demand for South African abalone as well as increase the bargaining power of the South African producers.

The live price has remained fairly stable over the last three years at US $39 /kg and this means that the farms can expect pressure on the live price (Loubser,

2005). In order to combat this, the farms need to increase their production and diversify their product. This will hopefully lead to a lower basket price (a traditional conceptualisation of a price index). The index measures the change in value of a fixed set of quantities - commonly described as a "fixed basket of goods and services" - between two periods, (OECD, 2003) and greater profitability in the industry. Another concern is the weaker US$ /R exchange which currently means lower margins and decreased profitability.

South Africa's position in the international abalone market is not only affected by the total supply-demand ratio of abalone, but also by the supply-demand ratio of the preferred abalone species (Gordon & Cook, 2001). While the demand for the desired *H. discus hannai* Ino. currently exceeds its supply, it is still considered among the best quality abalone on the market (Gerber, 2004; Loubser, 2005). A number of countries such as Chile, Ireland and Hawaii have also started to produce this species. For the moment, Mexico's *H. fulgens* Philippi, and South African *H. midae* are at the top of the price structure in China, fetching higher prices than New Zealand or Australian products (Oakes & Ponte, 1996). However, when the demand for *H. discus hannai* is met, then the price for *H. midae* may be greatly reduced. In addition DEAT are considering placing *H. midae* on Appendix III of CITES, which could adversely affect the industry (Willock *et al.* 2004).

Although South Africa is at present the leading supplier of cultivated abalone in new farming countries it is very apparent when comparing other countries production statistics to South Africa that there are major differences (See Table 2.15). South Africa, next to Mexico has the highest number of workers per ton. This could be seen as a major disadvantage. However, our large unskilled labor force means that the cost of labor, and thus its contribution to the overall running costs (22- 40 %, Figure 2.14) of an abalone farming operation is on par with that internationally (35 – 55 %). Development and expansion predicted within the South African industry is likely to keep South Africa as the number one producer till 2010. Although there are 22 rights holders, the fact that there are only 13 producing farms in 2006, and that this figure is unlikely to change by 2010, means that our dominance is likely to be overtaken by other countries with more rights holders e.g. Australia, Chile and New Zealand. In addition

these countries have the ability to farm abalone in sea cages which is far less capital intensive than land-based farming. Sea cage farming is not an option for South Africa due to our high wave energy coastline and the fact that farms are very vulnerable to poachers.

TABLE 2.15: South Africa's production statistics relative to other abalone producing countries (excluding Asia).

	South Africa [1;2]	Chile[3]	Australia[4]	New Zealand[5]	USA[6]	Mexico[7]
Laborers per ton	1.1		0.25	0.4	0.6	1.15
Tonnage (tons) 2005	840	205	350	3	239	25
Tonnage (tons) 2010 estimated	1200	1000	1000	150	400	100
Farm type L = Land Based SC = Sea Cage	L	L/SC	L/SC	L/SC	L/SC	L
# of existing farms	13	26	20	23	9	8

[1]Du Plessis, 2006
[2]This Study
[3]Flores, 2006
[4]Mc Linden, 2006
[5]Illingsworth, 2006
[6]Fields, 2006
[7]Vázquez, 2006

The abalone farming industry has indicated that it needs to operate within the constraints of a short response time in order to remain competitive in the international market and maintain a good relationship with buyers, to ensure that projected increases in supply are taken up. Many of the abalone farms felt that the "government was not involved in the industry" or that "its involvement distracted from the competitiveness of the industry" (Gerber, 2004). These views are reiterated on the role of MCM, the South African Bureau of Standards

(SABS) and the Department of Trade and Industry (DTI) (Gerber 2004). The reason for this is the difficulty with legislation and permits and the fact that for aquaculture in general there is no one-stop shop. At present, the legislation controlling Aquaculture is administered by three lead Departments:

a) The Department of Environmental Affairs and Tourism administers Acts that deal with the sustainable use of natural resources. These acts include: The National Environmental Management Act (Act 107 of 1998); The Marine Living Resources Act (Act 18 of 1998); The Biodiversity Act (Act 10 of 2004), and Sea Shore Act No. 21 of 1935 (to be repealed by the National Coastal Zone Management Act) and the Draft Policy for the Development of a Sustainable Aquaculture Sector in South Africa (DEAT 2006) which will be followed by a new Mariculture Act.
b) The Department of Agriculture administers Acts that deal with the sustainable use of Agricultural resources, zoo sanitary and phytosanitary control, the control over any genetic modifications to animal and plant resources used for food and agriculture. These acts include: the Conservation of Agricultural Resources Act (Act 43 of 1983 - to be repealed by the Sustainable Use of Agricultural Resources Act); the Agricultural Pests Act (Act 36 of 1983); the Animal Diseases Act (Act 35 of 1984); the Genetically Modified Organisms Act (Act 15 of 1997) and the Animal Improvement Act (Act 62 of 1998).
c) The Department of Water Affairs and Forestry (DWARF) in turn administers legislation that provides for the management of water resources under the The National Water Act (Act 36 of 1998).

Other Departments such as the Department of Land Affairs, Department of Provincial and Local Government, DTI and Department of Health also administer legislation that impacts directly or indirectly on Aquaculture, for instance – Laws pertaining to physical planning (e.g. The National Building Standards Act, 1977; The Development Facilitation Act 1995); Laws pertaining to town planning; Laws pertaining to the standards and requirements with which buildings must comply and Laws pertaining to the subdivision or classification of land (From Probyn, 1999, and Glavovic, 2000a, b, c; DEAT, 2006).

Recent improvements include developing a Marine and Freshwater Aquaculture legislation framework to facilitate an environmentally sustainable accelerated growth of the sector (DEAT, 2006), an institute to service mariculture (L. Botes, pers comm.), and the establishment of the Frontier Program to fund research in aquaculture as well as the research facility at the Sea Point Aquarium, implementing more projects on aquaculture research (Pitcher, Pers comm.). In the past, most of the research into the industry was funded within the AFASA. The Shellfish monitoring program and the veterinarian service have both been contentious issues for farmers due to the funding required for such programs and the independent monitoring required, which is at present a restriction for South African abalone entering the European market. Since 2005, the DTI and national government have funded these programs and are investigating control as an independent monitoring body as well as providing a liaison officer for the industry (Pitcher pers comm.).

Other constraints to the industry, as listed by the farmers, are the limits in the natural food supply (kelp) and the increasing costs associated with feeding. In 2000, a ton of kelp cost R500 while in 2005 the cost is R980 – R1 200 (US$ 159 – 194, April 2006) (farmers pers comm.). The cost and quality (increased SGR, decreased FCR, decreased costs) and disease performance of compound feeds is also a constraint. More recently the impact of HIV/AIDS on attrition of staff has become an increasing problem. To this end staff training on HIV and aquaculture expertise are now being addressed within the industry.

Access to transport and proximity to a processing or distribution centre and problems with the distribution of the product, (e.g. space on aircraft and no temperature controlled holds in aircraft) also reduce South Africa's competitiveness in the international arena.

In April 1988 and March 1989, South Africa experienced its two worst recorded abalone mortalities, on the shores of the Betty's Bay Nature Reserve on the South Coast. Among other marine fauna, an estimated 30 and 40 tons of abalone were washed ashore during the 1988 and 1989 events respectively. Not only were adult abalone affected, but 2 day-old larvae and spat (3 – 5 mm juveniles) also appeared severely affected. Associated with these mortalities were coastal blooms of a previously unrecorded dinoflagellate species *Karenia cristata* Botes, Sym *et* Pitcher, which imparted a dirty olive-green discolouration

in False Bay and Walker Bay (Botes *et al.* 2003a, b). Since then several farms have reported gross deformities and huge mass mortalities of abalone larvae linked with the presence of harmful algal blooms (HAB's) (Jansen, 2006).

Availability of large tracts of land with natural protection from excessive wave action and large quantities of unpolluted seawater (including low sediments and few or no HAB's) with the necessary infrastructure are the single biggest factors restricting the development of new abalone farms. Secondary considerations are the existence of the wild abalone fishery, and the presence and availability of a secure broodstock harvestable form the wild.

Previously disadvantaged individuals within the cultivated abalone and seaweed industries

In South Africa a new fisheries policy under the Marine Living Resources Act of 1994, contains an implicit "development" mandate reflecting the Act's three guiding principles, namely, *equity*, *sustainability* and *stability* (Britz *et al.* 2000). The prime goal of the Act is to ensure that a fair and equitable system of access to the fisheries stocks (transformation), while also ensuring the long term sustainability of the use of resources, both in the interests of conservation and in the interests of current and potential users of these resources. The Access Rights Technical Committee (which implemented the Act) had to increase access to fisheries resources to increase equity through admitting new entrants to the fishery. In cases where resources are being exploited at maximum, new entrants could only be admitted at the cost of past access holders. Preference was given to legal entities (South African companies, trusts or closed corporations) that were historically disadvantaged or owned/ co-owned and managed/ co-managed by historically disadvantaged individuals (HDI).

This is particularly relevant in the seaweed harvesting industry where prior to 1997 the seaweed industry was entirely in the hands of White companies. In 2001 – 2003 after the allocation of medium term rights 6 out of 14 rights holders were Black owned and managed (43 % transformed) (GPR, 2005). Female shareholding in the industry in 2004 amounted to 28 % (GPR, 2005). Since only 17 areas were held (6 out of 23 are simply not used because the resources

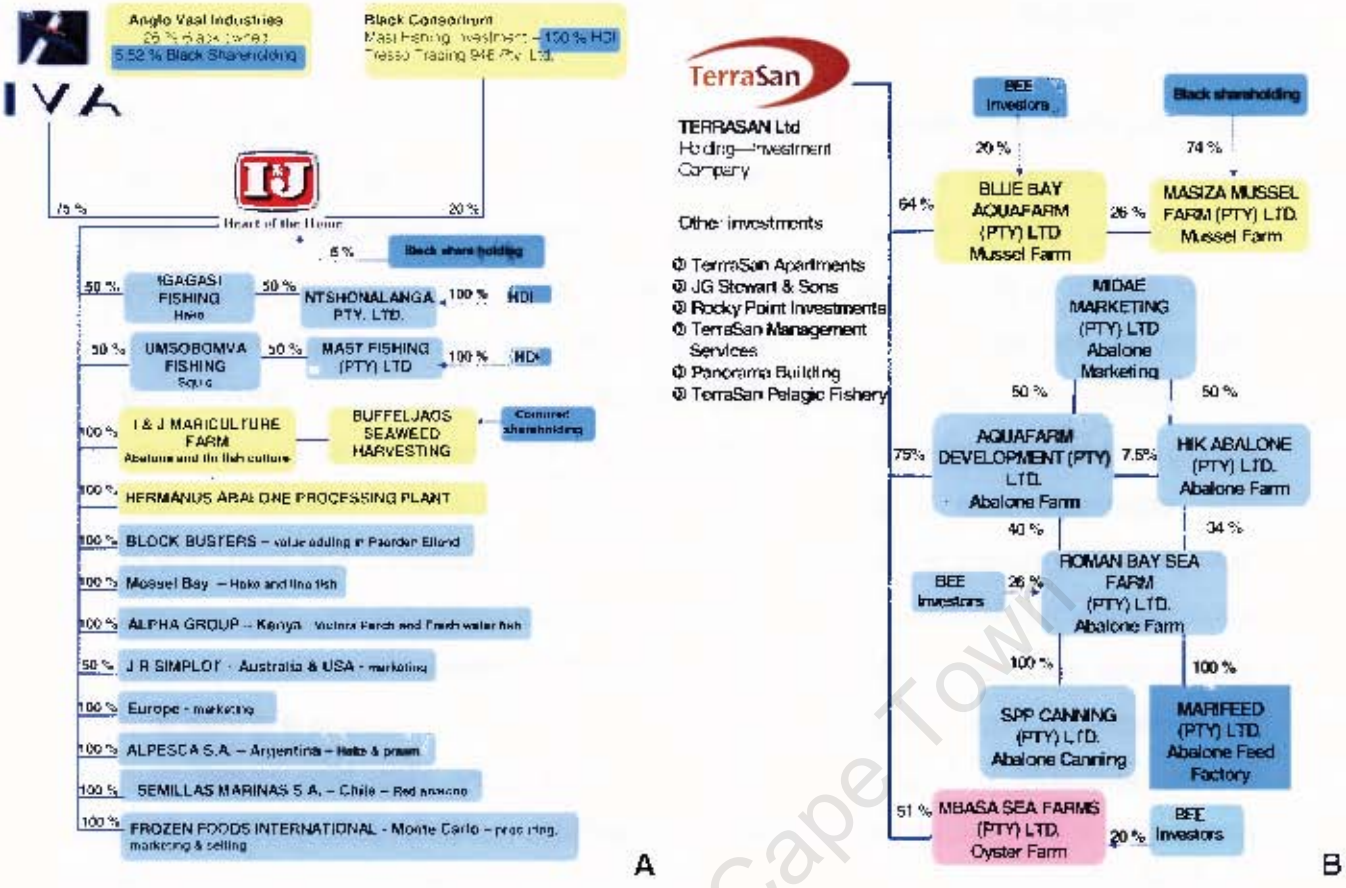


Figure 2.9 A & B: Holding company transformation and company structure of A – I & J and B – Terra san – Aquafarm, HIK and Roman Bay. Data from AVI report 2005. I & J report 2005, Du Plessis 2005; DEAT. 2002; DEAT 2004.

CONCLUSIONS

The total amount of abalone cultivated in 2000 was 100 tons and in 2005 740 tons, an increase of 740 % for a five year period. The industry employs over 770 people with a further 450 people employed in secondary supporting industries, 75 % of whom are unskilled or semi-skilled. Reasons for the industry expansion are the high price obtained for the product, and the long term commitment and investment especially with the long R & D period. AFASA is a strong and active organization and has 92 % of abalone farms as its members. The veterinary and health programs developed by the association and government ensure a good quality product with accountability. The farms are of economically viable sizes and have access to good water quality. The species itself is well accepted on the international market (in terms of taste and texture), the abalone also travel well and are hardy for cultivation. There is a cheap and readily available natural food source. The industry also has competitive production costs (electricity and labor). These data not only show the industry's success during infancy, but also its importance in the South African economy. It also illustrates the emphasis on sustainable growth of the farming industry as well as the impact and consequences that various activities and decisions have on the economy of these communities.

In order for this industry to continue, targeted government support is required, within the SABS, MCM and DTI. More effort needs to be placed into a sampling program that will service the entire shellfish mariculture industry (abalone, mussels and oysters) in order for the South African industry to gain access to the EU market. Promotion and marketing of the South African abalone on the international market is crucial to create a brand identity and ensure continued success and sales in an increasingly competitive international market. The streamlining of legislation and policies for aquaculture needs to be addressed hopefully in the new aquaculture legislation (DEAT 2006) to allow further expansion of the industry in order to maintain its place as the number 1 exporter of cultivated abalone outside of Asia.

CHAPTER 3A

DIMETHYLSULPHONIOPROPIONATE CONCENTRATIONS IN ABALONE (*HALIOTIS MIDAE*) AFTER CONSUMPTION OF VARIOUS DIETS

INTRODUCTION

Various groups of marine algae and some marine vascular plants produce significant quantities of the metabolite dimethylsulphoniopropionate (DMSP), a tertiary, non-volatile sulphonium compound (Malin & Kirst, 1997). Biological functions of DMSP in plants and algae may include acting as compatible solute in response to osmotic shock (Karsten *et al.* 1996; Kirst, 1996) or providing antioxidant properties (Stefels, 2000; Sunda *et al.* 2002). Acrylic acid, a breakdown product of DMSP, is known for its feeding deterrent properties and this has lead to suggestions of a possible role of DMSP in protecting against grazer damage (Sieburth, 1960; Van Alstyne *et al.* 2001, 2003). The DMSP production by plants and algae is under environmental control, and experimental work has shown that UV radiation (Slezak & Herndl, 2003). dissolved inorganic nitrogen (Dacey *et al.* 1987) and osmotic shock (Edwards *et al.* 1988b) may mediate DMSP production in the cells.

Dimethylsulphoniopropionate is released into the water column by grazing of phytoplankton cells by zooplankton (Dacey & Blough, 1987), by phytoplankton senescence (Nguyen *et al.* 1988), and by viral lysis of phytoplankton (Hill *et al.* 1998). Dimethylsulphide (DMS), is a highly volatile compound that has received much attention for its role in climate regulation (Andreae, 1990). (See Figure 3.1). Dimethylsulphide is subsequently cleaved from DMSP through enzymatic action (bacterial action) (Stefels, 2000). Dimethylsulphide is the main biogenic sulphur compound that is released into the atmosphere across the sea-air interface (Lovelock *et al.* 1972), and its oxidation produces aerosols that reduce the transmittance of light through the atmosphere, and affect cloud formation by acting as condensation nuclei (Andreae, 1990) (See Figure 3.1).

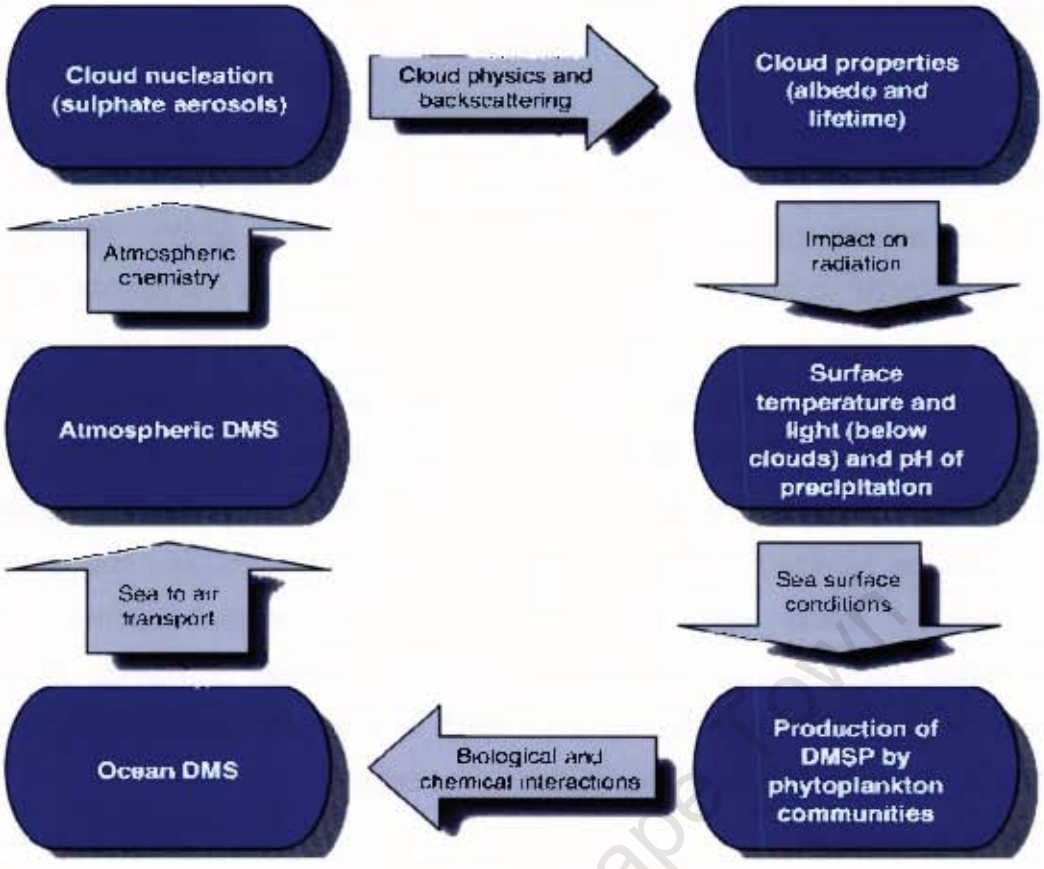


Figure 3.1: Simplified diagram of the global sulphur cycle (Lovelock 1988, 1991; Lovelock *et al.* 1972; Yoch 2002).

Dimethylsulphoniopropionate is not synthesised by marine animals but they acquire it from algae. When the compound is obtained through feeding on DMSP-rich micro- or macroalgal food sources it may subsequently be accumulated at high levels by some marine herbivores or filter feeders when provided with a DMSP-rich diet (Hill *et al.* 1995, 2000, 2004). One example of a macroalga with exceptionally high DMSP levels is *Ulva lactuca* which may attain the compound at concentrations of up to 8 - 103 $\mu g.g^{-1}$ (fresh) (Table 3.1) (Van Alstyne *et al.* 2003). Reports of animals fed diets containing high levels of DMSP (e.g. *Ulva)* cite increased growth rates, vigour and stress resistance (Nakajima, 1996; Naidoo *et al.* 2006). The current understanding is that DMSP is odourless and tasteless. However, DMS produced from the breakdown of DMSP is known to have strong tastes and odours at very low concentrations (Hill *et al.* 2000). When present in seafood in minute concentrations, DMS has a marked negative effect on the quality of the products. Brooke *et al.* (1968)

describe a distinct "off" petroleum-like or "seaweed- or kelp-like" smell (Cowan, 1988) in seafood products associated with the presence of DMS. Some marine products in which DMS has been implicated in affecting palatability include: the giant clams, *Tridacna maxima* and *T. squamosa* (Hill *et al.* 2000), the soft-shelled clam *Mya arenaria* (Brooke *et al.* 1968); the Pacific oysters, *Crassostrea gigas* (Ronald & Thomson, 1964); haddock, *Melanogrammus aeglefinus* (Mangan, 1959), and cod, *Gadus morhua* (Wong *et al.* 1967).

The ability of animals to cope with DMSP varies widely. Zooplankton such as copepods (Tang, 2001) do not retain DMSP but release it through defecation. Another method of DMSP elimination occurs in corals, and it involves the release of DMSP through mucus secretions following its production by symbiotic dinoflagellates (Broadbent *et al.* 2004). In giant clams DMSP is accumulated in the animals' tissues after being produced by zooxanthellae (Hill *et al.* 2000), and other filter-feeding bivalves and grazing gastropods accumulate DMSP in the flesh because they assimilate DMSP-rich food (Iida & Tokunaka, 1986). In instances where DMSP is retained and/or accumulated virtually no information is available about the mechanism behind its accumulation, metabolism or eventual elimination from the body tissue, although even the cursory information available shows that molluscs are distinctive (compared to other sorts of animals) in these respects.

Harvested kelp and to a smaller extent cultivated *Ulva* and *Gracilaria* are important feeds of aquacultured abalone in South Africa. The South African abalone industry has expressed concern about a bad taste and smell in some batches of cultivated abalone fed *U. lactuca* (R. Clark pers comm.). This problem seems to be exacerbated by the process of canning (Sea Plant Products cannery pers comm.). Based on published evidence from other seafood products (Iida *et al.* 1986), it was hypothesised that DMSP in the muscle of abalone meat may be converted to DMS during canning, a process that involves heating. In unprocessed meat, DMS evolves more slowly through an enzymatic process which is most probably mediated by microbial DMSP lyase (Ronald & Thomson, 1964). It is thus hypothesized that *Ulva* spp., which have been noted for their high DMSP content (Van Alstyne *et al.* 2003), is the

likely source of DMSP in abalone tissue, despite other seaweed feed also containing DMSP (Van Alstyne *et al.* 2003).

The overall objective of this research was to establish that *Ulva* was responsible for the bad taste in abalone fed an exclusive *Ulva* diet and to develop mitigation strategies for the negative effects imparted on cultured abalone because of the presence of *Ulva* in their diet. Dlaza (2006) and Naidoo *et al.* (2006) have demonstrated that *Ulva lactuca* provided as part of a mixed diet (both a compound and a natural diet) significantly enhances the gastropod's growth rate. Even removing *U. lactuca* may not eliminate the problem as Wong & Cheung (2000) and Van Alstyne *et al.* (2003) showed that other algal species also contain DMSP.

The main practical aim from this experiment is that farmers will be able to continue to use proven diets to produce high quality abalone meat without negative tastes and odours associated with DMS production from DMSP during processing. Research took place on commercial abalone farms situated on the south-west, west and south-east coasts of South Africa, which consented to provide live abalone and facilities for the experimental work.

The aims were to:

- Test for DMSP in cultivated abalone. The purpose of the initial tests were to determine a) If DMSP is present in abalone flesh b) if it was heterogeneously distributed within the tissues; and c) if DMSP is far more concentrated in certain locations which would be the best tissue type to use for future experiments. Past studies have been on whole tissues, and the data obtained represents the amount of DMSP per unit of whole-tissue mass. If the DMSP is in fact heterogeneously distributed between tissues, it could be far more concentrated in certain locations, and its location of effect could be a crucial factor for understanding its roles.
- Test DMSP concentrations in current abalone diets including compound diets which should not contain DMSP.
- Test for DMSP and DMS in canned abalone and for evolution over time, i.e. do concentrations increase with extended shelf life of the canned product?

- Test DMSP concentrations in various size classes of wild abalone and compare the results with concentrations in farmed abalone, as well as comparing partitioning between farmed and wild abalone.

A further aim is to:

- Develop a depuration phase during which abalone are taken off diets formulated for maximal growth rates (with *Ulva*) and placed onto an *Ulva*-free (i.e. low-DMSP) feed, both on the south coast and on the south-east coast and in three size classes of abalone (south east coast only).

Hypothesis: The ideal depuration diet is one which is low in DMSP. Changes in DMSP concentration should also occur more rapidly in smaller animals and decrease as they grow older. It is expected that the kinetics of DMSP metabolism depend on several factors, of which the two most important ones are water temperature and animal size. Metabolic rate increases exponentially with water temperature, and we expect DMSP to be accumulated and eliminated at a faster rate under the relatively warmer conditions experienced along the eastern Cape coasts than at colder temperatures along the south-west Cape coast. Of importance here too is that abalone growth rate is maintained at a maximum level during depuration within the constraints imposed by the lack of *Ulva* in the diet.

METHODS

DMSP/ DMS analysis

The measurement of DMS present in animal tissue (before and after death, canning, or other procedures) and feeds, was carried out by use of the purge-and-trap procedure of Kiene & Service (1991). The measurement of the total DMSP + DMS concentration was based on the method of Hill *et al.* (2000; 2004) for giant clams Tridacnidae, but GC-MS was used instead of a GC fitted with a chemiluminescence detector. This method involves placing 1 to 2 g of tissue samples in a glass vial containing 20 ml of analytical grade absolute methanol and sealed with a crimp top and Teflon-lined septum (Hill *et al.* 2000). Dimethylsulphoniopropionate was measured as DMS that was quantitatively produced from the cold alkali hydrolysis of DMSP (Dacey & Blough, 1987). The efficiency of DMSP extraction into methanol and the efficiency of conversion of DMSP to DMS were both investigated by Hill *et al.* (1995b). The reaction was effected by removing 1 ml aliquots of methanol, now containing dissolved DMSP, from the sample vials and reacting them with 25 ml of 2 NKOH at 2 °C for 24 hours in crimp-capped vials with septa. Dimethylsulphide in samples and the standard were measured in the headspace as described by Hill *et al.* (2000), but using gas chromatography-mass spectrometry (GC-MS) instead of GC with a flame photometric detector. The GC-MS comprised an Agilent GC 6 890 N and 5 973 inert Mass Selective Detector with a J&W GS-Q column (30 m long; 0.32 mm internal diameter). The MS was operated in EI SIM mode, and masses 46, 47, 61 and 62 were monitored for DMS and 43 and 58 were monitored for the internal standard. In order to measure the DMS produced inside the can, 10 ml of the brine in the cans was transferred to headspace vials and allowed to equilibrate for an hour before injecting the DMS sample into the GC. Standards were prepared in water for quantifying these samples. Using GC-MS ensured that DMS was positively identified. Dimethylsulphoniopropionate concentrations are expressed here as µg DMS per gram fresh tissue mass (µg g-1). The same method was used for determining the DMSP concentration in various algal and manufactured food sources fed to cultivated abalone. Five replicates were used per abalone sample and three replicates for each feed sample.

Animal preparation

Animals were selected from the experiments described in Chapter 5, or surveyed batches between 06h00 and 07h00 in the morning and were sealed in plastic bags containing sponge wetted with 100 ml of seawater and 100 % oxygen (Sales & Britz, 2001; Vosloo & Vosloo, 2006). Animals were transported by road (max. transport time *ca.* 10 hours) or air freighted (max. transport time *ca.* 18 hours) live to the lab for analysis, in the industry standard Styrofoam™ packaging (Cook & Ruck 1991). The maximum transport time of 18 hours, is equivalent to the minimum time required to transport the abalone from South Africa to the international market.

Temperatures within the containers varied from 16 – 23 °C and animals may have lost between 4 – 15 % of their live mass as water (Vosloo & Vosloo, 2006). Animals that were dead were removed from the batches to be sampled and the batches containing dead animals were noted. Animals were then patted dry and abalone body weight was recorded to the nearest 0.01 g, shell length was measured along the longest axis to the nearest 0.01 mm. Each animal was then shucked and the animal was decapitated. The gut and viscera were removed and 1 – 2 g of sample was sliced from the animal. In all cases except the partitioning experiment the tissue was removed from the top of abductor muscle (where abalone attaches to shell).

An experiment was performed to test sample preparation methods. Hypothesis: additional preparation of the removed tissue might result in more DMSP being available for reaction. Samples from 3 wild abalone were collected. The first were sections though the abductor muscle, the second were the same sized sections that were then diced in to (5 x 5 x 5 mm) cubes, the final sections were thin slices that were cubed and then compressed. A one way ANOVA showed that there was no significant difference between how the slices were obtained, and thus to reduce time, thin sections without any further preparations were used in all subsequent analyses.

DMSP Content of Feeds

The DMSP content of commonly used feeds was assayed in samples provided by the participating farms. These consisted of manufactured feeds (Abfeed®; Abfeed® K26; Taiwanese feed), and cultivated (*Ulva* and *Gracilaria*) and harvested (*Ecklonia maxima*). In the case of macroalgae, fresh samples were used at all times. These were placed in plastic bags and then sealed and placed on ice in a dark Styrofoam™ container and were transported immediately after collection. They were prepared for DMSP analysis as soon as they arrived at the University of Cape Town. As with the abalone, maximum transport time was *c.* 18 hours.

DMSP content of abalone raised on different feeds.

Abalone for the feed survey originated from brood stock kept at commercial hatcheries. The animals originated from the following farms: I & J; Abagold; Wild Coast abalone; West Coast abalone; Marine Growers; Jacobsbaai Sea Products and Aquafarm Development (See Figure 2.4, Chapter 2 for locations). The animals were maintained on the different farms and were fed according to the farms production routine using a variety of natural, cultivated and compound feeds. The animals for this survey were between 6 – 7 cm shell length, corresponding to an age of approximately 3.5 years. Husbandry information for this survey is not provided, as in many instances the farms requested that this information be kept confidential. This was a survey to obtain an indication of the range of DMSP concentrations found at the different farms as the animal were chosen by size and may have different ages due to differencing cultivation conditions.

DMSP Partitioning

To test if DMSP was being partitioned in the animals, wild and farmed animals (fed on a diet with a medium DMSP concentration) were sampled.

All farmed animals used in this assay were obtained from I & J. Immediately after settlement on microalgae, the abalone were place on an Abfeed® weaning diet for approximately 6 months. After this and at the weaning stage they were fed a mixture of *Ecklonia, Ulva, Gracilaria* and Abfeed®. The animals were then graded and sorted into baskets, then reared in the grow out section on the farm

on a mixed algal diet comprised of *Ulva*, *Gracilaria* and *Ecklonia* (25:25:50) every fourth week, and *Ecklonia* only for the rest of the time, until they reached a length of 6.7 ± 0.1 cm after about 3.5 years. 550 animals were placed in a basket with 24 baskets per tank. Each tank was fed 90 kg of seaweed a week.

Divers collected wild abalone from the Cape Point Nature Reserve on the Cape peninsular, South Africa in kelp beds in 2 – 18 m of water. The abalone were collected on a crustose coralline substrate or under the kelp holdfasts. Two separate collections were made. In the first, four size classes were selected: very small (3.9 – 6.1 cm shell diameter; 7.71 – 37.8 g total weight), small (7.5 – 8.5 cm; 80.68 – 102.06 g), medium (10.7 – 12.3 cm; 187.66 – 346.82 g), and large (16.1 – 17.0 cm; 700.11 – 1050.11g). Upon arrival at the laboratory, abductor muscle was dissected out and prepared for DMSP analysis. A one-way ANOVA tested the hypothesis that there was a difference in DMSP concentrations between the four size classes of wild-collected abalone.

In the second collection from the same site, 6 size classes were selected: extra small (3.2 – 3.5 cm; 3 – 6 g), very small (5.2 – 7 cm; 20 – 69 g); small (7.7 – 9.1 cm; 70 – 200 g); medium (12.8 – 14.2 cm; 300 – 499 g); large (13.9 – 14.5 cm; 500 – 600 g) and super large (16.9 – 18 cm; 900 – 1300 g).

Dimethylsulphoniopropionate concentrations were measured in four tissue types: abductor muscle, epipodial lobes, the top of the foot (obtained by taking thin sections), the bottom of the foot (obtained by taking thin sections): mucus and water lost through transportation from transport bags; and mucus and haemolymph production post decapitation.

A one-way ANOVA tested the hypothesis that there was a difference in DMSP concentrations between the six size classes or in their different tissue compartments. The rationale for this partitioning assay was twofold: first, it was hypothesised that there would be a difference in the level at which the different tissues accumulate DMSP; and second, if this hypothesis was accepted, I wanted to ensure that I consistently sampled the tissue with the highest DMSP concentration in the remainder of the study.

DMSP depuration/accumulation in canned and fresh abalone

The method used for the study of accumulation/depuration dynamics was similar to the one used by Naidoo *et al.* (2006), where batches of abalone were fed different diets (compound feeds and/ or macroalgae, including and excluding *Ulva* spp.) on a number of farms. A depuration diet is one that is, in all aspects, identical to the diet used initially, but without *Ulva* sp, while an accumulation diet is one that contains only *Ulva.*

Three experiments were set up to investigate accumulation and depuration dynamics. Two separate depuration experiments were set up, one on the south-west coast at Abagold and one on the south-east coast at Wild Coast Abalone. The main reason for this was due to access to feed. A secondary consideration was the difference in water temperature and the effects on depuration of DMSP. The ideal depuration diet was determined by considering the rate at which DMSP decreased from the animal tissues and growth rate.

For the south west coast depuration experiments, abalone were initially grown at Marine Growers Pty. Ltd. In Port Elizabeth, on a mixed diet comprised of *Ulva*, *Gracilaria* and Taiwanese compound feed for times ranging from 1049 to 1168 days. The variation in time the animals spent on the mixed diet resulted from using different batches of abalone, each hatched independently between 19th January 2001 and 13th January 2002. Nevertheless, despite the animals originating from separate batches, they were all treated identically in terms of feeding regime, stocking densities, and other general husbandry practices. Presenting *Ulva* as a dietary item to abalone during the initial grow-out phase allowed them to accumulate DMSP internally.

The subsequent depuration phase involved moving the animals from Marine Growers Pty. Ltd. to Abagold where they were fed *Ecklonia* only. At intervals from 0 to 477 days, the animals were taken off the *Ecklonia* diet and culled for DMSP determination. There was thus a range in time periods during which DMSP elimination could occur. The resulting DMSP concentration data were plotted against time off a DMSP-containing diet, a negative exponential function fitted and the parameters with associated standard errors estimated using non-linear regression in the software package Prism 4.

For the canning assay, the animals underwent the same treatments. However at Abagold the animals were taken off the kelp diet at intervals from 0 to 345

days after which they were shipped to the canning factory for processing. As contrasts there were also animals that remained on an *Ulva*-only diet (Wild Coast Abalone) and an *Ecklonia*-only diet (Abagold).

An additional canning experiment involved animals from the same batch fed a pure *Ulva* diet being canned into 20 cans and the contents being analysed in batches of 5 cans every 3 months. A one way ANOVA tested the hypothesis that initial DMSP evolution in the cans remained constant after canning through the can's shelf life.

The canning process at Sea Plant Products Cannery involved the live abalone being shucked and the head and viscera removed. The remaining foot and abductor muscle was then placed in a brine solution overnight, and the following morning the animals were washed and scrubbed by hand to remove all traces of "black skin". After this, the abalone were sorted by weight and placed into cans which were filled with water. For the purpose of the experiment, 5 abalone were placed into each can, although for proper production between 7 – 14 abalone can be placed in a can to make up a drained mass of 213 g. The mass of abalone in our cans ranged from 57 - 181 g with the average mass per can being 94 g. The cans were sealed and then went through a heating process lasting 10 minutes, after which they were properly sealed and then placed in a pressure cooker for 4 hours. After cooking the cans were allowed to cool down in a cold-water bath. They were then taken back to the laboratory. Dimethylsulphoniopropionate was measured in the brine solution and the meat. In addition, free DMS was measured in the brine solution.

Three different size classes of animals (15 g; 45 g and 75 g) initially from I & J, where they were fed a kelp only diet were flown to Wild Coast Abalone farm and were fed a cultivated *Ulva* only diet (accumulation experiment), while the same 3 size classes of animals from Wild Coast Abalone fed an *Ulva* only diet were fed an compound feed diet (depuration experiment). Sample abalone were drawn from these batches at approximately 90-day intervals and the rate of loss/ accumulation of DMSP from their tissues determined. Growth rates

(changes in length and mass with time) were measured at all sampling occasions.

Statistics

Statistical evaluation was performed using ANOVA, the source of variance being the diet the abalone had been fed and corresponding DMSP concentrations followed by Tukey HSD (honest significant difference) multiple comparison of means (Zar, 1999). *t*-tests were also used to determine differences between mucus and haemolymph concentrations. Mean values were considered significantly different at $p \leq 0.05$.

RESULTS

Partitioning

The concentration of DMSP varied with the tissue compartment analysed. In cultivated abalone the abductor muscle has significantly higher DMSP concentration than other tissues (ANOVA; $p < 0.001$; $d.f. = 4$), and DMSP was not detectible in the 'epipodial frill' (Figure 3.2). From this it was determined to use the abductor muscle in all future measurements. Firstly if DMSP was present then it would be in the abductor muscle. Secondly this is the part of the meat that is preferred when eaten and thus ultimately determines the abalone's taste. and thirdly to ascertain the maximum concentration in the flesh.

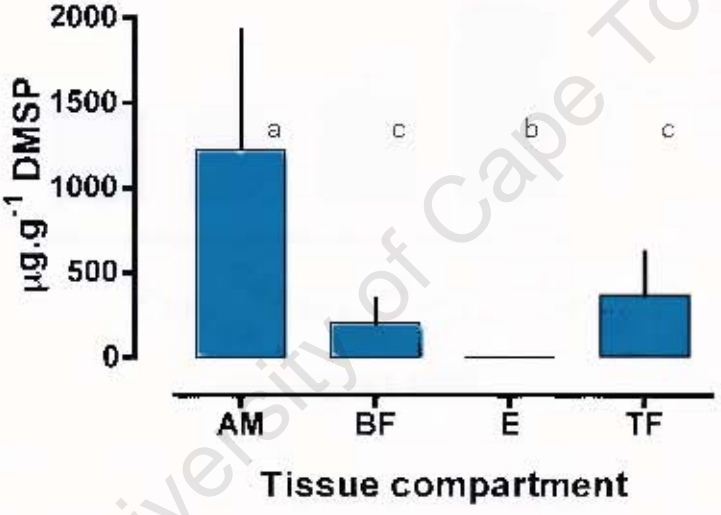


Figure 3.2: The partitioning of DMSP in abalone tissue compartments (fresh weight) (mean ± 1 SD). The animals were previously fed a diet comprised of 25% *Ulva lactuca*, 25% *Gracilaria gracilis* and 50% *Ecklonia maxima*. AM: abductor muscle; BF: bottom of foot; E: epipodial lobes; TF: top of foot.

The mucus and the haemolymph post-decapitation were analysed to look at possible routes for DMSP loss and transport. Mucus contained no detectable DMSP while the mucus and haemolymph mixture had DMSP concentrations ranging from (2.4 – 2.7 µg DMSP g^{-1}). A *t-test* showed that the DMSP contained in the haemolymph and mucus mixture, post decapitation was significantly higher than that in the mucus (T-test; d.f. = 3; $p < 0.05$). A

subsequent test looking at haemolymph only found concentrations ranging from 9.5 – 35.6 µg DMSP g^{-1}. This implies that DMSP is present in the haemolymph and also that DMSP loss is not through mucus loss as in corals.

There was a significant decrease in DMSP concentration with size in the abductor muscle tissue of wild-caught abalone (Figure 3.3) using linear regression ($F = 6.361$; d.f. = 1, 23; $p < 0.05$). As the size classes were varied the test was rerun and other tissue types were assayed (Table 3.1).

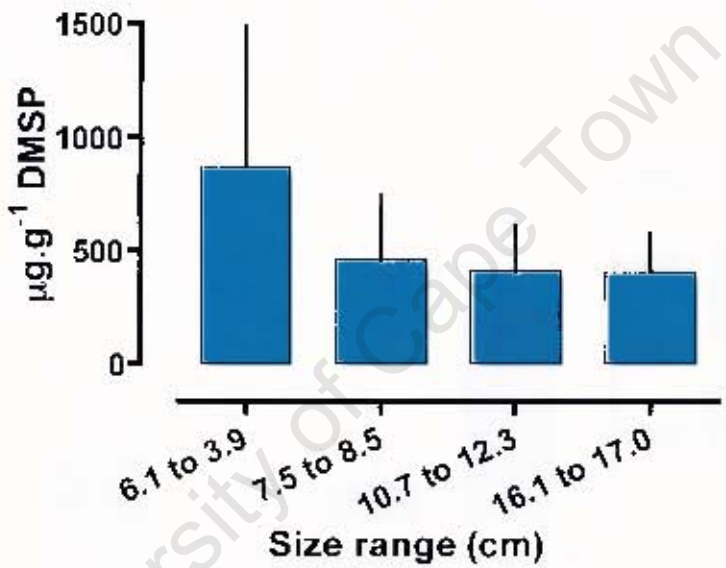


Figure 3.3: DMSP content (mean ± 1 SD) of abductor muscle of wild-caught abalone collected from the Cape Peninsula ($n = 5$).

TABLE 3.1: DMSP concentrations (μg DMSP g^{-1}) in cultivated abalone fed a mixed diet at I & J and wild abalone harvested from Cape Point Nature Reserve (n = 5). Figures are medium, followed by (range in brackets) and lastly standard deviation.

	Length (cm)	Weight (g)	Abductor muscle	Epipodial frill	Bottom of foot	Top of foot
Cultivated	**6.2 – 6.8**	47.0 – 52.6	605 (192 – 1011) ± 339	0.00 (0)	72 (14 – 143) ± 54	130 (32 – 193) ± 98
Wild						
very small	3.9 – 6.1	7.71 – 37.8	1076 (0 – 1593) ± 618			
small	7.5 – 8.5	80.7 – 102.1	274 (229 – 841) ± 282			
medium	10.7 – 12.3	187.7 – 346.8	436 (78 – 570) ± 201			
large	16.1 – 17.0	700.1 – 1050.1	418 (131 – 558) ± 173			
extra small	3.2 – 3.5	3 – 6	1123 (0 – 1670) ± 712	0.00 (0)		
very small	5.2 – 7	20 – 69	1090 (0 – 1623) ± 752	0.00 (0)		
small	7.7 – 9.1	70 – 200	230 (213 – 860) ± 294	0.00 (0)	28	48
medium	12.8 – 14.2	300 – 499	300 (131 – 884) ± 323	0.00 (0)	42	63
large	13.9 – 14.5	500 – 600	422 (70 – 581) 188	0.00 (0)	53	89
super large	16.9 – 18	900 – 1300	395 (143 – 560) ± 182	0.00 (0)	44	91

Feeds

The DMSP content of the diets used in this study ranged from 0 – 6977 µg DMSP g^{-1} (Table 3.2). *Ulva lactuca*, which is grown in marine aquaculture systems in South Africa as feed for cultivated abalone, may attain DMSP concentrations of up to 40.9 ± 6.8 mmol $kg^{-1}_{(fresh)}$ which is significantly higher than levels in other feeds (See Table 3.2). Formulated feeds had the lowest DMSP levels with the values ranging from undetectable to 0.2 ± 0.3 µg DMSP g^{-1}, indicating that a variability exists between different batches of the same feed. *Eklonia maxima* and *G. gracilis* had values ranging from 0.8 ± 0.3 to 26.8 ± 20.6 µg g^{-1}. The statistical analysis (excluding Abfeed™ K26 and Taiwanese feed, since these were used by one replicate farm) shows that DMSP levels vary significantly with feed (ANOVA; $p < 0.001$; d.f. = 3), but that the pattern of variation depends on which farm the samples were selected from (ANOVA; $p < 0.01$; *d.f.* = 6). This result indicates that there existed a large variability within the same species sourced or produced by different farms.

Table 3.2: DMSP content of various manufactured and natural feeds used on abalone farms in South Africa ($n = 3$). *Gracilaria* and *Ulva* are cultivated on the respective farms, and *Ecklonia* is harvested from populations in the vicinity of the farms.

Feed	Farm	DMSP content (µg g^{-1}) on a fresh mass basis (mean ± 1 SD)
Abfeed™	**Abagold**	0.0 ± 0.0
Abfeed™	**Aquafarm Development**	0.2 ± 0.3
Abfeed™ K26	**Abagold**	0.0 ± 0.0
Gracilaria	**Jacobsbaai Sea Products**	20.6 ± 24.8
Gracilaria	**Wild Coast Abalone**	23.8 ± 11.1
Ecklonia	**Abagold**	26.8 ± 20.6
Ecklonia	**Aquafarm Development**	0.8 ± 0.3
Ecklonia	**Jacobsbaai Sea Products**	3.4 ± 1.7
Ecklonia	**West Coast Abalone**	8.7 ± 12.2
Taiwanese feed – Tung lin brand	**Wild Coast Abalone**	0.0 ± 0.0
Ulva	**Jacobsbaai Sea Products**	5115 ± 906
Ulva	**Wild Coast Abalone**	6977 ± 1162

DMSP content of abalone raised on different diets

The levels of DMSP accumulation in abalone reared on different diets are shown in Table 3.3. When *U. lactuca,* alone, is provided as feed to abalone, DMSP accumulates in the abalone to a concentration of 16 162 ± 3 408 μg g^{-1}. In most normal situations *Ulva* is mixed with other macroalgal species, most notably *G. gracilis*, *E. maxima* and *Laminaria pallida* and some compound feeds, but even here the effect of DMSP/DMS may be noted to levels as high as 2 900 ± 1 084 μg g^{-1}. Abalone fed Abfeed®, a manufactured pellet that contains almost no DMSP (Table 3.2), accumulates DMSP at levels 43.5 ± 35.1 to 69.4 ± 43.0 μg g^{-1}. This is possible as there is a large amount of filamentous algae, diatoms and *Ulva intestinalis* L. available in the culture systems for the abalone to feed on. Dimethylsulphoniopropionate in *Ecklonia*-reared animals never exceeded 198 ± 73 μg g^{-1}, but levels of as low as 12.1 ± 4.7 μg g^{-1} were also measured. The addition of *Gracilaria* to the diet, together with *Ecklonia*, resulted in abalone DMSP levels falling within the range reported for an *Ecklonia* only diet

The DMSP biomagnification factor (*sensu* Lin and Liao, 1999) for abalone fed *Ulva* (calculated as $DMSP_{abalone}/DMSP_{Ulva}$) is 3.16 at Jacobs Bay Sea Products and 1.96 at Wild Coast Abalone. Similar values are difficult to calculate for abalone reared on *Ecklonia* because DMSP in kelp appears to be naturally quite variable (Table 3.2).

DMSP depuration

South-west coast

Changing the abalone diets from one which was high in DMSP (*Ulva* only) to one which was low in DMSP (*Ecklonia*), resulted in a decrease in the tissue DMSP concentration from 9194 ± 2722 μg g^{-1} on a fresh mass basis at the start of the experiment, to 1.9 ± 1.2 μg g^{-1} after 477 days (Figure 3.3). Note that the DMSP concentration at the start of the depuration phase (0 days) is indistinguishable from the DMSP content of abalone grown their entire lives on a high DMSP diet. Similarly, DMSP concentrations recorded about 300 days into the depuration phase are indistinguishable from that in abalone grown their entire lives on only *Ecklonia*. The rate of DMSP reduction follows a decreasing

exponential function in the form of $f_{(t)} = be^{-kt}$, where $f_{(t)}$ is the DMSP concentration as a function of time, t, b is the estimated DMSP concentration at $t = 0$, and $-k$ is the rate constant. Fitting this equation to the data yields $k = 0.0270 \pm 0.0050$ and $b = 9192 \pm 281$ $\mu g\ g^{-1}$ (Figure 3.4). The r^2 value of 0.9122 illustrates that the decrease of DMSP concentration with depuration time is significant and adequately described by the non-linear function.

Table 3.3: A survey of the DMSP concentration in abalone grown on diets comprised of various manufactured and natural feeds ($n = 5$).

Feeding regime	Farm	Live mass (g) at culling	Shell diameter (cm) at culling	DMSP content ($\mu g\ g^{-1}$) on a fresh mass basis (mean ± 1 SD)
Abfeed™ & *Ecklonia* (50:50); Abfeed™ only for last 3 wks	Abagold	33.5 ± 5.0	6.5 ± 0.4	100 ± 49
Abfeed™ K26 only	Abagold	41.4 ± 4.8	6.8 ± 0.3	46.2 ± 9.6
Abfeed™ K26 & *Ecklonia* (50:50); Afbfeed only for last 3 wks	Abagold	41.9 ± 9.4	6.7 ± 0.2	28.7 ± 20.0
Ecklonia only	Abagold	70.4 ± 7.0	7.4 ± 0.2	32.2 ± 11.7
Abfeed® and *Ecklonia*	AquaFarms	44.5 ± 1.6	6.5 ± 0.3	69.4 ± 43.0
Ecklonia only	AquaFarms	44.1 ± 4.2	6.3 ± 0.1	198 ± 73
Abfeed™ only (18 kg/wk, 3 × wk^{-1})	I&J	50.3 ± 7.4	6.5 ± 0.2	43.5 ± 35.1
Ecklonia only (90 kg/wk, 3 × wk^{-1})	I&J	50.0 ± 9.9	6.6 ± 0.4	26.1 ± 12.8
Mixed: *Ulva*, *Gracilaria* & *Ecklonia* (25:25:50) every fourth wk; rest of the time *Ecklonia* only	I&J	49.1 ± 2.4	6.5 ± 0.2	1584 ± 262
18 mo on farmed *Ulva*, then 6 mo on *Ecklonia*	Jacobsbaai Sea Products	48.9 ± 4.7	6.4 ± 0.3	34.5 ± 32.1
Ecklonia only	Jacobsbaai Sea Products	43.0 ± 4.5	6.3 ± 0.2	143 ± 80
Ecklonia & *Gracilaria* (25:75); 3 × wk^{-1}	Jacobsbaai Sea Products	44.2 ± 8.4	6.3 ± 0.4	68.3 ± 8.5
18 mo on *Ecklonia* & *Ulva*, then 6 mo onto *Ecklonia*)	Jacobsbaai Sea Products	37.3 ± 6.8	6.0 ± 0.3	146 ± 109
Farmed *Ulva* only	Jacobsbaai Sea Products	20.8 ± 2.1	5.3 ± 0.1	16162 ± 3408
Ecklonia & *Laminaria* (variable proportions)	West Coast Abalone	42.1 ± 5.4	6.4 ± 0.3	12.1 ± 4.7
Ulva	Wild Coast Abalone	38.9 ± 3.5	6.7 ± 0.2	13646 ± 4955
Ulva, *Gracilaria* & Taiwanese feed	Wild Coast Abalone	49.5 ± 6.7	6.9 ± 0.3	2900 ± 1084

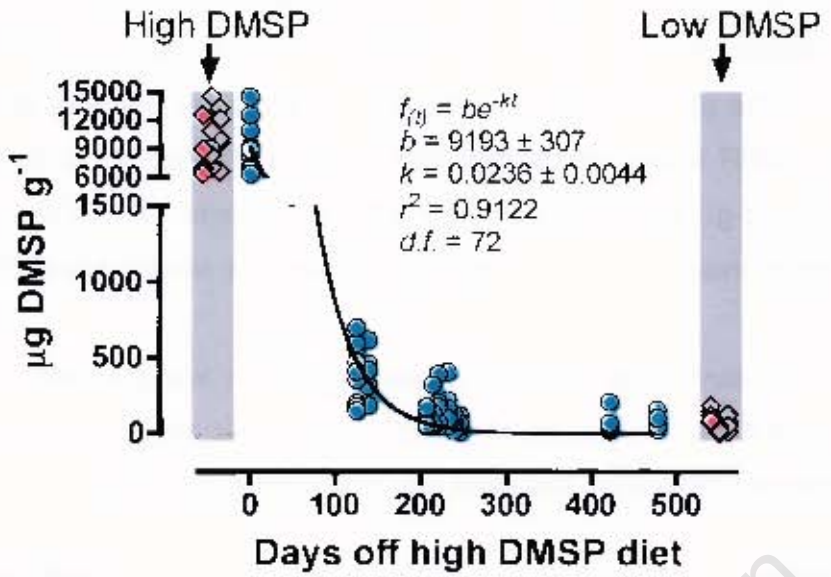


Figure 3.4: The depuration of DMSP from abalone tissue after taking them off a diet comprised predominantly of *U. lactuca* (high DMSP content) and placing them on a low DMSP diet. Data points indicate individual DMSP measurements of abductor muscle: the curve indicates the rate of DMSP depuration as a negative exponential function of time (n = 5). The 'High DMSP' group of data points indicates animals grown exclusively on *Ulva*, while the 'low DMSP' data points are from abalone raised on kelp only.

South-east Coast

The first measurements of accumulation (after 90 days) in the three size classes showed very high concentrations of DMSP. It is theorised that the period of maximum uptake is very fast on the southeast coast on the order of 1 – 2 months. Thus the experiment was terminated after only 2 measurements. The depuration experiment showed that values had also decreased to low values after 90 days, this experiment was also terminated after only 2 measurements.

DMSP production during canning

The amount of DMSP and DMS in the cans depended on the feeding regime of the canned abalone, including the length of the depuration phase. Those fed entirely on *Ulva* had the highest DMSP content in the meat (4404 ± 627 µg g^{-1})

and in the brine (76261 ± 18359 $\mu g\ g^{-1}$) as expected. In contrast, DMSP in cans containing abalone that were fed only *Ecklonia* had the lowest DMSP levels: 3.6 ± 0.8 $\mu g\ g^{-1}$ in the meat and 101 ± 11 $\mu g\ g^{-1}$ in the brine. During the course of depuration, DMSP levels decreased from 1473 ± 127 $\mu g\ g^{-1}$ in the meat and 34894 ± 4740 $\mu g\ g^{-1}$ in the brine to 24.2 ± 8.8 $\mu g\ g^{-1}$ and 727 ± 278 $\mu g\ g^{-1}$ in the meat and brine, respectively. Free DMS showed a similar trend (See Figure 3.5).

Cans from the same high DMSP containing batch were tested at 3 month intervals to see if concentrations of DMSP in the cans altered over time. There was no significant difference between any of the intervals tested.

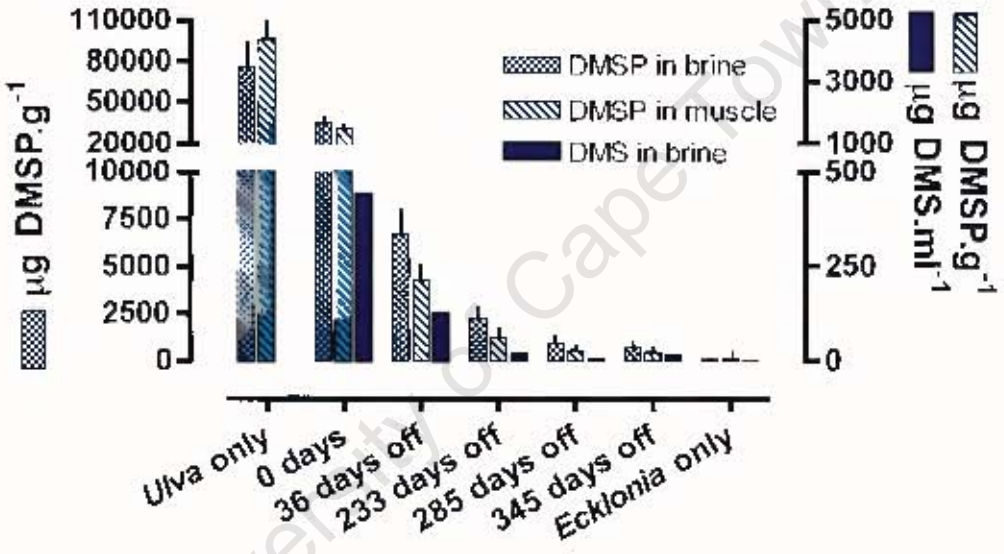


Figure 3.5: The effect of depuration of DMSP from abalone tissue after taking them off a diet comprised of a mixed diet which contained a large proportion of *U. lactuca* (high DMSP content) and placing them on an *Ecklonia* only diet (low DMSP) on the DMSP and DMS contents of canned abalone. DMSP concentrations were determined for abalone tissue and the canning liquid, and DMS concentrations were determined for the canning liquid only (n = 5). Abalone that were grown on only *Ulva* and only *Ecklonia* are presented for comparison.

DISCUSSION

Feeds

The level to which abalone accumulate DMSP is dependent on the concentration of DMSP in their diet. When *U. lactuca,* which can contain concentrations of up to 6 977 ± 1 162 $\mu g\ g^{-1}$ (Table 3.2; also see Van Alstyne *et al.* 2003), is provided as feed in isolation to abalone, DMSP accumulates in the abalone to a concentration of 80.0 ± 29.0 $mmol.kg^{-1}_{(fresh)}$, a value of about 1.4 % of the fresh mass of the animal. This is almost as high as the highest DMSP concentration yet reported for animal tissue (43.2 ± 3.9 $\mu mol\ g^{-1}$ in giant clams (Hill *et al.* 2000, 2004), vs. 34.5 ± 3.3 $\mu mol\ g^{-1}$ in abalone when expressed in equivalent units). The level of DMSP accumulation in cultivated abalone, where *Ulva* is used as one of the dietary items, is higher than that of wild abalone by one or two orders of magnitude. The normal situation in wild abalone is that DMSP occurs at levels ranging from 406 ± 174 to 870 ± 618 $\mu g\ g^{-1}$ (Figure 3.3 and Table 3.1). The data show that accumulation of DMSP is highly variable.

Feed combinations that contain macroalgae are preferable to those based solely on manufactured pellets, mainly because of the effect on growth rate (Dlaza 2005; Naidoo *et al.* 2006). The problem faced by abalone farmers that use *Ulva* spp. and other macroalgae as abalone feed is therefore how to reduce the highly noticeable effect of DMS on odour and taste of abalone meat. The easiest way to accomplish this is to completely remove DMSP-containing macroalgae from their diet, but there are several reasons why this is not an option. Firstly, all macroalgae currently used in abalone (Wong & Cheung, 2000; Van Alstyne *et al.* 2003). Seaweed-containing diets, especially those that contain *Ulva*, are preferable to those that include other macroalgae or compound food only (Robertson-Andersson, 2003; Gerber, 2004), mainly because they positively affect abalone growth rate as part of a mixed diet (Simpson, 1994; Simpson & Cook, 1998; Dlaza, 2006; Naidoo *et al.* 2006).

Some work suggests that DMSP could be the cause of the reported increased growth rate, vigour and stress resistance among animals fed a diet with a high DMSP content (Nakajima, 1996). DMSP is a naturally occurring analog of

betaine (Kiene *et al.* 1998), and since betaine is considered an effective feeding stimulant, DMSP might act as an agonist to betaine in the chemical stimulation of feeding behaviour (Lazo *et al.* 2000). Studies on South African farming systems have shown that diets with a greater DMSP content (*Ulva* > *Gracilaria* and kelp > compound feeds) result in faster abalone growth rates (Naidoo *et al.* 2006; Dlaza, 2006). These diets correspond to higher DMSP levels. At this stage it is safe to say that a feeding regime based on *Ulva* or other macroalgae enhances abalone growth rate above that obtained using compound feeds only, but the reason why it does remains unknown.

Secondly, the effect of algal diets on abalone growth has important ramifications on the economics of cultivation. Faster growth rates on algal diets translate to a greater abalone yield and biomass turnover on the farms. Also, higher farming costs are incurred because the compound feeds cost more than natural feeds (Chapter 2).

Thirdly, the market is based on the taste of wild abalone which contains DMSP in varying amounts, and DMS yielded during cooking would contribute to the characteristic abalone taste. Abalone fed manufactured feeds lack the taste and texture quality of abalone collected from the wild (Robertson-Andersson, 2003; Gerber, 2004; Chapter 3B). It is possible that this is because their DMSP content does not exceed 100 μg g^{-1} – about half the levels found in wild abalone. Dimethylsulphide is known as an important flavour compound in many seafood products (Hill *et al.* 2000). It has a taste-threshold in water at concentrations as low as 0.030 to 0.045 μg ml^{-1} (Bentley & Chasteen, 2004), and at concentrations of about 4 μg g^{-1} imparts a desirable 'clamlike' odour to the soft-shelled clam *Mya arenaria* (Brooke *et al.* 1968). The market is also based on the look (shell shape, length to width ratio and shell colour) of an abalone and several taste testers are able to differentiate between abalone placed on different diets and find an abalone fed compound feed (short, fat (Britz, 1996c) and pale shell and flesh) as being less desirable than one fed a more natural feed (long, thin (Britz, 1996c) and more golden in flesh colour with shell having bands of red, brown etc.). Thus, completely eliminating DMSP from the cultivated abalone would go against existing market preferences.

Canning

There is a negative side effect of having too much DMSP in abalone as it has the potential to affect the eating quality of the product. The bad smell experienced by the south east coast farms seems to be exacerbated through the process of canning and in severe cases, DMS produced inside the cans (in the brine) as result of heat treatment during the canning process may reach levels of up to 439 µg ml^{-1}. This is about 1000 times higher than the taste-threshold of DMS in water (Bentley & Chasteen, 2004).

Depuration

Preliminary data show that the compound is eliminated from abalone at a rate expressed as a negative exponential function of time (See Figure 3.4) after placement on a low DMSP containing diet on the south-west coast. On the south east coast both depuration and accumulation are assumed to occur much faster due to the warmer temperatures. Depuration strategies, developed by trial and error, have already been implemented on the south east coast farms (R. Clark Wild Coast Abalone and W. de Wet, Marine Growers pers comm.).

Variation within replicates

A study by Hill *et al.* (1995) showed that a large variation in the level of DMSP among individual mussels (*Mytilus edulis*) is normal. Our preliminary work on abalone has revealed several outlying measurements of DMSP concentrations among individual abalone within a batch, but because we relied on five replicate samples, it is not possible to state whether this is a normal characteristic or not. This was also compounded by the fact that it is not standard farm practice to keep animals of the same age in a batch, as batches are graded on size alone and not size and age. In addition it is known that within a batch of abalone there is a large variation in size and weight due to dominant feeders preventing other abalone from feeding. It can be noticed from Table 3.3 that DMSP concentrations in macroalgae used as abalone feed are extremely variable. This variation could be due to differences in the abiotic environment in aquaculture systems or in the field.

A major reason why physiological effects of DMSP accumulation in animals have not been investigated before is that only two studies (Hill *et al.* 2000; Broadbent *et al.* 2002), have shown that DMSP can accumulate in animals to levels sufficiently high for potential negative effects to actually occur. Hill *et al.* (2000) suggest that the Tridacnids, in which DMSP may reach similarly high levels under natural conditions as in cultivated abalone, may have developed biochemical adaptations to cope with the high concentrations. Such mechanisms must almost certainly also exist in corals because they evolved in symbiosis with DMSP-producing zooxanthellae. The fact that concentrations of DMSP in cultivated abalone are 9 – 18 times higher than in wild abalone raises questions as to whether abalone have developed physiological mechanisms to deal with high concentrations. Nishiguchi & Somero (1992) and Karsten *et al.* (1996) have shown that DMSP can negatively affect enzyme function. The question is if this is applicable to abalone?

DMSP in wild abalone

The natural feeding patterns of abalone change during different stages of their lifecycle (de Waal *et al.* 2003). This is not only due to an increase in their mouth size (Flemming *et al.* 1996), but also due to morphological changes in the abalone's radula as it grows (Kawamura *et al.* 2001; Daume & Ryan, 2004; Onitsuka *et al.* 2004). Changes in the diet result in changes in the gut micro-organisms, such as bacteria and enzymes within the digestive system of the abalone allowing them to better digest different types of macro-algae. As Table 3.3 illustrates macroalgae from the three divisions have varying concentrations of DMSP and abalone feeding on these different algae will accumulate DMSP to varying degrees. Barkai & Griffiths, (1986, 1987, 1988) showed that larger abalone have a higher percentage of *E. maxima* in their diets and this algae has a lower DMSP content than that of *Ulva* spp. which are commonly found in the gut contents of smaller abalone.

Loss of DMSP

Initially it was thought that DMSP was lost through mucus loss, as in corals. However the lack of detectable levels of DMSP in the mucus negates this. The fact that DMPS is present in the haemolymph may also explain the partitioning that is witnessed in the abalone. Figure 3.6 illustrates an abalone and its circulatory system. An open circulatory system is one in which blood flows from the arteries to the lacunar tissue spaces (LTS) and finally into venous sinuses before being collected in veins and returned to the heart for recirculation (Jorgensen *et al.* 1989). Russell & Evans (1989) describe the system as having characteristics of both an open and closed system but being more like a system of sinuses. Material exchange in such a system occurs as a result of bathing tissues by vascular fluid. Two pedal arteries run parallel and posteriorly through the pedal muscle (Jorgensen *et al.* 1989; Russell & Evans, 1989) extending almost the length of the foot. Branches extend laterally from these arteries and form a dense network of very fine blood channels or LTS varying in diameter from 10 – 50 µm. The LTS are irregular branching channels within the muscle and connective tissue matrix and are analogous to vertebrate capillaries (Jorgensen *et al.* 1989; Russell & Evans, 1989). The cephalic arterial sinus also feeds into the LTS along the anterior edge of the epipodium. Blood from the pedal LTS collects into vessels that run into the posterior pedal sinus (Jorgensen *et al.* 1989; Russell & Evans, 1989).

Blood supply and blood pressure play a mechanical role in feeding movements (Jorgensen *et al.* 1989; Russell & Evans, 1989). Blood flow to the foot muscle ranges from 6 - 10 ml 100 g^{-1} min^{-1}, the foot contributes 66 % of the mass to the animal yet only receives 27 % of the cardiac output, contrast this with the digestive gland which represents 6 % of the body weight but receives 13 % of the cardiac output at a rate of about 50 ml 100 g^{-1} min^{-1} (Jorgensen *et al.* 1989). The digestive gland may be the first receptor of DMSP in the abalone and from here it is possible that the DMSP is transported in the haemolymph through LTS in the foot. It is possible that the partitioning that is observed in Figure 3.2 is merely to do with the concentration of haemolymph containing DMSP occurring in those tissue types.

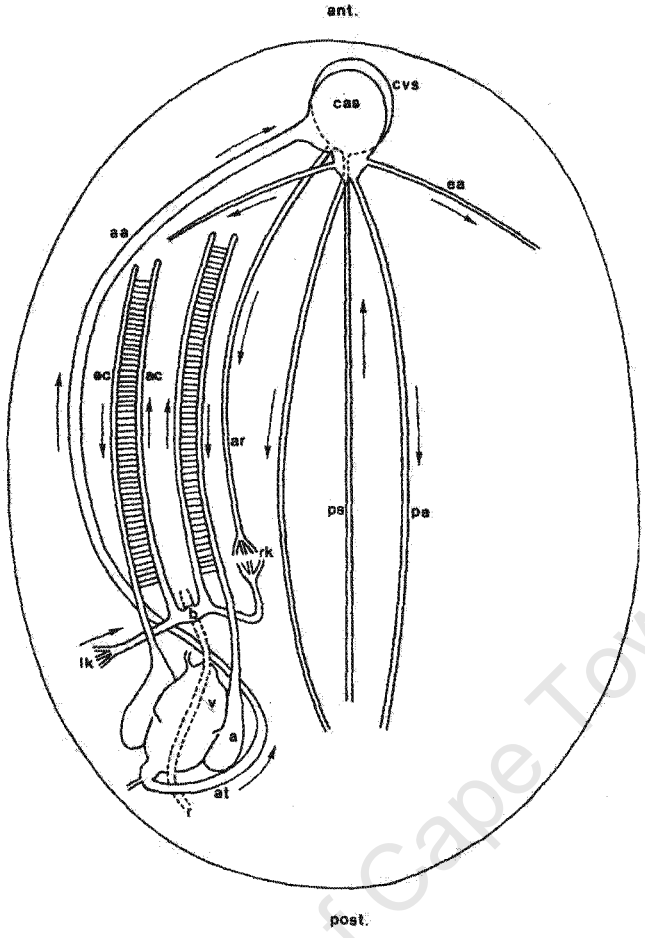


Figure 3.6: Diagrammatic representation of the major components of the cardiovascular system of an abalone. Arrows indicate direction of flow. Abbreviations: aa, anterior aorta; a, atrium; ac, afferent ctenidial vein; ar, afferent renal vein; at, common aortic trunk; cvs, cephalopedal venous sinus; ea, epipodial artery; ec, efferent ctenidial vein; lk, left kidney; pa, pedal artery; ps, posterioir pedal sinus; r, rectum; rk, right kidney; v, ventricle. From Russell & Evans (1989).

Palatability

We do not know at what concentration DMS becomes noticeable in abalone, or to what extent it contributes to the overall eating quality of abalone. The reduction of DMSP levels below that of wild abalone (*c.a.* 471 ± 339 µg ml^{-1}) should not be encouraged as the DMS yielded during cooking probably leads to the characteristic abalone taste. Abalone fed manufactured feeds also lack the taste and texture of wild abalone (Chapter 3B; Gerber, 2004). This is probably because the concentration of DMSP does not exceed 69.4 ± 43.0 µg ml^{-1},

although kelp-fed abalone have similar low concentrations. The market preference based on Gerber (2004) is wild > kelp > Abfeed®. At DMS concentrations of 4 μg g^{-1} the soft-shelled clam *Mya arenaria* has a "desirable clamlike odour" (Brooke *et al.* 1968). The values in canned abalone are much higher than this and such excessive concentrations also have an effect on palatability. Figure 3.7 is a conceptual diagram which illustrates the possible effects of DMSP concentrations in feed and how that would ultimately affect abalone taste, as we don't yet know at what concentration of DMS becomes noticeable in abalone or to what extent it contributes to the overall eating enjoyment of abalone.

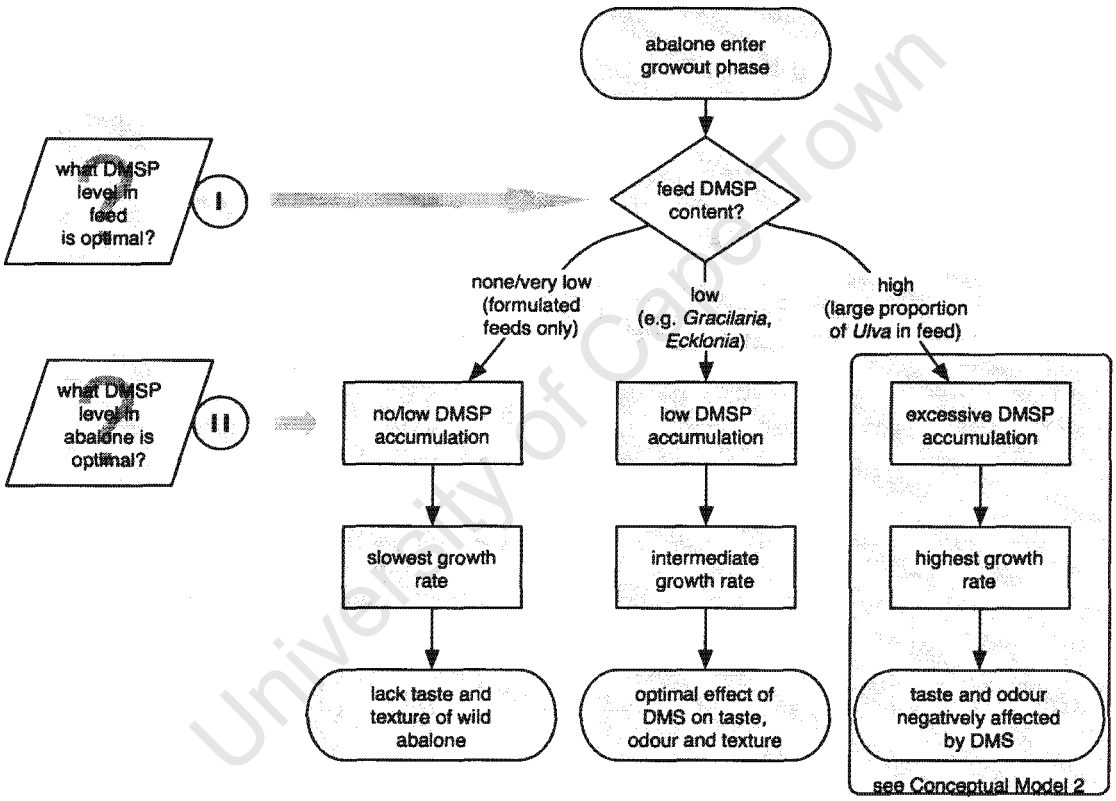


Figure 3.7: A conceptual model highlighting the entry of DMSP (initially present at three hypothetical concentrations in the feed) into abalone biomass. The subsequent consequence of the DMSP accumulated in the abalone tissue on growth rate and taste and odour quality of abalone meat is also indicated. The right-hand side of the diagram is expanded in Figure 3.9. The encircled numbers indicate possible control points where the negative effects of DMSP can be mitigated.

Probably the biggest problem faced by abalone farmers is how to reduce the negative effect on odour and taste of DMS in abalone tissue. Preliminary work shows that, given sufficient time, DMSP is eliminated from abalone tissue by

placing the animals on a diet free of DMSP (Figure 3.4). One mitigation strategy therefore is to implement a depuration (i.e. 'weaning') stage some time prior to processing (Figure 3.8). This will involve taking the abalone off the diet that has been optimised for rapid abalone growth rates (one that usually includes *Ulva*), and placing the animals on a diet with a very low DMSP content. Another possibility is to feed a high growth rate/ low DMSP containing diet (Figure 3.8). It is also possible that there may be a means of ridding abalone of DMSP in the canning process. Information on DMS concentrations in post-mortem abalone flesh, the dynamics of DMSP during processing, and its relation to taste and odour is central to developing methods for quality control of abalone meat. If all the tissue-DMSP in abalone fed on *Ulva* was quantitatively converted to DMS during processing (or post-mortem decay) it would be higher than the odour threshold of DMS in water (Brooke *et al.* 1968) by a factor of 1.51×10^7.

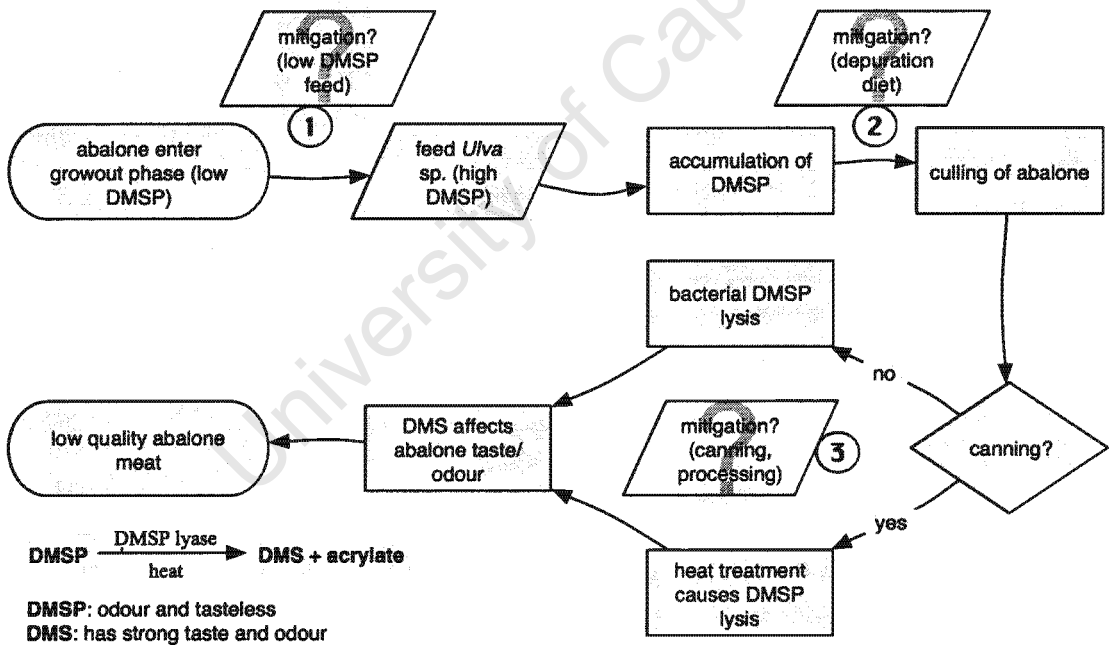


Figure 3.8: A conceptual model highlighting the entry of high levels of DMSP into abalone biomass, with subsequent effects on the taste and odour quality of abalone meat. This diagram is a more detailed account of the right-hand arm of the flow diagram in figure 3.18. The encircled numbers indicate possible control points where the negative effects of DMSP might be mitigated.

CONCLUSIONS

Taste and odour are subjective quality measures but they have to be objectively linked to DMS concentration in order to establish taste and odour thresholds for the compound. It is important that the 'off' odours are quantified in relation to DMS concentrations, not only because such quantification will allow for the consistent production of a good quality product, but also because it can be used as a measure of the effectiveness of mitigation strategies that will be developed to reduce DMSP accumulation in abalone tissue.

It seems likely that in the future more emphasis will be placed on cultivated seaweeds providing much of the nutritional need of farmed abalone. Benefits of farmed algae include reduced pressures on wild stock of kelp, decreased environmental pollution since algae also function as biofilter, lower costs of these feeds, etc. (See Troell *et al.* 2006, Neori *et al.* 2007). However, kelp will still be harvested and used by farms to which this resource is available. The future growth of the abalone industry is critically dependent on a continued supply of sufficient quantities of good quality macroalgae, and since these resources greatly affect the taste and odour quality of the final abalone product, emphasis needs to be placed on quality control issues of this material, especially in terms of DMSP levels. This is especially important as the market (easterners) perceives the product differently from the farmers and westerners.

For cultivated algae, such knowledge may be used to reduce DMSP levels in the seaweed through optimisation of the cultivation conditions in the aquaculture systems (in Figure 3.8). This may be achieved by adjusting parameters such as light intensity and/ or nitrogen content (Dacey *et al.* 1987; Edwards *et al.* 1988b; Slezak & Herndl, 2003). Similarly, an understanding of the environmental conditions that lead to variations in DMSP production in kelp (pre- and post harvesting) may be useful for obtaining kelp or even *Ulva* with a low DMSP content. These low DMSP feeds may then be used during the abalone growout phase in order to minimise DMSP accumulation by abalone and any consequent negative effects of DMS that might develop after culling and during processing.

CHAPTER 3B

THE EFFECT OF MACROALGAL AND COMPOUND FEEDS ON THE EATING CHARACTERISTICS OF CULTIVATED SOUTH AFRICAN ABALONE, *HALIOTIS MIDAE*

INTRODUCTION

Pungent tastes and odours are very important causing unpalatability of seafood products available on the market. Off flavours in aquacultured products may render them unsuitable for sale or consumption, and have repercussions for the aqualculture producer (Robin *et al.* 2006). Some of these highly noticeable flavours are caused by the release of the volatile sulphur compounds, such as dimethyl sulphide (DMS), during processing or decay. DMS, however, is not native to fish and shellfish flesh, but is produced through the lysis of dimethylsulfoniopropionate (DMSP) which accumulates in the consumer organism because of feeding on DMSP-containing foods (Hill *et al.* 1993; Smit *et al.* 2007; Chapter 3A), or because of the presence of DMSP-containing zooxanthellae, for example in tridacnids (Hill *et al.* 1995; Hill *et al.* 2000). DMS is known for its distinctly unpleasant odour. Seafood can develop an "off' petroleum (Brooke *et al.* 1968) or "seaweed- or kelp-like" (Cowan 1988) odour which has been linked to DMS production through the enzymatic (decay of dead, unprocessed products) or thermal breakdown (during cooking) of DMSP. In aquaculture there can be precise control over the types and quality of feeds used, allowing scope for optimising growth rates, taste and odour in the final product.

Abalone are herbivores and eat a variety of macroalgal species (Barkai & Griffiths, 1986). Some of these macroalgae contain large amounts of DMSP, and it is now know that this DMSP is incorporated into abalone tissue (Smit *et al.* 2007; Chapter 3A). The degree to which it accumulates depends on the concentration of DMSP in the macroalgal feeds (Smit *et al.* 2007; Chapter 3A). In South Africa, abalone farmers, particularly those who use *Ulva lactuca* as a feed source have expressed concern about a bad flavour in some batches of canned abalone; a similar problem has been noted in aquacultured abalone in Chile (Smit *et al.* 2007). It is important to understand how DMS-related flavours affect eating quality of cultivated abalone. If the abalone eating quality is poor, it is likely

that it will negatively affect the decision of the consumer to purchase the product, and lead to subsequent economic ramifications for the abalone industry. Since DMS is produced at high levels during canning of DMSP-containing abalone, and consumers eat both raw and cooked products, it is important to consider the effect of DMS on the eating experience of both raw and cooked abalone. However, eating quality is affected not only by taste and odour (i.e. the direct consequence of the volatile DMS), but also by appearance and texture (Robb *et al.* 2002). Data on eating experience are commonly gathered using general sensory analysis evaluation involving a panel of assessors that classify taste, odour and other eating characteristics according to a quantitative descriptive analysis (QDA) as summarised in ISO 13301 (International Organization for Standardization, 2002). The subjective characteristics obtained via taste tests can then be linked to DMS levels objectively determined for the product.

Chapter 3A and Smit *et al.* (2007) demonstrated how dietary DMSP levels relate to the accumulation of DMSP in fresh and processed abalone. This DMSP is cleaved into the highly volatile DMS during canning which is present in high quantities in the cans, and this introduces an unacceptable odour and taste characteristic of the seafood product.

The objective of this work was to investigate the eating and product quality of cooked and raw abalone fed a variety of different macroalgal and compound feeds commonly encountered on abalone farms in South Africa. These data were then compared to a 'control' of wild harvested abalone obtained from the Cape Peninsula. The overall objective of this research was to establish that *Ulva* was responsible for the bad taste in abalone fed an exclusive *Ulva* diet. Secondly to develop a rating method that will be used to determine an acceptable level of DMS in unprocessed and processed (canned) abalone meat.

METHODS

Dietary effects on canning yields

Cannery data was supplied by the farm Abagold on the South west coast, for the period April 2004 to May 2006. In March 2005 the farm changed their feeding regime from a kelp only diet to a Kelp and Abfeed® K26 combination. The farm wanted to understand how the change in diet affected their canning yields, and if this was only a dietary effect or were there other influences such as season and a minimum animal size for canning.

Source of abalone material

Three farms donated abalone for this taste trial. Jacobs Bay Sea Products (JSP) is located on the west coast in the Western Cape Province near Saldanha, Irvine & Johnson Cape Cultured Abalone (I&J) is located in the Western Cape Province near Gansbaai and Wild Coast Abalone is (WCA) located in the Eastern Cape Province near East London.

At JSP, the abalone were cultivated in brick and mortar flow through tanks. The animals were part of an experiment described by Naidoo *et al.* 2006 and were fed for 18 months according to the feeding regime described there. Eight feeding regimes were available from Naidoo *et al.* (2006) experiments. They had been fed on their original diet for a period of 18 months and then either remained on that diet or were fed a new diet for a period of 6 months.

At I&J, abalone were cultivated in moulded concrete flow through tanks. Animals were all spawned in January 2002 from the same spawning batch and were reared under normal hatchery conditions until being placed in the grow-out section of the farm where they were fed kelp only diet. These animals were graded in August 2003 and were redistributed into three experimental batches, each placed on a different feeding regime. The batches initially comprised 13 200 animals of 15 ± 2.5 g each, distributed over 24 baskets that were placed in a tank

(i.e 550 animals per basket). Animals were graded again in August 2004 when the stocking density was decreased to 450 animals per basket, while maintaining the same feeding rate and feed treatment. The animals remained in these conditions until June 2006. The first feeding regime consisted of a compound feed (Abfeed® K26) manufactured by Marifeed (Pty) Ltd, with an approximate protein composition of 26% made up of mainly soya, fish meal, starch and some dried kelp (*Ecklonia maxima*). Thirty three grams of Abfeed® K26 was fed three times a week to each basket of abalone. The second feeding regime was a kelp only diet where 90 kg were fed to each basket of abalone, with 60 kg being fed on a Monday and 30 kg on a Friday. The third feeding regime was a mixed diet consisting of kelp, *Ulva lactuca*, and *Gracilaria gracilis* in a ratio of 50:25:25, with feeding frequency as the kelp-only feeding regime. *Ulva lactuca* and *G. gracilis* were cultivated, whereas kelp was harvested from natural stocks along the Gansbaai coast.

At WCA abalone were cultivated in moulded raceway systems. The animals were spawned in November 2001. They were stocked at 250 animals per basket and were fed 20 kg per week of *U. lactuca*-only.

Divers collected wild grown abalone from the Cape Peninsula in 2 to 18 m of water. Here they occur in *Ecklonia* beds, usually on a crustose coralline algal substrate or among the kelp holdfasts, where their diets comprise drift *E. maxima* fronds, a biofilm of microalgae and some species of red and green algae (Barkai and Griffiths 1986). Animals of 16.1 – 17.0 cm shell diameter (700 – 1 050 g whole mass) were selected. The wild-collected animals were used as a control for the taste test. This was based on the assumption that the market preference is for wild abalone, so taste preferences are developed on this.

For the first taste test animals were raised on the following feeding regimes were available: 1) cultivated *Ulva* for 18 months then 6 months on Kelp; 2) cultivated *Ulva;* 3) Kelp & cultivated *Gracilaria*; 4) Kelp & Abfeed® K26 onto Kelp; 5) wild

harvested *Porphyra* onto Kelp; 6) Kelp & *Ulva* onto Kelp only; 7) Kelp only and 8) Abfeed® K26.

For the second taste test, animals were raised on the following five feeding regimes that included: 1) Abfeed® K26-only; 2) kelp-only; 3) mixed; 4) *Ulva*-only; and 5) wild-collected.

DMSP/ DMS analysis in animals and feed

The measurement of DMS present in animal tissue and feeds was the same as mentioned in Chapter 3A. The DMSP content of the feeds used was assayed in samples provided by the participating farms. These consisted of manufactured feeds Abfeed®, and cultivated (*Ulva* and *Gracilaria*) and harvested (*Ecklonia maxima*). In the case of macroalgae, fresh samples were used at all times. Five animals from each diet were randomly selected to measure DMSP concentrations.

Taste test

Taste and odour are subjective measures of quality that are objectively linked to DMS concentration in processed and unprocessed abalone meat. This allows us to establish taste and odour thresholds for the compound. The quantification of repellent 'off' odours is essential in allowing the maintenance of a consistently high quality product. These subjective characters are associated with eating quality and were assessed via three approaches. The first one involved panellists who are trained in terms of the ideal taste prescribed by representatives of the Asian marketing industry. The panellists were not familiar with the smell of DMS and were thus not able to relate the characteristics of the abalone to the compound (i.e. ranking was simply from 'very bad' to 'very good'). This approach also yielded objective measures that include mass loss of live animals during packaging and transport, shell hardness, survival rate, the ratio between the meat and guts, shell mass, mass loss during cooking, and the loosely defined term 'life force'.

The other approach was to obtain panellists who are familiar with abalone taste and represented a diverse group. These panellists would perform two tests, the first would assess their palate sensitivity and these scores could then be used to further discriminate the results from the second test, in which they would then be introduced to the smell of DMS.

Two separate taste tests were performed.

Japanese Nationals Taste Test

The first was done by 3 male Japanese nationals in Japan. Animals from 8 different diet treatments from the JSP Farm were exported to Japan. After the animals arrived in Japan and had their water loss reconstituted they were killed and then taste tested both raw and cooked (5 minutes of boiling in fresh water, no salt or seasonings added). Three testers assessed 8 abalone, 4 uncooked and 4 cooked abalone on the following points: colour of shell and meat, odour, taste, fillet yield of each diet both raw and cooked, hardness of the shell, flesh texture, palatability, overall quality, weight loss following export and Life Force.

Life Force is a subjective quality but for the purposes of this experiment the testers placed 10 abalone of each sample into a box filled with seawater at a constant temperature. They aerated the water and observed the abalone's behaviour compared to a traditional Japanese abalone *H. discus hanni* after the animals had recovered from transport stress.

The testing involved ranking the uncooked and cooked samples on a scale of odour, taste and overall quality. The standard industry method uses a scale from 1 – 6.

General taste test

The second taste test took place at the University of Cape Town (UCT) Club on UCT upper campus in Rondebosch and involved 51 panellists. Panellists included males (n = 30) and females (n = 20), ranging in age from 20 to 65 years. Prior to beginning the tests, tasters were given a short presentation on abalone

farming, and were instructed to use the test sheets with the QDA categories and ranks, and the triangle tests. The standard procedure for conducting taste tests involves using a panel of trained taste testers (ISO 13301). This was not possible for our study, because sufficient quantities of abalone are not generally available to allow for training of the test panel. To circumvent this problem, a panel whose members included those i) familiar with abalone (i.e. farmers who have access to farmed abalone, and who consume abalone on a regular basis); ii) representatives of the Asian abalone industry who represent the market where the abalone are exported to; and iii) members of the general public, 78% of whom have eaten abalone within the last year was constructed.

Processing and preparation of second taste test samples

Animals of all five feeding regimes were purged for 5 days prior to the taste test in culture facilities. Each sample was de-shelled and eviscerated, and washed in a tumbler machine at Sea Plant Products cannery in Hermanus. This was in accordance with standard preparation methods for canning. Wild abalone underwent an additional process of having the green bottom foot flesh thinly sliced to present a white sample and to prevent this flesh from adding to the taste, as it is thought to have a bitter taste and is traditionally removed in any canning or cooking process. The samples were then diced into cubes and placed into labeled boxes and bags. The samples were then transported on ice to the University of Cape Town where they were deep frozen in a -20°C blast freezer for 24 hours. The following 24 hours they were transferred to a 0°C freezer. On the morning of the trial they were thawed and all samples were washed in a tumbler machine to remove excess mucous (as a result of the freezing). Reference samples were taken to measure DMSP concentration. Diced particle size was also measured to the nearest mm from 10 randomly chosen cubes from each diet.

Plating

Black 6 division platter plates were used to allow for maximum contrast with the

abalone flesh. The raw samples were placed on the plates without any additional preparation. The cooked samples were placed in already boiling water and cooked for precisely 5 minutes, after which they were allowed to cool and plated at ambient temperature. Tasters were provided with still water for cleansing the palate between samples.

Triangle tests

Two triangle tests (Jacobsson *et al.* 2004) were performed, one for raw and another for cooked samples. Each triangle test involved presenting the panellist with three samples (consisting of 10 cubes) at a time, one of which was a different feeding regime from the other two. Paired combinations of samples, as well as positions of the three samples per plate, were randomly chosen by computer. Twelve sets were presented to the tasters: sets 1 – 6 were the raw samples, and 7 – 12 the cooked samples. Panellists were required to identify the odd sample out by odour and appearance alone, and were requested to supply reasons for their choice. Answers were indicated on the test sheets provided. The aim of this test was to assess the tester's palate sensitivity.

Quantitative descriptive analysis

A QDA test was developed according to the method outlined by (Nyambaka and Ryley 2004)). A list of sensory descriptive terms (Table 3.4) was determined in consultation with nine regular consumers (from the farming and research communities) of abalone.

Selection of panellists and panellist training

Immediately prior to the testing tasters were given a short presentation on abalone farming. They were also trained to use a 100 mm continuous line scale with two horizontal anchor descriptors at the ends. The "low" intensity anchor was at 0 mm and high intensity anchor at 100 mm. The tasters then indicated their response by marking horizontal lines for each given attribute. The marks were transformed into data by taking measurements (in mm) from the left anchor,

representing zero, on a scale of 0 and 10. In addition at the end of the test tasters were given a vial of DMS to smell containing 5 μmol DMS and were asked to rank the samples as to how the odour was similar to that of the vials contents

Statistical analyses

A General Linear Model (GLM) in STATISTICA V7, was used to find the % contribution of variables towards fillet yield in data supplied by Abagold. Mean values were considered significantly different at $p \leq 0.05$. The taste test dataset used in the multivariate analysis contained 20 variables of continuous data (converted to %). In order to test which predictor variables best separate the *a priori* known feed types (*Ulva*, *Ecklonia*, mixed, Abfeed®, wild) for asian vs. non-asian tasters and cooked vs. uncooked abalone, a discriminant analysis (Venables and Ripley, 1999) was used. To pre-screen the data one-way ANOVAs were applied in a stepwise fashion to identify and rank the variables whose values differ significantly between groups (Huberty, 1994); in the subsequent discriminant analysis only those variables whose *F*-values were greater than 2.5 (corresponding the $P < 0.05$) were included. The following variables were identified as responsible for causing much of the difference between feeds within these categories:

Category 1 (Asian): colour; bitterness (taste); mouth-feel (toughness); mouth-feel (hardness).

Category 2 (Non-Asian): colour; surface texture; aroma intensity; sweetness (taste); saltiness (taste); mouth-feel (hardness); DMS-like.

Category 3 (Cooked): colour; surface texture; saltiness (taste); freshness (taste); mouth-feel (toughness); mouth-feel (hardness); DMS-like.

Category 4 (Uncooked): colour; surface texture; aroma intensity; finger-feel (toughness); mouth-feel (hardness).

The discriminant analysis proceeded only with the variables identified above, independently for each of the four categories. For this, we used the discrimin

function of version 1.4-2 of the **ade4** package (Thioulouse *et al.*, 1997), and the lda function of the **MASS** package (Venables and Ripley, 2002), both of which were implemented in R 2.4.0 (Ihaca and Gentleman, 1996). Only the first two (in the case of Asian) or three (in the case of non-Asian, cooked, and uncooked) discriminant axes were maintained for interpretive purposes because they collectively explained more than 93% of the variation between feed groups within each category (or at least 10% per individual discriminant axis).

Table 3.4: Sensory attributes used in the quantitative descriptive analysis.

Attribute	Definition	Low anchor	High anchor
Appearance	**visual assessment only**		
Colour		Pale	Colourful
Surface structure		Non appealing	Appealing
Freshness	overall impression of freshness	fresh	Off
Aroma	**smell assessment only**		
Total intensity		weak	strong
		pleasant	unpleasant
		fresh	off/ rotten
		dislike	like
Taste	The total intensity of the flavour during the first five chews	unpleasant	pleasant
Sweetness		none	very
Sourness		none	very
Saltiness		none	very
Bitterness		none	very
Metallic		none	very
Freshness		fresh	off/ rotten
Texture			
Finger-feel toughness	by rubbing and squeezing the sample between the fingers and the thumb	tough	tender
Mouth-feel toughness	by biting down once with the molar teeth and evaluating the force required	tough	tender
	by biting down with the front teeth and evaluating the force required	hard	soft
	Chew the sample for only 2- 3 chews between your molar teeth and rub between the tongue and palate and assess the nature of the fibre	sinewous	non sinewous
	Chew the sample at a constant rate (e.g. 1 chew per second) and count the number of chews until ready to swallow.		
Overall acceptability		Dislike	like
DMS smell	Please ask for a vial of DMS and rank the samples according to the smell.	None	very

RESULTS

Taste test 1: (Japanese nationals only)

The results from the first taste test were inconclusive and the written report did not correspond with the values given. It was discovered, subsequent to the taste test, that the taste test was not a blind taste test but that the testers had been informed of the samples identity by the supplier. However, the identity that they were given for the samples was incorrect. This illustrated the need for a completely blind taste test. It is known that if a variety of unshucked abalone that have been fed different diets are displayed, it is relatively easy to distinguish, through shell colour, ones that have been fed an compound diet. This indicates that market preference is not solely through taste but also the appearance of the animals. The test did generate useful data regarding physical characteristics of abalone fed different diets (See Table 3.5) especially when comparing fillet yield which was significantly higher when the animals were fed either Abfeed® or an *Ulva* diet (ANOVA, df = 5, P > 0.05).

The figures for fillet yield, show that Abfeed® fed animals have a significantly higher fillet yield than abalone on other diets both when cooked and uncooked. (ANOVA; $p < 0.01$; *d.f.* = 87). Data supplied from Abagold shows that when the abalone's diet was changed from kelp 5 days a week to Abfeed® 4 times a week and kelp once a week, then the canned fillet yield increased by 10 % ($f = 69.387$; $p < 0.000001$) (Figure 3.9). The GLM explained 58.5 % of the variance ($p < 0.0000001$), of this the month in which the abalone was canned contributed 8.2 % ($p < 0.013$), size of abalone 32.1 % ($p < 0.0000001$), and diet 22.1 % ($p < 0.0000001$). Running the model again, using a feed size interaction explained 55.6 % of the variance ($p < 0.0000001$), of this month contributed 0.5 % ($p < 0.014$), size of abalone 26.7 % ($p < 0.0000001$), and diet 15.5 % ($p < 0.0000001$) while the feed size interaction explained 10.3 % of the variance ($p < 0.0000001$). A slight seasonal trend was found in that it is better to can in winter, in particular July (having significantly higher yields than 6 other months of the year), than in

late summer. The difference due to diet was highly significant with Abfeed® producing a better fillet yield than kelp ($f = 69.387$; $p < 0.000001$). The size of the abalone being canned also had a significant impact, with the minimum size required for canning being 100 g. Other factors that were not investigated in the model were growth rate of abalone and "overpack". Overpack occurs when more than 213 g meat is found per can once drained.

Table 3.5: Physical characteristics of abalone fed different diets from JSP (n= 8). Letters indicate significant differences.

Diet	% weight loss	Life force	Raw shell	Raw meat and guts	Cooked shell	Cooked guts	Cooked fillet
Farmed *Ulva* onto Kelp	4.0	1	27.2	72.8	28.4	18.2	53.4[a]
Farmed *Ulva*	1.9	2	25.33	74.67	32.67	21.35	46.04[c]
Kelp & *Gracilaria*	4.2	3	24.67	75.33	31.16	24.8	44.04[c]
Kelp & Abfeed® onto Kelp	2.9	4	25.44	74.56	30.55	22.39	47.06[c]
Porphyra onto Kelp	0.9	5	26.46	73.54	28.95	21.71	49.34[c]
Kelp & *Ulva* onto Kelp	4.3	5	24.35	75.65	30.9	21.61	47.49[c]
Kelp	1.9	6	27.9	72.1	29.23	20.23	50.54[b]
Abfeed®	2.9	6	21.93	78.07[a]	25.33	22.04	52.63[a,b]

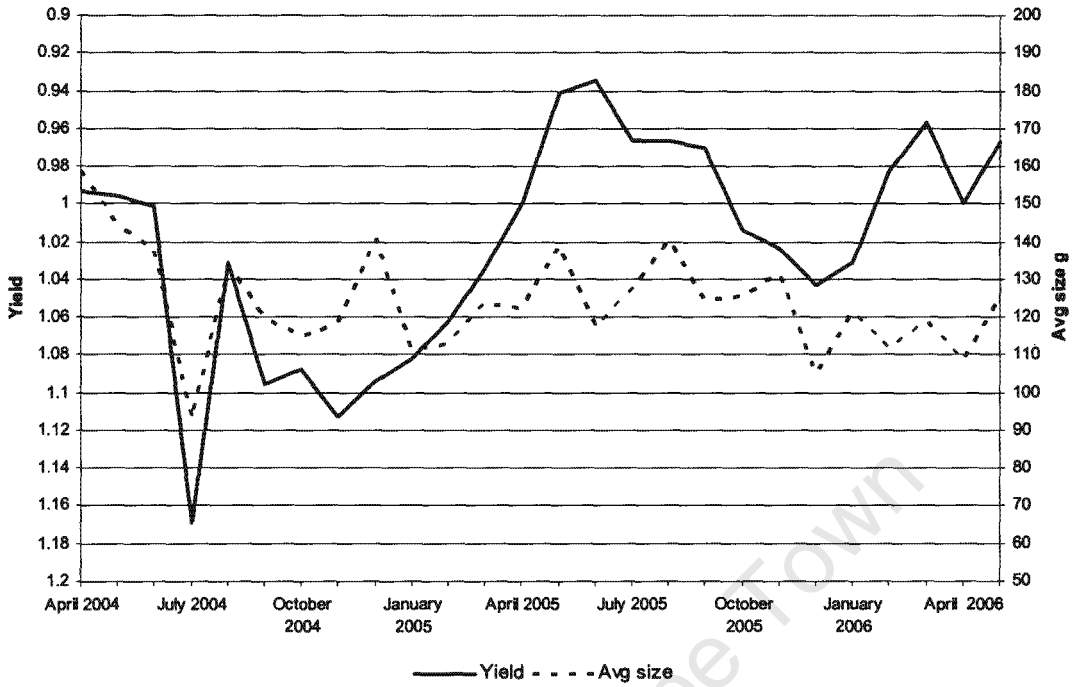


Figure 3.9: Fillet yield from Abagold canning data. Diet changed from a kelp only diet to a diet of four days Abfeed® and one day kelp in March 2005 (red arrow). Yields (LHS) are measured as live weight per can produced, so a value of 0.9 indicates 900 grams of equivalent live abalone to one can. The average size of the animals in the batch canned is indicated on the LHS.

Taste test 2

Of the 51 tasters only 42 people answered the question "Do you smoke?" Of these 28 % smoke (5 – 20 cigarettes per day). Of the 43 people that replied to the question "Do you have normal colour vision?" only 2 did not. Figure 3.10 shows the age frequency distribution of the tasters. 44 people answered the questions "How often have you eaten abalone?" and "How was it prepared?" (See Figure 3.11), thus we can assume that 86 % of the tasters had a preconceived idea of what abalone should taste like.

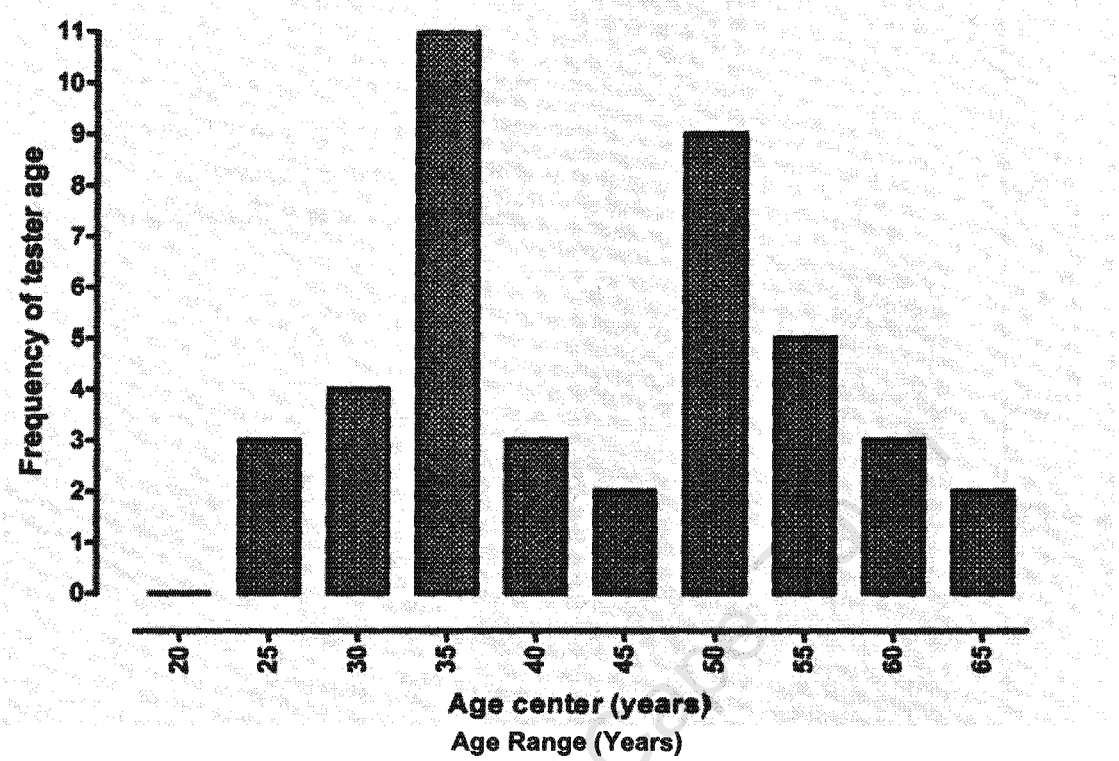


Figure 3.10: The age frequency distribution of tasters. n = 42 (out of 51 tasters)

Table 3.6 lists the size distribution of the food slices that were presented to the tasters. Wild abalone pieces were significantly larger than the other diets presented and this was due to the fact that finding more than 200 small and medium (100 – 200 g) wild abalone was impossible and thus the abalone used in the test were all greater than 750 g. The Abfeed® and mixed diet abalone came from experimental animals that were long past normal farm selling and grading weights and thus were larger than what is normal for a cultivated abalone (kelp and *Ulva* diets).

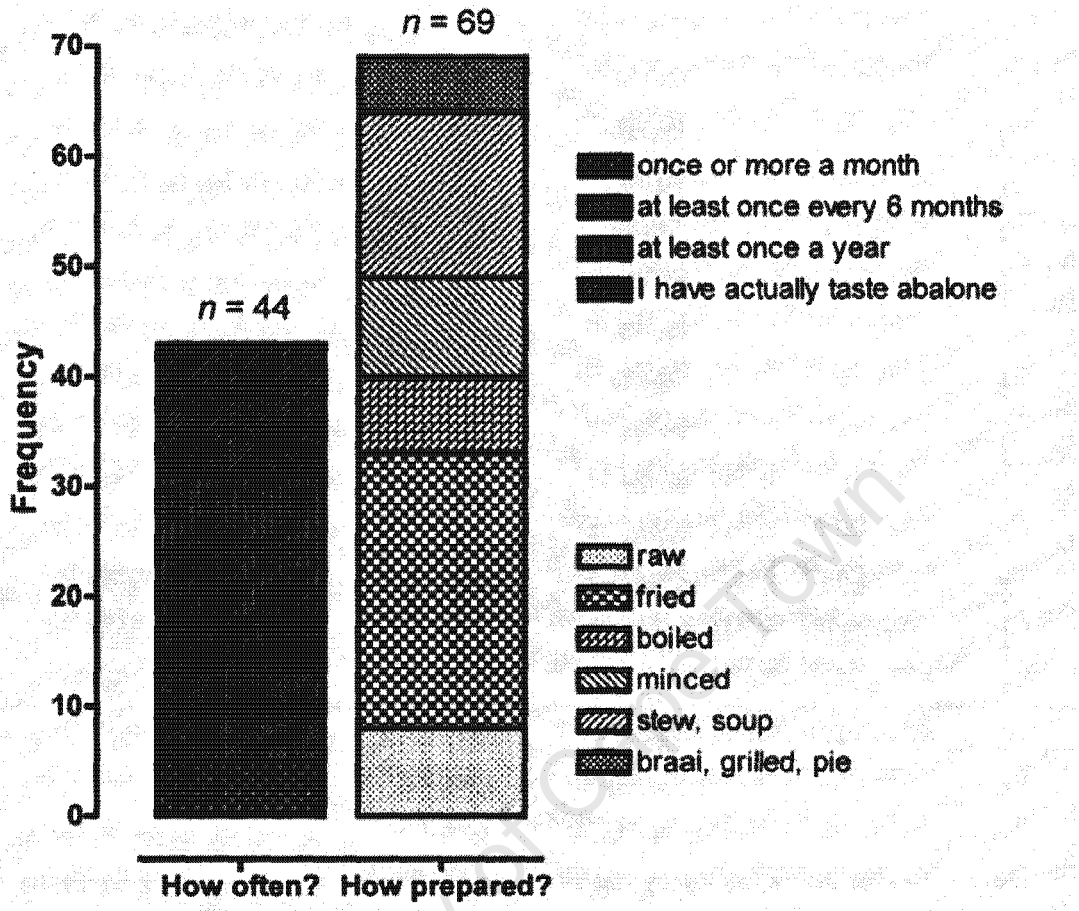


Figure 3.11: Frequency of eating abalone ("How often?") and method of preparation ("How prepared?"). Although 44 testers answered this question, the numbers of answers given for the preparation method exceed this figure because some respondents supplied more than one answer.

TABLE 3.6: Mean sizes of portions of abalone presented to tasters for each diet (n = 10).

Diet	Length	Height
Wild	4.24 ± 0.64^{a}	2.9 ± 0.43^{a}
Abfeed	2.52 ± 0.5	2.08 ± 0.27^{b}
Kelp	2.37 ± 0.33	1.93 ± 0.33
Ulva	2.35 ± 0.27	1.94 ± 0.22
Mixed	2.34 ± 0.23	1.76 ± 0.18^{b}

The results from the triangle test are shown in Figures 3.12; 3.13 and 3.14. All 51 testers responded. There was no significant difference in the number of correct answers supplied between cooked and uncooked samples. Figure 3.12 and 3.14 indicate that for the raw abalone the diets that were significantly difficult to distinguish through smell were wild abalone and Abfeed® fed animals and Abfeed® and kelp-fed animals. No correlations between numbers of correct answers and sample size were found indicating that the size of the sample presented to the tasters did not affect the ability to differentiate between samples. The cooked sample, diets that were difficult to differentiate between were wild and Abfeed® and mixed and *Ulva*. The easiest sample to differentiate was between the wild and *Ulva* diets both in the uncooked and cooked samples although in the cooked section of the test a far greater proportion of the tasters were able to correctly differentiate between these two diets.

Figure 3.15 shows the distribution of correct answers supplied according to the following categories (Asian, Farmers, Others). There is a normal distribution for others, a bimodal distribution for the Asian and a skewed distribution for the Farmers. The Figures show that the Asians and the farmers had a greater familiarity with the abalone smell and were more easily able to correctly identify the odd sample out in each of the sets.

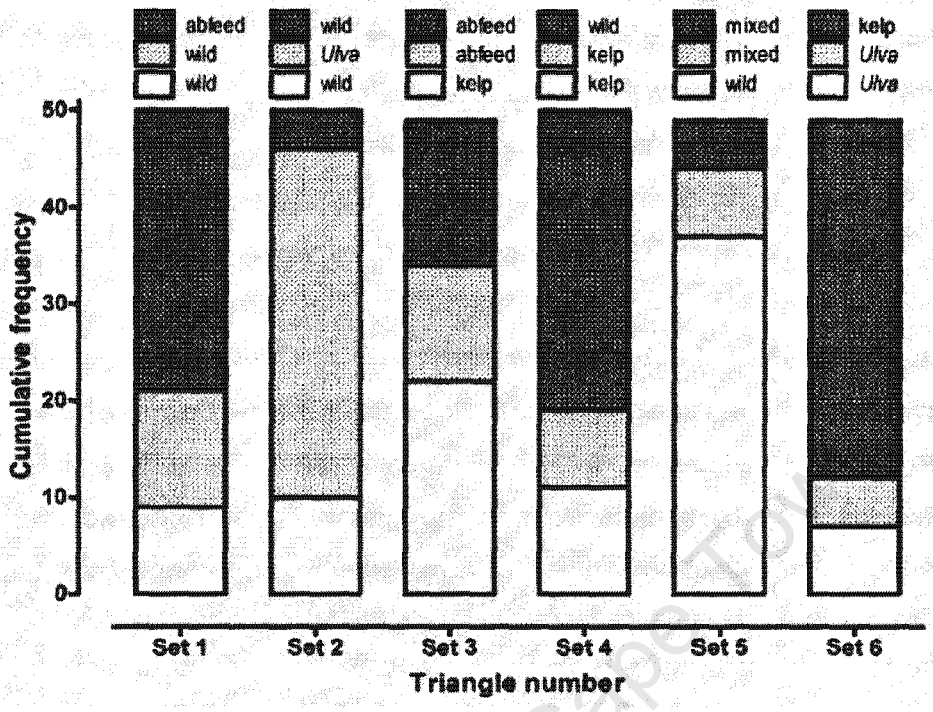
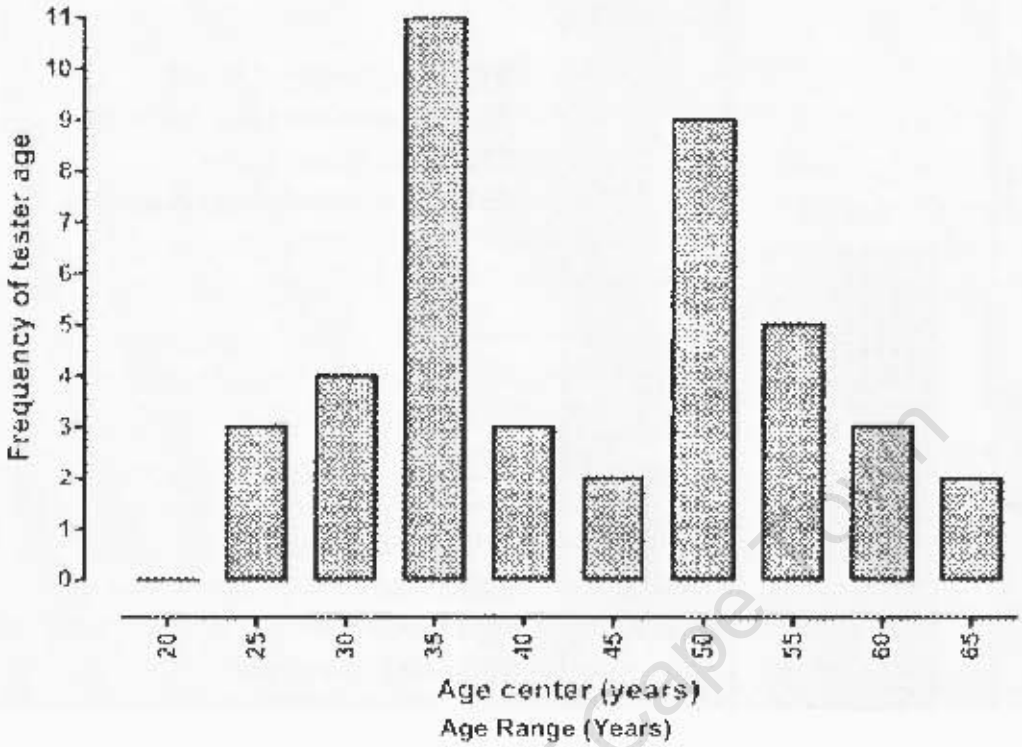


Figure 3.12: Triangle test with uncooked abalone. Sample identity is given at the top of the figure and bars indicate number of answers for each identity. The correct answer is the odd one out (n = 51).



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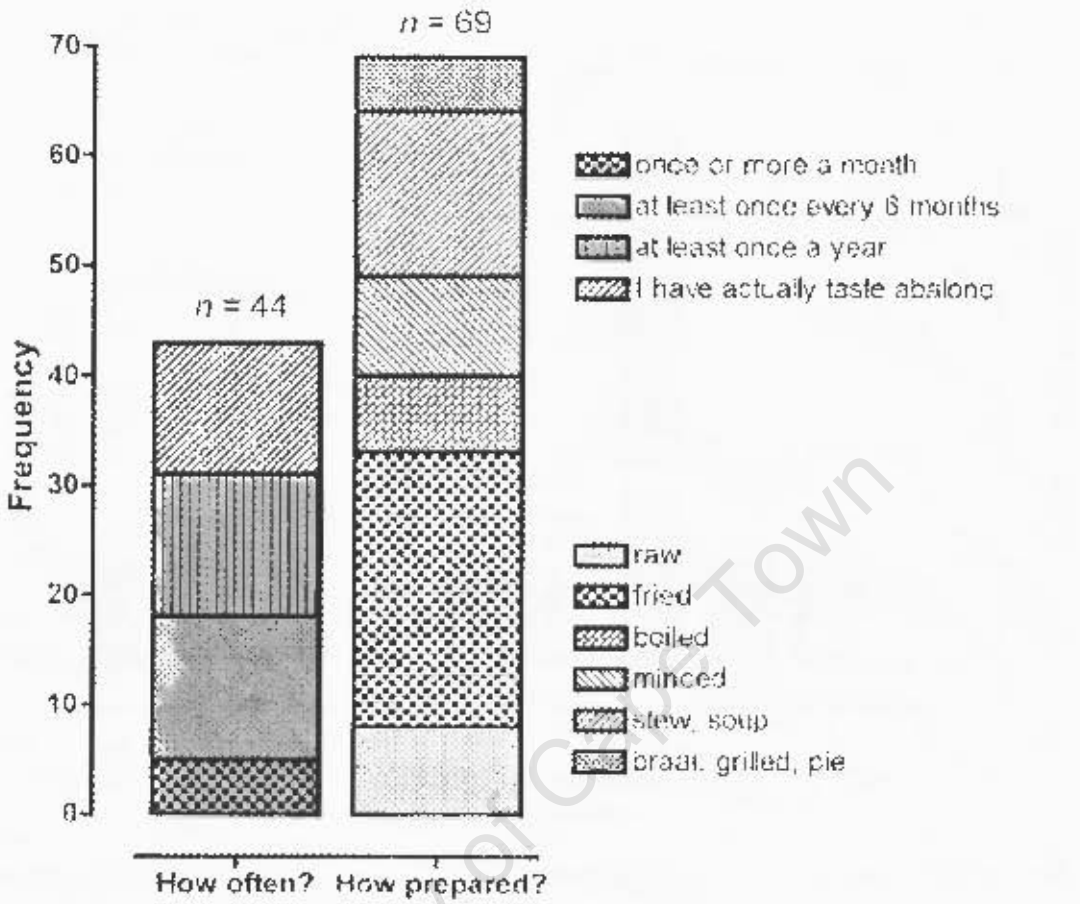

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Ulva	2.35 ± 0.27	1.94 ± 0.22
Mixed	2.34 ± 0.23	1.76 ± 0.18[b]

The results from the triangle test are shown in Figures 3.12; 3.13 and 3.14. All 51 testers responded. There was no significant difference in the number of correct answers supplied between cooked and uncooked samples. Figure 3.12 and 3.14 indicate that for the raw abalone the diets that were significantly difficult to distinguish through smell were wild abalone and Abfeed® fed animals and Abfeed® and kelp-fed animals. No correlations between numbers of correct answers and sample size were found indicating that the size of the sample presented to the tasters did not affect the ability to differentiate between samples. The cooked sample, diets that were difficult to differentiate between were wild and Abfeed® and mixed and *Ulva*. The easiest sample to differentiate was between the wild and *Ulva* diets both in the uncooked and cooked samples although in the cooked section of the test a far greater proportion of the tasters were able to correctly differentiate between these two diets.

Figure 3.15 shows the distribution of correct answers supplied according to the following categories (Asian, Farmers, Others). There is a normal distribution for others, a bimodal distribution for the Asian and a skewed distribution for the Farmers. The Figures show that the Asians and the farmers had a greater familiarity with the abalone smell and were more easily able to correctly identify the odd sample out in each of the sets.

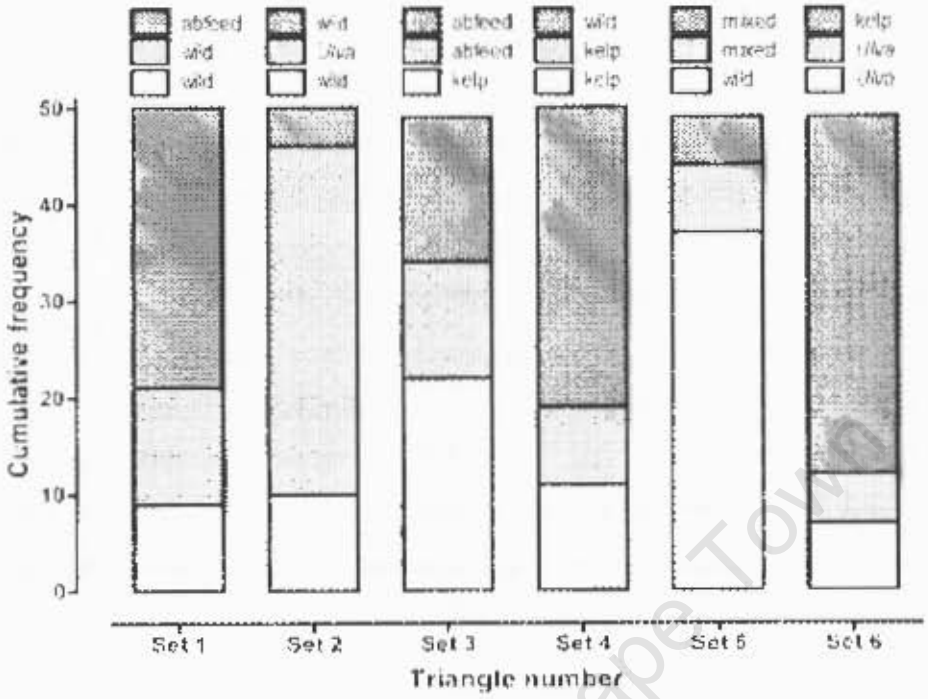

Figure 3.12: Triangle test with uncooked abalone. Sample identity is given at the top of the figure and bars indicate number of answers for each identity. The correct answer is the odd one out (n = 51).

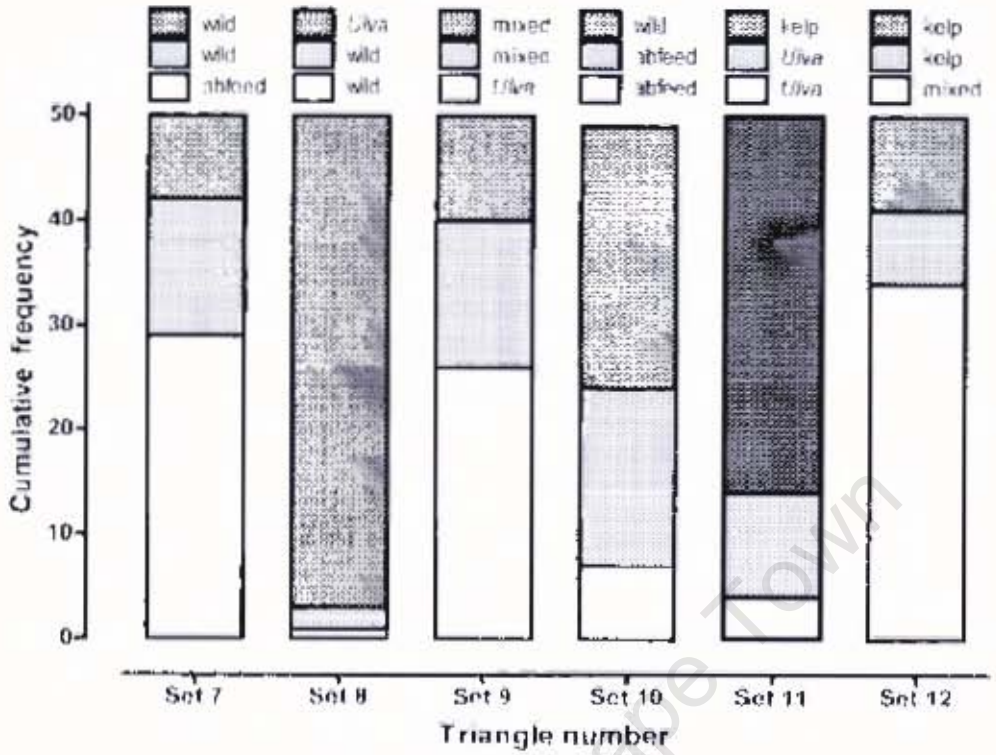

Figure 3.13: Triangle test with cooked abalone. Sample identity is given at the top of the figure and bars indicate number of answers for each identity. The correct answer is the odd one out (n = 51).

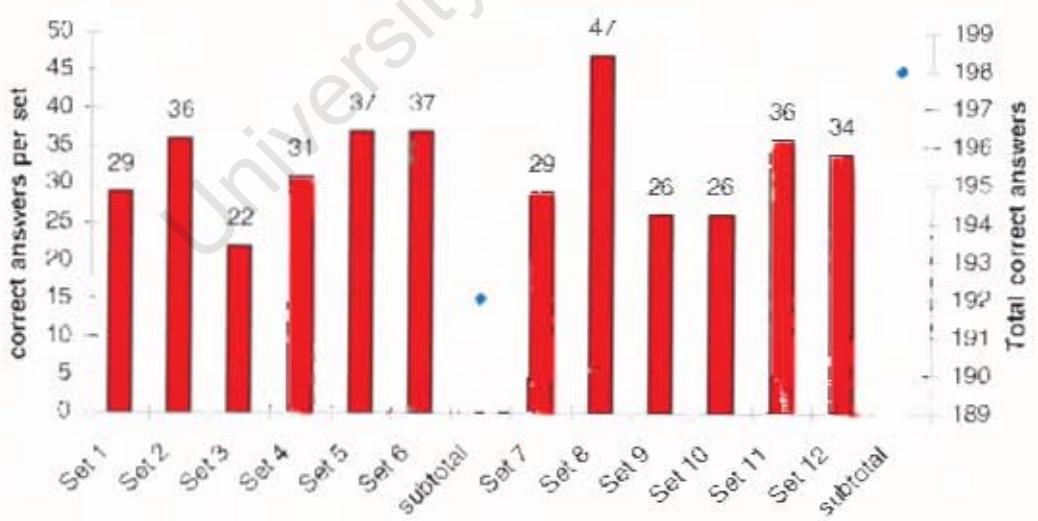

Figure 3.14: Number of correct answers supplied per set and sub-totals for cooked and uncooked sections of triangle test (n = 51).

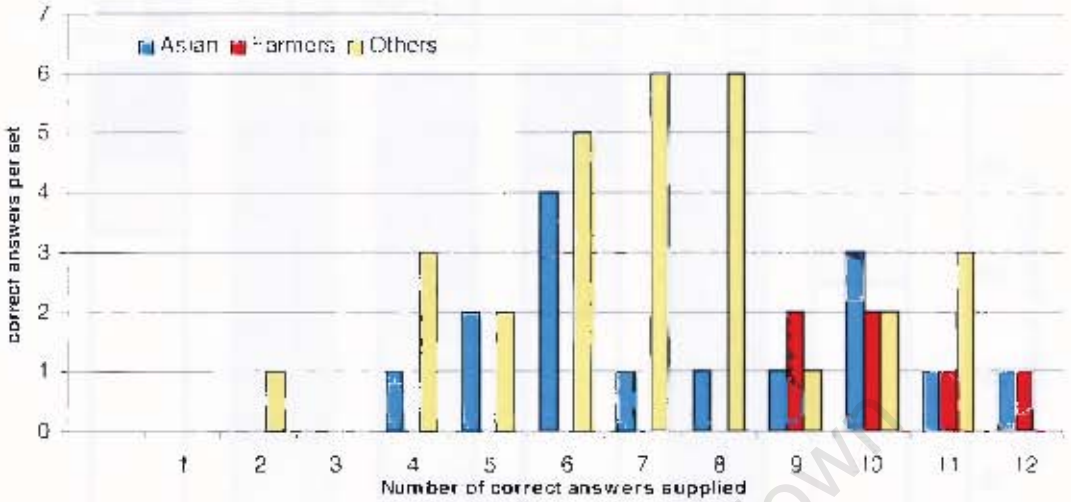

Figure 3.15: Distribution of correct answers supplied by Asians, farmers and other participants, for triangle test (n= 51)

The univariate approach showed that important features to differentiate between the different diets (Abfeed®, *Ulva*, kelp, mixed, wild) between Asians and non Asians set were colour and surface texture, but other variables also differed to a lesser extent (Table 3.7).

Surprisingly, whether the abalone were raw or cooked did not have a significant difference. Whether the tasters were Asian or not had a larger effect. No effect of smoking was present. A multivariate technique was used to make sense of the data: i.e. which combinations of things best explain differences among the feed treatments, or cooked/uncooked.

Using linear discriminant analysis on the entire data set, it was found that colour, surface texture, aroma (intensity), and taste (sweetness) explain most of the variation between the groups (63.5, 24.1, 8.8 and 3.5%, respectively) of all the variables tested. Once again, whether they were cooked or not did not have a significant effect (Figure 3.16).

TABLE 3.7: Features used to differentiate between the different diets between Asians and Non Asian testers.

Category 1 (Asian) (n= 15)	F value	**Category 2 (Non-Asian) (n = 36)**	F value
Colour	11.521	Colour	6.8578
Surface texture	4.8388		
Aroma intensity	4.7044		
Taste sweetness	3.0409		
Taste saltiness	2.7758		
Mouth hardness	2.8006	Mouth hardness	5.5743
DMS like	3.4976		
		Taste bitterness	2.8206
		Mouth toughness	2.7026

TABLE 3.8: Features used to distinguish between different diets using the entire data set between cooked and uncooked samples (n = 51).

Category 3 (Cooked)		**Category 4 (Uncooked)**	
Colour	14.198	Colour	5.0188
Surface texture	3.3838	Surface texture	7.4853
Taste saltiness	3.2618		
Taste freshness	3.8542		
Mouth toughness	4.3005		
Mouth hardness	2.8586	Mouth hardness	2.4665
DMS like	3.8299		
		Aroma intensity	4.7628
		Finger toughness	4.1692

Essentially, from the continuous data, only three of the 20 or so variables could be used to explain differences among the feed groups using the entire data set. In the continuous data whether the samples were cooked or not made no difference (Figures 3.17 – 3.18).

Category 1 (Asian): the first and second discriminant axes explained 58.76 % and 34.62 % of the variation; corresponding eigenvalues are 0.483 and 0.355, respectively;

Category 2 (Non-asian): the first, second and third discriminant axes explained 49.94 %, 29.99 % and 14.54 % of the variation; corresponding eigenvalues are 0.249, 0.1658 and 0.088, respectively;

Category 3 (Cooked): the first, second and third discriminant axes explained 55.45 %, 29.27 % and 12.10 %; corresponding eigenvalues are 0.409, 0.267 and 0.131, respectively;

Category 4 (Uncooked): the first, second and third discriminant axes explained 59.19 %, 25.73 % and 10.58 %; corresponding eigenvalues are 0.318, 0.168 and 0.077, respectively.

In Figures 3.16 – 3.18 the left panels show the 'centroids', or means, of each group after data in three dimensions (colour, aroma intensity, and surface texture) were reduced to two dimensions by conversion to eigenvalues. The right panels show which variables explain the differences among feed groups along the discriminant axes. In Figure 3.16 the texture and aroma are pulling in opposite directions, thus wild abalone have a strong smell and are tough. Abalone fed an *Ulva* only diet are colourful and tender. On the whole there is very little separation between the diets and the wild abalone diet has characteristics in each of the other diets. The *Ulva* is seen as more colourful than the other diets and the wild abalone have a stronger smell than the other diets.

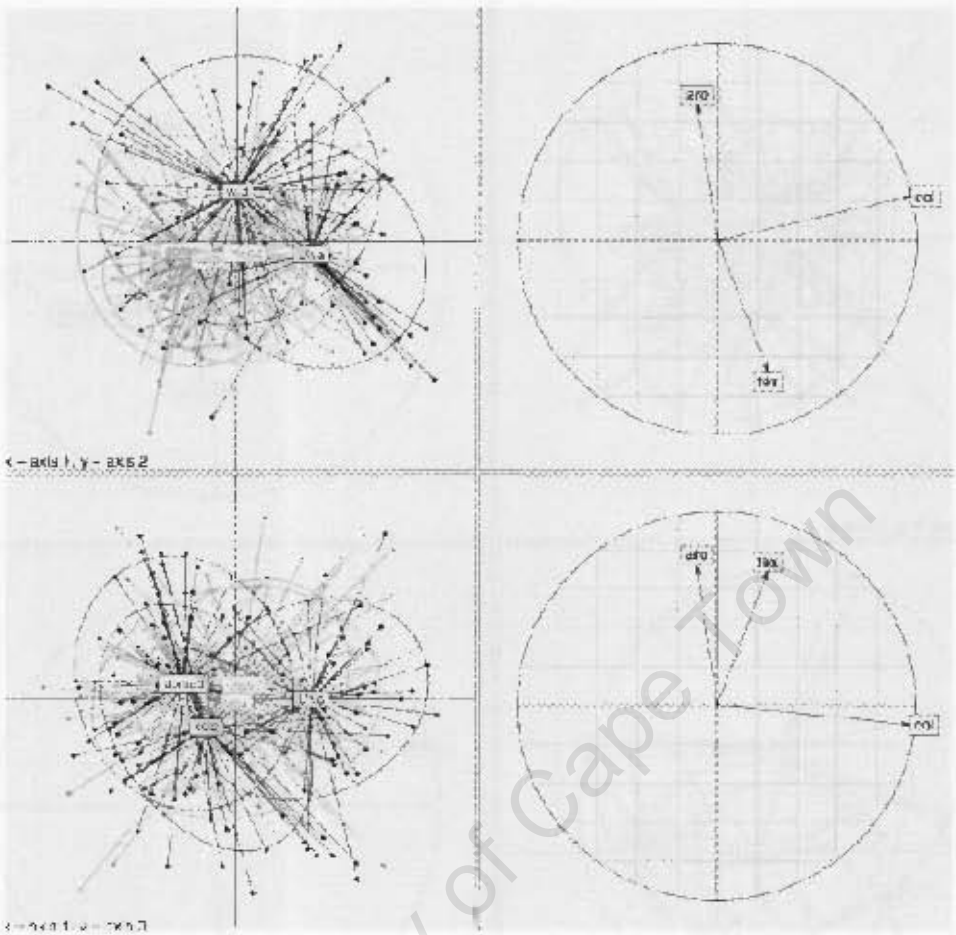

Figure 3.16 : Discriminant analysis of three variables using the entire data set – colour, surface texture and aroma intensity – using feeds as categorical variable. discriminant axis 1 vs. discriminant axis 2

From Figure 3.17, the data was split into the Asians and others, the figures show that there are clear differences in how the two groups evaluate the abalone. There is a greater separation in the diets in the Asian group and the characteristics used to generate this separation are different from the other group. The wild diet in the Asian category was virtually indistinguishable from the mixed diet, while colour played a role in separating the Abfeed© and *Ulva* diets.

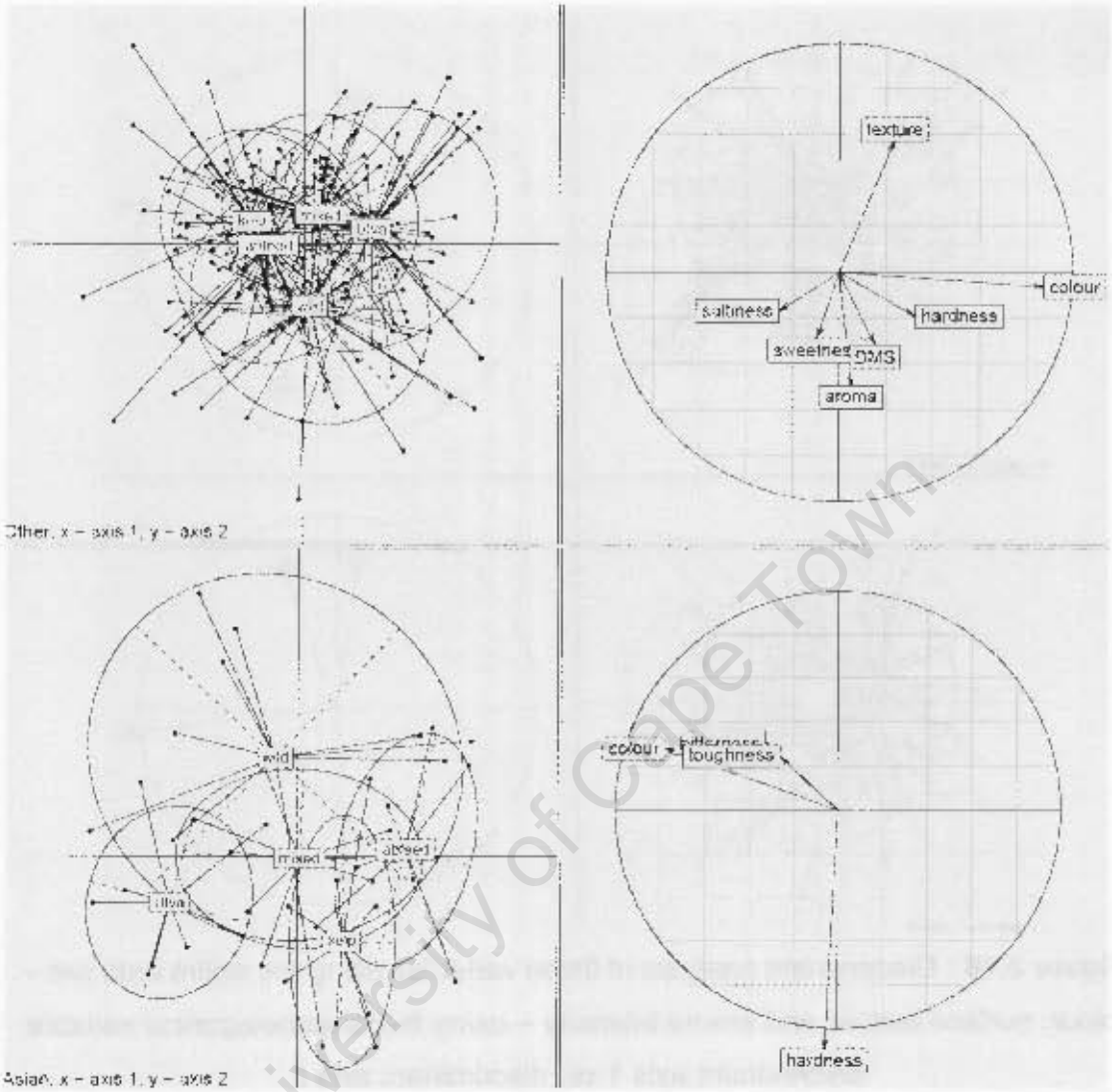

Figure 3.17: Discriminant analysis of variables – colour, surface texture, saltiness, sweetness, DMS aroma, hardness and aroma intensity in the "other" category and colour, toughness, bitterness and hardness in the Asian category using feeds as categorical variable. Data originating from Asian tasters were analysed independently from those of 'other' non Asian tasters. Axis 1 and 2 are shown.

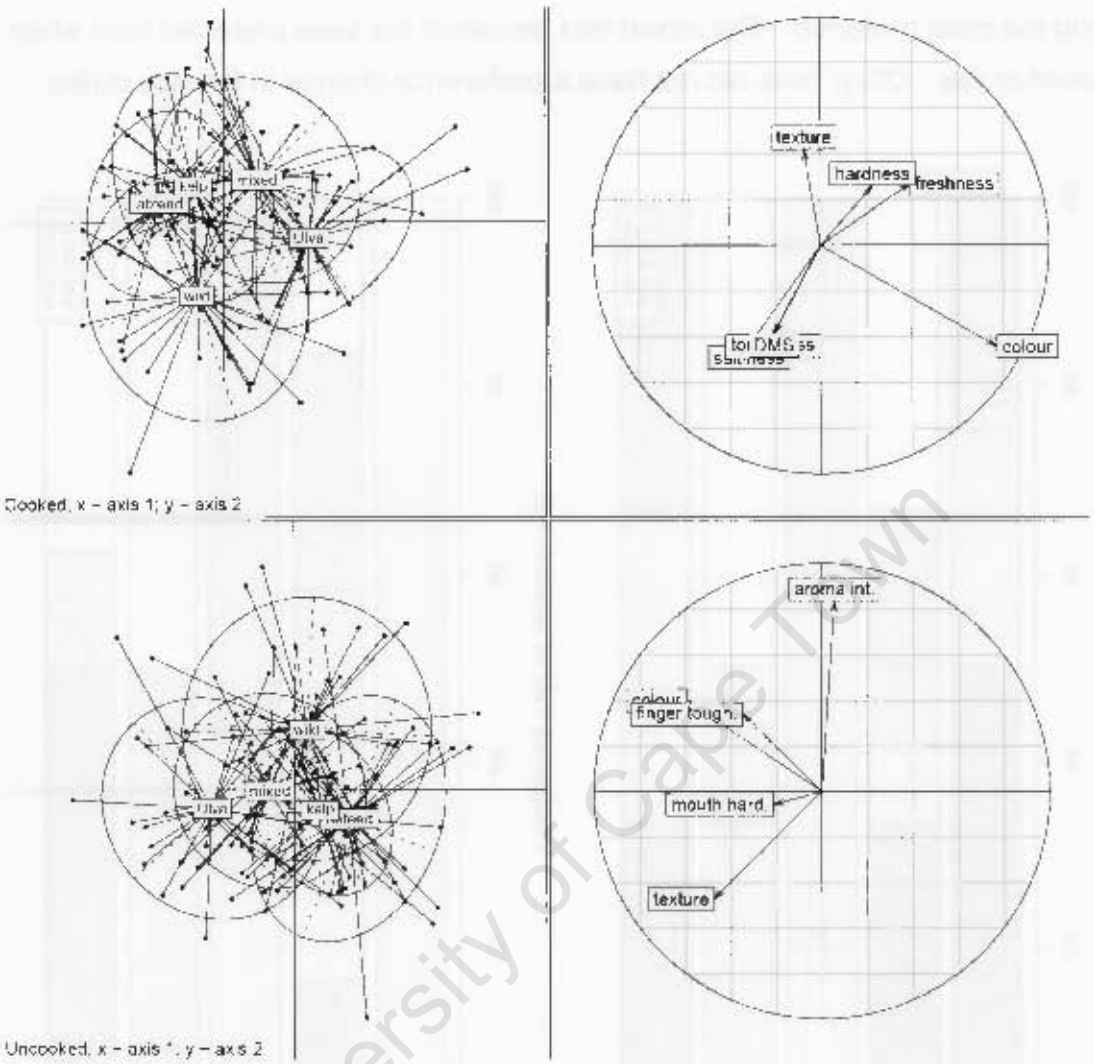

Figure 3.18: Discriminant analysis of the variables – colour, surface texture, hardness, freshness, DMS, toughness and saltiness in the cooked category and aroma intensity, finger toughness, colour, mouth hardness, and surface texture in the uncooked category – using feeds as categorical variable, using the entire data set. Axis 1 and 2 are shown.

Figure 3.18 illustrates that there is very little separation between diets whether they are cooked or uncooked. Figure 3.19 illustrates the final preference ranking of the different diets. In the uncooked samples, *Ulva* fed animals were the most preferred followed by wild animals. This preference switched in the cooked samples with the *Ulva* fed animals being the less preferred and the wild animals

being the most preferred. The mixed diet remained the least preferred both when cooked or raw. Other diets did not have a preference change in the two states.

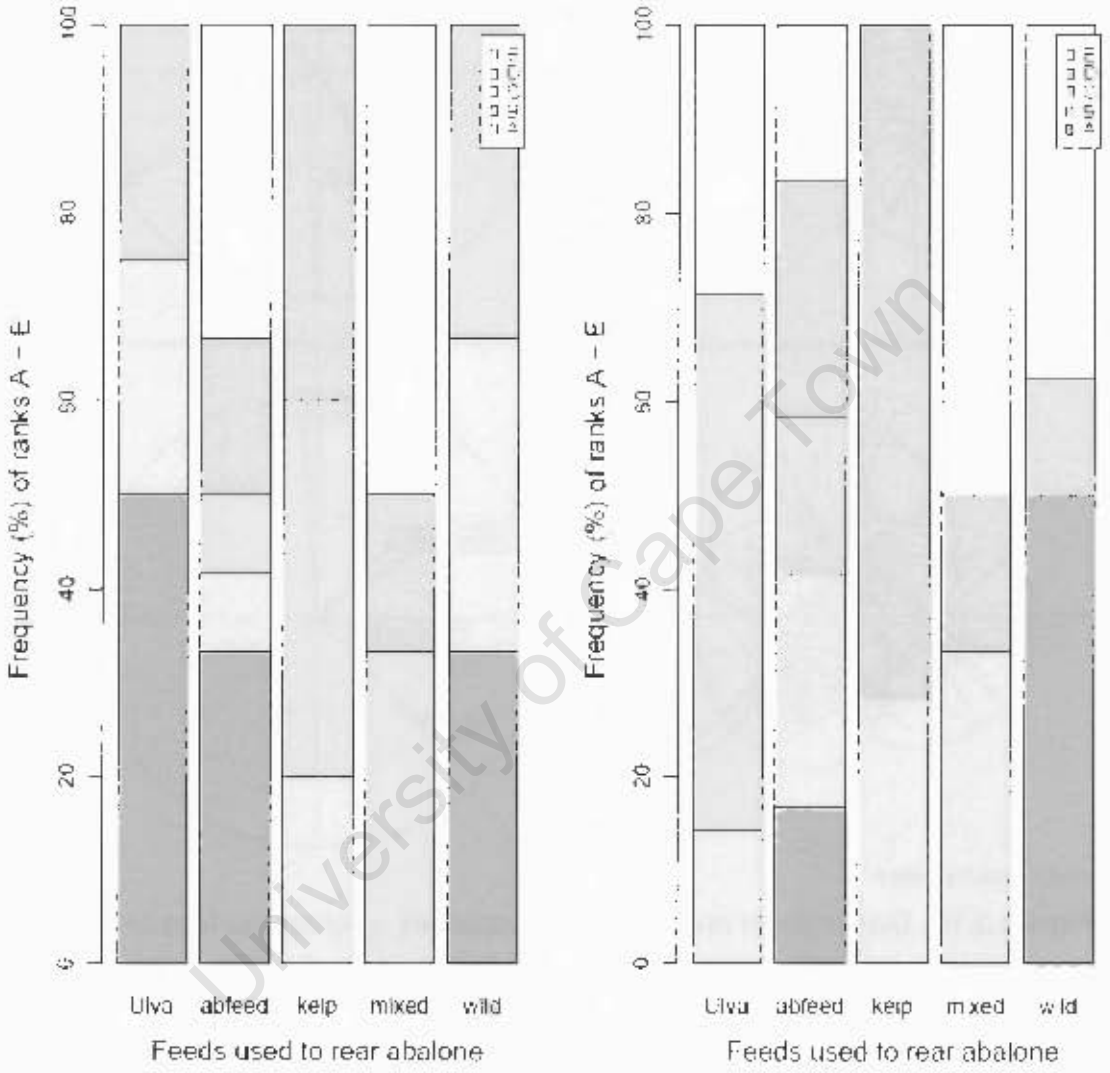

Figure 3.19: Frequency (%) of ranked preference of each of the abalone fed different diets cooked or uncooked with A = most preferred, E = least preferred. Uncooked ranking results on the left and cooked on the right.

DISCUSSION

Fillet yield

Both the canning data from Abagold and the fillet yield data from the first taste test showed that diet has a significant impact on fillet yield, with the compound diets having a more significant effect on fillet yield, than a single species macro-algal diet. From a farmers point of view this effect is important as it affects the amount of cooked product they can sell from their stock. A stock which has high moisture content (therefore low fillet yield) and a fast grow rate may be as profitable as a stock with low moisture content (high fillet yield) and a slower growth rate. It is also possible to alter the animals diets in the months prior to selling, so that the can be cultivated on the faster growth diets and then be converted on to a diet that improves their fillet yield. Fillet yield is not the only effect on the taste preference of the animals.

Colour

The flesh and shell colour also play important roles in the consumer acceptability/ palatability. In mussels, for example, the local South African mussel *Chromytilus meridionalis* Kraus has a dark chocolate coloured gonad in the females with the males being pale yellow, while the alien invasive *Mytilus galloprovincialis* (Lam.) (which is the mussel which is aquacultured and sold in South Africa) has a female gonad that is orange with the males being pale white (Branch *et al.* 2000). The local indigenous mussel cannot be marketed in South Africa due to the colour of the female gonads when the mussel is cooked, in that consumers perceive the brown gonad to be dirty or off, even though it has a richer flavour than other mussels eaten in blind taste tests (Blue Bay Aquafarm pers comm.). The local mussel market is based on the two types of markets; a historical market from Europe based on *M. galloprovincialis* created when Europeans settled in the Cape and a modern market based on the New Zealand green lipped mussel or Greenshell ™ mussel (*Perna canaliculus*), this market was established via extensive marketing and trade marking of the endemic New Zealand green lipped mussel both by the local producers and the New Zealand government. The fact

that this market is so large and is deemed difficult to get into by the South Africa producer of mussels is a testimony to how successful the marketing has been to change consumer perception to prefer the New Zealand mussel. The taste tests in this Chapter showed that there were differences in how the testers perceived the quality of the abalone, with the Asian tasters preferring a more colourful abalone over a paler one.

Criticisms of the taste tests

In the first taste test although we had a panel of trained tasters, which was not the case for the second test, the fact that the panellists had prior information as to the order of the diets meant the test was not completely blind or random, and this showed in the difference between the written report and the actual test samples results. In the second test it was not possible to obtain a trained panel of testers nor was it possible to train the panel as occurs in most taste tests (see ISO 13301). This was not possible due to a number of reasons. Abalone has not been available as a recreational catch for the last two years (see Chapter 2) in addition there are only a few restaurants that sell our local abalone (see Chapter 2) and these cater towards high end users and Asians. Thirdly the logistics and permit conditions to perform this taste test only allowed this researcher a limited number of wild abalone for the purposes of the taste test and these were all consumed in the second taste test. If a panel had to be trained the animal preparation and permits needed to deem the wild abalone safe for human consumption would have been costly and time consuming. It was for this reason that the triangle test was performed. The reason for this test was to use only data from panellists who correctly identified five or more of the odd samples out of the 12 triangle tests. These results would then be included in the analysis of the QDA dataset. Although this is not a significant proportion of correct answers, statistically the correct number of answers needed to be 8 or higher, doing this did give a large enough data set to analyse. The data set was then also broken up into Farmers, Asians and Others, as it was found that those panellists who worked with abalone on a daily basis had a significantly higher number of correct

answers than panellists other than Asians (see Figure 3.15). When these groups were analysed in the QDA it was found that the Farmers, Others & Asians used very different characteristics to distinguish the abalone (Robertson-Andersson *et al.* In press.). This is indicative of a difference in the market perception of a good abalone from a Farmers point of view and an Asian consumer point of view.

Preference

The final ranking test (see Figure 3.19) showed that there is a change in the preference of abalone when the samples were raw or cooked, this difference was more marked in the Asians than any other group and showed that the Asians preferred a stronger smelling/ tasting abalone in the raw state than in the cooked state. This test was particularly interesting in that in the raw state abalone high in DMSP were preferred over wild abalone (which is deemed to be the market preference), while in the cooked state wild abalone were preferred over any other diet. The results from this test are perhaps the most revealing of the taste test as this shows the market preference of the consumer and the changes in preference when cooked or raw still indicate that changes in DMSP concentrations are having an affect on taste perception.

CONCLUSIONS

Taste and odour are subjective quality measures and the taste trial showed that DMS could not be conclusively linked to the bad taste in abalone and that there were a number of other characteristics which consumers used to chose a good eating quality abalone. If we wished to illustrate the effect of DMSP in macro-algal diets on the eating quality of abalone we would have to have included a macroalgal diet (*Ulva*) free of DMSP. As this point in time this is not possible. It seems likely that in the future more emphasis will be placed on cultivated seaweeds and compound feeds providing much of the nutritional needs of farmed abalone. As these resources affect eating quality of the final abalone product, emphasis needs to be placed on quality control issues of this material, especially in terms of factors that negatively affect levels. This is especially important as the market (easterners) perceives the product differently from the farmers and westerners. The fact that there is a preference for abalone high in DMSP in the raw form, may illustrate another product niche for the South African abalone, in that the abalone fed an *Ulva* only diet could be marketed for the raw uncooked market or sushi market.

CHAPTER 4

A COMPARISON BETWEEN A FLOW THROUGH SYSTEM AND A COMMERCIAL GRAVEL-BED RE-CIRCULATING SYSTEM ON THE SPECIFIC GROWTH RATE AND HEALTH OF CULTIVATED ABALONE (*HALIOTIS MIDAE*)

INTRODUCTION

The majority of South African abalone farms operate on a flow through system with limited options for recirculation. These farms are exposed to a number of threats including harmful algal blooms (HABs), accidental discharges of pollutants (e.g. oil spills) and the application of more stringent water quality requirements to farm effluents in the future (Robertson-Andersson, 2003, Botes, 2003; Botes *et al.* 2003, 2004; Samsukal, 2004; Troell *et al.* 2006). A flow-through system (FTS) relies on an unlimited source of clean water for sustainability. The water has to have a high volume exchange rate (depending on stocking density and aquaculture intensity) so as to remove waste and oxygenate the cultivation system (Troell *et al.* 1999; Furey *et al.* 2003).

Recirculation systems with biofiltration are a means to reduce the above risks as well as having advantages such as the potential for temperature enhancement, lowered pumping costs (due to a reduced head and the re-use of water) and higher stocking densities, all of which help to increase a farms productive output (Losordo *et al.* 1998). There are however, disadvantages associated with such systems in that they are expensive to install, are technologically demanding and require constant monitoring to ensure operational efficiency. The latter is important due to risks associated with inefficient systems which may therefore compromise water quality e.g. increased toxic ammonia, increased nitrite conditions, reduced pH, CO_2 build up, elevated temperatures, increased salinity and the possible long term effect of pathogens, commensal or parasitic organisms retained in such systems.

This study aimed to monitor abalone growth rate and health across a range of size classes from two age cohorts, through regular tri-month sub-sampling in a re-circulating system with a biological filter. Growth rates of sub-samples were compared against tank biomass data obtained from farm grading data. In addition a seasonal snapshot of water quality in the two systems was compared by monitoring over three 72-hour periods (June 2004 - winter, September 2004 and January 2005 - Summer), in which temperature, pH, dissolved oxygen, ammonium, phosphate,

nitrate and nitrite were compared. The study period commenced in March 2004 and was completed in June 2005 to gain a complete seasonal data set.

Farm and tank design

Jacobs Bay is located along the West coast of South Africa (17° 53' 12.5" E, 32° 58' 2.5" S) approximately 120 km north of Cape Town (See Figure 2.4, Chapter 2). The farm Jacobs Bay Sea Products Pty. (JSP) is a land-based intensive mariculture operation of ± 11 ha situated on the point of Jacobs Bay and has been in operation since 1994. The farm cultivates mainly abalone, (*H. midae*), and previously cultivated turbot, (*Scopthalmus maximus* L.), oysters, (*Crassostrea gigas* Thunberg), *Gracilaria gracilis* and *Ulva lactuca.* The farm has an abalone stock of approximately 2.4 million abalone (± 76.8 tons), which range from spats to 6-year-old animals. Seventy seven tons of fresh harvested kelp (*Ecklonia maxima* and *Laminaria pallida*) are used each month as abalone feed. The abalone eat approximately 5 – 7 % of their body weight in kelp per day.

The farm is interested in testing a commercial recirculation system, primarily to counter low water temperatures (< 11 °C) due to coastal upwelling in summer (Largier & Boyd, 2001), which have a negative impact on abalone growth and farm productivity. The farm has a limited water intake ability, and the cost of laying new pipes to increase the water supply to the farm is significant. If the farm was able to recirculate its water supply or install a recirculation system, they could increase the amount of water available on the farm and thus increase the farm's abalone carrying capacity. HABs are common on the west cost and have been known to cause large abalone mortalities (Matthews & Pitcher 1996; Pitcher, 1998). HABs can be costly for the farm especially if the HAB tide event consists of the dinoflagellate *Alexandrium.* In such cases abalone that were ready for live sale have had to be canned as they were unsellable in the live form (Robertson-Andersson, 2003). Even though abalone are not filter feeders they were found to still carry the toxins in the viscera and on the skin epithelium, especially the epipodial fringe. At JSP, the abalone had to be canned for a year and a half following a HAB event and this resulted in a 30 % revenue loss for the farm (K. Ruck pers. comm.). The residues

racks (± 20 %). This gave a recommended weight per basket for different size classes of abalone (Bok, 2002).

FIGURE 4.1: Illustration of the Global Ocean AquaCycler cluster. The foam fractionator is in the centre of the two clusters.

Experimental animals

As abalone have a heterogeneous growth rate within a batch (Lee, 2004), genetically similar grow-out abalone were used for the experiment. Due to the large number of animals involved, two age cohorts (October 2000 and November 2000) were used and these cohorts were divided into a number of different size classes (See Table 4.1). There were no significant differences in either length or weight in the different size classes between the two systems. Both systems were fed a kelp only diet.

Water quality and physiochemical variables

Water parameters, monitored for both seawater systems described in this study, form the criteria for assessing relative advantages and disadvantages of these systems, as maintaining good water quality is essential for creating the best environment for abalone survival and growth. The motivation for choosing these water quality parameters is as follows. Temperature was chosen for its effect on abalone gametogenesis (Kikuchi & Uki, 1974). The range of water temperature for good

produced by HABs can also slow the growth rate of the animals and result in large spat and larval mortality (Botes *et al.* 2003, 2004; Jansen, 2005, 2006).

The Flow Through System (FTS)

Water from the sea is pumped directly into a top settling reservoir at a rate of 1 200 000 – 1 300 000 L hr^{-1}, and from here it is gravity fed to the bottom holding dams. Water can be pumped directly into the holding dams where it is heated by solar radiation (in summer). This is done to combat the low water temperatures experienced in summer when upwelling of cold water occurs off the west coast. The water turnover rate for the top-settling reservoir is 5.6 volumes d^{-1} and 4.5 volumes d^{-1} for the bottom 2 dams. From the dams, the water is pumped into the mixing tank (g) where it is distributed to the abalone tanks or returned back to the dams. The water is not filtered for particles before being distributed from the dam to the tanks. However, some settling does occur in the dam. The tanks are unlined concrete brick raceways, with 7 tanks in a raceway. Each tank has the following dimensions (1.5 x 1.5 x 6 m). Each tank receives 900 L h^{-1} ($\pm$ 100 L h^{-1}). Although the tanks are in a raceway each receives its own incoming water source, whereas waste water from 7 tanks is mixed into a common effluent channel.

The Global Ocean AquaCycler cluster

The Global Ocean AquaCycler cluster (GOA) is an all plastic abalone culturing unit consisting of two grow-out tanks (16 m^3) and a foam fractionator (Bok, 2002) (See Figure 4.1). It is driven solely by low-pressure air. The system biofilters water in an in-tank subsurface gravel (25 mm course gravel) biofilter with water aeration occurring via the water transport system (air lift pumps), while protein, fine particles and dissolved organics are "foamed" off in the foam fractionator (Bok, 2002). The tanks, and foam fractionator, underdrain through the gravel biofilter. Replacement water is either sand or drum filtered and fed into the culture system at a rate of 220 L h^{-1} ($\pm$ 120 L hr^{-1}) which equates to a turnover once every 105 hours. Sixteen baskets are suspended in each tank and the baskets are separated into a number of racks with a 50 mm spacing between each rack. Stocking density was determined by the ratio between the surface area of the shell relative to the surface area of the

growth of *H. midae* can be inferred from natural abalone distribution (13 – 19 °C) (Newman, 1969). Optimal temperature requirements are not as important as maintaining a constant temperature as sudden changes in temperature induce stress in abalone.

In aqueous solutions, ammonia exists in a pH, temperature, and salinity-mediated equilibrium between the unionized and ionized forms, of which the unionized form is the more toxic (Haywood & Wells, 1989; Russo & Thurston, 1991). Ammonia has been shown to affect the immune response of Taiwan abalone, *H. diversicolor supertexta* (Cheng *et al.* 2004) and kidney structure in greenlip abalone, *H. laevigata* (Harris *et al.* 1998a) and ultimately influence the growth of the abalone. A number of workers have investigated the influence of ammonia on the survival and growth of abalone (Harris *et al.* 1998b; Basuyaux & Matthieu, 1999; Hindrum *et al.*, 2001; Huchette *et al.* 2003). These studies concentrated mostly on the influence of ammonia on the growth of juvenile Australian abalone. Acute ammonia toxicity (i.e. lethal- and sub-lethal concentrations) in the South African abalone has only recently been investigated by Reddy-Lopata *et al.* (2006). They showed that tolerance to ammonia increased with increasing body size and age as well as prior exposure. Growth of juvenile abalone (1 – 2.5 cm shell length) when exposed to sub lethal levels of ammonia had a 58 % reduction in their specific growth rate (Reddy-Lopata *et al.* 2006). Nitrate concentrations tend to follow ammonium concentrations and can be used as an indication of prior ammonium concentrations, following bacterial nitrification.

pH is an inverse measure of the prevalence of hydrogen ions in water. Respiration of organisms in seawater produces carbon dioxide. In intensive mariculture operations, animal respiration can be excessive due to high bio-loads, increasing the concentration of hydrogen (H^+) ions and so lowering pH. At a pH below 7.6, calcium carbonate begins to dissolve in seawater (Hahn, 1989). At these pH levels, abalone shell begins to dissolve and they get 'shiny shell' syndrome. The shell of the abalone takes on a shiny appearance and in extreme cases, respiration vents join and form a slit down the length of the shell. The shell of the abalone can become paper-thin. If

the shell becomes punctured, the internal organs of the animal are exposed, making it vulnerable to damage (Hahn, 1989). Juvenile abalone are particularly susceptible to this (Hahn, 1989) and therefore close monitoring of pH in these recirculation systems is very important (Bok, 2002). Once the animals are larger than 40 mm shell length, they become far more resilient to low pH levels (Hahn, 1989). The toxicity of ammonia also increases at high pH levels (Boyd, 1990) due to increased evolution of hydrogen ions. pH levels can be controlled by adding a combination of sodium carbonate ($NaHCO_3$) and sodium bicarbonate (Na_2CO_3) to the water to maintain a pH of at least 7.9. Normal dosing quantities for the GOA system are around 1kg of sodium bicarbonate and 1 kg of sodium carbonate per cluster per day. The chemicals are mixed with freshwater. Exact dosing requirements can be calculated relative to the problem pH levels found at a particular facility.

Dissolved oxygen is an indicator which can be used to check both Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD). Decreases in dissolved oxygen would mean that BOD and/ or COD have increased. Ideally concentrations should be around 4 – 9 $mg.L^{-1}$ (Hahn, 1989).

Table 4.1: Starting sizes of experimental animals. Size classes were divided as follows: green, blue, red, white and pink (export).

	FTS		GOA	
10/2000	Weight (g)	Length (cm)	Weight (g)	Length (cm)
Green	22.96 ± 8.4	4.87 ± 0.6	18.40 ± 2.8	4.65 ± 0.2
Blue	32.10 ± 7.7	5.36 ± 0.4	29.43 ± 4.2	5.38 ± 0.3
Red	42.51 ± 10.9	5.94 ± 0.6	39.93 ± 7.3	5.96 ± 0.3
White	72.00 ± 9.7	7.16 ± 0.3	65.92 ± 9.3	7.24 ± 0.3
Pink	90.80 ± 3.6	7.84 ± 0.2		
11/2000				
Green	23.37 ± 3.0	4.53 ± 0.3		
Blue	34.60 ± 5.7	5.55 ± 0.5	29.95 ± 6.0	5.44 ± 0.3
Red	48.49 ± 8.3	6.21 ± 0.4	44.43 ± 8.8	6.19 ± 0.7
White	67.42 ± 9.6	7.10 ± 0.3	65.12 ± 8.8	7.30 ± 0.4

Abalone health

Terebrasabella heterouncinata Fitzhugh, Rouse (Fitzhugh & Rouse, 1999) is a member of the polychaete subfamily Fabracidae, and it is commonly referred to as a sabellid. Sabellids are known to cause problems in South African abalone farms among *H. midae*, especially when high abalone stocking densities were combined with poor hygiene and marginal water quality in culture tanks (Cook 1998; Ruck and Cook 1998, 1999; Sales & Britz 2000). Detrimental effects include significantly reduced growth rates, grossly deformed shells with the absence of respiratory aperture (gill pore) formation on the leading edge of the shell, decreased meat yields, increased mortality due to the inability of the abalone to right themselves when dislodged from their substrate, and reduced marketability (Bower, 2004). Heavy infestations (as observed in some abalone facilities) result in shells with a thickened leading edge that is very fragile and porous, due to the lack of prismatic calcite and the honeycombed affect caused by high polychaete populations. The shell of heavily infested abalone grows downward instead of outward as in normal abalone. Also, slower growing abalone appear to be more susceptible to heavy infections (Britz *et al.* 2005). A fast growing abalone can encapsulate a small number of sabellids and extend its shell beyond them. If a culture system can provide more stable and favourable temperatures for abalone growth, abalone in such a system may be able to cope with sabellid infestations.

METHODS

Subsamples and abalone growth

Samples were taken from tanks containing cohorts from 10/2000 and 11/2000 (Table 1) from January 2004 to June 2005. At each sampling 15 animals were removed from every basket in the tank. Before all weight measurements, abalone were blotted dry to remove excess water. Abalone body weight was recorded to the nearest 0.01 g, while shell length was measured along the longest axis to the nearest 0.01 mm. The sampling periods were designed to coincide with regular grading. Of the 104 660 animals that were part of the experiment at each monitoring period 4 320 animals were randomly sampled. In order to follow changes in growth rates individual baskets in both systems were sampled on a repeat basis.

There has been considerable discussion around which method to use to model abalone growth rates. Historically both linear (see review by Hahn, 1989) and cubic growth (Harris *et al.* 1997; 1998a) models have been used. Thus, within the literature conflict exists, regarding the practicality and complexity of these models. Reaburn & Edwards (2003) investigated these models with respect to abalone growth and stated that for weight measurements a growth model that allows for exponential growth "must be used, while for shell length measurements a linear model "may" be used. For this reason weight data was analysed using an exponential equation, this equation was then extended forward to 100 grams, as this is the weight at which the abalone are harvested. Regression analysis was used to determine the uniformity.

Grading

The grading was done on the farm as per normal farm grading methods. Animals were harvested from a tank, the basket number and age group recorded. Animals were then manually graded into different size classes and the weights of these size classes were recorded for a total biomass value. These data were recorded by farm personnel.

Condition factor

The condition factor is a concept that was developed to account for the relationship between the weight of the abalone per unit shell length (Britz, 1996a-c).

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

Where CF = condition factor, BW = the mean body weight and SL the mean shell length.

Physiochemical variables

Temperature, pH and dissolved oxygen were recorded daily by JSP and monitoring for the purpose of this report was done intensively during water sampling over a period of 72 hours. In addition temperature data loggers were placed in a global ocean tank and a flow through tank in August 2004. They recorded temperature every 15 minutes for 6 days (22^{nd} to 27^{th}).

Water quality

Water samples for ammonium, phosphate, nitrate and nitrite were taken during 72 hour experiments. Ammonium concentration was determined using the method described by Grasshoff *et al.* (1976), scaled down to a sample volume of 5 ml and reagent additions of 0.2 ml. Dissolved Inorganic Phosphate (PO_4^{3-}) concentration was determined using the method described by Grasshoff *et al.* (1976), with a slight modification in that samples and reagent amounts were reduced by a factor of 10.
Nitrate (NO_3^-) concentration was determined using the copper-cadmium method described by Nydahl (1976). Nitrite (NO_2^-) concentration was determined using the method described by Nydahl (1976). Ammonium and phosphate samples were taken in triplicate and analysed as such. Nitrate and nitrite were analyzed from a single sample. Testing was done for Total Ammonia Nitrogen (TAN) and then using a table to calculate Free un-ionized Ammonia Nitrogen (FAN) was calculated using the TAN concentrations, pH, temperature and salinity values following Bower & Bidwell (1978), and Emmerson *et al.* (1975).

Abalone health

Samples to measure abalone health were taken and analysed by the AFASA veterinarian (Dr. Anna Mouton) according to standard veterinary procedures (Mouton, 2004) and were analysed by her. This was then reported according to standard industry reports as shown in Table 4.2.

Feeding

For the duration of the experiment the animals in the GOA system were fed a kelp only diet, twice a week, with 60 kg of kelp being placed in each tank per week. In the FTS the animals were fed 40 kg of seaweed a week divided between a Monday and a Friday. Although the amounts are different the feeding ratio was done according to the biomass in each tank and was approximately 10 % of the biomass per day.

Statistical analysis

All data are expressed as means $\pm$ standard errors. The analysis for this study was done using STATISTICA V6.1. An initial analysis of co-variance was first tested with the baseline value of the outcome i.e. either length or weight used as a covariate. This was done to account for any differences in starting values. To test for actual differences ANOVAS were performed on the data. All data were regarded as significant at $p < 0.05$. Abalone health data were analysed using ranked statistics.

TABLE 4.2: Interpretation of health results

	Average sabellid score		**Average gonad development**	**Parasite status**	**General condition**		**Remarks**
Range and interpretation	**0**: absent	**0**	**Immature**:	Expressed as the percentage of sample infected.	Worst to best:		**Environmental stress**
	1: less than 10 on entire shell	**1**	only immature sex cells present		**very poor**	**1**	Is present (**1**) or absent (**0**).
	2: more than 10 on entire shell			The following are reported:	**poor**	**2**	
	3: tunnels superimposed on growth edge	**2**	**Moderate**:	**Coccidia**	**below standard**	**3**	
	4: tunnels completely cover more than 2/3 of growth edge.	**3**	mixture of developmental stages of sex cells	**Digestive gut protozoa**	**acceptable**	**4**	
				gut protozoa			
				Rickettsia	**satisfactory**	**5**	
		4	**Mature**:	**Shiny shells** are present or absent.	**good**.	**6**	
			gonad consists almost entirely of mature sex cells	***Polydora*** is expressed as the percentage of sample infected.			
Chief determinant	Shell examination		Histology of gonad	Histology of all organs	Histology of digestive gland		Histology
Mainly reflects	Shell condition		Sexual maturity	Parasite infection level	Nutrition		Water quality

RESULTS

Physical water quality parameters

Temperature

Temperature in the FTS correlated well with readings taken from a long term study from 2001 to 2003 (Robertson-Andersson, 2003) ($p < 0.05$). This showed that there were no anomalies present during the measurement period for this analysis.

Average temperatures in the GOA system in August 2004 were higher compared to the FTS. In addition the standard deviation and the temperature range was lower implying more constant temperatures in the GOA system (See Table 4.3). This was again illustrated in September 2004 by intensive 24 hour monitoring of temperature (See Figure 4.2). The diurnal pattern in temperature in the flow through system is very distinctive while the trend in the GOA system is more subdued. Temperatures in the GOA system were more constant than the FTS. The low temperatures in the FTS in the early morning are as a result of a single cold night and illustrate how the vulnerability of the FTS to external environmental temperatures

Table 4.3: Statistics from temperature data loggers in August 2004, temperature in °C (n = 1323)

	GOA	FTS
Average	14.2 ± 0.5	13.9 ± 1.6
min	12.93	9.42
max	15.23	16.76
range	2.30	7.34

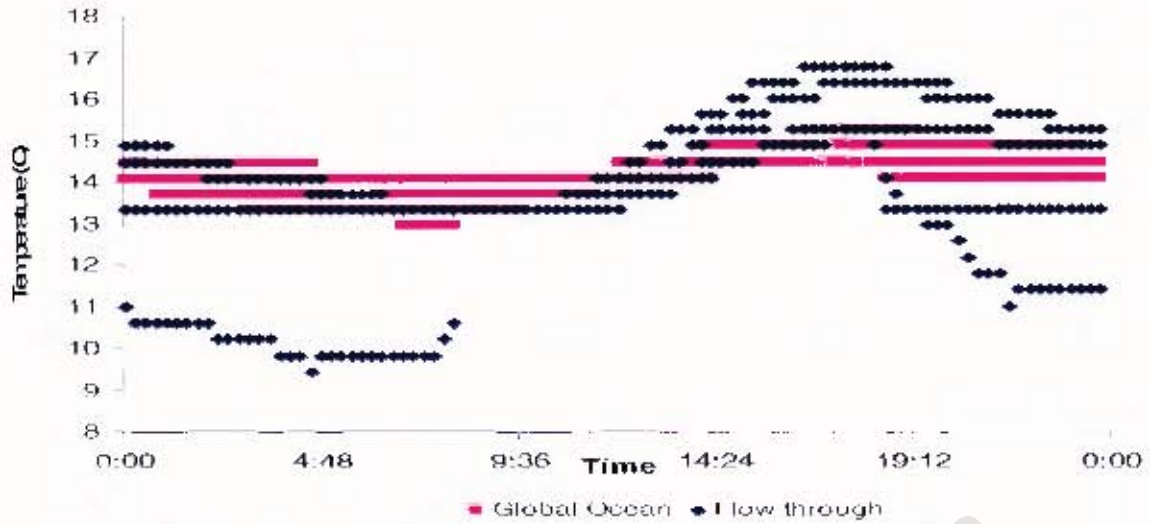

FIGURE 4.2: Intensive temperature monitoring on 9th - 12th September 2004 in a GOA system and FTS. Graph illustrates diumal variation in temperature over a 24 hour period. (n = 882).

A comparison was made of winter and summer physiochemical and water quality variables in the two systems, these would be the maximal and minimal temperatures that the systems would experience.

In summer the GOA system temperature was higher than that of the FTS (See Figure 4.3). This can be attributed directly to the high maximal air temperatures (greater than 28 °C) that were experienced at this time and the lack of water replenishment in the GOA system. The GOA system temperatures were significantly higher than those of the FTS (ANOVA, $p > 0.05$; $n = 9$; in 10 out of 14 cases). The GOA system showed less of an influence on night time air temperatures (8 °C) compared to the influence of summer day time temperatures. The summer range of temperatures in the GOA system was 2.8 °C vs. 4.9 °C in the FTS. In winter the temperature range in the GOA system was 3.1 °C vs. 2.5 °C in the FTS. In addition the GOA system had higher temperatures that the incoming seawater and the FTS.

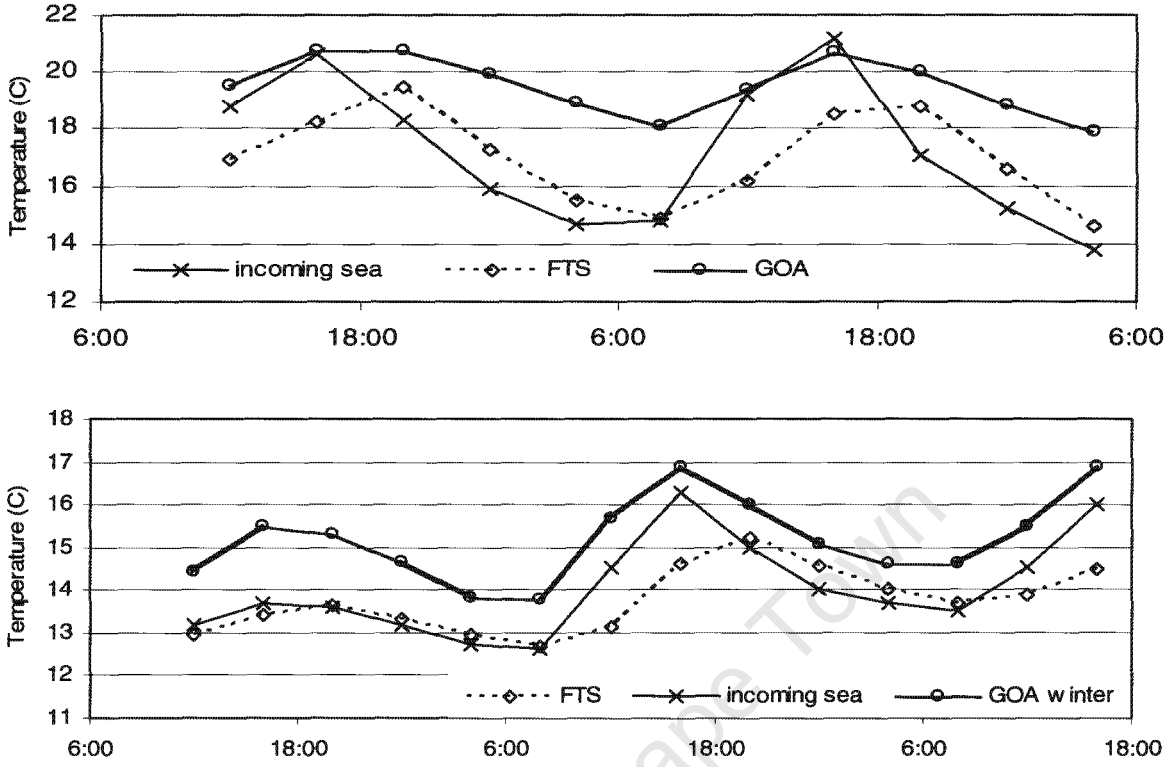

FIGURE 4.3: Summer (top) and winter (bottom) temperature (°C) variations (n = 363 for summer and 399 for winter). No error bars are shown to increase clarity but standard deviation was ≤ 0.6 ° C for all values

pH

pH in the GOA systems increased very rapidly in two of the clusters on the second morning during the summer measurement and it was noted that there was incomplete dissolving occurring of the dosing pills in these clusters (See Figure 4.4). This gave large standard errors for these measurements. This could account for the higher pH values shown in the summer pH measurements, as the pH in both systems was similar during the first day. pH was lower in both systems than in incoming sea water showing the effect of respiration on pH.

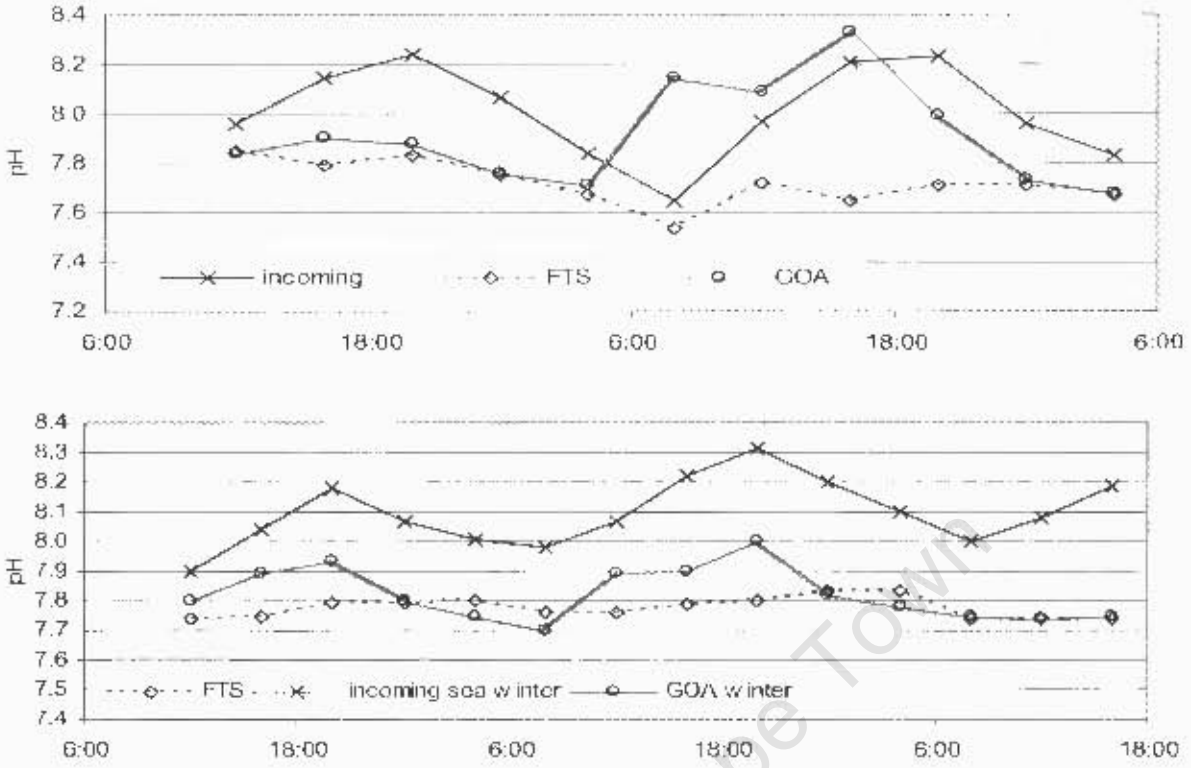

FIGURE 4.4: Summer (top) and winter (bottom) pH variations (n = 363 for summer and 399 for winter). No error bars are shown to increase clarity but standard deviation was ≤ 0.2 for all values.

Dissolved oxygen

There were significant differences in dissolved oxygen in the GOA system with and without kelp. Units in the GOA system with kelp had significantly lower values than those without kelp (ANOVA; $p < 0.05$; $n = 50$). This is not illustrated in the graphs shown. This difference was due to the availability of kelp and not due to changes in the feeding regime.

The GOA system had a higher BOD than the FTS (See Figure 4.5), particularly around 00h00 on the second night. Previous work on this farm in the FTS has shown that this time period corresponds to increased activity by the abalone (feeding and excreting) as well as an increase in the respiration demand of the kelp (Robertson-Andersson, 2003).

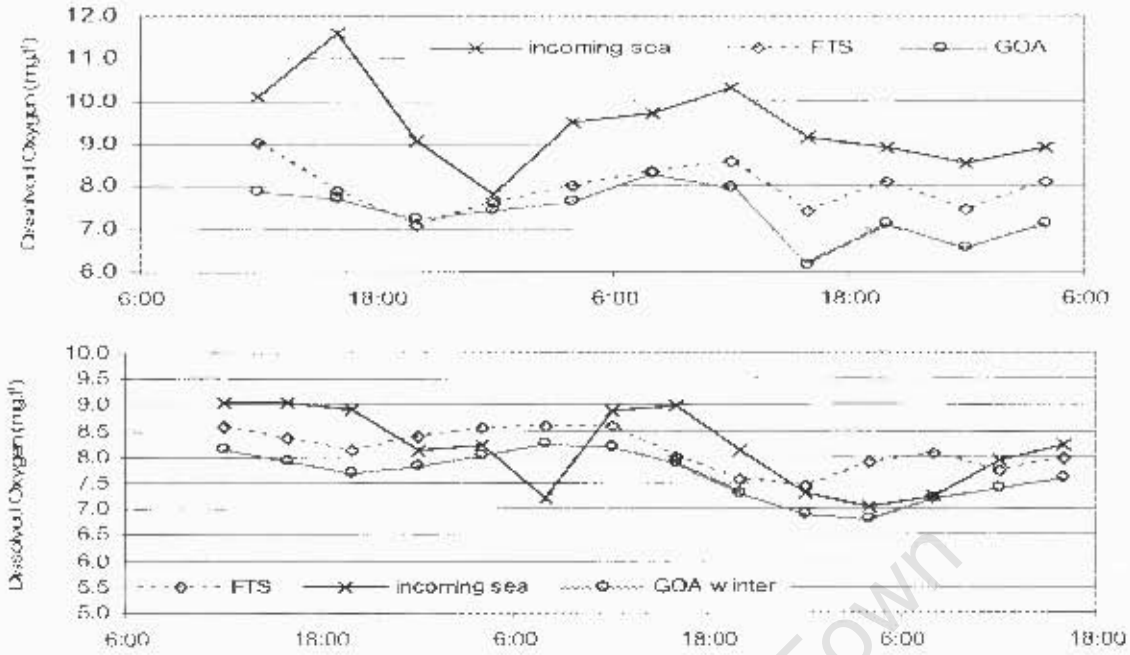

FIGURE 4.5: Summer (top) and winter (bottom) dissolved oxygen variations (n = 363 for summer and 399 for winter). No error bars are shown to increase clarity but Standard deviation was < 0.8 $mg.l^{-1}$ for all values.

Chemical water quality parameters

TAN

TAN concentrations in the FTS and in the incoming seawater correlated with those from a previous study (Robertson-Andersson, 2003). There was no significant difference between concentrations in seawater and the FTS. TAN concentrations were significantly higher in the GOA system (ANOVA; $p < 0.05$) (See Figure 4.7, TAN). Average concentrations in the GOA system were 24.25 (± 2.63) $\mu M\ L^{-1}$ with a maximum of 30.16 $\mu M\ L^{-1}$ and a minimum of 21.48 $\mu M\ L^{-1}$. The average concentration in the FTS and the incoming seawater was 3.20 (± 0.71) $\mu M\ L^{-1}$ and 3.95 (± 0.93) $\mu M\ L^{-1}$, respectively. The maximal concentration reached in the FTS was 4.34 $\mu M\ L^{-1}$ which was lower than the incoming seawater at 5.94 $\mu M\ L^{-1}$ and illustrates the uptake of TAN by the kelp (feed) in the system. There was a diurnal variation in TAN concentrations in the FTS and the GOA system and this is consistent with night time feeding and excretion by the abalone (See Figure 4.6, TAN)

Phosphate

Phosphate concentrations in the seawater and FTS also correlated well with previous work (Robertson-Andersson, 2003), with no significant difference between the two systems. Average phosphate concentrations were 6.25 (± 1.37); 3.85 (+ 0.46) and 2.06 (+ 0.22) μM L^{-1} for the GOA system, FTS and incoming seawater, respectively. Maximal concentrations recorded in the GOA system, FTS and incoming seawater were 8.29; 4.59 and 2.57 μM L^{-1}, respectively. An increase, although not significant, in phosphate concentrations in the GOA system and FTS was observed between the hours of 20h00 to 04h00, also consistent with feeding and excretion by the abalone (See Figure 4.6, Phosphate). Phosphate concentrations in the GOA system were significantly higher than in the FTS (ANOVA; $p < 0.05$ in 5 out of 8 cases).

Nitrate

There was no significant difference between the FTS and the GOA system with respect to nitrate concentrations. Average nitrate concentrations were 20.38 (±4.60); 17.44 (+ 3.06) and 10.48 (+1.58) μM L^{-1} for the GOA system, FTS and incoming seawater, respectively. Maximal concentrations recorded in the GOA system, FTS and incoming seawater were 28.26; 23.32 and 12.40 μM L^{-1}, respectively. Nitrate concentrations in the incoming seawater were significantly lower than in the GOA system and FTS. Nitrate also followed ammonium concentrations with a slight lag period and also showed evidence of feeding and excretion by the abalone (See Figure 4.6, Nitrate)

Nitrite

Nitrite levels in the incoming seawater and the FTS were not significantly different. Average nitrite concentrations were 2.39 (+ 0.56); 0.90 (+ 0.22) and 0.54 (+ 0.22) μM.L^{-1} for the GOA system, FTS and incoming seawater, respectively. Maximal concentrations recorded in the GOA system, FTS and incoming seawater were 3.54; 1.23 and 0.67 μM L^{-1}, respectively. Nitrite concentrations in the GOA system were significantly higher (ANOVA; $p < 0.05$ in all cases) (See Figure 4.6, Nitrite).

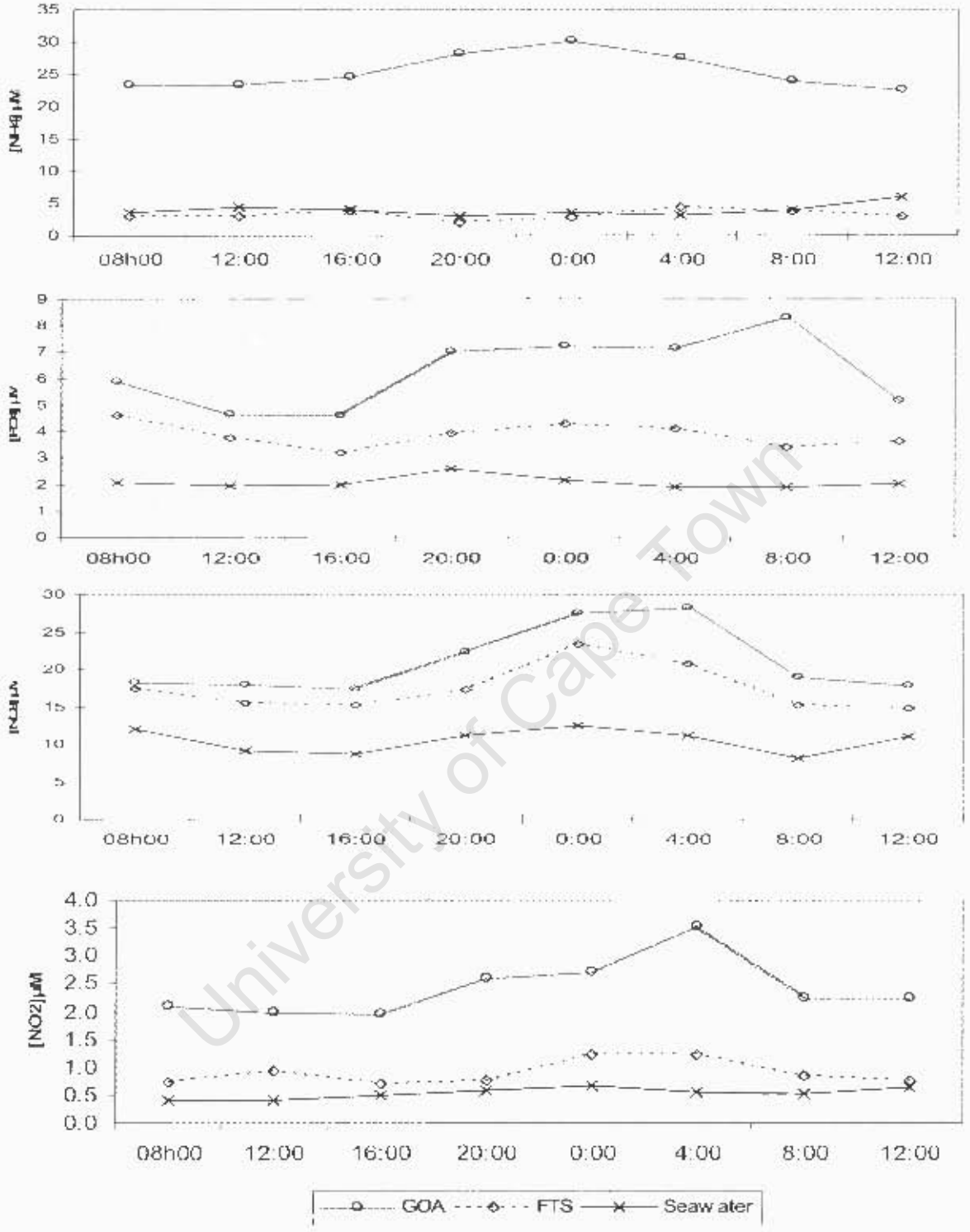

FIGURE 4.6: TAN, phosphate, nitrate and nitrite concentrations (μM/L) over a 28 hour period in September to illustrate diurnal variations in concentration (n = 32 for each). Standard deviations are not shown in the graphs for increased clarity.

FAN

Average FAN concentrations were 0.81, 0.11 and 0.06 μM L^{-1} for the GOA system, FTS and incoming seawater, respectively. Maximal concentrations recorded in the GOA system, FTS and incoming seawater were 1.63; 0.23 and 0.08 μM L^{-1}, respectively. The GOA system had greater fractions of FAN compared to both the incoming seawater and the FTS.

Abalone health

Sabellids - (See Figure 4.7)

In April 2004, the GOA system had considerably lower sabellid scores than the FTS, although the scores had increased since the start of the experiment. In September 2004, animals sampled from the GOA system had fewer sabellids than in the FTS. In addition the GOA system animals showed better shell growth than controls. No shell boring polychaetes were found. In October 2004, the animals in GOA system showed better shell growth and lower sabellid scores than those in FTS. Sabellid counts in the GOA system increased in June, possibly as a result of the increased presence of sabellid larvae in the water. The FTS animals had a log increasing sabellid ranking y = 352.2Ln(x) - 3713.3 with r = 0.9384; $p < 0.05$.

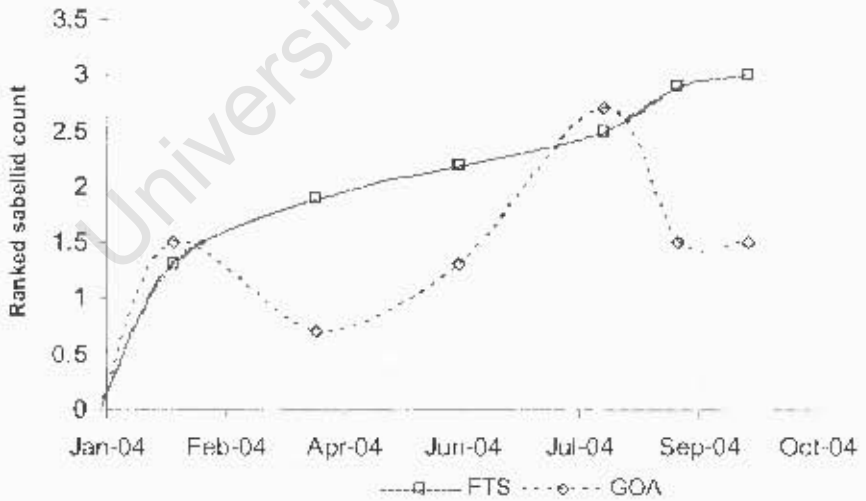

FIGURE 4.7: Ranked sabellid count in the GOA and FTS systems, see Table 2 for interpretation of ranking (n = 70).

General condition - (See Figure 4.8)

General condition decreased in the FTS in June and is linked to the presence of environmental stress at this time. There was some deterioration in water quality in both systems in June which resulted in a decrease in the health of the abalone in both systems. The general condition of the GOA system animals was poorer than that of the FTS animals for the duration of the experiment.

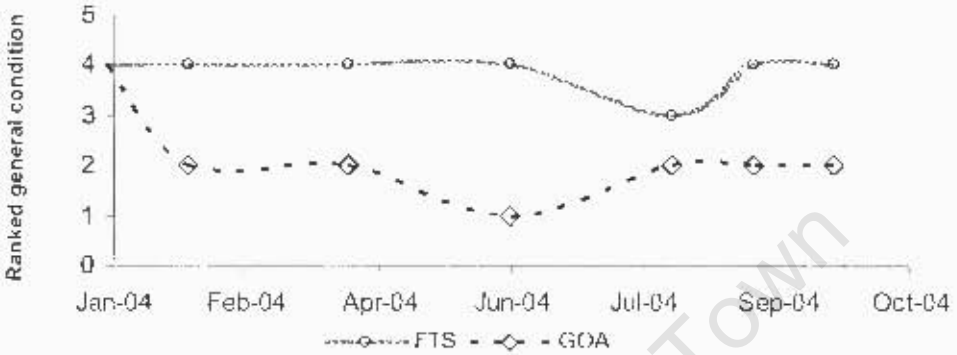

FIGURE 4.8: Ranked general condition in the GOA and FTS systems , see Table 2 for interpretation of ranking (n = 70 for both).

Environmental stress – (See Figure 4.9).

In February 2004, when comparing the GOA system animals to FTS ones, there was evidence of better water quality in the GOA group as a result of environmental stress being present in the FTS. No environmental stress was seen in either group in April 2004. In June 2004 both groups showed signs of environmental stress related to poor water quality. Environmental stress was then present in the GOA system for the remainder of the experiment.

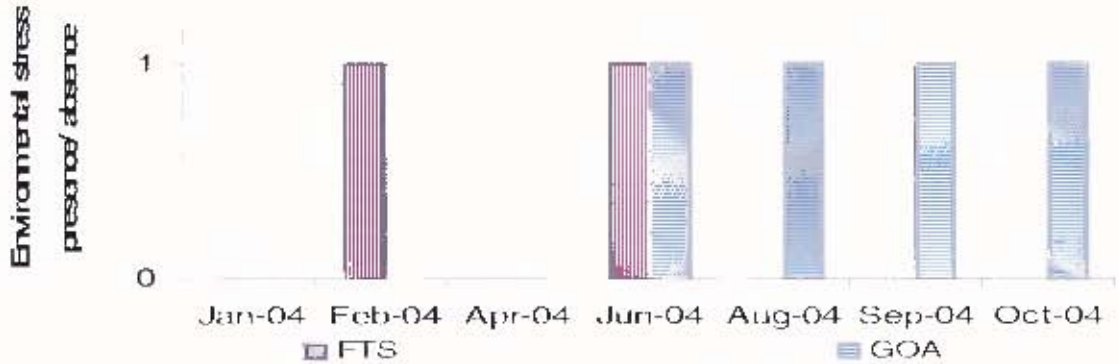

FIGURE 4.9: Environmental stress presence (1) /absence (0) scoring in the GOA and FTS systems see Table 4.2 for interpretation (n = 70 for both).

General histology – (See Figure 4.10)

In June 2004, multifocal epithelial hyperplasia or thickening was seen in the animals from the GOA system. It did not appear to occur in animals in the FTS. Lesions were also present in the GOA system animals. Coupled with this there was a decrease in the general condition of the animals (See Figure 4.8). In August 2004, the GOA system animals continued to show gill pathology, but this was different from the previous month. Whereas the previous sample was a hyperplastic or hypertrophic change, the animals in this sample showed congestion and inflammation. Lesions were again present in the sample. In September 2004, in the GOA system animals, gill lesions were absent, but muscle inflammation was seen in some individuals. These changes were not present in any FTS animals.

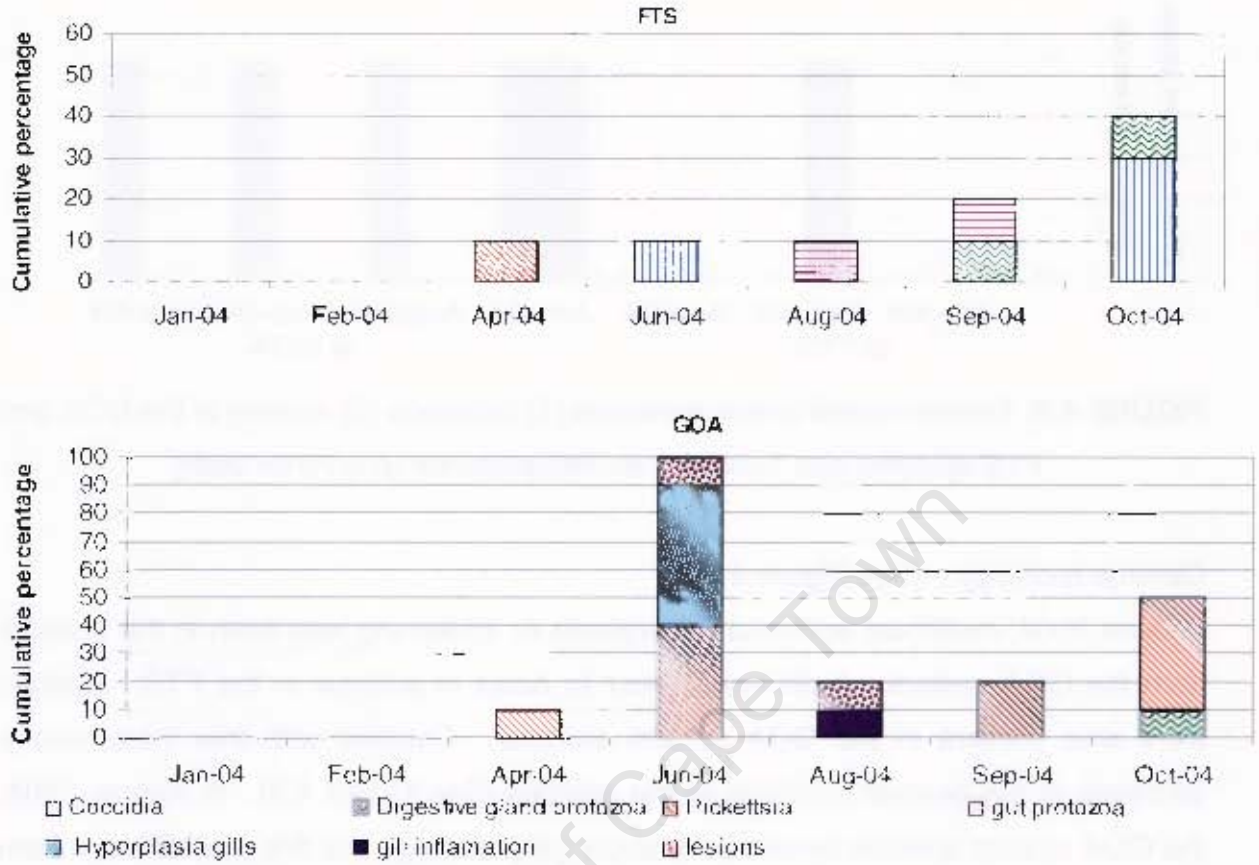

FIGURE 4.10: General parasitology of FTS and GOA animals as a percentage of the total sample affected/ infected, see Table 4.2 for interpretation (n = 70 for both).

Abalone SGR

Grading data

Farm supplied data from the grading were used to compare growth weight increases between sizes for the tri-monthly sub samples. There was no significant difference between weights in any of the size classes, or between the different cohorts or in the different systems for graded weights and sub-sample weights.

To obtain an idea of the performance of the systems on a biomass basis, graded data were used to compare weight increases in each size class. There was no significant difference in weight increase in any of the size classes in the 11/00 cohort (See Figure 4.11). In the 10/00 cohort in the green size class the GOA system had a much higher biomass increase in the November grading compared to the FTS (See Figure 4.12).

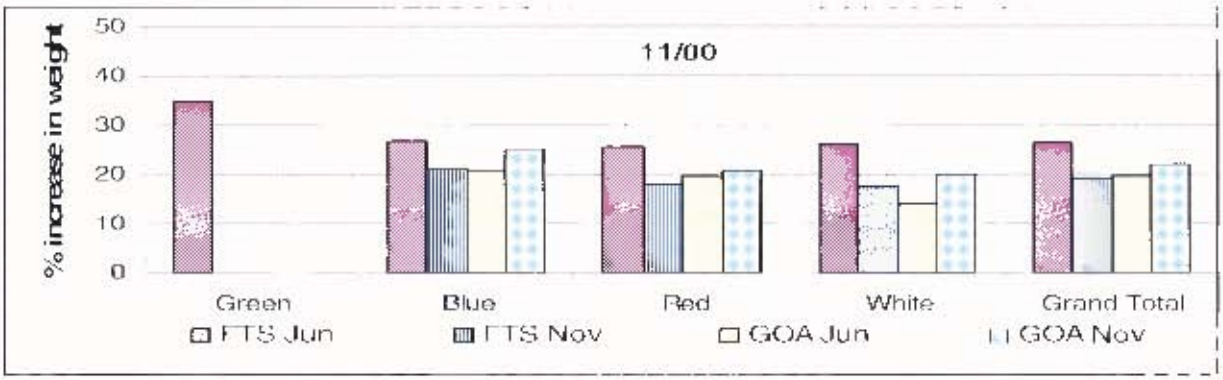

Figure 4.11: % increase in weight (kg) in each of the different size classes for the 11/00 cohort in the FTS and the GOA system after grading, using grading data.

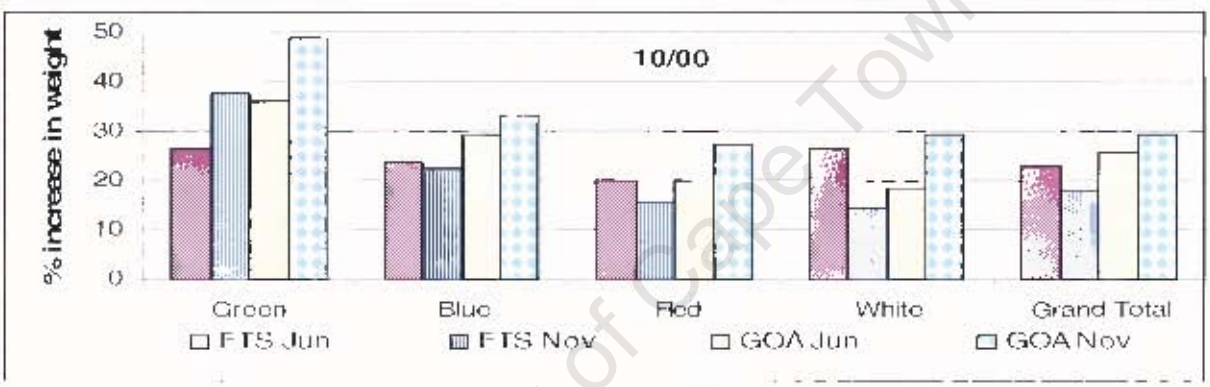

Figure 4.12: % increase in weight (kg) in each of the different size classes for the 10/00 cohort in the FTS and the GOA system after grading, using grading data.

Table 4.4 illustrates the differences in growth in grams between the two grading periods. The GOA system had higher weight increases in the second period between grading while the FTS had higher increases in the first grading period. There were no significant differences between any of these values.

TABLE 4.4: Grading data showing growth per month in grams with standard deviation in brackets for the two grading periods June and November 2004. Growth rates are calculated from farm data.

11/00	Size classes	June	November
FTS	Green	2.10 (0.3)	
	Blue	1.82 (0.6)	1.60 (1.1)
	Red	2.93 (1.2)	1.40 (1.1)
	White	3.40 (1.7)	1.86 (1.5)
	Pink		1.33 (0.3)
GOA	Blue	0.98 (0.6)	2.43 (0.4)
	Red	2.02 (1.2)	2.53 (0.8)
	White	2.46 (1.0)	4.53 (0.9)
	Pink		4.70 (0)
10/00	Green	0.83 (0.4)	0.74 (0.3)
FTS	Blue	1.66 (0.8)	0.68 (0.5)
	Red	1.49 (0.9)	1.44 (1.1)
	White	3.04 (2.8)	1.54 (0.7)
	Pink	3.8 (0)	2.87 (1.2)
GOA	Green	0.32 (0.6)	0.57 (0.7)
	Blue	2.30 (1.1)	1.29 (0.9)
	Red	1.61 (1.1)	1.94 (0.8)
	White	3.69 (0.9)	3.95 (2.8)
	Pink		2.1 (0)

Condition factor

There was no significant difference in condition factor between the groups of animals in the two systems. Both animals, as indicated by the condition factors (See Table 4.5) were short and fat and were putting on more muscle mass than shell growth. Both grading periods are shown due to the difference in growth rates in grams shown in Table 4.5. This indicates that in January to June there is greater meat production while in June to November there is more shell production.

TABLE 4.5: Sub-sample data showing changes in condition factors for the two grading periods June and November 2004.

11/00	Size classes	June	November
FTS	Green	1.34	1.28
	Blue	1.24	1.22
	Red	1.21	1.22
	White	1.23	1.2
GOA	Green	1.12	nd
	Blue	1.08	1.40
	Red	1.08	1.24
	White	1.06	1.07
10/00	Green	1.25	nd
FTS	Blue	1.20	1.26
	Red	1.21	1.25
	White	1.13	1.10
GOA	Green	1.13	nd
	Blue	1.18	1.08
	Red	1.14	1.06
	White	1.14	nd

Sub sample growth data

11/00 Cohort

No significant differences were found in weight increase in any size classes between the FTS and the GOA system. Figure 4.13 illustrates the increase in weight in each of the size classes over the measured period. Figure 4.14 illustrates the increase in shell over the same period. Linear regression analysis on trend lines showed that there was also no significant difference shell length increase rates by the individual size classes.

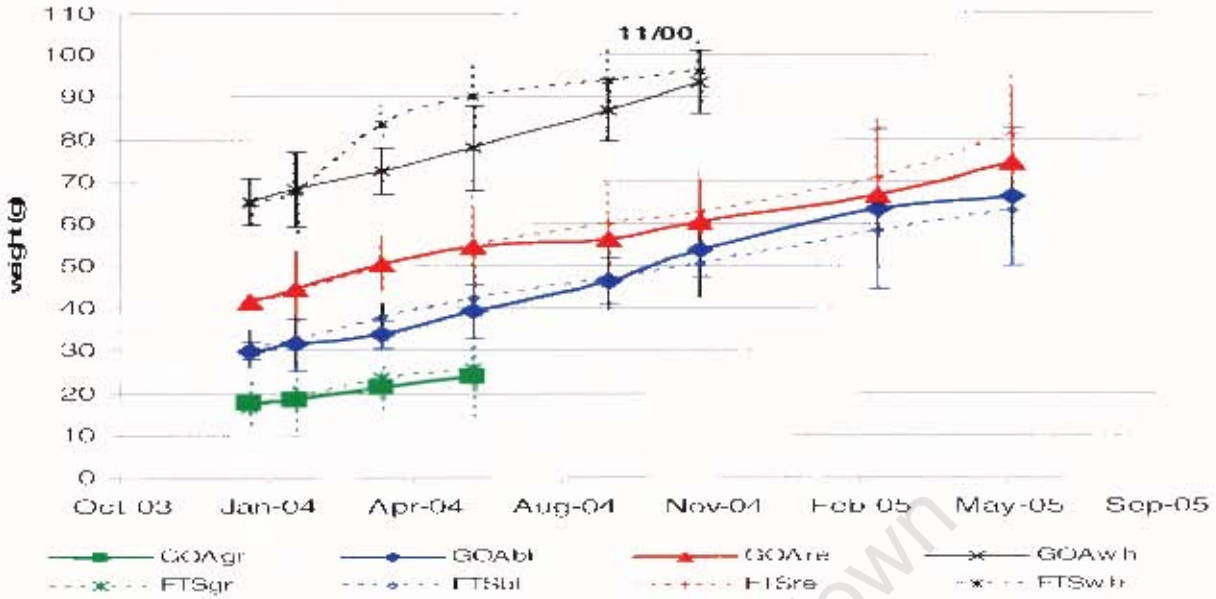

Figure 4.13: Mean increase in weight (g) in the 11/00 cohort in each of the different size classes for the entire measurement period. Data were obtained by tracking sub sampled baskets for the duration of the experiment. Standard deviations are shown (N = 16 480).

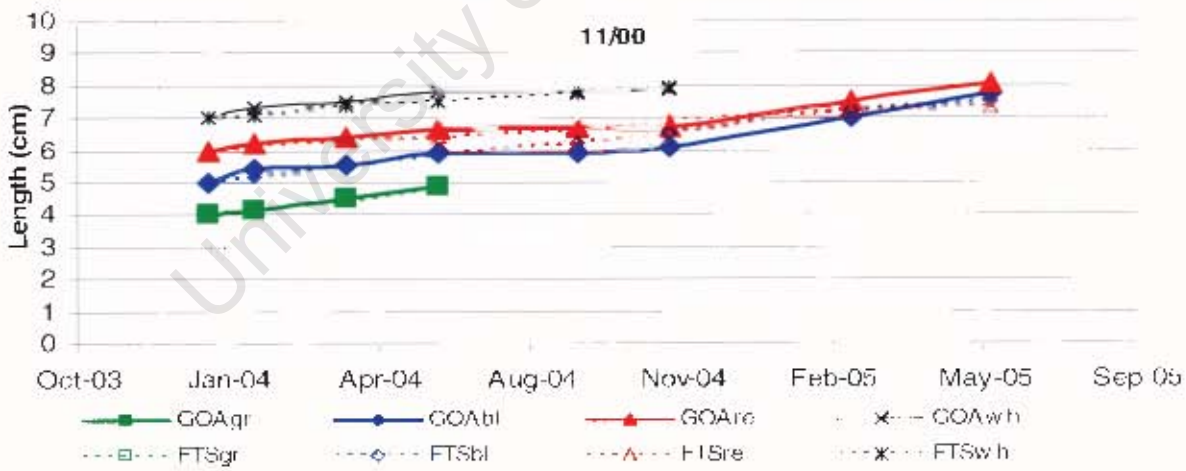

Figure 4.14: Mean increase in shell length (cm) in the 11/00 cohort in each of the different size classes for the entire measurement period. Data were obtained by tracking sub sampled baskets for the duration of the experiment. Standard deviations are not shown to increase clarity but were all less than 1 (N = 16 480).

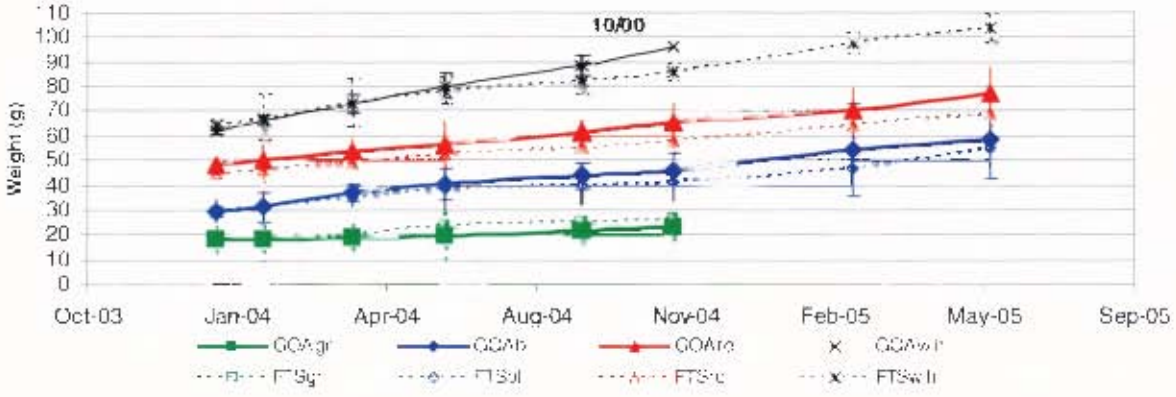

Figure 4.15: Mean increase in weight (g) in the 10/00 cohort in each of the different size classes for the entire measurement period. Data were obtained by tracking sub sampled baskets for the duration of the experiment. Standard deviations are not shown to increase clarity but were all less than 1 (n = 16 480).

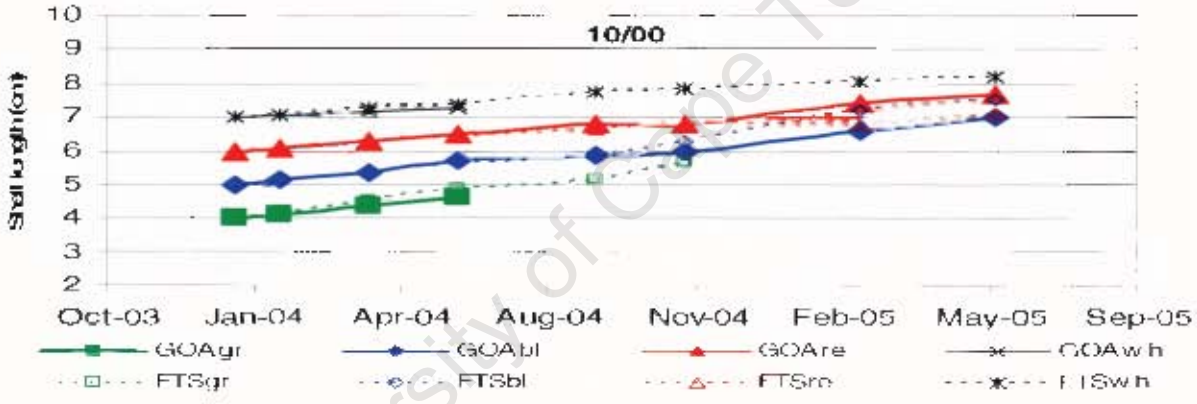

Figure 4.16: Mean increase in shell length (cm) in the 10/00 cohort in each of the different size classes for the entire measurement period. Data were obtained by tracking sub sampled baskets for the duration of the experiment. Standard deviations are not shown to increase clarity but were all less than 1 (n = 16 480).

10/00 Cohort

In the 10/00 cohort there were also no significant differences found in weight increase in any size classes between the FTS and the GOA systems. Figure 4.15 illustrates the increase in weight in each of the size classes over the measured period. Figure 4.16 illustrates the increase in shell over the same period. Regression analysis on trend lines showed that there was also no significant difference in shell length increase rates by the individual size class.

Table 4.6 shows the exponential regression equations with the R value, all of the equations were highly significant (d.f. = 164800; $p < 0.001$) indicating good agreement with the data. The regression equations were extended forward to see how long the abalone would take to reach 100 g, the weight at which they would be sold for export and how many days this would be after the start of the experiment. In the 10/00 cohort it appears that the GOA system increases the time that a small abalone would take to reach 100 grams by 23 – 68 % compared to the FTS, while the time taken for the larger size classes of abalone, would decrease by 27 – 30 %. However, the 11/00 cohort does not follow this trend and appears to increase the time taken by 16 – 37 %.

TABLE 4.6: Regression equations of abalone weight in the 10/00 and 11/00 cohorts with R value. The last column is a forward projection of the regression equations to calculate how long (in days) the abalone in that size class would take to reach 100 g.

Cohort	Exponential regression	R value	Time (d) to reach 100 g
10/00			
GOA green	$y = 9E\text{-}13e^{0.0028x}$	0.9814	2150
FTS green	$y = 2E\text{-}21e^{0.0043x}$	0.9818	1275
GOA blue	$y = 1E\text{-}20e^{0.0034x}$	0.9839	1110
FTS blue	$y = 9E\text{-}17e^{0.0021x}$	0.9848	901
GOA red	$y = 1E\text{-}13e^{0.0029x}$	0.9974	803
FTS red	$y = 1E\text{-}12e^{0.0026x}$	0.9975	965
GOA white	$y = 3E\text{-}22e^{0.0044x}$	0.9947	325
FTS white	$y = 1E\text{-}13e^{0.0029x}$	0.9924	458
11/00			
GOA green	$y = 9E\text{-}34e^{0.0021x}$	0.9963	838
FTS green	$y = 3E\text{-}47e^{0.0029x}$	0.9813	611
GOA blue	$y = 1E\text{-}26e^{0.0017x}$	0.9900	719
FTS blue	$y = 7E\text{-}22e^{0.0014x}$	0.9861	819
GOA red	$y = 4E\text{-}16e^{0.0012x}$	0.9818	796
FTS red	$y = 2E\text{-}19e^{0.0012x}$	0.9887	682
GOA white	$y = 2E\text{-}18e^{0.0013x}$	0.9997	363
FTS white	$y = 2E\text{-}20e^{0.0013x}$	0.9209	295

DISCUSSION

This study has analysed some of the factors that could be used to explain differences in between the FTS and the GOA system and have been summarised in Table 4.7, and have been described as positive, negative or neutral, when compared to the FTS. A negative value would indicate higher concentrations or values that would be detrimental to abalone health.

TABLE 4.7: Summary of effects of different variables tested between the FTS and the GOA system. A 0 symbol indicates no significant difference, A + symbol indicates a significant positive benefit for aquaculture, and a – symbol indicates a significant negative drawback for aquaculture.

		GOA	FTS
Abalone	Shell length	0	0
	Weight increase	0	0
	Time to harvest	0	0
	Condition Factor	0	0
Abalone health	Sabellid	+	
	General condition	-	
	Environmental stress	-	
Abalone histology	Gill inflammation	-	
	Coccidia		-
	Rickettsia	-	
	Gut protozoa		-
	Digestive gland protozoa		-
	Hyperplasia in gills	-	
	Lesions	-	
Water quality	TAN	-	
	FAN	-	
	PO_4^+	-	
	NO_2^-	0	0
	NO_3^-	0	0
Temperature	Average	+	
	Range	+	
pH	Average	0	0
	Range	-	
Dissolved oxygen	Average	-	
	Range	-	

Temperature monitoring of the two systems clearly demonstrates that the GOA system provides more stable and higher temperatures and therefore should provide a less stressful environment for the abalone on the west and South west coasts, however, this would not be the case on the south east coasts (e.g. Haga Haga). Air temperature had an influence on the temperatures in the GOA clusters, however, as the building surrounding the clusters was only partially complete during the time of intensive sampling it is thought that this would have less of an influence in the future. Spikes in pH in the GOA systems were directly attributed to incomplete dissolving of the dosing pills and shows the need for more intense management of these systems or a properly defined management protocol for farm workers.

This study showed that external events such as algal blooms and subsequent die-offs can place stress on the GOA system. In one case in winter when an algal bloom die off was experienced in the solar dams which feed the incoming water into the systems, the addition of kelp into the clusters significantly lowered the dissolved oxygen values between clusters. This indicates that dissolved oxygen should be monitored closely during periods of high biological oxygen demand.

Water quality experiments showed that TAN concentrations were significantly higher in the GOA system. Had these data been analysed without prior knowledge of abalone feeding and the effects of diets on water quality (see Chapter 5), these values may have indicated a possible problem with high ammonium concentrations in the GOA system. In the FTS being fed Abfeed® TAN levels decreased after feed had been in the trays for 18 hours (unpublished data). Over periods when there was no feeding, TAN values were the same as in a kelp-fed FTS. Reddy-Lopeta *et al.* (2006) demonstrated that abalone can be kept at high levels of ammonia if they have been acclimatized beforehand, although SGR can be reduced by up to 50 %. Growth rate comparisons between the two systems do not show increases as a result of the increased and stable temperatures in the GOA system. The growth rates in the GOA system may be decreased as a result of the high TAN levels. This assumption is assisted by the fact that all GOA animals experienced environmental stress, which was as a result of poorer water quality. Both nitrate and nitrite levels in

this study were substantially lower than the safe levels recorded for *H. tuberculata* (Basuyaux & Mathieu, 1999) and the chronic sublethal level (nitrite = 7.8 mg L^{-1}) recorded for the greenlip abalone, *H. laevigata* (Harris *et al.* 1997). Based on the assumptions made by Reddy-Lopata *et al.* (2006) the measured nitrite and nitrate levels probably had no influence on the stress of the abalone in this study.

In both systems the FAN concentrations never reached levels close to those reported by Reddy-Lopata *et. al.* (2006). In their study a FAN level of 8.7 $\mu g\ L^{-1}$ was lethal to juvenile abalone (1 – 2 cm shell length). Juvenile abalone are more sensitive to ammonia than the animals in this study and both systems contained FAN levels well below the sub-lethal level defined in their study (7.4 $\mu g\ L^{-1}$ FAN) for juveniles and animals of a similar size to this study had levels of 11.4 $\mu g\ L^{-1}$ FAN.

There were no significant differences found in weight increase or shell length increase between the two systems. The most interesting change is that higher growth rates occurred in the FTS during the first grading period, while in the GOA system these occurred during the second grading period. Water temperatures in the FTS are higher during the first grading period which was also found by Robertson-Andersson (2003; This Chapter) and it is well documented that higher temperatures are better for abalone growth rates (Bok, 2002). The reason for the change over of growth rates in the second grading period is possibly due to the more stable temperatures in the GOA system during summer when upwelling would have resulted in large temperature fluctuations in the FTS (see Chapter 6, for discussion of upwelling). During the same period the FTS temperatures were highly variable (Robertson-Andersson, 2003).

One drawback of this study, when comparing production of these two systems, was that it did not have access to data that illustrated how many animals of each system were removed for export. This is one of the disadvantages of doing applied research within a commercial system. Research often has to be made within farm secrecy policies and this made comparisons of total biomass between the systems

impossible and this may have an outcome on determining which of these two systems is more productive.

The condition factor (the relationship between the weight of the abalone per unit shell length) accounts for the amount of feed invested in developing both the body weight and shell length (Britz, 1996). This factor is important to farmers as it is used during the grading process for market purposes. Abalone that have a high condition factor tend to be fat and possess relatively short shells, reflecting that more nutrients were invested into increased body weight than into shell growth. The fact that there is no significant difference in condition factor between the two grading periods even though there were changes in SGR and weight, indicates that in January to June there is greater meat production while in June to November there is more shell production. Robertson-Andersson's (2003) data suggested that there was more phosphates in kelps from June to November. Phosphate is necessary for shell growth and is only obtainable through the diet. Britz (1996) showed that animals fed kelp tend to put on more shell mass, compared to other diets. The condition factor of all animals is close to one and thus both systems are producing short fat animals, which is desirable for export.

Abalone health in general showed that there was greater environmental stress in the GOA system with animals having a poorer general condition compared to those in the FTS system (See Figures 4.9 and 4.10). Multifocal epithelial hyperplasia or thickening is not common and has only rarely been found in animals from recirculation systems (A. Mouton, pers comm.). It does not appear to occur in animals cultivated in flow through systems. The cause is most probably related to water quality, possibly suspended solids. This is coupled with the animals showing environmental stress (See Figure 4.9). As a result these animals may be more susceptible to bacterial gill disease than animals cultivated in the FTS. Lesions present in the GOA animals, may be associated with leaching compounds from plastic tanks (A. Mouton, pers. comm.). Overall, parasitology was more diverse in the FTS with a lower overall infection (See Figure 4.10). The higher parasite diversity

are often associated with faster growing animals (N. Loubser, pers. comm.) with animals having large infection densities of a single parasite growing more slowly.

Reports commissioned by AFASA (Britz *et al.* 2005) showed that the generation interval of sabellids is between 3 – 5 months. This seems to be illustrated in Figure 4.8, with increases in sabellid counts occurring in tri monthly intervals. Fitting trend lines to the GOA sabellid data did not produce a good fit, and the trend line seems to indicate that there is a carrying capacity being reached, with the abalone in the GOA system being able to decrease the number of tunnels on the growing edge of the shell. This is demonstrated by the lack of growth curve fitting. The graph for the GOA animals followed a population ecology curve for carrying capacity (Manning & Dawkins, 1992), with the carrying capacity being 1.75, meaning 10 or more sabellids per shell. Increases above this figure coincide with the generation time of the sabellid. The FTS animals had at the end of the health trial, a sabellid score of 3 with tunnels being superimposed of the growth edge of the shell.

Coccidia-infected kidney epithelial cells become extremely hypertrophied and heavy infections appear to cause serious kidney damage. However, *H. midae* elicits no haemocytic response suggesting that it is not recognized as an invader by the abalone host (Friedman *et al.* 1995). The life cycle appears to be homoxenous (complete life cycle in one host; infection developed following exposure to water occupied previously by infected abalone). The parasite was implicated in black abalone mass mortalities (withering syndrome) in the late 1980s and early 1990s but is no longer considered to be the cause of this disease and is now thought to be benign in nature (Friedman, 1991; Steinbeck *et al.* 1992; Van Blaricom *et al.* 1993; Friedman *et al.* 1993, 1997, 1999; Kuris *et al.* 1994). Bower (2004), showed that despite heavy natural and experimental infections that have been observed in the field and as a result of laboratory experiments, no change in the condition of the abalone nor mortalities were observed. Renal *coccidia* have also been reported from *Haliotis midae* cultured in South Africa (Mouton, 2000a,b, Sales & Britz, 2000), also with no associated mortalities. The presence of *coccidia* in the FTS animals only, does not warrant much cause for concern. These results are different to those found

in Chapters 5 & 6, where all abalone fed a seaweed diet had *coccidia*. The absence of *coccidia* in the GOA system could warrant further study.

Rickettsia is a genus of intracellular prokaryote, with morphological characteristics of the class Proteobacteria, order Rickettsiales and family Rickettsiaceae. They are found in the epithelium of the intestinal tract (Gardner *et al.* 1995, Friedman *et al.* 2000a to d). *Rickettsia*-like organisms have also been reported in the digestive tract of abalone (*Haliotis midae*) from culture facilities in South Africa with no associated pathology (Mouton 2000a,b). *Rickettsia* can cause a lethal disease that affects all sizes of abalone and causes lethargy, retracted visceral tissues, atrophy of the foot muscle (thereby adversely affecting the ability of the abalone to adhere to the substrate) and is lethal. Elevated temperatures accelerated disease progression and decreased survival. At 18 to 20 °C, death usually occurs within one month of the appearance of the clinical signs. This disease is associated with mass mortalities of *H. cracherodii* (Gardner *et al.* 1995, Friedman *et al.* 2000a to d). At present, as there has been no associated pathology in *H. midae,* the prevalence of high *Rickettsia* counts in the GOA system animals is not cause for concern (Mouton, 2006). Should disease pathology develop, then animals in the GOA system would be highly susceptible.

CONCLUSIONS

The GOA system is the more stable system in terms of temperature and this has benefits for the abalone in that they are more easily able to cope with an increase in sabellid infestation. Although there were no signs of this in increased growth rates of the animals this could have been compounded by the high ammonium values experienced in the GOA system and resulting environmental stress and disease pathology experienced by this group of animals.

The study showed the need for more intensive management in the GOA system compared to the FTS. pH spikes in the system, caused by incomplete dissolving of the dosing pills is one of the main areas that should be monitored. On the plus side, analysis of the system as a whole, shows that fewer workers are required in such a system (0.6 workers per ton of abalone exported) than in a flow through system (1.1 – 1.62 workers per ton of abalone exported) (data from Chapter 2) and that running costs are lower (pumping and electricity). This needs to be contrasted with the higher initial capitol investment and the need for buildings to enclose the tanks, a study beyond the scope of this chapter.

Although water quality parameters show significant differences in concentrations, particularly of TAN in the GOA system, when these data are compared to existing data of water quality with different feeds (see Chapter 5) this difference cannot be used as a negative factor against the performance of the system. The difference between the FTS and the GOA system with regards to high TAN levels, is that in the GOA system TAN levels remained high during the measurement periods, while in a separate experiment in the FTS using a different feed they decreased with increasing time after feeding. This may explain the poor general condition of the GOA animals and the fact that they were experiencing environmental stress. Also long term exposure to the higher TAN concentrations could explain the fact that there was no real significant difference in growth rate performance between the FTS and GOA systems, as the high levels may have resulted in a decrease in growth rates.

The prevalence of high numbers of *Rickettsia*-like organisms in the GOA system is a cause for concern, even though no disease pathology has thus far been detected with these organisms.

The method of comparing weights between systems using data generated from grading is a sufficiently accurate way to record changes in growth rates. However, data that was not recorded in this method are length (mm) of the animals and thus it would be difficult to obtain a condition factor for this data. This can be solved by taking a sub sample of the graded animals and measuring both length and weight. If further studies are done in comparing the two systems it would be important to include all the animals removed from both systems for harvesting.

All the data in this report suggest that there is no significant difference in the physical performance of these two systems. But economic performance has not been evaluated in this study. The decreased susceptibility of the abalone to sabellid infections make the GOA system a more desirable system, due to improved shell condition and consumer acceptability (see Chapter 3B). On the west and south-west coasts, which are under the influence of upwelling in summer (See Chapter 6 for discussion of upwelling), the use of the GOA system could negate the need for solar dams to increase the incoming seawater temperatures and would result in a much lower temperature range for farms situated in these areas. If this system were to be used up the south-east coast, the water and air temperatures would be too high to allow such a system to operate to its full potential.

CHAPTER 5

A COMPARISON BETWEEN DIFFERENT DIETS, ON THE CULTURE CONDITIONS, GROWTH RATES AND HEALTH OF CULTIVATED ABALONE (*HALIOTIS MIDAE*)

INTRODUCTION

The growing abalone industry depends on a steady supply of feed resources. Today kelp makes up approximately 60 % of the utilised feed (by weight), the rest being mainly formulated pelletted feeds based largely on animal protein (e.g. fishmeal) (Chapter 2). As for most other aquaculture species diet is very important and it has been shown that different diets produce significantly different growth rates (Leighton, 1974; Barkai & Griffiths 1986, 1987, 1988; Britz, 1996; Guzmán & Viana, 1998; Shpigel *et al.* 1999; Boarder & Shpigel, 2001; Bautista-Teruel *et al.* 2003; Naidoo *et al.* 2006). Wild abalone have a preference for specific seaweeds and red algae are favoured by a number of different abalone species (Tutschulte & Connell, 1988; Shepherd & Steinberg, 1992; Stepto & Cook, 1993; Fleming, 1995). Abalone that feed on seaweed have high Food Conversion Ratio (FCR) values due to the high water content and relatively low protein content of macroalgae (Hahn, 1989). However, even if the success of a certain type of feed is mainly determined by growth performance, accessibility to the feed, price, and how the feed effects the culture environment (i.e. nutrient and oxygen concentrations, pest species density and parasites), are also important to the industry.

From a compound feed perspective, a good compound feed should have a good nutrient composition (Middlen & Redding, 1998, Nelson *et al.* 2002, Bautista-Teruel *et al.* 2003, Viera *et al.* 2005), be digestible (Sales & Britz, 2001, 2002, Gomez-Montes *et al.* 2003), manufactured with set processing techniques to ensure constant quality control (Booth *et al.* 2002, Sales & Britz 2002), have an optimum particle size for the animals mouth size and feeding method (Southgate & Partridge, 1998), be an optimum pellet size both for handling by the farmer and for feeding by the animal (Fleming *et al.* 1996), be attractive to the animal (Fleming *et al.* 1996, Sales & Janssens, 2004), and be palatable to the animal (Kautsky *et al.* 2001). A good diet that provides optimal growth should meet the nutritional requirements of the animals including the necessary carbohydrate (Nelson *et al.* 2002) and protein and amino and fatty acid ratios (Fleming *et al.* 1996, Guzman & Viana, 1998).
Advantages of compound diets are that their quality remains constant; they can be developed for specific life stages; they are easy to handle and are readily obtainable and they have a low FCR.

Development of compound diets is an active area of research in South Africa (see review by Sales & Britz, 2001). Although most of the South African farms are situated on the south-west coast where there is an abundant supply of kelp, compound diets are becoming more popular. Formulated aquaculture feeds provide a secure and guaranteed nutritional value (Britz, 1996; Britz & Hecht, 1997; Cook, 1998; Sales & Britz, 2001). This has the disadvantage to the farmer of being dependent on external fish supply, although a global commodity. It is unfortunate that this industry adds to a wasteful transformation of fish resources (Naylor *et al.* 1998). Compared to seaweed, formulated feeds offer convenience and cost benefits to farm management (Britz *et al.* 1994) if the cultivation system is designed for formulated feeds. The requirements of an abalone feed are that the water soluble nutrients remain in the feed and that the food particles remain bound together for at least 2 days (Flemming *et al.* 1996). A starch-bound dry pellet was developed in the early 1990's (Britz *et al.* 1994) and leaching from this pellet was 5% over 24hrs (Britz & Clayden, 1996). This was more cost effective than previous feeds, and it was shown that formulated diets produced a significantly better increase in shell length and weight in juvenile *H. midae* (5 - 8.54 mm shell length) than gel feeds (Knauer *et al.* 1995a,b).

A large amount of research has shown that natural seaweeds obtained from the wild do not deliver the same growth performance as compound diets (see review in Flemming *et al.*1996 and Sales and Britz, 2001). However, feeding a mixed algal diet fed on a rotation basis to abalone also improves growth rates above that fed a kelp only diet (Simpson & Cook, 1998; Nelson *et al.* 2002; Schneider *et al.* 2005; Naidoo *et al.* 2006). The abalone industry in South Africa was initially started on a kelp only diet as it was easy to access, low priced, was part of the abalone's natural diet and there were sufficient quantities readily available. However, seaweed diets are not constant throughout the year (Chapter 6). Their quality is variable and they deteriorate faster at high temperatures. Their FCR can be 3 times that of an compound diet. Supply (at present) is dependent on the weather. In addition, a kelp diet requires more labour for feeding than a compound diet. They do, however, impart a more natural taste and color to the abalone (Chapter 2). Any diet that is to be used on a farm must therefore outperform a kelp diet, not only in growth but in cost and quality aspects as well.

Today many farms themselves invest in research and development to find suitable combinations (ratios) of feeds and even new formulations. The following are but a few local feed alternatives that exist: kelp (*Ecklonia maxima* and *Laminaria pallida*), red seaweeds (e.g. *Gracilaria* spp., *Gelidium* spp.; *Porphyra* spp..), green seaweeds (e.g. *Ulva* spp.) and formulated feeds such as Abfeed® (fishmeal as the primary protein source) and Midae Meal™ (an all seaweed based formulated feed) (from questionnaires, see Chapter 2). Due to logistic and cost implications reasons no kelp, either fresh or dry is imported. Many brands of international formulated feed (e.g. Adam & Amos Abalone Foods and Eyre Peninsula Aquafeeds) are being tested locally. Although abalone prefer fresh kelp (Simpson, 1994), dried kelp pellets are also being tested as a feed source. Only two companies presently supply dried kelp locally - Taurus Products (Pty) Ltd, and Rivonia and Kelp Products (Pty) Ltd, Simon's Town.

Compound feeds

Abfeed® (Marifeed Pty Ltd, South Africa) is a formulated feed containing fishmeal (55 %), starch, *Spirulina* spp. (10 %), vitamins and minerals (Fleming *et al.* 1996). The approximate analysis of Abfeed® is 34.6 % protein, 43.3 % carbohydrates, 5.3 % fat, 1.2 % Crude fibre, 5.7 % ash and $\pm$ 10 % moisture (Marifeed Pty Ltd, pers. comm.). A cheaper, low protein (26 %) form of Abfeed® is currently also in production. Presently Abfeed® is only sold locally, although the product is being tested abroad (e.g. Australia, Chile, Taiwan and New Zealand) against a host of other formulated feeds (Marifeed Pty Ltd, pers. comm.).

The only commercially available all-seaweed based feed in SA is a formulated dried feed called "Midae Meal MM-1c" (Eric-Piet (Pty) Ltd, Luderitzbucht, Namibia) being manufactured for Taurus Products (Pty) Ltd. The ingredients are mainly *Laminaria pallida* and *Ecklonia maxima* (stipes and fronds) but it also contains *Gracilaria* spp., *Gelidium* spp. (including the epiphyte *G. vittatum*), and *Porphyra capensis.* The wet seaweed: dry pellets ratio is 6 - 7:1 and protein content is around 18 % which gives the abalone meat a natural taste, colour and smell. Sabellid infections have apparently not been observed with this feed and the shell color is apparently also positively affected (Taurus Products pers. comm.). Although widely exported to the Far East, this feed has, however, had limited success in a series of growth trials,

producing poor growth rates in abalone when compared against other formulated feeds (Matschke, pers. comm.; Dlaza, 2006). The feed is, however, being further developed (Taurus Products, pers. comm.)

Other seaweed species

Other seaweed species used by the industry as feed include *Gracilaria* sp., *Gelidium* sp., *Plocamium corallorhiza* (Turner) Harvey, *Zonaria subarticulata* (Lamouroux) Papenfuss, *Ulva* spp. and *Porphyra* spp. (Chapter 2). These seaweeds are used in very low quantities and are generally only fed to the brood stock. This is due to the fact that they occur either in very low quantities or their supply is very erratic (Griffin *et al.* 1999). One farm has a seaweed concession to collect drift seaweeds from the south west coast which they feed to their grow-out stock. Two abalone farms feed cultivated *Ulva* and *Gracilaria* as they have no access to kelp resources (Chapter 2).

Relatively recent investigations into marine eutrophication have shown that seaweeds are extremely useful in removing large proportions of dissolved inorganic matter (total ammonium nitrogen, free ammonia nitrogen, dissolved inorganic phosphorus etc.) from aquaculture effluent (e.g. Neori, 1996). A number of *Ulva* species, for example, are able to remove up to 90 % of dissolved nitrogen from aquaculture effluent (Neori *et al.* 1998) increasing their protein content as much as 10 times (Shpigel *et al.* 1999; Boarder & Shpigel, 2001; Robertson-Andersson, 2003; Robertson-Andersson *et al.* 2006; Chapter 6). Farm-grown, enriched *Ulva* has subsequently been shown to improve growth in, for example *H. tuberculata* (Neori *et al.* 1998; Shpigel *et al.* 1999), *H. discus hannai* (Shpigel *et al.* 1999), *H. roei* (Boarder & Shpigel, 2001), and more recently *H. midae* (Naidoo *et al.* 2006).

Growth performance

Research on performance of different diets has mainly dealt with single seaweed species diets in culture (mainly kelp), and more recently also performance of formulated feed (animal based and seaweed based) (Simpson & Cook, 1998). However, wild *H. midae* generally feed on a broad selection of algae, normally with at least two species being found in the gut at any one time (Barkai & Griffiths, 1986; 1987; Britz, 1991), although the macroalgal preference of species varies worldwide depending on habitat and macroalga availability (Dunstan *et al.* 1996; Nelson *et al.*

2002). This implies that abalone typically select more than just a single species and preferentially choose a mixture of algae. Naidoo *et al.* (2006) tested the effects on growth of various diets on *H. midae*, including mixed diets consisting of kelp with combinations of both green seaweed *Ulva* sp. and red seaweed *Gracilaria* sp. These diets were also compared with fish based Abfeed®. First, Naidoo *et al.* (2006) showed that dried kelp in any form (blades, stipes, and pellets) produced poor growth when compared against a host of fresh seaweed treatments, including fresh kelp. Secondly, they showed that fresh kelp, fortified with protein enriched farm-grown – *U. lactuca* (which has an average protein content of 33.4 % when grown in abalone waste, and 36.6 % when grown in turbot waste as opposed to 3.7-19.9 % in wild seaweed (Robertson-Andersson, 2003; Robertson-Andersson *et al.* 2006)) performed best. The Naidoo *et al.* (2006) growth trials support a host of other studies (Owen *et al.* 1984; Day & Fleming, 1992; Fleming, 1995, Simpson & Cook, 1998) that have shown that "mixed" diets produce better growth rates than single-species diets.

Nutrient release

Abalone are known to be inefficient feeders (Sales& Britz, 2001) with approximately 63 % of the energy from feed consumed being lost in faeces. Bredberg (2003) studied the differences in nutrient release from abalone excretion being fed on three different seaweed diets. Her data showed that concentrations of Total Ammonia Nitrogen (TAN) and Dissolved Inorganic Phosphorus (DIP) were significantly different in the different diets, and that excretion of TAN from the abalone correlated well with the nitrogen content of the seaweeds.

There have been numerous studies showing that different species of algae contain different amounts of nitrogen and phosphorus and that cultivation of these seaweeds especially integrated cultivation, can increase nutrient amounts in the seaweeds (Fourie, 1994; Simpson 1994; Friedlander and Levy, 1995; Smit, 1997; Hampson, 1998; Wilson, 1999; Steyn, 2000; Robertson-Andersson 2003; Njobeni, 2006; Robertson-Andersson *et al.* 2006). Positive correlations between dietary protein content and ammonium excretion have also been demonstrated in many species of fish (Hardy & Gatlin, 2002). For example Ballestrazzi *et al.* (1994) found that ammonia excretion of European sea bass *Dicentrarchus labrax* Lin. increased linearly with the protein level of the diet. Médale *et al.* (1995) also found that higher

digestible protein relative to digestible energy resulted in increased ammonia excretion in rainbow trout *Oncorhynchus mykiss* Wlabaum. These findings are of importance in re-circulation systems where increased concentrations of nutrients being released can have a negative impact on the water quality of the culture systems and a corresponding negative impact on the cultivated organisms.

Parasitic sabellid worm

The parasitic sabellid worm (*Terebrasabella heterouncinata*) is widespread in South Africa and infests a range of host gastropods (Cook & Ruck, 1998). The sabellid is associated with high stocking densities and poor water quality in abalone farms (Chalmers, 2002). High stocking densities and poor water quality due to insufficient flushing have also been identified as factors influencing infestation rates (Oaks & Fields, 1996). Farmers have noted higher infestation rates on Abfeed® fed abalone and it is suggested that the diet could be an important factor causing these high infestation levels (Chalmers, 2002). Research has shown that good farm design and animal husbandry can reduce infestations and keep the infestation levels low (Chalmers, 2002).

Farm design

Gansbaai is located along the south coast of South Africa approximately 140 km east of Cape Town. The farm I & J Cape Abalone Mariculture Pty, Ltd., is a land-based intensive mariculture operation situated at Danger Point and has been in operation since 1994. The farm is situated on 11 ha of land and cultivates primarily abalone (*H. midae*). The abalone stock consists of spat and approximately 7.2 million grow-out abalone (± 240 tons), which range from 1- to 6-year-old animals.

The farm consists of two platforms. The first platform has seaweed tanks for these experiments connected to it, while the second platform is currently being completed. In both platforms seawater is drawn from the sea into a pump house and then pumped directly into a header tank at a rate of 1 200 m^3 hr^{-1}. From there it is gravity fed to either the abalone tanks or the seaweed tanks. The water is filtered through a 100 μm screen filter before entering the tanks. The water turnover rate for the header tank is 5 - 6 volume exchanges per day (V E d^{-1}). Under normal farm conditions, effluent water from platform 1, is returned directly to the sea. When the

farm recirculates its water, effluent water is channelled to the re-circulation dam. Before going into the dam the water is drawn over a conveyor filter which removes about 85 % of the water-borne faeces. The recirculation dam holds approximately 2 500 m^3 of water, and here some of the particulates are settled out due to sediment traps (a series of low walls in the dam which slow water motion and allow for sedimentation of particles). From the dam, about 1 000 to 1 500 m^3 hr^{-1} is re-circulated around the farm before returning to the dam from where excess wastewater is returned to the sea.

Abalone farming is leading growth in the mariculture industry in South Africa. The industry production is still predicted to expand to 1200 tons by 2010. Several factors have led to this development and the demand for farmed abalone as a product is increasing. This development has stimulated research into abalone digestive physiology, the application of animal feed science principles to abalone, abalone feeding behaviour, and the optimization of the utilization of both natural and formulated diets under intensive culture conditions (see Britz, *et al* 1994; Fleming *et al*. 1996; Sales & Britz, 2001). Most of the research has been laboratory-based under very controlled conditions and looking at single cause and effect relationships. Few studies have looked at research on a multidisciplinary scale, such as this one. This has mainly been due to the scale and time required for such research. This research looked at the effects of different diets not just on the animals but also on the culture environment. Chapter 3 has shown that the diet ultimately affects the taste of cultured product and the eastern market identifies the product differently than the western market. This study was initiated to monitor abalone SGR and health of abalone fed different diets in two separate cohorts, through regular monthly sub-sampling. SGR sub samples were compared against tank biomass data obtained from farm grading data. In addition water quality was monitored over four 72 hour periods, to compare temperature, pH, dissolved oxygen, TAN and phosphate. The main study period commenced in September 2003 and was completed in May 2005, with the first cohort stocked using 10 - 15 g animals. This was done to gain a complete seasonal data set and allow for seasonal overlap.

The second study period looked at SGR between kelp fed animals and mixed diet animals only (this was due to the poor performance of the Abfeed® fed animals in the first trial). This study commenced in July 2005 and ran until January 2006 using 20 – 25 g animals. Additional measurements including particle size range and sediment amount, mobile fauna diversity and abundance, bacterial loading as well as water nutrient concentrations were performed by other researchers within the system set up for this thesis and these will be commented on.

MATERIALS AND METHODS

Diets

The experiment was performed in the farms moulded concrete flow-through tanks (6.6 m l X 2.08 m w X 0.88 m d; ± 12 000 L). The three diets used were Abfeed®, kelp and mixed diet (See Table 5.1 for feed amounts and protein content).

The control for this study was the kelp-fed abalone. Each of these treatments in both experiments consists of 3 replicates of each treatment i.e. there were three Abfeed® fed tanks, three kelp fed tanks, three mixed diet fed tanks. The tanks had 12 volume exchanges per day and the bottom of each tank was v-shaped.

Mixed seaweed diet

Since 1998 I & J has been cultivating both *Gracilaria* and *Ulva*. The *Gracilaria gracilis* (Iyer, 2001) was obtained from both Saldanha Bay, RSA and from Luderitz, Namibia, while *Ulva* (predominately *U. lactuca* and *U. capensis*) was obtained from free-living populations in Gansbaai harbour and Simons Town harbour. Both seaweeds were stocked at an initial density of 2 kg m^{-2} (optimum stocking density obtained from experiments in Robertson-Andersson, 2003). Both were fed 100 g of nutrient supplements once weekly and the water turnover rate was ± 12 V E d^{-1}. The fertilizer used is a combination of Maxiphos® (a commercial phosphorus fertilizer) and ammonium sulfate. *Gracilaria* was fertilized with a ratio of 10 parts Maxiphos to 1 part ammonium sulfate, while for *Ulva* the ratio was 6:1. Both seaweeds were pulse fertilized at night. This pulse feeding serves two purposes. Firstly it helps to combat epiphytic growth (Ryther *et al.* 1981; Friedlander & Ben-Amotz, 1991; Friedlander, 1992) and secondly it allows for maximum absorption of the nutrients by the algae. The luxury uptake of nutrients by *Gracilaria* at night has been documented by Friedlander & Ben-Amotz, (1991) and Friedlander (1992), while experiments within the system showed that luxury uptake also occurred in *Ulva* (Robertson-Andersson, 2003 and Duke *et al.* 2005 confirmed luxury uptake by *Ulva* spp. is possible). The algae were harvested bi-weekly. The harvested material was then fed to the grow-out abalone bigger than 50 mm, where it lasted for 2 days before being completely consumed by the abalone.

Abfeed® and kelp diets

In 2005 the farm used 20 tons of Abfeed®. There were two different types used on the farm. A smaller pellet (10 mm) feed was given to the animals in the weaning section and they were grown on this diet for a year. The animals in the grow-out section received a larger pellet (50 mm) and were fed 3 times a week. The proximate analysis of Abfeed was given in Chapter 2. The farm uses about 20 tons of kelp per week in the grow-out sections. About 1 800 tons of kelp (*Ecklonia maxima*), per year, was required for the whole farm.

Table 5.1: Feed type and amount fed (for grow-out abalone) as well as average protein content.

Feed	Amount per basket per week	Feeding days	Protein content
Abfeed®	33 kg	Monday, Wednesday, Friday	34 & 26 %[A]
Kelp	90 kg	Monday, Friday	7.8%[B]
Mixed diet	90 kg kelp for 3 weeks and in the 4th week 45 kg kelp, 22 kg *Ulva* and 22 kg *Gracilaria*	Monday, Friday	33 %[C]

[A] Sea Plant Products Abalone formulated feed Document

[B] Simpson, 1994; Simmons, 1990

[C] Robertson-Andersson, 2003 values for *Ulva* and *Gracilaria* only, not for total diet

Experimental animals

As abalone have a heterogeneous growth rate (Lee, 2004), broodstock spawned in October 2001 were used for the first experiment. There were no significant differences in either length or weight between the different replicates in the first experiment. In the second experiment, using animals spawned in November 2002, there was a difference in size and lengths between treatments, although this was not significant.

Water Quality

Temperature, pH and dissolved oxygen were recorded daily by farm employees and monitored more intensively during the three 72 hours water sampling periods. Only data for September 2003 will be discussed in detail although maximal and minimal ranges for the January 2004- summer and June 2004 – winter will be reported. The 72 hour water quality testing was begun on the third day of a cleaning cycle (i.e. 3 days after all sludge had been removed from the floor of the tank). This was done to allow the system to settle after the weekly cleaning and allow for a possible maximum build up of nutrients in the system. Water quality experiments were also not performed directly after a weekend as the abalone go through a two day forced fasting. No additional feed was added into the tank between a Friday feed and the Monday afternoon feed.

During monitoring for physiochemical variables, water samples for ammonium phosphate nitrate and nitrite were taken and analysed according to the methods listed in Chapter 4. Free ammonia nitrogen was calculated according to the methods in Chapter 4. Bredberg (2003), Potgieter (2005) and Lindström (2006) used the same methods described in Chapter 4 to analyse water samples. For the purpose of comparison with published concentrations, values were converted from µg N (or P) L^{-1} to µmol N (or P) L^{-1}. When necessary, concentrations in reference literature were also converted to µmol N (or P) L^{-1}.

Sub samples

Samples were taken from tanks from January 2004 to June 2005. At each sampling 50 animals were randomly removed from each tank. Weights and lengths were recording as per the methods described in Chapter 4. Of the ± 118 800 animals that were part of the experiment at each monitoring period 450 were sampled. Regression analyses were done according to the protocol described by Reaburn & Edwards (2003).

Grading and stocking densities

The grading was done in September 2004 using farm grading methods. Animals were harvested from a tank and were mechanically graded into different size classes and the weights of these size classes were recorded for a total biomass value.

These data were recorded via a grading computer, and were compared to sub sample biomass estimates. This method of grading was considerably more accurate than that employed at JSP (Chapter 4). The end result of graded animals from both cohorts was unavailable at the experiments termination, due to farm policy.
At the initiation of both experiments abalone were stocked at 550 animals per basket with 24 baskets per tank, thus each treatment consisted of 39 600 animals. After the grading in September 2005 animals were stocked at 400 animals per basket with approximately 28 800 animals per treatment.

Condition factor

The condition factor was calculated according to the method in Chapter 4.

Abalone health

Forty five animals from the experimental systems, three groups of five from each replicate, were collected and analysed by the AFASA veterinarian (Dr Anna Mouton) and reported according to standard industry reports (Mouton, 2004) as shown in Table 4.2 in Chapter 4. The results were analysed using ranked statistics.

Sediments

Sediment loading and particle fractionation of the water column and settled sediment were tested and reported in Potgieter (2005) and Brandt (2006). The methods they used are as follows: Particle load as well as particle fractionation in incoming, outgoing and tank water were measured and sampled during the weekly tank cleaning. Since it was impossible to clean all tanks on the same day the cleaning of the treatments were separated in time. Sea-water was collected separately to ensure that it would be representative for the particulate conditions in the sea on the same day. Water for particle load was sampled just before the weekly cleaning, at 20 cm from the surface in the middle of the tank and between the baskets. To determine the particle load 1-2 L of water from each sample were syringe filtered through burned and pre-weighed glass-fiber filters (47 mm). The filters were frozen and later dried to constant weight at 70 °C. The dry weight was then measured. For particle fractioning 5 L of each sample was sieved through a set of sieves with different mesh sizes (50, 40, 30 and 20 µm). The particles were collected using distilled water and then syringe filtered through burned and pre-weighed filters (25

mm) separately for the different fractions (20 - 30, 30 - 40, 40 - 50 and 50 - 1 000 µm). The filters were then frozen and later dried to constant weight at 70 °C at UCT. Sediment load was measured during the cleaning of the abalone tanks. Water from the tank was drained leaving an accumulated layer of sediment at the bottom of the tank. Sediment deposits from baskets and feeder trays were washed down with water, side walls were scrubbed. The sediment rich bottom water was then transferred to 500 L tanks. Three sub-samples were after intensive mixing collected from these tanks. Potgieter (2005), measured total sediment loads by syringe filtering 3 sub samples of 100 ml through 25 mm Whatman Fiber Glass filter paper and then drying the samples at 70 °C at UCT. Brandt (2006) used an alternative method and estimated total sediment load according to the following methods: one L of each sample was put in a plastic Imhoff-container and left for 24 hours, after which the water was decanted with a tube and the remaining sediment was transferred to containers (sediment that stuck on the Imhoff container was removed with distilled water and added to the sample), frozen, then dried at 70 °C in the laboratory at UCT.

Bacteria

A once of study was conducted by Flodin (2005), during September to November 2004. Samples were taken in the morning on the last day of the 7-day feeding and cleaning cycle at the same time as sediment, water quality and mobile fauna samples. Water samples were collected in sterile screwcapped 10 ml bottles some 50 cm below the water surface. For sediment samples, loose sediment was scraped from the bottom of the tank into a sterile screw-capped tube and excess water was removed after allowing the sediment to settle. Samples were carefully homogenized and mixed with sterilized artificial seawater (3%) using Vortex. All samples were processed immediately after collection. 10 fold serial dilutions with sterilized artificial seawater (3%) were applied to all samples. 100 µL was plated in triplicates on TCBS (Difco) and MA (marine agar). In water samples, where concentrations of bacteria were suspected to be low, 20 – 100 ml of the water sample was filtered using 0,45 µm pore size Microfil V Filtration Device from Millipore. The filters were then seeded onto duplicates of TCBS plates. Alternatively 1 ml of the water sample was processed using the pour-plate method. The inoculated plates were incubated at 25 °C and after 48 h the colony-forming units (CFU) were counted. For water and sediment samples CFU ml, were calculated per water and sediment, respectively.

Mobile macro fauna

A once off study was conducted by Hansen (2006), during September 2004. During tank drainage a plankton-net (<100 µm mesh) was held under the tank outlet to collect fauna. After drainage the remaining sediment was then sucked out via a pump filling a 400 L container. From this a 50 L sample was randomly taken with a 5 L bucket with constant stirring. In addition to the tank sampling, intake seawater to the farm was sampled at 3 occasions during low, mid and high tide (n = 9). The sediment samples and the material collected in the plankton-net were sieved through a 1 mm mesh to separate the macrofauna from fauna and sediment particles of a smaller size. A sub-sample was taken when the density of animals and detritus load was very high. The animals were thereafter sorted to higher taxonomic groups alive and then preserved in 95 % ethanol for more detailed identification. The sorted and identified animals were dried in an oven at 60 °C to constant weight and weighted. Taxa with extremely low biomass were given a minimum weight of 1*10-4 g. Air-breathing isopods (e.g. *Ligia dilatata* Brandt), which easily escaped during sampling, had to be excluded. Sessile macrofauna that had to be scraped off the tank walls were also excluded.

Statistical analysis

All data were expressed as means ± standard errors. The analysis for this study was done using STATISTICA V6.1. For SGR data an initial analysis of co-variance was first tested with the baseline value of the outcome i.e. either length of weight used as a covariate. This was done to account for any differences in starting values. To test for actual differences ANOVAS were performed on the data. All data were regarded significant at $P < 0.05$. For physiochemical and water nutrients one-way and Factorial ANOVAs were performed. All values for nitrate and ammonium were transformed logarithmically to obtain homogeneity of variance (tested by using the Cochran Test). Due to a lag phase in the length and weight data of abalone, data were forced to a common mean and the rate increase in the original data was used to create the curves in the common mean data.

RESULTS

Abalone growth rates

September 2003 to May 05

All diets reflected a positive correlation between body weight gain and increase in shell length using linear regression analysis (see Table 5.2). At the termination of the experiment there was no significant difference in length or weight increase between kelp and mixed diet. This was also evident in that animals fed these diets would take between 612 and 624 days to reach 100 g, a difference of less than two weeks (Table 5.2). The average length and weight increases between these two diets were very similar (see Table 5.2). The desired length increases on the farm on a per month basis is 2.15 mm month^{-1}. All three diets were well short of this target with the Abfeed® diet performing significantly worse in terms of weight and length increases (ANOVA; $p < 0.05$, $df = 299$), and taking approximately 200 more days to reach a harvestable size (Table 5.2). At the experiment initiation and after the September 2004 grading there is a 2 month period when all diets performed at the same rate, indicating that there is at least a two month acclimatization phase (see Figures 5.1 and 5.2). If the data after the September grading were used and forced to a common mean, then in December and January, the mixed diet outperformed the kelp diet. The condition factor of all the diets was close to 1 (See Table 5.2), meaning that the animals produced from the tested diets had short shell length and large mass. The Abfeed® condition factor was significantly lower than the other diets (ANOVA, $p < 0.05$).

Table 5.2: Size and weight per month and changes in condition factor of abalone fed a mixed, kelp only and an Abfeed® diet. The symbol * denotes significant differences between feeds ($P < 0.05$). The r^2 value denotes the significance of the regression line used to calculate the average length and weight increases. The exponential regression equation is given as well as the R value (significance of the fit) and the time taken for the abalone on those diets to reach 100 g.

	MIX DIETS		ABFEED®		KELP	
	Length (mm)	Weight (g)	Length (mm)	Weight (g)	Length (mm)	Weight (g)
Average	1.50	3.32	1.12*	2.02*	1.42	3.10
r^2 value	0.97; $p < 0.05$		0.94; $p < 0.05$		0.96; $p < 0.05$	
Exponential regression	$y = 3E\text{-}44e^{0.0027x}$		$y = 1E\text{-}30e^{0.0019x}$		$y = 7E\text{-}45e^{0.0028x}$	
R value	0.9582; $p < 0.05$		0.9034; $p < 0.05$		0.9728; $p < 0.05$	
Time (d) to 100 g	612		624		821	
Condition factor						
Begin	1.05		1.05		1.05	
End	1.23		1.16		1.23	

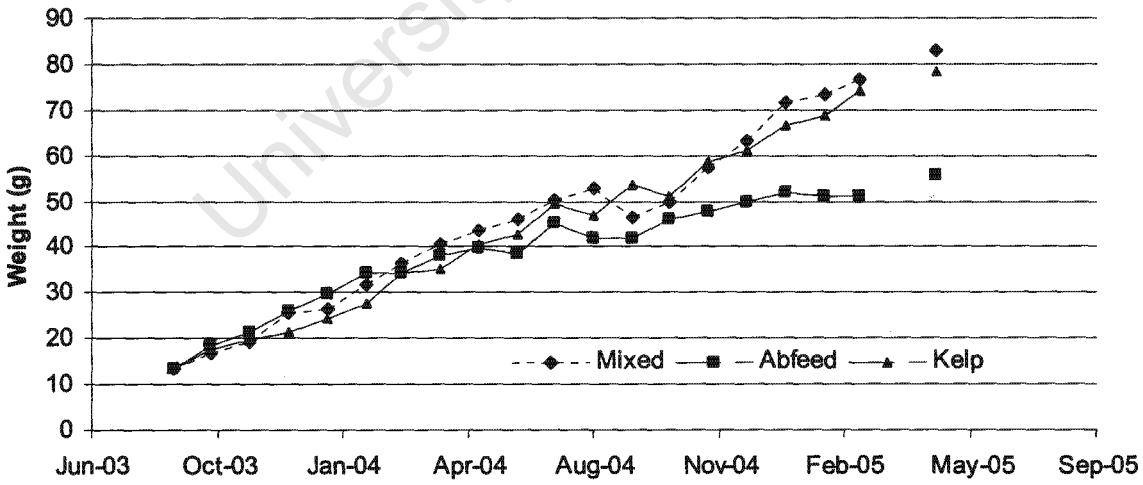

Figure 5.1: Weight (g) increase of abalone fed a mixed, kelp and Abfeed® only diet from September 2003 to May 2005 (n= 1400).

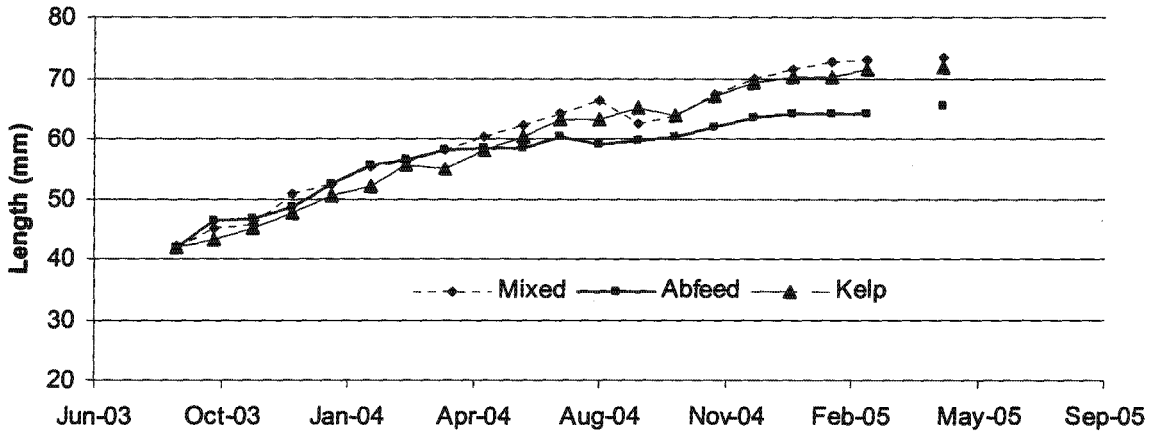

Figure 5.2: Length (mm) increase of abalone fed a mixed, kelp and Abfeed® only diet from September 2003 to May 2005 (n= 1400)

June 2005 to January 2006

Both diets reflected a positive correlation between body weight gain and shell length increase using linear regression analysis (see Table 5.3). A mixed diet produced a larger and heavier animal than a kelp only diet, and it would take 70 days faster to reach harvest weight than a kelp fed animal (see Table 5.3). The average monthly growth rate increases in length were lower than the farm optimum of 2.15 mm month^{-1}. Although the starting values were different, with the mixed diet experiment being stocked with a slightly larger animal, if the data were taken to a common mean (see Figures 5.3 and 5.4), the rate increase was greater in the mixed diet experiment. In both treatments there was an acclimatization period which lasted for approximately two months. The percentage increase in biomass for the mixed experiment was 97.56 % vs. 79.78 % for the kelp diet. This means that the biomass in the mixed diet increased by 86.9 kg over the experimental period while the kelp diet increased by 71.1 kg. A frequency distribution of size classes showed that the kelp diet produced a normal distribution while the mixed diet had a bimodal distribution (See Figure 5.5). There was no significant difference in the condition factor of the animals at the termination of the experiment and the values were close to 1 (See Table 5.3).

Table 5.3: Average increase in size and weight per month of abalone fed a mixed diet vs. a kelp only diet from June 2005 to January 2006. The r^2 value denotes the significance of the regression line used to calculate the average length and weight increases. The exponential regression equation is given as well as the R value (significance of the fit) and the time taken for the abalone to reach 100 g.

DATE	MIX DIETS		KELP	
	Size (mm)	Weight (g)	Size (mm)	Weight (g)
2005/08/19	1.60	2.97	2.23	3.63
2005/09/20	2.44	3.37	1.31	0.53
2005/10/18	2.78	3.38	2.36	4.02
2005/11/18	1.73	3.52	1.20	2.91
2005/12/29	1.43	5.77	2.65	5.08
2006/01/18	1.66	4.10	0.41	2.64
Average	**1.94**	**3.85**	**1.69**	**3.14**
r^2	**0.96**; $p < 0.05$		**0.95**; $p < 0.05$	
Exponential regression	$y = 5E\text{-}64e^{0.0039x}$		$y = 3E\text{-}54e^{0.0033x}$	
R value	0.9995; $p < 0.05$		0.9925	
Time (d) to 100 g	393		470	
Condition factor				
Begin	1.1		1.12	
End	1.19		1.17	

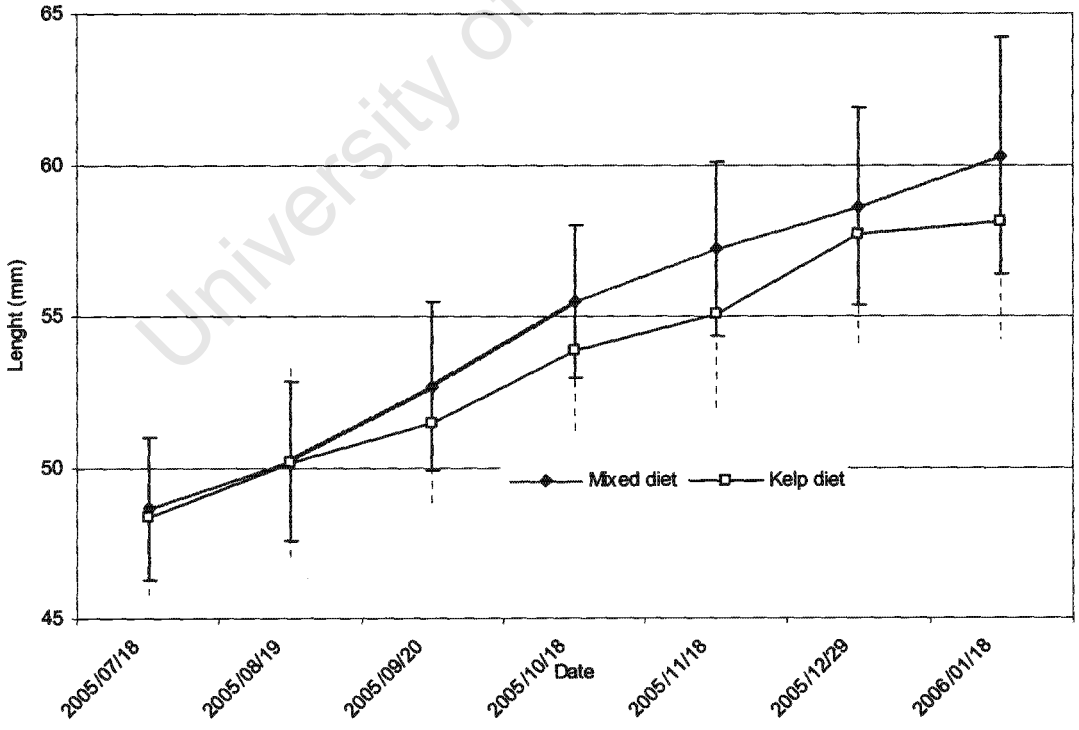

Figure 5.3: Length (mm) increase of abalone fed a mixed diet vs. a kelp only diet from June 2005 to January 2006 (n= 1400).

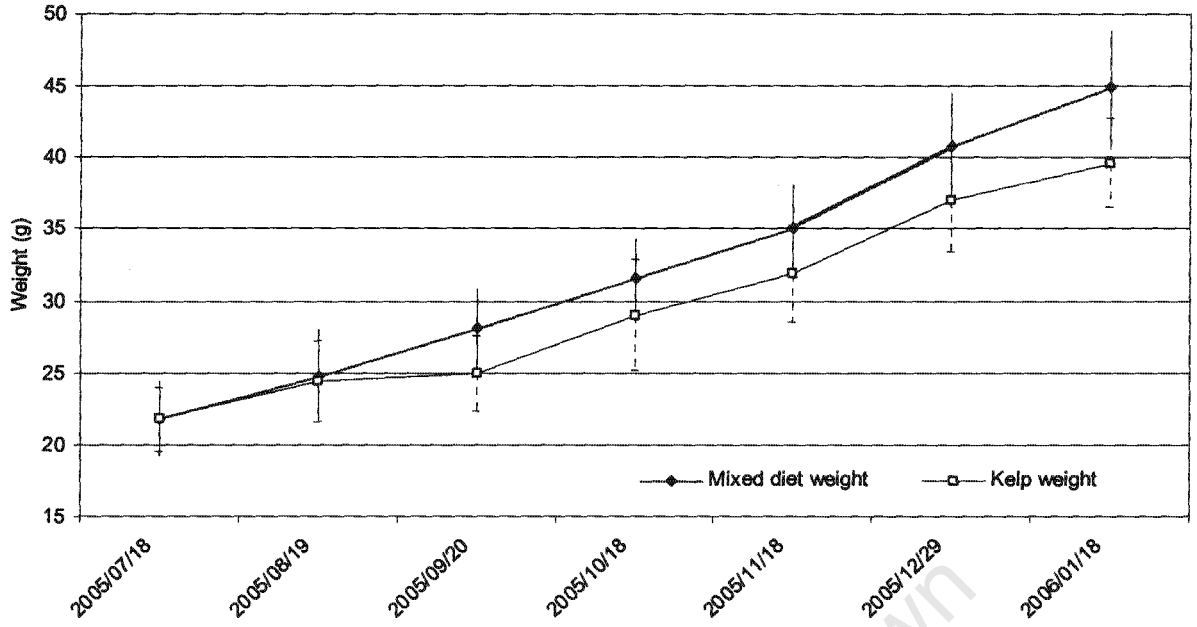

Figure 5.4: Weight (g) increase of abalone fed a mixed diet vs. a kelp only diet from June 2005 to January 2006 (n= 1400).

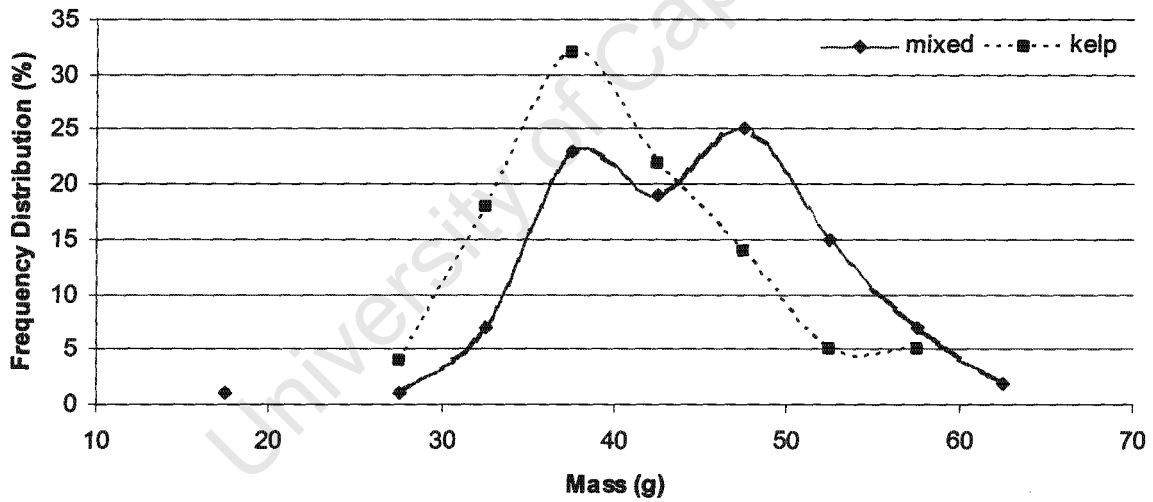

Figure 5.5: Frequency distribution of weights following final measurements in January 2006 of abalone fed a mixed diet vs. a kelp only diet from June 2005 to January 2006 (n= 200).

Abalone health

Sabellids – Refer to figure 5.6

In February 2004 the kelp fed (control) animals had a significantly lower (T-test; $p < 0.05$; df = 8) sabellid infestation prevalence and intensity compared to the Abfeed® fed animals, with the mixed diet being intermediary. Sabellid scores increased in all groups in April 2005, with animals on Abfeed® worst affected. In June 2004, sabellid score decreased with the kelp animals having a significantly lower sabellid score. In November 2004, sabellid scores were significantly higher in the Abfeed® animals (T-test; $p < 0.05$; df = 8) and these animals showed little indication of shell growth. No shell boring polychaetes were found in any of the samples. The farm diet is a mixture of kelp and Abfeed®, this may explain why the sabellid rankings are similar in the farm and the tested Abfeed® diets.

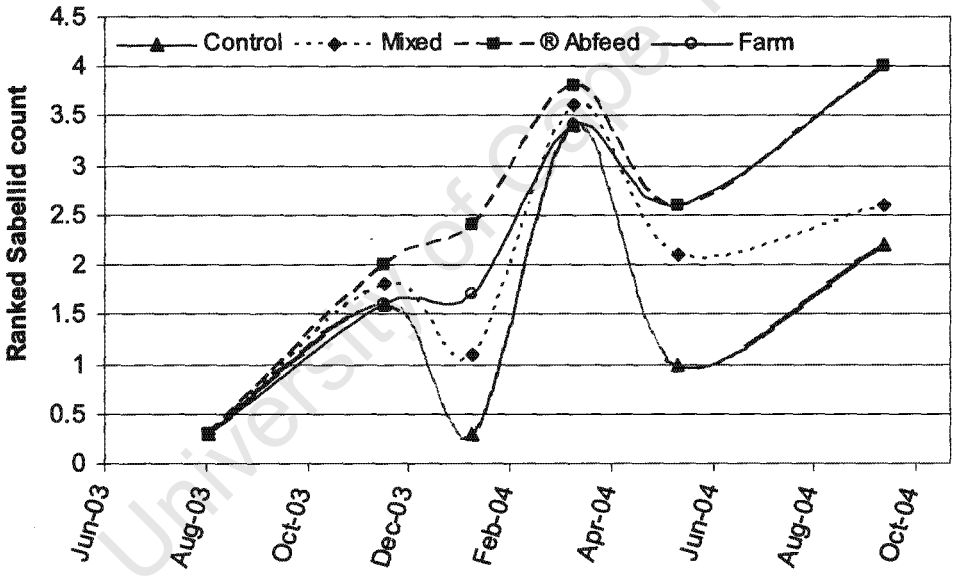

FIGURE 5.6: Mean sabellid count of animals fed 3 different diets with farm health status data for comparison. (n = 3).

General condition – Refer to Figure 5.7

In February 2004 the mixed diet and Abfeed® fed animals had a better general condition than the kelp control fed animals. In April 2004 nearly all animals had shiny shells compared with their February shell condition. The Abfeed® fed animals showed loss of condition which is most likely due to nutritional stress. The kelp control diet animals had the best general condition in June 2004 and good shell growth was present in all the kelp groups with growth being less pronounced in the

mixed diet and little growth being observed in the Abfeed® groups. In November there was little indication of shell growth in the Abfeed® fed animals, while animals from the other treatments appeared to be increasing in length.

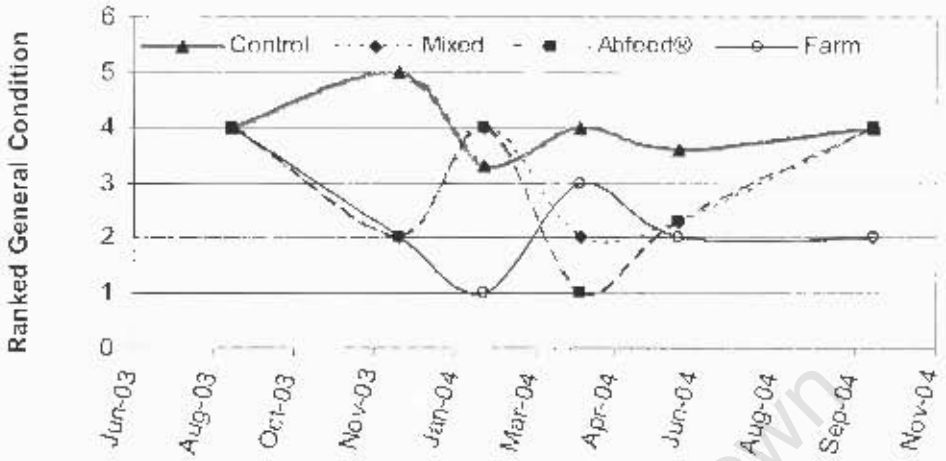

FIGURE 5.7: Mean general condition of animals fed 3 different diets with farm health status data for comparison. (n = 3).

Environmental Stress – Refer to Figure 5.8

Abfeed® fed animals showed signs of environmental stress from December 2003 and onwards with March 2004 being an exception. The mixed diet animals only showed signs of environmental stress in December 2003. The health reports of farm animals showed that signs of environmental stress were present from March 2004 until the experiment was terminated.

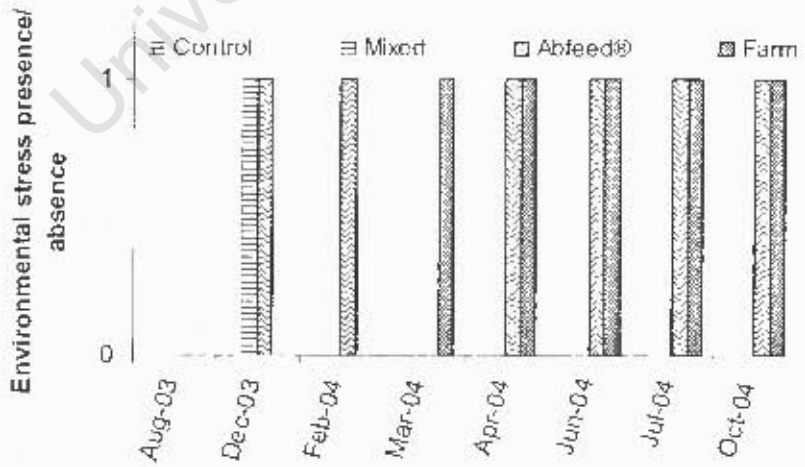

FIGURE 5.8: Presence or absence of environmental stress in animals fed 3 different diets with farm health status data for comparison (n = 3). 1 = environmental stress present, 0 = environmental stress absent.

Ranked gonad condition – Refer to Figure 5.9

Abfeed® fed animals had more advanced gonad development than any other diet. This result is not dissimilar to the farm animals of the same age.

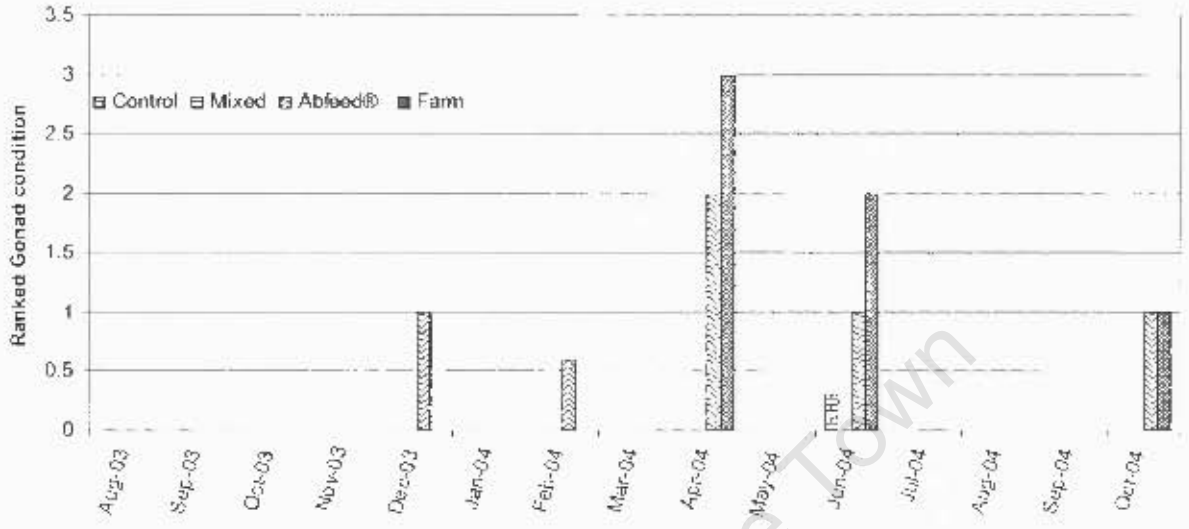

FIGURE 5.9: Ranked gonad condition as an indicator of abalone sexual maturity in animals fed 3 different diets with farm health status data for comparison (n = 3).

General Histology – Refer to Figure 5.10

From December 2003 until April 2004 the Abfeed® animals had no internal parasites whereas all treatments receiving natural feeds were infested with *Rickettsia*. The Mixed and kelp fed animals had gut protozoa present while there were no gut protozoa in the Abfeed® fed animals.

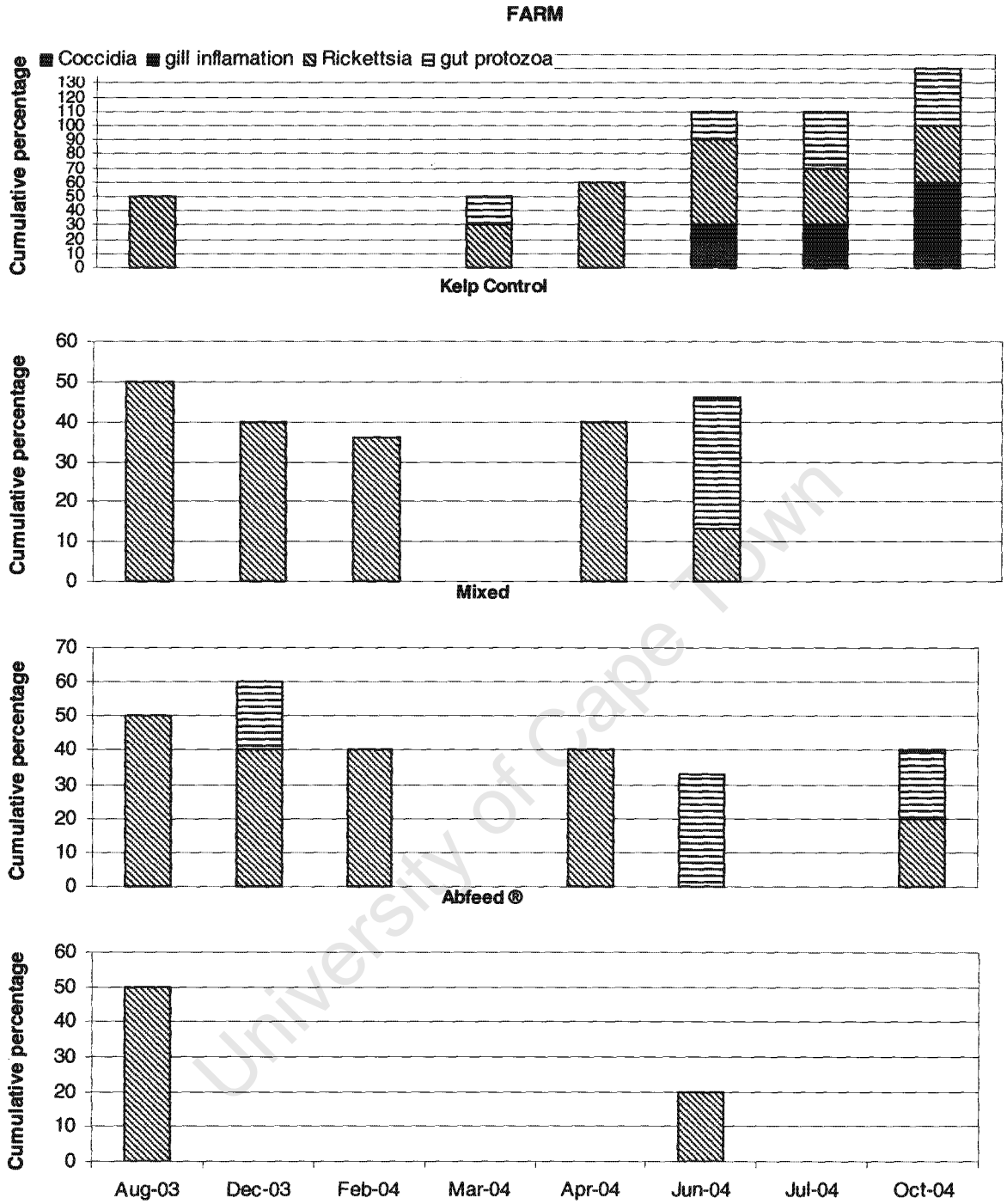

FIGURE 5.10: Cumulative percentage of parasites found during a histological examination in animals fed 3 different diets with farm health status data for comparison (n = 3).

Water Quality

Physiochemical variables

Temperature

There was no significant difference in temperatures between the three diets treatments in summer, winter or spring. The seawater in the Abfeed® treatment experienced an increase in the average temperature above that of the incoming seawater by half a degree to 16 °C in winter and spring at night. The average summer water temperature was 15.5 °C. Maximal temperatures recorded in the treatments were 19.5 °C and the minimal being 14.4 °C. In winter the average temperature was 15.4 °C with the maximum being 16.3 °C and the minimum being 15 °C. This is a much more stable temperature range in winter compared to the summer measurements. Spring temperature ranges were similar to winter ranges. The temperatures in all seasons and in all diets had a diurnal rhythm being highest at 16h00 and lowest at 00h00.

Dissolved oxygen

In summer, there was no significant difference in dissolved oxygen values between the treatments, but there was a significant difference between the treatments and the incoming seawater ($p < 0.05$). There was a decrease in dissolved oxygen values between 20h00 and 00h00, which is an indication of higher activity, possibly as a result of feeding by the abalone at these times. Average dissolved oxygen concentrations for summer were 7.84 ($\pm$ 0.48) mg L^{-1} for the three treatments compared to 9.1 ($\pm$ 0.66) mg L^{-1} for incoming seawater. In winter dissolved oxygen values decreased between 16h00 and 20h00. This is when the abalone are assumed to start feeding. This is earlier than in summer and is probably triggered by sunset. The value of the decrease was greater in the Abfeed® treatment 1.2 mg L^{-1} vs 0.8 mg L^{-1} for mixed and 0.5 mg L^{-1} for kelp treatments. This is a well known phenomenon as eating protein rich feeds requires greater oxygen demand than lower protein feeds. The mixed treatment had significantly lower dissolved oxygen values ($p < 0.05$) than the incoming seawater, the Abfeed® and the kelp control treatment, indicating that there was a significantly higher Biological Oxygen Demand (BOD). After Abfeed® had been placed in the tanks at around 09h00 on the second day of the monitoring the dissolved oxygen decreased until it too was significantly lower ($p < 0.05$) (16h00 – 08h00 on the second day) compared to the kelp treatment.

The average value of dissolved oxygen in the mixed treatment was 6.9 mg L^{-1} compared with 7.6 for kelp and 7.3 mg L^{-1} for Abfeed® treatments. This trend was repeated in September and is shown in Figure 5.11.

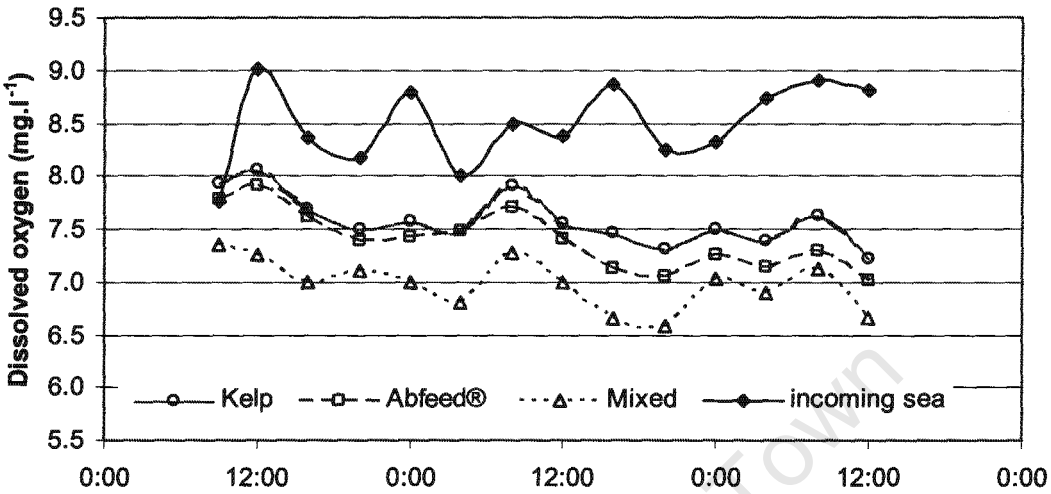

FIGURE 5.11: Dissolved oxygen (mg L^{-1}) in the seawater of tanks fed 3 different diets with incoming seawater for comparison (n = 3) in September 2003.

pH

There were no significant differences between any of the treatments during summer. Average pH for the three treatments was 7.82 with a maximal 8.06 of and a minimal of 7.56. This was lower than the incoming seawater which averaged 7.85 with a maximum of 8.24 and a minimum of 7.6. In winter and spring, the pH of the mixed treatment was significantly lower than the kelp or Abfeed® treatments. Aside from the minimum pH being 7.6, there was no real indication of acidic conditions (pH below 7.6) that would lead to the shiny shells being noticed in the health examinations. The pH showed a diurnal rhythm being higher at 16h00 and lower at 00h00. The pH in winter in the mixed treatments was significantly lower ($p < 0.05$) than the other two treatments. pH in winter showed a diurnal rhythm with a peak around noon and a decrease between 20h00 and 00h00. Average pH in the kelp and Abfeed® treatments were 7.59 (± 0.29) with a maximum of 8.32 and a minimum of 6.83. The pH in the mixed treatment averaged 6.98 (± 0.31), with a maximum at 7.5 and a minimum of 6.27. The average pH in the incoming water was 8.49 (± 0.31) which was significantly higher than all treatments ($p < 0.05$). In spring, the pH in the

mixed treatment was significantly lower than the other two treatments ($P < 0.05$) (See Figure 5.12).

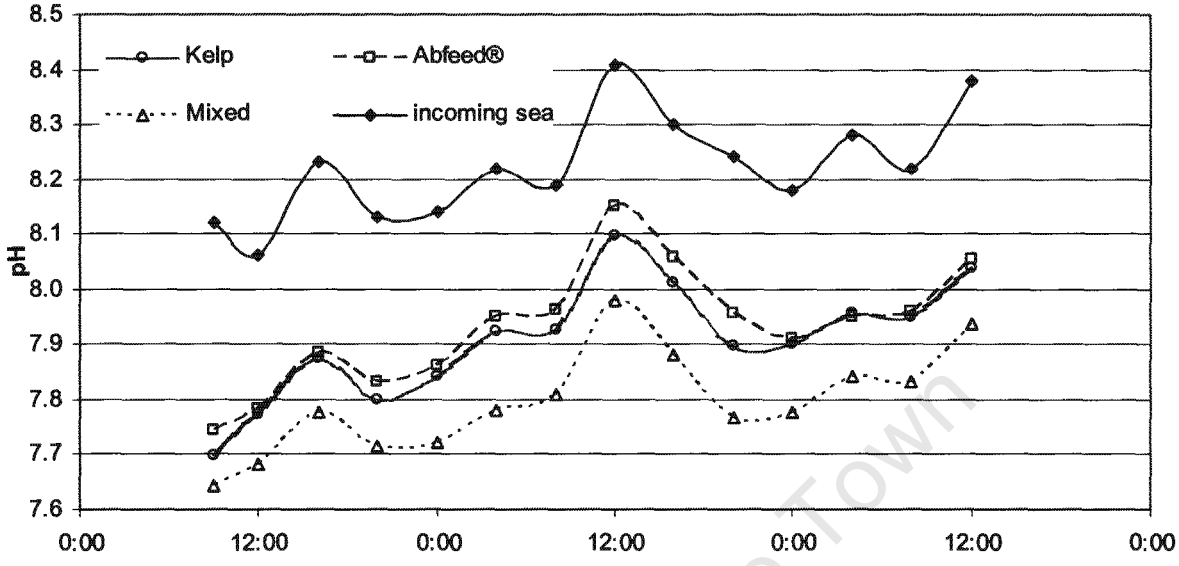

FIGURE 5.12: pH of seawater in tanks fed 3 different diets with incoming seawater for comparison (n = 3) in September 2003.

Water nutrients

As general trends, magnitudes and values obtained from the other seasons were similar to the values obtained in September, only the September 2003 values will be commented on. These values will also be used to compare between studies by Bredberg (2003), Potgieter (2005) and Lindström (2006), which took place in September 2002, September 2004 and September 2005, respectively.

Average TAN concentrations for the duration of the experiment were for kelp treatment 3.32 (± 1.3) µmol L^{-1}, mixed 3.49 (± 1.3) µmol L^{-1}, Abfeed® 10.27 (± 4.0) µmol L^{-1} and incoming seawater 3.75 (± 0.78) µmol L^{-1} (See Figure 5.13). TAN concentrations in the Abfeed® treatment were significantly higher after the feed had been placed in the tanks. They remained high with a slight peak at 20h00 following a decrease to ambient levels of other treatments during the day, then increased significantly ($p < 0.05$) above day time concentrations at 20h00 the following night. Both kelp and mixed treatments decreased TAN concentrations below ambient incoming seawater during the day. This result is consistent with that found by

Bredberg (2003) and Potgieter (2005). Although in the study by Bredberg (2003), the kelp diet generated significantly lower TAN concentrations compared to both an *Ulva* and *Gracilaria* only diets.

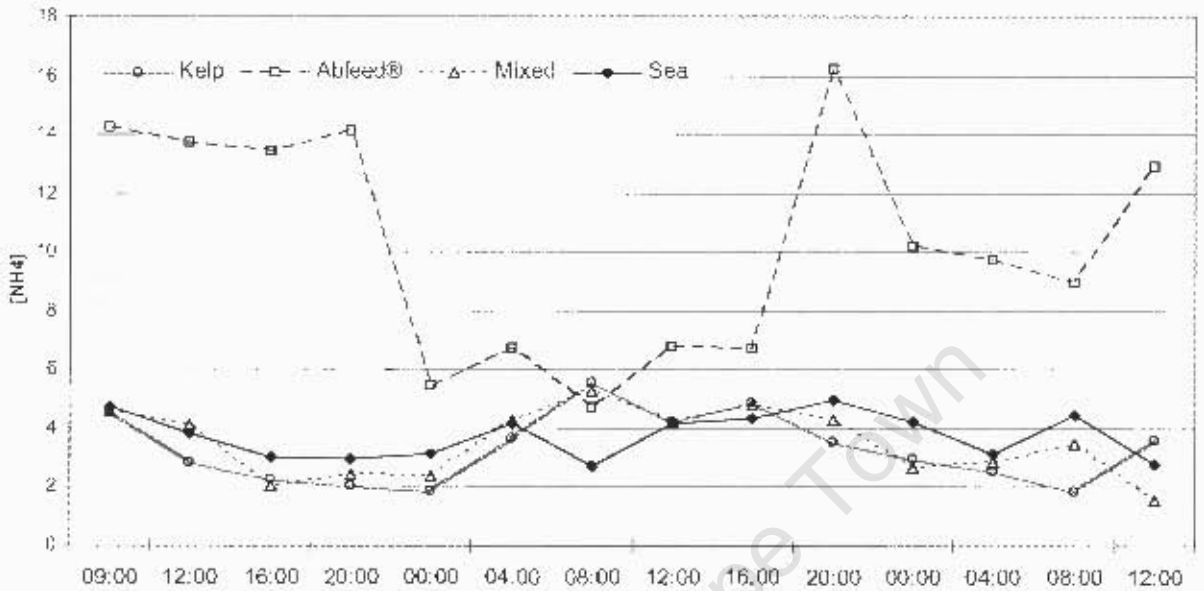

FIGURE 5.13: Mean TAN concentrations (µmol L^{-1}) in tanks fed 3 different diets with incoming seawater for comparison (n = 3).

Dissolved Inorganic Phosphate (DIP) concentrations for the duration of the experiment are for the kelp 0.88 (+ 0.4) µmol L^{-1}, mixed 1.23 (± 0.5) µmol L^{-1}, and Abfeed® treatments 1.18 (+ 0.8) µmol L^{-1} and incoming seawater 0.64 (± 0.4) µmol L^{-1} (See Figure 5.14). DIP concentrations in the Abfeed® treatment were significantly higher than the other diets at 20h00 the first night and at 04h00 the second night ($p < 0.05$; df = 11). The mixed diet had significantly higher DIP concentrations than the kelp diet over the whole experiment ($p < 0.05$) and the Abfeed® diet during the second day.

Nitrate had the higher concentration in the different treatments and nitrite which is in a transition state in seawater, was only present in low concentrations. The nitrate concentrations for the duration of the experiment are for kelp (3.47 ± 0.9) µmol L^{-1}, mixed (3.9 + 0.98) µmol L^{-1}, and Abfeed® treatments (3.86 ± 1.14) µmol L^{-1} and incoming seawater (3.07 + 0.9) µmol L^{-1} (See Figure 5.15). The Abfeed® treatment

produced the highest nitrate concentrations, followed by the mixed treatment. During the day there was evidence of nitrate uptake by the kelps in the kelp treatment. As with TAN and DIP, the nitrate concentrations peaked at 20h00 on the first night and at 00h00 on the second night.

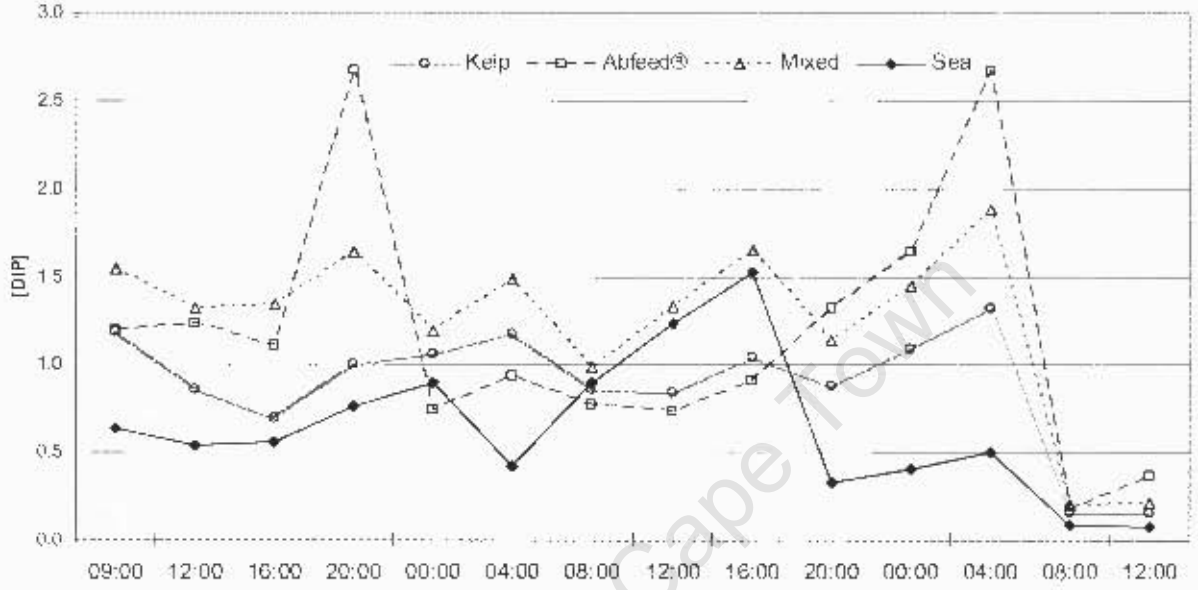

FIGURE 5.14: Mean DIP (µmol L^{-1}) of tanks fed 3 different diets with incoming seawater for comparison (n = 3).

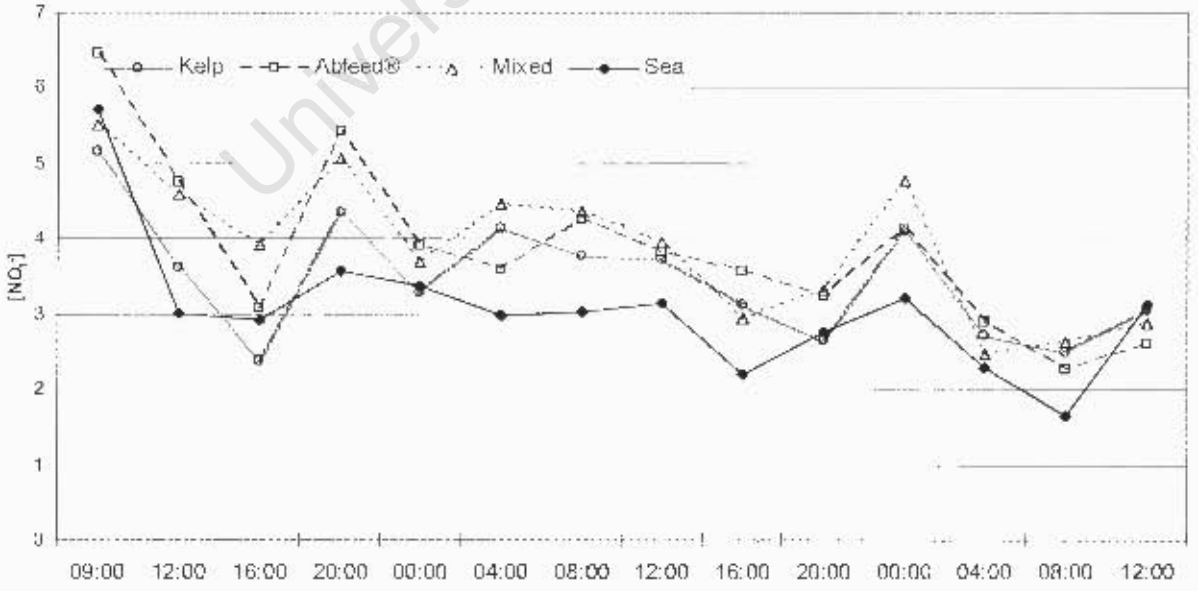

FIGURE 5.15: Mean nitrate concentrations (µM) in tanks fed 3 different diets with incoming seawater for comparison (n = 3).

The nitrite concentrations for the duration of the experiment are for kelp (0.63 ± 0.5) µmol L^{-1}, mixed (0.75 + 0.5) µmol L^{-1}, and Abfeed® treatments (0.92 + 0.5) µmol L^{-1} and incoming seawater (0.84 ± 0.5) µmol L^{-1} (See Figure 5.16). Abfeed® had significantly higher concentrations just post feeding ($p < 0.05$; df = 11). Potgieter (2004) found similar results and concentrations. There is also evidence of nitrite uptake by the kelp and *Gracilaria* and *Ulva* during the day. Nitrite increased significantly in all treatments at 00h00, compared to values at 20h00 ($p < 0.05$: df = 23).

Values for ammonia in summer are higher due to the higher seawater temperatures (see Table 5.4). While in winter, with the lower seawater temperatures, the values are lower. All treatments experienced two peaks in ammonia concentrations, one at 12h00 and the other at 20h00. The peak at 12h00 is due to the fact that pH peaks at 12h00 and although temperature in the tanks peaks at 16h00 the combination of the high temperature and high pH result in a greater amount of ammonia being present in the water. Incoming seawater showed a diurnal rhythm with peak values at 16h00.

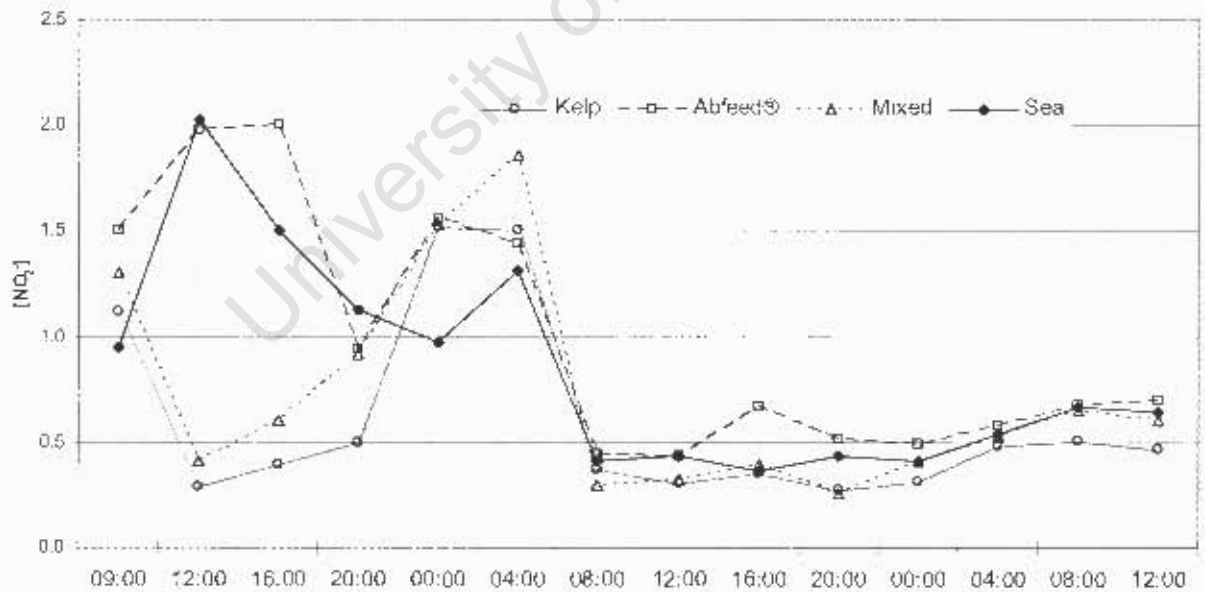

FIGURE 5.16: Mean nitrite concentrations (µmol L^{-1}) in tanks fed 3 different diets with incoming seawater for comparison (n = 3).

Table 5.4: Average (standard deviation), maximal and minimal values for ammonia (µmol L^{-1}) in summer and winter in tanks of abalone fed a mixed, kelp only and an Abfeed® diet. These values are shown as they are the highest and lowest of the all 72 hour experiments.

	Average	Max	Min
Summer			
Kelp	1.04 (± 0.71)	2.88	0.26
Abfeed®	2.61 (± 1.31)	5.25	1.00
Mixed	0.80 (± 0.47)	1.75	0.20
Seawater	1.49 (± 0.52)	2.46	0.77
Winter			
Kelp	1.58 (± 0.88)	3.36	0.81
Abfeed®	2.29 (± 1.23)	5.80	1.00
Mixed	0.62 (± 0.26)	1.21	0.37
Seawater	0.83 (± 0.24)	1.24	0.54

Sediments

Both sediment studies showed that the particle concentration in the incoming seawater was highly variable (90.3 mg L^{-1} ± 20.5 in 2005 (Potgieter, 2005) and 48.2 mg L^{-1} ± 30.7 in 2006 (Brandt, 2006)). Particle fractioning showed a higher percentage of larger particles (Brandt, 2006) (see Table 5.5).

TABLE 5.5: Average weight of particles in different size classes from incoming seawater from Potgieter (2005).

Particle size fraction	Average mg.l^{-1}
50 < µm	2.1 (± 2.7)
40 – 50 µm	0.8 (± 1.2)
30 – 40 µm	1.5 (± 1.9)
20 – 30 µm	1.0 (± 1.1)

Suspended particle concentration

Potgieter, (2005), showed that there was no significant difference in the amount of particles suspended in the water column of any of the diets and that the particle loading varied from 102.6 – 107.6 mg L^{-1}. The particle fractions occurring in the suspended sediments were also not significantly different, however the Abfeed® treatment contained more particles in the 20 – 30 μm size range (See Figure 5.17).

Accumulation

The amounts of organic and inorganic materials accumulating on the tank bottom in abalone baskets and on the tank walls was significantly higher compared to the suspended particle load (Potgieter, 2005; Brandt, 2006). This was expected as we were comparing a biomass that accumulated over time with a concentration in the water column. However, if taken over a week the total sediment load leaving the tanks through the over flow would be 30 – 41 times more compared to when the tanks was cleaned.

Potgieter (2005), found a significant difference in particle accumulations of the bottom sediment loads of the different diets. Particles greater than 50 μm were more dominant in the accumulated sediments. The Abfeed® diet also had the greatest number of particles within the 20 – 30 μm size range (see Figure 5.18). The mixed diet resulted in significantly more sediment being accumulated compared to both the Abfeed® and kelp diets, with the least accumulation in the kelp treatment (see Table 5.6).

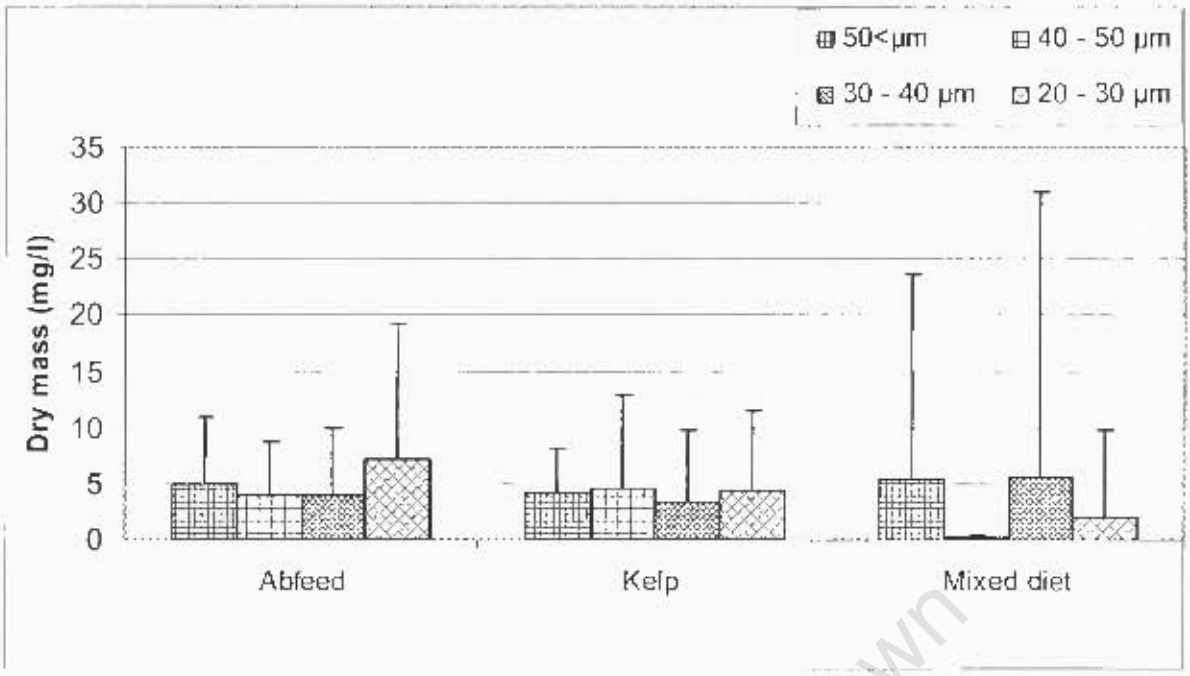

Figure 5.17: Particle fraction of suspended sediments in the three treatments (data from Potgieter, 2005).

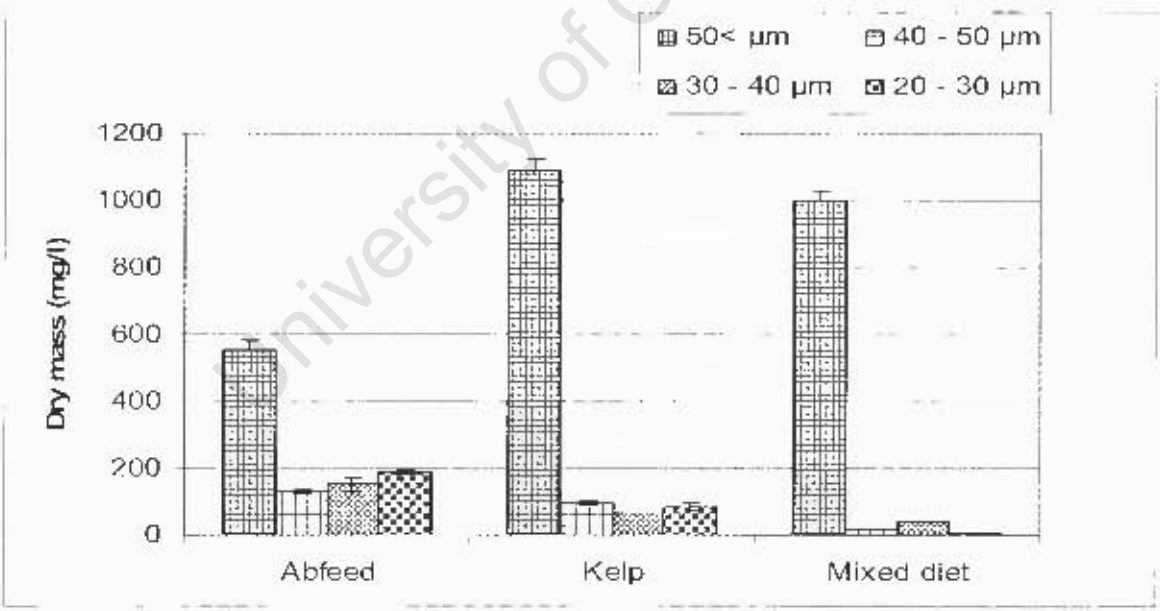

Figure 5.18: Particle fraction of bottom sediments in the three treatments (data from Potgieter, 2005).

TABLE 5.6: Amount of bottom sediment per tank in 400 L of bottom sediment water, and sediment production calculated per tank, per week, to an estimate of 500 tanks per year (data in second column from Potgieter, (2005).

Diet	$mg.l^{-1}$	kg / tank / wk	kg/ tank /yr	Tons/yr/farm of 500 tanks
Abfeed	434 ± 34.4	1.74	90.4	45.18
Kelp	274.7 ± 28.2	1.10	57.2	28.58
Mixed diet	594.8 ± 32.4	2.38	123.7	61.9

Total loading

If the amount of bottom sediment per L were multiplied by the number of litres of liquid making up the bottom sediment then the amount of sediments that can be produced by a 500 tank farm feeding a particular diet can be illustrated as in Table 5.6. A farm feeding a mixed seaweed diet in 500 tanks would produce almost 62 tons of sediment a year from the cleaning of the tanks alone. While the sediment leaving via the overflow would be equal to 1653 tons

Bacteria

Counts of viable heterotrophic bacteria (VHB) and Vibrio-like bacteria (VLB) in both the sediment and water column showed that significantly more bacteria was found in the sediment ($1.5 \times 10^7 - 2 \times 10^8$ CFU/ml VHB; $3 \times 10^6 - 4 \times 10^7$ CFU/ml VLB) compared to the water column ($4.6 \times 10^3 - 1.1 \times 10^4$ CFU/ml VHB; $7 \times 10^2 - 2.6 \times 10^3$ CFU/ml VLB) (Flodin, 2005). Flodin (2005) showed that there is a build-up of bacteria in the sediment during the weekly feeding and cleaning cycle. Abfeed® had higher levels of both bacteria types compared to the kelp fed system but due to the high variance no overall significant differences could be detected.

Mobile macro-fauna

The mean density of mobile macrofauna was 2.17 g DW (± 1.75), 11 648 individuals (± 10 960) per tank in the Abfeed® fed treatment; and 1.99 g DW (± 0.21 SD), 8703 individuals (± 3 594) per tank in the kelp-fed treatment. In total, 28 faunal taxa were identified from all samples. The diversity was significantly higher in the Abfeed® (16.0 ± 4.0 taxa) compared to the kelp-fed system (9.7 ± 3.2 taxa) ($p < 0.05$). The total faunal density and diversity was significantly higher in the tanks compared to an equal volume of intake seawater ($p < 0.001$). The most abundant taxa in the tanks

was the amphipod *Paramoera capensis* (Dana) and the tanaids (*Tanaidacea* spp.) together with polychaetes, especially the *Nereid* spp. Other abundant taxa were the amphipod *Hyale* sp. and the isopod *Janiopsis palpalis* Barnard, as well as other (unidentified) amphipods. These taxa contributed 81 – 99 % of the total mobile macrofauna biomass in the tanks.

DISCUSSION

Abalone growth rates

Contrary to published results on growth performance of Abfeed® (Britz *et al.* 1994; Britz, 1996a-c; Britz & Clayden, 1996; Knauer *et al.* 1996; Viana *et al.* 1996; Britz & Hecht, 1997; Britz *et al.* 1997a,b; Erasmus *et al.* 1997; Doeschate *et al.* 2000; Sales & Britz, 2001 & Dlaza, 2006), the results in this Chapter show the Abfeed® diet to be the poorest in terms of growth.

Fleming *et al.* 1996 and Mai *et al.* 2001 stated that most abalone farmers would be satisfied with a growth of 2.01 – 3 mm per month. However, this relates to tropical abalone that grow much faster than the temperate species *H. midae*. These results show that on all diets abalone failed to reach these growth rates as well as the target set by the farm of 2.15 mm.month^{-1}. The farm target may be rather optimistic.

In both cohorts there was an acclimatization phase where the abalone grew at the same rates before the dietary effects could be detected. This period seems to be as long as two months and occurred after grading. The difference between the mixed diet and the kelp diet seems to be related to a seasonal change. In both cohorts at similar times of the year (around spring) the mixed diet out-performed the kelp only diet, while at other times of the year the situation was reversed. This could indicate that the abalone have different dietary requirements at different times in the year. In the second cohort, the mixed diet produced 10 kg more abalone per treatment than the kelp based diet over the same period.

Abalone health

Sabellids

Chalmers (2002) found that the sabellid infestation intensity and occupation level are not influenced by the growth rate of abalone. Although the Abfeed® fed abalone were growing slower, they were not necessarily more susceptible to sabellid infestation. However, slow growing abalone had sabellids that were larger and produced more eggs and larvae than faster growing abalone. Chalmers (2002) study also showed a positive influence of Abfeed® on sabellids. Abalone fed Abfeed® had a greater sabellid intensity and contained larger adults with a greater

base width than sabellids from a kelp fed abalone. He concluded that the effect of slow growing abalone fed a more nutritious diet synergistically affected the sabellids infection densities in combination with the slow abalone growth rates. These results seem to be confirmed in the context of this study, where the diet is affecting the sabellid population more than the growth rate of the abalone. The reason for this is that the sabellid infestation in the Abfeed® animals is similar to that found on the rest of the farm, thus the slow growth is not the only reason for the high infestations on these animals.

The loss of general condition in the Abfeed® animals is more likely due to insufficient access to feed rather than reflecting feed quality. Towards the end of the experiment there was no difference in the general condition of any of the animals being fed the different diets. The loss of general condition in the seaweed fed animals from January to April is probably related to a decrease in the kelp quality around this time (see Chapter 6).

Environmental stress was evident in the Abfeed® fed animals for 10 months of the year. This type of stress can be caused by poor water quality (i.e. high nutrient concentrations) or high particulate concentration, both of which are higher in the Abfeed® diet. If this result is taken in context with the whole farm, then other farm animals of a similar age are also experiencing this type of stress. Therefore it is likely that another factor such as a high farm stocking density couple with insufficient access to the feed could be the stressor.

The advancement of gonad maturity could reflect the increased nutritional value in the Abfeed® diet. It is understood that animals which receive better nutrition reach sexual maturity sooner (Hahn, 1989). In addition, gonad maturity increased in December 2003, April 2004 and October 2004 corresponding with the spawning seasons of wild abalone (Hahn, 1989). An animal that reaches sexual maturity sooner is undesirable in a cultivation situation, as the animals partitions more resources into gonad development rather than somatic growth (Hahn, 1989).

There was a clear difference in the general histology between the different diets with the Abfeed® animals not having any gut protozoa. Mouton (2006a,b) has seen this

before and has said that the gut protozoa are a consequence of a seaweed diet, although this has not been tested. The animals in the experiment had fewer parasites compared to animals of a similar age on the rest of the farm. This is probably due to the more intensive management that these animals received during the course of the experiment.

Water quality

The results obtained from the experiments at I & J were consistent with those found in a similar diet experiment set up at Jacobs Bay Sea Products (JSP) (unpublished data), with the Abfeed® fed animals producing significantly higher TAN and DIP concentrations than the kelp fed animals. Results from this experiment was not reported in this thesis as the experiment had to be terminated after a 6 month period as the Abfeed® fed animals were decreasing in condition and weight. In effect they were starving. This was due to the feeding behaviour of the animals, which caused feed to be inaccessible to the animals shortly after feeding.

The peak in TAN and DIP values in the evening has been noted in a previous study (Robertson-Andersson, 2003). Although these values are often not significantly different from the overall values, other studies such as Lindström, (2006) and JSP, (unpublished data) have noticed this trend although it has occurred earlier or later in the evening, depending on the season. Work done by Robertson-Andersson (2003) suggested that these peaks may be due to feeding activity as abalone are known to be nocturnal feeders. In the present study, these peaks were occurring at different times in the different seasons thus suggesting that they occur at specific times after sunset. In addition, the abalone do not start feeding on the feeder trays at this time as seen by counts of abalone at 20h00 present on the top of the feeder trays in September. Out of 30 trays, on average, there was 1 (± 3) animal on the feeder trays, while at 00h00 there were on average 16 (± 6) animals. A laboratory behavioral study seems to confirm this observation (Shipton, 1999). Knauer *et al.* (1995a,b), showed that although appearance rate of juvenile *H. midae* (5.00 ± 8.54 mm shell length) in glass tanks was about 80 % between 19h00 and 23h00, fewer than 7 % of the animals were actively feeding. Thus, rather than reflecting a feeding peak, these peaks could hypothetically arise from the abalone egesting their guts

prior to feeding. This occurs at least twice in the night with a second peak occurring a few hours before sunrise. This would be a useful direction for future research.

TAN

In oxygenated, unpolluted seawater, TAN rarely exceeds 5 µmol N L^{-1} (DWAF, 1996). However, in deep stagnant water, e.g. the Black Sea, the amount of TAN can be as high as 150 µmol N L^{-1} (DWAF, 1996). Hydrated NH_4^+ ions are non-toxic and serve as a nutrient to primary producers (Boyd, 1990; 1998). Samsukal (2004), found that abalone farm effluent had values for TAN in effluents ranging between 0.44 and 19.25 µmol N L^{-1}. Department of Water Affairs and Forestry (DWAF) government guidelines for ammonium in unpolluted waters must not exceed 43 µmol N L^{-1} (DWAF, 1996). The values for ammonium fall well within the range found by other researchers working locally (Robertson-Andersson, 2003; Samsukal, 2004; Potgieter, 2005) and do not exceed the DWAF values. Abfeed® produces significantly higher ammonium peaks than the other diets and this could be a reason for the environmental stress seen in these animals. The peaks correspond to days of feeding and it would be interesting to see how values change with more frequent feeding.

Bredberg (2003) also found that larger animals excreted less than smaller animals. The animals reported here, average 15 g in weight which is similar to small animals in the study by Bredberg (2003). This could explain why nutrient values in the winter study in 2004 were slightly lower than those obtained here.

The TAN measurements included both free ammonium ions (NH_4^+) and free ammonia (NH_3). In seawater at normal pH, free ammonia (NH_3) is only 3 – 5 % of the total value. NH_3 is regarded as toxic because it is uncharged and lipid soluble. The DWAF values for ammonia for the South African Coastline must be below 43 µmol $N.L^{-1}$). Ammonia toxicity is expressed as total ammonia (the sum of NH_3 and $NH4^+$) in the environment, and increases with water pH, chloride and calcium concentrations (Randall & Tsui, 2002). Exposure of fish to high concentrations of ammonia causes an increase in gill ventilation, hyperexcitability, convulsions and then death (Boyd, 1990). Levels of ammonia were significantly higher in the Abfeed® diet tanks and this is a result of the increased ammonium due to the high protein

content in the feed. The lower ammonia concentrations in the seaweed based feeds have been shown to be due to seaweed photosynthesis/respiration and subsequent nutrient uptake (Bredberg, 2003; Robertson-Andersson, 2003). Bredberg (2003) showed that if *Ulva*, *Gracilaria* and kelp were placed in the same containers as small and large abalone compared to containers without abalone the difference in TAN values was significantly lower in containers with seaweeds. The containers were sealed and were not open to the atmosphere indicating that uptake occurred without light.

All levels for ammonium are well below the sub lethal limits for abalone (Reddy-Lopata *et al.* in press). Reddy-Lopata *et al.* (in press) showed that farmed abalone can adapt to high concentrations of ammonium but this has cost in terms of decreased growth rates of the abalone. It is important for farmers to not only maintain ammonia concentrations below toxic levels but also to keep them low to ensure optimum growth.

Nitrite (NO_2)

Nitrite occurs in seawater as an intermediate compound in the microbial reduction of nitrate or in the oxidation of ammonia and is usually present in very low concentrations (DWAF-vol.1, 1996). There is little record of natural occurrences of nitrites in the open-ocean. Mean concentrations of nitrite-N, for the west coast average 0.3 µmol N L^{-1} (Hutchings & Andrews, 1980; Chapman and Shannon, 1985; Mitchell-Innes & Walker, 1991; Largier & Boyd, 2001) and for the south coast 0.2 µmol N L^{-1} (Boyd *et al.* 1985; Largier *et al.* 1992; Probyn *et al.* 1994). Safe nitrite levels for *H. laevigata* are above 5 mg L^{-1} (Basuyaux & Mathieu, 1999) with a chronic sublethal level of 7.8 mg L^{-1} (Harris *et al.* 1997). Since these levels are not known for the South African abalone, we can assume that since the levels in this study were below these, it was unlikely that there was nitrite stress to the abalone in any of the diets. Samsukal (2004), found that abalone farm effluent had values for nitrites in effluents ranging between 0.15 - 1.10 µmol N L^{-1}. Nitrite impairs the ability of fish blood to transport oxygen-methemoglobinemia (Russo, 1985; Boyd, 1990, 1998). Oxygen is transported in fish blood by the respiratory pigment hemoglobin. The iron in hemoglobin is present in the ferrous (Fe II) state and methemoglobin is formed. Methomoglobin is not capable of combining reversible oxygen and thus sufficiently

high concentrations can cause hypoxia and death. Nitrite oxidizes hemoglobin to methomoglobin thus increasing the amount of methomoglobin present and impairing oxygen transport by blood (Russo, 1985; Boyd, 1990, 1998).

Nitrate (NO_3)

Nitrate is the final oxidation product of nitrogen compounds in seawater and is considered to be the only thermodynamically stable oxidation level of nitrogen in the presence of oxygen (DWAF-vol. 1, 1996). Nitrate concentrations usually increase with depth. Nitrate –N concentrations on the west coast are on average 1.17 µmol N L^{-1} with upwelled waters having an average of 20 µmol N L^{-1} (Hutchings & Andrews, 1980; Chapman and Shannon, 1985; Mitchell-Innes & Walker, 1991; Largier & Boyd, 2001). The south coast average is 5.79 µmol N L^{-1}. (Boyd *et al.* 1985; Largier *et al.* 1992; Probyn *et al.* 1994). Safe nitrate levels for *H. laevigata* are between 100 – 250 mg L^{-1} (Basuyaux & Mathieu, 1999). Samsukal (2004), found that abalone farm effluent had values for nitrates in effluents ranging between 4.92 and 21.71 µmol N L^{-1}. Values from Robertson-Andersson (2003) for abalone farm effluent were also in this range as were the values in this study and all are well below the safe levels found for the greenlip abalone. Nitrate is considered essentially non-toxic and there are few reported studies of its toxicity in aquaculture (Russo, 1985; Boyd, 1990, 1998).

DIP (PO_4)

Phosphorus is normally found in the sea in the form of suitable inorganic phosphorus i.e. reactive phosphate, particulate and inorganic forms. The mean concentration of reactive phosphate in sea water has been estimated at 2 µmol P L^{-1} (DWAF-vol.1, 1996). On the west coast the average concentration is 1.71 µmol P L^{-1}) and in upwelled waters 1.51 µmol P L^{-1} (total phosphorus) (Hutchings & Andrews, 1980; Chapman and Shannon, 1985; Mitchell-Innes & Walker, 1991; Largier & Boyd, 2001). On the south coast the average concentration of total phosphorus is 1.19 µmol P L^{-1}(Boyd *et al.* 1985; Largier *et al.* 1992; Probyn *et al.* 1994). Samsukal (2004), found that abalone farm effluent had values for phosphates in effluents ranging between 0.65 - 6.04 µm P L^{-1} and dissolved organic phosphate values ranging between 0 - 1.86 µm P L^{-1}. Dissolved organic phosphate results from this study are similar to Potgieter (2004) comparing a once off measurement during the

day, with daily DIP concentrations in the mixed diet treatment being significantly higher than kelp or Abfeed® fed treatments. Bredberg (2003) found that abalone fed *Gracilaria* excreted significantly more phosphate than when they were fed *Ulva* or kelp. Phosphate content in cultivated *Gracilaria* was higher in comparison to cultivated *Ulva* (Njobeni, 2006; Robertson-Andersson, 2003). This is possibly due to the differences in the ratio of phosphates used in the fertilizers for each seaweed rather than differences in phosphate uptake rates.

The values recorded in this study are below the mean phosphate sea levels except for the mixed diet which is above this level. This could be due to the fertilization of the seaweed tanks.

Physiochemical variables

The oxygen data may appear on the supersaturated side, this can be explained as air is bubbled into the tanks on a continuous basis would therefore help in raising dissolved oxygen concentrations. In summer there was no effect on any physiochemical variable from using the different feeds. In winter the BOD of the mixed diet treatment was higher than the other treatments. This is surprising as the normal situation is for BOD to increase with a high protein diet (Boyd, 1990) which in this case, is Abfeed®. This trend was also experienced with the data obtained for JSP (unpublished data). The decrease in dissolved oxygen at night is due to the increased demand for oxygen by the abalone while feeding. This decrease occurs later than the peaks mentioned in the nutrients and it is likely that this is a true reflection of abalone feeding activity. This decrease occurs earlier in winter than in summer. The increase in temperature above ambient incoming seawater in the Abfeed® treatment at night may be due to an increase in the metabolic rate of the abalone due to the nutritional value of the feed. This trend was also seen at JSP (unpublished data). The more stable temperature seen in winter has always been used by farmers to explain the better growth rates at this time. The data in this Chapter indicate that highest growth rates occur in September and October with above-average growth occurring in winter. The decreased pH (by ca. 0.1 of a pH unit) in the mixed diet in winter coupled with the decreased dissolved oxygen concentrations is an indication that metabolic activity in the abalone in this diet is higher than the other diets at this time. The decrease in dissolved oxygen means

that carbon dioxide production would increase through increased respiration, this would result in the production of more carbonic acid thus lowering the pH.

Suspended solids and bottom sediments

There have been several studies completed on feed formulation and digestibility in abalone (Britz, 1995; Shipton 1999; Sales, 2001) but few studies on suspended particle concentrations in abalone tanks resulting from feed derived wastes. Detritus is a major source of food for many polychaete worms including *Terebrasabella. heterouncinata* (Fauchald & Jumars, 1979). The sabellid worm is a filter feeder and sorts particles according to size distribution (Fitzsimons, 1965; Bock & Miller, 1997), and may be capable of changing its feeding pattern in response to changes in particulate composition and size (Chalmers, 2002). Fauchald & Jumars (1979) suggested that sabellids selected particles exclusively on size. Kiørboe *et al.* (1980) and Bacon *et al.* (1998) showed that particle selection was based on organic quality. Bock & Miller (1997), showed that particle organic coating functions as a cue that moderates both feeding mode and behaviour of sabellids, while the nitrogen or organic content of the particle increased the ingestion rate (Kiørboe *et al.* 1980; Bacon *et al.* 1998). Selective rejection of nutritionally poor particles or inorganic particles to increase the quality of ingested particles has also been observed in bivalves (Kiørboe *et al.* 1980; Bacon *et al.* 1998). Shields *et al.* (1998) and Ruck (2000) showed that microcapsules ranging between 3 and 30 μm, and particles up to 35 μm were found in the gut of the sabellid *T. heterouncinata*. It is suggested that the preferred feeding size range of the sabellid is smaller than 40 µm, although it may ingest larger particles (Ruck, 2000; Chalmers, 2002).

Both Chalmers (2002) and Potgieter (2005) showed that the average weight and particle size composition in the tank water column differed significantly between abalone being fed kelp and those being given compound feeds. Kelp and the mixed treatments had a greater fraction of large particles (> 50 μm) (Potgiter, 2005). Chalmers (2002) found that this large size class was predominately made of particles > 100 μm in diameter, which is outside the feeding range of the sabellid. Chalmers (2002) showed that the feeding activity of the sabellid was disrupted when they came into contact with particles larger than 50 µm.

Potgieter's (2005) study showed that abalone fed on a kelp diet produced more suspended solids (SS) of 40 – 50 μm and that the Abfeed® diet produced more SS in the size range of 20 – 30 μm. The mixed diet generated the lowest SS concentration mainly within the size range of 30 – 40 μm. The suspended particle concentration in the water column was not significantly different between the diets and this may mean that that the water column has reached loading capacity. A reason for this is that the aeration within the tanks was equal as well as the flow rates and therefore the water motion (velocity) within the tanks was similar.

Chalmers (2002) and Potgieter (2005) showed that there was a significant difference in the protein content of the feeds, with the Abfeed® having a significantly higher protein and energy content. In a mixed diet the seaweed is fertilized but protein levels are not as high as with an Abfeed® diet but are higher than a kelp diet (Robertson-Andersson, 2003). Chalmers (2002) showed that the protein and energy composition was consistent within large (> 100 μm) and small (> 100 μm) particles. Chalmers (2002) showed that the size of the abalone had no effect on protein or energy content of the particles generated.

If we assume that energy content per particle per diet is similar irrespective of particle size, then the Abfeed®, followed by the mixed diet, generates the ideal particle size range and highest particle energy and protein for the sabellid worm. This seems to be reflected in the sabellid data. In addition, Sorokin (1973) suggested that sabellids may be concentrating on filtering the smaller size range particles composed of bacterio-, myco- and small phytoplankton which are readily assimilated. Abalone farmers have noted that Abfeed™ decomposes faster than kelp. The small nutritious particles could be attractive for bacterial colonization due to their high surface area to volume ratio, thus resulting in a richer food source to the sabellid. The bacterial studies by Flodin (2005) in the same system seem to confirm this observation with significantly higher bacterial densities in the Abfeed® diet.

Chalmers (2002) showed that the sabellids in an Abfeed® diet had significantly larger morphometrics than kelp fed sabellids, reflecting a better feed quality. Chalmers (2002) also showed that abalone diet had a significant effect on the infestation level by sabellids, with a significantly greater number of sabellid tubes being found along

the growing edge of Abfeed® fed abalone, this was also shown in this study with the Abfeed® diet having the highest sabellid infection levels of the three diets. However, sabellids on a kelp diet reached sexual maturity 2 months sooner than on an Abfeed® diet as well as reaching a greater size (Chalmers, 2002). Diet did not have an effect on the fecundity of the sabellids (Chalmers, 2002).

Aquaculture waste outputs are very variable and are dependent upon the species being cultivated and the environment in which they are cultured (Boyd, 1990, 1998). Wastewater outputs from land based aquaculture usually consist of dilute farm effluents. They may also include concentrated farm sediments (Tacon & Forster, 2003). The suspended particulate matter (SPM) consists of organic and inorganic material. In abalone farm effluents SPM consists of eliminated faeces and uneaten food, i.e. mainly organic in nature. The extent of the impact of suspended solids (SS) depends on the nature of the waste and the extent to which it accumulates, which in turn is influenced by the location of the farm and farm management practices. The south coast is a less energetic regime than that of the Benguela system and the dispersion of particulate matter is determined by the fluctuating component of the current (Stigebrandt *et. al.* 2004).

Negative effects of SPM in aquaculture include: decreased light penetration (Lloyd, 1987), toxicity (Newcombe & Macdonald, 1991), mechanical and abrasive impairment of gills resulting in cultured organisms death (Lloyd, 1987). It can also lead to decreased reproduction (in fishes), impaired feeding behavior (in species that use visual cues), and smothering of benthic organisms (Hogg & Norris, 1991). Clogging of filter feeding apparatus (Newcombe & Macdonald, 1991, Metzeling *et.al.* 1995), stress, behavioral changes (Doeg & Milledge, 1991) have also been seen a result of increased SPM. High concentrations of SPM can also alter habitats e.g. by filling the interstices of the substrate (Campbell & Doeg, 1989) and influencing both the decomposition and availability of detrital material, with consequent impacts on the availability of food for many macroinvertebrates (Metzeling *et al.* 1995). These effects occur both in the culture rearing environment as well as the waste receiving environment.

Potgieter (2005) showed that the bottom sediments concentration and bottom particulate fraction size differed significantly between the three treatments with the kelp and mixed treatment having a larger proportion of larger particles (> 50 µm). The large particles present in the bottom sediments may be attributed to the abalones' feeding behaviour. Abalone grazing on a kelp diet break off larger particles when feeding, due to the rasping nature of their proboscis. In the Abfeed® tanks, bulldozing of pellets and break-up of the Abfeed® pellets could lead to the larger particles, although aggregation and bacterial process could also contribute. In the mixed diet, *Gracilaria's* structure is such that when the abalone feed they break a section off and whole strands of the seaweed may be lost to the bottom sediments. The total bottom sediment build up gives an indication of the potential pollution levels of the particular diet and the potential amount of bacteria build up. A study by Samsukal (2004) looked at sediment composition of effluent water from 7 abalone farms from around the South African coast. She found that the average concentration of particulate matter in the > 63 μm fraction was 8.36 mg L^{-1} (3.24 - 18.80 mg L^{-1}) in the effluent during normal operation and 12.27 mg L^{-1} during cleaning - a significant increase. The range of particulate matter in the < 63 μm fraction was 0.71 - 21.10 mg L^{-1}. This was a significant difference between normal farm flow though conditions and when tanks were being cleaned. The difference between SS in the water column and bottom sediments in Potgieter (2005) study also confirms this finding although the amounts were lower.

A study done by Brandt (2006) looked at the carbon and nitrogen composition of the bottom sediment and found that the average carbon content of the bottom sediment from a kelp based diet was 14,06 % (min. 12.26 %; max. 15.38 %) of the total dry weight while the average nitrogen content was 1.47% (min. 1.12 %; max. 1.74 %) of the total dry weight. Thus a 500 tank farm feeding kelp only would produce 4.01 tons of carbon and 0.42 tons of nitrogen over a year. A study done by Chalmers (2002) looked at the protein differences between kelp and Abfeed® sediment, multiplying using the conversion factor (6.25 see Chapter 6), kelp produced 2.52 % N while the Abfeed® sediment produced 4.70 % N. This means a 500 tank farm feeding Abfeed® only would produce 2.12 tons of N a year. This is four times the amount produced by a kelp based diet. The values calculated in this study are for 400 L of bottom sediment per tank using Potgieter's (2005) and Brandt's (2006)

data. Potgieter (2005) calculated values were lower than Brandt (2006) but were done using a different sediment collection method (See Table 5.7). If the sediment amount in the continuous outflow water was used, and using the same percentages of nutrients as the bottom sediments, a kelp diet would produce 231 tons of carbon and 24 tons of nitrogen while an Abfeed® diet would produce 78 tons of nitrogen.

Incoming seawater SS was highly variable. A likely reason for this variability is due to the inlet pipe for seawater being located close to a kelp bed. Kelps undergo fragmentation from the frond tips and thus release structural components as particulate matter and a dissolved fraction as well as cell contents into the water column (Newell *et al.* 1980). In addition there is an upwelling cell in the Walker Bay area. Upwelling and the energetic nature of the coastline are known to cause increased SPM (Stigebrandt *et al.* 2004).

Table 5.7: Production in tons of sediment from a farm consisting of 500 tanks feeding different diets

	Potgieter (2005)	**Brandt (2006)**
kelp	26.0	32.4
Abfeed®	28.6	
mixed	31.2	

Bacteria

Higher levels of bacteria and higher counts of vibrios in the sediment, compared to the water column, show that the sediment could serve as a reservoir for bacteria (Flodin, 2005). The build-up of bacteria in the sediment during the weekly feeding and cleaning cycle is consistent with other studies of bacterial water quality in abalone tank cultures (Lizarraga-Partida *et al.* 1998). Abfeed®, which is based on fish products and soy proteins, is more likely to promote bacterial growth than the other diets used in this Chapter as it generates more nutrient rich wastes, compared to kelp (Chalmers, 2002; Potgieter, 2005; Brandt, 2006). Pathogenic bacteria might not be the only problem in the culture systems, as high levels of heterotrophic bacteria can lead to lower oxygen levels and even cause oxygen depletion.

Mobile macro-fauna

The difference in the number of species between an Abfeed® and kelp fed system was probably due to the higher number and energy of Abfeed® particles (Potgieter, 2005; Brandt 2006) and higher bacterial loading (Flodin, 2005). Also more of the particles were smaller (< 30 μm) in the Abfeed® system meaning they have a higher surface area to volume ratio. The Abfeed® detritus is rich in energy (Chambers, 2002; Brandt, 2006) and this means that it can support a richer fauna diversity and be more attractive for filter and detritus feeders.

CONCLUSIONS

This study has analysed some of the factors that could be used to explain differences in abalone growth rates due to feeds, as well as the influence of feeds on tank environmental culture conditions. These have been summarised in Table 5.8 and have been described as positive, negative or neutral, or no data (ND), when compared to the kelp based diet. A negative value would indicate higher concentrations or values that would be detrimental to the abalone.

TABLE 5.8: Summary of effects of different variables tested between the Kelp, Mixed and Abfeed® diets in a flow through system. A 0 symbol indicates no significant difference, A + symbol indicates a significant positive benefit for aquaculture, a – symbol indicates a significant negative drawback for aquaculture and ND indicates no data. Alternate values are for Experiment 1/ Experiment 2.

		Mixed	Kelp	Abfeed®
Abalone	Shell length	0/ +	0	-
	Weight increase	0/ +	0	-
	Time to harvest	0/ +	0	-
	Condition factor	0	0	0
Abalone health	Sabellid	0	+	--
	General condition	0	+	-
	Environmental stress	-	0	--
	Gonad histology	0	-	--
Abalone histology	*Ricketsia*	-	-	+
	Gut protozoa	--	-	+
Bacteria	Water column	ND		-
	Sediment	ND		-
Sediments	Suspended	0	0	0
	Bottom accumulation	--	0	-
	Nitrogen waste production	ND		-
Mobile macrofauna	Densities	ND		-
	Taxa	ND		-
Water quality	TAN	0	0	-
	FAN	+	0	-
	PO_4^+	-	0	--
	NO_2^-	0	0	0
	NO_3^-	0	0	0
Temperature	Average	0	0	0
pH	Average	-	0	0
Dissolved oxygen	Average	0	0	-

This chapter has shown how diets can affect the general histology of the animals, the prevalence of pests and other fauna, source point pollution and abalone growth. The ultimate goal of this Chapter was not to decide which is the "best" diet to use but to gain an understanding of all the variables at play in the culture environment and how these variables interact with each other. From the data presented in this Chapter, it could be assumed that the Abfeed® diet is the poorer of the three. Discussions with other farmers who use this product say that management, water quality, culture system design and stocking density are all factors which affect this diet's performance and that their growth rates using this product can reach 2.5 mm a month. Abfeed® as a diet has several advantages over a kelp diet, the first the low FCR 1:1 for Abfeed® vs 12.5 - 15.1 for kelp (Chapter 2; ABFEED, 2006). The feed cost alone to produce 1 kg of abalone on kelp is ZAR 14.40 (US $ 2.36; April 2006) while on Abfeed it is ZAR 14.25 (US $ 2.33; April 2006) (ABFEED, 2006). In addition labour savings are also large, a 140 ton abalone farm feeding kelp would need to handle 80 tons of kelp a week, while the same farm feeding only Abfeed® would only need to handle 10 tons a week. As mentioned in Chapter 2 Abfeed® diets give a better fillet yield both when canning and after cooking. Despite this, the conclusion from the current research is that an Abfeed® diet fed in isolation is not the optimum diet for this culture system design.

In the last 5 years a large amount of research on feeds has been done in South Africa. This research has had two main thrusts - a seaweed based diet from cultivated seaweeds and a compound diet. Rather than being contradictory in nature I believe that this research has presented the farms with options for the best feed. This is seen in that 8 of the farms use a combination of compound feed and a natural seaweed diet.

Future development

New developments in feed research in South Africa include looking a gut bacteria in *H. midae* capable of hydrolysing a variety of complex polysaccharides (laminarin, carboxymethylcellulose, alginate, agarose, carrageenan) in algae. (Erasmus *et al.* 1994, 1997). Erasmus (1996) showed that endogenous polysaccharases of abalone fed either *E. maxima* or *G. gracilis (ex. verrucosa)* varied in response to diet. A probiotic research programme to use bacteria to enhance feed digestion in abalone

is underway at the University of Cape Town. Bacterial isolates included in diets containing *E. maxima* and *G. gracilis* extracts have been shown to improve growth in abalone (Doeschate *et al.* 2000). The "probiotic" can be added to compound food and is an advantage as the feed is more nutritious for the abalone. Future developments for Abfeed® diets in South Africa are temperature specific diets especially ones for higher temperatures, and size specific diets, especially for the grow-out abalone as well as decreasing the dependence of the compound diet on fish meal and reducing the protein content of the feed (Jones *et al.* 2006).

CHAPTER 6

A COMPARISON BETWEEN A TRADITIONAL FLOW THROUGH ABALONE CULTURE UNIT AND AN INTEGRATED SEAWEED /ABALONE RECIRCULATING UNIT

INTRODUCTION

The single most important factor affecting abalone cultivation is water quality (Fallu, 1991). Water is not only a source of essential substances, but serves as a waste disposal system as well. Usually life processes, such as those experienced in intensive abalone aquaculture, lead to degradation of water quality. Poor water quality may contain substances that are toxic/ deleterious to abalone or may not contain sufficient quantities of essential substances. This is because abalone cultivated at high stocking densities may contribute to localized eutrophication by waste production, through uneaten feed, faeces and excretion that are rich in dissolved nutrients and particulate matter. Further, artificial cultures may enhance the available concentrations of nutrients. The cultures may also cause changes between nitrogen and phosphorus, which may trigger the development of undesirable toxic algal blooms (Folke *et. al.* 1994). High concentrations of ammonium and nitrite are the most toxic nutrients to abalone and the environment (Fallu, 1991; Hahn, 1989; Folke *et. al.* 1994).

Methods for using seaweeds for treating effluents from enclosed land-based mariculture systems were introduced in the mid 1970's (Haines, 1975). Waste nutrients can be considered in integrated multi trophic aquaculture (IMTA) as a resource for the auxiliary culture of plants (Chamberlane & Rosenthal, 1995; Brzeski & Newkirk, 1997; Copin & Yarish, 1998; Troell *et al.* 1999a,b; Buschmann *et al.* 2001; Neori *et al.* 1991, 1996, 1998, 2003, 2004). Closed recirculation systems with bacterial filter units are being used on some abalone farms in South Africa. The disadvantages of such systems are that they are capital intensive and rather complex (see Chapter 4).

Ulva lactuca can be used as a biofilter as it is efficient in removing ammonia from the water. Cohen & Neori (1991), Shpigel & Neori (1996), Shpigel *et al.* (1997) and Neori *et al.* (1998) studied the possible utilization of *U. lactuca* as a natural filter for fish pond effluents from a fish mariculture farm in Eilat, Israel. As this farm is situated close to an oligotrophic gulf, there is a possibility of eutrophication occurring due to

runoff (Cohen & Neori 1991; Shpigel *et al.* 1997). *Ulva lactuca* is used to filter excess ammonia and other inorganic nutrients from the water before it is returned to the gulf of Aqaba. Cohen & Neori (1991) and Shpigel *et al.* (1997) found that 1 kg wwt m^{-2} of *U. lactuca* can remove over 90 % of the ammonia from fish effluents at inflow rates of 10 µmolesLhr^{-1}. Thus 10 m^2 of *U. lactuca* can remove over 90 % of the ammonia produced by 1 kg of fish feed or 75 kg of fish (Shpigel *et al.* 1997). Because both the fish and *U. lactuca* can be sold, this is an environmentally sound and economically beneficial relationship.

Ulva and *Gracilaria* were used as biofiltration organisms in South Africa for the purposes of integration (Fourie, 1994; Smit, 1997; Hampson, 1998; Morgan, 2000; Steyn, 2000; Miller, 2001; Robertson-Andersson, 2003; Njobeni, 2006). Other experiments elsewhere have shown that they are safe for human consumption and can be used as feed for macroalgivores such as abalone and sea urchins (Neori *et al.* 2004). These seaweeds have high nutrient uptake capacities and industrial culture technologies for their production are well known (Martinez-Aragon *et al*, 2002). The integration of abalone /seaweed recirculation systems could bring many benefits for the abalone industry in South Africa. Preliminary results from small-scale recirculation experiments, carried out at some South African farms, showed promising results (Anderson, 2003; Robertson-Andersson, 2003; Njobeni, 2006).

The potential benefits of seaweed/ abalone recirculation systems include the following:

- They allow the farm to be less dependent on continual seawater input;
- Pumping costs are decreased when compared to two separate farms;
- They are less vulnerable to other coastal environmental factors such as HAB's or oil spills;
- Recirculated water is of higher temperature and can be less variable than incoming seawater which can have a positive effect on the growth of abalone and seaweeds, particularly on coasts with cooler ambient sea water temperature regimes;
- These systems release fewer nutrients into coastal waters;

- The high specific growth rate and the ability of *Ulva* to grow in eutrophic conditions, as well as its nutritional content, have made it a good candidate for animal feed and for use as a biofilter (DeBusk *et al.* 1986; Shpigel & Neori, 1996; Neori *et al.* 1998);
- Reduced dependence on kelp as a feed source for abalone;
- A constant supply of nutrient rich feed (Robertson-Andersson, 2003); and
- The ability to market the abalone as being fed a completely natural diet and the farm participating in environmentally friendly aquaculture, thus potentially allowing the abalone to be marketed as being "organic abalone" and have an organic certification which allows for product and market niche-ing.

Disadvantages of such a recirculation system are:

- Potentially increasing the particulate concentration, with subsequent negative effects on abalone health;
- Increasing the residence time of the water can, potentially, result in the build up of :
    - Toxic constituents both in the water and in the abalone
    - Invertebrate fauna
    - Bacterial densities
    - Pest species and parasites

Developing recirculation and integrated abalone farming systems could potentially increase the occurrence of disease in the culture system, and thereby reduce benefits of these systems. With the rearing of two different hosts in the same facility there is a possibility of contagion of pathogens between them (Starliper & Morrison, 2000). Recirculation closes the culture system and pathogenic bacteria can become a problem due to the lower water exchange rate and longer water retention time.
High numbers of pest species and parasites can also negatively impact the cultivated aquaculture species due to grazing on seaweeds (Nicotori, 1977; Shacklock & Croft, 1981; de Oliveira *et al.* 1989; Buschmann *et al.* 2001; Smit *et al.* 2003), or stressing the cultured animals, and in the case of abalone, macro fauna have been known to

consume living abalone (Kuris & Culver, 1999; Chalmers, 2002; farmers' pers. comm.).

Long-term growth rates, health, water quality, physiochemical variables and sediment production of abalone in a recirculation system vs. abalone in a flow-though system have not yet been included in any comprehensive analysis that also covers economical aspects. This study was initiated to monitor abalone specific growth rates (SGR) and health of abalone fed a single diet (kelp only) in two cohorts, through regular monthly sub-sampling in a 25 % and a 50 % recirculating unit, with a flow-through unit as a control. Sub samples were compared against tank biomass data obtained from farm grading data. In addition water quality was monitored over four 72 hour periods, to compare temperature, pH, dissolved oxygen, ammonium and phosphate. The study period which incorporated several smaller studies (bacteria, mobile macro-fauna and sediments) commenced in September 2003 at 25 % recirculation and was completed in May 2005. This was done to gain a complete seasonal data set and allow for seasonal overlap. A second cohort was placed in the tanks in late June 2005 and was cultivated until January 2006 with the recirculation rate being 50 %. Small scale experiments were run with these animals looking at the effects of higher recirculation rates (50 % and 75 %) on water quality and sediment production.

MATERIALS AND METHODS

Experimental setup

The experiment was performed at I & J (see Chapter 5) in the farms concrete flow through tanks (6.6 m l x 2.08 m w x 0.88 m d; ± 12 000 L). The experimental units were designed and built in August 2003, and consisted of 3 separate integrated units and three separate flow-through units. In September 2003 the units were stocked with abalone 15 g (± 2.5 g) in weight, from the same brood stock. The abalone culture tanks contained 24 Ivey Blue up™ baskets and there were 550 abalone per basket. In September 2004, all the abalone of the experiment were graded and the stocking density was reduced to 450 animals per basket. Ninety kg of fresh kelp per week, were fed to the animals, for the duration of the experiment (60 kg on Mondays and 30 kg on Fridays). The average protein content of the kelp was 7.8 % (Simpson, 1994; Simmons, 1990). The abalone tanks were drained and cleaned once a week as per farm cleaning methods.

The 6 experimental seaweed tanks were 5 m x 1 m surface area and 0.6 m deep with an outlet 17cm from the top. They were made of white PVC lining (to facilitate easy cleaning and to reduce light absorption) supported on a frame. The PVC lining was rounded on the bottom. The tanks were aerated by a 30 mm PVC pipe that ran along the bottom centre of the tank. Holes (3 mm) were spaced evenly every 250 mm along the pipe and the air was supplied by a Howard & Donkin channel blower. The seaweed tanks were stocked with a starting biomass of 10 kg (2 kgm^2 stocking density) of *Ulva lactuca* per tank and were harvested every 14 days to measure growth rates. The tanks were shaded with a 50 % shade cloth from September to February. Four additional seaweed tanks that were being fed fertilized abalone waste water at 12 volume exchanges per day without recirculation (approximately double the water volume that each integrated seaweed tanks received), were used to compare seaweed growth rates and seaweed tissue properties. The fertilizer consisted of a 100g mixture of Maxiphos® and ammonium sulphate in a ratio of 1:6. Three abalone tanks were on a flow-through system receiving 6 000 L per hour (Figure 6.1). Three additional abalone tanks had their waste water gravity-fed to two seaweed tanks each. After the water passed through the 2 seaweed tanks 25 % (1

500 L) of it was pumped by a 2 KW pump back to the abalone tank from which it originated, 4 500 L h^{-1} (75 %) of seawater was supplied from the same source as the flow-through units (Figure 1). The overflow water exited the units from the seaweed tanks. The recirculation ratio of 25 % was chosen for the long term monitoring. A small scale study done, over 72 hours investigating physico-chemical variables at 50 % recirculation, showed that the dissolved oxygen concentration very low and this may have detrimental effects on the abalone in a long term study (see Harris et al., 1999).

Flow rates, stocking densities and other parameters relevant to *Ulva lactuca* cultivation in abalone effluent in the available seaweed tanks had been determined by Robertson-Andersson (2003). The data from this study were used in the design and set-up of the integrated system, in conjunction with abalone cultivation parameters (as determined by the existing abalone cultivation conditions). The integrated units were set up with a ratio 20 kg of abalone to 1 kg of seaweed based on the limitations of the abalone cultivation and optimization of seaweed growth and biofiltering efficiency as determined by Robertson-Andersson (2003).

Figure 6.1: The integrated seaweed abalone unit at I & J. Arrows show direction of water flow.

Abalone sub-samples

Samples were taken from tanks from January 2004 to May 2005 at 25 % recirculation and from June 2005 to January 2006. At each sampling 50 animals were randomly removed from every basket in the tank. Length and weight measurements were in accordance with the methods listed in Chapter 4. Of the 104 660 animals that were part of the experiment at each monitoring period, 300 were randomly sampled.

Grading

Abalone grading data were only available for September 2004. The grading was done on the farm as per grading methods. Animals were harvested from a tank, the tank number and age group recorded. Animals were then mechanically graded into different size classes and the weights of these size classes were recorded for a total biomass value. The values from this grading were then compared to monthly size frequency distributions. The sub-sample weight data and the differences between the two biomass values were then calculated.

Condition factor

The condition factor was measured according to the equation (from Britz, 1996a-c) listed in Chapter 4.

Abalone health

5 sub-samples of abalone were removed from every experimental tank, every three months by Dr. A. Mouton. The health of these animals were analysed using the standard South African veterinarian procedures and data was reported in commercial health reports (Mouton, 2004). Veterinary aspects investigated included: counting the number of sabellid *Terebrasabella heterouncinata* tunnels on the growing edge of the shell; investigating the gonad histology to determine gonad maturity; a histological examination of all organs to determine parasite status (expressed as the percentage of sample infected); whether or not the animals were experiencing some form of environmental stress (expressed as either being present or absent); and a histological examination of the digestive gland to determine the general condition of the abalone (see Table 4.2 in Chapter 4). In addition, animals from both the flow-

through units and the 25 % recirculation (Recirc.) units were compared with animals from the farms own health reports.

Water quality and physiochemical variables

Temperature, pH and dissolved oxygen were recorded daily from January 2004 to January 2006, for long term monitoring in the flow-through abalone tanks, the seaweed tanks, incoming seawater and the integrated seaweed/ abalone tanks at 08h00, 12h00 and 16h00. Monitoring for the purpose of this report was done intensively during three, 36 hour periods in September 2003 – spring, January 2004 - summer and June 2004 - winter. Only data for September 2003 will be illustrated although maximal and minimal ranges for the summer and winter will be reported. During this water quality monitoring period water samples for ammonium, phosphate, nitrate and nitrite were taken every 4th hour and analysed according to the methods described in Chapter 4.

The 72 hour water quality testing begun on the third day of a cleaning cycle (i.e. 3 days after all sludge had been removed from the floor of the tank). This was done to allow the system to settle after the weekly cleaning and allow for a possible maximal build up of nutrients in the system. Water quality experiments were also not performed directly after a weekend as the abalone go through a two day forced fasting. No additional feed was added into the tank between a Friday feed and the Monday afternoon feed.

During monitoring for physiochemical variables, water samples for ammonium and phosphate were taken and analysed according to the methods listed in Chapter 4. FAN was calculated according to the methods in Chapter 4. Bredberg (2003), Potgieter (2005) and Lindström (2006) used the same methods described in Chapter 4 to analyse water samples.

Seaweed growth rate

Seaweed Specific Growth Rate (SGR in % wet weight day^{-1}) and yield (Y = g wet wt m^{-2} d^{-1}) was determined according to Evans (1972) and calculated as:

$$SGR\ \% = 100 \times [\ln(W_t / W_0)] / (t_t - t_0)$$
$$Y = [(W_t - W_0) / t] / SA$$

Where W_0 and W_t are initial and final wet weights (wt) in grams, t_0 t_t are initial and final times in days, respectively, and SA is the surface area.

Because a brown algal epiphyte *Myrionema strangulans* Greville was found detrimental to seaweed SGR if present in high numbers by Robertson-Andersson (2003), a modification of the Braun-Blanquet (B-B) percentage cover-abundance scale was developed to measure percentage cover of *M. strangulans* on individual thalli. An *Ulva* thallus was placed on a white board and then the percentage coverage of *M. strangulans* was estimated. Five thalli were taken from each seaweed tank. The range of the B-B values given as percentage cover is as follows: 0 % coverage = 1; 1 - 10 %coverage = 2; 11 - 25 % coverage = 3; 26 - 50 % coverage = 4; > 50 %coverage = 5.0

Seaweed tissue nitrogen & phosphorus

Samples were taken from the 25 % seaweed recirculation tanks and from fertilized tanks to record dry to wet weight ratios and for biochemical analysis. After each weighing, the seaweed samples collected were washed in distilled water, and visible epiphytes and epifauna were removed. After washing, the samples were spun in a salad spinner for 1 minute, weighed on an OHAUS electronic balance to 2 decimal places, oven dried (70 °C, 72 hours – as per Duke *et al.* 1989a,b) and then reweighed. The dried seaweed was ground using a mechanical grinder with a maximum mesh size of 1 mm. The powder was stored in sealed glass jars in a desiccator at room temperature.

Total nitrogen was determined using the micro-Kjeldahl technique (Solorzano, 1969). The protein content was determined by multiplying the N concentration obtained from the micro-Kjeldahl technique by a factor of 6.25, based on the protein N content of 0.16 $g.g^{-1}$ from methods described by Fleurence *et al.* (1995). It must be mentioned

that the conversion factor used, although commonly accepted, includes N not in the form of protein but intracellular reserve pools of N as well (Fleurence *et al.* 1995). Therefore the micro-Kjeldahl method used would tend to overestimate the actual protein content. Phosphate (PO_4^{3-}) concentration was determined using the tri acid digestion method described by Murphy & Riley (1962).

Bacteria

A once off study on bacteria was done by Flodin (2005) and these methods are reported in Chapter 5. Seaweeds were sampled by cutting pieces of *Ulva* and placing them inside a sterile screw-capped tube. Pieces were cut to roughly make up 1 ml of the tube and rinsing the seaweed with sterilized artificial seawater (3%) was done to remove bacteria present in water. Seaweed samples were dried at 65°C for four days and CFU/g dry weight was calculated.

Mobile and sessile macro fauna and flora

A once off study of mobile macrofauna was done by Hansen (2006), and these methods are reported in Chapter 5. During harvests, notes were made of the species of epifauna and flora that occurred in the tanks. If they were collected, they were preserved in 10 % formaldehyde in seawater, until they could be identified. Because the herbivorous gastropod, the Cape keyhole limpet, *Fissurella mutabilis* (Sow.), was found to be so devastating to the seaweed in previous studies (see Robertson-Andersson, 2003; Hansen *et al.* 2006;Njobeni, 2006), a modification of the Braun-Blanquet (BB) percentage cover-abundance scale was developed to compare numbers of keyhole limpets, rather than measuring weights and ratios. A 25 cm quadrat was placed on the side walls of the recirculation tanks and kelp fed tanks and numbers of limpets in the quadrats were noted. The range of the BB values given as percentage cover is as follows:

0 limpets present = 1; 1 – 3 limpets = 2; 4 – 7 limpets = 3; 8 – 10 limpets = 4; > 10 limpets = 5.

Sediments

Sediment loading and particle fractionation of the water column and settled sediment were tested by Potgieter (2005) and Brandt (2006). The methods they used are listed in Chapter 5.

Environmental events

Long term temperature monitoring showed that the units were vulnerable to external environmental events, that were explained using a series of satellite images, data and tools obtained from www.remarinesa.org.za of Sea Surface Temperatures (SST).

Statistical analysis

All data were expressed as means ± standard errors. All data were tested for normal distribution. The analysis for this study was done using STATISTICA V6.1. For abalone growth data, an initial analysis of co-variance was first tested with the baseline value of the outcome, i.e. either length or weight used as a covariate. This was done to account for any differences in starting values. Where starting data were non-similar the data were forced to a common mean and the absolute means were adjusted by the difference. Graphs where this was applicable are listed in the text. To test for actual differences ANOVAS were performed on the data. All data were regarded significant at $p < 0.05$.

For physiochemical and water nutrients data, one-way and factorial ANOVAs were performed after verifying normal distribution and homogeneity of variances. All values for nitrate and all ammonium analyses comparing "flow-through units" and "25 % Recirc." and comparing "seaweed" and "25 % Recirc." were transformed logarithmically to obtain homogeneity of variance with the Cochran Test. Long term physiochemical variables were analysed by 5 and 10 point running means and ratios of these means (e.g. (variable a) over (variable b)).

RESULTS

Abalone growth rates

September 2003 to May 2005 – 25 % recirculation

There was a two month phase in the beginning of the experiment in which there were no differences in abalone growth or length between the two systems, indicating that the experimental animals had a two month acclimatization phase. Average length increases in the 25 % Recirc. units were 1.42 mm a month (mth^{-1}) vs. 1.38 mm m^{-1} in the control flow-through units for the duration of the experiment. Abalone in the 25 % Recirc. units grew best in autumn and winter, with growth rates of over 4 mm mth^{-1} being measured in these months. Abalone weight increases in the 25 % Recirc. units were 3.1 g mth^{-1} vs. 2.8 g mth^{-1} in the flow-through units. There were no significant differences between the systems in either abalone length or weight from September 2003 until September 2004. This was two months after the September grading where the stocking density was reduced. In October and November 2004, abalone in the 25 % Recirc. unit had significantly higher growth rates than the flow-through units (ANOVA; $p < 0.05$; df = 198). During the summer months abalone in the flow through units started to grow faster with the end result being that was there no significant difference in weight or length between either unit (see Figures 6.2 and 6.3). Regression analysis of length vs. weight showed a positive correlation ($y = 2.0951x - 80.497$; $r^2 = 0.9613$; $p < 0.05$ for the abalone in the FTU and $y = 1.9889x - 76.006$; $r^2 = 0.9074$; $p < 0.05$ for the abalone in the 25 % Recirc. units). Exponential curve fitting to the weight data showed that abalone in the 25 % Recirc. units ($y = 3E\text{-}44e^{0.0027x}$; $R = 0.9582$; $p < 0.05$) would reach harvest size (100 g) 35 days sooner than the FTU ($y = 3E\text{-}40e^{0.0025x}$; $R = 0.9557$; $p < 0.05$).

The farm graded biomass increased from September 2003 to September 2004 by 275 % (144 kg) and 256 % (134 kg) in the 25 % Recirc. and the Control flow-through units, respectively. Using the sub sample data to calculated biomass and multiplying it to the total number of animals, both units increased in biomass by 285 % (149 kg). This was a 2.16 % underestimate for the 25 % Recirc. units and a 5.12 % overestimate for the flow-through units compared to the data from the grading. From

September 2004 to May 2005 the calculated biomass increase in the flow-through units was 81 % (77 kg) and 73 % (119 kg) in the 25 % Recirc. units, which was not significantly different.

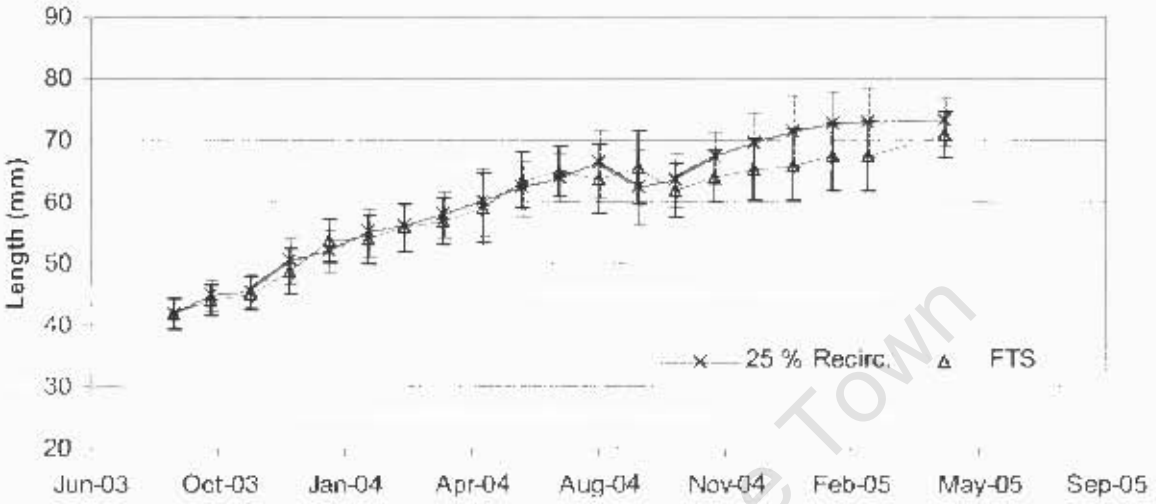

Figure 6.2: Length (mm) increase of abalone in a 25 % Recirc units vs. flow-through units on a kelp only diet from September 2003 to May 2005 (n= 4 000).

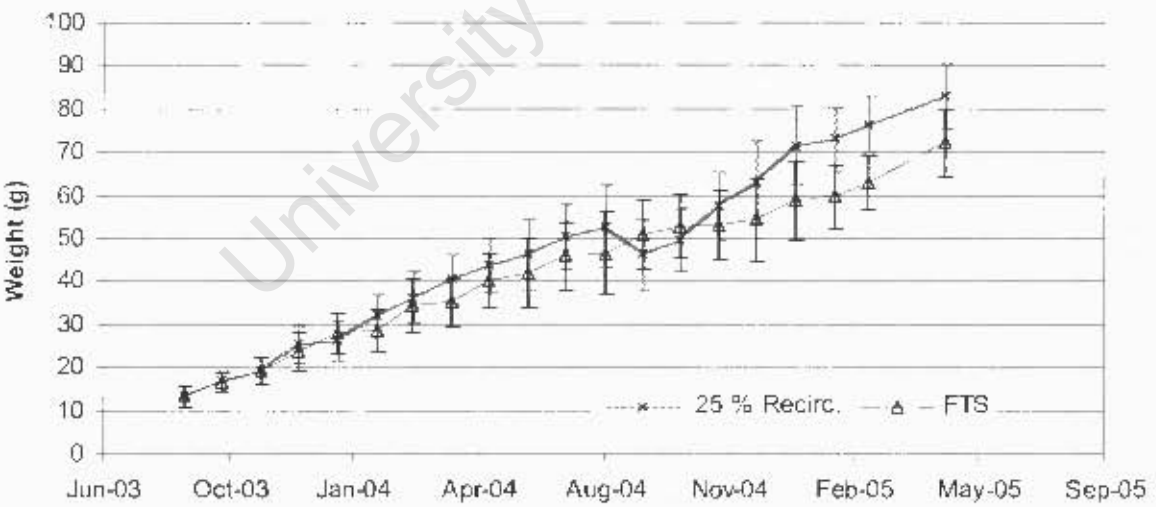

Figure 6.3: Weight (g) increase of abalone in a 25 % Recirc. units vs. flow-through units on a kelp only diet from September 2003 to May 2005 (n= 4 000).

A frequency distribution of weights from the grading done in September 2004 and the final weight measurements show that there was no significant difference in the mean greatest size of the abalone (see Figure 6.4).

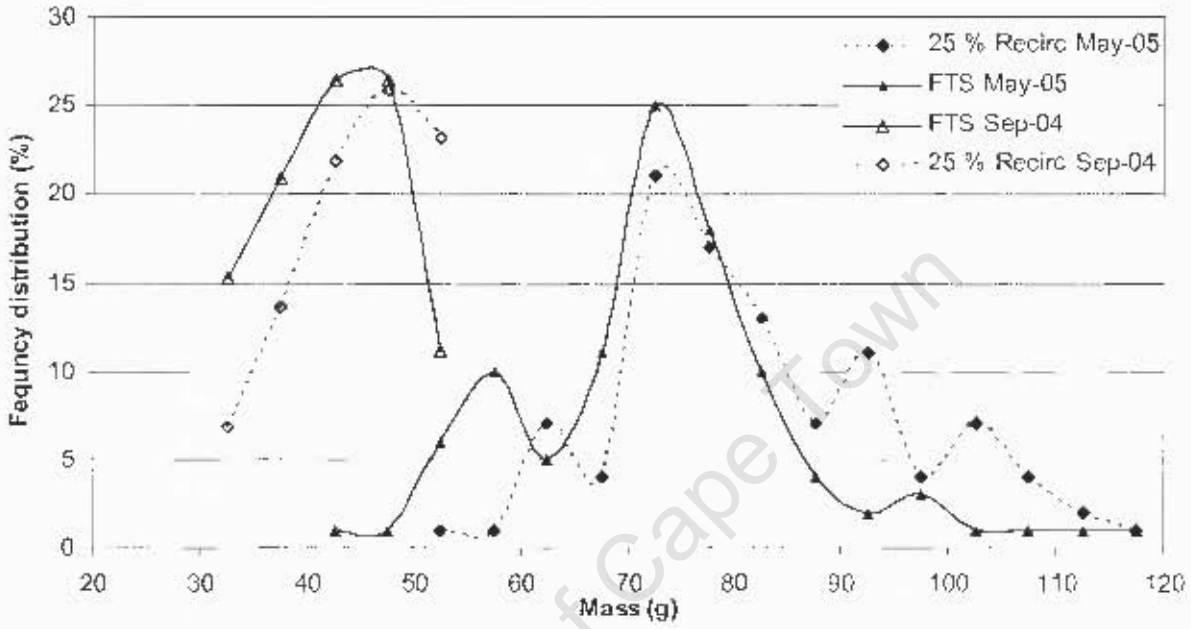

Figure 6.4: Frequency distribution of weights following final measurements in May 2005 and of first grading in September 2004 of abalone in a 25 % Recirc units vs. flow-through units from September 2003 to September 2004 fed a kelp only diet (n= 100).

June 2005 to January 2006 – 50 % recirculation

The animals in this experiment were not the same starting size so the data were forced to a common mean and the graphs represent this. As with the 25 % Recirc. experiment, abalone growth rates for the initial two months showed no significant difference. The average monthly increases in size are shown in Table 6.1. There was no significant difference in abalone length at the end of the experimental period (see Figure 6.5). The weight of the animals showed a significant difference with the 50 % Recirc. animals being heavier than the flow-through units (ANOVA; $p < 0.05$; df = 198) (see Figure 6.5). Regression analysis of length vs. weight showed a significant positive relationship. Exponential regressions of weight showed that

animals the 50 % Recirc. would reach harvest weight 77 days sooner than the animals in the FTU (see Table 6.1).

Table 6.1: Average increase in size and weight per month of abalone in flow-through units vs. a 50 % Recirc units fed a kelp only diet. Table includes a length vs. weight regression analysis and an exponential regression analysis of weight and time to reach harvest size from size at experiment initiation.

DATE	50 % RECIRC		CONTROL	
	Size	Weight	Size	Weight
2005/08/19	2.22	1.79	2.23	3.63
2005/09/20	1.16	1.44	1.31	0.53
2005/10/18	1.85	4.80	2.36	4.02
2005/11/18	1.04	3.13	1.20	2.91
2005/12/29	2.03	3.09	2.65	5.08
2006/01/18	2.67	5.81	0.41	2.64
Average	**1.83**	**3.35**	**1.69**	**3.14**
Weight vs length	$y = 1.9346x - 73.856$		$y = 1.8335x - 69.182$	
r^2	0.9778: $p < 0.05$		0.9752: $p < 0.05$	
Weight exponential regression	$y = 5E\text{-}64e^{0.0030x}$		$y = 3E\text{-}54e^{0.0033x}$	
R value	0.9995: $p < 0.05$		0.9925: $p < 0.05$	
Time to reach 100 g	393		470	

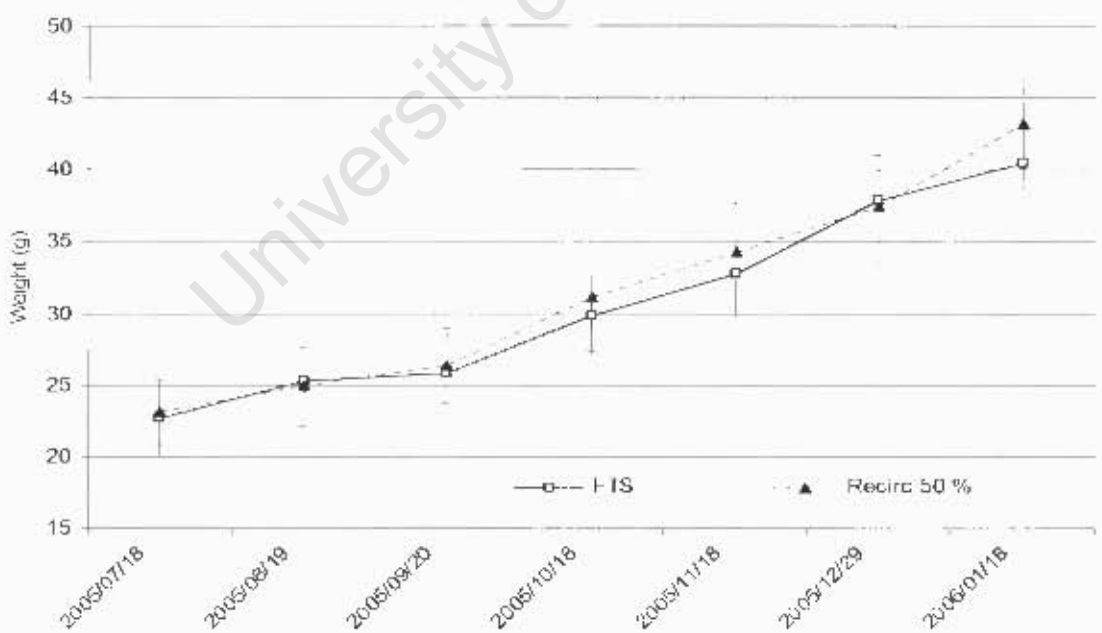

Figure 6.5: Weight (g) increase of abalone in a 50 % Recirc unit vs. flow-through units on a kelp only diet from July 2005 to January 2006 (n= 1 400). Data have been altered to show common means for starting values.

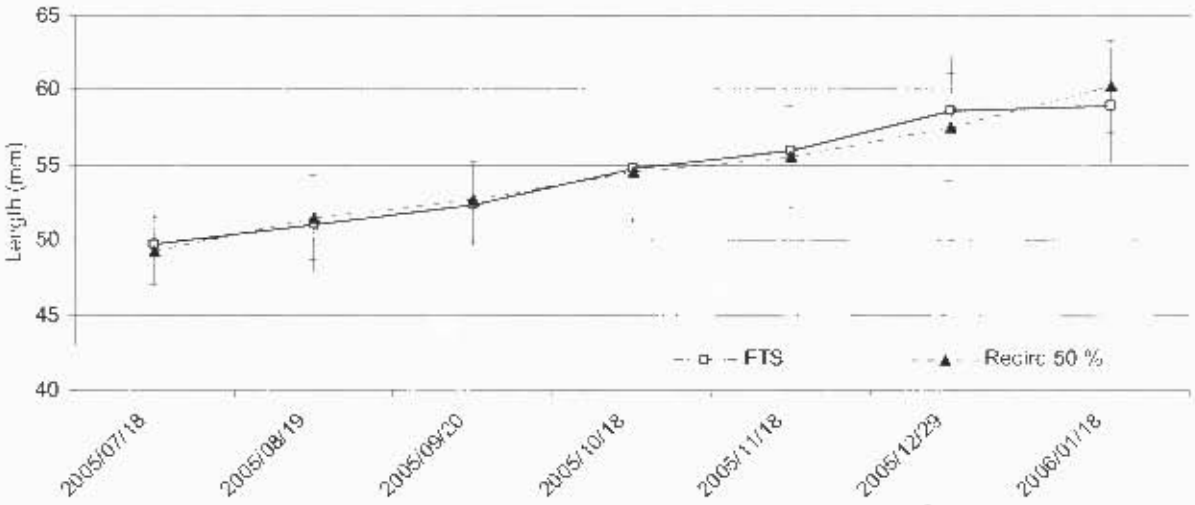

Figure 6.6: Length (mm) increase of abalone in a 50 % Recirc. units vs. flow-through units on a kelp only diet from July 2005 to January 2006 (n= 1 400). Data have been altered to show common means for starting values.

A frequency distribution of weights at the end of the experiment shows that the 50 % Recirc. units had a higher percentage of animals in a larger size class compared to the flow-through units (47.5 g vs. 37.5 g) (see Figure 6.7).

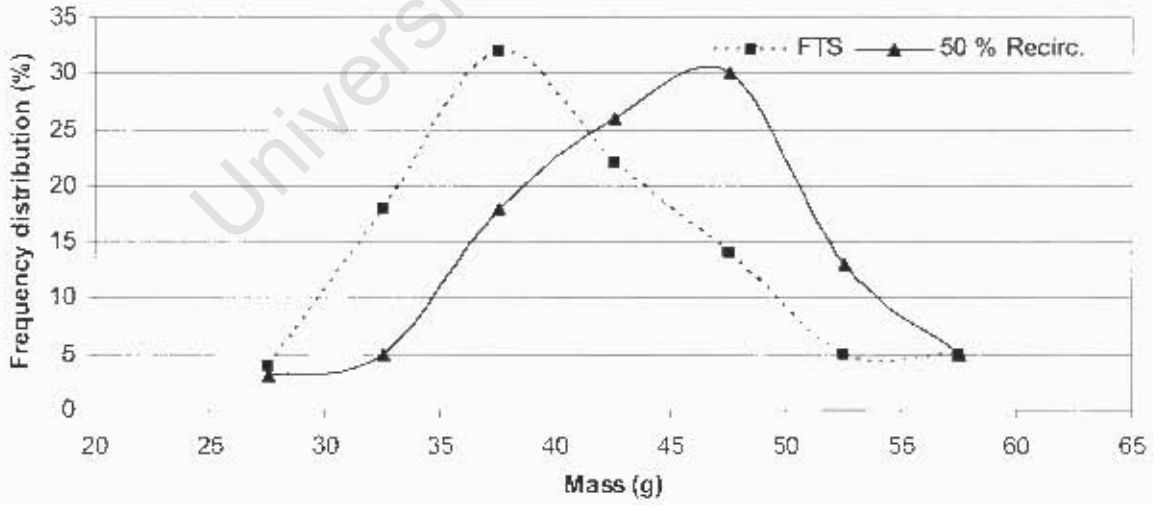

Figure 6.7: Frequency distribution of weights following final measurements in January 2006 of abalone in a 50 % Recirc units vs. flow-through units from July 2005 to January 2006 fed a kelp only diet (n= 200).

Abalone health

Sabellids – Refer to Figure 6.8

February 2004 saw the flow-through units having a significantly lower sabellid infestation prevalence and intensity (ANOVA; $p < 0.05$; $df = 8$). In April 2004, sabellid scores were high in both systems, and evidence of shell boring polychaetes was found in the recirculation animals only. Sabellid scores decreased in all units in June but were significantly lower in the flow-through units (ANOVA; $p < 0.05$, $df = 8$). The last health measurement showed an increase in sabellid counts. No evidence of shell boring polychaetes were found.

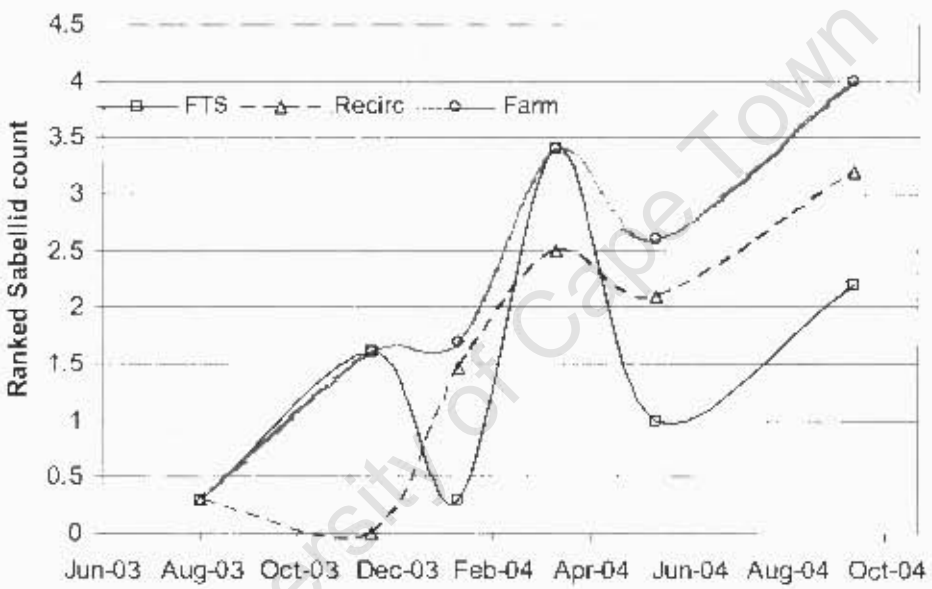

FIGURE 6.8: Sabellid count of animals in a 25 % Recirc. units vs. flow-through units with farm health status data for comparison. (n = 3).

General condition – Refer to Figure 6.9

In April 2004, animals in both systems had shiny shells, indicative of poor water quality. Evidence of good shell growth was present in June in the flow-through units and growth was moderate to good in the 25 % Recirc. units. Towards the end of the experiment the general condition of the 25 % Recirc. units animals improved with the result that there was no significant difference in general condition between either of the two systems. The condition of animals in both systems was significantly better compared to that of the animals on the rest of the farm (ANOVA; $p < 0.05$; $df = 8$).

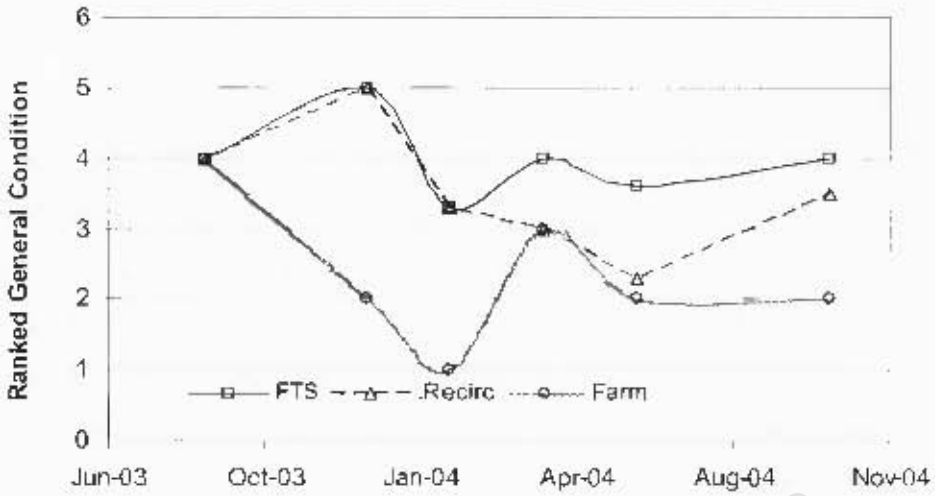

FIGURE 6.9: General condition of animals in a 25 % Recirc. units vs. flow-through units with farm health status data for comparison (n = 3).

Environmental Stress – Refer to Figure 6.10

The only month in which abalone in the 25 % Recirc. units showed signs of environmental stress in their digestive gland histology, was in December 2003. This corresponded to high temperatures (20.5 °C) and high pH (8.00) in the 25 % Recirc. system.

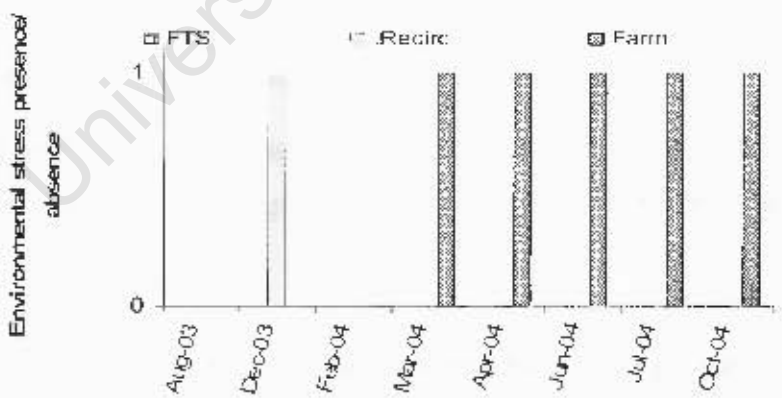

FIGURE 6.10: Presence or absence of environmental stress in animals in 25 % Recirc. units vs. flow-through units with farm health status data for comparison (n = 3).

Ranked Gonad condition – Refer to Figure 6.11

There were no significant differences in gonad condition, except in the last measurement made in the unit (ANOVA; $p < 0.05$; df = 5). This is a measure of the sexual maturity of the animals and is of critical importance for management. Compared with animals in the rest of the farm whose gonads showed a mixture of developmental stages of sex cells, the gonad development was lower for recirculation units with only immature sex cells present, while the animals in the flow though units remained immature. The high gonad maturity of the farm animals in April and October 2004 correspond to natural spawning seasons of wild abalone (Hahn, 1989).

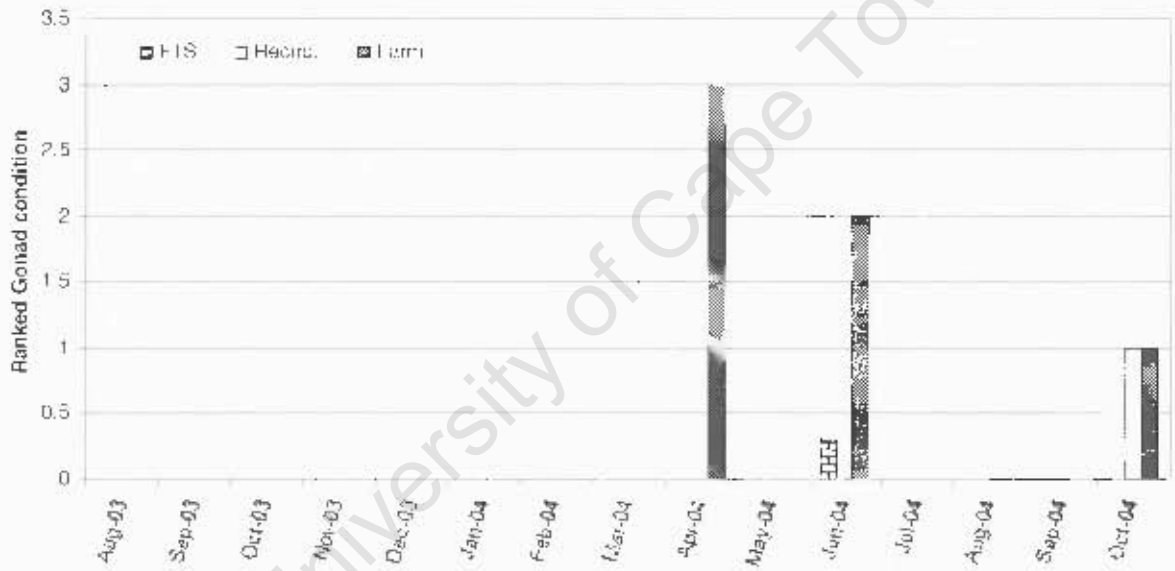

FIGURE 6.11: Gonad condition as an indicator of abalone sexual maturity in 25 % Recirc. units vs. flow-through units with farm health status data for comparison (n = 3).

General Histology – Refer to Figure 6.12

Histological examinations of the animals showed no differences between systems, with both having gut protozoa present (10 – 50 % infected - due to the kelp diet) and the presence of a *Rickettsia*-like intracellular prokaryote (10 – 30 % infected). Histologically, the animals in both treatments were in a better condition than the rest of the farm animals (50 – 100 % infected with *Coccidia*, *Rickettsia*, gut protozoa and

gill inflamation). The animals in the 25 % Recirc. units did not show any pathology after February 2004.

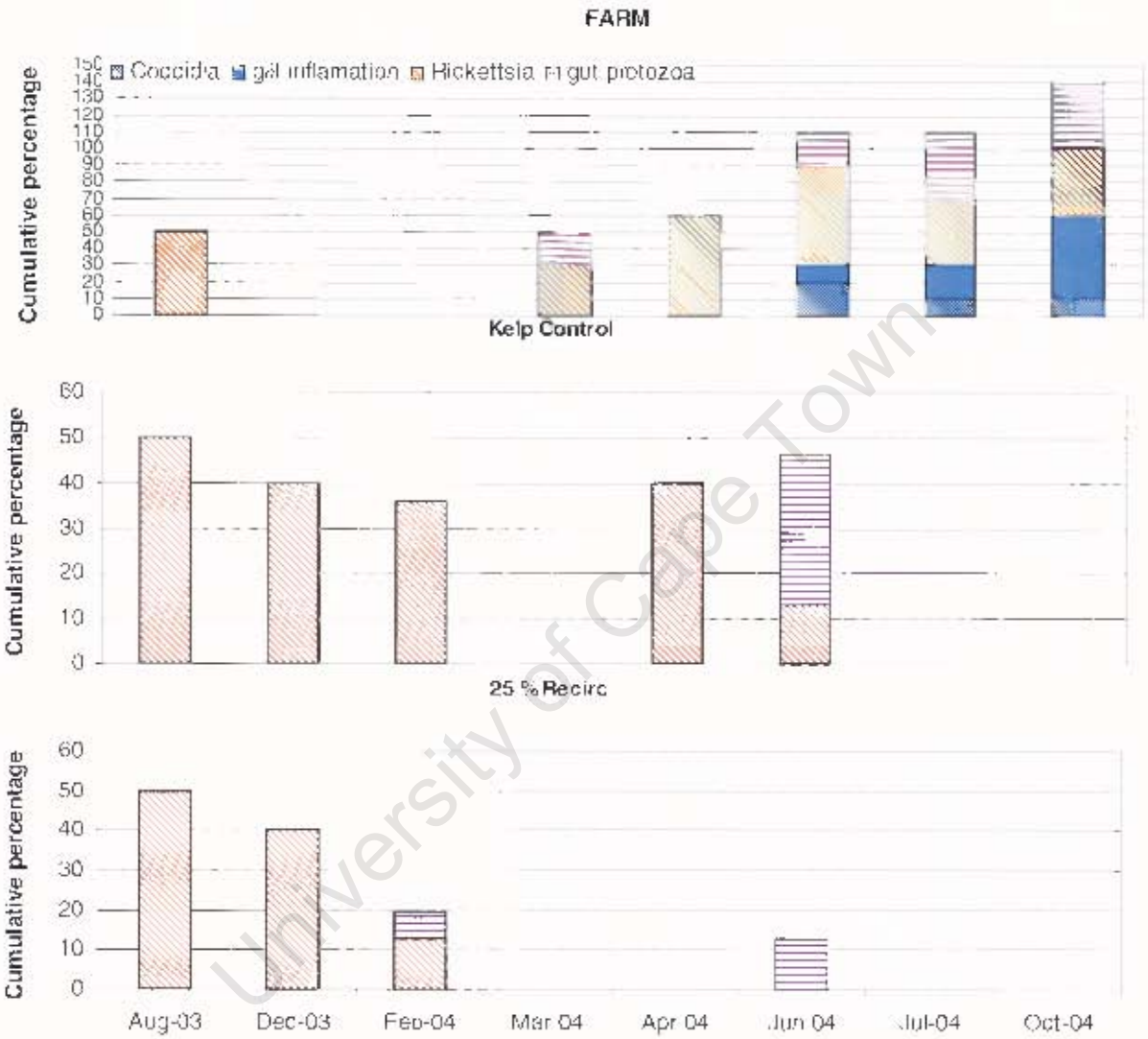

FIGURE 6.12: Cumulative percentage of parasites found during histological examinations in animals in 25 % Recirc. units vs. flow-through units with farm health status data for comparison (n = 3).

Water quality

Physiochemical variables at September 2003 to May 2005

Temperature

The seaweeds and 25 % Recirc. abalone units showed a clear diurnal temperature rhythm with the highest temperatures in the tanks being recorded at 16h00 in summer, winter and spring and lowest temperatures being recorded at 00h00 (see Figure 6.13). There was a very close correlation with the 25 % Recirc. abalone units being affected by the temperature in the seaweed tanks ($df = 42$; $r = 0.85$; $p < 0.0001$). In January 2004 (summer), the temperatures in 25 % Recirc. abalone units (18.1 °C average) was significantly higher than the ambient seawater (16.25 °C average) as well as the control flow-through units (16.5 °C average) (ANOVA; $df = 42$; $p < 0.05$). The range of temperature was 5 °C for both systems.

In June 2004 (winter), during the day, the seaweed tanks helped to increase the temperature of the 25 % Recirc. abalone units above that of the incoming seawater and the flow-through units. At night the seaweed tanks lost a lot of heat, and this helped to significantly decrease the temperature in the 25 % Recirc abalone units (ANOVA; $df = 28$; $p < 0.05$) compared to the flow-through units. The average temperature in the 25 % Recirc. tanks (14.6 °C) was lower than the flow-through units (15.4 °C). The minimal temperature experienced in the 25 % Recirc. tanks was 13.4 °C vs. 14.8 °C in the flow-through units. The maximal daily temperature range was greater in the 25 % Recirc. abalone units (3 °C) compared to the flow-through units (1.9 °C). September 2003 (spring), temperatures were similar to winter and showed that the 25 % recircultion units lost heat through the seaweed tanks at night, while during the day they imparted heat to the recirculation units.

Dissolved oxygen

In January, the dissolved oxygen in the 25 % Recirc. units was significantly lower (6.7 mg L^{-1} average) than the flow trough units (7.7 mg L^{-1} average) or the seaweed units (8.9 mg L^{-1} average) (ANOVA, $df = 42$; $p < 0.05$). Although the dissolved oxygen in the seaweed tanks was high this oxygen was not being transferred to the 25 % Recirc abalone units. In addition, during periods of darkness and particularly around 04h00 in the 25 % Recirc. abalone units, the dissolved oxygen concentration

decreased to below 5.4 mg L^{-1} and was significantly lower than the flow-through abalone units (ANOVA, df = 28; $p < 0.05$).

In June, the dissolved oxygen in the 25 % Recirc. abalone units ranged from 7.5 to 6.3 mg L^{-1} with an average of 6.9 mg L^{-1}compared to the flow-through units which ranged from 8.32 to 6.83 mg L^{-1} with an average of 7.56 mg L^{-1}. The latter was significantly higher (ANOVA; df = 28; $p < 0.05$). The oxygen in the seaweed tanks had a significantly higher concentration (10.1 – 7 mg L^{-1}; average 8.28 mg L^{-1}) (ANOVA; df = 42; $p < 0.05$). The dissolved oxygen values were higher than those found in summer and the range for all treatments was smaller when compared to the summer values. Spring dissolved oxygen concentrations were similar to winter and the seaweeds showed a diurnal rhythm in oxygen production (see Figure 6.14). Between 20h00 and 04h00 the 25 % Recirc abalone units had significantly lower dissolved oxygen values compared to the flow-through units 6.3 mgL^{-1}.

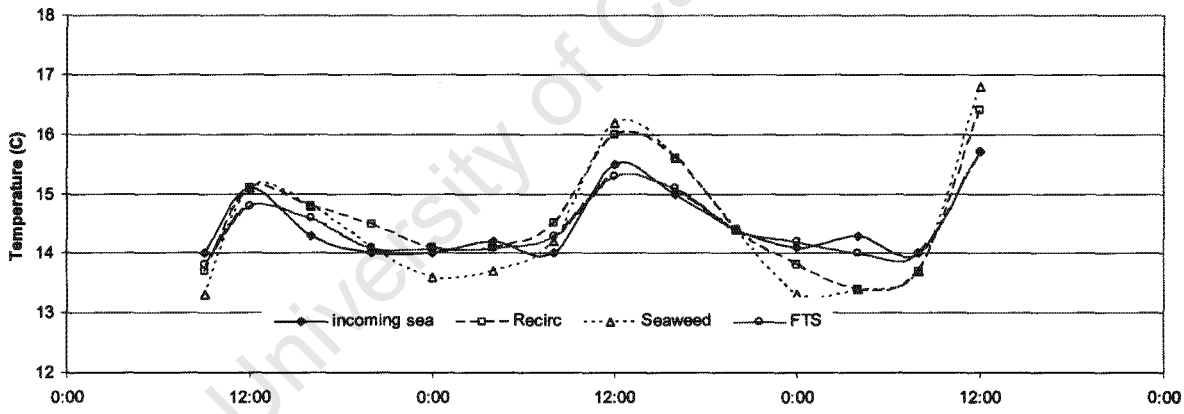

FIGURE 6.13: Temperature (°C) in September 2003 of 25 % Recirc. abalone units flow-through units, seaweed tanks and incoming seawater for comparison (n = 3).

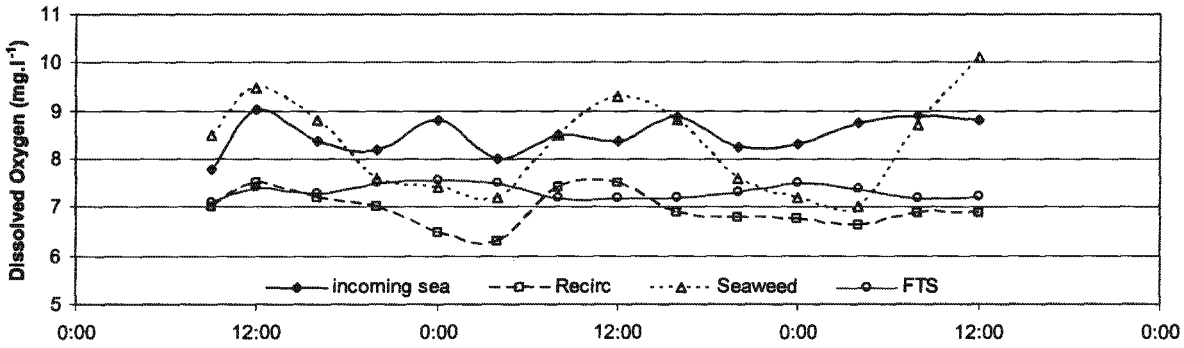

FIGURE 6.14: Dissolved oxygen (mg L^{-1}) in September 2003 of 25 % Recirc. abalone units, flow-through units, seaweed tanks & incoming seawater for comparison (n = 3).

pH

The pH in all tanks, and at all three seasons, showed a clear diurnal rhythm, but was exacerbated in the 25 % Recirc. abalone units and the seaweed tanks . There were no significant differences in pH between the flow-through units and the 25 % Recirc. units in any of the seasons. In the flow-through units the pH ranged from 7.73 - 8.18 with an average of 7.9, while the 25 % Recirc. abalone units ranged from 7.8 - 8.2 with an average of 8.0. The seaweed tanks had a higher variability (7.8 - 8.4) with an average of 8.01.

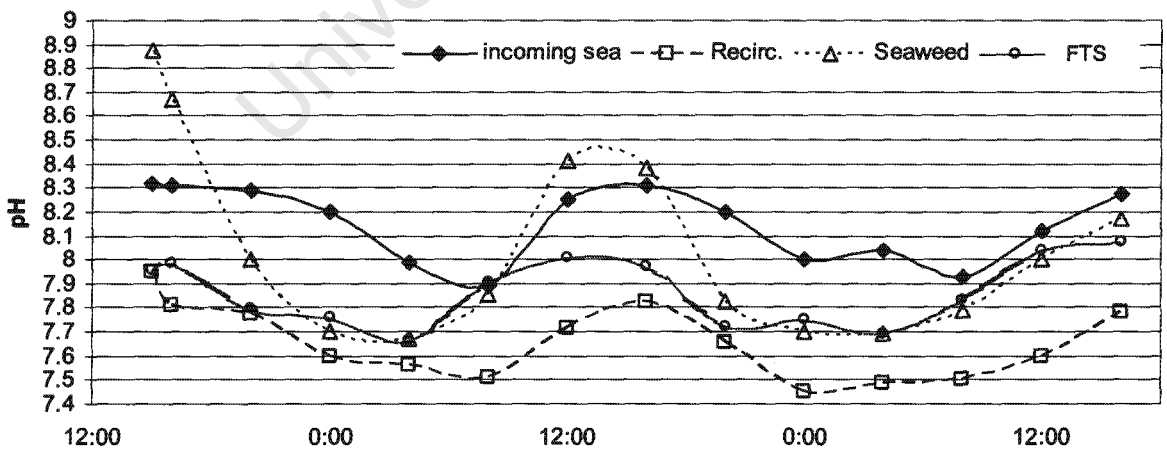

FIGURE 6.15: pH in September 2003 of 25 % Recirc. abalone units, flow-through units, seaweed tanks and incoming seawater for comparison (n = 3).

Long term physiochemical variables

Long term physiochemical variables are shown in Figures 6.16 – 6.20. These show the average daily temperature, pH and dissolved oxygen contents in the seaweed, flow-through units and the 25 % and the 50 % Recirc. abalone units. Dissolved oxygen at 25 % Recirc. in the abalone units, was on average 4 % (± 0.5) lower than the flow-through units. Temperature was 1 % (± 0.2) higher and pH was 1 % (± 0.1) lower in the 25 % Recirc. abalone units as compared to the flow-through units over the whole experimental period. Dissolved oxygen at 50 % Recirc. was 9 % (± 6) lower than the flow-through units. Temperature was 2 % (± 0.3) higher and pH was 2 % (± 1) lower in the 25 % Recirc. abalone units compared to the flow-through units. The flow-through units temperature was 1 % (± 0.3) higher than the incoming seawater through out the experimental period.

There was an inverse relationship between temperature and oxygen in the 25 % Recirc. abalone units and the flow-through units with less oxygen being available in the water at higher temperatures ($y = -0.3985x + 1.3383$; $df = 24834$; $r = 0.2$; $p < 0.05$). On average the dissolved oxygen in the 25 % Recirc abalone units was 33 % (± 6) lower than in the seaweed tanks with the pH being 2 % lower (± 0.6). The seaweed tanks and the 25 % Recirc. abalone units had temperatures that did not significantly differ from each other. There was a positive correlation between temperature and dissolved oxygen ($y = 0.1244x + 0.6401$; $df = 24834$; $r = 0.25$; $p < 0.05$), due to the production of oxygen when the seaweeds were photosynthesising.

At 50 % Recirc., the temperature in the 50 % Recirc. abalone units was 3 % (± 0.4) higher and pH was 1 % (± 1) lower than the flow-through units. The temperature between the 50 % Recirc. and the seaweed tanks were similar. The pH was 2 % (± 0.2) lower in the 50 % Recirc. abalone units and the dissolved oxygen was 36 % (± 7) lower than the seaweed tanks.

Environmental events

In Figures 6.16– 6.20 six environmental events occurred: Theses events can be divided into two types:

Event A: from 26th February until 10th March 2004 – advection;

Event B: 18th March to the 26th April 2004 – advection;
Event C: 21st April to 4th May 2004 – warm water intrusion;
Event D: 24th January to 7th February 2005 – warm water intrusion;
Event E: 22nd February to the 3rd of March 2005 – advection; and
Event F: 17th of March until the 1st of April 2005 - advection.
All are caused by a decrease in wind-forcing, which leads to the event formation. The more common event (4 out of 6) is a decrease in upwelling and increase in solar advection resulting in increased temperatures and high nutrient availability of coastal waters. The other type of event is a warm water intrusion over the Western Agulhas Bank (WAB) and Agulhas eddies.

An event such as D occurs in summer when the south easterly winds are blowing. These winds set up an upwelling cell in the Walker Bay area (see Figure 6.16; light blue) (Shannnon, 1985). The average incoming water temperature at Danger Point was 16.1 °C. The Walker Bay upwelling cell coincides with the presence of an Agulhas jet stream (Bang, 1973; Bang & Andrews, 1974), and an "Agulhas front" running predominantly westwards above the 35th parallel South. This is thought to exist due to a combination of the shelf edge and coastal flows. Around the 26th of January 2005, there was a decrease in the wind speed and thus the wind forcing. This resulted in the breakdown and west ward migration of the Agulhas front towards Danger Point (Bang, 1973; Bang & Andrews, 1974) resulting in warm oligotrophic Indian Ocean water reaching Danger point and an Agulhas Ring/Eddie event (Largier *et al.* 1992). The temperature at Danger point had risen to 19.9 °C. Further migration of the Agulhas front shorewards towards Quoin Point occurred (1/02/05). The migration of the stream and subsequent weakening jet allowed warm oligotrophic Indian Ocean water to intrude into Walker Bay. Further east, an Agulhas ring was visible on the Agulhas shelf. The farm experienced incoming seawater temperature increasing 4.8 °C above the average to 23.4 °C (the highest recorded temperature for 2005). Coupled with this the pH and the dissolved oxygen in the water decreased by 1.06 points and 1.8 mgL^{-1} respectively at the height of the event. Seaweed production decreased by 4.5 kg per tank (Figure 6.29). On the 2nd of February, 2005, the south easterlies began to blow, and by the 4th this had resulted

in the formation of an upwelling cell to the east of Cape Point. Temperatures at Danger Point remained above 20 °C from the 30th of January until the 4th of February 2005. The south easterly wind continued to blow, strengthening and causing south eastward migration of the Agulhas front and the resulting decrease in SST in Walker Bay. A warm water intrusion over the western Agulhas bank (WAB), such as Event C, is seen as a rise in temperature of 3.7 ° C and decrease in dissolved oxygen by 1 mg L^{-1}. There was also a 0.3 increase in pH, as well as a 7 kg decrease in seaweed production per tank (see Figures 6.16 and 6.18 – 6.20 and 6.29).

In Event F, the wind-forcing was decreased by the 15th of March but the Agulhas front still persisted. The SST near Danger Point increased to a high of 19 °C. This was more due to advection of surface waters and northwards displacement of surface waters from the front back to the inshore region. There was no intrusion of water over the WAB and the warm water still remained nutrient-rich. By the 17th of March, the SST showed an increase in the surface waters in Walker Bay. Water temperatures at Danger point increased to 18.2 °C. Seaweed production during this event increased by 2.5 kg per tank (see Figures 6.18 – 6.20 and 6.29). Upwelling events can be seen by sharp decreases in temperatures over a short time scale. The lowest temperature recorded at Danger Point (during this study) occurred in an upwelling event in August 1st 2003 with the temperature being 11.5 °C.

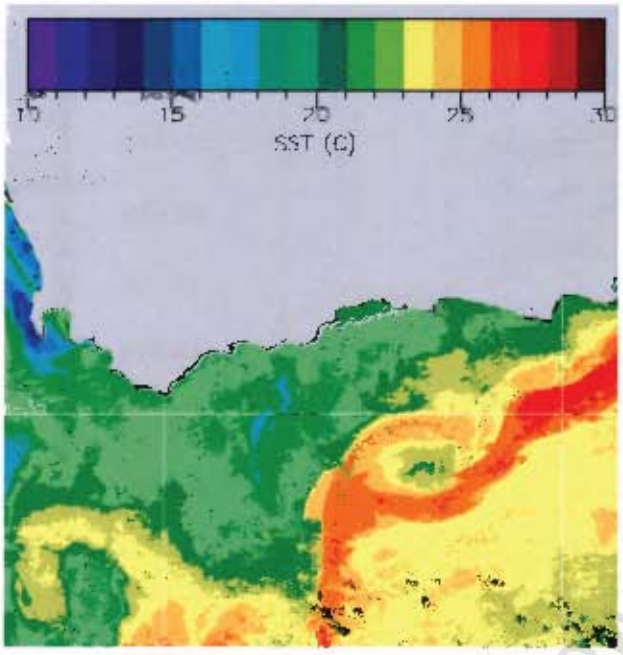

FIGURE 6.16: Sea surface temperatures on the 28th April 2004 - Event C – note the presence of an Agulhas ring on the bottom left. Warm (green) water is intruding past Cape Agulhas and into Walker Bay.

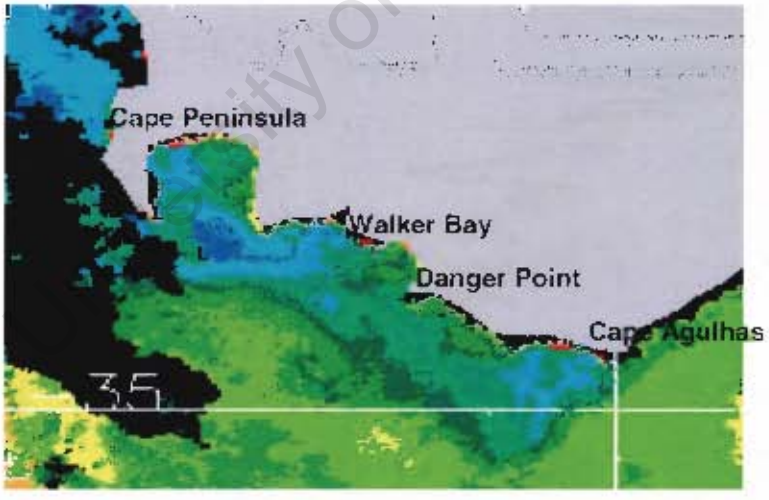

FIGURE 6.17: Sea surface temperatures on the 17th March 2005. the Agulhas front is visible as a green strip running west wards from Cape Agulhas. Warm surface water is visible in the bay as green.

FIGURE 6.18: Average daily temperature (°C) of 25 % and 50 % Recirc. abalone units, seaweed tanks and flow through units from 6th February 2004 to 17th January 2006. The gap is where the experiment was graded and restocked and recirculation increased to 50 %. Letters denote large scale environmental events: Event A: from 26th February until 10th March 2004 – advection; Event B: 18th March to the 26th April 2004 – advection; Event C: 21st April to 4th May 2004 – warm water intrusion; Event D: 24th January to 7th February 2005 – warm water intrusion; Event E: 22nd February to the 3rd of March 2005 – advection; and Event F: 17th of March until the 1st of April 2005 - advection. Sharp decreases in ambient seawater temperatures are indicative of upwelling events.

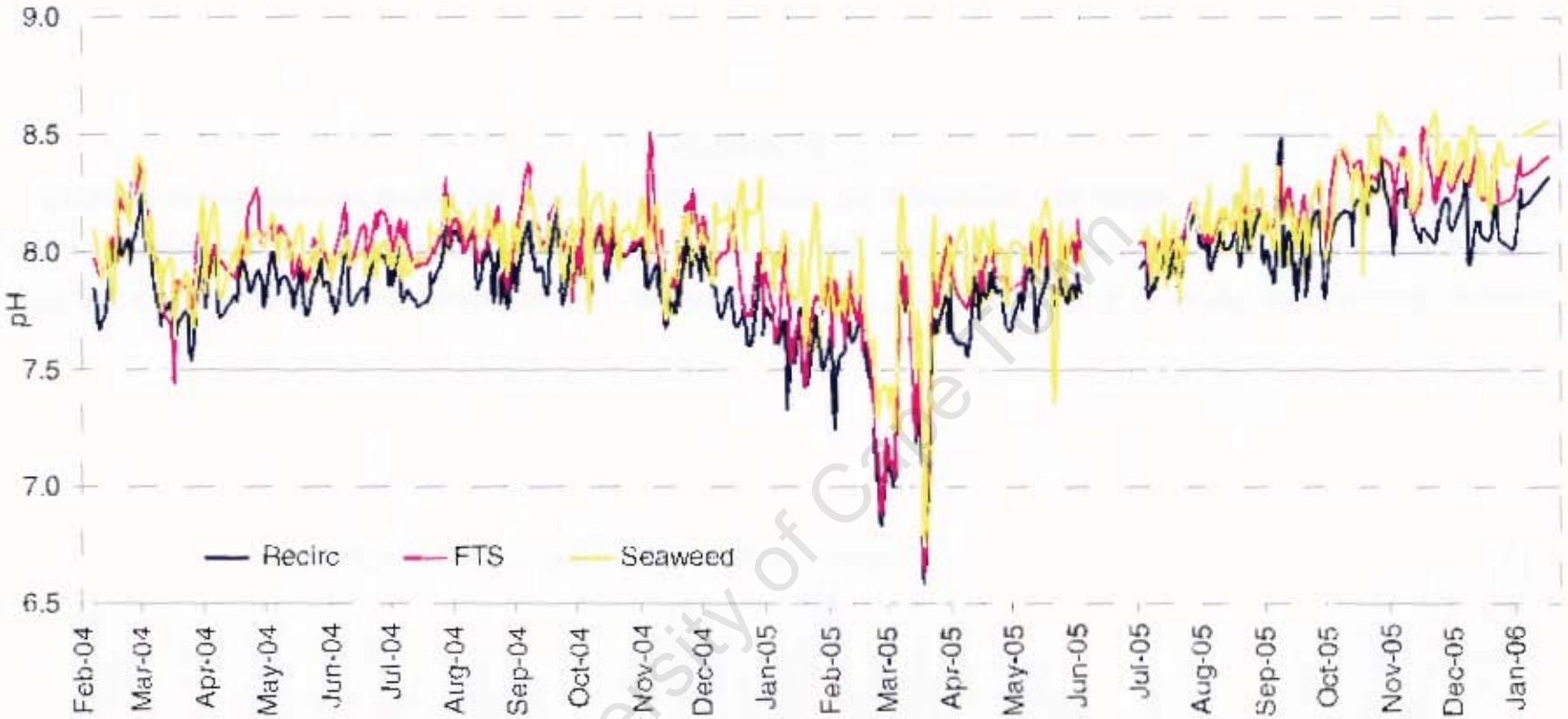

FIGURE 6.18: Average daily pH of 25 % and 50 % Recirc. abalone tanks, seaweed tanks and flow through units from 6th February 2004 to 17th January 2006. The gap is where the experiment was graded, restocked and recirculation increased to 50 %.

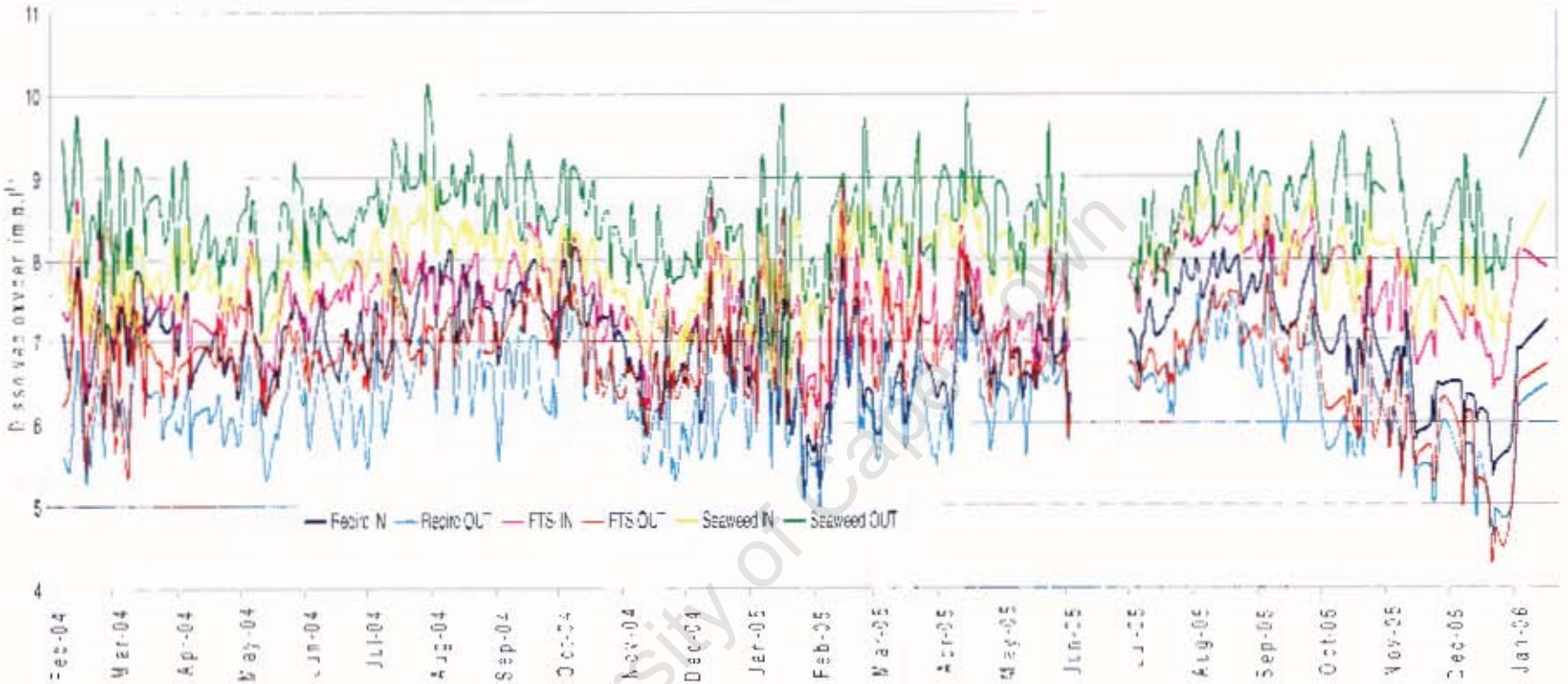

FIGURE 6.20: Average daily dissolved oxygen concentrations ($mg.l^{-1}$) in a 25 % and 50 % Recirc. abalone units, seaweed tanks and flow-through units from 6^{tr} February 2004 to 17th January 2006. Values are shown for both the incoming water to the tanks as well as water exiting the tanks. The gap is where the experiment was graded, restocked and recirculation increased to 50 %.

Water Nutrients

Mean TAN concentrations for the flow-through units were 3.32 µmol L^{-1} (+ 1.3), for the 25 % Recirc. units 2.0 µmol L^{-1} (+ 1.2), for the seaweeds tanks 0.48 µmol L^{-1} (+ 0.6) and for the incoming seawater 3.89 µmol L^{-1} (± 0.8). TAN values at 25 % recirculation did not accumulate, by contrast mean TAN values in the flow-through units were significantly higher than the 25 % Recirc. units (ANOVA; df = 12; $p < 0.05$). The seaweed unit had significantly lower mean TAN concentrations (ANOVA; df = 12; $p < 0.05$). As with Chapter 5, peaks in TAN concentrations occurred at 20h00 and 00h00 (see Figure 6.21).

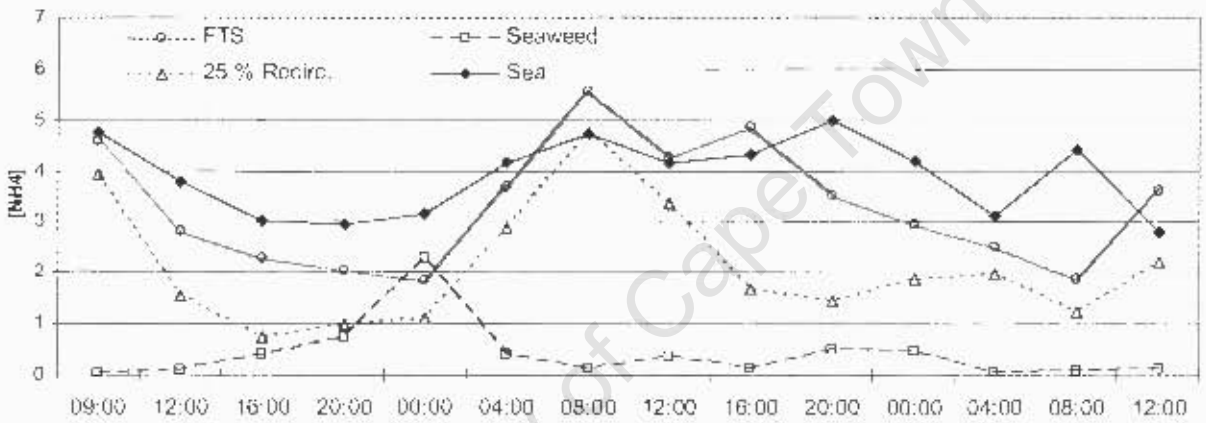

FIGURE 6.21: Mean TAN concentrations in flow-through units, 25 % Recirc. abalone units, seaweed tanks and incoming seawater for comparison (n = 3).

Mean DIP concentrations for the flow-through units were 0.88 µmol L^{-1} (+ 0.4), for the 25 % Recirc. abalone unit 1.17 µmol L^{-1} (± 0.2), for the seaweeds 1.28 µmol L^{-1} (+ 0.4) and for the incoming seawater 0.64 µmol L^{-1} (+ 0.4). There were small peaks in concentrations at 00h00 and 04h00 (See Figure 6.22). There was no accumulation of DIP under recirculation. The seaweed tanks also had significantly lower phosphate concentrations compared to the flow-through units (ANOVA; df = 12; $p < 0.05$).

Mean nitrate concentrations for the flow-through units were 3.47 µmol L^{-1} (± 0.9), for the 25 % Recirc. abalone units 1.17 µmol L^{-1} (± 0.2), for the seaweeds tanks 0.25 µmol L^{-1} (± 0.2) and for the incoming seawater 3.07 µmol L^{-1} (± 0.9). There were

small peaks in concentrations at 20h00 and 00h00 (See Figure 6.23). Mean flow-through units concentrations were significantly higher than 25 % Recirc., which were in turn significantly higher from the seaweed concentrations (ANOVA; df = 12; $p < 0.05$). There was no accumulation of nitrate under recirculation (See Figure 6.23).

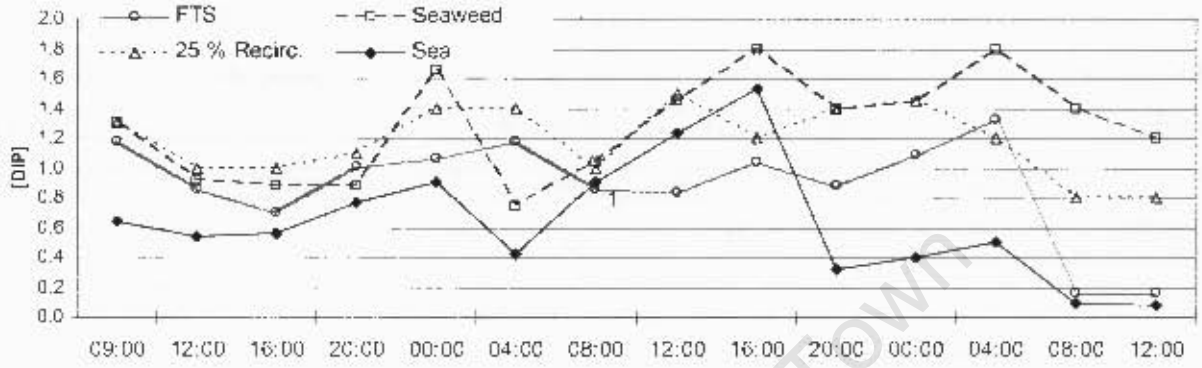

FIGURE 6.22: Mean DIP concentrations in flow-through units, 25 % Recirc. abalone units, seaweed tanks and incoming seawater for comparison (n = 3).

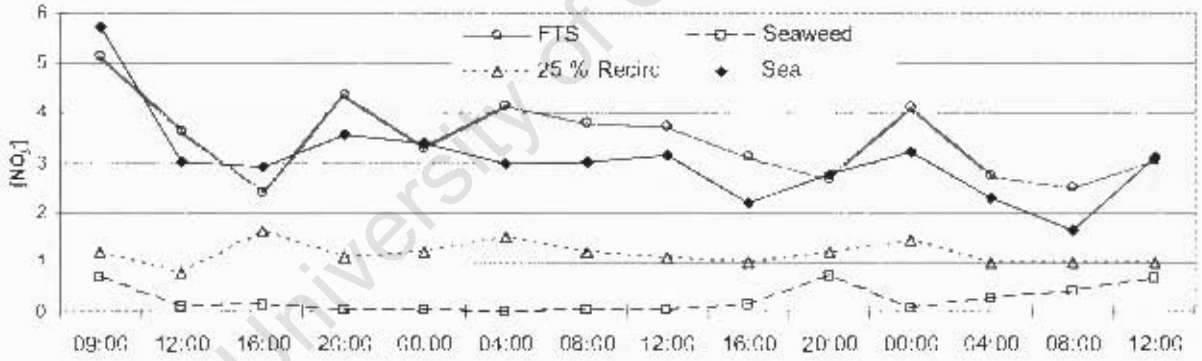

FIGURE 6.23: Mean nitrate concentrations in flow-through units, 25 % Recirc. abalone units, seaweed tanks and incoming seawater for comparison (n = 3).

Mean nitrite concentrations for the flow-through units were 0.63 µmol L^{-1} (+ 0.46), for the 25 % Recirc. unit 0.22 µmol L^{-1} (± 0.05), for the seaweeds 0.08 µmol (± 0 06) and for the incoming seawater 0.84 µmol L^{-1} (± 0.50). There was greater fluctuation in the flow-through units with small peaks in concentrations at 00h00 and 04h00 (see Figure 6.24). The flow-through units had significantly higher average concentrations than the seaweeds and the 25 % Recirc. abalone units (ANOVA; df = 6; $p < 0.05$).

The seaweeds tanks had significantly lower average concentration than the 25 % Recirc. abalone units (ANOVA; df = 12; $p < 0.05$). There was no accumulation of nitrite under recirculation.

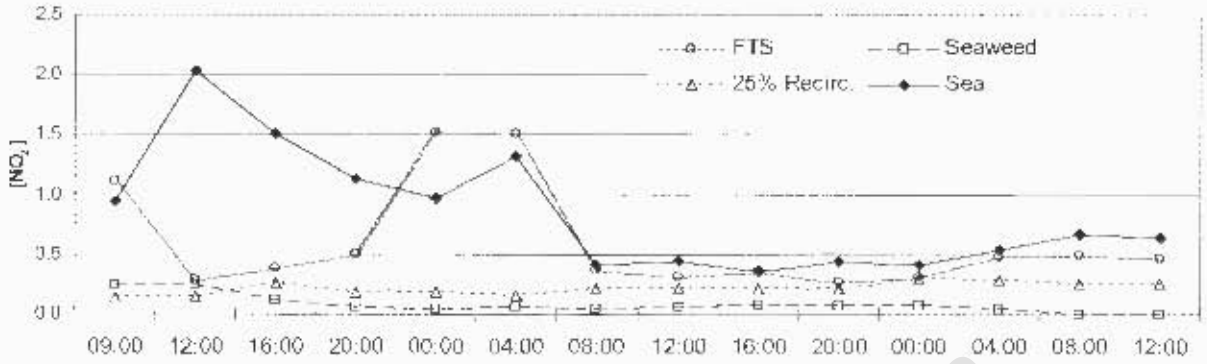

FIGURE 6.24: Mean nitrite concentrations in flow-through units, 25 % Recirc. abalone units, seaweed tanks and incoming seawater for comparison (n = 3).

Table 6.2 lists ammonia concentrations for summer and winter. In summer the pH and temperature are high, allowing more ammonia to be formed. In the winter seaweed uptake is reduced (due to seasonal and low light effects), thus allowing for the potential build up of ammonia.

Table 6.2: Average, (Standard deviation), minimum and maximum values for ammonia (µmol L^{-1}) in summer and winter of in a 25 % Recirc. abalone units and flow-through units.

	Average	Max	Min
Summer			
Flow-through units	1.04 (± 0.71)	2.88	0.26
25 % Recirc. abalone units	0.8 (± 0.47)	1.75	0.2
Winter			
Flow-through units	1.58 (+ 0.88)	3.36	0.81
25 % Recirc. abalone units	0.62 (± 0.26)	1.21	0.37

Table 6.3 lists the nutrient uptake efficiency. It was measured as the average percentage decrease in nutrients (as defined by Buschmann *et al.* 2001; Chopin *et al.* 2001) between the flow-through units and the 25 % Recirc. units and then

between the seaweeds and the Recirc. units. During this study, phosphate was not removed by the seaweeds. This is in contrast to Lindström (2006) whose results are illustrated for comparison. Nitrogen in the seaweed tanks, running at 25 % recirculation, was almost completely removed in nitrate and nitrite forms. Even at 75 % recirculation, there was very high nitrogen uptake efficiency. There was also a large difference in the uptake efficiency between the abalone units on flow through vs. recirculation. Thus demonstrating that the amount of nutrients removed by the seaweeds decreased nutrients in the whole system as compared to a flow-through system.

Table 6.3: Uptake efficiency (%) of dissolved nutrients from this study and Lindström (2006). Seaweed biomass in this study was 2 kg m^2 and for the 75 % study was 5 kg m^2. Average, (Standard deviation), minimal and maximal values are shown.

	NH_4		PO_4		NO_3		NO_2	
25 % Recirc abalone vs. flow-through units abalone	38.06 (18)	32.5 – 85.4	0		64.8 (11.1)	33.1 - 77.9	48.8 (25.2)	5.1 – 89.4
Seaweeds vs 25 % Recirc	80.8 (22.6)	24.3 – 99.5	0		77.7 (24.5)	30 - 100	75.7 (13.7)	53.4 - 100
Lindström (2006) 25 %	42	50 - 94	14	5.5 - 51	76	57 - 95	67	29 - 100
Lindström (2006) 75 %	35	19 - 70	3	1.2 - 13	85	70 - 99	58	12 - 100

Sediments

As in Chapter 5, two studies were performed on the amount and type of sediments found in the tanks in September 2004 (Potgieter, 2005) and in September 2005 (Brandt, 2006).

Suspended particle loads

Potgieter (2005) showed that there was no significant difference in the suspended solids in the water column between the two systems either in the total load (105.3 mg L^{-1} flow-through units vs. 90.6 mg L^{-1} 25 % Recirc. abalone units) or in the size fractionation (See Figure 6.25). This was also true of the Brandt (2006) study who studied the unit at 25 %, 50 % and 75 % recirculation rates. The values found by Potgieter (2005) were similar to those found by Brandt (2006) (see Table 6.4). Brandt (2006) found no significant differences between the flow-through units and the Recirc. units or between the different recirculation rates. Both studies found that there was no increase in the particle fractions between the two systems with the larger size class (> 50 μm) having the highest percentage of the total. In addition Brandt (2006) did not find any significant differences between particle size fractionation at higher recirculation rates. In a seven day period between 55 and 72 kg of sediments would be released through the overflow water from both systems.

Table 6.4: Particle load in mg L^{-1} between the flow-through units and the Recirc. units run at 25, 50 and 75 % recirculation rates (Data from Brandt, 2006).

Particle load mean values mg/litre	Flow-through units	25 % Recirc.	Flow-through units	50 % Recirc.	Flow-through units	75 % Recirc.
In tank	162 ± 145	63 ± 19	64 ± 11	72 ± 21	77 ± 61	65 ± 14
Outflow	97 ± 104	81 ± 40	50 ± 13	92 ± 22	59 ± 15	89 ± 35
Algae		79 ± 22		56 ± 23		91 ± 46
Seawater	24 ± 1	37 ± 11	56 ± 20	43 ± 5	55 ± 12	74 ± 71

Accumulation

The amounts of settled sediments were significantly more than the suspended particle load (Potgieter 2005; Brandt 2006). Potgieter (2005), found a significant difference between the bottom sediment loads of the two units. The particle fractionation showed that the flow-through units had significantly more of the larger particles (> 50 μm and 40 – 50 μm and 20 – 30 μm) ($p < 0.05$), while the 25 % Recirc. abalone units had significantly more particles in the 20 – 30 μm fraction (see Figure 6.26). The flow-through units would produce 45.45 kg of sediment a year vs. the 41.64 kg produced by the 25 % Recirc. units in bottom sediments alone.

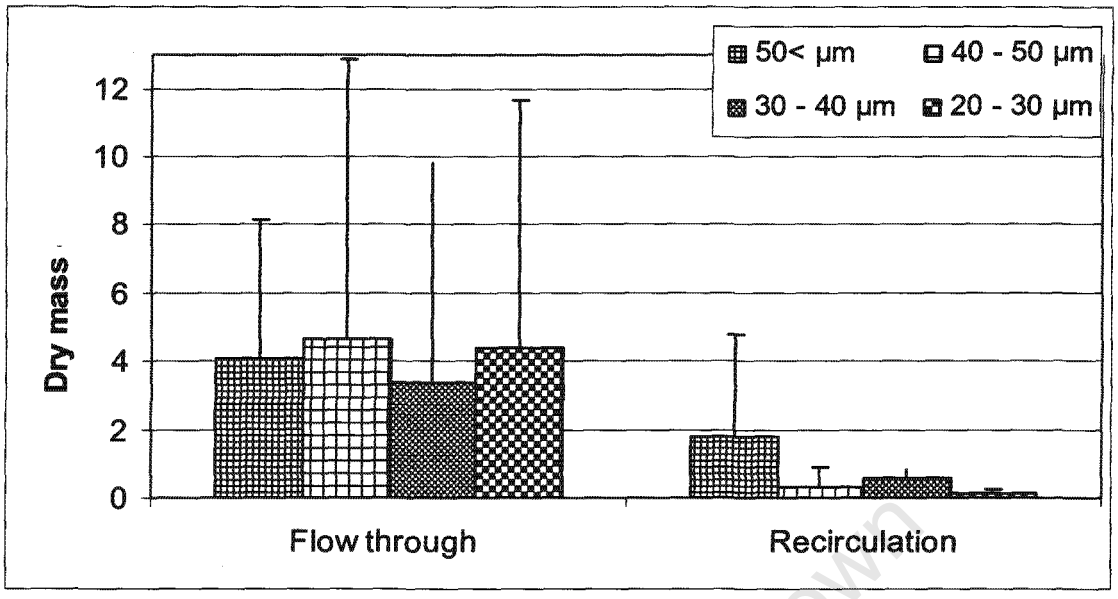

Figure 6.25: Particle fraction (mg L^{-1}) of water column suspended particles between the flow-through units and the 25 % Recirc. abalone units (data from Potgieter, 2005).

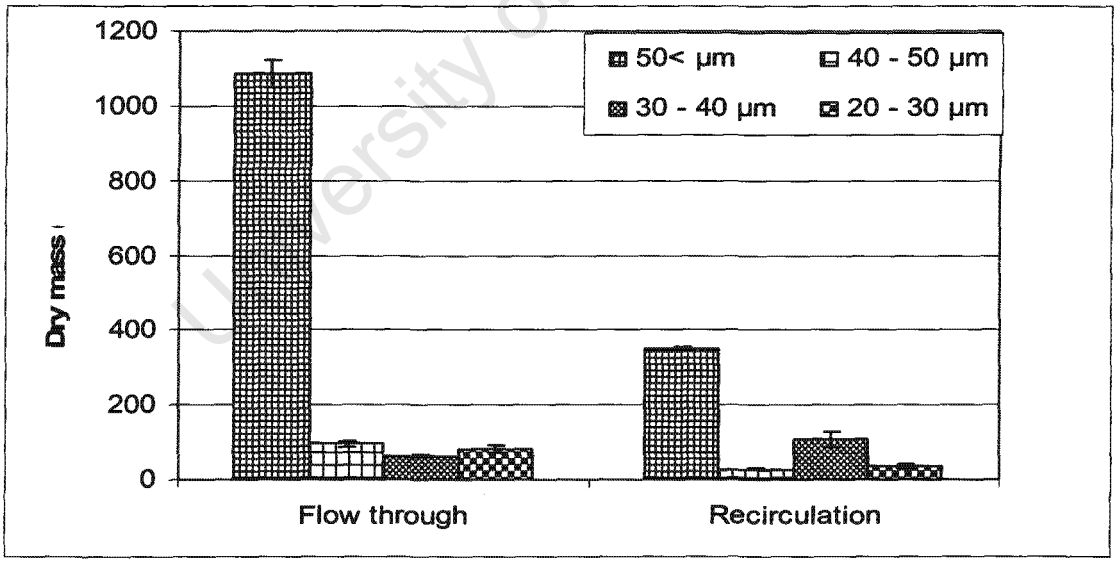

Figure 6.26: Particle fraction (mg L^{-1}) of bottom sediments between the flow-through units and the 25 % Recirc abalone units (data from Potgieter, 2005).

TABLE 6.5: Amount of bottom sediment per tank in 400 L of bottom sediment water, and sediment production calculated per tank per week to an estimate of 500 tanks per year (data in second column from Potgieter, 2005).

Diet	mg L^{-1}	kg/tank/wk	kg/tank/yr	Tons/yr/farm of 500 tanks
25 % Recirc. abalone units	200.2 ± 34.4	0.801	41.64	20.82
Flow-through units	218.5 ± 28.2	0.874	45.45	22.72

Particle build up in the water column between the two units over a seven day period

Potgieter (2005) looked at the build up of particles in the water column over a 7 day period and compared the two systems (Figure 6.27). She showed that there was no significant difference in the suspended solid loads between the two units. The flow-through units did not have a significant difference in the daily suspended solid load increase. Regression analysis showed that the concentration of particles in the flow-through units and the 25 % Recirc. units remained constant over the 7 day period despite the loading between different days being significant in the 25 % Recirc. unit. Potgieter (2005) also looked at the particle fractionation in the two units and found no significant differences. The greatest load in both units was in the largest particle size class.

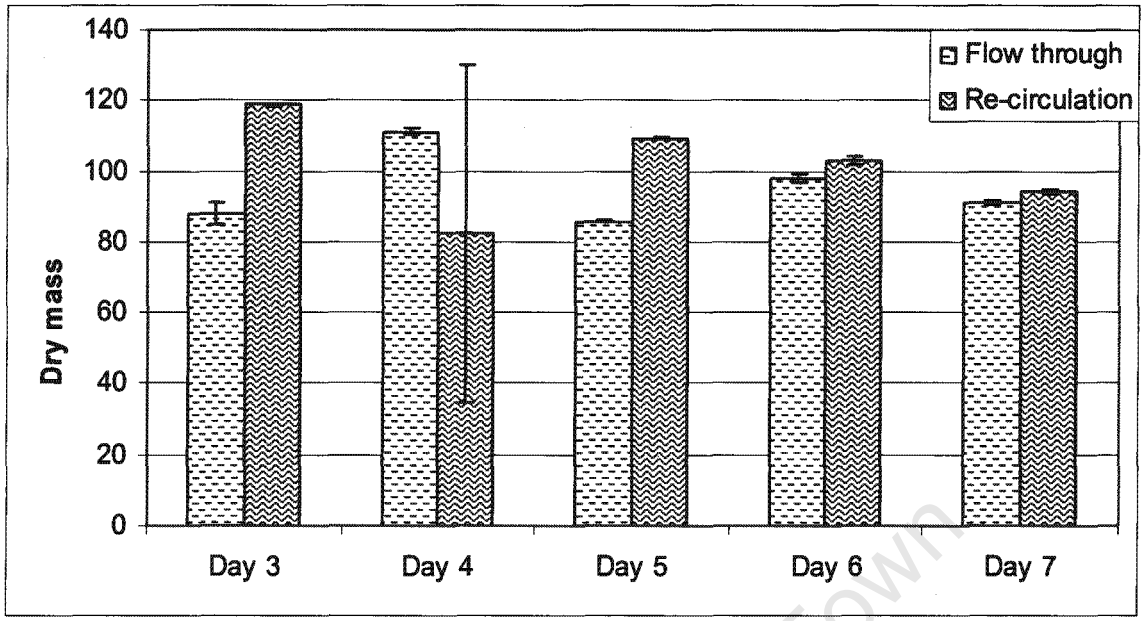

Figure 6.27: Daily suspended particle concentration (mg L^{-1}) over a 7 day cleaning cycle between flow-through units and 25% Recirc. abalone units in the water column (n = 6). Standard error bars are represented.

Carbon and nitrogen content in particle load

Brandt (2006) found that the average carbon content in the particles was 0.15 % of the dry weight while the average nitrogen content was 0.02 %. She did not find any differences in either carbon or nitrogen content in the particle load between the flow-through units or the 25 % Recirc. abalone units at any of the recirculation rates tested.

Bacteria

Flodin (2005) found that significantly more bacteria were found in the sediment (1.5 X 10^7 – 2 X 10^8 CFU/ml VHB; 3 X 10^6 – 4 X 10^7 CFU/ml VLB) compared to the water column (4.6 X 10^3 – 1.1 X 10^4 CFU/ml VHB; 7 X 10^2 – 2.6 X 10^3 CFU/ml VLB) in both units. Flodin (2005) showed that there were no differences in bacterial water quality between flow-through units and 25 % Recirc. units. He concluded that higher recirculation rates could lead to more bacteria build up. In addition, when comparing bacterial concentrations from seaweeds in the seaweed tanks connected to the 25 % Recirc. units and seaweeds grown in fertilized waste water from the recirculation

dam, no significant difference was found. Seaweed samples from the 25 % Recirc. units had significantly lower concentrations of VLB relative to VHB compared to both the sediment and water column samples (Flodin, 2005).

Mobile and sessile macro-fauna

Hansen (2005) looked at mobile macro-fauna densities and diversity in the flow-through units vs. the 25 % Recirc. abalone units. The mean density of mobile macro fauna was 2.19 g DW (± 0.67) or 8232 individuals (± 1 104) per tank in the 25 % Recirc. units and 2.17 g DW (± 1.75), 11 648 individuals (± 10 960) per tank in the flow-through units. In total, Hansen (2005), identified 28 faunal taxa from all samples (See Appendix C). The diversity (including unidentified taxa) was not significantly different between the 25 % Recirc. abalone units and the flow-through units. Thirty four mobile and sessile fauna were found in the seaweed tanks alone (See Appendix C). If common species are removed, the combined unit supports approximately 48 taxa. The total faunal density and diversity was significantly higher in the tanks compared to an equal volume of intake seawater (Hansen, 2005). The most abundant taxa in the tanks were the amphipod *Paramoera capensis* (Dana) and tanaids (*Tanaidacea* spp.) together with polychaetes, especially the *Nereid* spp. Other abundant taxa were the amphipod *Hyale* sp. and the isopod *Janiopsis palpalis* Barnard, as well as other (unidentified) amphipods. These taxa contributed 81 – 99 % of the total mobile macrofauna biomass in the tanks (Hansen, 2005).

The modified B-B scale of keyhole limpet numbers shows a seasonal trend in number, with an increase around September (see Figure 6.28).

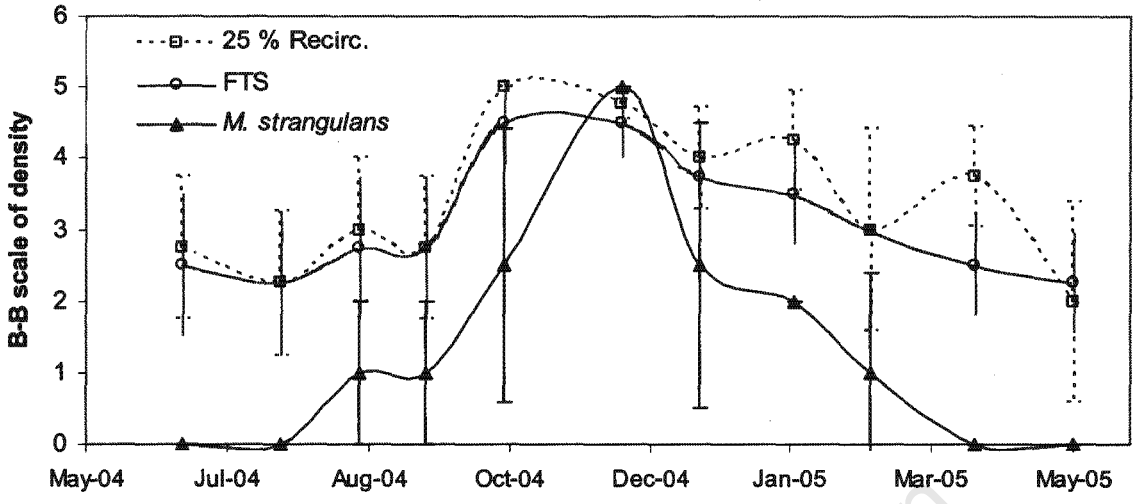

Figure 6.28: A modified B-B scale of density of keyhole limpets in the 25 % Recirc. seaweed tanks (n = 6) and the flow-through seaweed tanks (n = 4) as well as the percentage cover of *Ulva* thalli by *M. strangulans* from the seaweed tanks.

Seaweed production, nutrient and water content

There was a seasonal trend in SGR of the seaweeds with highest SGR (6.25 % d^{-1} ± 3.4) and yields (0.12 – 2 kg m^2 d^{-1}) occurring in September through to February in the 25 % Recirc. seaweed tanks. The high standard error was due to low growth rates in some tanks, which experienced a *M. strangulans* Greville infestation (see Figure 6.28). The SGR in June and July, was lower (1.59 % d^{-1} ± 2.1) with an average of 3.2 % d^{-1} with yields of 0.0 – 0.03 kg m^2 d^{-1}. *Ulva* production per tank (see Figure 6.29) shows that during the winter months the algae did not increase in biomass but rather declined. At 25 % Recirc. rate there was no significant difference in algal production between the fertilized and the 25 % Recirc. tanks. When the recirculation rate was increased to 50 % the production from the seaweed tanks was significantly higher in September, October and November 2005 (ANOVA; $p < 0.05$; df = 10). Other decreases in SGR were explained earlier due to environmental events.

Table 6.6 lists the proportions of N and P in the seaweeds at different seasons in the spring and summer months. The N content in the seaweeds was very low. This corresponded with high SGR, in addition, the water content in the seaweeds was higher from October 2001 to February 2002 (late spring to late summer) and

decreased in March 2002 to August 2003 (autumn to winter). Phosphate content in the seaweeds increased in the autumn and winter months.

Work done by Björnsäter & Wheeler (1990) showed that there is a relationship which exists between tissue N and P for *Ulva* in the North East Pacific. This relationship was used to determine N or P limitation in our cultivated seaweeds. The higher phosphate contents in July indicate that the seaweeds switched to being nutrient sufficient for most of the year to being nitrogen limited.

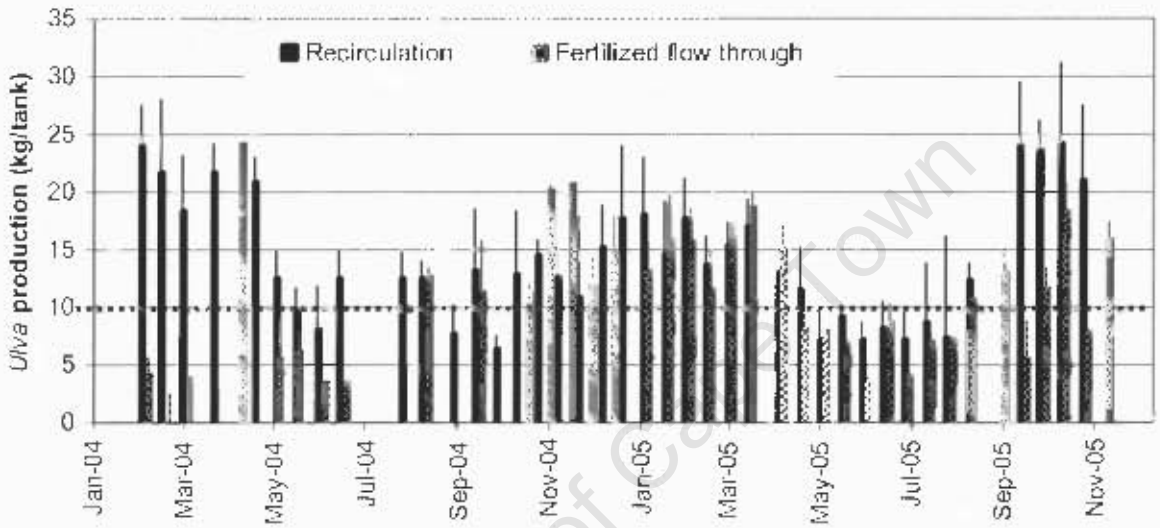

FIGURE 6.29: *Ulva* production per tank (n = 6) from January 2004 to May 2005 at 25 % Recirc. and from June 2005 to December 2005 at 50 % Recirc. The fertilized flow through tanks are for comparison and received 12 volume exchanges per day (n = 4).

Table 6.6: Tissue N and P concentrations in seaweed samples from 25 % Recirc. tanks and fertilized flow-trough tanks. Values with an asterisk are significantly higher between treatments; lettered values are significantly different within treatments. (n = 6 for Recirc. tanks and n = 4 for fertilized flow-through tanks. Std. Dev. are in brackets.)

		September 2003	December 2004	April 2004	July 2004
P (mg.g)	25 % Recirc	0.65 (0.40)	0.80* (0.01)	2.27^{b} (0.20)	3.78^{a} (0.66)
	Fertilized flow-through tanks	1.05^{c} (0.13)	0.66 (0.03)	2.33^{b} (0.08)	3.15^{a} (0.49)
N (mg.g)	25 % Recirc	11.51 (1.91)	13.98 (2.44)	45.02^{a*} (6.06)	43.80^{a} (5.13)
	Fertilized flow-through tanks	22.54^{c*} (2.48)	14.96 (6.6)	36.04^{b} (0.43)	51.64^{a*} (2.27)
N:P ratio	25 % Recirc	17.71 (2.66)	17.53 (2.75)	20.05* (4.42)	11.67 (0.64)
	Fertilized flow-through tanks	21.46 (1.4)	22.48 (8.84)	15.48 (0.36)	11.59 (7.76)
SGR (%.d^{-1})	25 % Recirc	2.33 (3.79)	3.53 (0.73)	1.62 (0.88)	- 0.85 (1.71)
	Fertilized flow-through tanks	- 0.89[1] (2.96)	3.28 (0)	2.14 (3.69)	- 5.73 (2.15)
% water	25 % Recirc	88.06 (0.45)	88.31 (0.12)	85.93 (0.38)	85.89 (1.15)
	Fertilized flow-through tanks	87.99 (0.65)	89.25 (0.92)	85.62 (0.17)	85.79 (0.85)

[1] Low growth rates reported due to a loss of seaweed biomass due to a *M. strangulans* infestation.

DISCUSSION

Abalone SGR

This study did not find a significant difference in abalone growth rates at the conclusion of the experiment. Thus abalone growth rates were neither positively nor negatively affected by being cultivated in a recirculation system. Abalone growth rates were similar to those obtained on the farm under normal culture conditions. Fleming *et al.* (1996) and Mai *et al.* (2001) stated that most abalone farmers would be satisfied with a growth of 2 – 3 mm per month. This, however, relates to tropical abalone, which grow much faster than the temperate species, *H. midae*. There was some indication that growth rates were significantly different at certain times of the year in the different units, but this did not affect the outcome of the experiment. It is possible that the 1 and 2 % increase in temperature in the recirculation units was too low to positively affect growth rates. The low recirculation ratio could also account for the lack of negative effects on abalone growth rates.

Bacteria

Micro-organisms such as bacteria in aquaculture units play a major role in productivity, nutrient cycling, water quality, disease control and environmental impact of the effluent (Moriarty, 1997). High levels of heterotrophic bacteria are of particular concern as they can decrease dissolved oxygen values and cause disease. An increase in the occurrence of bacterial diseases caused by the genus *Vibrio* has been noted in the abalone aquaculture industry (Bower, 2003). The genus *Vibrio* contains several species that are pathogenic to marine vertebrates and invertebrates (Lizarraga-Partida *et al.* 1998; Tantillo *et al.* 2004). Species of vibrio cause problems in humans (e.g. *V. cholerae* causes cholera and *V. parahaemolyticus* causes waterborne gastroenteritis in humans following consumption of contaminated seafood.), fish and crustaceans and other mollusks (see Li *et al* 1998; Lizarraga-Partida *et al.* 1998; Bower, 2003; Lee *et al.* 2003; Tantillo *et al.* 2004). The bacteria also develop resistance to prolonged use of antibiotics and diseases can spread to wild abalone populations (Li *et al.* 1998). Diseases in abalone farming could, besides causing problems with antibiotic resistance and abalone diseases for both wild and

cultured abalone, be a direct health problem constituting possible health risks for humans handling abalones.

The water temperature of the water is considered the most important factor controlling the abundance of pathogenic *Vibrio* (Lee *et al.* 2001). Temperatures above 20 °C, increase densities of *Vibrio* (Tantillo *et al.* 2004) and seem to be required for the development of diseases such as withering syndrome in abalone, exposed to "*Candidatus* xenohaliotis californiensis" (Moore *et al.* 2001). In addition, the *Vibrio* are more prevalent during summer months when the temperature is higher. Some species of *Vibrio* may not develop during the winter (Cavallo & Stabili, 2002). Higher counts of VLB in a recirculation unit could therefore be expected with warmer water, especially if temperatures were to reach 19°C or above.

Integrating seaweed like *Ulva*, used as bio-filters in the unit, could possibly lower the bacterial levels in the unit (Tan *et al.* 1999; Pang *et al.* 2006). By the same token seaweeds are known to have unique microbial communities on their thalli and a relatively high level of microbial heterogeneity in an integrated system could help to reduce the vulnerability of the farmed animals (Pang *et al.* 2006). Studies by Zhang *et al.* (2001) and Pang *et al.* (2006) showed that the density of bacterial population in recirculating water of an abalone culture unit, were higher with more nutrients in the water (due to compound feeds). Zhang *et al.* (2001) could not show that bacteria were inhibited by *Ulva pertusa.* Pang *et al.* (2006) showed that *Gracilaria textorii* decreased *Vibrio* counts while increase total bacterial counts, although this was not true of *Grateloupia filicina, Ulva lactuca* and *Chondrus chrispus.* Besides lowering bacterial levels by removing nutrients, seaweed could also have a direct positive effect on the bacterial water quality. Feed enriched with butanolic extracts of seaweeds, such as *Ulva lactuca,* have been shown to boost survival, increase growth rate and lower *V. parahaemolyticus* load on shrimp *Penaeus indicus* juveniles (Immanuel *et al.* 2003). Dimethylsulfoniopropionate is thought to have antibacterial properties (Van Alstyne *et al.* 2001, 2003) and the increase in DMSP concentrations from cultivated seaweeds (Chapter 3) could mean that the seaweeds in a recirculation unit are playing a greater role in antibacterial qualities against

pathogenic bacteria such as *V. parahaemolyticus,* which would make an integrated unit with *Ulva* even more beneficial.

Co-cultivation of different organisms into the same culture unit, could on the other hand, also have negative effects such as increasing the risks for transmission of pathogenic bacteria. One organism could serve as a host of the disease and transmit bacteria to other organisms in the unit (Starliper & Morrison, 2000). Practices where farming seaweeds grown in abalone wastewater, are being used as abalone feed, could potentially inoculate the entire farm with pathogenic bacteria. Flodin (2005), showed that the seaweed, even if cultivated in wastewater, had relatively low numbers of bacteria In this system counts of *Vibrio,* compared to viable heterotrophic bacteria were low, thus this scenario is unlikely.

Abalone health

Compared to the normal farm situation where most of the farm animals showed signs of environmental stress, the lack of environmental stress in the animals from the whole experiment is thought to be an artefact of much better management of the experimental unit when compared to the rest of the farm.

Gonad Index

This is a measure of the sexual maturity of the animals and is of critical importance for management. A mature gonad may make up 15 % to 20 % of an animals body weight in a fully mature individual (Hahn, 1989). One of the hypotheses was that a recirculation unit would cause an increase in the gonad index due to the increased temperatures meaning that animal energy was going to reproductive growth and not somatic growth. The increase in gonad index in the recirculation unit also corresponded with a natural increase due to wild spawning seasons (Hahn, 1989). Thus this is not an effect of the animals being in the recirculation unit.

Seaweed nutrient content

The protein content of *Ulva* grown in the recirculation units is less than the 44 % reported by Goldburg & Triplett (1997) in their recirculation system. Our results fall within the protein range found to be most beneficial to *H. midae* (36 – 44 % protein) (Shipton, 1999; Britz, 1996; Sales & Britz, 2001). Average tissue protein values obtained for this study (36.6 %, from the 25 % Recirc. and 33.4 % from the fertilized flow-through tanks) were much higher than wild harvested *Ulva* (3.7 – 24 %) (Smith & Young, 1954, Nisizawa *et al.* 1987; Simpson, 1994; Castro-Gonzales *et al.* 1996; Simpson & Cook, 1998; Wilkinson, 2001; Wong & Cheung, 2001). *Ulva* has positive effects on abalone growth when used as a food source (Naidoo *et al.* 2006). However, the tissue N content in this study and that of Lindström (2006) did not reach up to values reported by Schuenhoff *et al.* (2003) with 5 - 7% N in dry weight in *Ulva* spp. tissue.

During summer, the seaweeds experienced higher SGR but lower tissue N. This finding agrees with previous reports (Duke et al., 1986; 1987; 1989a,b), of an inverse relationship between SGR and tissue nitrogen, but is in contrast with studies by Neori *et al.* (1991). This result may have been confounded as a result of the *M. strangulans* infestation.

Work done by Björnsäter & Wheeler (1990), showed that there is a relationship which exists between tissue N and P for *Ulva fenestrata* in the North East Pacific. A ratio of 16 – 24 indicated that the seaweeds were nutrient sufficient. In this study the cultivated seaweeds ratios fall within the low end of this range. Chopin *et al.* (1996) argue that one should be careful in using the mean values determined for different species from different locations as a useful approximation. These differences may be related to differences in the nutrient requirements of the different species

Previous stocking density experiments with *Ulva* spp (Robertson-Andersson, 2003). showed that nutrient tissue content decreases with increased seaweed biomass. Lindström (2006) found that despite the higher seaweed biomass during 75% recirculation, the C and N tissue content of the seaweeds was significantly higher

compared to 25% recirculation. There was no statistical difference between seaweed controls and seaweeds in recirculation. In the experiments performed by Robertson-Andersson (2003) it was found that the seaweeds had a two week acclimatization period with higher SGR in the second week, which decreased tissue nitrogen. The study by Lindström (2006) did not repeat this experiment and it is possible that the values would decrease if the experiment was run for a longer period. Robertson-Andersson (2003) found that of the 4 different stocking densities tested (1 – 4 kg m^2), maximal uptake per gram of seaweed occurred at 5 kg/ tank.

Tissue nitrogen results are consistent with those found by Robertson-Andersson (2003). Seasonal changes in tissue N and P can be explained by coastal oceanographic process. Boyd *et al.* (1985) showed that there is an increase in nitrogen values in the seawater in the Walker Bay area (close to I & J) in autumn and winter. Phosphate content in the seaweeds increased in the autumn and winter months, as found by Robertson-Andersson (2003) and corresponds with a seasonal increase in phosphate concentrations in the Walker bay area (Hutchings & Andrews, 1980; Chapman & Shannon, 1985; Mitchell-Innes & Walker, 1991). Reasons for the increase in coastal waters are probably related to processes occurring in the early winter such as die offs of plankton or stirring of sediments by early winter storms (Hutchings & Andrews, 1980; Chapman & Shannon, 1985; Mitchell-Innes & Walker, 1991).

Two essential minerals required for abalone shell growth are calcium and phosphorus. Waterborne calcium can be readily absorbed across the gill epithelium and thus dietary calcium is poorly utilized (Phillips *et al.* 1958). In contrast the uptake rate of phosphorus is very low: less that 0.001 % of the rate of uptake of calcium (Phillips *et al.* 1958). This together with the extremely low levels of waterborne P (Hutchings & Andrews, 1980; Boyd *et al.* 1985; Chapman and Shannon, 1985; Mitchell-Innes & Walker, 1991; Probyn *et al.* 1994; Largier & Boyd, 2001), means that the satisfaction of the P requirements of the abalone needs to be met by the diet (Sales & Britz, 2000). Recirculation has the effect of increasing phosphate concentrations to above those in the incoming seawater. This may facilitate

increased phosphate absorption by the abalone and account for the faster growth rates in this unit.

Seaweed SGR

This chapter has shown how the seaweed SGR is either positively or negatively affected by large scale (nutrient upwelling and advection or intrusion) environmental events in the adjacent environment. In addition, their SGR was also affected by events on a tank scale. One of the most notable negative effects on seaweed growth rates was the presence of a *M. strangulans* infestation which occurred in spring and persisted over the summer months. This alga forms small round epiphytic crusts ('brown spots') on the surface of *Ulva* and as *Myrionema* ages, the centre of the crusts disintegrates, apparently encouraging break-up of the host thallus. The decrease in *Ulva* SGR was accompanied by increasing occurrence of *M. strangulans* on the algae (a relationship previously seen and described in Robertson-Andersson, 2002, 2003; Robertson-Andersson & Wilson, 2004). The increase in *M. strangulans* is known to correlate with a linear decrease in SGR (Robertson-Andersson, 2003). Another negative effect on seaweed growth rates in the same period was the increased presence of the Cape keyhole limpet and its effect as a seaweed grazer (Hansen *et al.* 2006). These events can change long term seasonal trends in SGR and have other effects such as altering nutrient uptake and tissue nitrogen composition.

The research in this paper is complicated due to the effects of shading the seaweed treatments with a 50 % shadecloth. Research done by Robertson-Andersson (2003) looked at the effects of seasonal shading in the same seaweed tanks over the same seasonal period and compared this to unshaded tanks. The results showed that the tanks that were shaded had a significantly higher SGR than the unshaded tanks mainly due to an infestation of *M. strangulans* causing the complete loss of the seaweed culture. Results also indicated that there were no significant differences in any of the physiochemical variables tested or differences in the water quality, when seaweed cultures were shaded or unshaded (Robertson-Andersson, 2003). The maximal benefit, in terms of SGR and decreased *M. strangulans* presence, occurred

when the seaweed tanks were shaded from September to February (Robertson-Andersson, 2003). Additional experiments looked at the type of shade cloth used. No change in *M. strangulans* percentage coverage was obtained using a 20 % shadecloth, while an 80 % shadecloth decreased SGR significantly (Robertson-Andersson, 2003). As the primary goal of this research was to maintain the seaweed as biofilters, it was deemed necessary to shade the seaweed tanks, to maintain the recirculation system.

The SGR showed a seasonal trend and was highest in summer. The SGR's observed in this study were similar to those found by Robertson-Andersson (2003) in the abalone treatment, but lower than those obtained in the literature. However, in most cases, smaller tank sizes were used (see, Duke *et al.* 1989a,b; Björnsäter & Wheeler, 1990). Work done by Robertson-Andersson (2003), showed that there is a decrease in SGR when scaling up tank sizes. Growth rates for *U. fenestrata* Postels & Ruprecht under experimental conditions were 16 % wwt d^{-1} (Björnsäter & Wheeler, 1990) while *U. curvata*, grew at 52 % wwt d^{-1} (Duke *et al.* 1989a,b). Neori *et al.* (1991) grew *U. lactuca*, 18.6 % wwt d^{-1} in their study and Lapointe & Tenore, (1981) obtained SGR for *U. fasciata*, of 36 % wwt d^{-1}. The high SGR for *U. curvata* occurred in a short term laboratory study, while the low growth rate for *U. fenestrata* may be attributed to the lower water temperature used (13 °C) compared to other experiments (Björnsäter & Wheeler, 1990). The SGR obtained by Neori *et al.* (1991) were also low and could also be due to the scaling up of the tanks sizes as the other studies were in experimental small scale systems.

Physiochemical variables

Over the long term the integrated unit raised the water temperature by 1 % at 25 % recirculation and 2 % at 50 % recirculation. During the short term studies in September by myself and Lindström (2006), the flow-through units had higher night-time temperatures, while the 25 % Recirc. tanks were losing heat at night, by being connected to the seaweed tanks which had a minimum of 13.3 °C. Lindström (2006) found that when the recirculation ratio was increased to 75 % the water temperature in the 75 % Recirc. tanks decreased further to 12.1 °C. This means that the seaweed

tanks were losing more heat or were less able to retain the heat at higher recirculation in the winter periods, due to the seaweed tanks having a lower latent heat capacity and no insulation. They were more vulnerable to cooler night time temperatures. This effect was seen in smaller tanks where night time temperatures dropped as low as 5 °C (Robertson-Andersson, 2003). During the day the tanks heated up very quickly and were able to raise the temperature of the recirculating abalone tanks, while the flow-through units was dependent on incoming seawater temperatures and would remain low in an upwelling event. Schuenhoff *et al.* (2003) showed that an integrated fish/abalone/seaweed culture unit with 50 % recirculation in Israel using *Ulva lactuca* as the biofilter, also experienced heat-loss during winter recirculation. The construction of a greenhouse cover over the biofilter tanks prevented this heat loss. They found maximal heat-loss in early mornings and maximal heat-gain around 16h00, the same pattern experienced in this study and in Lindström (2006). The temperature ranges experienced in the seaweeds and the abalone were sufficient for the seaweeds 'growth needs (Duke *et al.* 1989a,b) and were within the optimum physical range for abalone (Britz *et al.* 1997).

It is generally thought that connected seaweed tanks should be able to provide higher dissolved oxygen concentrations to the recirculating culture unit (Troell *et al.* 2003; Neori *et al.* 2004). Schuenhoff *et al.* (2003) found that water recirculated through seaweed tanks raised dissolved oxygen levels and that this could support fish tanks without additional oxygen "at times of highest oxygen demand". Neori *et al.* (1996) studied a land-based fish/seaweed recirculation unit and found that connected seaweed tanks contributed to the oxygen balance in the fish compartment by significantly slowing the rate of oxygen depletion. However in this study, at 25 % recirculation dissolved oxygen was 4 % lower and at 50 % recirculation it was 9 % lower compared to a flow-through units. In the short term studies by myself and Lindström (2006), the oxygen concentration at both 25 % and 75 % recirculation were significantly lower compared to the flow-through units. This was despite the fact that the seaweed tanks in the recirculation unit had produced oxygen even at high temperatures and had 33 % more oxygen than the Recirc. abalone tanks. The data showed that the dissolved oxygen in the seaweed tanks was not being transferred to

the abalone tanks. Various theories were proposed and changes were made to the unit from August 2003 to December 2003 to try to improved oxygen concentrations. The loss of oxygen was not due to bacterial depletion (this was tested and disproved – unpublished data). Two mixing units were designed and built a) to increase the mixing time to allow the oxygen to become more saturated and b) to increase oxygen through tumbling. Both designs failed in their objectives and it is thought that the seaweeds were supersaturating the oxygen in the tanks and that during the pumping this oxygen was being blown off and was not remaining in the recirculated water. A larger unit with a longer water retention time might improve these values. Despite the nontransferal, values obtained for dissolved oxygen concentrations are above critical concentration levels required for abalone at night and at 75% recirculation (Lindström, 2006). At higher recirculation ratios in spring and summer time, when the temperature in the incoming seawater is higher (intrusion event) and air temperature high (the abalone unit picks up heat from warm air being blown into the tank for aeration) the oxygen holding capacity will be low. This is likely to be a critical failing of the unit. In addition, at night when there is a large amount of seaweed in unit, the oxygen demand will be high and this will also stress the unit. This was seen by Lindström (2006) when she ran the unit at high seaweed stocking density and high recirculation ratios. Robertson-Andersson (2003) showed that a high stocking density (4 kgm^2), compared to lower densities, exhibited the highest concentrations of dissolved oxygen at day time and the lowest at night time, due to photosynthesis and respiration. If the problem with dissolved oxygen transportation can be solved, the higher dissolved oxygen concentrations in the seaweed tanks would be able to support the recirculating abalone tanks with additional oxygen at night time or during the day in spring and summer. In the farmer's favour, and present unit's favour, is the fact that abalone can extract 56 % of the oxygen from the water pumping over their gills. This is high if compared to *Murex* spp and sedentary lamellibranchs, which extract 38 % and 5 – 9 %, respectively (Morton, 1967).

Water nutrients

Lindström (2006) and Potgieter (2005) found similar TAN concentrations at 25 % Recirc. (see Table 6.7). Lindström (2006) found peaks in ammonium concentrations at similar times to this study. At 75 % Recirc. she found that there was no significant difference in TAN concentrations between the two systems (see Table 6.7).

The low ammonium, nitrate and nitrite concentrations in all four studies under recirculation (Table 6.7) indicates that biofiltration by the seaweeds is occurring.

The DIP results are slightly different form those of Lindström (2006), who found that at 25 % Recirc. the flow-through units had significantly lower concentrations than the 25 % Recirc. unit (see Table 6.7). At 75 % recirculation, the mean DIP concentration was 2.3 μmol L^{-1}, while in a tank running at 100 % recirculation the mean concentration was 9.9 μmol L^{-1}. Potgieter (2005) also found higher phosphate at 25 % Recirc (see Table 6.7). Lindström (2006) found no significant differences in concentrations between any of the treatments, and there was no accumulation of DIP in the recirculating unit compared to the controls at 75 % recirculation (Table 6.7).

Lindström (2006) found that at 25 % recirculation the seaweeds significantly lowered nitrate concentrations over the period of the study and this resulted in the seaweed units concentrations being significantly lower while the flow-through units was significantly higher. In her study the seawater had the highest concentrations (see Table 6.7). These results were also consistent at the 75 % recirculation rate (See Table 6.7).

Potgieter (2005) also found nitrite concentrations to be higher in the flow-through units. Lindström (2006) also found a greater fluctuation in the flow-through units, as well as having significantly higher concentrations (compared to the 25 % Recirc. unit). The seaweed concentrations were significantly lower than the other treatments. At 75 % recirculation, the flow-through units was again significantly higher compared to the 75 % Recirc. units which was in turn significantly higher than the seaweed units. At 75 % recirculation there was no significant accumulation of nitrite in the 75 % Recirc. units. Lindström (2006), had values for ammonia at both 25 % and 75 % recirculation that ranged from 0 to 1.68 μM.

TABLE 6.7: Nutrient concentration ranges from 4 studies, 3 at 25 % recirculation and one at 75 % recirculation, the first was in September 2003, the second in September 2004 and the third and fourth in September 2005. Concentrations are shown for a flow-through units, 25 % Recirc abalone units, seaweed tanks and incoming seawater for comparison (n = 3).

	This study 25 % µmol L^{-1}	Potgieter (2005) 25 % µmol L^{-1}	Lindström (2006) 25 % µmol L^{-1}	Lindström (2006) 75 % µmol L^{-1}
TAN				
Seaweeds	0.01 – 1.6		0.03 – 3.84	0.17 – 0.92
Recirc. units	0.5 – 3.4	0.8 - 4.1	0.17 – 1. 55	0.59 – 1.22
Flow-through units	0.8 – 4.6	1.2 – 3.1	0.44 – 7.7	0.54 – 1.03
Seawater	2.0 – 2.7	1.1 – 1.8	0.72 – 1.82	0.83 – 1.45
PO_4				
Seaweeds	0.7 – 2.0		1.06 – 2.19	4.10 – 6.64
Recirc. units	0.8 – 1.5	1.8 - 2.2	1.67 – 2.43	4.3 – 6.7
Flow-through units	0.1 – 2.0	1.75 - 2.0	0.82 – 1.58	4.28 – 6.59
Seawater	0.08 – 1.53	1.1 – 1.8	0.32 – 0.84	0.17 – 0.83
NO_3				
Seaweeds	0.27 – 0.72		0.15 – 5.38	0.01 – 0.36
Recirc. units	0.22 – 1.6		2.01 – 4.52	0.98 – 1.50
Flow-through units	1.79 – 5.56		3.63 – 7.57	3.99 – 6.45
Seawater	0.92 – 5.71		3.77 – 9.31	4.96 – 6.73
NO_2				
Seaweeds	0 – 0.25		0 – 0.17	0 – 0.29
Recirc. units		0.16 – 0.33	0.16 – 0.30	0.09 – 0.33
Flow-through units	0.23 – 1.62	0.06 – 0.18	0.18 – 0.46	0.33 – 0.58
Seawater	0.36 – 2.03	0.06 – 0.2	0.09 – 0.43	0.28 – 0.71

The constant low tissue nutrient and water nutrient values in the seaweed tanks at 25 % recirculation in all studies and at 75% recirculation (Lindström, 2006), could be due to N-starvation of the seaweeds, which results in fast ammonium uptake. Rapid nutrient uptake is typical of N-starved or N-depleted seaweeds and results in rapid uptake rates of large quantities of ammonium (Lobban & Harrison, 1985; 1994). At higher stocking densities (25 kg seaweed/ tank) such as that tested by Lindström

(2006) and high recirculation ratios (75 %), the seaweed biomass effectively used all ammonium, nitrate and nitrite supplied, thus keeping concentrations at steady low values.

Robertson-Andersson (2003) showed that a density of 3 kg m^2 (same tanks as present study) resulted in maximal nutrient removal. For ammonium, the removal efficiency ranged between 73 – 97 % during day time and 77 – 85 % during night time. Lindström (2006) showed that day time ammonium removal was greater at 25 % recirculation (50 – 94 %, with an average reduction of 42 %) than at 75 % recirculation (19 – 70 %, and averaged 35 %) (see Table 6.3). Neori *et al.* (1998) studied an integrated abalone/ *Ulva lactuca* culture unit in Israel, and concluded that the seaweed removed 58 % of the nitrogen input to the unit. The lower ammonium-reduction experienced by Lindström (2006) in this unit at 75 % recirculation could be due to high seaweed densities.

The low ammonium, nitrate and nitrite concentration in the seaweed tanks in this study and in Lindström's (2006) study at 25 % and 75 % recirculation in the same system indicates that the seaweeds are using all available nitrogen. This supports the notion of N-starvation, since seaweeds are known to prefer ammonium before nitrate (Lobban & Harrison, 1985; 1994), and a good supply of ammonium would result in less reduction of nitrate and nitrite. An indication of this is Lindström's (2006) results in which a decrease of nitrate and nitrite reduction occurs at 75 % recirculation (Table 6.7). If the unit was set with optimal seaweed densities for nutrient removal, then at higher recirculation ratios, a larger reduction of ammonium and less reduction of nitrate would likely occur. This is preferable since ammonium has the potential of becoming toxic to abalone in the form of ammonia.

Phosphate was the only nutrient analyzed that showed significantly higher concentrations in recirculating tanks compared to the flow through seaweed tanks. In this study there was no uptake of phosphate by the seaweeds and in Lindström (2006) phosphate uptake decreased at 75 % recirculation. The Lindström (2006) study is in contrast to that of Robertson-Andersson (2003), where the seaweeds at a

stocking density of 15 kg seaweed/ tank reduced phosphate between 88 – 92 % during the day, and 71 – 84 % at night. Lindström (2006) showed less efficient phosphate uptake by the seaweeds. Reduction at 25% recirculation was 5.5 – 51 %, with an average of 14 %, and at 75 % recirculation it ranged between 1.2 and13 %, with an average of 3.4 % (Table 6.3). The limited capacity of the seaweeds to reduce high concentrations of phosphate again points to N-limitation in the unit (Troell *et al.* 2003). If we look at Lindström's (2006) study, at 25 % recirculation phosphate depletion occurs, while at 75 % recirculation, with doubled stocking density, the seaweed used up all available dissolved nitrogen and little phosphate reduction occurred. Schuenhoff *et al.* (2003) show reduced N/P ratios in an integrated fish/abalone/seaweed culture and suspected N-limitation in the unit. Robertson-Andersson, (2003) presented figures on N/P ratios in *Ulva* spp. tissue, cultivated on the I & J farm, and showed that the seaweeds were nitrogen limited in late winter to early spring (July – September), the same time period as this study and Lindström (2006). This study, as well as those of Potgieter (2005) and Lindström (2006) could not find any evidence of accumulation of nutrients in the recirculating unit even at higher recirculation ratios.

If water quality biofiltration is the main goal of integrated aquaculture using seaweeds, it may be necessary to starve the seaweeds to obtain maximal reduction of the water nutrient concentrations (Troell *et al.* 2003; Neori *et al.* 1998, 2003, 2004). In a 1-stage biofilter, such as the one in our study, a large biofilter area is required to strip nutrients efficiently, hence the high seaweed to abalone ratio. Our system could have been made more efficient if we had used a multi-stage biofilter with the two seaweed tanks in series rather than in parallel as was done in the study by Neori et al. (2003). Reasons for having the seaweed tanks in parallel and not in series, was to prevent transfer of potential diseases, pathogens and epiphytic algae harming the biofilter. *Mironema strangulans* can completely decimate the seaweed biofilter and if the seaweed tanks were in series, transfer of this alga would result in both seaweed tanks having infestations. By having the two tanks in parallel one tank could be infected while the other could remain unaffected, thus maintaining the biofiltration capacity of the system. Both Lindström (2006) and Robertson-Andersson (2003) –

found that in this system - it is important to optimize seaweed densities to obtain maximum nutrient reduction. Nitrogen starved seaweeds do not provide a good quality feed (Neori *et al.* 1998, 2004; Troell *et al.* 2003). By altering the seaweed stocking density one can change the products of a recirculation system to optimise either seaweed biofiltration efficiency or seaweed quality and growth. Biofiltration efficiency can change over the cultivation period of the seaweeds, this was one of the reasons for the short harvesting period of 14 days, Robertson-Andersson (2003) found optimal nutrient efficiency occurred when the seaweeds were stocked at 3.5 kg m^2 and that nutrient efficiency decreased at 4.5 kg m^2. By stocking the tanks with an initial biomass of 2 kg m^2 and harvesting them more frequently, we were able to maintain a high nutrient efficiency over the 14 day period regardless of season. Another means of changing biofiltering efficiency is to alter the water flow rates, with low flow rates having high biofiltering efficiencies and low biomass production (Buschmann *et al.* 2001). The flow rates in the seaweed tanks in our recirculation system were low due to the low recirculation ratio. When flow rates were increased by increasing the recirculation ratio biofiltration efficiency decreased (Lindström, 2006). For normal operating conditions a balance that provides for both a high biofiltation capacity and a high biomass needs to be found (Neori *et al.* 1998; Troell *et al.* 2003).

Sediments

Suspended solids management is a key factor in determining the success of recirculation units (Chen *et al.* 1993). Chen *et al.* (1993) state that suspended solids concentration should not exceed 15 mg L^{-1} for recirculating units. In this study the surface particle concentration was greater than 90 mg L^{-1}, which is 6 times this limit. Chen *et al.*'s (1993) study was based on a gravel bed drawdown biofilter, which is highly sensitive to clogging by particulates. Sources for particulate matter to enter the unit are from the feed and faeces, sand from beach-cast kelp, from the seawater and from the integrated seaweeds (mainly larger pieces). The easiest and most effective ways to decrease the nutrient and sediment loading are to improve the feed and feeding (Makinen *et al.* 1988), to minimize resuspension of particulate matter and proper cleaning processes.

Both Potgieter (2005) and Brandt (2006) found that the total tank accumulations of the flow-through units and recirculation units were not significantly different from each other even at higher recirculation (50 and 75 %). This is an important result because it provides evidence to dismiss the notion that the recirculation units accumulate particles to a higher degree. Both studies showed that there were no significant differences in particle size fractionation in the water column, total particle load, or the loading of 35 µm or smaller fractions. In addition, the total particle load did not increase with higher recirculation, nor the loading of 35 µm or smaller fractions. The carbon content of the particle load between a flow-through system and a recirculation system also remained similar. Since sabellids prefer organic particles smaller than 35 µm as feed (Chalmers, 2002), these results indicate that, at least from a feed perspective, kelp fed recirculating units do not favour sabellids. This is supported in the sabellid count data for both systems. There are two possible reasons for similar suspended solid particle loads in the water column of both systems: a) the particles effectively get trapped in the sediment, either in the abalone tank or the recirculation unit (seaweed tanks) or b) the water velocity in the tanks is similar and the water column has simply reached its sediment load carrying capacity. Potgieter (2005) showed that there was no accumulation of sediments in the water column over the 7 day cleaning period further indicating that there is a set carrying capacity. Brandt (2006), showed that temperature had an effect on sediment production with higher temperatures producing more sediments. As both studies were done at similar times of the year, the seasonal production of sediments has not been investigated.

If sediment builds up constantly throughout the year, then a 500 tank farm feeding a kelp only diet, would release between 23 – 93 tons of sediment a year (see Table 6.8). If we assume that Brandt's (2006) figures for carbon and nitrogen remain constant (1000 l of sediment per tank per cleaning cycle), and that the sediment load does not change over time, then 1.0 – 1.7 kg of nitrogen and 9.7 – 16.6 kg of carbon are released from every tank annually, or in a 500 tank farm 0.5 - 0.85 tons of nitrogen and 4.85 – 8.3 tons of carbon are released annually. In addition to fertilizing the coastal waters in the vicinity to the farm outfalls, with nitrogen and phosphorous, an increase in particulate organic matter dropping to the sea bottom consumes

oxygen during the break-down processes (Cripps & Bergheim, 2000). However, South Africa has a high energy coastline and the water is well mixed (Troell *et al.* 2006), so this effect is likely to be small. A study by Sankar (2005), looking at the effect of abalone farm effluent on the rocky shore community, found that rocky shore species composition close to the outfall did not differ significantly from that 15 m away from the outfall.

Table 6.8: Estimation of sediment build up by a 500 tank farm over a year period on a kelp only diet in a recirculating unit and a flow-through units.

	Potgieter (2005) 25 % Recirc.	**Brandt (2006) 25 % Recirc.**	**Brandt (2006) 50 % Recirc.**	**Brandt (2006) 75 % Recirc.**
Recirc. units	23.4	28.92	93.08	36.14
Flow-through units	26	40.56	60.58	55.9

Volume, dry weight, carbon and nitrogen content of sediment

Brandt (2006) found significant differences in the volume of sediment produced at different recirculation rates. The difference did not follow a linear trend with higher recirculation rates having more sediments. In fact, the lowest sediment load was found in the 75 % Recirc. (14.8 liters per tank) tanks vs. 23.5 liters per tank at 50 % recirculation. There were no significant differences in the dry weight between the flow-through units and recirculation units or between the different recirculation rates. Brandt (2006) did not find significant differences between the carbon and nitrogen contents of the bottom sediments or between the treatments or the different recirculation rates. The average carbon content was 14.06 % and the average nitrogen content was 1.47 %.

Table 6.9: Differences in volume (L), dry weight (kg), % C and % N of sediments between flow-through units and a recircualtion units at varying recirculation rates (data from Brandt, 2006).

Sediment load	Flow-through units	25 % Recirc.	Flow-through units	50 % Recirc.	Flow-through units	75 % Recirc.
L	19.89 ± 8.35	20.03 ± 3.52	23.47 ± 6.02	52. 49[a] ±5.79	22.48 ±1.48	14.80 ± 2.64
kg	1.56 ± 0.78	1.39 ± 0.49	2.33 ±0.55	3.58 ±1.05	2.15 ±0.42	1.39 ±0.31
% C	14.44 ± 1.59	14.92 ± 6.18	12.26 ± 1.18	13.63 ± 4.14	13.73 ± 1.51	15.38 ± 3.41
% N	1.61 ± 0.17	1.63 ± 0.70	1.12 ± 0.10	1.32 ± 0.46	1.42 ± 0.18	1.74 ± 0.43

Mobile and sessile macro-fauna

A mobile macro-fauna population can easily become established in a recirculation unit via association with the cultured species, feed input (fauna coming in with the kelp as it is not cleaned before being placed in the tanks), or introduction from the surrounding ecosystem through the seawater intakes as few filters are used. Within recirculation units there is, theoretically, a higher potential for build up of mobile macro-fauna densities, since the fauna can circulate with the water and may stay long enough to reproduce. This can have positive and negative impacts on the target aquaculture species. Positive effects may be anticipated from the conversion of particles to dissolved nutrients, which can be taken up by the cultivated algae as a nutrient source, through grazing on epiphytes and microfilms (Shacklock & Doyle, 1983; Brawley & Fei, 1987; Anderson *et al.* 1998a; Klamermans *et al.* 2002). This may increase seaweed growth and reduce labour cleaning work.

No significant differences in densities or taxa of mobile macrofauna between the 25 % Recirc. and the flow-through units were found by Hansen (2005). In addition, numbers of *F. mutabilis* were not significantly different between treatments, which was contrary to what was expected. Thus, recirculation at this low water exchange rate does not influence the mobile fauna densities. The cleaning of the abalone tanks in the unit every 7th day and the harvesting of the seaweed tanks every 14th day is also likely to prevent the build up of a mobile fauna population in these tanks. A higher recirculation rate is also unlikely to result in a change in densities as there is no corresponding increase in detritus load (Brandt, 2006). None of the taxa found in the tanks are pest species and most are detritivores. However the amphipod *Hyale*

sp. is largely a herbivore and several of the other amphipod species found may graze on both micro and macro algae. The keyhole limpet *F. mutabilis* is known to graze on *Ulva* and *Gracilaria* by trapping floating thalli in cultivated seaweed tanks (Robertson-Andersson, 2003). It becomes more problematic in the unit in September when large numbers of it are found (Robertson-Andersson 2003; Hansen *et al.* 2006, this Chapter). Fortunately, it can be controlled through the use of fresh water washing of the tanks and seaweeds for longer than 20 minutes, which is not harmful to the seaweeds (Robertson-Andersson, 2003; Smit et al., 2003; Hansen *et al.* 2006). Other problematic isopod species, such as *Paridotea reticula* Barnard, have been found in *G. gracilis* seaweed tanks on the farm (Smit *et al.* 2003; Njobeni, 2006, Hansen *et al.* 2006). A 3 hour freshwater treatment has been successfully used to control this pest isopod (Smit *et al.* 2003). This exposure had a minor effect on seaweed growth.

Environmental events

The South African coastline can be typically divided into 3 major coastal regions: west coast (cold temperate), south coast (warm temperate) and east coast (subtropical) and two transition zones. The Benguela upwelling regime is fed by the South Atlantic current and by the leakage from the Indian Ocean or Agulhas Current (Shannon, 2001). The west coast is characterized by coastal upwelling – the process whereby cold subsurface water is brought up from depths greater than 200 m to the surface near the coast as a consequence of long shore south easterly winds (Shannon, 2001). Basically, long shore winds displace warm surface water equatorwards and as a consequence of the earths rotation, offshore, resulting in a drop in sea-level against the coast and an uplift of water from below (and alongshore) to correct the imbalance (Shannon, 2001). This is caused by a shift in the South Atlantic High Pressure (SAHP) cell in spring to a more southerly position. This results in a strong pressure gradient and strong south easterly winds found off Cape Point. These are the winds that drive coastal upwelling (Hart & Currie, 1960).

The I & J farm, located on the Western Agulhas Bank is subject to intermittent wind-driven coastal upwelling, particularly northward of prominent capes (including Danger Point) (Boyd *et al.* 1985; Probyn *et al.* 1994). On the Western Agulhas Bank, the upwelling season starts later (i.e. summer) when the South Atlantic High Pressure cell is far enough south to ridge around the African subcontinent and drive southeasterly winds along the Western Agulhas Bank (Jury, 1988). The SAHP ridge is not a persistent feature, thus the episodic nature of the upwelling (Lagier *et al.* 1992). Oceanographic response (flow structure and upwelling) in this area is largely two dimensional and is primarily driven by long shore variation in topography and wind field (Lagier *et al.* 1992). The structure named "Agulhas front", moving westwards from Cape Agulhas is an 18 °C thermal front which contains colder water and nutrients inshore of it (Lagier *et al.* 1992). This front clearly marks longshore variations in upwelling associated with Cape Agulhas. There is a measured jet in roughly the same position (Bang & Andrews, 1974). Upwelling and subsequent containment of water inshore results in higher nutrient levels inshore of the front with lower to negligible levels above the front and offshore of the front (Lagier *et al.* 1992). Although the upwelling is episodic the Western Agulhas Bank the subsurface thermoclines remain tilted upwards towards the coast throughout the upwelling season (Boyd *et al.* 1985). When the wind-forcing weakens in this upwelled unit warm surface water moves shorewards at the surface but the isotherm still remains tilted. The nutrient rich waters are still contained inshore of this front. This results in warm nutrient rich water in the Walker Bay area. If the thermocline deepens, then a warm water intrusion of the Agulhas current water occurs particularly over the outer shelf (Boyd *et al.* 1985; Probyn *et al.* 1994). This results in very high temperatures with low nutrient concentrations (Lagier *et al.* 1992). The SGR of seaweeds in the recirculation unit was either positively or negatively affected by these environmental events. This was surprising as the unit was designed on data from a previous study (Robertson-Andersson, 2003) and these effects had not been investigated in that study.

Environmental pollution through recirculation

Laws (1993), defined eutrophication "...as a natural process whereby there is a gradual accumulation of nutrients and organic biomass accompanied by increased levels of production". Kerr & Ryder (1992) state that nutrient enrichment acts first to change the character and size composition of primary producers. These changes are more easily anticipated in small, enclosed, systems (e.g. lakes) than in larger marine ecosystems, due to marine ecosystems having rapid dispersion of nutrients caused by physical stress (wind, wave, and currents), as well as the sheer size of the recipient system (Nixon, 1988). However, Viviani (1992) argued that the intensification of aquaculture has influenced water quality of marine ecosystems leading to an increase in toxic phytoplankton blooms. In 1984, an event in the Faroe Islands provided the first proof that a relationship between aquaculture pollution and red tides exists, a *Gonyaulax excavata*, bloom resulted in massive fish mortalities and one case of Paralytic Shellfish Poisoning (Viviani, 1992).

The ultimate goal in water quality management in South Africa is "...to keep the water resources suitable for all designated uses..." (South African Constitution). In order to achieve this goal, the Receiving Water Quality Objectives approach has been adopted (DWAF-vol.1, 1996). This approach set targets for the South African Coastal zone: "Waters should not contain concentrations of dissolved nutrients that are capable of causing excessive or nuisance growth of algae or other aquatic plants/ reducing dissolved oxygen concentration below the target range indicated for dissolved oxygen." "The concentration of suspended solids should not be increased by more than 10 % of the ambient concentration." Samsukal (2004) investigated water quality variables from the outfalls of 7 abalone farms. The data ranges she generated are shown in Table 6.10. All of the data collected in the recirculation unit in this Chapter and by Lindström (2006) and Potgieter (2005) falls well within these ranges.

Sankar's (2005) study on the effects of rocky shore communities in and near effluent flow showed that species in the effluent flow are functionally subtidal species (*Cladophora flagelliformis* (Suhr) Kuetzing; *Plocamium rigidum* Bory in Belanger;

Grateloupia sp.; *Hypnea spicifera* (Suhr) Harvey in J. Agardh; *Botrycarpa prolifera* Greville; *Streblocladia camptoclada* (Montagne) Falkenberg and various species of *Actiniaria*). The outfall also shifted some species up the shore (e.g. *E. maxima* moved 32 cm vertically). Thus the presence of the water from the effluent was more important than the nutrient content in determining species composition. The only discernable effect of the increased amount of sediments was that filter feeding worms *Gunnarea capensis* (Schm.) formed dense mats which trapped sediments and their distribution was limited to areas inside the outfall only.

Table 6.10: The range of water quality found in 7 abalone farm effluent outfalls (data from Samsukal, 2004).

Nutrients	**umol N or P L^{-1}**
Ammonium	0.44 - 19.25
Nitrite	0.15 - 1.10
Nitrate	4.92 – 21.71
Inorganic phosphate	0.65 - 6.04
Dissolved Organic Nitrogen	0 - 14.25
Dissolved Organic Phosphorous	0 - 1.86
Suspended Matter	**mg L^{-1}**
>63	3.24 - 18.80
<63	0.71 - 21.10

CONCLUSIONS

This study has analysed some of the factors that could be used to explain differences between a flow through cultivation unit (for seaweed and abalone) and a recirculating unit. These have been summarised in Table 6.11 and have been described as positive, negative or neutral, or no data (ND), when compared to the flow through unit. A negative value would indicate higher concentrations or values that would be detrimental to aquaculture.

All studies performed on a 25 % Recirc. unit showed there were no adverse effects on the abalone or the culture environment by running the unit at 25 % recirculation (this Chapter, Flodin, 2005; Hansen, 2005; Potgieter, 2005; Brandt, 2006; Lindstörm, 2006). The relatively low recirculation rate is probably the reason for this.

Integrated seaweed recirculation is a viable option when considering a large scale commercial recirculated abalone farm.

TABLE 6.11: Summary of effects of different variables tested between the FTS and the Recirc. systems. A 0 symbol indicates no significant difference, A + symbol indicates a significant positive benefit for aquaculture, and a – symbol indicates a significant negative drawback for aquaculture. Alternate values are for Experiment 1/ Experiment 2.

		FTS	25 % Recirc.	50 % Recirc.	75 % Recirc.
Abalone	Shell length		0	0	
	Weight increase		0	+	
	Time to harvest		0	+	
	Condition factor		0	0	
Abalone health	Sabellid		0		
	General condition		0		
	Environmental stress		-		
	Gonad histology		-		
Abalone histology	*Ricketsia*	-			
	Gut protozoa		0		
Bacteria	Water column		+		
	Water column build up		0		
	Sediment		+		
	Seaweed		0		
Sediments	Suspended load		0	0	0
	Suspended particle fractions		0/ +	0/ +	0/ +
	Bottom accumulation	-	0	0	0
	Bottom accumulation fractions		0	0	0
	Water column build up		0	0	0
	Carbon & Nitrogen contents		0	0	0
Mobile macrofauna	Densities		0		
	Taxa		0		
Water quality	TAN		+	+	+
	FAN		+	+	+
	PO_4^+		0	0	0
	NO_2^-		+	+	+
	NO_3^-		+	+	+
Temperature	Average		- and +/ +	- and +/ +	-
	Range		-	-	-
pH	Average		0	0	0
Dissolved oxygen	Average		-	-	-
Seaweeds	SGR		0	0	0
	Key hole limpet density		0	0	
	Tissue N		+	+	+
	Water content		0	0	0

CHAPTER 7

CONCLUSIONS

South Africa has a relatively recent history of seaweed aquaculture. Most of the current seaweed resource use is based on material from the wild (Critchley & Rotmann, 1991). Commercial interest into seaweeds in South Africa developed through shortages imposed by World War II, through a lack of algal derived colloids (see Isaac, 1942; 1953; Isaac *et al.* 1943; Isaac & Molteno, 1953; Anderson *et al.* 1989; Critchley & Rotmann, 1991; Critchley *et al.* 1998). Cultivation of seaweeds in South Africa was not started until the 1990's and occurred using *Gracilaria* (See Rotmann, 1987; Anderson *et al.* 1989; Dawes, 1995; Anderson *et al.* 1996a; Anderson *et al.* 1999; Wakibia *et al.* 2001).

Research into land based cultivation of seaweeds began with several Masters and honours projects at South African Universities looking at seaweed cultivation in abalone waste water in the eastern cape (Fourie, 1994; Smit, 1997; Hampson, 1998; Steyn, 2000, Morgan, 2000; Miller, 2001; Robertson-Andersson, 2003; Njobeni, 2006). These projects looked at cultivating the following seaweed species *Gracilaria gracilis*, *Ulva capensis*, *Ulva facsiata, Ulva rigida, Ulva lactuca, Gelidium* spp. and *Gacilariaopsis* spp. Of these initial species investigated it was concluded by the researchers that it was possible to cultivate all the species except *Gelidium* in addition integrated cultivation was feasible. Other seaweed species that have been looked at for potential cultivation are *Ecklonia maxima*, *Aeodes* spp., *Suhria* spp., *Mazzella* spp., *Falkenbergiia* spp., *Ulva* spp., *Porphyra* spp. and *Gracilaria* spp. (Bolton, pers. comm.).

All of the research into land based seaweed cultivation prior to this thesis was done on placing the seaweeds in the effluent stream with no recirculation of the seaweed outflow water. The initial scope of the project was to investigate whether or not seaweed cultivation of *Gracilaria* and *Ulva* were possible on the west and south west coasts of South Africa, and whether it was possible to integrate this into an aquaculture farming facility.

Following on from the success of these projects (see Robertson-Andersson, 2003; Njobeni, 2006) an integrated seaweed abalone recirculating system was designed and built at I & J and this system formed the basis of this PhD. In addition, this system was used to perform several small-scale experiments by a number of researchers. These experiments were designed to look at individual aspects of the overall system; e.g. sediment production, bacteria concentrations, mesoherbivore densities, water quality at varying recirculation rates, feeding frequency, abalone health, most of which has been commented on in Chapters 5 & 6.

Doing this type of research on a commercial facility *in situ* has had several advantages and disadvantages. The fact that this thesis was completed under industrial conditions is both it's biggest advantage and disadvantage. The study was exposed to pre-existing conditions on the farms (i.e. the abalone although grown in separate tanks from the farm animals were exposed to practices which could lead to the transfer of parasites from the farm animals to the experimental animals). The experiment was also not monitored on a daily basis by a single person and responsibility for the system was delegated to a person other than the researcher. Even though the experiment occurred under farm conditions, it was not a true reflection of what occurs on a farm. This can be seen in that the parasite density and prevalence was much greater on the farm compared to the experiment.

An advantage of this study is that it is a commercial application of lab-based science, although on a pilot scale. However, a commercial, large scale, integrated recirculating system's performance can only be inferred from a study such as this one, although more accurately than a laboratory based study. There is the possibility that the results from this thesis are only applicable to the farms on which the research took place and this questions the relevance of this research to the greater scientific community. Seasonal and temporal effects of the system could be investigated in contrast with a laboratory based study.

Replicates on a large and even pilot scale are difficult as they are expensive. This is why there were only three replicated in Chapter 4 - 6. The animals and tanks were in effect donated by the farm and the farm lost cultivation space that would normally have been used. The Abfeed® animals in the feed experiment were of such a poor quality that they had to be canned and could not be sold in the live form, which was a revenue loss for the farm. Research hypotheses for science are often different to the questions that the farm wants answered. Research into diets and effects of diets on growth rates does not take into account economics. The Abfeed® diet although the worst performer, was the cheapest, in terms of labor required for feeding. The feed quality was consistent throughout the year and there were no problems with weather affecting supply.

The main issue with an integrated recirculation system is optimal functioning, and the definition of optimal. The organisms that are part of the integrated system have different physiological processes taking place which have different requirements and optima (Chopin *et al.* 2001). As a result optimizing the efficiency of the system is complex as there are conflicting objectives, e.g. must the system be efficient at bio-filtering or must it be efficient at providing a good quality feed (Chopin & Yarish, 1998). For example our system was shown to be nitrogen limited, but this resulted in a high nutrient uptake efficiency. One of the easiest ways to change this in our system is to change the water flow (Lindström, 2006) or the feed source being fed to the abalone (Chapter 5). Changing these two variables will result in changes in the nutrient flux in the water, resulting in changes in the nutrient uptake efficiency of the system and the seaweeds tissue nitrogen contents. Neori & Shpigel (1999) demonstrated that seaweeds grown in an integrated system increased the overall profitability of the aquaculture operation per cultivation unit as well as per resource unit. When the service of improving water quality is recognized and quantified this type of system will significantly improve the success of an aquaculture operation.

A systems approach was initially used to place this thesis in context with current literature. Figure 7.1 tries to illustrate how this thesis fits in with the three interrelated aspects of the sustainability of an aquaculture system: namely production technology, social and economic aspects, and environmental aspects (after Edwards, 1994). This thesis has shown that the seaweeds can reduce nutrient loads in the effluent and in the process increase their biomass. The seasonal and long term data generated in this thesis can be extrapolated to a larger commercial scale system. The thesis looked at the socio-economic context of abalone farming in South Africa. The introduction of integrated seaweed/ abalone farms will facilitate further expansion of the abalone industry in South Africa without placing increasing strain on the natural environment (e.g. kelp harvesting and dissolved organic loading). The seaweed harvesting industry would benefit from increased demand, as well as the canning industry. The system has been shown to be as productive as existing flow-through systems. This thesis shows that integrated seaweed/ abalone recirculation aquaculture is feasible and that there are a number of benefits to abalone farmers for incorporation with existing facilities.

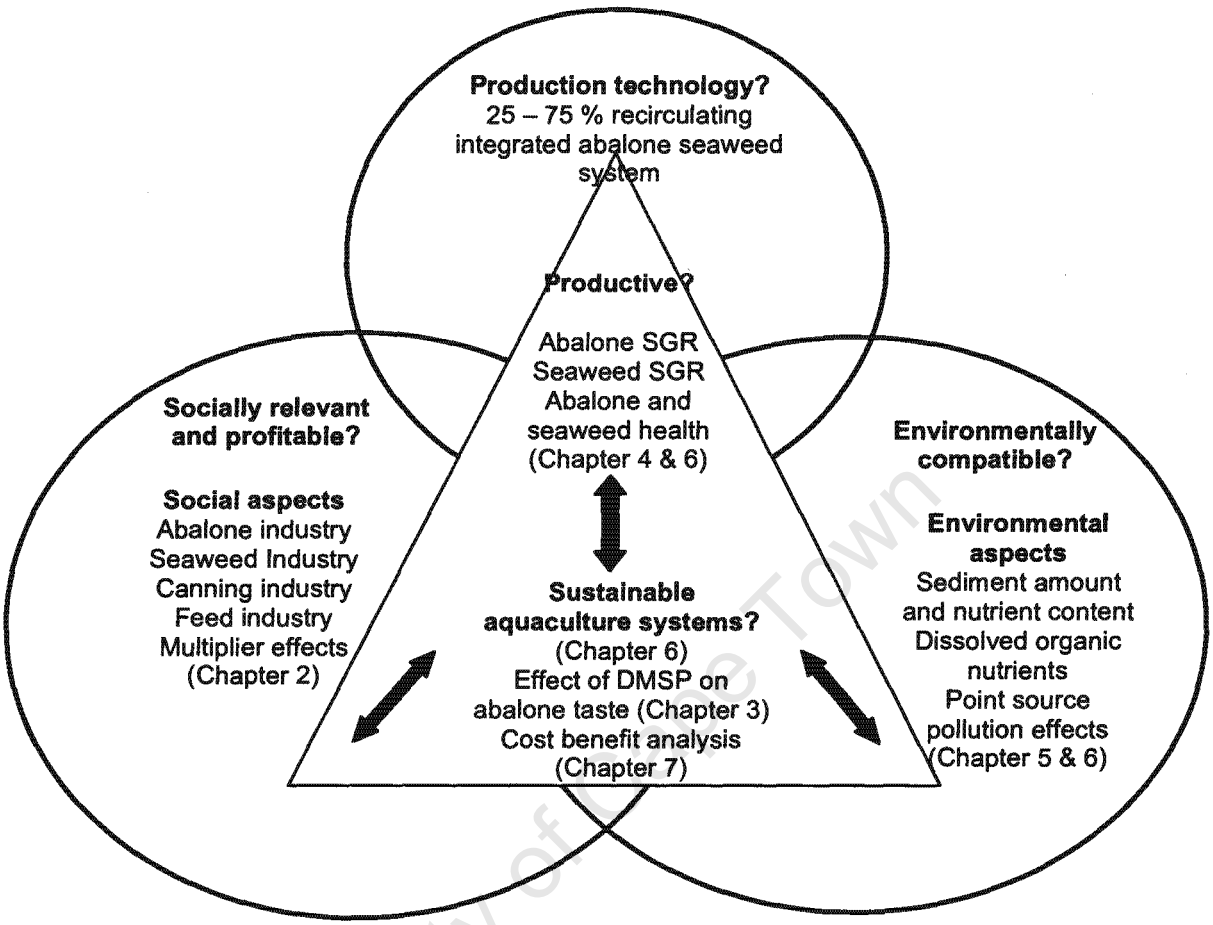

Figure 7.1: Revisiting the three interrelated aspects of the sustainability of an aquaculture system: production technology, social and economic aspects, and environmental aspects within the context of this thesis (after Edwards, 1994).

Physical cost savings of such as system

The I & J farm has, based on this research, built its second platform capable of cultivating 120 tons of abalone as an integrated recirculating seaweed /abalone system. The commercial scale seaweed cultivation occurs in paddle ponds. There are 4 currently working and are stocked with *U. lactuca* and *U. capensis*. The ponds are 8 m X 30 m with a volume of 162 m^3 due to a sloping depth from 0.75 m to 0.55 m to facilitate easy drainage during harvesting. The seaweeds are circulated by means of a current caused by a paddle wheel, which is driven by a 3 KW electric motor with a chain drive. The ponds are lined with a high

density (750 micron) polyethylene liner, to facilitate easy cleaning and maximize light reflection. The ponds are harvested every 22 working days. The water exchange rate is about 13.3 volume exchanges per day and the water the ponds receive is abalone waste water from about a 120 ton abalone platform. The water is drum filtered through a 100 µm drum filter prior to entering the ponds. The water is recirculated back to the abalone platform after collecting in a sump. Based on research by Robertson-Andersson (2003) the preferred water exchange rate for maximum nutrient removal and best seaweed growth rates was between 12 - 20 VE d^{-1}. The volume of water moving through one pond in one day in the current system is 162 m^3 X 13.3 VE = 2 160 m^3 d^{-1}. The abalone platform water supply is - 500 m^3 h^{-1} X 24 = 12 000 m^3 d^{-1}. In the system as it is currently working 8 640 m^3 of water for the system is being passed though the seaweed ponds which equated to 72 % of the water in the platform being passed through a biofilter. If we assume a nutrient uptake efficiency of 80.8 % (Chapter 6, Table 6 of TAN by the seaweeds in the effluent water, then 50.18 % of the total volume of water being used has 80.8 % of the TAN removed in a 24 hour period.

Feed

A single pond at the I & J system is able to produce 1.2; 2.3 and 4 tons of *Ulva* in winter, spring and summer, respectively, every 22 working days (I & J farm data). The cost of 1 tone of kelp ranges from ZAR 950 – ZAR 1 250 (US$ 154 – 203; April 2006) per ton with a FCR of between 1: 12.5 – 17 (I & J farm data). This has a direct rand value of R 132 000 (assumptions 120 tons produced in a year at a value of ZAR1 100 (US$ 179; April 2006)). Cultivated *Ulva* has an FCR of 1: 3 – 5 (I & J farm data) due to its higher protein content and this means that the equivalent feed value is ZAR 478 500 (US$ 77 679; April 2006) (assumption a 3.6 FCR ratio).

Pumping costs

Due to the reduced head heights when pumping the total savings from having this system is an average of 20 KW h^{-1} (based on I & J system schematics, calculated by SS pumps). Electricity costs per KVA are around 16 c per unit (US$ 0.03; April 2006) (bulk usage for Gansbaai district, ESKOM, November 2006). If we assume that the power correction factor for a pump is equal to 1, then 1 KVA is equal to 1 KW. A 20 KW saving over a year would equal ZAR 20 032 (US$ 3 252; April 2006).

Cost of a pond

In 2005 the cost to build a single pond with the components listed in Table 7.1 was ZAR 84 240. Thus the cost of the four ponds built at I & J was met by their savings in the feed cost in the first year alone.

TABLE 7.1: Cost breakdown of components required to construct one seaweed paddle pond (38 x 8 m) in 2005.

	Costing	Cost per pond
Concrete casted	40000	
Sand floor (compacted)	1400	
HDPE white liner 750 micron 2 X 6 X 38 m	8800	
Coverstrips 8 mm X 40 mm X 120 m HDPE	1500	
S/S screws 40 mm X 4.2 mm X 500/pond	225	
Elec motor and gearbox 3 KW & chain & sprockets	10400	5200
Paddle and bearings	9000	4500
Cover for elec motor (fibreglass)	450	225
Channel @ R650/m X 18	11700	
Sluice gate for incoming water	350	
Civils to level out & compact platform @ R25/m2	6840	
Elec cable for supply and D. B.	3500	
TOTAL	**94165**	**84240**

To conclude I ask what is the largest aquaculture product in South Africa by weight? With 38 seaweed paddle ponds in South Africa each currently producing

an average of 2.3 tons of seaweed a month, current seaweed production is 1 048 tons of seaweed a year and all of this is through integration with abalone effluent. This is 300 tons more than the 2006 abalone production.

ACKNOWLEDGEMENTS

AND

REFERENCES

ACKNOWLEDGEMENTS

I am grateful to the National Research Foundation (NRF), the Swedish and South African Collaborative Programme, The Marine & Coastal Management Fronteir Programme, The SANCOR Student Travel Grant and SIDA for funding this research.

I wish to thank a number of people without whose help I would have been unable to complete this project. To my supervisors Prof. J. J. Bolton, Associate Prof. M. Troell & Dr. Trevor Probyn, many thanks for their assistance and guidance. To Dr. R. J. Anderson, Dr A. J. Smit and Dr. C. Halling, thank you for your words of wisdom. To S. Peal thank you for analysing all those smelly samples. To Dr. A. Mouton thank you for the histology analysis. To Viva caterers thank you for making my taste test such a success.

Thanks must also be extended to the all the managers of farms and seaweed harvesting companies in particular, to the people from I & J farm, namely: Nick Loubser, Lawrence Ansara; Juan Dormehl and Lize Schoonby and to all the other staff who helped with the harvesting and experiments and donation of animals and facilities for experiments.

To the people on the Jacobs Baai farm especially, Kevin Ruck, Cecila Viljoen and Jonathan Venter a big thank you for all your hard work and help with the data collection as well as animals. To Dr. Piere Hugo form Abagold thank you for your time and animals. To Richard Clark on Wild Coast Abalone thank you for your time and help as well as the supply of abalone.

Thanks to too Roger Krohn, Louise Jensen and Gavin Johnsten (HIK), Ferdie Endeman (AQUAFARM) and Denis Whyte (WEST COAST ABALONE).

To Andrea Plos thanks for listening and waking up during the 72 hour experiments, thank you for your help with the diving and when panicked calls came in to help prepare 600 animals you were there. To Chris and Derek of the seaweed unit thank you for you help in collecting (or should I say decimating the Simons town seaweed population).

Finally to my family, I owe a special thank you for their tolerance, love understanding and never-ending support in all my endeavours. A big thank you to my mother for her support and help in looking after my children. Lastly, Coral and Reece thank you for your understanding when mummy was late, tired and dirty, for your smiles and the way you make me laugh.

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APPENDICES

Appendices

APPENDIX A

List of permits obtained for the duration of this thesis

Permits obtained for the duration of this thesis

All animals were transported under Permits V1/10/5/1; 62311; from the Department of Environmental Affairs and Tourism (RSA) and Movements of Live Moluscan Shellfish in South Africa permits: 01919, 01700, 01552 and 01431.

Animals were cultivated under Permits ref no. V1/1/3/3/2; 50679, V1/1/4/1 and V1/1/5/1 from the Department of Environmental Affairs and Tourism.

Animals were killed following the protocol outlined in Animal Experimentation Committee Application 2004/V17/DR from 2004 to 2006.

Wild animals were collected under the following permits V1/1/5/1 from the Department of Environmental Affairs and Tourism (RSA) from 2003 to 2006.

Permission to collect marine organisms under the auspices of the UCT Zoology department was obtained from 2003 to 2006 as per sections 2b and 1.1 of the collection permits ref no V1/1/5/1 and V1/1/3/3/2.

Additional collection and transport permits V1/1/3/3/2 from the Department of Environmental Affairs and Tourism (RSA) form 2004 to 2006. An addition to this permit was obtained in 2006 for the purposes of the taste trial.

Permission to enter Table Mountain National Parks for research proposes was obtained from the South African National Parks to collect abalone from the Cape Point Nature Reserve via SCUBA was obtained for July to August 2005 and July 2006.

Exemption to dive in a marine protected area for scientific purposes only was obtained under Department of Environmental Affairs and Tourism ref number V1/1/5/1 for 2006 following the promulgation of the new diving regulations under Section 79 of the Marine Living Resources Act (Act 18 of 1998).

SABS Health Certificates for abalone for human consumption were obtained for the taste trial Ref no 06/251 of 25 May 2006 and 5333/LA/147/0.

Abalone Health Certificates for the animals used in the taste trail were as follows 03/074; 03/097; 04/117; 04/145; 04/163 and 05/197.

The Taste test met all of UCT Science Ethics Committee requirements for research performed on human subjects.

Appendices

INDEMNITY FORM

1. I, _____, the undersigned **("the Indemnity Grantor")** in my personal capacity as a major adult over the age of 21 years; wish to participate in the abalone tasting event **("the Tasting Event")** to be held at the UCT Club on 31 July 2006 hereby acknowledge, agree and undertake in favour of the University of Cape Town, its directors, employees, representatives and agents ("**the Indemnified Persons**") that:

   1.1 the Indemnity Grantor is fully aware that the eating of shellfish may cause an allergic reaction in some people and that the Grantor fully accepts all the risks associated with eating shellfish at the Tasting Event;

   1.2 the Indemnity Grantor hereby releases the Indemnified Persons from all liability and holds each and all of the Indemnified Persons harmless against all claims, damages, injuries, losses, deaths, expenses and liabilities arising out of or in any way connected with tasting shellfish and/or other foods or beverages at the Tasting Event without limitation:

      1.2.1 any personal injury or loss of life;

      1.2.2 any loss of support, maintenance or other claims or damages arising from or connected with any personal injury or loss of life to the Indemnity Grantor;

   whether arising out of strict liability, statute or otherwise and whether caused by the negligence or gross negligence on the part of the Indemnified Persons or any other person or otherwise.

2. Each clause of this deed of indemnity is independent and severable from all other clauses.

3. The acknowledgements, agreements and undertakings in this indemnity shall be deemed to be made in favour of the directors, employees, representatives and agents of the University of Cape Town, capable of acceptance at any time.

4. Each element of the release from liability and/or indemnity in respect of each cause or activity covered by this release from liability and/or indemnity shall be separate and severable from the other elements.

5. This indemnity shall in all respects be governed by the laws of the Republic of South Africa, and all disputes, actions and other matters arising in connection therewith shall be determined in accordance with such laws.

SIGNED on 31 July 2006

Witness:	**INDEMNITY GRANTOR**
_____	_____
Signature	Signature
_____	_____
Name (print)	Name (print)

Appendices

APPENDIX B

List of Acts consulted in the writing of this thesis

Acts

The following Acts were consulted in the writing of this thesis.

White Paper on the Conservation and Sustainable Use of South Africa's Biological Diversity, published in the *Government Gazette* on 28 July 1998.
The Constitution Act 108 of 1996 section 24
White Paper on the Conservation and Sustainable Use of South Africa's Biological Diversity
World Conservation Strategy of the United Nations Convention on Biological Diversity and Climate Change
The National Environmental Management Act 107 of 1998
Integrated Environmental Management Guidelines Series
Fisheries Policy Development Commission 1994
A Marine Fisheries Policy for South Africa 1996
The Marine Living Resources Act (Act 18 of 1998) and Regulation gazette 6284
Sustainable Coastal Development in South Africa 2000
Sea Shore Act No. 21 of 1935 to be repealed by the National Coastal Zone Management Act.)
Maritime Zone Act 15 of 1994
United Nations Convention on Law of the Sea 1982
Environment Conservation Act 73, 1989
Crown land
Admiralty zones
National Water Act 36 1998
Agricultural Pests Act 36 1983
Animal Diseases Act 35 1984
Genetically Modified Organisms Act 15, 1997
Public Health Act 63, 1977
Foodstuffs and Disinfectants Act 54, 1972
Standards Act 29, 1993
The Biodiversity Act (Act 10 of 2004)

Conservation of Agricultural Resources Act (Act 43 of 1983) to be repealed by the Sustainable Use of Agricultural Resources Act once promulgated.)

Animal Improvement Act (Act 62 of 1998)

The National Building Standards Act, 1977

The Development Facilitation Act of 1995