

Setting optimal diet ratios of kelp (*Ecklonia maxima* (Osbeck) Papenfuss) and formulated feed, and tests of a recirculating system using *Ulva* (Chlorophyta) as a biofilter, in South African abalone (*Haliotis midae*) cultivation.

By

Kishan Sankar

Thesis presented for the degree of Master of Science

Department of Botany (Faculty of Science)

University of Cape Town

April 2010

Co-Supervisors: Prof. J.J Bolton

: Dr D.V. Robertson–Andersson

University of Cape Town

University of the Western
Cape

The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.

DECLARATION

I know the meaning of plagiarism and declare that all of the work in the document, save for that which is properly acknowledged, is my own unaided work and has not been submitted in this or any form to another university.

Experimental work discussed in this thesis was carried out under the supervision of Prof. J.J. Bolton Botany department, University of Cape Town and Dr D.V. Robertson–Andersson Biodiversity and Conservation Biology, University of the Western Cape.

Water nutrients performed with the assistance of Dr. T. Probyn Marine and Coastal Management. All abalone histology and health examinations were conducted by Dr. A. Mouton.

Kishan Sankar

Department of Botany, University of Cape Town

April 2010

Acknowledgments

I am grateful to the National Research Foundation (NRF), the Swedish and South African Collaborative Programme, SANCOR and SIDA for funding this research.

I would like to thank my supervisors Prof. J. J. Bolton and Dr D.V. Robertson-Andersson for all your assistance. To Dr. A. Mouton thank you for the histology analysis and to Dr. Trevor Probyn, thank you for your assistance with the water nutrient analyses. I would like to thank Dr Colleen Moloney for assistance with statistics.

To the members of the Phycology lab and the MCM Seaweed Unit based at UCT, thank you for your constant support and guidance over these past few years.

Thanks must also be extended to the people from I & J farm, namely: Nick Loubser, Lawrence Ansara and Lize Schoonby and to all the other staff who helped with the experiments.

To Clement J. Arendse, thank you for all your help during the sampling period, from our many days in abalone excrement, to our days of sleep deprivation during the 72 hour water nutrient experiments.

To my friends and family thank you for all your endless love and support. A special thanks must be said my parents and sister, I would not be where I am today without you and to my grandparents, thank you for taking me into your home, and taking care of me over the past few years, it was greatly appreciated.

Abstract

Land-based abalone aquaculture in South Africa, based on the local species *Haliotis midae*, started in the early 1990's and has grown rapidly in the last decade. This has led to numerous studies into various aspects of farming *H. midae*. There are two aims of this study, (i) to test the relative benefits of different ratios of kelp and the formulated feed Abfeed[®] K26 (feed component), (ii) to test the suitability of farming *H. midae* with 50 % and 75 % recirculation rates (recirculation component) in an integrated system using the green seaweed *Ulva* as a biofilter. Aims for both components were to investigate the performance of the systems in terms of health of abalone, growth rates of abalone, sediment loadings and water quality. Both components were conducted on Irvin & Johnson Ltd Cape Abalone farm in Gaansbaai, 140 km east of Cape Town.

Both experiments were stocked with 550 abalone per basket, from three spawning cohorts (January, February and March 2004), with a starting abalone weight range of 10 – 30 g. The feed component of the experiment consisted of three treatments, with three replicates. The percentage of Abfeed[®] K26 fed per day was 0.6 % BW d⁻¹, and the percentage of Kelp (*Ecklonia maxima*) fed per day was 7 % BW d⁻¹. The abalone in the Abfeed[®] 3 diet was fed 2.7 kg of kelp on Mondays and 180 g of Abfeed[®] K26 on Fridays; the abalone in the Abfeed[®] 4 diet was fed 2.08 kg of kelp on Mondays and 225 g of Abfeed[®] K26 on Thursdays; and the abalone in the Abfeed[®] 5 diet was fed 1.4 kg of kelp on Mondays, 135 g Abfeed[®] K26 on Wednesdays and 180 g Abfeed[®] K26 on Fridays. There was no significant difference between the weights (ANCOVA F = 0.05, DF = 264 and p > 0.05) and lengths (ANCOVA F = 0.117, DF = 264 and p > 0.05) of abalone in all three dietary treatments at the termination of the experiment. There was no significant difference in water nutrients, physico-chemical variables and sediment loadings between all three dietary treatments. The health recordings showed all the abalone had significant environmental stress and all the diets had sabellid infections. This study showed that increasing the levels of Abfeed[®] K26 had no effects on abalone growth rates and did not negatively effect the culture environment

The recirculation component of the experiment was conducted using 50 % recirculation for a six month period, and for a week at 75 % recirculation. The recirculation system consisted of abalone tanks connected to two seaweed tanks containing *Ulva lactuca*. Three flow-through units (FTUs), which did not recirculate water, were used as controls for this experiment. Abalone were fed 2.7 kg of kelp fed on Mondays and 180 g of Abfeed[®] K26 fed on Fridays, which was equivalent to the Abfeed[®] 3 diet in the feed component of this experiment. There was no significant difference between the lengths (t-test $t = 0.495$, $df = 178$ and p -value 0.621) and weights (t-test $t = 0.726$, $df = 178$ and p -value 0.468) of the abalone at the termination of the experiment in the 50 % Recirculation system and FTUs. Testing for differences between the two population regression coefficients (ANCOVA) also showed no significant differences between abalone lengths ($t = 0.048$, $df = 176$, $p > 0.05$) and weights ($t = 0.055$, $df = 176$, $p > 0.05$) in the Recirculation system and FTU's. Abalone in both the FTUs and 50 % Recirculation system had sabellid infections and also showed significant environmental stress. There were no significant differences between the pH, temperature and dissolved oxygen of the FTUs and 50 % Recirculation system. At 75 % recirculation the Recirculation system had a significantly higher temperature than the FTUs. There were no significant differences between the total ammonium nitrogen and phosphate concentrations for the 50 % and 75 % Recirculation systems and their respective FTUs. There were no significant differences between the nitrate levels recorded for the 50 % Recirculation system and the FTUs. The nitrate concentrations recorded in the 75 % Recirculation system were significantly lower than the FTUs ($p < 0.05$). Long term physico-chemical recordings show dissolved oxygen in the 50 % Recirculation abalone units to be on average 1.89 % (± 0.02) higher than the FTUs. pH in the 50 % Recirculation abalone units, was on average 0.84 % (± 0.004) higher than the FTUs. Temperature in the 50 % Recirculation abalone units, was on average 0.54 % ± 0.01 (0.09°C) higher than the FTUs. There was no significant difference between the FTUs and 50 % and 75 % recirculation systems water column sediments and bottom sediments accumulation. This experiment shows that it would be suitable to grow abalone in a 50 % Recirculation system, as there were no significant differences between the FTU and 50 % Recirculation system in terms of water nutrients, physico-chemical variables, sediment accumulation and abalone health. The cost savings

of pumping and the benefits of seaweed production may make it more profitable to use a recirculating system. For the 75 % Recirculation system, the only worrying factor is the low dissolved oxygen that was recorded during the experiment. If it is possible to improve the dissolved oxygen levels, the 75 % Recirculation system would be also be a viable option.

Table of Contents

Chapter 1: Literature Review

1.1 World aquaculture	1
1.1.2 World seaweed aquaculture	2
1.1.3 World abalone aquaculture	4
1.1.4 Important parameters for abalone culture	5
1.2 The South African context	6
1.2.1 South African Seaweed industry	7
1.2.2 South African Seaweed aquaculture	8
1.3 Abalone in South Africa	10
1.3.1 South African Abalone aquaculture	11
1.3.2 Problems with abalone health in South Africa	13
1.4 Compound Feeds	16
1.5 Integrated aquaculture	20
1.6 Seaweed choice for efficient biofiltration	23
1.7 Integrated aquaculture in South Africa	24
1.8 Objectives of thesis	28

Chapter 2: Setting optimal diet ratios of kelp (*Ecklonia maxima* (Osbeck) Papenfuss) and formulated feed (Abfeed[®] K26) for *Haliotis midae* cultivation

2.1 Introduction	31
2.1.1 Aims	33
2.2 Materials and Methods	34
2.2.1 Study site	34
2.2.2 Experimental animals	35
2.2.3 Diets	36
2.2.4 Sub Samples	37

2.2.5 Daily Increment in Shell Length	38
2.2.6 Specific growth rates	38
2.2.7 Condition factor	38
2.2.8 Physico-chemical variables and Water nutrients	39
2.2.9 Sediments	40
2.2.10 Abalone health	41
2.2.11 Statistical analysis	43
2.2.12 Estimated time to harvest	44
2.3 Results	45
2.3.1 Growth	42
2.3.2 Growth parameters	50
2.3.3 Estimated time to harvest	52
2.3.4 Condition factor	53
2.3.5 Water nutrients	54
2.3.6 Physico-chemical variables	58
2.3.7 Sediments	61
2.3.8 Abalone health	61
2.4 Discussion	64
2.4.1 Growth	64
2.4.2 Water Nutrients	66
2.4.3 Physico-chemical variables	68
2.4.4 Sediments	69
2.4.5 Abalone health	70
2.4.6 Cost assessment	71
2.5 Conclusion	72

Chapter 3: Abalone (*Haliotis midae* Lin.) cultivation at high recirculation rates (50 – 75 %) using a seaweed and formulated feed fortified diet, with *Ulva lactuca* L. as a biofilter

3.1 Introduction	73
3.1.1 Aims	77
3.2 Materials and Methods	78
3.2.1 Study site	78
3.2.2 Experimental design	78
3.2.3 Experimental setup	80
3.2.4 Experimental animals	81
3.2.5 Diets	81
3.2.6 Sub samples	81
3.2.7 Daily Increment in Shell Length	82
3.2.8 Specific growth rates	82
3.2.9 Condition factor	82
3.2.10 Physico-chemical variables and Water nutrients	83
3.2.11 Sediments	83
3.2.12 Abalone health	84
3.2.13 Statistical analysis	84
3.2.14 Estimated time to harvest	85
3.3 Results	86
3.3.1 Growth	86
3.3.2 Estimated time to harvest	92
3.3.3 Condition factor	93
3.3.4 Abalone health	94
3.3.5 Physico-chemical variables	96
3.3.6 Water nutrients	102
3.3.7 Long term Physiochemical variables	107
3.3.8 Sediments	110
3.4 Discussion	111
3.4.1 Growth	111

3.4.2 Condition factor	112
3.4.3 Abalone health	112
3.4.4 Physico-chemical variables	114
3.4.5 Water nutrients	116
3.4.6 Sediments	121
3.5 Conclusion	121
Chapter 4: General discussion	123
Chapter 5: References	129

List of acronyms

AFASA	Abalone Farmers Association of Southern Africa
CF	Condition factor
DGP	Digestive gland protozoa
DISL	Daily Increment in Shell Length
DO	Dissolved oxygen
FAN	Free un-ionized Ammonia Nitrogen
FTUs	Flow through units
HAB	Harmful algal blooms
MCM	Marine and Coastal Management
MSY	maximum sustainable yield
RLP	Rickettsia-like prokaryote
SGR	Specific Growth Rates
SPM	suspended particulate matter
TAN	Total Ammonia Nitrogen
WS	Withering Syndrome

Introduction

1.1 World aquaculture

Aquaculture is believed to have originated some 4000 years ago in the Asian region (Reay, 1979; Ackefors et al., 1994; Hecht & Britz, 1990; Joseph, 1998). Freshwater aquaculture was initiated before the more recent practice of marine aquaculture developed (Lee, 1998). There has been a major increase in aquaculture over the last few decades, with worldwide aquaculture production growing by an average of 8.8 % a year between 1950 and 2004, to 59.4 million tons in 2004, worth more than US\$ 70 billion (FAO, 2006). The major factors responsible for the expansion in aquaculture include: an increase in the demand for aquaculture products and the incapacity of global capture fisheries to expand seafood production (Neori et al., 2004; Bolton, 2006); an increase in available areas for aquaculture (through improved technologies); improvement of culture techniques; increase in the number of species under cultivation; genetic enhancement of aquaculture species; development and improvement of formulated feeds and improved control and understanding of disease of culture species (Li, 1999). For aquaculture in marine environments (by weight), 44 % is seaweed production, 46 % molluscs, 8.7 % fin-fish farming and 1 % is from farming crustaceans (FAO, 2004). Aquaculture accounts for almost 50 % of the world's food fish and, given the projected population growth over the next two decades, it is estimated that at least an additional 40 million tones of aquatic food will be required by 2030 to maintain the current per capita consumption (FAO, 2006). With the diminishing availability of fresh water, most of this growth will take place in the marine environment (Neori et al., 2004).

Of the total world wild fish catch, Africa contributes 6 % (570 000 tons) while South Africa contributes 9 % of Africa's contribution amounting to 0.5 % to the total world catch (AASA, 2004). Africa contributes < 1 % of the world aquaculture production and South Africa is < 1 % of that (FAO, 2006). As the human population continues to grow and the demand for seafood continues to increase, the need to expand aquaculture production will increase accordingly. The FAO (2001) cautions that an increase in aquaculture production will depend on new research and improved management practices. The major issues that need to be addressed are problems with access to proper technology and financial resources, together with environmental impacts and diseases (FAO, 2001). In order for aquaculture to continue and grow, it must be based on relevant species choices and sound technologies more relevant to developing countries (Naylor et al., 2000; Williams et al., 2000; Hambrey 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).

1.1.2 World seaweed aquaculture

Worldwide algal aquaculture has been estimated to be worth US\$ 5 - 6 billion (Wikfors & Ohno, 2001). The largest proportion of the industry is macroalgal production for human food in Asia (Wikfors & Ohno, 2001). Brown and red seaweeds dominate the production, especially nori (*Porphyra* spp.), kombu (*Laminaria japonica* Aresch.) and wakame (*Undaria pinnatifida* Harv.) (Wikfors & Ohno, 2001). Nearly 70 % of world aquaculture takes place in China, and a further 22 % in other Asia-Pacific countries (FAO, 2006).

In Europe and the United States the historical use of cultivated seaweeds is less direct, with phycocolloids (carrageenan, agar and alginates) being used in processed foods (Zemke-White & Ohno, 1999). Carrageenan is extracted from red seaweeds (*Kappaphycus*, *Chondrus*, *Eucheuma*, *Gigartina*, *Iridaea*), agar from *Gracilaria* and *Gelidium*, and alginates from *Macrocystis*, *Laminaria* and other kelps and fucoids. (Wikfors & Ohno, 2001). The use of seaweeds for human food is growing in the western world, with the increased popularity of 'sushi bars' (Bolton, 2006).

Marine macroalgae form dense stands on the well-illuminated rocky margin of all continents (Lüning & Pang, 2003). It is this accessibility to seaweeds that has allowed them to be utilised and farmed by man for hundreds of years as food and fodder, particularly in the Far East (Lüning & Pang, 2003). At present there are approximately 200 species of seaweeds used worldwide (Zemke-White & Ohno, 1999), of which about 10 species or genera are intensively cultivated, such as the brown algae *Laminaria japonica* and *Undaria pinnatifida*, the red algae *Porphyra*, *Eucheuma*, *Kappaphycus* and *Gracilaria* and the green algae *Monostroma* and *Ulva* (Wikfors & Ohno, 2001). Currently the major driver for seaweed cultivation is for human consumption, while new research and the potential for new algal products and novel uses of seaweeds are acting as stimulants to encourage more research and development of seaweed cultivation (Lüning & Pang, 2003).

1.1.3 World abalone aquaculture

Abalone are among the most highly valued seafoods in the world, with the prime demand in Asian countries where abalone products form part of traditional cuisine and ceremony (Britz,

1996). Numerous recent authors report approximately 90 species of abalone world wide (e.g. Jarayabhand & Paphavasit, 1996; Elliot, 2000; Selvamani et al., 2001; Bester et al., 2004; Sales & Janssens, 2004) however this number is subject to debate, but only 15 species are of economic importance with respect to fisheries and aquaculture (Sales & Janssens, 2004). Abalone are found mainly along rocky shores of almost all continental shelves, with the exception of the western coast of South America and eastern North America (Imai, 1977; Hahn, 1989a; Nash, 1991; Brown & Murray, 1992; Lindberg, 1992; Lee & Vacquier, 1995; Geiger, 2000). Temperate species of abalone were observed to be larger than tropical species (Imai, 1977). This is believed to be due to the higher abundance of food and oxygen in temperate waters compared to that of tropical waters (Fleming et al., 1996). Abalone are found in the lower intertidal zone and sublittoral fringe of rocky shores (Newman, 1969; Hooker & Morse, 1985; Prince & Shepherd, 1992; Miller & Lawrenz-Miller, 1993; Richards & Davis, 1993; Preece & Mladenov, 1999; Geiger, 2000), the subtidal (Hooker & Morse, 1985; Prince & Shepherd, 1992; Preece & Mladenov, 1999; Geiger, 2000) and the deep open ocean (Hooker & Morse, 1985; White, 1995).

The global production of abalone reached 22,600 metric tonnes (including poaching of 3700 metric tonnes) in 2002 (Troell et al., 2006), 8600 metric tonnes of this was farmed and the total value of the production was estimated as approximately US\$ 0.8 billion (Gordon & Cook, 2004). Due to a decrease in wild fisheries and an increase in demand, a rapid development of abalone cultivation took place in the 1990s, in the USA, Mexico, South Africa, Australia, New Zealand, Japan, China, Taiwan, Ireland, and Iceland (Hahn, 1989; Gordon & Cook, 2001). China is the largest producer in the world with over 300 farms and a total production of approximately 4500 metric tonnes (Gordon & Cook, 2004).

1.1.4 Important parameters for abalone culture

“Water quality” is usually defined as the suitability of water for the survival and growth of aquatic organisms (Boyd 1982). There are a host of water quality parameters that are important to consider for optimum cultivation of any aquatic organism. These include:

- organic waste (faeces, metabolic, feed, fertilizers, etc.) (Rychly 1980; Pillay 1992, 1994; Goldberg et al. 2001)
- pH (McDonald 1983; Beveridge 1987; Randall 1991; Swann 1992; Barnabé 1994; Harris et al. 1999a)
- pathogens (Lester & Davis 1981; Bower 1987; Pillay 1992, 1994; Douillet & Langdon 1994; Bachère et al. 1995; Nakatsugawa et al. 1999; Rengpipat et al. 2000; Olafsen 2001; Nicolas et al. 2002; Bower 2003; Macey & Coyne 2005).
- salinity (Alabaster & Lloyd 1980; Brix 1983; Chen 1984; Beveridge 1987; Higashi et al. 1989; Anderson 1990; Fallu 1991; Landau 1992; Jarayabhand & Phapavisit 1996; Boarder & Shpigel 2001; Edwards 2003; Wuenschel et al. 2004)
- dissolved gasses (oxygen, carbon dioxide, nitrogen, hydrogen sulphide) (Marcello & Strawn 1973; Sylvester 1975; Spotte 1979; Boyd 1982; Elston 1983; Chen 1984, 1989; Sebert et al. 1984; Innes & Houlihan 1985; Beveridge 1987; Boyd & Watten 1989; Fallu 1991; Pillay 1992; Ackefors et al. 1994; Harris et al. 1997; Boyd 1998; Harris et al. 1999a, b; Hindrum et al. 2001; Parker 2002; Cheng et al. 2004a; Lopez & Tyler 2006; Camargo et al. 2007)

Dissolved nutrients such as ammonia also affect water quality. Ammonia is the principal nitrogenous compound by aquatic animals (Colt & Armstrong, 1981) and being toxic to fish, crustaceans and mollusks, can limit production in aquaculture (Epifanio & Srna, 1975; Wickins, 1976; Russo, 1985; Allan et al., 1990; Russo & Thuston, 1991). In solution,

ammonia exists in a pH and temperature mediated equilibrium between the unionized and ionized forms, with unionized ammonia considered more toxic (Russo & Thurston, 1991). Ammonia induces detrimental changes in tissue structure, cell function, blood chemistry, osmoregulation, disease resistance, growth and reproductive capacity (Colt & Armstrong, 1981; Russo, 1985; Jeney et al., 1992).

1.2 South African coastal ecosystems

South Africa has a coastline of approximately 3000 km, which is bordered by two oceans, the Indian Ocean to the east and Atlantic Ocean to the west (Anderson et al., 2006). This has led to one of the world's richest seaweed floras (Bolton & Stegenga, 2002). The east coast of South Africa is influenced by the warm Agulhas Current, which has its origins in the South Equatorial Current of the Indian Ocean (Anderson et al., 2006). The biota of the east coast of South Africa has generally been referred to as 'sub-tropical' (Anderson et al., 2006). The west coast of South Africa is influenced by cool upwelled water from the northward flowing Benguela Current which originates at the Sub-tropical Convergence, and comprises mainly Atlantic Central water (Shannon, 1985). The south coast from Cape Agulhas to Port Elizabeth has only sporadic upwelling, with generally warm-temperate conditions (Bolton & Anderson, 1997). The maximum tidal range on the South African coasts is about 2 m (Anderson et al., 2006). The coastline is subjected to intense wave action and there are few very sheltered natural embayments, the Saldanha / Langebaan system on the west coast being an exception (Schils et al., 2001). Southern African kelp beds are present on rocky coasts from just west of Cape Agulhas (the southern tip of Africa), around the southwest coast as far as northern Namibia (Anderson et al., 1997). In the south the dominant species in shallow water (0 to 8

m) is *Ecklonia maxima* (Osbeck) Papenfuss, with a sub-canopy of *Laminaria pallida* Greville ex J. Agardh which becomes the dominant species at depths from about 8 to 20 m, and replaces *E. maxima* in inshore beds on the northern west coast and in Namibia (Anderson et al., 1997).

1.2.1 The South African seaweed industry

The history of commercial exploitation of seaweed resources in South Africa started with the collection of beach cast *Gracilaria gracilis* and *Ecklonia maxima* (EM), which have been dried and exported for the production of agar and alginates (initially for gunpowder) since World War II (Anderson et al., 1989). Three species of *Gelidium* have been harvested, since the mid-1950s: *Gelidium pristoides*, *G. abbottiorum* and *G. pteridifolium* for agar (Isaac & Molteno, 1953; Anderson et al., 1989; Critchley & Rotmann, 1992; Anderson et al., 2003; Tronchin et al., 2003).

The demand for kelp in South Africa has fluctuated widely, with periodic slumps due to falls in international prices and production (Anderson et al., 2006; Troell et al., 2006). The first harvesting of kelp cut from beds started in the 1970's and was used for a liquid stimulant for agricultural crops (Anderson et al., 1989; Levitt et al., 2002; Kelpak[®], 2007). The growing demand for kelp by the abalone industry has greatly increased harvesting (Anderson et al., 2003). According to estimates done by coastal management authorities (Marine and Coastal Management, Department of Environmental Affairs and Tourism, Cape Town) the maximum sustainable yield (MSY) of the kelp was approached in 2003 for two areas; from Quoin Point to Cape Hangklip and in the Cape Columbine area (Anderson et al., 2003). The pressure on

kelp harvesting has since decreased from 6,000 t kelp harvested in 2004 to around 3,000 t in 2006 due to the increase use of formulated feeds for the abalone industry (Anderson et al., 2007; Bolton et al., in press).

Harvesting of kelp for abalone feed involves cutting the fronds from the floating canopy at 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 top of the stipe. The whole “head” of kelp is then pulled aboard, leaving the stipe and holdfast to die. Recovery of the kelp biomass then requires the growth of new sporophytes. Ashore, the fronds are then cut off and the primary blade discarded (Troell et al., 2006).

A non-lethal harvesting technique is also being used to commercially harvest kelp. This method excises only the distal parts of the secondary blades, leaving at least 20 – 30 cm of their bases (with the basal meristems intact) attached to the primary blade (Troell et al., 2006). The primary blade and stipe are left undamaged and can thus be repeatedly reharvested, and the yield of frond material over time was reported to be about five times higher than if the sporophytes were killed (Levitt et al., 2002).

1.2.2 South African seaweed aquaculture

Sea-based cultivation of the red seaweed *Gracilaria* was first researched in South Africa in the early 1990's (Engledow & Bolton, 1992; Anderson et al., 1996). To supplement the beach cast harvests, in 1989 South African researchers started *Gracilaria* growth experiments, using seaweed anchored with stakes to the bottom of Saldanha Bay (Anderson et al., 2006). In the early 1990s the then Sea Fisheries Research Institute started experiments on raft cultivation of

Gracilaria in Saldanha Bay (Anderson et al., 1996). These growth trials were relatively successful, with the *Gracilaria* growing well through most of the year, although there were problems with poor growth rates in summer when surface water became oligotrophic (Anderson et al., 2006). The seaweed resources in South Africa were described by Anderson et al., 2003, as being fully used, with future developments dependent on mariculture.

More recently, the increase in abalone farming in South Africa has renewed the interest in seaweed cultivation, with *Gracilaria* and *Ulva* being cultivated in tanks on the farms to supplement the abalone feed (Anderson et al., 2006). This land-based seaweed cultivation has the potential to expand rapidly in the future, if the demand for abalone feed starts to exceed kelp supplies.

Two farms in the Eastern Cape use raceways to cultivate the majority of their own feed (Anderson et al., 2006). This development occurred out of necessity, because large kelps (*Ecklonia maxima* and *Laminaria pallida*) do not occur in the Eastern Cape (Anderson et al., 2006). Farms in the Western Cape have also since begun to cultivate *Gracilaria* and *Ulva* to supplement their abalone feed (Robertson–Andersson, 2003; Robertson–Andersson et al., 2008; Bolton et al., in press). Over 1,000 t fresh weight of *Ulva* was cultivated on South African abalone farms in 2007, primarily for feed, but in one case to allow partial recirculation by nutrient removal (Bolton et al., in press).

Marine Growers near Port Elizabeth began experimental studies in the cultivation of initially *Gracilaria* (Fourie, 1994; Smit, 1997; Hampson, 1998) and later *Ulva* (Steyn, 2000) for feed addition. Since then, Wild Coast Abalone at Haga Haga near East London has become world leaders in *Ulva* paddle pond cultivation producing \pm 960 tons of cultivated seaweed per annum (pers comm. R. Clark, Manager Wild coast abalone). In 1992, the farm built a series of

32 large-scale raceways growing seaweed in effluent from the flow-through abalone tanks, and using paddle wheels for water movement, with dimensions of 40 m × 10 m and a depth of 0.5 – 0.75 m (Bolton et al., in press). Initially both *Gracilaria* and *Ulva* were cultivated, but for the last few years they have been used to grow *Ulva* (Bolton et al., in press). This integrated flow through abalone / *Ulva* system produces ca. 2.5 t *Ulva* per pond per month throughout the year (Bolton et al., in press).

1.3 Abalone in South Africa

There are six species of *Haliotis* in South Africa: *H. midae*, *H. parvum* (Lin.) *H. spadicea* (Donovan), *H. queketti* (Smith), *H. speciosa* (Reeve), *H. pustulata* (Reeve) (Sales & Britz, 2001). *Haliotis midae*, locally known as “perlemoen”, is the only species of interest for cultivation and it can reach a maximum size of about 200 mm shell length at an age of over 30 years in the wild (Newman, 1968). It is found naturally in the rocky coastal waters from Cape Columbine on the west coast to just north of Port St Johns in Transkei on the southeast coast (Newman, 1965; Muller, 1986; Wood, 1993). The abalone live in the area below the low tide mark to approximately 25m depth, but mainly occur at depths of 2 – 10 m and within *Ecklonia maxima* kelp beds (Tarr, 1992). Juvenile abalone also live in the subtidal zone, usually using small rocks and sea urchins as a refuge from predators (Tarr, 1989; SANCOR, 1996; Tarr et al., 1996; Day, 1998).

Haliotis midae is an opportunistic herbivorous species with a nocturnal pattern of grazing behaviour, tending to remain inactive by day and move about at night (Muller, 1984; Barkai & Griffiths, 1987; Knauer et al., 1995; Wood & Buxton, 1996). *Haliotis midae* consumes a

wide variety of algal species that are abundant in their vicinity (Barkai & Griffiths, 1986; Wood & Buxton, 1996). Although taking a wide range of algae, the dominant food item of mature *H. midae* in nature is kelp *E. maxima* and *L. pallida* on the southwest coast, with *Plocamium corallorhiza* (Turner) on the south coast (Newman, 1968, 1969; Barkai & Griffiths, 1986; Tarr, 1987; Wood, 1993; Britz, 1996b; Wood & Buxton, 1996). The diets of smaller size classes of abalone are similar, except that *Ulva* spp. is taken in larger proportion (Barkai & Griffiths, 1986). This might be explained by the fact that both *Ulva* spp. and small abalone tend to be more abundant in shallower water (Barkai & Griffiths, 1986).

1.3.1 South African abalone aquaculture

South Africa has become the largest abalone producer outside Asia (FAO, 2004) and over-exploitation of wild abalone stocks by poaching and high market prices have been the main drivers for its cultivation (Troell et al., 2006). Access to relatively cheap labour, together with favourable coastal water quality and infrastructure, facilitated rapid growth of the abalone industry in South Africa (Troell et al., 2006). The abalone fisheries in South Africa have existed since 1949. Cultivation started by successful spawning of captured specimens in 1981 to produce spat and juveniles (Genade et al., 1985, 1988). There are, currently, 22 commercial abalone rights holders with 13 producing farms in South Africa with an estimated investment of R346.5 million, with productions of 527 tons in 2003 and 750 in 2004 (Robertson-Andersson 2007). The majority of farms are located along the South African southwest and west coasts (Rothman et al., 2006). This is largely due to the abundance of the main food source (kelp) that occurs so abundantly along these shores (Simons, 1990; Simpson & Cook,

1998;). Farm production is concentrating on an average abalone size of 100 mm after 5 years (Sales & Britz, 2001).

According to AFASA (Abalone Farmers Association of Southern Africa) the expansion of the industry will continue. However, the lack of suitable coastal land and the dependence on wild harvest of kelp for feed may restrict further development in some areas (Troell et al., 2006). The development of nutritionally complete compound pelleted feeds is seen, by a number of analysts, as being fundamental for the expansion of abalone farming (Hahn, 1989c; Fallu, 1991; Britz et al., 1994). Dry pelleted formulated feeds are being used in Japan, China, Australia, New Zealand and South Africa (Abfeed[®]) (Britz et al., 1994; Fleming et al., 1996). Seaweed is not necessarily cheaper than formulated feeds as savings are offset by the cost benefits achieved from low feed ratio and a shorter production cycle offered by pelleted feeds (Marifeed, 2007). Despite costs, the major advantage of pelleted feed lies in their reliability and convenience from a farm management point of view (Marifeed, 2007). Harvesting and the use of natural kelp in abalone farming is dependent on sea conditions. This complicates farm management and adds to financial risks. Another advantage in favour of formulated feeds is that there is less restriction on the location of the farms, as kelp does not grow naturally in the Eastern Cape, the use of pelleted feeds and the cultivation of seaweeds for feed make farming in this area possible (Robertson-Andersson, 2007). Cultured seaweed can be produced at a much better quality, as feed, than natural stocks. For example, the protein content of *Ulva* has been increased to over 30 % of dry weight in South African systems (Robertson-Andersson, 2003, 2007), and experimental trials showed that kelp plus cultured seaweed addition (*Gracilaria* and *Ulva*) produced as much abalone growth as compound feed (Naidoo et al.,

2006). Thus, cultivated seaweed is of a better and more controllable quality than feeds such as kelp (Bolton et al., in press).

1.3.2 Problems with abalone health in South Africa

The first serious health issue to affect production of *H. midae* was the infestation by a sabellid polychaete, *Terebrasabella heterouncinata* (named by Fitzhugh & Rouse, 1999), an obligate symbiont which forms burrows in the shells of numerous gastropods (Ruck & Cook, 1998; Fitzhugh & Rouse, 1999). *Terebrasabella heterouncinata* are simultaneous hermaphrodites that brood clutches of up to seven offspring throughout the year, in favourable conditions (Culver et al., 1997). The young *T. heterouncinata* leave the parental burrow and crawl to the growth edge of the shell where the host covers it with nacreous shell as it forms a new burrow (Culver et al., 1997). Heavy infestation of abalone leads to deformation and weakening of the shell, a reduction in growth, or the death of the abalone (Fitzhugh, 1996; Oakes & Fields, 1996; Culver et al., 1997; Ruck & Cook, 1998). Numerous methods have been tried to treat worm infestation, including coating the shell with wax; exposing the worms to encapsulated toxins that can be ingested by *T. heterouncinata* but not by abalone; anaesthetizing the worms and then exposing them to fresh water or ultrasound, biocontrol agents and heat treatment (Culver et al., 1997; Leighton, 1989; Shields et al., 1998; Loubser & Dormehl, 2000). However, these methods were either too impractical to be implemented on a large scale, or the worms were resistant to the treatment as they could retract into the safety of their burrows (Oakes & Fields, 1996; Leighton, 1989). In California, where *T. heterouncinata* was accidentally introduced in the late 1980's (Ruck & Cook, 1998), abalone farmers are able to eradicate the worm by culling or isolating infested abalone (Oakes & Fields, 1996; Culver et

al., 1997). This method of treatment is not practical in South Africa as *T. heterouncinata* is endemic and therefore will continuously be introduced via incoming sea water (Simon et al., 2004). Therefore, sabellid populations in South African abalone farms must be controlled and maintained at a level that does not negatively affect growth or appearance of the abalone (Simon et al., 2004). To manage the sabellid infestation in South Africa it is therefore important to understand factors determining sabellid infestation rate. Salinity, water temperature and sediment composition appear to be the main factors influencing sabellid abundance (Laukner, 1983). Other factors including abalone size, growth rate, diet, stocking density and season have been cited as influencing the infestation rate of *T. heterouncinata* (Oakes & Fields, 1996; Clayden, 2000).

In South Africa cultured abalone are usually fed a pelleted diet, Abfeed[®] S34 for juveniles and Abfeed[®] K26 for grow – out animals (> 30 mm) (Britz, 1995, N. Loubsher pers comm.), *Ecklonia maxima* or a mixture of these feeds. Abfeed[®]S34 has a high protein and lipid content and was specifically formulated to promote abalone growth (Britz, 1995; Simon et al., 2002). Abfeed[®] S34-fed abalone, seem to be more susceptible to sabellid infestation than kelp-fed abalone (Clayden, 2000). Chalmers (2002) showed that sabellids on Abfeed[®] S34-fed abalone displayed a higher production of eggs and larvae than those on kelp-fed abalone. This is most likely due to the nutritional value of abalone faecal waste on which the sabellids presumably feed, as the faeces produced by Abfeed[®] S34-fed abalone contained significantly higher levels of protein and energy (Chalmers, 2002). Due to this and the fact that protein is the most expensive component of the diet a lower protein feed was developed (Marifeed, 2007). This was K26 and contains 26 % protein with the rest of the components being identical to S34

(Marifeed, 2007). Further research is being undertaken in the development of a diet containing only 18 % protein.

A size effect also appears to be present as abalone larger than 45 mm in length seem to be more susceptible to infestation by *T. heterouncinata*, and grow better on kelp than on Abfeed® (Simon et al., 2004). 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 (Britz et al., 2005). Abalone growth is influenced by stocking density and increased stocking density has been shown to result in slower growth and a higher *T. heterouncinata* infestation level (Clayden, 2000). Improvements in hygiene and husbandry in tanks have considerably reduced the problems that this infection has recently caused in South African abalone farms (Cook, 1998).

Another problem facing the global abalone industry is Withering Syndrome (WS). Withering Syndrome is caused by a *Rickettsia*-like prokaryote (RLP). *Rickettsia* is a genus of intracellular prokaryote, with morphological characteristics of the class Proteobacteria, order Rickettsiales and family Rickettsiaceae (Gardner et al., 1995; Friedman et al., 2000a to d). *Rickettsia*-like prokaryote lines and infects the epithelial cells of the digestive tract of the host abalone (Gardner et al., 1995; Caceres-Matinez et al., 2000). *Rickettsia*-like prokaryote infection results in the degeneration of the digestive gland which leads to reduced appetite and metabolic efficiency, causing starvation that probably leads to death (Friedman et al., 1997, 2000b; Gardner et al., 1995; Shields et al., 1998). Elevated temperatures accelerated disease progression and decreased survival (Friedman et al., 1997; Moore et al., 2000). Friedman et al. (2003) tested the use of Oxytetracycline (OTC) as a treatment for WS infected abalone and

found it to be an effective treatment for WS. However, the persistence of OTC residues in the gut of treated abalone needed further research (Friedman et al., 2003). A RLP organism has been reported in the digestive tract of *H. midae* from abalone farms in South Africa, with no associated pathology (Mouton 2000a, b) and as yet WS has not been reported in South Africa.

1.4 Compound Feeds

In South Africa there is currently one company producing compound feeds: Marifeed PTY. LTD. produces Abfeed[®] (Marifeed, 2007), although several feeds are in development by various other companies (Robertson-Andersson pers comm.). Abfeed[®] is the main supplier of compound feeds to the abalone farming industry. Abfeed[®] (Marifeed Pty Ltd, South Africa) is a formulated feed containing mainly fishmeal, soya bean meal, starch, vitamins and minerals. It previously contained '*Spirulina* spp.' (*Arthrospira* spp.) but not currently due to supply problems. Abfeed[®] S34 contains about 34 % protein, 43 % carbohydrates, 5 % fat, 1 % crude fibre, 6 % ash and 10 % moisture (Marifeed Pty Ltd, pers comm.). A cheaper, low protein (26 %) and kelp (*Ecklonia maxima*) containing form of Abfeed[®] K26 formulated for large abalone (>50 mm shell length) is currently also in production (Marifeed Pty Ltd, pers comm.). For the current experiments Abfeed[®] K26 was used.

One negative attribute of Abfeed[®] is that it uses fish meal as a source of protein, which has the disadvantage to the farmer of being dependent on external fish supply, although a global commodity (Tacon, 2005; Deutsch et al., 2007).

A good formulated feed should have the following attributes:

- Reliability (Fallu, 1991; Dixon, 1992; Britz, 1993, 1995; Lyon, 1995; Tahil & Junio-Menez, 1999).

- Convenience and ease of application (Dixon, 1992; Britz, 1993, 1995; Britz et al., 1994).
- Balanced nutritional composition (Lyon, 1995; Britz, 1996a, b; Fleming et al., 1996; Viana et al., 1996; Mai et al., 2001; Montaña-Vargas et al., 2005).
- Produce high growth rates (Britz, 1993, 1995, 1996a, b; Britz et al., 1994; Lyon, 1995; Viana et al., 1996).
- Have low food conversion ratios (FCR) (Britz, 1993, 1995, 1996b; Knauer, 1994).
- Be digestible (Lyon, 1995).
- Have optimum particle size and feeding methods (Fleming et al., 1996).
- Be attractive to abalone (Fleming et al., 1996).
- Provide optimal growth rates, by meeting nutritional requirements (i.e. carbohydrate, proteins and amino and fatty acid ratios) (Fleming et al., 1996).
- Be easy to store and transport (Britz, 1993, 1995; Lyon, 1995).
- Be available year-round (Britz et al. 1994; Lyon, 1995).

Formulated feeds offer convenience and cost benefits to farm management (Britz et al., 1994). 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 (Fleming et al., 1996).

Abalone will readily eat a nutritionally poor diet when no alternative feed is offered. This will affect abalone performance and nutrient waste, and thus the profit margin will be far from optimal (Fleming et al., 1996). Formulated feed should retain water soluble nutrients in the feed, and food particles should remain bound together for at least 2 days (Fleming et al., 1996). Formulated feed should also be cost-effective to make it viable for farmers to use and at the same time meet the nutritional requirements for abalone growth (Fleming et al., 1996).

tuberculata. Peck et al. (1987) found mucus production formed a large part of the budget (from 23.3 % to 29.1 % of consumption). Barkai & Griffiths (1988) reported that the energy loss in the form of ammonia excretion is negligible in *H. midae*, accounting for < 1 % of consumption. Therefore, the energy digested by abalone is a good approximation to the amount which is available for growth, reproduction and maintenance. Maintenance is defined as the amount of energy required to maintain the animal in a state of zero growth (Fleming et al., 1996). Formulated feeds need to have energy in the diet in excess of this amount, in order for growth to occur.

It is important that tank design and feed formulation be developed simultaneously to achieve optimal feed availability and water quality (Fleming et al., 1996). According to Fleming et al. (1996) the following husbandry requirements should be met by a feed.

- The amount of uneaten feed is minimised;
- Uneaten feed does not pollute the tank;
- Animals are feeding to satiation;
- Animals do not expend significant amounts of energy searching for feed;
- Feed and faeces do not occur in the same place in the tank.

Research groups in South Africa have designed tank systems with associated water circulation and aeration regimes in an attempt to meet some of these requirements (Britz, 1993).

A major drawback with the use of formulated feeds in culture systems is the problem of feed decomposition and the subsequent rapid deterioration in water quality (Cuthbertson, 1978). Meat-based and fishmeal-based diets are particularly susceptible to such decomposition, particularly in warmer water (Fleming et al., 1996).

1.5 Integrated aquaculture

A problem with aquaculture in the past is that it has focused on a single species; this has led to environmental degradation. In order for aquaculture to become environmentally friendly and competitive it needs to be based on balanced ecosystem approach (Folke et al., 1994; Naylor et al., 2000; Chopin et al., 2001). Integrated aquaculture has therefore been suggested to increase production, sustainability and revenues (Folke & Kautsky, 1989; Chamberlaine & Rosenthal, 1995; Naylor et al., 2000; Chopin et al., 2001; Troell et al., 1999a, b, 2003).

This can be achieved by increasing productivity per unit of feed, which can reduce production cost (Neori et al., 2004). In an integrated aquaculture system the waste nutrients are used as a resource, for the auxiliary culture of plants, or animals which feed on particulates (Chamberlaine & Rosenthal, 1995). Seaweeds that remove nutrients from effluent water in mariculture operations, known as biofilters, have been applied successfully in numerous parts of the world (Neori et al., 1996; reviewed in Neori et al., 2004)

The new plant biomass can have numerous functions:

- Fed back to culture organisms
- Sold for human consumption
- Phycocolloids
- Agrichemicals
- Pharmaceuticals

A culture system that diversifies its products by integrating the culture animal with an extractive algal culture, and with organisms that feed on these algae, therefore makes sense, not only ecologically, but also economically (Anon., 2003). An example is the farming of seaweed in abalone effluent and feeding the seaweed back to the abalone.

Therefore the benefits of integrated aquaculture include:

- Remove and recycle toxic metabolites by using recirculating systems.
- Increase production of specific co-cultured extractive species (e.g. seaweeds).
- Increase overall productivity of the resources of feed, water and fossil energy (Krom et al., 2001).
- Potential for temperature enhancement, in culture systems where increased temperature would have a positive effect on growth rates (Bolton, 2006; Bolton et al., in press; Troell et al., 2006; Robertson-Andersson, 2003; 2007; Robertson-Andersson et al., 2008).
- Lowered pumping costs (due to the re-use of water) (Neori et al., 2004; Bolton, 2006; Bolton et al., in press; Robertson-Andersson, 2007).
- Release fewer nutrients into coastal waters (Krom, 1986; Shpigel & Neori, 1996; Neori et al., 1998; Robertson-Andersson, 2003, 2007; Robertson-Andersson et al., 2008).
- Reduced dependence on harvested seaweeds (i.e. kelp) (Neori et al., 1998, 2004; Robertson-Andersson, 2003, 2007; Troell et al., 2006; Anderson et al., 2007).
- Production of quality feed, for example, *Ulva* grown in eutrophic conditions has higher nutritional content, which improves its quality as an animal feed (De Busk et al., 1986; Shpigel & Neori, 1996; Neori et al., 1998).

There are also potential disadvantages associated with using a recirculating system which include:

- High costs of installation (Bolton et al. in press).
- These systems are technologically demanding and require constant monitoring to ensure operational efficiency (Robertson-Andersson, 2007).

- Potential for deterioration of water quality, e.g. increased toxic ammonia, increased nitrate and nitrite, reduced pH, CO₂ build up, elevated temperatures and increased salinity (Robertson-Andersson, 2007; Robertson-Andersson, et al., 2008).
- Potential for retention of parasitic organisms in the system (Nicotri, 1977; Shacklock & Croft, 1981; De Oliveira et al., 1989; Buschmann et al., 2001; Smit et al., 2003).

Future mariculture technologies could attain sustainability by integrating waste generating (fed) and cleaning (extractive) organisms in each farm (Buschmann et al., 2001; Chopin et al., 2001). This could be achieved by the use of filter feeding shellfish to remove particulate organic nutrients and the use of algae to extract dissolved inorganic nutrients (Neori et al., 2004). Thus, when integrated with fed aquaculture, the extractive organisms turn wastes into productive resources (Rawson et al., 2001, 2002; McVey et al., 2002). The new integrated aquaculture systems will use multiple species from different trophic levels for reducing wastes and costs (through recycling of wastes), while increasing total productivity (in weight and in value) with respect to feed input and pollution output (Troell et al., 2003). In an integrated aquaculture system the cost of feed proteins constitutes the largest item (Neori et al., 2004). A large proportion of the fed proteins are excreted and eventually end up as dissolved ammonia, which algae are able to recapture and convert into useful protein-rich biomass (Neori et al., 1991, 1996). “An efficient algal-based integrated mariculture farm maintains optimal standing stocks of all the cultured organisms, considering the respective requirements of each for water and nutrients and the respective rates of excretion and uptake of the important solutes by each of them. This allows the profitable use of each of the culture modules with minimum waste” (Neori et al., 2004).

1.6 Seaweed choice for efficient biofiltration

The choice of seaweed species for inclusion in an integrated aquaculture system, were identified by Neori et al., (2004) these seaweeds should meet a number of basic criteria:

- High growth rate and tissue nitrogen concentration.
- Ease of cultivation and control of life cycle.
- Resistance to epiphytes and disease-causing organisms.
- Match between the ecophysiological characteristics and the growth environment.

When choosing seaweed for an integrated system it is important to choose a native species, as non-native species could have negative effects on the environment. If the principal focus is the process of bioremediation, then nutrient uptake and storage and growth are the primary determinants (Neori et al., 2004). Growth rate is defined, to a large extent, by morphology (Littler & Littler, 1980); generally speaking, the higher the ratio of surface area to volume, the faster the specific growth rate (Neori et al., 2004). Thin sheet morphology has a higher growth rate than a more fleshy habit (Neori et al., 2004). A seaweed species must grow very well in high nutrient concentrations, in order to be an effective biofilter. To take up ammonia and nitrogen at a high rate, fast-growing seaweed should be able to build up a large biomass N content (Neori et al., 2004).

The ideal choice for the seaweed biofilter is one that also has a market value (Sahoo et al., 2002). In the case of farming seaweeds in abalone effluent, the seaweeds are fed back to the abalone, adding value. A large body of research exists for the genus *Ulva*; these flat sheet morphotypes have high growth rates as well as high nitrogen contents, making them very good candidates for remediation (Cohen & Neori, 1991; Neori et al., 1991). Their life cycle

and its controls are generally well known (Neori et al., 2004). Another important factor to include when choosing seaweed as a biofilter is the resistance to epiphytes. Epiphytes are pest seaweeds and microalgae that use the cultured seaweed as substrates and compete for nutrients and light (Neori et al., 2004). Fast growing opportunistic seaweeds, such as *Ulva*, suffer from epiphytes only when they get stressed and do not grow at their usual fast rate (Robertson-Andersson, 2003; Neori et al., 2004). The fact that *Ulva* is often the main epiphyte in monocultures of other seaweed makes *Ulva* the preferred biofilter seaweed genus (Neori et al., 2004). One possible drawback is that the *Ulva* itself does not have a market value (Bolton et al. in press).

1.7 Integrated aquaculture in South Africa

A number of studies involving abalone have been conducted using mixed diets (containing more than one feed) (Owen et al., 1984; Day & Fleming, 1992; Fleming, 1995; Simpson & Cook, 1998; Naidoo et al., 2006; Daume et al., 2007) and rotational diets (Simpson & Cook, 1998). A study by Naidoo et al. (2006) showed that kelp plus cultured seaweed addition (*Gracilaria* and *Ulva*) produced as much abalone growth as compound feed. In addition, Dlaza et al. (2008) showed that supplementation with fresh wild seaweed enhances the growth of abalone reared on formulated feeds. All of the above studies have confirmed that variation in the diet often results in enhanced abalone growth rate.

From 1992 to present, there has been a considerable amount of research looking at the land based cultivation of seaweeds and abalone in South Africa, initially at 2 abalone farms on the southeast coast which did not have access to kelp and were also limited in artificial feeds due

to bloating caused by the warm water temperature in the area (Robertson-Andersson et al., 2007). Most of these studies looked at cultivation in flow-through systems, utilising *Ulva* spp. and *Gracilaria* spp.

Research on seaweed recirculation systems (Robertson-Andersson, 2007; Robertson-Andersson et al., 2008), demonstrated no significant differences in abalone health or growth rates, sediment build up and composition, mobile macro fauna densities and species between a 25 % recirculation system (fed a seaweed diet) and the normal flow-through units. Transfer of oxygen generated by the seaweeds to the abalone tanks was poor, resulting in the recirculated abalone tanks having lower (33 %) dissolved oxygen concentrations than a comparable flow-through abalone unit. Seaweed nutrient content and specific growth rates in the units were comparable to seaweeds cultivated in fertilized effluent. Indications were that at the low recirculation ratio the seaweeds in the units were nutrient limited and that there were no negative effects to the abalone being cultivated in the recirculation unit at 25 % recirculation ratio. Robertson-Andersson (2007) therefore concluded that higher levels of recirculation would be possible.

Following on from the Robertson-Andersson (2007) and Robertson-Andersson et al. (2008) studies, I & J Cape cultured abalone built a 120 ton abalone and 105 ton seaweed integrated recirculating system (Bolton, 2006). The success of the recirculating system at I & J has proved to be due to the multipurpose role of the *Ulva* raceways in the system (Bolton et al., 2008). “The *Ulva* not only provides abalone feed and reduces ammonia and bacteria levels in the effluent, allowing partial recirculation (thus slightly reducing pumping costs due to reduced pumping height) but also, especially due to the enhanced light-absorbing properties of the seaweed, increases the temperature of the abalone culture tanks” (Bolton et al., 2008).

As most farms in South Africa (including I & J) occur where ambient seawater temperatures are suboptimal for abalone, this thus increases the growth rate of the abalone, and hence increases profits (Robertson-Andersson, 2007; Robertson-Andersson et al., 2008; Bolton et al., 2008). A number of student theses have been produced recently investigating recirculation on abalone farms.

Brandt (2006) performed sediment trials, over a 7 day cleaning cycle, in the same experimental set up as the current study using a kelp only diet, with 25, 50 and 75 % Recirculation systems. Brandt's (2006) study showed that the total particle load did not increase with higher recirculation rates. Brandt (2006) therefore concluded that it would be feasible to farm abalone fed a kelp diet at a recirculation rate of up to 75 %.

Potgieter (2005) had two components of investigation in the same experimental system as this study. Potgieter's (2005) first experiment investigated abalone tank water of three different diets (Abfeed[®], Kelp and a Mixed algal diet) fed to abalone in a flow-through tank system as compared to a 25 % Recirculation system (abalone fed a kelp diet). Potgieter's (2005) second experiment compared the build up of suspended particulate matter and nutrient concentrations over a 7 day cleaning cycle in a 25 % Recirculation system versus flow-through units (FTUs). Potgieter (2005) found no significant difference in the nutrient concentrations of the Recirculation system and FTUs. There was also no significant difference in the particle concentrations between the flow-through system and the 25 % Recirculation system. Potgieter (2005) showed that a 25 % Recirculation system would not result in deterioration of tank water quality.

Lindström (2006) recorded water nutrients and physico-chemical variables in 25 % and 75 % Recirculation systems, fed a kelp diet, in the same experimental setup as the current study. Lindström (2006) showed that the temperature was higher in the FTUs, compared to

As most farms in South Africa (including I & J) occur where ambient seawater temperatures are suboptimal for abalone, this thus increases the growth rate of the abalone, and hence increases profits (Robertson-Andersson, 2007; Robertson-Andersson et al., 2008; Bolton et al., 2008). A number of student theses have been produced recently investigating recirculation on abalone farms.

Brandt (2006) performed sediment trials, over a 7 day cleaning cycle, in the same experimental set up as the current study using a kelp only diet, with 25, 50 and 75 % Recirculation systems. Brandt's (2006) study showed that the total particle load did not increase with higher recirculation rates. Brandt (2006) therefore concluded that it would be feasible to farm abalone fed a kelp diet at a recirculation rate of up to 75 %.

Potgieter (2005) had two components of investigation in the same experimental system as this study. Potgieter's (2005) first experiment investigated abalone tank water of three different diets (Abfeed[®], Kelp and a Mixed algal diet) fed to abalone in a flow-through tank system as compared to a 25 % Recirculation system (abalone fed a kelp diet). Potgieter's (2005) second experiment compared the build up of suspended particulate matter and nutrient concentrations over a 7 day cleaning cycle in a 25 % Recirculation system versus flow-through units (FTUs). Potgieter (2005) found no significant difference in the nutrient concentrations of the Recirculation system and FTUs. There was also no significant difference in the particle concentrations between the flow-through system and the 25 % Recirculation system. Potgieter (2005) showed that a 25 % Recirculation system would not result in deterioration of tank water quality.

Lindström (2006) recorded water nutrients and physico-chemical variables in 25 % and 75 % Recirculation systems, fed a kelp diet, in the same experimental setup as the current study. Lindström (2006) showed that the temperature was higher in the FTUs, compared to

the Recirculating system, for both recirculation ratios. Temperature in the Recirculation system appeared to be governed by the temperature in the Seaweed tanks. Oxygen concentration was lowest in the recirculation abalone tanks, and highest in Seaweed tanks, pointing at an existing problem with dissolved oxygen transport from the Seaweed tanks to abalone tanks with the recirculating water. Dissolved nutrient analyses showed that the seaweed were capable of adequately removing ammonium, nitrate, nitrite and phosphate from the Recirculation system, even at the 75 % recirculation ratio. High phosphate values during 75 % recirculation could be due to seaweed N-limitation. Lindström (2006) concluded that seaweed functions well as biofilter and that the Recirculation system was capable of reducing the effluent concentrations and maintain, or sometimes even improve, water quality compared to control

Key questions which arose from the aforementioned studies include:

1. How would a mixed diet of Kelp and Abfeed[®] K26, commonly used in the industry, affect the growth rates of abalone?
2. How would the Kelp and Abfeed[®] K26 diet affect the culture environment in terms of water nutrients, physico-chemical variables, abalone health and sediment loadings?
3. How would a higher protein diet of kelp and Abfeed[®] K26 affect abalone growth rates of abalone in higher recirculation systems? Would the higher protein diet affect water nutrients, physico-chemical variables, abalone health and sediment loadings in the higher recirculation systems?
4. How would the seaweeds in the recirculation system perform in terms of nutrient uptake?

1.8 Objectives of this thesis

This thesis followed on from the work done by Potgieter (2005), Dlaza (2006), Brandt (2006), Lindström (2006) Robertson-Andersson (2007) and Robertson-Andersson et al. (2008), and had two components of investigation. These were based on questions arising from the above mentioned work.

The first component of this study was to address key questions 1 and 2 by testing between different ratios of kelp / formulated (Abfeed[®] K26) mixed diet on out grown abalone, to find which ratio gives the best growth rates. These two feeds were chosen as they were the two most popular dietary choices by abalone farmers in South Africa for out grown abalone, and are often fed in combination, where kelp is available. The use of two feeds should also enhance growth rates as a mixed diet was used; this combination was shown to work well with juvenile abalone fed a higher protein feed (S34, 34 % protein) and kelp by Dlaza et al. (2008).

The second component of this study was to address key questions 3 and 4 by testing the effects of an integrated abalone / seaweed aquaculture system, with the abalone cultivated in 50 % and 75 % recirculation systems, using a mixed diet of kelp and Abfeed[®] K26. Robertson-Andersson (2007) and Robertson-Andersson et al. (2008) concluded that the seaweeds were nutrient limited in their existing system at 25 % recirculation when the abalone were fed seaweeds only. In addition, it was thought that a higher protein feed would place additional oxygen demands on the system and therefore its performance at higher recirculation rates needed to be assessed.

The performance of the various feed combinations and recirculation rates were assessed in terms of health of abalone, growth rates of abalone, sediment loadings and water quality in a long term study, supplemented by several short term studies.

This study was carried out *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 or in a more controlled research environment.

Chapter 2

Setting optimal diet ratios of kelp (*Ecklonia maxima* (Osbeck) Papenfuss) and formulated feed (Abfeed[®] K26) for *Haliotis midae* cultivation

2.1 Introduction

Cultivation of abalone began in South Africa in the 1990's (Sales & Britz, 2001; Troell et al., 2006), and the industry has since grown to be the largest producer outside of Asia (FAO, 2004). Due to a decline in natural stocks and a total moratorium on wild abalone harvest (DEAT, 2007), farmed abalone now dominates the export market in South Africa (Troell et al., 2006), other than poaching (Steinberg, 2005).

In the wild, kelp (*Ecklonia maxima* and *Laminaria pallida*) provide the primary food source for *H. midae* on the southwest coast (Barkai & Griffiths, 1986). However, abalone are known to consume a wide variety of seaweeds in large quantities to fulfil their nutritional requirements (Barkai & Griffiths, 1986).

Kelp is used as the major feed component for farmed abalone in South Africa, making up approximately 60 % of the utilised feed by weight (Robertson-Andersson, 2007). Alternatives to kelp harvesting are the cultivation of seaweeds such as *Ulva* and *Gracilaria* on abalone farms and the feeding of formulated feeds. Seaweed cultivation has proved to be successful on a large scale at Wild Coast Abalone who have become world leaders in *Ulva* cultivation (Bolton et al., 2008) and on a smaller scale at the Irvine & Johnson (I & J) Mariculture farm, in Gansbaai (Robertson-Andersson, 2003, 2007; Robertson-Andersson, et al., 2008). However, the logistical supply problems, abalone export weight loss and additional labour costs associated with the feeding of fresh kelp, has led to the South African abalone industry becoming increasingly reliant upon formulated diets (Hahn, 1989b; Anonymous, 1991; Britz et al., 1994; Britz, 1996b; Anderson et al., 2007).

Utilising formulated feeds is seen as being necessary by some analysts for the abalone industry to continue to expand and make a significant contribution to the global abalone yield (Britz, 1996a; Sales & Britz, 2001; Troell et al., 2006).

Development of formulated diets is an active area of research in South Africa. Formulated feeds provide a secure and guaranteed nutritional value (Britz, 1996; Britz & Hecht, 1997; Cook, 1998; Sales & Britz, 2001). However, farmers are disadvantaged by being dependent on external fish production (Tacon, 2005, Deutsch et al., 2007). Formulated feeds, when compared to natural sources, offer convenience and cost benefits to farm management (Britz et al., 1994). The primary aim in the development of a formulated feed for abalone must be to maximise growth rates by matching the diet specifications as closely as possible to the abalone's requirements for nutrients, and for the least possible cost (Fleming et al., 1996). This will result in maximum efficiency of nutrient use and maximum profit margins, with minimum ecological impact without affecting abalone quality (Fleming et al., 1996).

The growing demand for kelp by the abalone industry has greatly increased harvesting (Anderson et al., 2003). This has created pressure on the kelp resource with the maximum sustainable yield of the kelp being reached in two areas in 2003 (Anderson et al., 2003). It is therefore important that formulated feeds be used in order for the South African abalone industry to continue to grow. As a formulated feed has become a necessity for the growth of the abalone industry in South Africa, what is the best ratio of Abfeed[®] K26 to kelp to be used?

2.1.1 Aims

The aim of this experiment was to establish the ratio of Kelp and formulated feed (Abfeed[®] K26) needed for the best growth rate. In this experiment three ratios of kelp and Abfeed[®] were used to establish the best feeding ratio. In these treatments the regular weekly diet (high kelp / low Abfeed[®]) administered by the farm, used as a control, was tested against a lower kelp / higher Abfeed[®] diet and a very low kelp / very high Abfeed[®] diet (see Table 1). Kelp and Abfeed[®] were chosen as they are the two most popular dietary choices by abalone farmers in South Africa. Subsequent aims were to investigate the performance of the different diets in terms of health of abalone, sediment loadings, physico-chemical characteristics and water nutrients.

2.2 Materials and Methods

2.21 Study site

This project took place in Gansbaai, at the I & J Cape Abalone Mariculture farm, which is 140 km east of Cape Town (34°37'S, 19°28'E). I & J Cape Abalone Mariculture is a land-based intensive mariculture operation, which has been in operation since 1994. The farm cultivates *H. midae*, Kob, both the Dusky and Silver (*Argyrosomus japonicus* Temminck & Schlegel and *A. inodorus* Griffiths & Heemstra), Yellowtail (*Seriola lalandi* Valenciennes) and Red Roman (*Chrysoblephus laticeps* Valenciennes). Quantities of gracilarioids and *Ulva* spp. (*U. lactuca* and *U. capensis*) have also been cultivated since 1998. The *Ulva* species vary seasonally, due to specific species preferences (Robertson-Andersson 2003).

Seawater was drawn from the sea into a pump house and then pumped directly into a header tank (5000 m³) on platform 1 (the section of the farm where the experiment occurred), at a rate of 1 200 m³. hr⁻¹. The water was then gravity fed to either the abalone tanks or the seaweed tanks. The water was 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. Effluent water from platform is returned directly to the sea.

2.2.2 Experimental animals

Animals for this experiment were spawned in 3 consecutive months, January, February and March 2004. Animals had to be taken from 3 spawning dates to fill the experimental setup, which required a total of 118 800 abalone (see Figure 1). All animals ranged between 10 - 30 grams at the beginning of the experiment. An average of 18 grams was used to calculate feed ratios. Abalone were stocked at 550 animals per Ivey Blue up™ basket, with 24 baskets in each tank. The tank dimensions were 6.6 m long X 2.08 m wide X 0.88 m deep; *ca.* 12 000 L. Each feed treatment consisted of 3 tanks, which gives a total of 39 600 abalone used for each of the three treatments (see Figure 1). For this experiment animals from the three spawning dates were distributed between the nine tanks, but remained separated by the baskets. Hence animals from the three spawning dates are nested twice, first into treatments and the second into tanks.

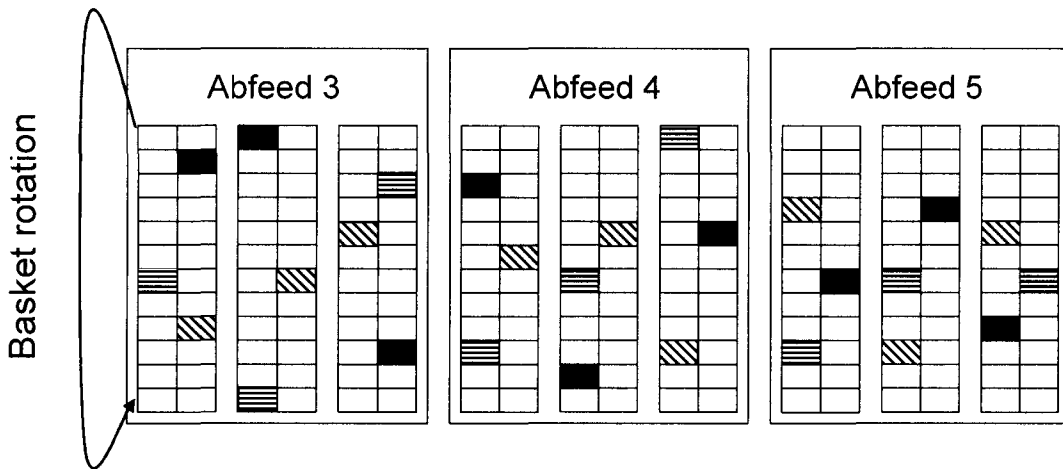


Figure 1: Diagram of experimental setup

Marked blocks represent the initial placement of the abalone baskets that were repeatedly sampled once a month from May to November 2006. Black baskets represent placement of the January spawned cohort, horizontal lines represent the placement of the February spawned cohort baskets and diagonal lines represent the placement of the March spawned cohort baskets. Baskets were rotated once a week during the weekly cleaning cycle, with the first basket in the tank being removed; all the baskets were moved one place forward in the tank, before placing the first basket in the last position of the tank.

2.2.3 Diets

Table 1 illustrates the three different ratios of Abfeed[®] K26 and kelp used for this experiment. The percentage of Abfeed[®] K26 fed was 0.6 % of the body weight per day, and the percentage of Kelp (EM) fed per day was 7 % of the body weight. These values were calculated from farm data. A weekly feeding regime was calculated using proportions of the above daily values, with feeding days determined by farm practice.

Table 1: Diet names, feed ratios and weekly feeding regime per basket

Diets	Feed ratio per week	Weekly feeding per basket	
Abfeed [®] 3	Abfeed [®] 3 / Kelp 4	Monday	Kelp = 2.7 Kg
		Friday	Abfeed = 180 g
Abfeed [®] 4	Abfeed [®] 4 / Kelp 3	Monday	Kelp = 2.08 Kg
		Thursday	Abfeed = 225 g
Abfeed [®] 5	Abfeed [®] 5 / Kelp 2	Monday	Kelp = 1.4 Kg
		Wednesday	Abfeed = 135 g
		Friday	Abfeed = 180 g

The weekly feed ratios above were calculated as follows:

$\text{Kg to feed} = (\text{kg animals}) \times (\text{nr. of days}) \times (\% \text{ body mass fed per day}).$

E.g. Abfeed[®] 3 treatment:

Abfeed [®] :	Kg	= 712.8 x 3 x 0.6 %
		= 12.8304
		= 4.2768 kg per tank
		= <u>0.178 kg per basket</u>
Kelp:	Kg	= 712.8 x 4 x 7 %
		= 199.5 kg
		= 66.5 kg per tank
		= <u>2.7 kg per baskets</u>

The control for this study was Abfeed[®] 3 diet as this is the weekly diet the farm currently uses.

2.2.4 Sub samples

Samples were taken from tanks from May 2006 to November 2006, at monthly intervals. At each sampling, 10 animals were randomly sampled from each marked basket (see Figure 1). Thirty animals were sampled from each spawning cohort and tank for each of the three diets, therefore a total of 90 animals were sampled for each of the three dietary treatments (see Figure 1).

Of the 118 800 animals that were part of the experiment at each monitoring period, 270 were sampled (0.23 % of the animals). Abalone were blotted dry to remove excess water before weight measurements were recorded. 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. In order to follow changes in growth rates individual baskets were sampled on a repeat basis (see Figure 1).

2.2.5 Daily Increment in Shell Length (DISL)

Daily Increment in Shell Length was calculated using the formula of Zhu et al. (2002)

$DISL (\mu\text{m} / \text{day}) = [(SL_t - SL_i) / t] \times 1000$, where SL_t = the final mean shell length (mm),

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

2.2.6 Specific Growth Rates (SGR)

SGR was calculated using the following formula $SGR = ((\ln(W_t) - \ln(W_i))/t) \times 100$

Where SGR is the specific growth rate (percentage body weight per day)

$\ln(W_t)$ is the natural log of the mean final weight of abalone, $\ln(W_i)$ is the natural log of the mean initial weight of the abalone and t is the time in days.

2.2.7 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.

2.2.8 Physico-chemical variables and Water nutrients

Physico-chemical variables (temperature, dissolved oxygen and pH) and water samples to measure ammonium and phosphate were taken during two 72 hour experiments (9 - 11 October 2006 and 10 - 12 November 2006 as per the methods listed in Robertson-Andersson et al 2008). This was done at the end of the weekly cleaning cycle, Friday 8 am till Monday 4 am, to get the maximum build up of dissolved nutrients present in the system. Physico-chemical variables were recorded and water samples were collected every four hours during the 72 hour periods. The samples were syringe-filtered through Whatman GF/B glass microfibre filters to remove any sediment in the water samples. Samples were frozen on the farm for later analysis. The same procedure was followed as for previous studies in this system (see Potgieter, 2005; Brandt, 2006; Lindström, 2006; Robertson-Andersson, 2007 and Robertson-Andersson et al., 2008).

Ammonium concentration was determined using the method described by Grasshoff et al. (1976), scaled down to a sample volume of 5 ml. Dissolved Inorganic Phosphate (PO_4) 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.

Ammonium and phosphate samples were taken in triplicate and analysed as such. Testing was performed for Total Ammonia Nitrogen (TAN), from which Free un-ionized Ammonia Nitrogen (FAN) was calculated using the TAN concentrations, pH, temperature and salinity values following the method of Bower & Bidwell (1978), and Emerson et al. (1975) methods.

2.2.9 Sediments

Sediment load was measured during the cleaning of the abalone tanks. As it was not possible to clean all the tanks in one day, a cleaning rotation was set up where three tanks from the same diet were cleaned per day. Daily litre samples of inlet water were taken to account for any differences in sediment entering the tanks. Daily one litre samples were also taken a week prior to the cleaning of the tanks, to measure sediment entering the tanks from the incoming water. The water column was sampled at the base of the abalone basket, approximately 50 centimetres below the water surface. Water was collected by placing a hose pipe attached to a stick to a depth of 50 centimetres, suction was created on the hose pipe, which allowed water to flow, a one litre sample was then taken. To sample bottom sediment, 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 sea water and the side walls were scrubbed and rinsed. The sediment rich bottom water was then pumped in to a 600-litre tank. After intensive mixing, a one litre sub sample was collected. This procedure was repeated twice for each tank due to each tank being sub-divided down the middle by a concrete wall. Once collected, all the samples were frozen and taken to the laboratory, where samples were thawed and placed into plastic Imhoff-containers and left for 24 hours. The water was decanted with a tube and the remaining sediment transferred to a foil container, and any excess sediment in the Imhoff-container was washed into the sample with distilled water. Samples were dried at 70 °C for 72 hours before being weighed in the laboratory.

2.2.10 Abalone health

Forty-five animals from the experimental systems, three groups of fifteen sampled from each dietary treatment, from the February spawned cohort were collected and analysed by Dr Anna Mouton, the AFASA (Abalone Farmers Association of Southern Africa) veterinarian, and reported according to standard industry reports (Mouton, 2006). These samples were analysed for parasite status, sabellid score, shell condition, gonad development, condition score and environmental stress score. These results are presented according to the industry standard. Results were interpreted using Table 2.

TABLE 2: Interpretation of health results (table from Robertson-Andersson 2007)

	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		

2.2.11 Statistical analysis

Unless otherwise stated, all graphs are expressed as means \pm SE.

The growth data were analyzed by two methods,

(1) Analysis of variance (ANOVA's) were performed at the end point of the experiment (using abalone lengths and weights recorded in November) to test for significant differences between the abalone growth measurements in the three diets, at the termination of the experiment. The growth data were analysed by treatment (where all 90 abalone per dietary treatment were combined (see Figure 1)) and by spawning cohort (where the 30 abalone per spawning cohort were combined for each dietary treatment (see Figure 1)).

(2) Analysis of covariance (ANCOVA's) were performed as described in ZAR (1999) on the entire six month growth data set (calculations of ANCOVA's are located in the Appendix Tables 1 - 8). The natural log of the weight recordings were performed prior to the ANCOVA testing. The ANCOVA testing was performed to test for significant differences between the regression equations calculated, over the six month growth trial, from the abalone weights and lengths in the three diets. The ANCOVA testing differs from the ANOVA testing as it takes into account the entire six month data set, where as the ANOVA testing only uses the final recordings to test for significant differences.

ANOVA's were also performed over the two 72 hour periods for the physico-chemical variables and water nutrients; this indicates significant differences over the 72 hour period. ANOVA's were performed using the statistical program STATISTICA V8. All

data were regarded significant at $p < 0.05$. If the assumptions of the ANOVA's (normality and equal variance) were not met the non-parametric Kruskal Wallis ANOVA test, was applied. Comparisons after ANOVA were made using the post hoc Tukey test to individualise specific differences, comparisons after the Kruskal Wallis ANOVA test were made with the multiple comparisons test (Zar, 1999).

2.2.12 Estimated time to harvest

Estimated time to harvest was calculated from the six month weight recordings. The weight recordings were natural log transformed and projected (using a linear growth equation) to 4.605 which is the natural log of 100 grams. The growth graphs with a 95 % confidence interval and growth equations are located in the Appendix Figures 1 – 4.

2.3 Results

2.3.1 Growth

At the termination of the experiment there was no significant difference between the weights of abalone in the three diets (ANOVA $F(2, 267) = 3.37, p = 0.035$) (see Figure 2). There were significant differences between the lengths of abalone in the three treatments, ANOVA $F(2, 267) = 5.0085, p = 0.007$. A post hoc Tukey test indicated that the abalone in the Abfeed[®] 3 treatment were significantly longer than abalone in the Abfeed[®] 4 ($p = 0.04$) and Abfeed[®] 5 ($p = 0.01$) treatments (see Figure 3).

When broken down into spawning cohorts (see Figures 4 and 5), the abalone in the Abfeed[®] 5 January spawned cohort were significantly heavier than abalone in the Abfeed[®] 3 and Abfeed[®] 4 January spawned cohorts, Kruskal-Wallis test: $H(2, N = 90) = 12.127, p = 0.002$. Abalone in the February spawned cohort in the Abfeed[®] 3 treatment were significantly heavier compared to the abalone in the Abfeed[®] 5 treatment, Kruskal-Wallis test: $H(2, N = 90) = 8.94, p = 0.011$. Abalone in the March spawned cohort in the Abfeed[®] 3 treatment were significantly heavier than the abalone in the Abfeed[®] 5 treatment, ANOVA $F(2, 87) = 9.98, p < 0.001$.

There were no significant differences between the lengths of the abalone in the January spawned cohort in the three treatments (Fig. 5). In the February spawned cohort the abalone in the Abfeed[®] 3 treatment were significantly longer than abalone in the Abfeed[®] 5 treatment, ANOVA $F(2, 87) = 4.2512, p = 0.01$. In the March spawned cohort the

abalone in the Abfeed[®] 3 treatment were significantly longer than abalone in both the Abfeed[®] 4 and the Abfeed[®] 5 treatments, ANOVA $F(2, 87) = 9.0947, p < 0.001$.

The ANCOVA results indicated that the growth rates in terms of weights and lengths were equal between the abalone in the three treatments (Weight, $F = 0.05, DF = 264$ and $p > 0.05$) and (Length, $F = 0.117, DF = 264$ and $p > 0.05$), (see Appendix Table 1 and 2 for calculations).

The ANCOVA weight results for the three spawning cohorts all indicated that the growth rates were equal for the three treatments. January ($F = 0.03, DF = 84$ and $p > 0.05$), February ($F = 0.04, DF = 84$ and $p > 0.05$) and March ($F = 0.03, DF = 84$ and $p > 0.05$) (see Appendix Table 3 - 5 for calculations).

The ANCOVA length results for the three spawning cohorts all indicated that the growth rates were equal for the three treatments. January ($F = 0.01, DF = 84$ and $p > 0.05$), February ($F = 0.09, DF = 84$ and $p > 0.05$) and March ($F = 0.05, DF = 84$ and $p > 0.05$) (see Appendix Table 6 - 8 for calculations).

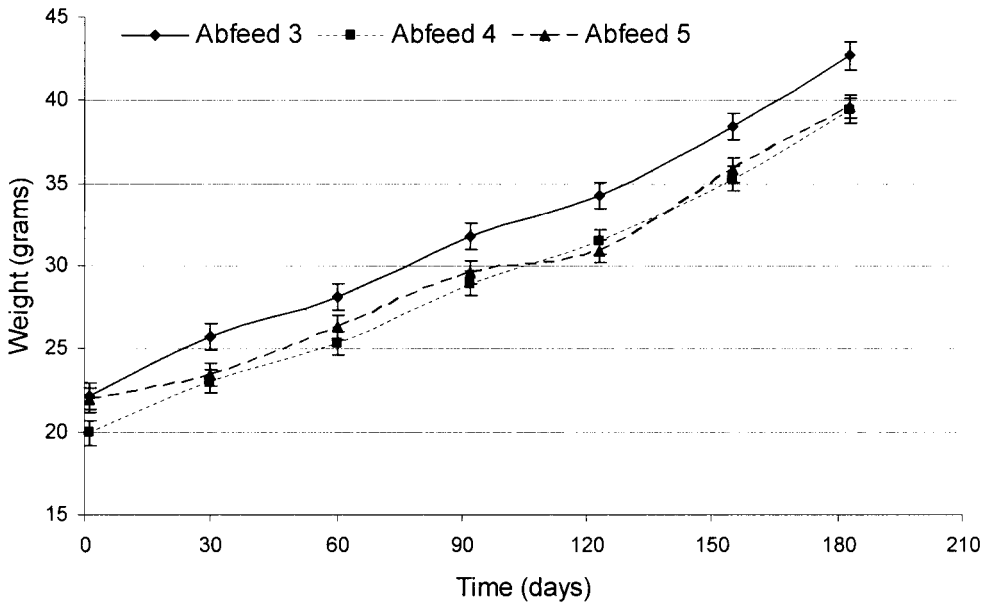


Figure 2: Average abalone growth in body weight in the three treatments
 Ninety abalone were sampled each month for the three dietary treatments from May to November 2006.

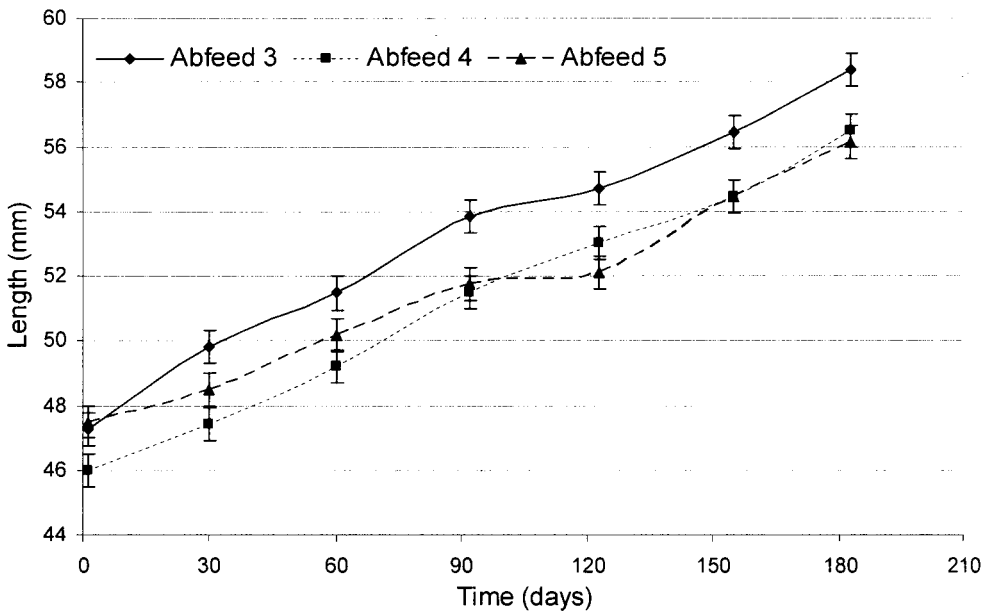


Figure 3: Average abalone growth in shell length in the three treatments
 Ninety abalone were sampled each month for the three dietary treatments from May to November 2006.

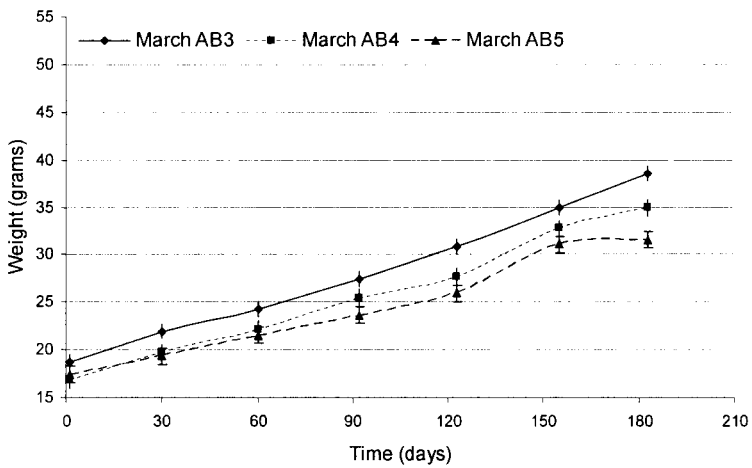
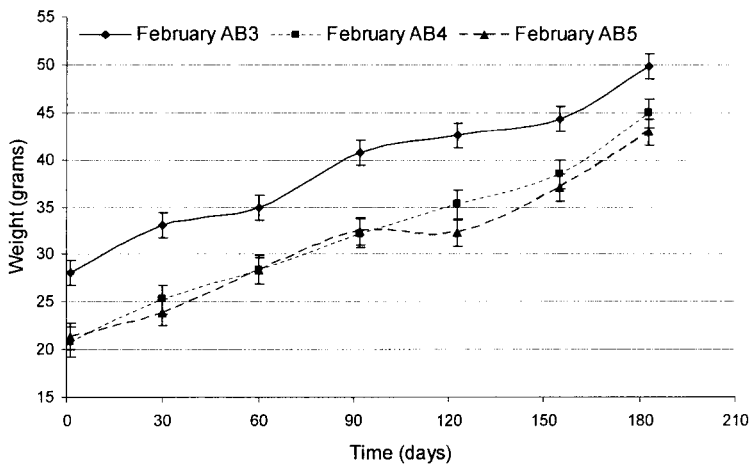
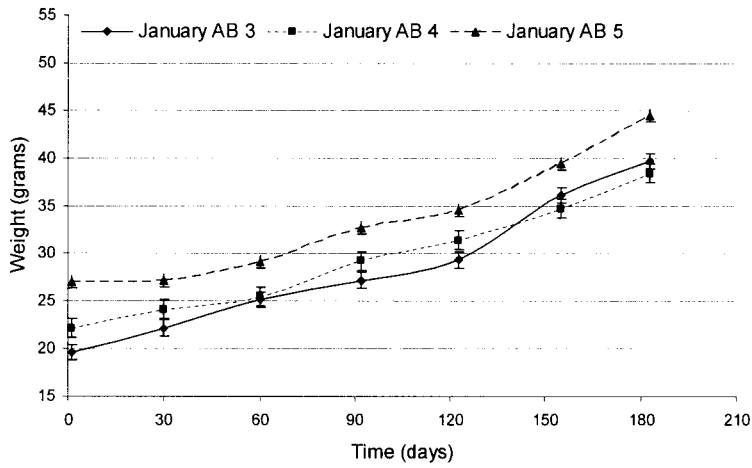


Figure 4: Average abalone growth in body weight for the three spawning cohorts
 Thirty abalone were sampled each month for the three dietary treatments from May to November 2006.

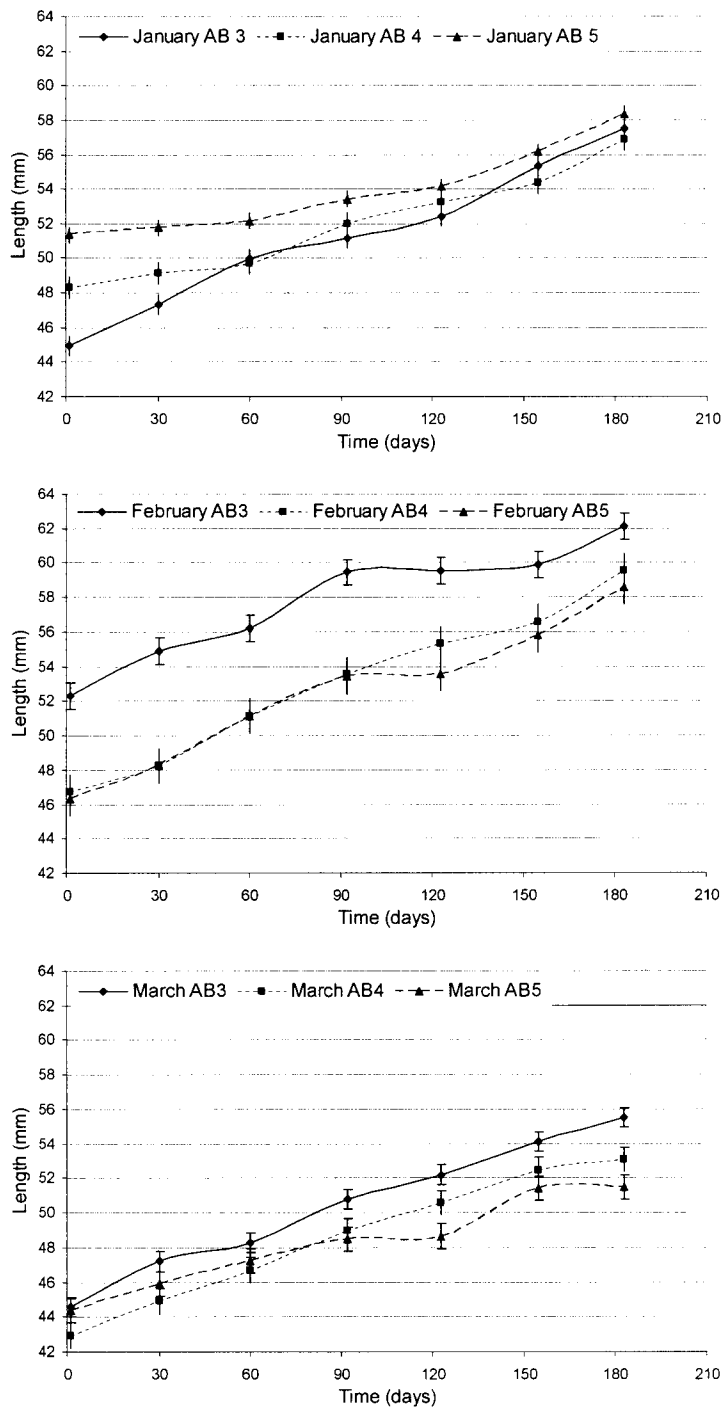


Figure 5: Average abalone growth in shell length for the three spawning cohorts
 Thirty abalone were sampled each month for the three dietary treatments from May to November 2006.

2.3.2 Growth parameters

Over the trial period the abalone in the Abfeed[®] 3 had higher (i) mean weight gain (1.05 – 2.82 grams), (ii) mean shell length gain (0.62 – 2.49 mm), (iii) DISL (3.36 – 13.58 $\mu\text{m.d}^{-1}$) when compared to abalone in the Abfeed[®] 4 and Abfeed[®] 5 respectively (see Table 3). The abalone in the Abfeed[®] 4 diet had a higher SGR (0.014 - 0.049 %) when compared to the Abfeed[®] 3 and Abfeed[®] 5 diets respectively.

Abalone in the January spawned cohort in the Abfeed[®] 3 had higher (i) mean weight gain (3.9 – 2.7 grams), (ii) mean shell length gain (4.01 – 5.55 mm), (iii) DISL (21.93 – 30.36 $\mu\text{m.d}^{-1}$), (iiii) SGR (0.086 – 0.114 %) when compared to abalone in the Abfeed[®] 4 and Abfeed[®] 5 diets respectively (see Table 3).

Abalone in the February spawned cohort in the Abfeed[®] 4 had higher (i) mean weight gain (1.65 – 2.56 grams), (ii) mean shell length gain (2.98 – 0.57 mm), (iii) DISL (16.3 – 3.15 $\mu\text{m.d}^{-1}$), (iiii) SGR (0.108 – 0.04 %) when compared to abalone in the Abfeed[®] 3 and Abfeed[®] 5 diets respectively (see Table 3).

Abalone in the March spawned cohort in the Abfeed[®] 3 had higher (i) mean weight gain (2.41 – 5.61 grams), (ii) mean shell length gain (0.81 – 3.87 mm), (iii) DISL (4.46 – 17.2 $\mu\text{m.d}^{-1}$), when compared to abalone in the Abfeed[®] 4 and Abfeed[®] 5 diets respectively (see Table 3). Abalone in the Abfeed[®] 4 diet had higher SGR (0.006 – 0.074 %) when compared to abalone in the Abfeed[®] 3 and Abfeed[®] 5 diets respectively.

Table 3: Growth parameters of abalone from the three treatments and spawning cohorts

Treatment: all three spawning cohorts combined

		Abfeed [®] 3	Abfeed [®] 4	Abfeed [®] 5
Treatment	Mean initial weight (g)	22.14	19.92	21.92
	Mean final weight (g)	42.7	39.4	39.6
	Mean weight (g) gain	20.53	19.48	17.71
	Mean initial length (mm)	47.3	46.0	47.5
	Mean final length (mm)	58.4	56.5	56.1
	Mean length (mm) gain	11.13	10.51	8.64
	DISL	60.82	57.46	47.24
	SGR	0.358	0.372	0.323
January Cohort	Mean initial weight (g)	19.6	22.17	26.98
	Mean final weight (g)	39.73	38.4	44.4
	Mean weight (g) gain	20.13	16.23	17.43
	Mean initial length (mm)	44.91	48.31	51.75
	Mean final length (mm)	57.5	56.89	58.36
	Mean length (mm) gain	12.59	8.58	7.04
	DISL	68.81	46.88	38.45
	SGR	0.386	0.3	0.272
February Cohort	Mean initial weight (g)	28.1	20.81	21.39
	Mean final weight (g)	49.81	44.91	42.94
	Mean weight (g) gain	21.7	24.11	21.55
	Mean initial length (mm)	52.29	46.73	46.31
	Mean final length (mm)	62.12	59.54	58.55
	Mean length (mm) gain	9.83	12.81	12.24
	DISL	53.73	70.03	66.88
	SGR	0.312	0.42	0.38
March Cohort	Mean initial weight (g)	18.73	16.79	17.41
	Mean final weight (g)	38.48	34.9	31.56
	Mean weight (g) gain	19.76	18.11	14.15
	Mean initial length (mm)	44.56	42.92	44.37
	Mean final length (mm)	55.52	53.07	51.46
	Mean length (mm) gain	10.96	10.15	7.09
	DISL	59.92	55.46	38.72
	SGR	0.393	0.399	0.325

Daily increment increase in shell length (DISL – $\mu\text{m}\cdot\text{d}^{-1}$), specific growth rate (SGR - % body weight. day^{-1}), length and weight gained by the abalone from the three treatments (all three spawning cohorts combined) and weight and length gained by the abalone in the 3 spawning cohorts.

2.3.3 Estimated time to harvest

When all the spawning dates were combined, the estimated time to harvest was very similar for the Abfeed[®] 3 and Abfeed[®] 4 diets with a ten days difference in the estimated time to harvest (time taken to reach 100 g). The Abfeed[®] 5 diet performed slightly worse with the estimated time to harvest a month later than the other two diets (see Table 4 and Appendix Figure 1).

The January spawned cohort:

The abalone in the Abfeed[®] 3 diet had the shortest estimated time to harvest, with 73 days and 53 days shorter time compared to the Abfeed[®] 4 and Abfeed[®] 5 diets respectively (see Table 4 and Appendix Figure 2).

The February spawned cohort:

The abalone in the Abfeed[®] 4 diet had the shortest estimated time to harvest which was 30 and 32 days shorter than the Abfeed[®] 3 and Abfeed[®] 5 diets respectively (see Table 4 and Appendix Figure 3).

The March spawned cohort:

The abalone in the Abfeed[®] 3 diet had the shortest estimated time to harvest which was 13 and 87 days shorter than the Abfeed[®] 4 and Abfeed[®] 5 diets respectively (see Table 4 and Appendix Figure 4).

Table 4: Estimated time to harvest (days)
 Treatment: all three spawning cohorts combined

	Abfeed [®] 3	Abfeed [®] 4	Abfeed [®] 5
Treatment	247	257	289
January cohort	249	322	302
February cohort	237	207	239
March cohort	244	257	331

Estimated number of days to harvest was estimated using the entire six month growth data, an exponential trend line was fitted to the data and projected to 100 grams (see Appendix Figures 1 - 4).

2.3.4 Condition factor

Treatment: (all three spawning cohorts combined)

The abalone in the Abfeed[®] 5 diet had significantly higher CF than the abalone in the Abfeed[®] 4 diet ANOVA ($F(2, 267) = 6.425, p = 0.001$) (see Table 5).

January cohort:

The abalone in the Abfeed[®] 5 diet had significantly higher CF compared to both the Abfeed[®] 3 and Abfeed[®] 4 diets. ANOVA ($F(2, 87) = 10.029, p < 0.001$) (see Table 5).

February cohort:

The abalone in the Abfeed[®] 3 diet had significantly higher CF compared to both the Abfeed[®] 4 and Abfeed[®] 5 diets ANOVA ($F(2, 87) = 7.105, p = 0.001$) (see Table 5).

March cohort:

The abalone in the Abfeed[®] 5 diet had significantly higher CF compared to the abalone in the Abfeed[®] 3 diet ANOVA ($F(2, 87) = 4.1812, p = 0.0184$) (see Table 5).

Table 5: Condition factors ($\text{g}\cdot\text{mm}^{-1}$) for the diets and spawning cohorts (standard deviation)
Treatment: all three spawning cohorts combined

	Abfeed [®] 3	Abfeed [®] 4	Abfeed [®] 5
Treatment	1.273 (0.140)	1.23 (0.126)	1.297 (0.134)
January	1.221 (0.122)	1.198 (0.165)	1.341 (0.105)
February	1.322 (0.164)	1.244 (0.123)	1.251 (0.129)
March	1.232 (0.095)	1.246 (0.085)	1.314 (0.159)

2.3.5 Water Nutrients

Total Ammonia Nitrogen

The TAN concentrations were highly variable, with peaks occurring between 8 pm and 4 am (see Table 6 and Figure 6). The major TAN peak for the Abfeed[®] 4 diet occurred the first evening of recording around midnight, and thereafter the values decreased. This is due to the Abfeed[®] 4 diet being fed with 225 g of Abfeed[®] K26 on Thursday; whereas the other two diets were fed 180 g of Abfeed[®] K26 on Friday. Peaks in the Abfeed[®] 5 diet occurred at midnight of day one, 8 am and 8 pm of day two, and a smaller peak at 12 am on day three (see Figure 6). The Abfeed[®] 3 diet had lower concentrations of TAN compared to the other two diets; the peaks between 8 pm and 4 am were also absent.

Table 6: The Total Ammonia Nitrogen concentrations μml^{-1} recorded over two 72 hour experiments.

(i) 9 - 11 October 2006 and (ii) 10 - 12 November 2006

(i)

TAN	Average	Min	Max
Inlet	1.99	0.89	3.70
Abfeed [®] 3	4.26	0.17	6.58
Abfeed [®] 4	6.62	3.61	13.75
Abfeed [®] 5	6.88	0.48	11.32

(ii)

TAN	Average	Min	Max
Inlet	1.99	0.31	5.42
Abfeed [®] 3	5.10	2.09	9.35
Abfeed [®] 4	5.12	2.30	7.25
Abfeed [®] 5	7.09	5.00	10.82

Analysis of variance (ANOVA's) was performed over the two 72 hour periods, the ANOVA for the October recordings indicated that there were significant differences between the treatments $F(3, 176) = 4.77, p = 0.003$, a post-hoc Tukey test showed that the Abfeed[®] 4 treatment had significantly higher TAN values when compared to both the inlet ($p = 0.01$) and Abfeed[®] 3 treatment ($p = 0.02$). The ANOVA for the November recordings indicated that there were significant differences between the treatments Kruskal-Wallis test: $H(3, N = 180) = 63.23, p < 0.001$. A post-hoc multiple comparison test showed that the inlet had significantly lower TAN values when compared to all three treatments, the Abfeed[®] 3 treatment was significantly lower than the Abfeed[®] 5 treatment and the Abfeed[®] 4 treatment was significantly lower than the Abfeed[®] 5 treatment ($p < 0.05$).

Table 7: Maximum Free un-ionized Ammonia Nitrogen concentrations μml^{-1}

DATE	ABFEED [®] 3	ABFEED [®] 4	ABFEED [®] 5
October	0.588	0.264	0.376
November	0.261	0.151	0.029

Maximum FAN values are shown in Table 7. The readings recorded in October are much higher than those recorded in November. The Abfeed[®] 3 diet had the highest of the three diets, in both the October and November recordings. FAN concentrations are affected by temperature, salinity and pH, and all these factors contributed to the difference in FAN concentrations in October and November.

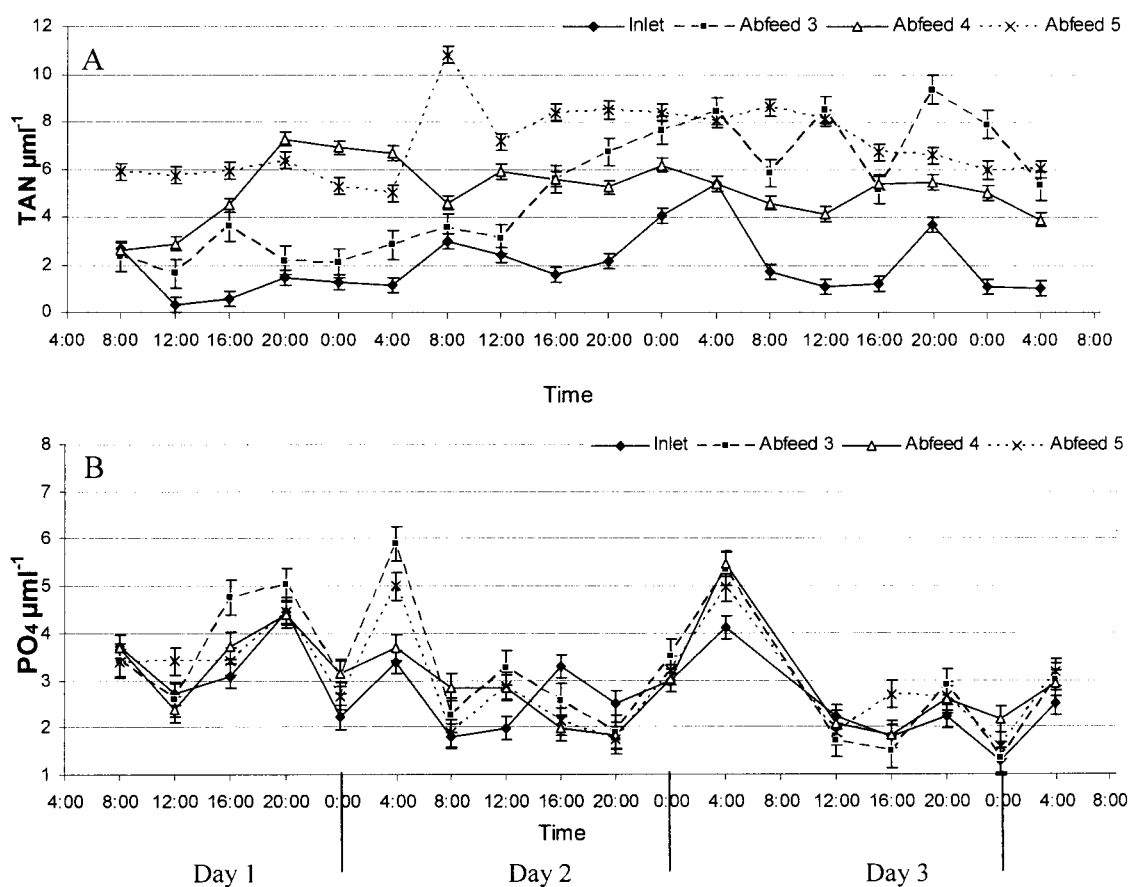


Figure 6: Total Ammonia Nitrogen (A) and Phosphate (B) concentrations (μml^{-1}) in the inlet water and all three diets (n =162 each). Experiment began on Friday morning at 8 am (day 1). Abfeed[®] 4 diet was fed 225 g of Abfeed[®] K26 on Thursday (the day before the start of recordings); whereas the other two diets were fed 180 g of Abfeed[®] K26 on Friday (the first day of recordings).

Phosphate concentrations:

The phosphate concentrations peaked at 6 pm of day 1 and 4 am of day 1, 2 and 3. The peaks are synchronized with inlet peak values, with the exception of a small peak in the inlet at 4 pm of day 2 (See Figure 6 B and Table 8).

**Table 8: The Phosphate concentrations μml^{-1} recorded over two 72 hour experiments.
(i) 9 - 11 October 2006 and (ii) 10 - 12 November 2006**

(i)

Phosphate	Average	Min	Max
Inlet	2.72	1.28	4.46
Abfeed [®] 3	3.17	1.33	5.89
Abfeed [®] 4	2.97	1.83	5.46
Abfeed [®] 5	3.00	1.59	5.00

(ii)

Phosphate	Average	Min	Max
Inlet	3.05	1.05	7.38
Abfeed [®] 3	3.20	0.90	6.80
Abfeed [®] 4	3.40	1.47	6.65
Abfeed [®] 5	3.39	1.18	7.41

Analysis of variance (ANOVA's) was performed over the two 72 hour periods. The ANOVA's indicate that there were no significant differences between the phosphate concentrations in October ($F(3, 176) = 0.24, p = 0.86$) and November ($F(3, 166) = 0.650, p = 0.58$).

2.3.6 Physico-chemical variables

pH

There were no significant differences between the pH of the three dietary treatments, but the pH in the inlet water was significantly higher than all the dietary treatments (ANOVA: $F(3, 176) = 26.85$, $p < 0.001$). Average pH (see Figure 7 A) for the three diet treatments was 7.9 with minimum of 7.64 and a maximum of 8.19. This was significantly lower ($p < 0.05$) than the incoming seawater which had an average pH of 8.3 with a minimum of 8.02 and a maximum of 8.54. The pH showed a diurnal rhythm being highest at 4 pm and lowest at 8 am (see Figure 7 A).

Temperature

There was no significant difference between the temperatures in the three dietary treatments and between the inlet and the three diet treatment temperatures ANOVA $F(3, 176) = 0.527$, $p = 0.66$ (see Figure 7 B). The inlet temperature had an average of 17 °C with a minimum of 15.7 °C and a maximum of 18.3 °C. The average temperature of the three diet treatments was 17.2 °C with a minimum of 15.7 °C and a maximum of 18.7 °C. The drop in daily temperature that occurred on day 3 was due to lower ambient temperature. The temperature values showed a diurnal pattern which was synchronized to the pH.

Dissolved Oxygen

There was no significant difference in DO concentrations between the three dietary treatments. The DO in the dietary treatments were significantly lower than the inlet water (ANOVA $F(3, 176) = 51.314, p < 0.001$) (see Figure 7 C). The inlet had an average DO of 4.85 mg l^{-1} with a minimum of 4.60 mg l^{-1} and a maximum of 5.21 mg l^{-1} . The three diets had an average DO of 4.09 mg l^{-1} with a minimum 3.62 mg l^{-1} and a maximum 4.48 mg l^{-1} . There was a decrease in DO at 4 pm, which corresponds to the highest temperature and pH during the day.

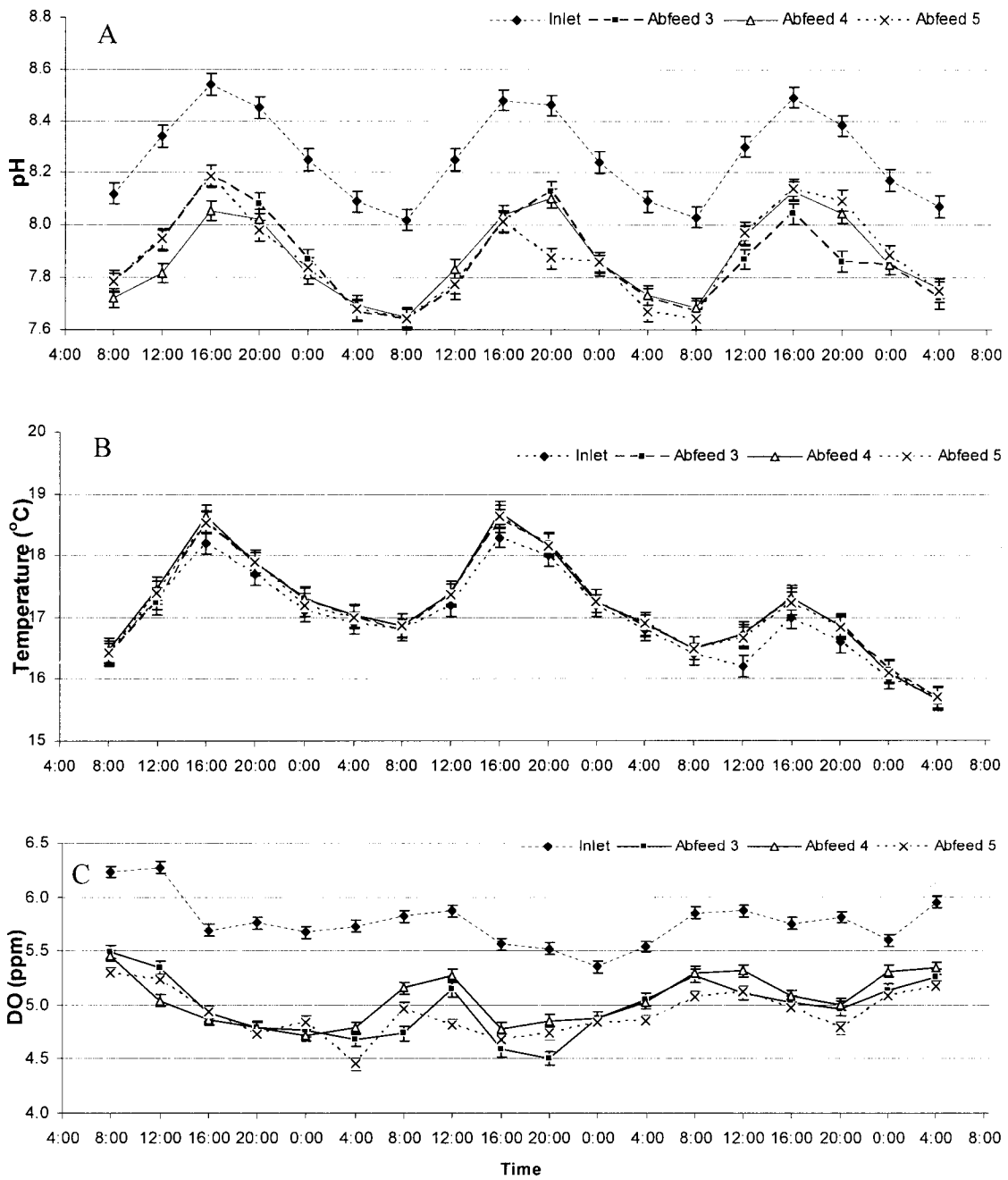


Figure 7: pH (A), Temperature (B) and Dissolved oxygen (C) recorded (n = 162 each)

2.3.7 Sediments

There were no significant differences between the sediments in the water column of the three diets (see Table 9), Kruskal-Wallis test: $H(2, N = 9) = 0.6222$ $p = 0.7326$. The Abfeed[®] 3 diet had the highest water column sediment followed by the Abfeed[®] 4 and Abfeed[®] 5 diets respectively. No significant differences were found between the bottom sediments accumulation of the three diets (see Table 9) Kruskal-Wallis test: $H(8, N = 18) = 6.456140$ $p = 0.5963$. The Abfeed[®] 3 diet had the highest bottom sediment load per litre, followed by the Abfeed[®] 5 and Abfeed[®] 4 diets respectively.

Table 9: Average water column sediment and bottom sediment accumulation per liter (grams) (\pm Standard deviation)

	Abfeed [®] 3	Abfeed [®] 4	Abfeed [®] 5
Average water column sediment	1.743 \pm (0.666)	1.455 \pm (0.257)	1.306 \pm (0.293)
Average Bottom sediment accumulation	6.67 \pm (1.666)	5.05 \pm (0.539)	5.51 \pm (0.509)

2.3.8 Abalone health

There were no significant differences between the sabellid score present on the abalone in all the diets, as identified by Dr Anna Mouton, the AFASA veterinarian, with average sabellid scores of between 1 and 2. Abalone in all the diets showed significant environmental stress. This is reflected in the bad condition scores and bad shell condition scores with the abalone all having poor to very poor shell conditioning. Abalone in all the diets had immature sex cells or moderate gonad development; this was expected due to immature abalone being used in the experiment (see Table 2 and Table 10).

Table 10: Abalone Health values (standard deviation); see Table 2 for key

Diets	Sabellid score (Average)	Shell condition (Average)	Gonad development (Average)	Cum. Condition score (Sum)	Cum. Environmental stress score (Sum)
Abfeed ³	1.5 (0.4)	2.7 (0.6)	1.2 (0.3)	5	22
Abfeed ⁴	1.4 (1.4)	1.7 (0.6)	1.3 (0.3)	6	18
Abfeed ⁵	1.8 (0.5)	2.0 (1.4)	1.5 (0.9)	31	15

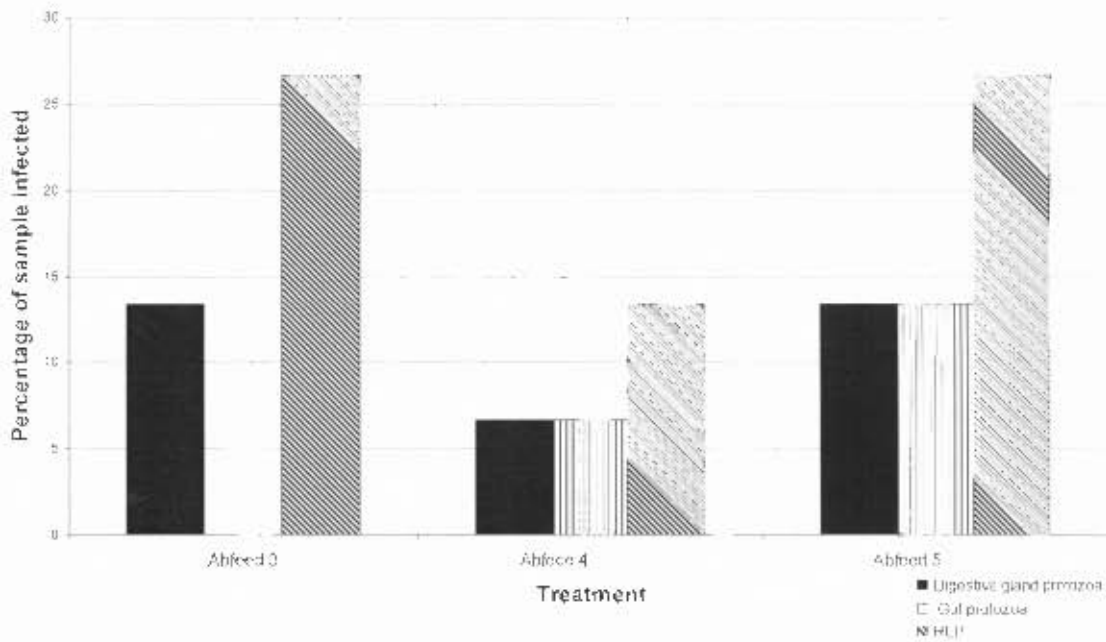


Figure 8: Digestive gland protozoa, Gut protozoa and *Rickettsia*-like prokaryote in the three diet treatments

Digestive gland protozoa (DGP) were present in all the diet treatments, with 13 % of the abalone having DGP in the Abfeed[®] 5 and Abfeed[®] 3 diets, abalone in the Abfeed[®] 4 diet treatment had less with 6,5 % of the sample having DGP (see Figure 8).

Gut protozoa were present in the Abfeed[®] 5 and Abfeed[®] 4 diets only, with 13 % and 6.5 % of the abalone having the gut protozoa (see Figure 8).

All the treatments had RLP present; the Abfeed[®] 5 and Abfeed[®] 3 diets had the same level of infection with 26.5 % of the abalone having the RLP. The abalone in the Abfeed[®] 4 diet had a lower rate of infection (13 %) (see Figure 8).

2.4 Discussion

2.4.1 Growth

The initial growth results seemed to be conflicting, as the ANOVA's show significant differences between (1) the abalone lengths in the treatments (2) the abalone weights of all three spawning cohorts (3) abalone length in the February and March spawned cohorts, whereas the ANCOVA's showed no significant difference between weights and lengths of abalone in all three treatments and all three spawning cohorts. In order to address the conflicting results, ANOVA's were performed on the initial weights and lengths recorded in May 2006, to determine if the initial weights and lengths were significantly different from each other. These results indicated that there were significant differences between the weights and lengths of the treatments and spawning cohorts. Therefore the significant differences identified by the ANOVA's of the final (November 2006) recording were a result of the significant differences being carried through the experiment from the initial recordings (see Figures 2 - 5). The size difference of animals at the start of the experiment was an artefact of working in a commercial environment, where it was not possible to be as accurate as working under laboratory conditions. The ANCOVA's were therefore a better indicator as to how the treatments affected the growth of the abalone. Therefore there was no significant overall difference between the three treatments in terms of weight and length increase over the experimental time period. The difference in initial size could also explain the difference in SGR and the difference

in estimated time to harvest as larger animals would be able to have higher weight increase in comparison to smaller animals.

This study concludes a large body of research performed on abalone diets in a land based aquaculture farms; Table 11 below presents the length increases per month from Naidoo et al. (2006) and Robertson-Andersson (2007).

Table 11: Comparison of abalone length increase (mm) per moth from three studies using different diets

Diets	Naidoo et al. 2006	Robertson-Andersson 2007	Current study
Kelp	1.65	1.69	
kelp, Ulva and Gracilaria	1.98	1.96	
Abfeed®	1.47		
Abfeed® 3			1.86
Abfeed® 4			1.75
Abfeed® 5			1.44

The abalone in the Abfeed® 3 and Abfeed® 4 diets had higher monthly length increase compared to those recorded for the kelp diet by Robertson-Andersson (2007) (Robertson-Andersson worked on the same farm, with abalone of similar size and stocking density to the current study). Abalone in all the diets in the current study had lower monthly length increases in comparison to abalone in Robertson-Andersson (2007) mixed diet treatment. It is important to note that growth factors are influenced by numerous factors, mentioned above; and therefore these comparisons should be used as an estimate rather than a direct comparison. Naidoo et al. (2006) conducted feed trials with *H. midae*, with a number of different diets, at the Jacobsbaai Sea Products farm on the west coast of South Africa,. The abalone used in their study had a starting weight of 7.22 - 7.92 g, with a stocking density of 500 abalone per basket. Their results showed that a mixed diet (*Gracilaria*

gracilis, *Ulva lactuca* and kelp) had the best growth rates with monthly abalone length increase of 1.98 mm per month (a higher rate than in the current study). Naidoo et al.'s (2006) results were very similar to those of Robertson-Andersson, 2007 for the mixed diets. Numerous abalone farms are currently farming *Gracilaria* spp. and *Ulva* spp. (Bolton, 2006; Troell et al., 2006; Robertson-Andersson, 2007, Robertson-Andersson et al., 2008), and including these seaweeds into the current diet of kelp and Abfeed® K26 could possibly increase growth rates. This needs further research.

The CF is the relationship between the weight of abalone per unit shell length and takes into account amount of feed invested in developing both the body weight and shell length. The CF of abalone in the Abfeed® 5 diet was the highest of the three diets (see Table 5). The CF of abalone in the Abfeed® 5 diet was significantly higher than the abalone of the Abfeed® 4 diet. This indicates that abalone in the Abfeed® 5 diet invested more into weight than into length, which is more desirable for the market.

2.4.2 Water Nutrients

ANOVA's over the 72 hour periods indicate that there was significant difference between the Total Ammonia Nitrogen (TAN) concentrations in the three diets. The TAN concentrations were highly variable as shown by the significantly higher TAN concentration in the October Abfeed® 4 treatment recording and the significantly higher TAN concentration recorded in the Abfeed® 5 treatment in the November recordings (see Figure 6 A and Table 8). In an aquaculture system the level of nitrogenous wastes

excreted by aquatic animals is probably the most important parameter, once oxygen levels are maintained (Colt & Armstrong, 1981). Within the nitrogenous wastes of abalone, ammonia is the major component of the protein catabolism (Kinne, 1976; Colt & Armstrong, 1981; Russo & Thurston, 1991). Chronic exposure can lead to slower growth and ultimately mortality in the cultured animals, thus limiting production in aquaculture (Basuyaux & Mathieu, 1999; Hargreaves & Kucuk, 2000; Russo & Thurston, 1991; Reddy-Lopata et al., 2006). The proportions of Free un-ionized Ammonia Nitrogen (FAN) and TAN in solution depend on temperature, pH and to a lesser extent on salinity (Huchette et al., 2003). Concentrations of FAN increase with elevated temperature and pH values and decrease with higher salinities (Downing & Merckens, 1955). Thus, TAN at low pH is usually toxic only in overwhelming quantities whereas at high pH, much smaller amounts may be lethal (Warren, 1962). The maximum FAN concentrations recorded in the abalone tanks of this experiment were $0.588 \mu\text{ml}^{-1}$ for the Abfeed[®] 3 diet, $0.264 \mu\text{ml}^{-1}$ for the Abfeed[®] 4 diet and $0.376 \mu\text{ml}^{-1}$ for the Abfeed[®] 5 diet. These values were far lower than the recommended safe concentrations of $7.4 \mu\text{ml}^{-1}$ established for similar sized *H. midae* by Reddy-Lopata et al. (2006), and should therefore have little effect on the growth rate.

2.4.3 Physico-chemical variables

There was no significant difference between any of the physico-chemical variables in the three diets recorded over the 72 hour period (see Figure 7). The diurnal patterns in the temperature data were linked to those of the inlet water (which is at ambient sea temperature), and both of these were influenced by air temperature. The slight increase in temperature in the abalone tanks was due to both the biological activity of the abalone and the radiation from the sun. Britz et al. (1997), showed that the physiologically optimal temperature range for *H. midae* growth is 12 - 20 °C (with an absolute optimum of 18 °C). All the dietary treatments in the current experiment fell within the optimal temperature range between 15.5 and 19 °C. The temperature measurements were recorded in the warmer summer months and would be close to the maximum the system would be exposed to.

The pH showed diurnal patterns, rising with increasing temperature (see Figure 7 A and 7 B). As the temperature increases so will the biological activities of the abalone, including increased respiration which produces carbon dioxide. Carbon dioxide dissolved in seawater forms carbonic acid by affecting the chemical balance between hydrogen ions, bicarbonate and carbonate, thus lowering the pH. The pH values recorded in the experiment ranged between 7.64 - 8.19, which fell within the target range of 7.5 – 8.2 of the I & J Abalone farm.

The DO recorded in the abalone tanks of all dietary treatments was lower than that of the inlet water, as expected, due to the respiring abalone in the tanks. Robertson-Andersson et al. (2008) found a decrease in DO at night, due to higher abalone activity. This trend

was not evident in the current study. The lowest DO recorded in the current study occurred at 4 pm, which corresponds to the higher temperature readings (see Figure 7).

2.4.4 Sediments

There was no significant difference between the suspended particulate matter (SPM) of the three diets. This may mean that the water column has reached loading capacity. A possible reason for this may be due to aeration and flow rates in the tanks being equal and therefore the water motion (velocity) within the tanks was similar (Robertson-Andersson, 2007). Accumulation of bottom sediments were significantly higher when compared to SPM, which was expected as the comparison is between sediments accumulated over time with the concentration in the water column. No significant differences were found for bottom sediments accumulation for the three dietary treatments. This was in contrast to the findings of Potgieter (2005), who found a significant difference in particle accumulations of the bottom sediment loads. This may be due to the same dietary components (Kelp and Abfeed[®] K26) being used in all three diets in the current study, whereas Potgieter (2005), used diets consisting of Abfeed[®] only; Kelp only and a Mixed algal diet. Negative effects of SPM include: mechanical and abrasive impairment of gills resulting in the cultured organism's death (Lloyd, 1987), decreased light penetration (Lloyd, 1987), impaired feeding behaviour, and smothering of benthic organisms (Hogg & Norris, 1991), clogging of filter feeding apparatus (Newcombe & Macdonald, 1991; Metzeling et al., 1995), stress and behavioural changes (Doeg & Milledge, 1991). As there were no significant differences between the two higher levels of Abfeed[®] in the diet

(Abfeed[®] 4 and Abfeed[®] 5 diets) and the control, it can be concluded that increasing the ratio of Abfeed[®] in the diet had no effect on the sediment loadings.

2.4.5 Abalone health

Abalone from all diets were infected with the sabellid polychaete, *T. heterouncinata*, which is endemic to South Africa (Ruck & Cook, 1998; Fitzhugh & Rouse, 1999) (see Table 10). This is a problem that needs to be managed on South African farms due to the ubiquity of *T. heterouncinata*. There was no significant difference in the infection rate of *T. heterouncinata* between the three dietary treatments. Therefore the increased level of Abfeed[®] K26 in the diet had no effect on sabellid numbers in the I & J system. Abalone in all three diets had poor shell development and showed significant signs of stress, identified by Dr Mouton. Stress can be caused by numerous conditions including diet, stocking density, free un-ionized Ammonia Nitrogen and suspended particulate matter concentrations. As abalone in all the dietary treatments, including the control, showed signs of stress, diet is probably not the major factor inducing stress in the I & J system. The effects of the digestive gland protozoa and Gut protozoa are thought to be benign in nature and should have little effect on the abalone growth rates (see Figure 8). The conditions for the control (Abfeed[®] 3) were the same as the rest of the farm. As abalone in the Abfeed[®] 3 dietary treatment showed signs of stress it can be assumed that abalone on the rest of the farm would have also been stressed.

2.4.6 Cost assessment

In addition to the factors mentioned above it is also important to take cost of the diets into account.

Cost values obtained from Marifeed (Pvt) Ltd 2007

- Wet kelp at ZAR1.07 per kg
- Abfeed[®] at ZAR 14.50 per kg (dry feed)

Table 12: Cost assessment per basket (calculated using 550 abalone per basket, with an average weight of 18 grams).

Diets	Kelp / Abfeed [®] per week (grams)	Rand per week	Rand per month	Rand per year
Abfeed [®] 3	2700 / 180	2.88 + 2.61	22.00	285.94
Abfeed [®] 4	2080 / 225	2.22 + 3.26	21.95	285.38
Abfeed [®] 5	1400 / 315	1.49 + 4.57	24.26	315.40

The Abfeed[®] 5 diet cost per basket per year is ZAR 29.46 and ZAR 30.02 more than the Abfeed[®] 3 and Abfeed[®] 4 diets respectively (see Table 12). Abfeed[®] 3 and Abfeed[®] 4 diets costs were very similar with there being a ZAR 0.56 difference in price per basket per year. Scaled up to a 500 tank farm (with 24 baskets per tank); the farm would save ZAR 6720 per year using the Abfeed[®] 4 diet as compared to the Abfeed[®] 3 diet.

2.5 Conclusion

There was no significant difference in terms of abalone growth in terms of weight and length increase over the trial period. The higher levels of Abfeed[®] K26 in the diet did not have any significant affect on sediment loadings, abalone health, physico-chemical variables and water nutrients. It is therefore possible to use higher ratios of Abfeed[®] K26 in the diet with out any negative effects to abalone growth and the culture environment.

Chapter 3

Abalone (*Haliotis midae* Lin.) cultivation at high recirculation rates (50 – 75 %) using a seaweed and formulated feed fortified diet, with *Ulva lactuca* L. as a biofilter

3.1 Introduction

Reducing negative environmental impacts from aquaculture activities is a key issue for ensuring long-term sustainability of the aquaculture industry (Troell et al., 2003). Integrated multi-trophic aquaculture has been suggested as a means of reducing negative effects and increasing production and sustainability of aquaculture (Folke & Kautsky, 1992; Newkirk, 1996; Brzeski & Newkirk, 1997; Naylor et al., 2000; Lüning & Pang, 2003; Troell et al., 2003; Ridler et al., 2007). Integrated multi-trophic aquaculture is the culture of fed organisms combined with the culture of organisms that extract either dissolved inorganic nutrients (i.e. seaweeds) or particulate organic matter (i.e. shellfish), thus balancing the biological and chemical processes (see review in Buschmann et al., 2001; Chopin et al., 2001; Ridler et al., 2007). The by-product wastes from one resource become inputs for another (Shpigel & Neori, 1996; Troell et al., 2006; Chopin, 2006; Ridler et al., 2007). This can be achieved by using a recirculating system with a biofilter (Neori et al., 1996). Recirculating systems have the potential to reduce the effects of harmful algal blooms (HAB's) (Pitcher et al., 2002), accidental discharges of pollutants and reduce the amount of dissolved nutrients that are released into the environment (Krom, 1986; Robertson-Andersson, 2003; Samsukal, 2004).

Water is the medium that transports the pollutants from the animal culture to the biofilter (Neori et al., 2004). In integrated systems, water from aquaculture ponds recirculates through seaweed biofilter ponds, where waste organic matter is broken down and dissolved nutrients are taken up (Neori et al., 2004). Seaweeds have a high capacity for

nutrient uptake and can themselves be valuable products (see Neori, 1996; Chopin et al., 2001; Troell et al., 2003; Neori et al., 2004). Seaweeds can assimilate as much as 90 % of the ammonium produced by intensive fish culture (see Cohen & Neori, 1991; Neori et al., 1991, 1996, 2000; Jimenez del Río et al., 1996; Buschmann, 1996). This is a great benefit in intensive fish aquaculture where three quarters of the proteins fed to the fish are excreted and eventually end up as dissolved ammonia. (Neori, et al., 2004). Seaweed biofilters have the added benefits of increasing dissolved oxygen in the system as nighttime oxygen consumption by seaweeds is much lower than their daytime oxygen production due to photosynthesis (Neori et al., 2004). For this experiment *Ulva lactuca* was chosen, as it fulfilled the requirements outlined in Chapter 1, Section 1.6. Farm-grown *U. lactuca* has been shown to have higher protein content than wild material (Robertson-Andersson, 2003; Robertson-Andersson et al., 2006), growing better in abalone effluent than in natural seawater (Fourie, 1994; Friedlander & Levy, 1995; Smit, 1997; Hampson, 1998; Steyn, 2000; Robertson-Andersson, 2003). *Ulva* spp. remove dissolved nitrogen from the abalone effluent which results in an increase in both its phosphorus and protein content (Neori et al., 1991, 1996; Robertson-Andersson, 2003, 2007; Robertson-Andersson et al., 2006). These factors make *U. lactuca* a valuable biofilter with high nutrient uptake efficiencies (Cohen & Neori, 1991; Chopin et al., 2001; Troell et al., 2003; Robertson-Andersson, 2003).

This experiment follows on from the work of Robertson-Andersson (2007) and Robertson-Andersson, et al. (2008), on abalone / seaweed recirculation systems, fed a kelp only diet. Robertson-Andersson (2007) and Robertson-Andersson, et al. (2008) showed no significant differences in abalone health or growth rates, sediment build up

and composition, mobile macro fauna densities and species between a 25 % recirculation system (using seaweeds as biofilter) and flow-through units. Transfer of oxygen generated by the seaweeds to the abalone tanks in these studies was poor, resulting in the recirculating abalone tanks having 33 % lower dissolved oxygen concentrations than a comparable flow-through abalone unit. Seaweed nutrient content and specific growth rates in the units were comparable to seaweeds cultivated in fertilized effluent (SGR = $3.2 \pm 3.4 \text{ \%} \cdot \text{d}^{-1}$; Yield = $0.2 \pm 0.19 \text{ kg} \cdot \text{m}^2 \cdot \text{d}^{-1}$). Robertson-Andersson (2007) and Robertson-Andersson et al. (2008) showed that seaweeds were nutrient limited and that there were no negative effects to the abalone being cultivated in such a recirculation unit at 25 % recirculation. Robertson-Andersson therefore concluded that higher levels of recirculation might be possible. Questions which arose from Robertson-Andersson's studies include:

- How will abalone growth be affected by higher recirculation rates and how would higher recirculation rates affect the culture environment?
- Would a higher protein diet have any effect on abalone growth and the culture environment at higher recirculation rates?

3.1.1 Aims

This experiment was conducted to test the effects of an integrated abalone / seaweed aquaculture system at higher recirculation rates in comparison to Robertson-Andersson (2007) and Robertson-Andersson et al. (2008). Abalone were cultivated with 50 % and 75 % recirculation through *Ulva* tanks, using a mixed diet of Kelp and Abfeed[®] K26. This study investigated abalone performance in the system by monitoring abalone health and growth rates, sediment loadings, physico-chemical variables and water nutrients.

3.2 Materials and Methods

3.2.1 Study site

This project took place in Gansbaai at the I & J Cape Abalone Mariculture farm (see Chapter 2 for details).

3.2.2 Experimental design

This study was initiated to monitor abalone growth and health when fed a mixed diet of kelp and Abfeed[®] K26 (equivalent to the Abfeed[®] 3 diet in the previous chapter), through regular monthly sub-sampling in a 50 % Recirculation system, with flow-through units (FTUs) as a control. Flow-through units are abalone tanks without recirculation. In addition water quality was monitored over a 72 hour period, to compare temperature, pH, dissolved oxygen, TAN, nitrate and phosphate. Small scale experiments were run using a 75 % Recirculation system to test the effects on water nutrient, physico-chemical variables and sediment production in the 75 % Recirculation system.

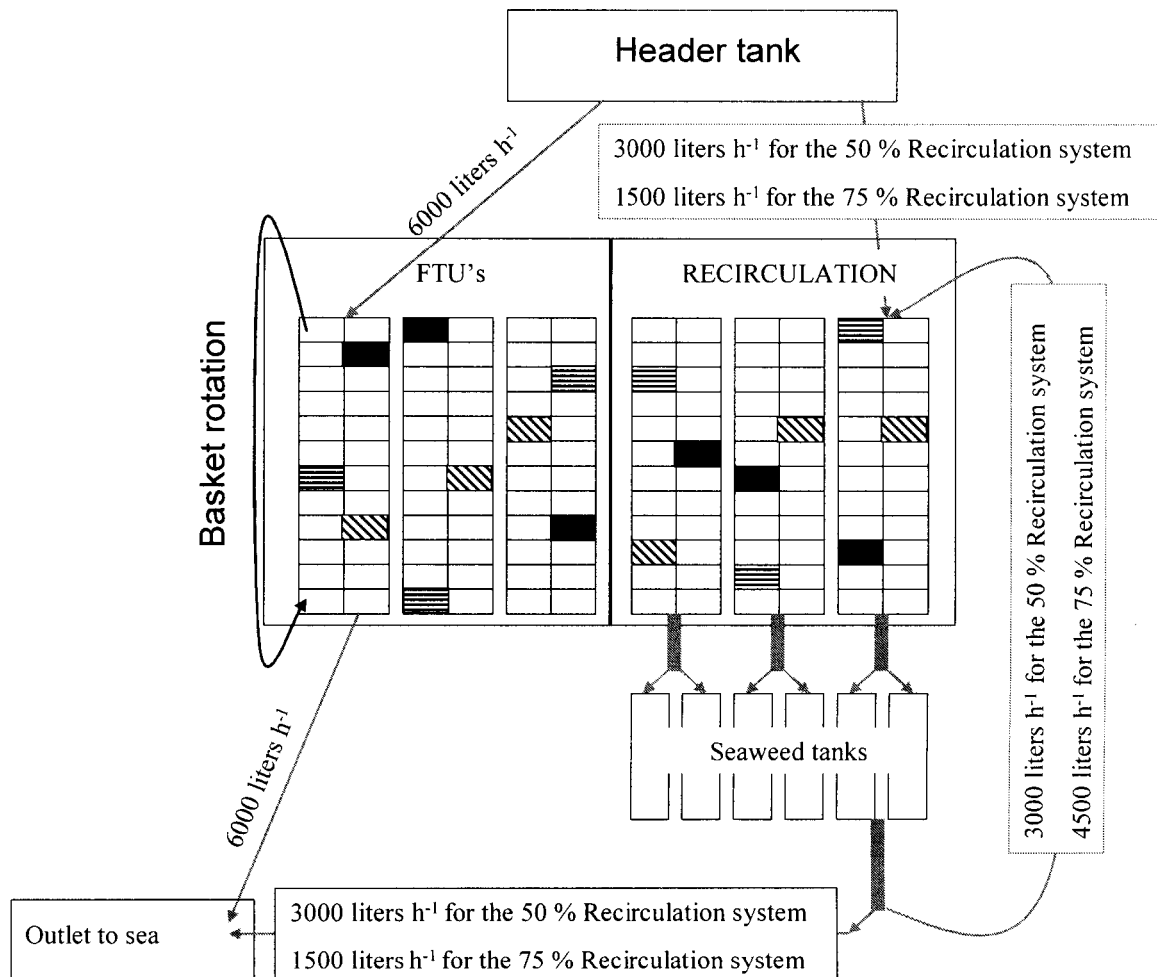


Figure 1: Diagram of experimental setup

Marked baskets represent placement of the abalone baskets that were repeatedly sampled once a month from May to November 2006. Black baskets represent the initial placement of the January spawned cohort, horizontal lines represent the initial placement of the February spawned cohort baskets and diagonal lines represents the initial placement of the March spawned cohort baskets. The baskets were rotated once a week during the weekly cleaning cycle, with the first basket in the tank being removed; all the baskets were moved one place forward in the tank, before placing the first basket in the last position of the tank. The FTUs received 6 000 litres of water per hour from the header tank, this water was drained through the outlet to the sea. The 50 % Recirculation system received 3 000 per hour from the Header tank and 3000 litres per hour from the seaweed tanks, 3000 litres per hour was released from the seaweed tanks out to sea. The 75 % Recirculation system received 1 500 litres per hour from the Header tank and 4 500 litres per hour from the seaweed tanks, 1 500 litres per hour was released from the seaweed tanks out to sea.

3.2.3 Experimental setup

This experiment was performed at I & J, in the farm's concrete flow-through tanks (see Chapter 2 for details). The abalone tanks received water from their inlets at a rate of 3000 litres per hour per tank; the same amount of water was received from the Seaweed tanks at 50 % recirculation (see Figure 1). During 75 % recirculation the abalone tanks received 4500 litres per hour from the Seaweed tanks and 1500 litres per hour from the inlets (see Figure 1). The Seaweed tanks were gravity-fed from the abalone tanks, with two Seaweed tanks being fed from one abalone tank. After the water passed through the two Seaweed tanks: (i) In the 50 % Recirculation system, 3000 litres of it was pumped by a 2 KW pump back to the abalone tank from which it originated and 3000 litres was released to the sea. (ii) In the 75 % Recirculation system, 4500 litres of it was pumped by a 2 KW pump back to the abalone tank from which it originated and 1500 litres was released to the sea (see Figure 1). The same experimental setup for the seaweeds / abalone recirculation system was used by Potgieter (2005), Brandt (2006), Lindström (2006), Robertson-Andersson (2007) and Robertson-Andersson et al. (2008). The six experimental Seaweed tanks were 5 m x 1 m surface area and 0.6 m deep with an outlet 17 cm from the top. They were made of white PVC lining (to facilitate easy cleaning and to reduce light absorption) supported on a steel 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 diameter) 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 kg m^{-2} stocking density) of *Ulva*

lactuca per tank and were harvested every 14 days. The overflow water exited the units from the Seaweed tanks.

3.2.4 Experimental animals

Animals for this experiment were spawned in 3 consecutive months; January, February and March 2004 (see Chapter 2 for details).

3.2.5 Diets

The same diet was used for both the flow through units (FTUs) and the Recirculation system, with 2.7 kg of kelp fed on Monday and 180 grams of Abfeed[®] K26 fed on Friday (the same diet as the Abfeed[®] 3 diet in the previous chapter).

3.2.6 Sub- sampling of abalone

Samples were taken from tanks from May 2006 to November 2006, at monthly intervals (see Chapter 2 for detail). Ninety abalone were sampled for both the 50 % Recirculation system and FTUs (see Figure 1). Of the 79200 animals that were part of the experiment at each monitoring period, 180 were sampled (0.23 % of the animals were sampled).

3.2.7 Daily Increment in Shell Length (DISL)

Daily Increment in Shell Length was calculated using the formula of Zhu et al. (2002)

$DISL (\mu\text{m} / \text{day}) = [(SL_t - SL_i) / t] \times 1000$, where SL_t = the final mean shell length (mm),
 SL_i = the initial mean shell length (mm) and t = the feeding trial period in days.

3.2.8 Specific Growth Rates (SGR)

SGR was calculated using the following formula $SGR = ((\ln (W_f) - \ln (W_i))/t)*100$

Where SGR is the specific growth rate (percentage body weight per day)

$\ln (W_f)$ is the natural log of the mean final weight of abalone, $\ln (W_i)$ is the natural log of the mean initial weight of the abalone and t is the time in days.

3.2.9 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).

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

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

3.2.10 Physico-chemical variables and Water nutrients

Physico-chemical variables (temperature, dissolved oxygen and pH) and water nutrient samples were recorded as for the previous chapter. The 50 % recirculation recordings were measured from the 9 -11 October 2006, and the 75 % recirculation recordings from the 10 - 12 November 2006. Long term physico-chemical variables were recorded daily from May to November 2006, for long term monitoring in the flow-through abalone tanks, the seaweed tanks, incoming seawater and the 50 % recirculation abalone tanks daily at 12h00. Long term physico-chemical variables were measured in order to track the change over the experimental trial period.

Nitrate was also recorded in this portion of the experiment. Nitrate concentration was determined using the copper-cadmium method described by Nydahl (1976).

Seaweed uptake rates were calculated as the percentage difference for nitrate, phosphate and TAN between the seaweed tanks and the abalone recirculating tanks.

3.2.11 Sediments

Sediments were recorded as for Chapter 2. A cleaning rotation was not set up for these sediment recordings, as it was possible to clean all six tanks on the same day.

3.2.12 Abalone health

Thirty animals from the experimental systems, three groups of five (from both the FTUs and 50 % Recirculation system) from the February spawned group were collected and analysed for parasite status, Sabellid score, shell condition, gonad development, condition score and environmental stress score by Dr Anna Mouton, the AFASA veterinarian, and reported according to standard industry reports (Mouton, 2006). See Chapter 2, Table 2 for the interpretation of the health results.

3.2.13 Statistical analysis

Unless otherwise stated, all graphs are expressed as means \pm SE. The growth data were analyzed in two methods

- (1) t-tests were performed on the end point of the experiment, if the assumptions of a t-test were not fulfilled (unequal variance and normality), the non parametric Kolmogorov-Smirnof two-sample test was performed. The Kolmogorov-Smirnof two-sample test was chosen as an alternative due to the samples having a normal distribution but unequal variances.
- (2) ANCOVA testing, tests for differences between two regression equations (see Zar, 1999). The regression equations were created from the entire six month growth data set; see Appendix tables 9 – 16 for calculations.

ANOVA's were also performed over the two 72 hour periods for the Physico-chemical variables and Water nutrients (see Chapter 2 for detail). Long term physico-chemical

variables were analyzed using 10 point running means and presented as percentage difference over the study period.

3.2.14 Estimated time to harvest

The same procedure was used as for Chapter 2 (see Appendix Figures 5 – 8).

3.3 Results

3.3.1 Growth

At the termination of the experiment there was no significant difference between the weights (t-value 0.726, df = 178 and p-value 0.46) and lengths (t-value 0.495, df = 178 and p-value 0.62) of abalone between the FTUs and the Recirculation system (see Figure 2 and 3). When broken down into spawning cohorts (see Figures 4 and 5), there was no significant difference between the weights of the three spawning cohorts in the FTUs and the Recirculation system (Kolmogorov-Smirnov two sample test $p > 0.1$). There was no significant difference between the lengths of the abalone in all three spawning cohorts in the FTUs and the Recirculation system (January and February Kolmogorov-Smirnov two sample test $p > 0.1$, and March cohort t-test, t-value 0.966, df = 58 and p-value 0.34).

The ANCOVA's also showed no significant differences between the weights and lengths of abalone in the Recirculation system and FTUs (abalone weights $t = 0.055$, df = 176, $p > 0.05$) (abalone lengths $t = 0.048$, df = 176, $p > 0.05$) (see Appendix Tables 9 and 10 for calculations). When broken down into spawning cohorts there were also no significant differences between the weights of abalone in the two treatments (January cohort weights $t = 0.039$, df = 56, $p > 0.05$, February cohort weights $t = 0.081$, df = 56, $p > 0.05$ and March cohort weights $t = 0.0241$, df = 56, $p > 0.05$) (see Appendix Tables 11 – 13). There were no significant differences between the abalone lengths in the three spawning cohorts in the FTUs and Recirculation system (January cohort lengths $t = 0.077$, df = 56,

$p > 0.05$, February cohort lengths $t = 0.107$, $df = 56$, $p > 0.05$ and March cohort lengths $t = 0.102$, $df = 56$, $p > 0.05$) (see Appendix Tables 14 – 16).

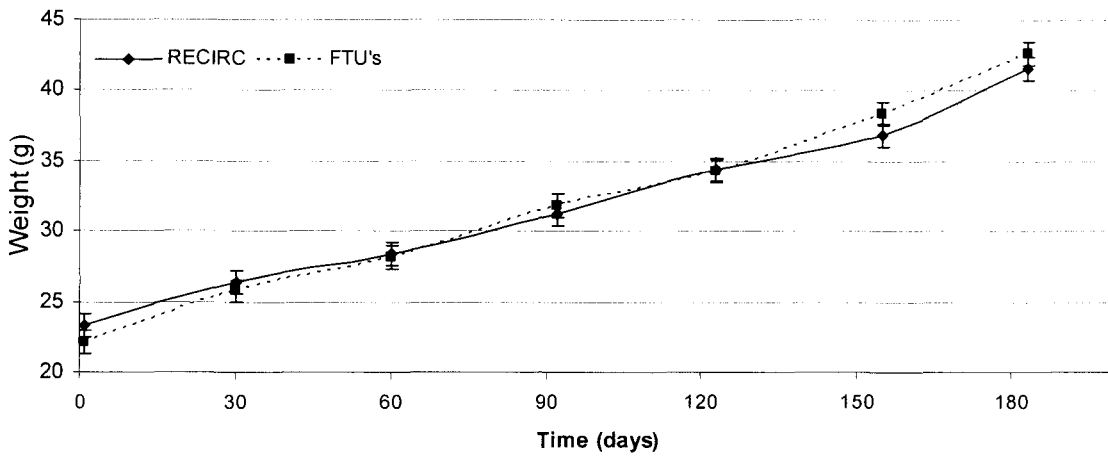


Figure 2: Average abalone weight from July to November in the Flow through units (FTUs) and Recirculation system (Recirc) (n = 180).

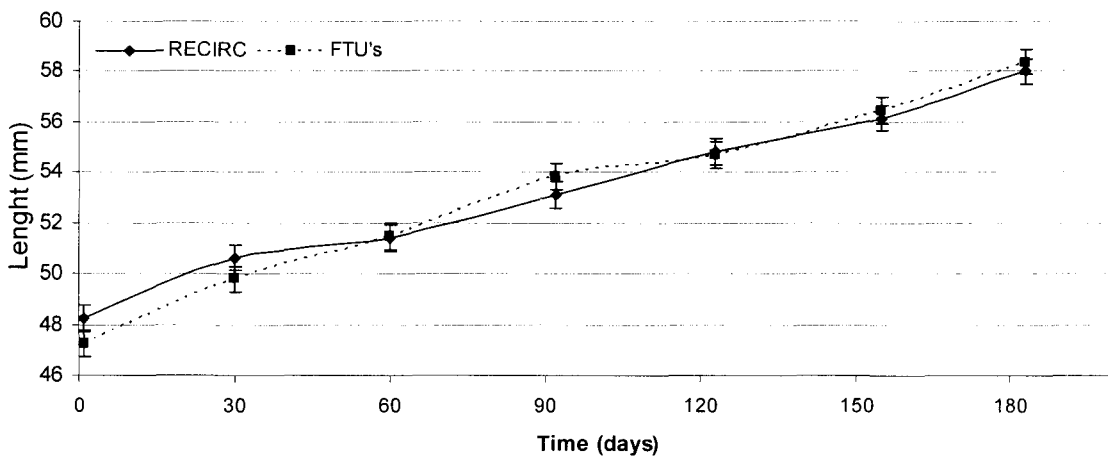


Figure 3: Average abalone length from July to November in the Flow through units (FTUs) and Recirculation system (Recirc) (n = 180).

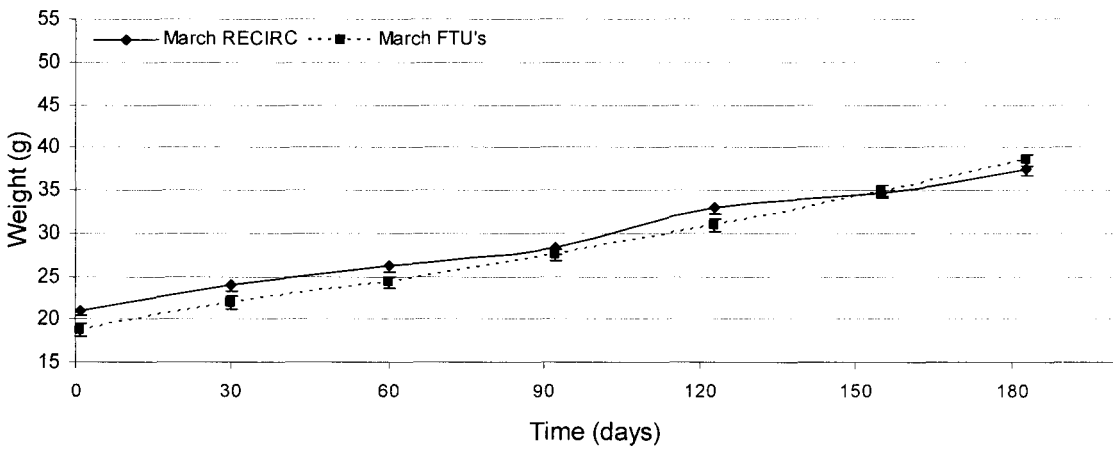
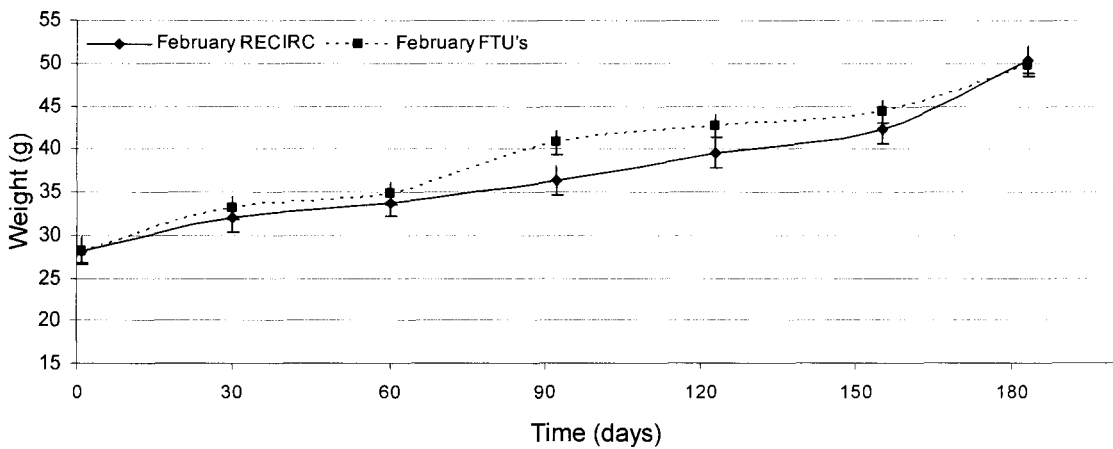
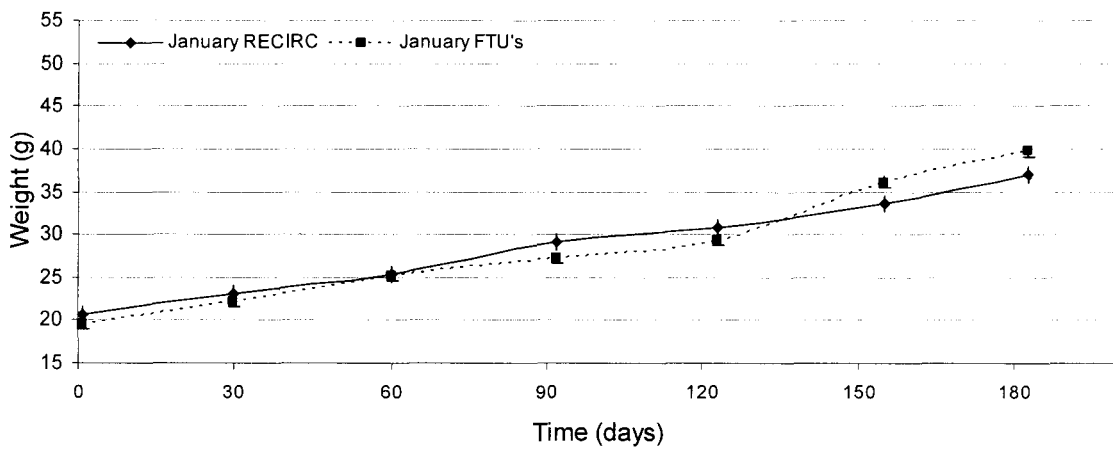


Figure 4: Average abalone weight from July to November for the three spawning cohorts in the Flow through units (FTUs) and Recirculation system (Recirc) (n = 60 each)

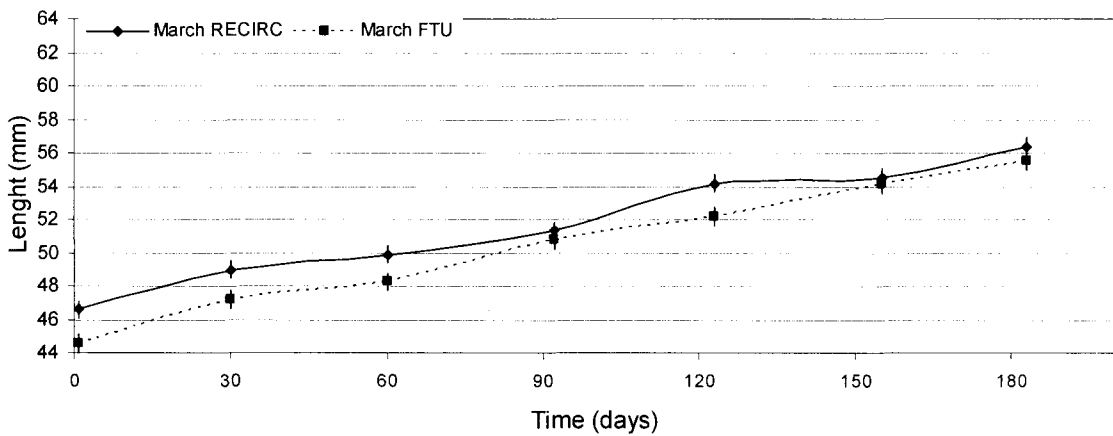
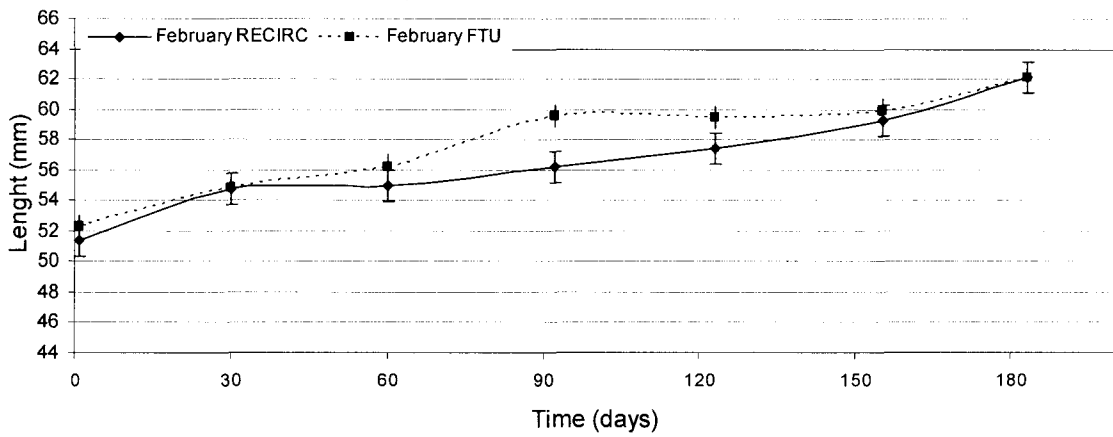
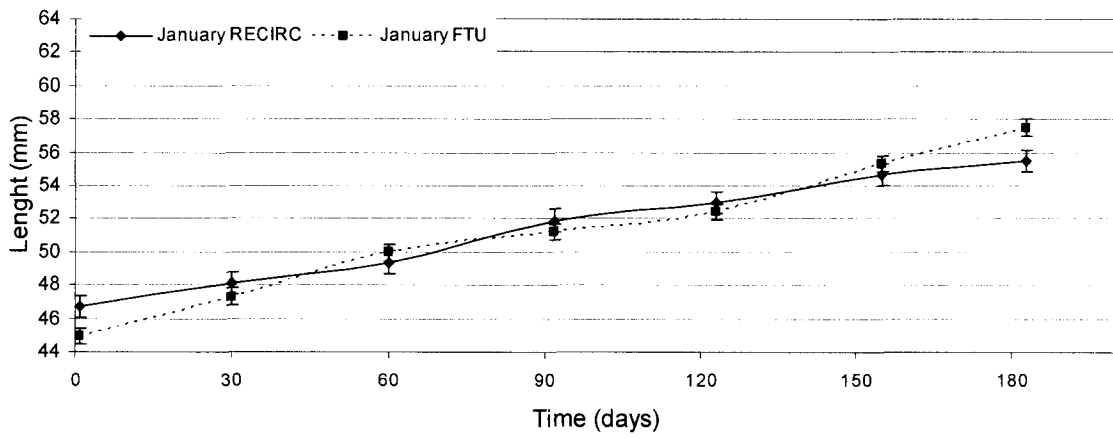


Figure 5: Average abalone length from July to November for the three spawning cohorts in the Flow through units (FTUs) and Recirculation system (Recirc) (n = 60 each)

Over the trial period the abalone in the FTUs had higher (i) mean weight gain (2.17 grams), (ii) mean shell length (1.33 mm), (iii) DISL ($7.4 \mu\text{m.d}^{-1}$) and (iiii) SGR (0.04% body weight. day^{-1}) when compared to abalone in the Recirculation system (see Table 1). Abalone in the January spawned cohort FTUs had higher (i) mean weight gain (3.68 grams), (ii) mean shell length (3.79 mm), (iii) DISL ($20.74 \mu\text{m.d}^{-1}$) and (iiii) SGR (0.065% body weight. day^{-1}) when compared to abalone in the Recirculation system (see Table 1). Abalone in the February spawned cohort Recirculation system had higher (i) mean weight gain (0.54 grams), (ii) mean shell length (0.87 mm), (iii) DISL ($4.79 \mu\text{m.d}^{-1}$) and (iiii) SGR (0.006% body weight. day^{-1}) when compared to abalone in the FTUs (see Table 1). Abalone in the March spawned cohort FTUs had higher (i) mean weight gain (3.37 grams), (ii) mean shell length (1.16 mm), (iii) DISL ($6.24 \mu\text{m.d}^{-1}$) and (iiii) SGR (0.078% body weight. day^{-1}) when compared to abalone in the Recirculation system (see Table 1).

Table 1: Growth parameters of abalone from the two treatments and spawning cohorts

Treatment: all three abalone spawning cohorts combined

		RECIRC	FTUs
Treatment	Mean initial weight (g)	23.25	22.14
	Mean final weight (g)	41.61	42.7
	Mean weight (g) gain	18.36	20.53
	Mean initial length (mm)	48.2	47.3
	Mean final length (mm)	58	58.4
	Mean length (mm) gain	9.8	11.13
	DISL	53.42	60.82
	SGR	0.318	0.358
January cohort	Mean initial weight (g)	20.59	19.6
	Mean final weight (g)	37.05	39.73
	Mean weight (g) gain	16.45	20.13
	Mean initial length (mm)	46.7	44.91
	Mean final length (mm)	55.5	57.5
	Mean length (mm) gain	8.8	12.59
	DISL	48.07	68.81
	SGR	0.321	0.386
February cohort	Mean initial weight (g)	28.17	28.1
	Mean final weight (g)	50.41	49.81
	Mean weight (g) gain	22.24	21.7
	Mean initial length (mm)	51.4	52.29
	Mean final length (mm)	62.1	62.12
	Mean length (mm) gain	10.7	9.83
	DISL	58.52	53.73
	SGR	0.318	0.312
March cohort	Mean initial weight (g)	20.99	18.73
	Mean final weight (g)	37.38	38.48
	Mean weight (g) gain	16.39	19.76
	Mean initial length (mm)	46.6	44.56
	Mean final length (mm)	56.4	55.52
	Mean length (mm) gain	9.8	10.96
	DISL	53.68	59.92
	SGR	0.315	0.393

Daily increment increase in shell length (DISL – $\mu\text{m}\cdot\text{d}^{-1}$), specific growth rate (SGR - % body weight. Day^{-1}), length and weight gained by the abalone from the two treatments (all three spawning cohorts combined) and weight and length gained by the abalone in the 3 spawning cohorts.

3.3.2 Estimated time to harvest

With all three spawned cohorts combined, the abalone in the FTUs had a shorter estimated time to harvest by 45 days when compared to abalone in the Recirculation system (see Table 2, Appendix Figure 5). The abalone in the FTUs January spawned cohort had a shorter estimated time to harvest by 63 days when compared to the abalone in the January spawned cohort in the Recirculation system. The abalone in the FTUs February spawned cohort had a shorter estimated time to harvest by 20 days when compared to the abalone in the February spawned cohort in the Recirculation system. The abalone in the March spawned cohort in the FTUs had a shorter estimated time to harvest by 63 days when compared to the abalone in the Recirculation system March spawned cohort (see Table 2, Appendix Figures 6 -8).

Table 2: Estimated time to harvest
Treatment: all three abalone spawning cohorts combined
See Appendix Figures 5 - 8

	RECIRC	FTUs
Treatment	292	247
January cohort	312	249
February cohort	257	237
March cohort	307	244

3.3.3 Condition factor

Treatment:

The abalone in the FTUs had a significantly higher CF compared to the abalone in the Recirculation system, when the three spawned cohorts were combined ANOVA: $F(1, 198) = 6.400$, $p = 0.01$ (see Table 3).

January cohort:

There were no significant differences between the CF of the abalone in the FTUs and abalone in the Recirculation system for the January spawned cohort ANOVA: $F(1, 58) = 0.001$, $p = 0.98$ (see Table 3).

February cohort:

The abalone in the FTUs had a significantly higher CF compared the abalone in the Recirculation system for the February spawned cohort ANOVA: $F(1, 78) = 6.73$, $p = 0.01$ (see Table 3).

March cohort:

There were no significant differences between CF of the abalone in the FTUs and the abalone in the Recirculation system for the March spawned cohort ANOVA: $F(1, 58) = 2.52$, $p = 0.11$ (see Table 3).

Table 3: Condition factors ($\text{g}\cdot\text{mm}^{-1}$) for the FTUs and 50 % Recirculation system and spawning cohorts (standard deviation)

	FTUs	50 % Recirculation
Treatment	1.273 (0.14)	1.226 (0.122)
January cohort	1.221 (0.122)	1.221 (0.105)
February cohort	1.322 (0.164)	1.26 (0.122)
March cohort	1.232 (0.095)	1.186 (0.126)

3.3.4 Abalone health

Both the FTUs and 50 % Recirculation system had sabellid infections. There was no significant difference between abalone sabellid scores recorded in the FTUs and 50 % Recirculation system, as identified by Dr Mouton. (see Table 2 (in chapter 2) and Table 4 below).

Abalone in both the FTUs and Recirculation system showed significant environmental stress. This is reflected in the bad condition scores and bad shell condition scores with the abalone all having poor - very poor shell conditioning (see Chapter 2, Table 2 and Table 4 below). Both treatments had immature sex cells or moderate gonad development; which was expected due to immature abalone being used in the experiment. Abalone in both the FTUs and Recirculation system had digestive gland protozoa (DGP) present, and the FTUs had a higher percentage of DGP present (13.5 %) (see Figure 6). The Recirculation system had less, with 6 % of the sample having DGP. Rickettsia-like prokaryotes were also present in the abalone in both the FTUs and Recirculation system, with the FTUs having a higher percentage of 26 %, compared to the abalone in the Recirculation system which had 7 % of the sample being infected. Gut protozoa and *Coccidia* were only present in the Recirculation system, with 7 % of the sample being infected (see Figure 6).

Table 4: Abalone health and Sabellid score (standard deviation). See Chapter 2, Table 2 for the key to interpret the results.

Treatment	Sabellid score (average)	Shell condition (average)	Gonad development (average)	Cum. Condition score (sum)	Cum. Environmental stress score (sum)
FTUs	1.5 (0.4)	2.7 (0.6)	1.2 (0.3)	5	22
Recirc	2.1 (0.8)	2.3 (0.6)	1.5 (0.0)	13	24

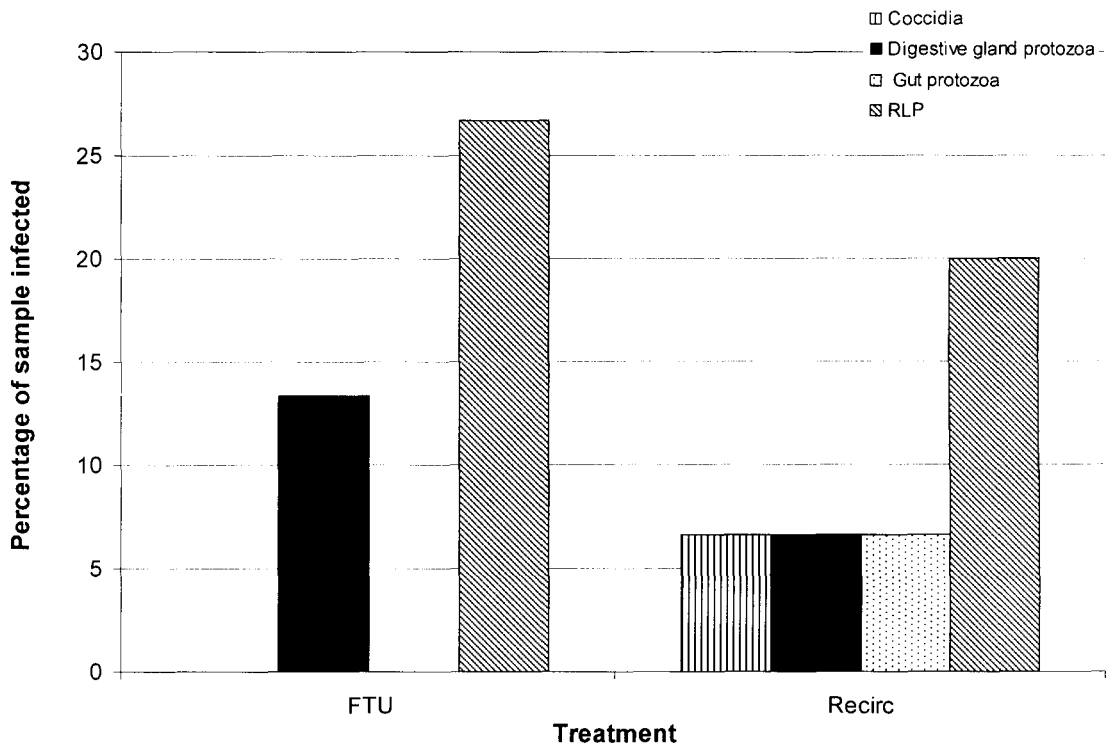


Figure 6: Digestive gland protozoa, Gut protozoa and Rickettsia-like prokaryote
 FTU - Flow through units and Recirc - 50 % Recirculation system

3.3.5 Physico-chemical variables

50 % Recirculation Dissolved Oxygen

There was no significant difference in DO concentrations between the FTUs and Recirculation system (see Table 5 and Figure 7 A). The inlet and Seaweed tanks had significantly higher DO concentrations compared to both the FTUs and 50 % Recirculation system (ANOVA $F(3, 176) = 63.775, p < 0.001$).

Table 5: The average, minimum and maximum dissolved oxygen (ppm) recorded over 72 hours from the 9 - 11 October 2006 at 50 % Recirculation

	Average	Min	Max
Inlet	5.77	5.35	6.27
Seaweeds	5.58	5.14	6.27
50 % Recirculation	5.03	4.60	5.53
FTUs	4.98	4.5	5.49

50 % Recirculation pH

There was no significant difference between the pH values in the FTUs and Recirculation system (see Table 6 and Figure 7 B). The pH in the inlet and Seaweed tanks were significantly higher than both the FTUs and the 50 % Recirculation system (ANOVA $F(3, 176) = 26.852, p < 0.001$).

Table 6: The average, minimum and maximum pH recorded over 72 hours from the 9 - 11 October 2006 at 50 % Recirculation

	Average	Min	Max
Inlet	8.27	8.02	8.54
Seaweeds	8.01	7.75	8.35
50 % Recirculation	7.87	7.62	8.15
FTUs	7.87	7.64	8.19

50 % Recirculation Temperature

There was no significant difference between the temperatures of the FTUs and Recirculation system (see Table 7 and Figure 7 C). There was no significant difference between the inlet and both the FTUs and 50 % Recirculation system. The Seaweed tanks had a significantly higher temperature compared to the inlet and the FTUs (ANOVA $F(3, 176) = 4.47, p = 0.004$ (see Figure 6 C).

Table 7: The average, minimum and maximum temperature (°C) recorded over 72 hours from the 9 - 11 October 2006 at 50 % Recirculation

	Average	Min	Max
Inlet	17.0	15.7	18.2
Seaweeds	17.7	15.9	19.8
50 % Recirculation	17.3	15.7	19.0
FTUs	17.1	15.7	18.6

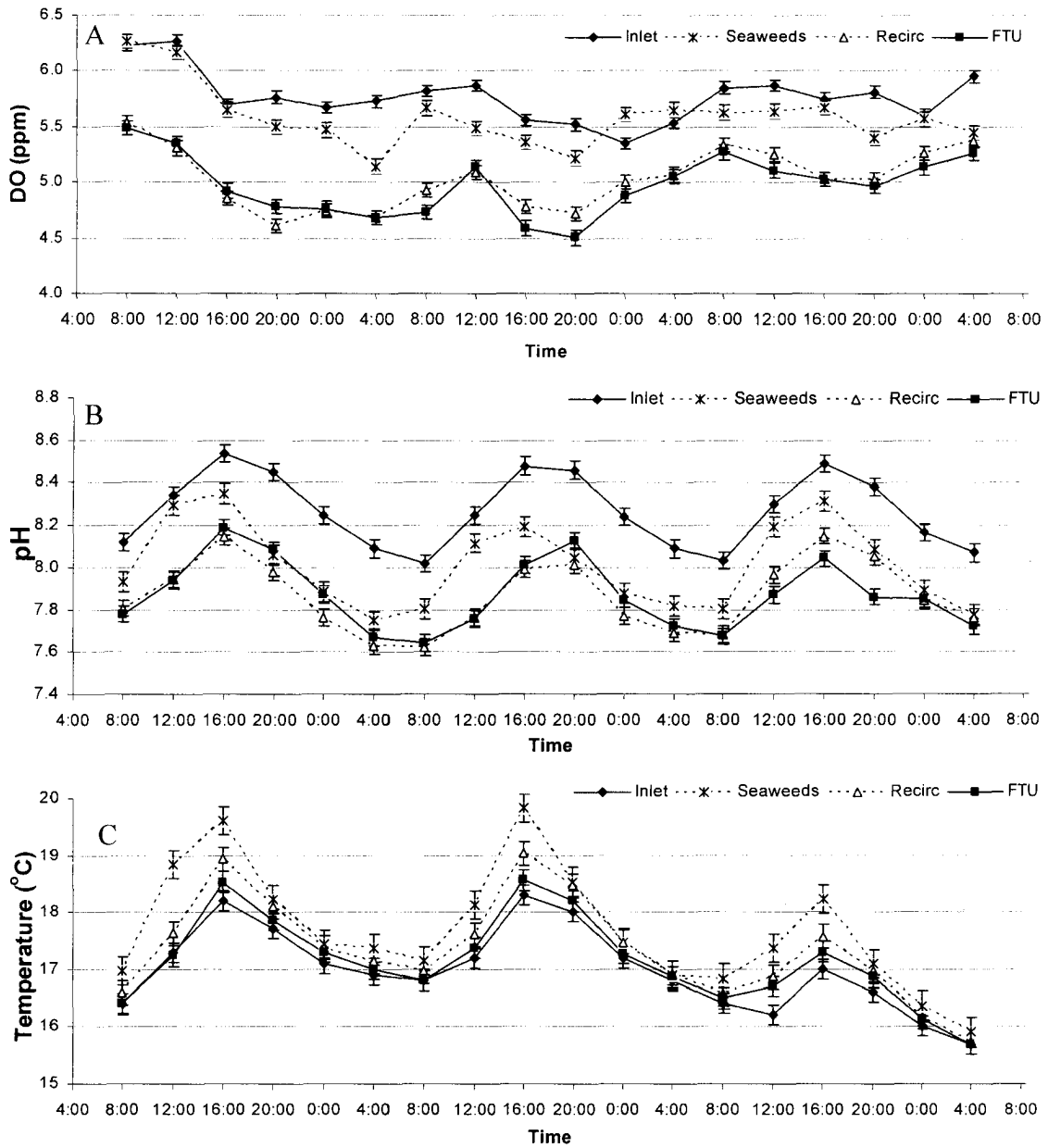


Figure 7: Physico-chemical variables recorded over a 72 hour period, at 50 % recirculation in September to illustrate diurnal variations in concentration (n = 180 each).

75 % Recirculation Dissolved Oxygen

The inlet had significantly higher DO concentrations when compared to the Seaweed tanks and both treatments. The Seaweed tanks had significantly higher DO values compared to both treatments and the FTUs had significantly higher DO values compared to the 75 % Recirculation system (ANOVA $F(3, 176) = 95.65, p < 0.001$) (see Table 8 and Figure 8 A).

Table 8: The average, minimum and maximum dissolved oxygen (ppm) recorded over 72 hours from the 10 - 12 November 2006 at 75 % Recirculation

	Average	Min	Max
Inlet	4.84	4.60	5.21
Seaweeds	4.62	4.08	4.94
75 % recirculation	3.86	3.27	4.35
FTUs	4.08	3.72	4.43

75 % Recirculation pH

There was no significant difference in pH values between the FTUs and the 75 % Recirculation system (see Table 9 and Figure 8 B). There was no significant difference in pH between the inlet water and both the FTUs and 75 % Recirculation system. There was also no significant difference between the pH in the Seaweed tanks and both the FTUs and 75 % Recirculation system.

Table 9: The average, minimum and maximum pH recorded over 72 hours from the 10 - 12 November 2006 at 75 % Recirculation

	Average	Min	Max
Inlet	7.70	6.50	8.50
Seaweeds	7.49	6.60	8.40
75 % Recirculation	7.44	6.50	8.00
FTUs	7.50	6.50	8.10

75 % Recirculation Temperature

The 75 % Recirculation system and the Seaweed tanks had a significantly higher temperature compared to the FTUs and inlet water (ANOVA $F(3, 176) = 26.835$, $p < 0.001$) (see Table 10 and Figure 8 C).

Table 10: The average, minimum and maximum temperature (°C) recorded over 72 hours from the 10 - 12 November 2006 at 75 % Recirculation

	Average	Min	Max
Inlet	14.86	13.40	16.60
Seaweeds	17.31	14.90	20.30
75 % Recirculation	16.78	13.70	19.70
FTUs	15.10	13.50	16.90

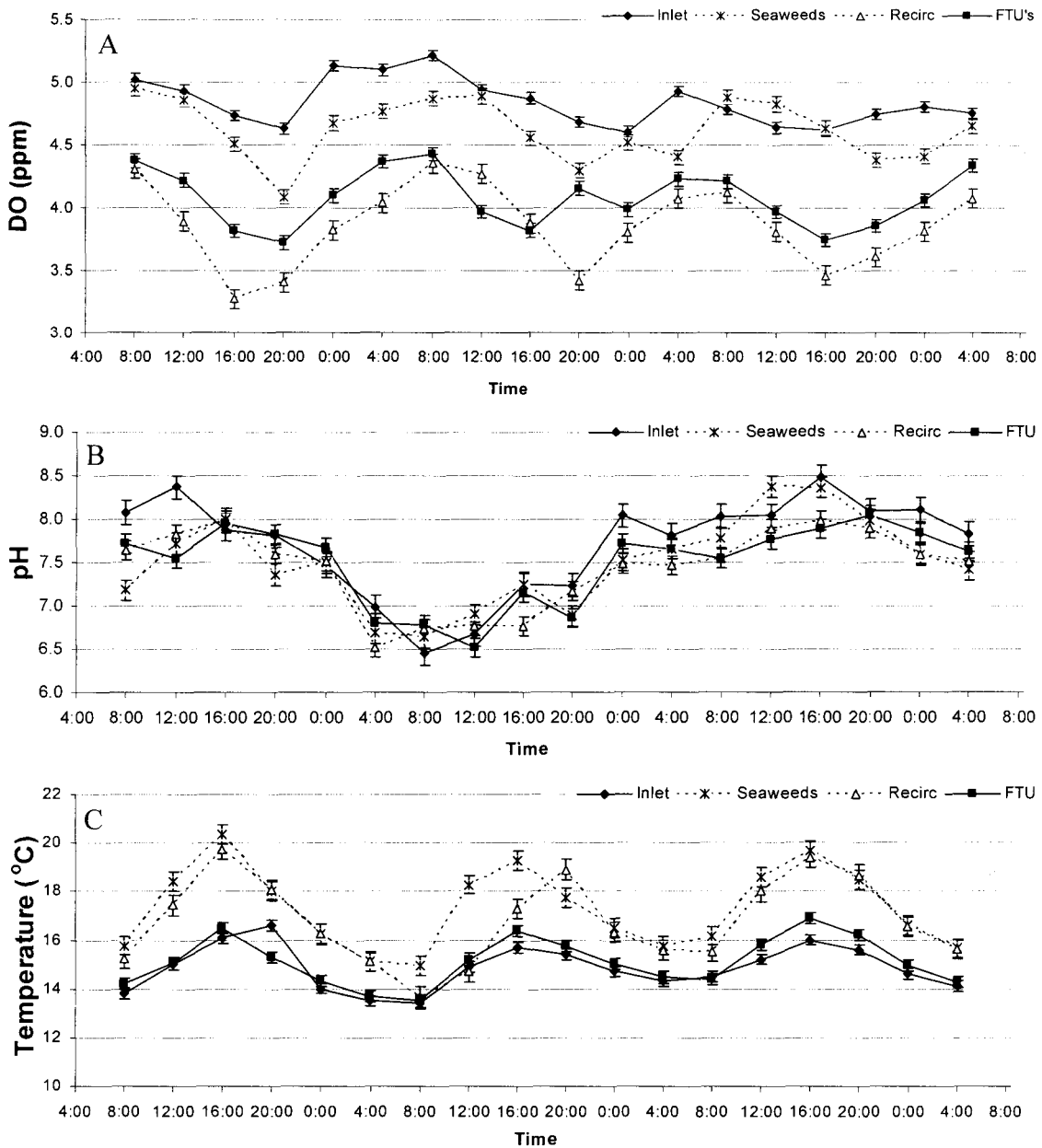


Figure 8: Physico-chemical variables recorded over a 72 hour period, at 75 % recirculation in September to illustrate diurnal variations in concentration (n = 180 each).

3.3.6 Water Nutrients

Total Ammonia Nitrogen:

The TAN concentrations were highly variable (see Table 11 and Figure 9). There was no significant difference between the FTUs and Recirculation system, in both the 50 % and the 75 % Recirculation systems ($p > 0.05$). The inlet and Seaweed tanks had significantly lower TAN concentrations for both the 50 % Recirculation system (Kruskal-Wallis test: $H(3, N = 180) = 73.408$ $p < 0.001$) and 75 % Recirculation systems (Kruskal-Wallis test: $H(3, N = 180) = 84.291$ $p < 0.001$). The TAN reached higher concentrations in the 75 % Recirculation system, which also occurred in the FTUs.

Table 11: The average, minimum and maximum TAN concentrations (μml^{-1}) recorded over two 72 hour periods. (i) The 50 % recirculation was recorded during the 9 - 11 October 2006 and (ii) the 75 % recirculation was recorded during the 10 – 12 November 2006

(i)

TAN	Average	Min	Max
Inlet	1.99	0.89	3.70
Seaweeds	1.53	0.57	2.95
50 % Recirculation	3.63	0.89	6.56
FTUs	4.26	0.17	6.57

(ii)

TAN	Average	Min	Max
Inlet	1.99	0.31	5.42
Seaweeds	1.52	0.37	5.13
75 % Recirculation	4.67	1.93	8.53
FTUs	5.10	1.63	9.35

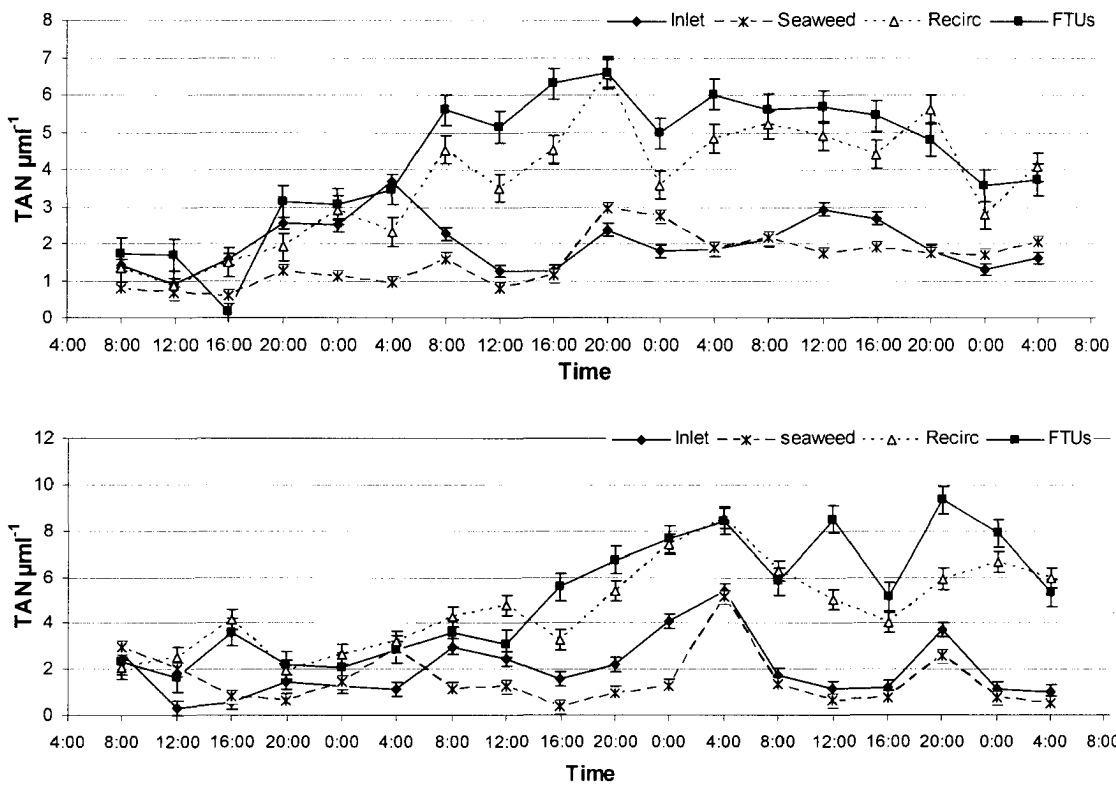


Figure 9: TAN concentrations over 72 hour period (n =162 each)

Top: 50 % recirculation recorded from the 9 – 11 October 2006

Bottom 75 % recirculation from the 10 – 12 November 2006

Maximum FAN concentrations were higher in the FTUs compared to both the 50 % and 75 % Recirculation systems (see Table 12).

Table 12: Maximum FAN concentrations $\mu\text{m l}^{-1}$

	Seaweeds	Recirculation	FTUs
50%	0.076	0.170	0.588
75%	0.073	0.112	0.261

Phosphate concentrations:

There was no significant difference between the phosphate concentrations recorded in the FTUs and 50 % Recirculation system (see Table 13 and Figure 10). The phosphate concentrations were significantly higher in the Seaweed tanks and 75 % recirculating abalone tanks when compared to the inlet and FTUs (Kruskal-Wallis test: $H(3, N = 170) = 23.41$ $p < 0.001$).

Table 13: The average, minimum and maximum phosphate concentrations (μml^{-1}) recorded over two 72 hour periods. (i) The 50 % recirculation was recorded during the 9 -11 October 2006 and (ii) 75 % recirculation was recorded during the 10 – 12 November 2006

(i)

Phosphate	Average	Min	Max
Inlet	3.05	1.05	7.38
Seaweeds	3.41	1.04	7.48
50 % Recirculation	3.57	1.22	7.68
FTUs	3.20	0.90	6.80

(ii)

Phosphate	Average	Min	Max
Inlet	2.72	1.29	4.46
Seaweeds	4.41	1.74	8.52
75 % Recirculation	4.59	2.24	8.48
FTUs	3.17	1.33	5.89

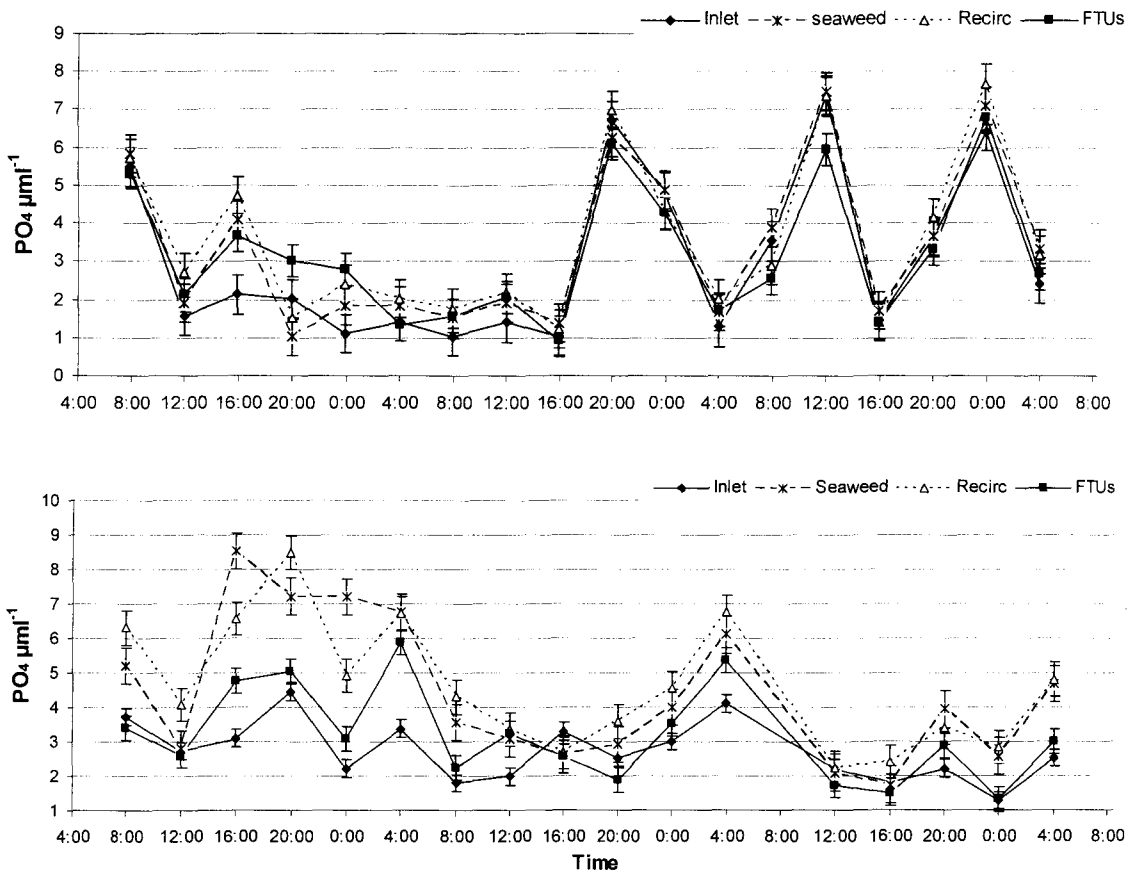


Figure 10: Phosphate concentrations over 72 hour period (n =162 each)
 Top: 50 % recirculation
 Bottom 75 % recirculation

Nitrate concentrations

There was no significant difference between the nitrate concentrations in the FTUs and the 50 % Recirculation systems (see Figure 11 and Table 14). The Seaweed tanks had significantly lower nitrate concentrations compared to the inlet water, the FTUs and the abalone tanks in the 50 % Recirculation system (ANOVA $F(3, 155) = 16.36, p = 0.00$). In the 75 % Recirculation system the Seaweed tanks and the recirculating abalone tanks had significantly lower nitrate concentrations compared to the FTUs and the inlet water. The

Seaweed tanks had a significantly lower nitrate concentration compared to the 75 % Recirculation system. The Seaweed tanks had extremely low values for nitrate ($< 0.5 \mu\text{ml}^{-1}$) (Kruskal-Wallis test: $H(3, N = 167) = 144.902, p < 0.001$).

Table 14: The average, minimum and maximum nitrate concentrations (μml^{-1}) recorded over two 72 hour periods. (i) The 50 % recirculation was recorded during the 9 - 11 October 2006 and (ii) 75 % recirculation was recorded during the 10 – 12 November 2006

(i)				(ii)			
Nitrate	Average	Min	Max	Nitrate	Average	Min	Max
Inlet	5.48	1.75	13.34	Inlet	9.80	7.04	12.56
Seaweeds	2.20	0.40	7.69	Seaweeds	0.40	0.21	0.81
50 % recirculation	4.52	1.32	13.15	75 % recirculation	2.96	2.25	4.17
FTUs	5.42	1.89	12.95	FTUs	9.54	6.74	11.58

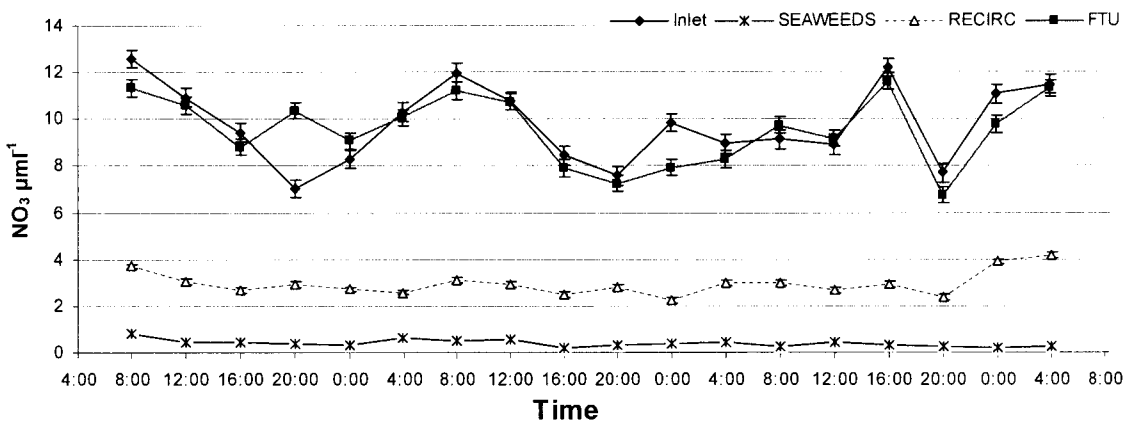
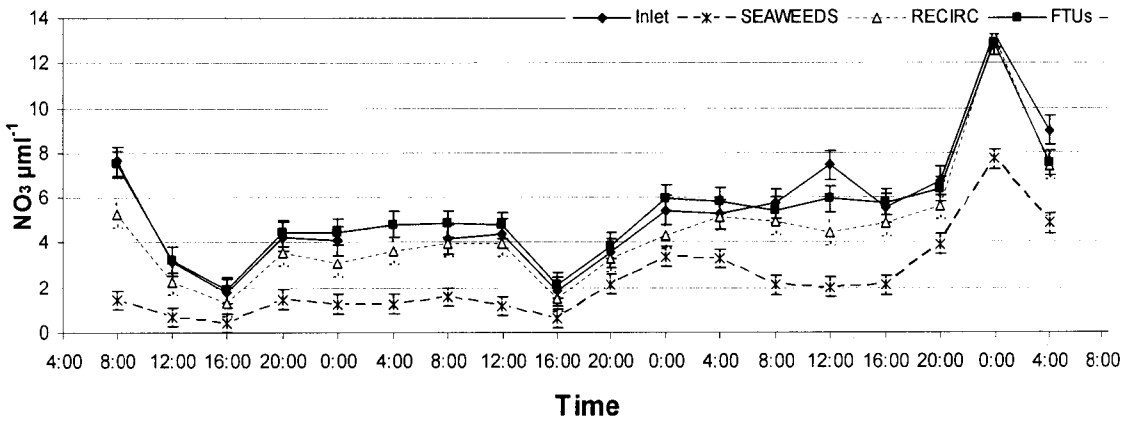


Figure 11: Nitrate concentrations over 72 hour period (n =167 each)
 Top: 50 % recirculation
 Bottom 75 % recirculation

Seaweed nutrient uptake rates for the 50 % and 75 % Recirculation systems are shown in Table 15.

Table 15: The percentage nutrient uptake recorded over 72 hours by the Seaweed tanks at (i) 50 % (9 - 11 October 2006) and (ii) 75 % recirculation (10 – 12 November 2006)

(i)				(ii)			
50 %	Average	Min	Max	75 %	Average	Min	Max
Nitrate	65.92	43.42	80.81	Nitrate	95.51	92.82	97.34
TAN	43.31	-246.15	85.03	TAN	63.15	27.34	93.39
Phosphate	-5.81	-51.24	65.3	Phosphate	-36.25	-133.42	5.66

Negative values indicate production

3.3.7 Long term physico-chemical variables

Long term physico-chemical variables are shown in Figures 12 - 14. These show the average daily DO, pH and temperature contents in the Seaweed tanks, FTUs and the 50 % recirculating abalone tanks.

Dissolved oxygen in the recirculating abalone tanks, was on average 1.89 % (± 0.02) higher than the FTUs. The DO was on average 19.8 % (± 0.03) higher in the Seaweed tanks than the recirculating abalone tanks (see Figure 12).

pH in the recirculating abalone tanks, was on average 0.84 % (± 0.004) higher than the FTUs. The pH was on average 0.36 % (± 0.003) higher in the Seaweed tanks than the recirculating abalone tanks (see Figure 13).

Temperature in the recirculating abalone tanks, was on average 0.54 % (± 0.01) higher than the FTUs. The temperature was on average 0.23 % (± 0.01) higher in the Seaweed tanks than the recirculating abalone tanks. Temperatures in the header tank was on

average 7.3 % (± 0.02) lower than the Recirculation system and 6.7 % (± 0.03) lower than the FTUs (see Figure 14).

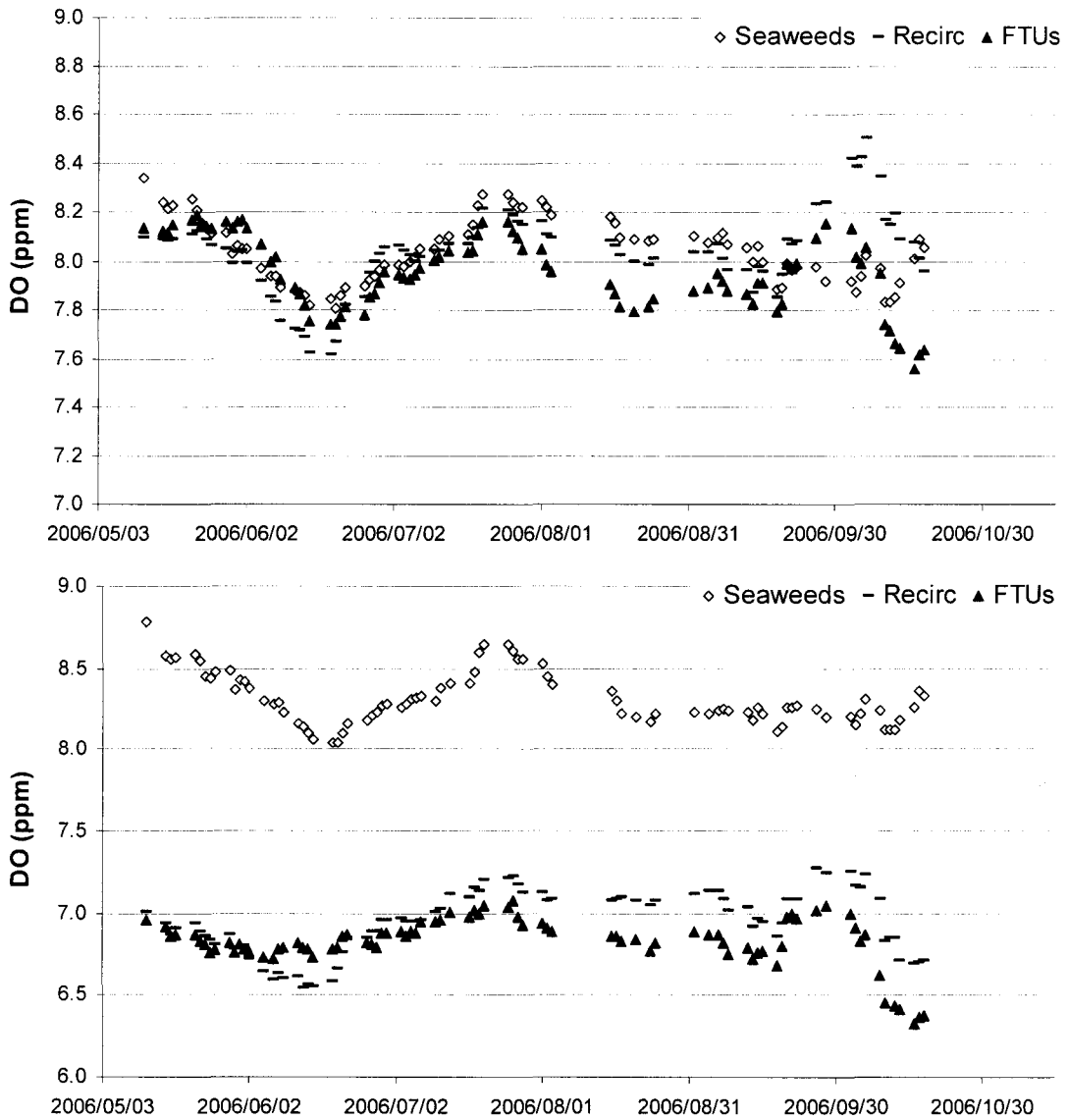


Figure 12: Dissolved Oxygen 10 Point running mean from May to November 2006

Top: Dissolved Oxygen from the inlet of the tank recorded at 50 % recirculation.

Bottom: Dissolved Oxygen from the Outlet of the tank recorded at 50 % recirculation.

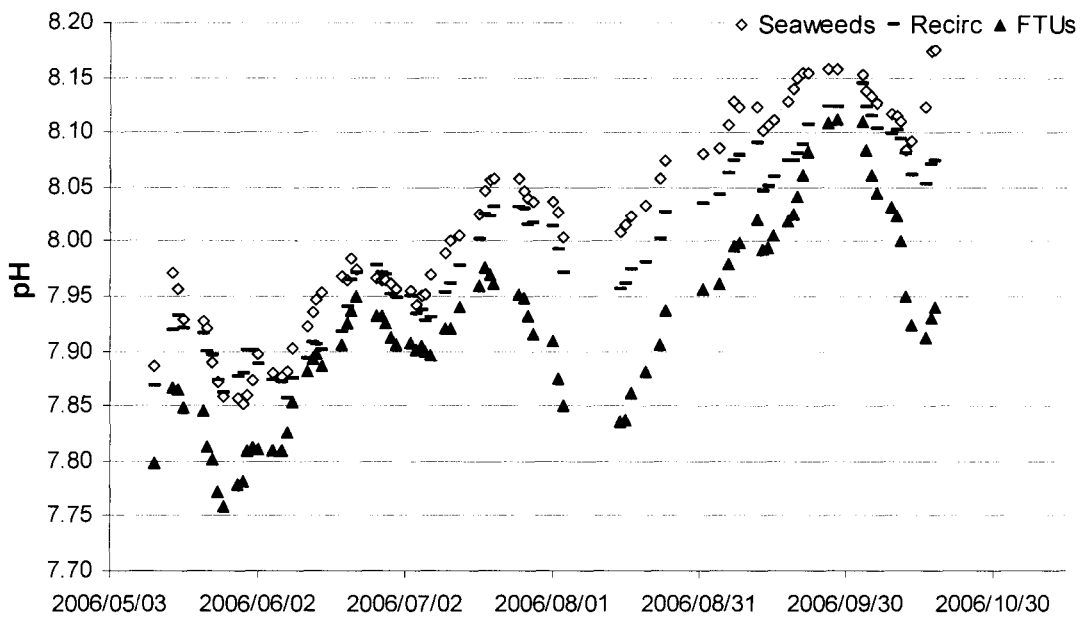


Figure 13: pH 10 Point running mean from May to November 2006
Recorded at 50 % recirculation

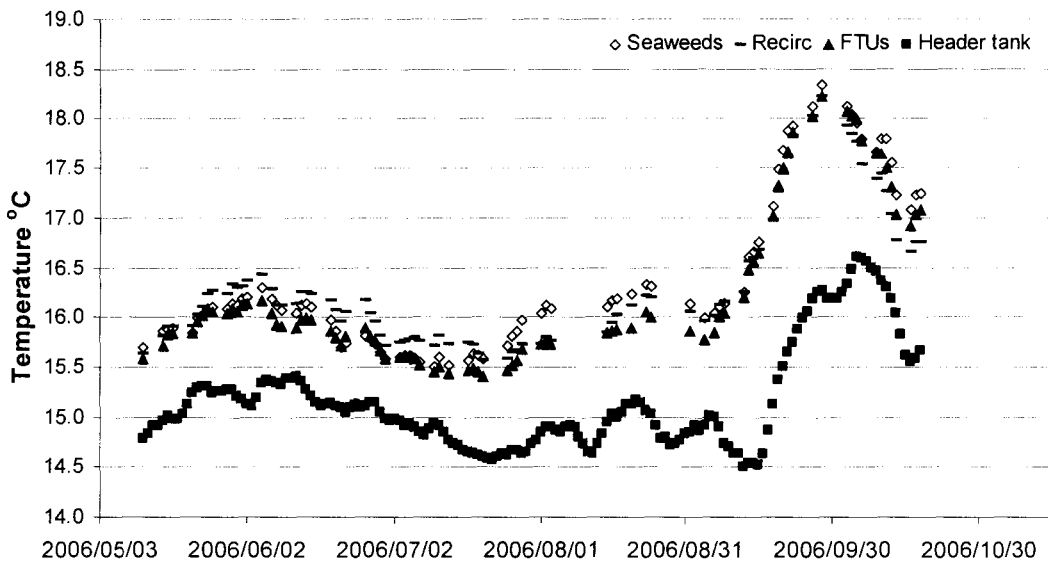


Figure 14: Temperature 10 Point running mean from May to November 2006
Recorded at 50 % recirculation.

3.3.8 Sediments

There was no significant difference between the FTUs and 50 % Recirculation systems water column sediments and bottom sediment accumulation ($p > 0.05$) (see Table 16 and 17). There was also no significant difference between the sediments in the 75 % Recirculation system and FTUs in both the water column and bottom sediment accumulation (see Table 16 and 17) ($p > 0.05$).

Table 16: The average water column sediment (g l^{-1}) at 50 % and 75 % Recirculation systems (standard deviation)

	Seaweeds	Recirculation	FTUs
50 % recirculation	0.6 (0.35)	1.1 (0.44)	0.97 (0.43)
75 % recirculation	1.02 (0.40)	1.02 (0.20)	1.2 (0.14)

Table 17: The average bottom sediment accumulation (g l^{-1}) at 50 % and 75 % Recirculation systems (standard deviation)

	Recirculation	FTUs
50 % recirculation	2.52 (0.17)	2.73 (0.66)
75 % recirculation	1.36 (0.47)	1.98 (0.57)

3.4 Discussion

3.4.1 Growth

The ANOVA of the growth data at the end point of the experiment failed to show any significant differences between abalone weights and lengths in the 50 % Recirculation system and FTUs for the treatments or for the three spawning cohorts. Testing for differences between the two population regression coefficients (ANCOVA's) also showed no significant differences between the weights and lengths of abalone in the 50 % Recirculation system and FTUs for both the treatments and spawning cohorts. There were therefore no significant differences between abalone growth in terms of weight and length in the FTUs and 50 % Recirculation system, even though the abalone in the FTUs had a higher SGR (0.04 % body weight. day⁻¹), higher DISL (DISL 7.4 $\mu\text{m}\cdot\text{d}^{-1}$) and shorter estimated time to harvest (45 days) (see Table 1 and Table 2).

Fleming et al. (1996) and Mai et al. (2001) state that most abalone farmers would be satisfied with a growth of 2 – 3 mm per month and the I & J farm strives for a growth rate of 2 mm per month. The abalone length increase per month for this project was 1.85 mm per month for the FTUs and 1.63 mm per month for the 50 % Recirculation system. Abalone in Robertson-Andersson's (2007) study had monthly length increments of 1.83 mm for a 50 % Recirculation system and 1.69 mm for the FTUs fed a kelp diet. As both studies failed to reach the goal of 2 mm per month, there may have been factors other than the diet and recirculation rate that were limiting growth in the I & J system.

3.4.2 Condition Factor

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). Abalone that have a high condition factor tend to be meaty and possess relatively short shells, reflecting that more nutrients were invested into body weight than into shell growth. Abalone in both the FTUs and the 50 % Recirculation system had CF higher than one, indicating that more feed was invested into body weight gain compared to shell length increase, which is desirable for the abalone market. Abalone in the FTUs had a significantly higher CF compared to abalone in the 50 % Recirculation system, indicating that the abalone in the FTUs were investing more into body weight gain, than abalone in the 50 % Recirculation system (see Figure 3).

3.4.3 Abalone Health

There was no significant difference between the sabellid scores present in the FTUs and Recirculation system (see Table 4). Therefore a recirculation ratio of 50 % did not have any effect on sabellid numbers in the I & J system. There was a greater diversity of parasites in the Recirculation system with a lower overall infection rate (see Figure 6). *Coccidia* infections were only recorded in the Recirculation system; this infection is believed 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). The abalone health results obtained in this study are a once off snapshot with very low percentage coverage

(0.01 %) and thus a larger sample for abalone health could possibly indicate different infection rates. The percentage sampling coverage for abalone health on the farm is far lower than that of the current study, with approximately 60 animals being sampled for the entire farm at each sampling period. Thus, we were satisfied with the health results reported here and feel that they represent a true reflection of abalone health in the tested systems. Due to the high value of abalone and the destructive nature of the test, it is impractical to take larger samples of abalone.

Rickettsia-like prokaryotes have been reported in the digestive tract of abalone (*H. midae*) from culture facilities in South Africa with no associated pathology (Mouton, 2000a, b). The RLP was present in both the FTUs and the Recirculation system, with the FTUs having a higher percentage of infection. *Rickettsia* elsewhere in the world has been shown to cause a lethal disease that affects all sizes of abalone and causes lethargy, retracted visceral tissues and atrophy of the foot muscle (Friedman, et al., 2003; Mouton, 2006). At present as there has been no associated pathology in *H. midae*, and the prevalence of high RLP counts is not cause for concern (Mouton, 2006). Abalone in both the FTUs and the Recirculation system showed significant environmental stress. This may be the cause of the less than optimal length increments recorded in this study and previous studies in the I &J system.

3.4.4 Physico-chemical variables

Dissolved Oxygen

The DO in both the FTUs and Recirculation system was lower than that of the inlet water and Seaweed tanks as expected, due to the respiring abalone in the FTUs and recirculating abalone tanks, and the seaweeds producing oxygen in the Seaweed tanks during the day. There was no significant difference between the DO concentrations recorded in the FTUs and 50 % Recirculation system (see Figures 7A). This indicates that although there is oxygen being produced in the Seaweed tanks, the oxygen production was not high enough to increase the DO in the recirculating abalone tanks. The most likely reason for this is the small size of the Seaweed tanks in comparison to the recirculating abalone tanks. At the higher 75 % recirculation rate the Recirculation system had significantly lower DO compared to the FTUs, the Seaweed tanks also had significantly lower DO compared to the inlet (see Figure 8 A), dropping to minimum of 3.27 (which is lower than the targeted DO rang 4 – 9 ppm of the I & J farm). The drop in DO was probably caused by increased water temperatures. This indicates that a 75 % recirculation rate will not be suitable at the temperatures recorded in this study (16.78 – 19.70 °C). The drop in DO could be a contributing factor to the high levels of stress recorded in both the Recirculation system and FTUs (see Table 4). The long term DO data recorded in Figure 12 show the same trends as above, with there being little DO transfer between the Seaweed tanks and the 50 % recirculating abalone tanks.

pH

The average pH for the 50 % Recirculation system fell within the targeted pH range of the I & J farm (7.5 - 8.2) (Farm operating procedures). The pH of the 75 % Recirculation system had values that exceeded target values, but this occurred in both the Recirculation system and the FTUs (see Figures 7 and 8). The increase in pH was therefore not due to the rate of recirculation, but was influenced by other factors such as water temperature. An increase in pH affects the equilibrium of TAN and FAN, producing more FAN at lower pHs, which is toxic to abalone. This, however, was not a problem in either the 50 % or 75 % Recirculation systems as the FAN concentrations recorded were far lower than safe level of $7.4\mu\text{gl}^{-1}$ established for cocktail abalone (5 - 8 cm shell length) by Reddy-Lopata et al. (2006), and therefore the low pH's should have little effect on abalone growth rates. Long term pH reading taken daily from May to November 2006 by the farm staff also fell within the targeted pH range of I & J farm (see Figure 13). There is a gradual increase in pH recordings from May to November that corresponds with the temperature increase over the time period (see Figures 13 and 14).

Temperature

The temperature in the Seaweed tanks was significantly higher than that of the 50 % recirculating abalone tanks, but there was no significant difference between the temperatures of the FTUs and the 50 % recirculating abalone tanks (see Figure 7). This indicates there was little heat transfer from the Seaweed tanks to the 50 % Recirculating

abalone tanks. Temperature in the 75 % Recirculation system was significantly higher than the temperatures in the FTUs, indicating that there is better heat transfer and retention with the 75 % Recirculation system when compared to the 50 % Recirculation system (see Figures 7 and 8). The long term data showed little difference 0.54 ± 0.01 % (0.09 °C) between the temperatures of the 50 % Recirculation system and the FTUs (see Figure 12). The lack of heat transfer from the Seaweed tanks to the abalone tanks may have been caused by the smaller size of the Seaweed tanks in comparison to the abalone tanks (abalone tanks = 6.6 m long x 2.08 m wide x 0.88 m deep; $\pm ca$ 12 000 L; Seaweed tanks = 5 m x 1 m surface area and 0.6 m deep; $\pm ca$ 3 000 L) or the lack of insulation in the Seaweed tanks. The Seaweed tanks would therefore build up heat faster during the day, and would lose heat faster at night, in comparison to the abalone tanks. Similar trends were documented by Robertson-Andersson et al. (2008). Since the termination of this experiment, I & J has built larger seaweed raceways, and have used a 50 % recirculation rate. I & J have reported better heat retention in the larger system and better DO transfer than was recorded in this system (N. Loubser, I & J manager pers. comm).

3.4.5 Water Nutrients

A maximum of 85.03 % of the TAN was taken up by the Seaweed tanks in the 50 % Recirculation system and a maximum TAN of 93.39 % was removed by the Seaweed tank in the 75 % Recirculation system (see Tables 14 and 15); these values are close to the maximum ammonium uptake of 90 % reported in previous studies (see Cohen & Neori, 1991; Neori et al., 1991, 1996, 2000; Jimenez del Río et al., 1996; Buschmann,

1996). Even though there was a maximum TAN uptake of 85.03 in the 50 % Recirculation system and 93.39 % in the 75 % Recirculation system, there were no significant differences between the TAN concentrations of the FTUs and the 50 % and 75 % Recirculation systems. This indicates that the seaweeds are effective biofilter with respect to TAN removal from both the 50 % and 75 % Recirculation systems. 'Ammonia is highly toxic to the South African abalone, *H. midae*, but this sensitivity decreases with size (age). Abalone can adapt to sub-lethal levels of ammonia, and such acclimatization reduces their ammonia sensitivity. Sub-lethal levels of ammonia cause, however, a substantial reduction in growth' (Reddy-Lopata et al., 2006). Reddy-Lopata et al. (2006) showed that it is essential to keep ammonia levels low for animal health, and viability of abalone farming, and suggested a safe FAN level for *H. midae* of below 7.4 $\mu\text{g l}^{-1}$. The FAN concentrations recorded in this experiment fall within the recommended safe concentrations established by Reddy-Lopata et al. (2006) and it can therefore be deduced that FAN concentration would have little effect on abalone growth in either the 50 % or 75 % Recirculation systems (see Table 12).

There were no significant differences between the phosphate concentrations recorded between the 50 % Recirculation systems and the FTUs. (see Figure 10). The fluctuations in phosphate concentrations in the 50 % Recirculation system are most likely due to fluctuations external to the system, as the fluctuations occurred in the inlet water. It was noted that the timing of the high and low phosphate levels recorded in the experiment corresponded to the spring tides which occurred during the sampling period (SATT, 2006). A potential explanation for this pattern may be the large quantities of birds which excrete onto the rocks surrounding the intake pump. The spring high tides may have

washed the guano into the water, from which the farm draws its water source, resulting in higher phosphate values. A similar phenomenon has been noted with temperature, on hot sunny days the rocks heat up at low tide and at high tide, the incoming water is warmed by up to 5°C (N. Loubser, I & J manager pers. comm.). There is more phosphate retention in the 75 % Recirculation system which is indicated by the significantly higher phosphate levels in the Seaweed tanks and recirculating abalone tanks. This indicates that during this study it was not possible to demonstrate phosphate uptake by the seaweeds (see Table 15).

There were no significant differences in the nitrate concentrations between the FTUs and 50 % Recirculation system. The nitrate concentration in the Seaweed tanks were significantly lower than in the 50 % recirculating abalone tanks, indicating that the Seaweed tanks are taking up the available nitrate (average of 66 %). In the 75 % Recirculation system the nitrate concentrations were significantly lower than those recorded in the FTUs. The nitrate concentrations in the Seaweed tanks were significantly lower than abalone tanks in the 75 % Recirculation system, indicating that the Seaweed tanks were taking up the majority (up to 95.5 %) of the nitrate in the Recirculation system, and may actually be nitrogen limited, evident by the low nitrate levels in the Seaweed tanks (see Table 15).

High variability in nutrients in the inlet seawater has been shown by Potgieter (2005), Lindström (2006), Robertson-Andersson (2007), and the current study (with the inlet water having TAN concentrations ranging from 0.72 - 5.42 $\mu\text{mol.l}^{-1}$, phosphate concentrations ranging from 0.08 - 7.38 $\mu\text{mol.l}^{-1}$ and Nitrate concentrations ranging from 0.92 - 13.3 $\mu\text{mol.l}^{-1}$) (see Table 18). The nutrients recorded in the inlet water in the

current study were higher than those in the previous studies. The TAN for the three previous studies had a range of 0.72 – 2.7, compared to 0.31 – 5.42 in this study. The phosphate in the previous studies had a range of 0.08 – 1.8, compared to the 1.05 – 7.38 in this study. The nitrate recorded in the three previous studies had a range of 0.92 – 9.31, compared to the 1.75 – 13.34 in this study. The lower ammonium and nitrate concentrations in all six studies under recirculation indicates that significant biofiltration by the seaweeds is occurring (see Table 18). Recirculation has the effect of increasing phosphate concentrations to above those in the incoming seawater, in all six studies (see Tables 13, 15 and 18).

TABLE 18: Nutrient concentration ranges from 5 studies using different recirculation rates. The first was in September 2003, the second in September 2004, the third and fourth in September 2005 and fifth October 2006 and sixth November 2006. Concentrations are shown for a flow-through units (FTUs), recirculation abalone units, seaweed tanks and incoming seawater for comparison (n = 3). Table adapted from Robertson-Andersson (2007).

	Robertson-Andersson (2007) September 2003 25 % $\mu\text{mol.l}^{-1}$	Potgieter (2005) September 2004 25 % $\mu\text{mol.l}^{-1}$	Lindström (2006) September 2005 25 % $\mu\text{mol.l}^{-1}$	Lindström (2006) September 2005 75 % $\mu\text{mol.l}^{-1}$	This study October 2006 50 % $\mu\text{mol.l}^{-1}$	This study November 2006 75 % $\mu\text{mol.l}^{-1}$
TAN						
Seaweeds	0.01 - 1.6		0.03 - 3.84	0.17 - 0.92	0.57 - 2.9	0.37- 5.13
Recirc. Units	0.5 -3.4	0.8 - 4.1	0.17 - 1.55	0.59 - 1.22	0.86-6.56	1.93- 8.53
FTUs	0.8 - 4.6	1.2 - 3.1	0.44 - 7.7	0.54 - 1.03	0.17 -6.57	1.63- 9.35
Seawater	2.0 - 2.7	1.1 - 1.8	0.72 -1.82	0.83 - 1.45	0.89 - 3.70	0.31- 5.42
PO₄						
Seaweeds	0.7 -2.0		1.06 - 2.19	4.10 - 6.64	1.04-7.48	1.74-8.52
Recirc. Units	0.8 -1.5	1.8 - 2.2	1.67 - 2.43	4.3 - 6.7	1.22- 7.68	2.24-8.48
FTUs	0.1 - 2.0	1.75 - 2.0	0.82 -1.58	4.28 - 6.59	0.90- 6.80	1.33 -5.89
Seawater	0.08 - 1.53	1.1 - 1.8	0.32 - 0.84	0.17 - 0.83	1.05- 7.38	1.29- 4.46
NO₃						
Seaweeds	0.27 - 0.72		0.15 - 5.38	0.01 - 0.36	0.40-7.69	0.21-0.81
Recirc. units	0.22 -1.6		2.01 - 4.52	0.98 - 1.50	1.32-13.15	2.25-4.17
FTUs	1.79 - 5.56		3.63 - 7.57	3.99 - 6.45	1.89-12.95	6.74-11.58
Seawater	0.92- 5.71		3.77 - 9.31	4.96 - 6.73	1.75-13.34	7.04-12.56

3.4.6 Sediments

The suspended particle concentration in the water column was not significantly different between FTUs and either the 50 % and 75 % Recirculation systems (see Table 16). This suggests that the water column has reached loading capacity. A possible reason for this may be due to aeration and flow rates in the tanks being equal, and consequently the water motion (velocity) within the tanks was similar (Robertson-Andersson, 2007). There were also no significant differences between the FTUs bottom sediment accumulation and both the 50 % and 75 % Recirculation systems (see Table 17). This is a good indication that the weekly cleaning cycles are enough to remove the sediment accumulations. This study showed that higher recirculation rates do not affect sediment loadings. Similar results were found by Brandt (2006), using the same system, feeding a kelp diet with 25 %, 50 % and 75 % Recirculation systems.

3.5 Conclusion

This experiment showed that it would be suitable to grow abalone using a 50 % Recirculation system fed a mixed kelp and Abfeed[®] K26 diet, as there were no significant differences between the growth rates of the abalone in terms of weight and length in the FTUs and 50 % Recirculation system. There were also no significant differences in terms of the following factors: water nutrients, physico-chemical variables, sediment accumulation and abalone health. In the 75 % Recirculation system, the only worrying

factor is the low DO that was recorded during the experiment. If it is possible to increase the dissolved oxygen levels, the 75 % Recirculation system would also be a viable option.

Chapter 4

General discussion

The research from this thesis showed that

- (1) Changing the ratio of the two most popular feeds (*Ecklonia maxima* and Abfeed[®] K26) had little effect on the growth rates of abalone.
- (2) The increase ratio of Abfeed[®] K26 in the diet also had no effects on the culture environment.

The South African abalone industry is predicted to double in the next 5 years (Robertson-Andersson, 2007). In 2007 kelp use for abalone was 5 305 707 tons wet weight and in 2008 increased to 5 429 279 tons wet weight (data courtesy of MCM). In order for the abalone industry to continue to grow, other sources of feed needed to be used. It is therefore important to continually research different aspects of the abalone diet to ensure optimal growth rates and reduce the pressure on the kelp resource.

This thesis has proven that increased levels of Abfeed[®] do not significantly affect the growth rates of abalone. It is therefore possible for the abalone industry to continue to grow at the current rate and at the same time reduce the dependence on the kelp resource by increasing the level of Abfeed[®] in the diet.

Recommendations for the farm would be to use the cheapest of the three diets, as there was no significant difference in terms of growth and effects to the culture environment. This would need to be assessed according to current Abfeed[®] and kelp prices.

In order to encourage growth in aquaculture, it has been given high priority by the South African Government, with a new policy for marine aquaculture gazetted on 7 September 2007 (Anon, 2007). In this policy document, proposed research and technology development programmes include: abalone culture support programme, integrated aquaculture, seaweed research and nutrition (Anon, 2007). This thesis falls in line with government objectives as it researches aspects of integrated aquaculture.

The second component of the experiment demonstrated that it would be suitable to grow abalone in a 50 % Recirculation system with a kelp and Abfeed[®] K26 diet, as there were no significant differences between the FTUs and 50 % Recirculation system in terms of the following factors: abalone growth, water nutrients, physiochemical variables, sediment accumulation and abalone health.

For the 75 % recirculation system, the only worrying factor is the low dissolved oxygen that was recorded during the experiment, if it possible to improve the dissolved oxygen levels, the 75 % recirculation would be also be viable option. The other option would be to reduce the recirculation rate at higher temperatures.

Recommendations for the farm would be to use the 50 % Recirculation system as it suitable to use for *H. midae* farming. The 75 % Recirculation system would also be suitable, with constant monitoring of the dissolved oxygen in the system.

Modern fish mariculture is increasingly criticised for its non-sustainability (Neori et al., 2007) as it is almost exclusively practiced in flow-through systems, discharging effluents

without pollutant removal (Naylor et al., 1998). Recirculating aquaculture systems can overcome many of aquaculture's economic and environmental limitations as they combine good regulation of the water quality characteristics with high fish yields, low water use and minimal nutrient export (Neori et al., 2007). The current study has similar findings to a large body of literature that has shown integration of seaweeds with the primary culture organism can bring many benefits to overall farm performance (see Brzeski & Newkirk, 1997; Troell et al., 1999a, b; Buschmann et al., 2001; Neori et al., 1991, 1996, 1998; Robertson-Andersson et al., 2008).

Research and development over three decades has brought integrated land-based aquaculture technology to a commercial reality in South Africa. Through algal biofilters, integrated aquaculture recycles nutrients into profitable products, while restoring water quality (Neori et al., 2004). Recirculating systems can increase the temperature of the water and positively affect abalone growth rates (Bolton, 2006; Robertson-Andersson, 2007; Robertson-Andersson et al., 2008). The finding of this study showed that the temperature increase in a 50 % Recirculation system at I & J did not significantly affect the growth rates of abalone. The 75 % Recirculation system at I & J showed significantly higher temperatures than the FTUs, and this can have a positive effect on the growth rate on the cooler west and southwest coasts, as suggested by Bolton (2006), Robertson-Andersson (2007) and Robertson-Andersson et al. (2008).

Recirculation may have negative effects which include increased sediments, parasites and pest species in the system, adversely affecting abalone health and growth rates. Water quality in an integrated system could also be poorer than in a flow through system

(Robertson-Andersson, 2007; Robertson-Andersson et al., 2008). This study has proven that these negative effects were not significant at 50 % recirculation rate in the I & J system fed a diet of Abfeed[®] and kelp, and should make integrated systems more popular for the rest of the aquaculture industry.

Future research should include:

- To combine the current diet tested (Abfeed[®] K26 and kelp), with *Ulva* spp. which farms are increasingly producing (Robertson-Andersson et al., 2008; Bolton et al., in press). This would allow farms to be less reliant on external food sources.
- Testing the effects of higher recirculation rates for short periods of time. It would be useful for the farms to be able to run at 100 % recirculation during times of harmful algal blooms (HAB); this would stop the abalone from being affected by the HAB organisms. Blooms of toxic algae occur frequently on the west coast of South Africa (Pitcher, 1998).
- Adding other extractive organisms to the system, such as mussels or oysters, that could feed on the particulate waste products of the abalone.
- Testing the suitability of other seaweeds as a biofilter and abalone feed, or possibility as a commercial crop.

The adaptability of South African abalone farmers and their willingness to try new species have been successfully combined to produce the current commercial abalone / seaweed integrated systems, which appear to be unique worldwide (Neori et al., 2007; Bolton et al., in press). There are two major success stories where successful integrated

abalone / seaweed have been implemented (i) at Wild Coast abalone, where the lack of available kelp and the real or perceived benefit of feeding live seaweed was the major drivers in the use of an integrated flow-through abalone / seaweed system. (ii) The I & J Recirculation system, where the bioremediation aspect of the seaweed has added to the economic benefits, by both reducing pumping costs and increasing water temperature in the abalone tanks (Robertson-Andersson, 2007; Bolton et al., in press).

The success of the abalone industry has been enhanced by the collaboration between scientific institutions and abalone farmers. It is important to continue this relationship to ensure the abalone industry continues to grow and develop in South Africa

References

- AASA – 2004. Aquaculture Association of Southern Africa's homepage:
<http://www.aasa-aqua.co.za/>. Last accessed 11 May 2008.
- Ackefors, H.; Huner, J.V.; Konikoff, M., 1994. Introduction to the general principles of Aquaculture. The Haworth Press, Inc, United States of America. 172 pp.
- Alabaster, J.S.; Lloyd, R., 1980. Water quality criteria for freshwater fish (2nd Ed). Butterworth, London, 297pp.
- Allan, G.L.; Marguire, G.B.; Hopkins, S.J., 1990. Acute and chronic toxicity of ammonia to juvenile *Metapenaeus macleayi* and *Panaeus monodon* and the influence of low dissolved-oxygen levels. Aquaculture **91**: 265 – 280.
- Anderson, D.P., (1990) Immunological indicators: effects of environmental stress on immune protection and disease outbreaks. American Fisheries Society Symposium **8**: 38-50.
- Anderson, R.J.; Bolton, J.J.; Molloy, F.J.; Rotmann, K.W.G., 2003. Commercial seaweed production and research in southern Africa. Proceedings of the 17th International Seaweed Symposium. Oxford University Press. pp. 1 – 12.
- Anderson, R.J.; Carrick, P.; Levitt, G.J.; Share, A., 1997. Holdfasts of adult *Ecklonia maxima* provide refuges from grazing for recruitment of juvenile kelps, Mar. Ecol. Prog. Ser. **159**: 265 – 273.
- Anderson, R.J.; Levitt, G.J.; Share, A. 1996. Investigations for the mariculture of *Gracilaria gracilis* at Saldanha Bay, South Africa. J. Appl. Phycol. **8**: 421 – 430.

- Anderson, R.J.; Rand, A.; Rothman, M.D.; Share, A.; Bolton, J.J., 2007. Mapping and quantifying the South African kelp resource, *Afri. J. of Mar. Sci.* **29** (3): 369 – 378.
- Anderson, R.J.; Rothman, M.D.; Share, A., Drummond, H., 2006. Harvesting of the kelp *Ecklonia maxima* in South Africa affects its three obligate, red algal epiphytes. *J. Appl. Phycol.* **18**: 343 - 349.
- Anderson, R.J.; Simons, R.H.; Jarman, N.G., 1989. Commercial seaweeds in South Africa: A review of utilization and research, *S. Afr. J. Mar. Sci.* **8**: 277 – 299.
- Anonymous, 1991. Nutrition worries for American abalone farmers. *Fish Farm. Int.* **18** (3): 28.
- Anonymous, 2003. Blue revolution, the promise of fish farming: a new way to feed the world. *Economist* August 9, 2003.
- Anonymous, 2007. Government Gazette, Republic of South Africa Vol. 507, Number 30263: 3 – 20. Department of Environmental Affairs and Tourism. General Notice 1109 Marine Living Resources Act (18 / 1998): Policy for the Development of a Sustainable Marine Aquaculture Sector in South Africa.
- ARC, 1981. The Nutrient Requirements of Pigs. Agricultural Research Council,
- Bachère, E.; Mialhe, E.; Noël, D.; Boulo, V.; Morvan, A.; Rodrigues, J., 1995. Knowledge and research prospects in marine mollusc and crustacean immunology. *Aquaculture* **132**: 17-32.
- Barkai, R.; Griffiths, C.L., 1986. Diet of the South African abalone *Haliotis midae*. *S. Afr. J. Mar. Sci.* **4**: 37 - 44.
- Barkai, R.; Griffiths, C.L., 1987. Consumption, absorption efficiency, respiration and excretion in the South African abalone *Haliotis midae*. In: Payne, A.I.L.; Gullard,

- J.A.; Brink, K.H. (eds) *The Benguela and Comparable Ecosystems*. S. Afr. J. Mar. Sci. **5**: 523 - 529.
- Barkai, R.; Griffiths, C.L., 1988. An energy budget for the South African abalone *Haliotis midae* Linnaeus. J. Molluscan Studies. **54**: 43 - 51.
- Barnabé, G., 1994. Aquaculture: Biology and Ecology of cultured species. Ellis Horwood Ltd, Great Britain, 403pp.
- Basuyaux, O.; Mathieu, M., 1999. Inorganic nitrogen and its effects on growth of the abalone *Haliotis tuberculata* (Linnaeus) and the sea urchin *Paracentrotus lividus* (Lamarck). Aquaculture **174**: 95 - 107.
- Bayne, B.L.; Newell, R.C., 1983. Physiological energetics of marine molluscs. In: K.M. Wilbur and A.S.M. Saleuddin (Editors), The Mollusca. Part 1. Vol. 4. Academic Press, New York, pp. 407 - 515.
- Bester, A.E.; Slabbert, R.; D'amato, M.E., 2004. Isolation and characterization of microsatellite markers in South African abalone (*Haliotis midae*). Molecular Ecology Notes **4**: 618 - 619.
- Beveridge, M.C.M., 1987. Cage aquaculture (1st edition). Fishing News Books Publishers, Great Britain, 346pp.
- Boarder, S.J.; Shpigel, M., 2001. Comparative performances of juvenile *Haliotis roei* fed on enriched *Ulva rigida* and various artificial diets. J. Shellfish Res. **20**(2): 653-657.
- Bolton, J.J., 2006. Do we have the vision to integrate our marine aquaculture? Commentary. S. Afr. J. Mar. Sci. **102**: November / December 2006.

- Bolton, J.J.; Robertson-Andersson, D. V.; Shuuluka, D.; Kandjengo, L., 2008. Growing *Ulva* (Chlorophyta) in integrated systems as a commercial crop for abalone feed in South Africa: a SWOT analysis. *J. Appl. Pycol.* In press. DOI 10.1007/s10811-008-9385-6.
- Bolton, J.J.; Anderson, R.J., 1997. Marine vegetation. In: *Vegetation of Southern Africa*. Cowling, R.M.; Richardson, D.M.; Pierce, S.M. (Eds.), Cambridge University Press. pp. 348 – 370.
- Bolton, J.J.; Stegenga, H., 2002. Seaweed biodiversity in South Africa. *S. Afr. J. Mar. Sci.* **24**: 9 - 18.
- Bower, C.E.; Bidwell, J.P., 1978. Ionization of ammonia in seawater: Effects of temperature, pH and salinity. *J. Fish. Res. Board Can.* **35**: 1012 - 1016.
- Bower, S.M., 1987. *Labyrinthuloides haliotidis* n. sp. (Protozoa: Labyrinthomorpha), a pathogenic parasite of small juvenile abalone in a British Columbia mariculture facility. *Canadian J. Zoology* **65**: 1996-2007.
- Boyd, C.E., 1982. Water quality management for pond fish culture, Vol. 9. *Developments in Aquaculture and Fisheries Science*, Elsevier, New York, 318pp.
- Boyd, C.E., 1998. Pond water aeration systems. *Aquacultural Engineering* **18**: 9-40.
- Boyd, C.E.; Watten, B.J., 1989. Aeration systems in aquaculture. *Reviews in Aquatic Sciences* **1**(3): 425-472.
- Brandt, M., 2006. A re-circulating integrated abalone / seaweed culture system in South Africa: effects on particulate and sediment load. *Minor Field Study*, Stockholm University. 24pp.

- Britz, P.J., 1993. The role of pelleted feeds in the commercialisation of abalone aquaculture. Paper presented at "Aquaculture '92". Second Congress of the Aquaculture Association of southern Africa, Rhodes University. September 1992, Proceedings of the Aquaculture Association of Southern Africa **1**: 155 - 157.
- Britz, P.J., 1995. The nutritional requirements of *Haliotis midae* and development of a practical diet for intensive abalone culture. PhD. thesis, Rhodes University, South Africa.
- Britz, P.J., 1996a. Effect of dietary protein level on growth performance of South African abalone, *Haliotis midae*, fed fishmeal-based semi-purified diets. Aquaculture. **140**: 55 - 61.
- Britz, P.J., 1996b. The suitability of selected protein sources for inclusion in formulated diets for the South African abalone, *Haliotis midae*. Aquaculture **140**: 63 - 73.
- Britz, P.J.; Clayden, C., 1996c. Evaluation of the growth rate of feed conversion ratio of *Haliotis midae* fed formulated practical diets. Proceedings of the Aquaculture Association of Southern Africa. **5**: 68 - 73.
- Britz, P.J.; Hecht, T., 1997. Effect of dietary protein and energy level on growth and body composition of South African abalone, *Haliotis midae*. Aquaculture **156**: 195 – 210.
- Britz, P.J.; Hecht, T.; Knauer, J.; Dixon, M.G., 1994. The development of an artificial feed for abalone farming. S. Afr. J. Mar. Sci. **90**: 7 - 8.
- Brix, O., 1983. Blood respiratory properties in marine gastropods. In: Brix, O. (ed) Environmental Biochemistry and Physiology, Volume 2: The Mollusca, Academic Press, London, pp 51-76.

- Brown, L.D.; Murray, N.D., 1992, Genetic relationships within the genus *Haliotis*. In: Shepherd, S.A.; Tegner, M.J.; Guzman del Proo, S.A. (eds). Abalone of the World. Biology, Fishery and Culture. Fishing News Books, London. pp. 19 - 23.
- Brzeski, V.; Newkirk, G., 1997. Integrated coastal food production systems - a review of current literature. *Ocean Coast. Manag.* **34**: 66 – 71.
- Buschmann, A.; Troell, M.; Kautsky, N., 2001. Integrated algal farming: a review. *Cah. Biol. Mar.* **43**: 615 – 655.
- Buschmann, A.H., 1996. An introduction to integrated farming and the use of seaweeds as biofilters. *Proceedings of the 15th International Seaweed Symposium.* **15**: 326 – 327.
- Camargo, J.A.; Alonso, A.; Sagarin, R.D., 2007. Inorganic nitrogen pollution in aquatic ecosystems: causes and consequences. In: Cleveland CJ (ed) *Encyclopedia of Earth. Environmental Information Coalition, National Council for Science and the Environment, Washington DC, 4pp.*
- Chalmers, R., 2002. An investigation into the feeding biology and factors influencing the population dynamics of *Terebrasabella heterouncinata* (Polychaeta: Sabellidae) a problematic tube-dwelling polychaete in farmed abalone in South Africa. MSc. thesis, Rhodes University, South Africa.
- Chamberlane, G.; Rosenthal, H., 1995. Aquaculture in the next century: opportunities for growth, challenges for stability. *World Aquac. Soc. Mag.* **26**(1): 21 - 25.
- Chen, H-C., 1984. Recent innovations in cultivation of edible molluscs in Taiwan, with special reference to the small abalone *Haliotis diversicolor* and the hard clam *Meretrix lusoria*. *Aquaculture* **39**: 11-27.

- Chen, H-C., 1989. Farming the small abalone, *Haliotis diversicolor supertexta*, in Taiwan. In: Hahn KO (ed) Handbook of Culture of Abalone and other marine gastropods. CRC Press, Inc, United States of America, pp 265-283.
- Cheng, S.Y.; Chang, Y.H.; Chen, J.C., 2004a. Lethal levels of dissolved oxygen for *Haliotis diversicolor supertexta* at different salinity levels. J. Shellfish Res. **23**(2): 569 - 573.
- Cheng, S-Y.; Chang, Y-H.; Chen, J-C., 2004. Lethal levels of dissolved oxygen for *Haliotis diversicolor supertexta* at different salinity levels. J. Shellfish Res. **23**(2): 569-573.
- Cheng, W.; Hsiao, I.S.; Hsu, C.H.; Chen, J.C., 2004b. Change in water temperature on the immune response of Taiwan abalone *Haliotis diversicolor supertexta* and its susceptibility to *Vibrio parahaemolyticus*. Fish and Shellfish Immunology. **17**: 235 - 243.
- Chopin, T., 2006. Integrated multi-trophic aquaculture. What it is, and why you should care...and don't confuse it with polyculture. Northern Aquaculture. **12**: 4.
- Chopin, T.; Buschmann, A. H.; Halling, C.; Troell, M.; Kautsky, N.; Neori, A.; Kraemer, G.P.; Zertuche-González, J.A.; Yarish, C.; Neefus, C., 2001. Integrating seaweeds into marine aquaculture systems: A key towards sustainability. J. Phycol. **37**: 975 – 986.
- Clayden, C., 2000. Management considerations in the presence of the threat from the sabellid polychaete *Terebrasabella heterouncinata*. In. Simon, C. A.; Kaiser, H.; Britz, P.J., 2004. Infestation of the abalone, *Haliotis midae*, by the sabellid, *Terebrasabella heterouncinata*, under intensive culture conditions, and the influence of infestation on abalone growth. Aquaculture. **232**: 29 – 40.

- Cohen, I.; Neori, A., 1991. *Ulva lactuca* biofilters for marine fishponds effluents. Bot. Mar. **34**: 475 - 482.
- Colt, J.E.; Armstrong, D.A., 1981. Nitrogen toxicity to crustaceans, fish, and molluscs. In: Allen LJ, Kinney EC (eds) Proceedings of the Bio-Engineering Symposium for Fish Culture. Fish Culture Section of the American Fisheries Society, FCS Publication 1. pp. 34 - 47.
- Commonwealth Agricultural Bureaux, Farnham Royal.
- Cook, P.A., 1998. Aquaculture in South Africa: Current Status and Future potential. Regional Developments. University of Chicago. pp. 467 – 471.
- Cook, P.A., 1998. The current status of abalone farming in South Africa. J. Shellfish Res. **17**(3): 603 - 602.
- Critchley, A.T.; Rotmann, K.W.G., 1992. Industrial processing of seaweeds in Africa.
- Culver, C.S., Kuris, A.M., Beede, B., 1997. Identification and management of the exotic sabellid pest in California cultured abalone. California Sea Grant College Program. University of California, La Jolla, CA Publication No. T - 041.
- Cuthbertson, A., 1978. The Abalone Culture Handbook. Tech. Rep. No. 1, Tasmanian Fisheries Development Authority, Australia.
- Daume, S.; Davidson, M.; Ryan, S.; Parker, F., 2007. Comparison of rearing systems based on algae or formulated feed for juvenile greenlip abalone (*Haliotis laevis*). J. Shellfish Res. **26**(3): 729 - 735.
- Day, E., 1998. Ecological interactions between abalone (*Haliotis midae*) juveniles and echinoids (*Parechinus angulosus*) off the southwest coast of South Africa. Ph.D. thesis, University of Cape Town, South Africa.

- Day, R.W.; Fleming, A.E., 1992. The determinants and measurement of abalone growth. Abalone of the World, Biology, Fisheries & Culture. *In*: Shepherd S. A.; Tegner M. J, Guzmán Del Prío (Eds). Abalone of the World, Biology, Fisheries and Culture. Blackwell Scientific Publications, Oxford. pp.169 - 181.
- De Busk, T.A.; Blakeslee, M.; Ryther, J.H., 1986. Studies on the outdoor cultivation of *Ulva lactuca*. L. Bot. Mar. **29** (5): 381 – 386.
- De Oliveira, E.C.; De Paula, E.J.; Berchez, F.A.S., 1989. Essays on the cultivation of tropical red seaweeds in tanks. *In*: de Oliveira, E.C. and Kautsky, N. (Eds.). Workshop – Cultivation of seaweeds in tanks. University of St. Paulo / International Foundation for Science, Brazil. 79 - 87.
- DEAT 2007. :<http://www.mcm-deat.gov.za/mariculture/> last accessed March 2008
- Deutsch, L.; Gräslund, S.; Folke, C.; Troell, M.; Huitric, M.; Kautsky, N.& Lebel, L., 2007. Global Environmental Change. **17**: 238 – 249.
- Dixon, M.G., 1992. The effect of temperature and photoperiod on the digestive physiology of *Haliotis midae*. MSc. thesis, Rhodes University, South Africa.
- Dlaza, T.S., 2006. Growth of juvenile abalone under aquaculture conditions. MSc. thesis, University of the Western Cape, South Africa.
- Dlaza, T.S.; Maneveldt, G.W.& Viljoen, C., 2008. The growth of post-weaning abalone *Haliotis midae* (Linnaeus) fed commercially available formulated feeds supplemented with fresh wild seaweed. Afr. J. Mar. Sci. **30**(1): 199 - 203.
- Doeg, T.; Milledge, G., 1991. Effect of experimentally increasing concentrations of suspended sediment on macroinvertebrate drift. Aus. J. Mar. & Freshwater Res. **42**: 519 - 26.

- Douillet, P.A.; Langdon, C.J., 1994. Use of a probiotic for the culture of larvae of the Pacific oyster (*Crassostrea gigas* Thunberg). *Aquaculture* **119**: 25-40.
- Downing, K.M., Merkens, J.C., 1955. The influence of dissolved oxygen concentration on the toxicity of unionized ammonia to rainbow trout (*Salmo gairdnerii* Richardson). *Ann. Appl. Biol.* **43**: 243 – 246.
- Edwards, S., 2003. Short communication: Assessment of the physiological effect of altered salinity on greenlip (*Haliotis laevis*) and blacklip (*Haliotis rubra*) abalone using respirometry. *Aquaculture Res.* **34**: 1361-1365.
- Elston, R., 1983. Histopathology of oxygen intoxication in the juvenile red abalone, *Haliotis rufescens* Swainson. *J. Fish Diseases* **6**: 101-110.
- Emerson, K.; Russo, R.C.; Lund, R.E.; Thurston, R.V., 1975. Aqueous ammonia equilibrium calculations: effect of pH and temperature. *J. Fish. Res. Board Can.* **32**(12): 2379 – 2383.
- Engledow, H.R.; Bolton, J.J., 1992. Environmental tolerances in culture and agar content of *Gracilaria verrucosa* (Hudson) Papenfuss (Rhodophyta, Gigartinales) from Saldahha Bay, S. Afr. *J. Bot.* **58**. 263 - 267.
- Epifanio, C.E.; Srna, R.F., 1975. Toxicity of ammonia, nitrate ion, and orthophosphate to *Mercenaria mercenaria* and *Crassostrea virginica*. *Mar. boil.* **33**, 241 – 246.
- Fallu, R., 1991. Abalone Farming. Fishing News Books Publishers, Great Britain. 195pp.
- Fallu, R., 1991. Abalone Farming. Fishing News Books Publishers, Great Britain, 195pp.
- FAO, 2001. GESAMP (IMO/FAO/UNESCO-IOC/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection).

- Planning and management for sustainable coastal aquaculture development. Rep. Stud. GESAMP. **68**: 90.
- FAO, 2004. The state of the world fisheries and aquaculture 2004 Rome, Italy.
- FAO, 2006a. Fishery Information, Data and Statistics unit (FIDI). Fishery statistical collections. FAO Rome, Italy.
- FAO, 2006b. Food and Agriculture Organization (2006). State of World Aquaculture 2006. FAO Fisheries Technical Paper 500. Rome, Italy.
- Fitzhugh, K., 1996. A polychaete threatens California's abalone culture industry. Terra 33 (4), 4 – 5.
- Fitzhugh, K.; Rouse, Q. W. 1999. A remarkable new genus and species of fan worm (Polychaeta: Sabellidae: *Sabellinae*) associated with some marine gastropods. Invertebrate Biology. **118**: 357 - 390.
- Fleming, A.E., 1995. Growth, intake, feed conversion efficiency and chemosensory preference of the Australian abalone, *Haliotis rubra*. Aquaculture. **132**: 297 - 310.
- Fleming, A.E.; Van Barneveld, R.J.; Hone, P.W., 1996. The development of artificial diets for abalone: A review and future directions. Aquaculture. **140**: 5 - 53.
- Folke, C.; Kautsky, N., 1989. The role of ecosystems for a sustainable development of aquaculture. Ambio **18**: 234 - 243.
- Folke, C.; Kautsky, N., 1992. Aquaculture with its environment: prospects for sustainability. Ocean Coast. Manag. **17**: 5 - 24.
- Folke, C.; Kautsky, N.; Troell, M., 1994. The cost of eutrophication from salmon farming: Implications for policy. J. Environ. Manag. **40**: 173 - 182.

- Fourie, A.M., 1994. Tank culturing of *Gracilaria verrucosa* for use as a food source for abalone. MSc thesis. University of Port Elizabeth. South Africa.
- Friedlander, M.; Levy, I., 1995. Cultivation of *Gracilaria* in outdoor tanks and ponds. *J. Appl. Phycol.* **7**, 315–324.
- Friedman, C.S., 1991. Coccidiosis of California abalone, *Haliotis* spp. (Abstract) *J. Shellfish Res.* **10**: 236.
- Friedman, C.S.; Andree, K.B.; Beauchamp, K.A.; Moore, J.D.; Robbins, T.T.; Shields, J.D.; Hedrick, R.P., 2000d. '*Candidatus Xenohaliotis californiensis*', a newly described pathogen of abalone, *Haliotis* spp., along the west coast of North America. *Inter. J. Syst. Evol. & Micro.* **50**: 487 - 855.
- Friedman, C.S.; Andree, K.B.; Robbins, T.T.; Shields, J.D.; Moore, J.D.; Beauchamp, K.A.; Hedrick, R.P., 2000c. "*Candidatus Xenohaliotis californiensis*," a newly described bacterial pathogen and etiological agent of withering syndrome found in abalone, *Haliotis* spp., along the west coast of North America. (Abstract) *J. Shellfish Res.* **19**: 513.
- Friedman, C.S.; Robbins, T.; Jacobsen J.L.; Shields, J.D., 2000a. The cellular immune response of black abalone, *Haliotis cracherodii* (Leach), with and without Withering Syndrome. (Abstract) *J. Shellfish Res.* **19**: 514.
- Friedman, C.S.; Robbins, T.; Jacobsen, J.L.; Shields, J.D. 1999. Examination of the cellular immune response of black abalone, *Haliotis cracherodii* with and without Withering Syndrome. (Abstract) *J. Shellfish Res.* **18**: 322.
- Friedman, C.S.; Robbins, T.T.; Moore, J.D.; Shields, J.D.; Andree, K.B.; Beauchamp, K.A.; Antonio, D.B.; Hedrick, R.P., 2000b. "*Candidatus Xenohaliotis californiensis*",

- a newly described bacterial pathogen and etiological agent of abalone withering syndrome. (Abstract) *J. Shellfish Res.* **19**: 645.
- Friedman, C.S.; Roberts, W.; Kismohandaka, G.; Hedrick, R.P., 1993. Transmissibility of a coccidian parasite of abalone, *Haliotis* spp. *J. Shellfish Res.* **12**: 201 - 205.
- Friedman, C.S.; Thomson, M.; Chun, C.; Haaker, P.; Hedrick, R.P., 1997. Withering syndrome of the black abalone, *Haliotis cracherodii* (Leach): water temperature, food availability, and parasites as possible causes. *J. Shellfish Res.* **16**: 403 - 411.
- Friedman, C.S.; Trevelyan, G.; Robbins, T.T.; Mulder, E.P.; Fields, R., 2003. Development of an oral administration of oxytetracycline to control losses due to withering syndrome in culture red abalone *Haliotis rufescens*. *Aquaculture.* **224**: 1 - 23.
- Gardner, G.R.; Harshbarger, J.C.; Lake, J.L.; Sawyer, T.K.; Price, K.L.; Stephenson, M.D.; Haaker P.L.; Togstad, H.A., 1995. Association of prokaryotes with symptomatic appearance of withering syndrome in black abalone *Haliotis cracherodii*. *J. Invert. Path.* **66**: 111 - 120.
- Geiger, D.L., 2000. Distribution and biogeography of the *Haliotidae* (Gastropoda: Vetigastropoda) world-wide. *Bolletino Malacologico.* **35**: 57 - 120.
- Genade, A.B.; Hirst, A.L.; Smit, C.J., 1985. Observations on the spawning and rearing of the South African abalone *Haliotis midae* (Linn.). *S. Afr. J. Mar. Sci.* **6**: 3 - 12.
- Genade, A.B.; Hirst, A.L.; Smit, C.J., 1988. Observations on the spawning, development and rearing of the South African Abalone *Haliotis midae* (Linn). *S. Afr. J. Mar. Sci.* **6**: 3 - 12.

- Goldburg, R.J.; Elliott, M.S.; Naylor, R.I., 2001. Marine aquaculture in the United States: Environmental Impacts and Policy Options. Pew Oceans Commission, Arlington, Virginia, 33pp.
- Gordon, H.R.; Cook, P.A., 2001. World abalone supply, markets and pricing: Historical, Current and Future. *J. Shellfish Res.* **20**(3): 567 - 570.
- Gordon, H.R.; Cook, P.A., 2004. World abalone fisheries and aquaculture update: supply and market dynamics. *J. Shellfish Res.* **23**: 935 – 939.
- Grasshoff, K.; Ehrhardt, M.; Kremling, K. 1976. Methods of seawater analysis. Weinheim. Germany. 419pp.
- Hahn, K.O., 1989a. Survey of the commercially important abalone species in the world. In: Hahn, K.O. (ed) *CRC Handbook of Culture of Abalone and other marine gastropods*. CRC Press, Inc, United States of America. pp. 3 - 11.
- Hahn, K.O., 1989b. Artificial induction of conditioning (Gonad maturation). In: Hahn K.O. (ed) *CRC Handbook of Culture of Abalone and other marine gastropods*. CRC Press, Inc, United States of America. pp 41 - 51.
- Hahn, K.O., 1989c. Nutrition and growth of abalone. In: Hahn KO (ed) *CRC Handbook of Culture of Abalone and other marine gastropods*. CRC Press, Inc, United States of America. pp. 135 - 156.
- Hahn, K.O., 1989d. Larval development of Abalone. In: Hahn, K.O. (ed) *CRC Handbook of Culture of Abalone and other marine gastropods*. CRC Press, Inc, United States of America. pp. 71 - 99.

- Hambrey, J.; Tuan, L.A.; Thuong, T.K., 2001. Aquaculture and poverty alleviation: II. Cage culture in coastal waters of Vietnam. *World Aquac.* pp. 32, 34 – 38, 66 – 67.
- Hampson, S., 1998. Evaluation of effluent water from an abalone mariculture system as a culture medium for two strains of *Gracilaria gracilis*. M.Sc.thesis. University of Port Elizabeth. South Africa.
- Hargreaves, J.A., Kucuk, S., 2000. Effects of diel un-ionized ammonia fluctuation on juvenile hybrid striped bass, channel catfish, and blue tilapia. *Aquaculture* **195** (1 – 2), 163 – 181.
- Harris, J.O.; Maguire, G.B.; Edwards, S.J.; Hindrum, S.M., 1997. Effect of Nitrite on growth and oxygen consumption for juvenile greenlip abalone, *Haliotis laevis* Donovan. *J. Shellfish Res.* **16**(2): 395-401.
- Harris, J.O.; Maguire, G.B.; Edwards, S.J.; Johns, D.R., 1999b. Low dissolved oxygen reduces growth rate and oxygen consumption rate of juvenile greenlip abalone, *Haliotis laevis* Donovan. *Aquaculture* **174**: 265-278.
- Harris, J.O.; Maguire, G.B.; Handlinger, J.H., 1998. Effects of chronic exposure of greenlip abalone, *Haliotis laevis* (Donovan), to high ammonia, nitrite, and low dissolved oxygen concentrations on gill and kidney structure. *J. Shellfish Res.* **17**: 683 - 687.
- Harris, J.O.; Maguire, G.B.; Edwards, S.; Hindrum, S.M., 1999a. Effect of pH on growth rate, oxygen consumption rate, and histopathology of gill and kidney tissue for juvenile greenlip abalone, *Haliotis laevis* Donovan and blacklip abalone, *Haliotis rubra* Leach. *J. Shellfish Res.* **8**(2): 611-619.

- Hecht, T.; Britz, P., 1990. Aquaculture in South Africa, History, Status and Prospects. Aquaculture Association of South Africa, Pretoria. 55pp.
- Higashi, R.M.; Fan, T.W.M.; MacDonald, J.M., 1989. Monitoring of metabolic responses of intact *Haliotis* (abalones) under salinity stress by ^{31}P surface probe localized NMR. J. Exp. Zoology **249**: 350-356.
- Hindrum, S.; Burke, C.; Edwards, S.; Johns, D., 2001. Effects of combined exposure to elevated ammonia and low dissolved oxygen levels in greenlip (*Haliotis laevis* Donovan) and blacklip (*H. rubra* Leach) abalone. 1. Growth and mortality data from simulated systems failure. J. Shellfish Res. **20**(1): 679-684.
- Hogg, I.D. and Norris, R.H., 1991. Effects of runoff from land clearing and urban development on the distribution and abundance of macroinvertebrates in pool areas of a river. Australian J. Mar. and Freshwater Res. **42**; 507 – 518.
- Hooker, N.& Morse, D.E., 1985. Abalone: The emerging development of commercial cultivation in the United States. In: Huner, J.V.; Brown, E.E., (eds) Crustacean and mollusk cultivation in the United States. AVI Publishing, Westport, CT, pp. 365 - 413.
- Huchette, S.M.H.; Koh, C.S.; Day, R.W., 2003. Growth of juvenile blacklip abalone (*Haliotis rubra*) in aquaculture tanks: effects of density and ammonia. Aquaculture. **219**: 457 - 470.
- Imai, T., 1977. Aquaculture in shallow seas: progress in shallow sea culture. Amerind Pub., New Delhi, Part IV.

- Innes, A.J.; Houlihan, D.F., 1985. Aerobic capacity and cost of locomotion of a cool temperate gastropod: a comparison with some Mediterranean species. *Comparative Biochemistry and Physiology* **80**: 487-493.
- Isaac, W.E.; Molteno, C.J., 1953. Seaweed resources of South Africa. *J. S. Afr. Bot.* **19**: 85 – 92.
- Jarayabhand, P.& Paphavasit, N., 1996. A review of the culture of tropical abalone with special reference to Thailand. *Aquaculture*. **140**: 159 - 168.
- Jarayabhand, P.; Paphavasit, N., 1996. A review of the culture of tropical abalone with special reference to Thailand. *Aquaculture* **140**: 159-168.
- Jeney, G.; Nemesòk, J.; Jeney, Z; Olah, J., 1992. Acute effect of sublethal ammonia concentrations on common carp (*Cyprinus carpio* L): II. Effect of ammonia on blood plasma transaminases (GOT, GPT),GIDH enzyme activity and ATP value. *Aquaculture* **104**, 149 – 156.
- Joseph, M.M., 1998. Mussel and oyster culture in the Tropics. In: De Silva, S.S. (ed) *Tropical Mariculture*. Academic Press. California. pp. 309 - 360.
- Kelpak[®], 2007. Available at <http://www.kelpak.com/products.asp?contentPlaceID=7>.
Last accessed March 2008.
- Kinne, O., 1976. *Marine Ecology*. Vol. **3**. Wiley, London. 1293pp.
- Knauer, J., 1994. Development of an artificial weaning diet for the South African abalone *Haliotis midae* (Haliotidae: Gastropoda). MSc. thesis, Rhodes University, South Africa.

- Knauer, J.; Hecht, T.; Britz, P.J., 1995. A note on the feeding behaviour and growth of juvenile South African abalone, *Haliotis midae*, fed on an artificial weaning diet. S. Afr. J. Mar. Sci. **91**: 91 - 93.
- Krom, M.D., 1986. An evaluation of the concept of assimilative capacity as applied to marine waters. *Ambio* 15, 208–214.
- Krom, M.D.; Neori, A.; van Rijn, J.; Poulton, S.W.; Davis, I.M., 2001. Working towards environmentally friendly marine farming. *Ocean Chall.* **10**, 22 – 27.
- Kuris, A.M.; Schmitt, R.J.; Hedrick, R., 1994. Abalone wasting disease: role of coccidian parasites and environmental factors. In: California Sea Grant Biennial Report of Completed Projects 1990 - 92. California Sea Grant College, La Jolla, CA. pp. 120 - 123.
- Landau, M., 1992. Introduction to aquaculture. John Wiley and Sons, Inc., Singapore, 440pp.
- Laukner, G., 1983. Diseases of Mollusca: Bivalvia. In: Kinne, O. (Ed.), Diseases of Marine Animals. Introduction to Scaphopoda, vol. II. Biologische Anstalt Helgoland, Hamburg, pp. 805 – 817.
- Lee, C.S., 1998. Culture of marine finfish species of the Pacific. In: De Silva, S. S., (ed) Tropical Mariculture. Academic Press. California. pp. 361 - 380.
- Lee, Y-H.; Vacquier, V.D., 1995. Evolution and systematics in Haliotidae (Mollusca: Gastropoda): inferences from DNA sequences of sperm lysine. *Mar. Biol.* **124**: 267 - 278.
- Leighton, D.L., 1989. Abalone (genus *Haliotis*) mariculture on the North American Pacific Coast. *Fish. Bull.* **87**: 689 - 702.

- Lester, R.J.G.; Davis, G.H.G., 1981. A new *Perkinsus* species (Apicomplexa, Perkinsea) from the abalone *Haliotis ruber*. *J. Invertebrate Pathology* **37**: 181-187.
- Levitt, G.J.; Anderson, R.J.; Boothroyd, C.J.T.; Kemp, F.A., 2002. The effects of kelp harvesting on its regrowth and the understorey benthic community at Danger Point, South Africa, and a new method of harvesting kelp fronds. *S. Afr. J. Mar. Sci.* **24**: 71 - 85.
- Li, S., 1999. Availability of areas for further aquaculture production. In: Svennevig, N.; Reinertsen, H.; New, M., (eds) *Sustainable Aquaculture: Food for the future?* Proceedings of the second international symposium on sustainable aquaculture / Oslo / Norway / 2-5 November 1997. pp. 29 - 42.
- Lindberg, D.R., 1992. Evolution, distribution and systematics of Haliotidae. In: Shepherd SA, Tegner MJ, Guzman del Proo SA (eds) *Abalone of the World. Biology, Fishery and Culture*. Fishing News Books, London. pp. 3 - 18.
- Lindström, A., 2006. Unpublished manuscript, Water quality in a re-circulating abalone/seaweed system, masters thesis, Stockholm University pp. 1 – 31.
- Littler, M.M. & Littler, D.S., 1980. The evolution of thallus form and survival strategies in benthic marine macroalgae. *Am. Nat.* **116**: 25 – 44.
- Lloyd, D.S., 1987. Turbidity as a water quality standard for salmonid habitats in Alaska. *N. Am. J. Fisheries Management.* **7**: 34 - 45.
- Lopez, L.M.; Tyler, P., 2006. Energy budget of cultured female abalone *Haliotis tuberculata* (L.) *J. Shellfish Res.* **25**(2): 385-389.

- Loubser, N.C.; Dormehl, N., 2000. The use of ultrasound in the treatment of sabellid infestations in South African abalone. 4th International Abalone Symposium, University of Cape Town, South Africa.
- Lüning, K.; Pang, S., 2003. Mass cultivation of seaweeds: current aspects and approaches. *J. Appl. Phycol.* **15**: 115 - 119.
- Lyon, G., 1995. Aspects of the physiology of the South African abalone, *Haliotis midae* L., and implications for intensive abalone culture. MSc. thesis, Rhodes University, South Africa.
- Macey, B.M.; Coyne, V.E., 2005. Improved growth rate and disease resistance in farmed *Haliotis midae* through probiotic treatment. *Aquaculture* **245**: 249-261.
- Mai, K.; Wu, G.; Zhu, W., 2001. Abalone, *Haliotis discus hannai* (Ino), can synthesize MYO-Inositol De Novo to meet physiological needs. *Journal of Nutrition.* **131**: 2898 - 2903.
- Marcello, R.A.; Strawn, K., 1973. Cage culture of some marine fishes in the intake and discharge canals of a steam-electric generating station, Galveston Bay, Texas. *Proceedings of the Annual World Mariculture Society* **4**: 97-112.
- Marifeed, 2007. Available online at: <http://abfeed.com/mari-resrep2.htm>. Last accessed September 2008.
- McDonald, D.G., 1983. The effect of H⁺ upon the gills of freshwater fish. *Canadian J. Zoology* **61**: 691-703.
- McVey, J.P.; Stickney, R.R.; Yarish, C.; Chopin, T., 2002. Aquatic polyculture and balanced ecosystem management: new paradigms in seafood production. In:

- Stickney, R.R.; McVey, J.P. (Eds.), Responsible Marine Aquaculture. CABI Publishing, Oxon. pp. 91 – 104.
- Metzeling, L.; Doeg, T.; O'Connor, W., 1995. The impact of salinisation and sedimentation on aquatic biota. Conservation Biodiversity: Threats and Solutions (Eds.) Bradstock, R.A.; Auld, T.D.; Keith, D.A.; Kingsford, R.T.; Lunny, D.; Silertsen, D.P. Surrey Beatty & Sons, London. pp. 126 – 136.
- Miller, A.C.; Lawrenz-Miller, S.E., 1993. Long-term trends in black abalone, *Haliotis cracherodii* (Leach), 1814, populations along the Palos Verdes Peninsula, California. J. Shellfish Res. **12**(2): 195 - 200.
- Montaño-Vargas, J.; Viana, M.T.; D'Abramo, L.R.; Shimada, A.; Vásquez-Peláez, C., 2005. Growth and energy utilization of juvenile pink abalone *Haliotis corrugata* fed diets containing different levels of protein and two starch:lipid ratios. J. Shellfish Res. **24**(4): 1179 - 1185.
- Moore, J.D.; Robbins, T.T.; Friedman, C.S., 2000. Withering syndrome in farmed red abalone *Haliotis rufescens*: thermal induction and association with a gastrointestinal Rickettsiales-like prokaryote. J. Aquat. An. Health **12**, 26 – 34.
- Mouton, A. 2006. Veterinary health reports for Abalone farms details of methods used and results determination. AFASA report afa06290.
- Mouton, A., 2000. Health management and disease surveillance in abalone, *Haliotis midae*, in South Africa. (Abstract) J. Shellfish Res. **19**: 526.
- Muller, S., 1984. Studies on the ecology, feeding growth and reproduction of *Haliotis spadicea* (Donovan), 1808. MSc. thesis, University of Port Elizabeth, South Africa.

- Muller, S., 1986. Taxonomy of the genus *Haliotis* in South Africa. Transactions of the Royal Society of S.A. **46**: 69 - 77.
- Naidoo, K.; Maneveldt, G.; Ruck, K.; Bolton, J.J., 2006. A comparison of various seaweed-based diets and formulated feed on growth rate of abalone in a land-based aquaculture system. J. Appl. Phycol. **18**: 437 - 443.
- Nakatsugawa, T.; Nagai, T.; Hiya, K.; Nishizawa, T.; Muroga, K., 1999. A virus isolated from juvenile black abalone *Nordotis discus discus* affected with amyotrophia. Diseases of Aquatic organisms **36**: 156-161.
- Nash, C.E., 1991. Introduction and background to aquaculture. In: Nash, C.E. (ed) Production of aquatic animals: Crustaceans, Molluscs, Amphibians and Reptiles. Elsevier Science Publishers, Netherlands. pp. 1 - 18.
- Naylor, R.; Goldberg, R.; Primavera, J.; Kautsky, N.; Beveridge, M.; Clay, J.; Folke, C.; Lubchenco, J.; Mooney, J.; Troell, M., 2000. Effect of aquaculture on world fish supplies. Nature. **405**: 1017 – 1024.
- Neori, A., 1996. The type of N supply (ammonia or nitrate) determines the performance of seaweed biofilters integrated with intensive fish culture. Israeli J. of Aquaculture-Bmidgeh. **48**(1): 19 – 27.
- Neori, A., Shpigel, M.; Ben-Ezra, D., 2000. A sustainable integrated system for culture of fish, seaweed and abalone. Aquaculture. **186**: 279 - 291.
- Neori, A.; Chopin, T.; Troell, M.; Buschmann, A.H.; Kraemer, G.P.; Halling, C.; Shpigel, M.; Yarish, C., 2004. Integrated aquaculture: rationale, evolution and state of the art

- emphasizing seaweed biofiltration in modern aquaculture. *Aquaculture*. **231**: 361 – 391.
- Neori, A.; Cohen, I.; Gordin, H., 1991. *Ulva lactuca* biofilters for marine fish pond effluents. II. Growth rate, yield and C:N ratio. *Bot. Mar.* **34**: 483 – 489.
- Neori, A.; Krom, M.; Ellner, S.; Boyd, C.; Popper, D.; Rabinovitch, R.; Davison, P.; Dvir, O.; Zuber, D.; Ucko, M.; Angel, D.; Gordin, H., 1996. Seaweed biofilters as regulators of water quality in integrated fish-seaweed culture units. *Aquaculture*. **141**(3-4): 183 – 199.
- Neori, A.; Ragg, N.L.C.; Shpigel, M. 1998. The integrated culture of seaweed, abalone, fish and clams in modular intensive land-based systems: II. Performance and nitrogen partitioning within an abalone (*Haliotis tuberculata*) and macroalgae culture system. *Aquac. Eng.* **17**: 215 – 239.
- Neori, A.; Troell, M.; Chopin, T.; Yarish, C.; Critchley, A.; Buschman, A., 2007. The need for a balanced ecosystem approach to blue revolution aquaculture. *Environment* **49**: 37 – 43.
- Newcombe, C.P.; MacDonald, D.D., 1991. Effects of suspended sediments on aquatic ecosystems. *North American J. Fish. Management*. **11**: 72 – 82.
- Newkirk, G., 1996. Sustainable coastal production systems: a model for integrating aquaculture and fisheries under community management. *Ocean Coast. Manag.* **32**: 69 – 83.
- Newman G.G., 1968. Growth of the South African abalone *Haliotis midae*. Investigational Report of the Division of Sea Fisheries, South Africa. **67**: 1 – 24.
- Newman, G.G., 1965. South African Shipping News and Fishing Industry Review.

- Newman, G.G., 1969. Distribution of abalone (*Haliotis midae*) and the effect of temperature on productivity. Investigational Report of the Division of Sea Fisheries, South Africa. **74**: 1 - 7.
- Nicolas, J.L.; Basuyaux, O.; Mazurié, J.; Thébault, A., 2002. *Vibrio carchariae*, a pathogen of the abalone *Haliotis tuberculata*. Diseases of Aquatic Organisms **50**: 35-43.
- Nicotri, M.E., 1977. The impact of crustacean herbivores on cultured seaweed populations. Aquaculture. **12**: 127 – 136.
- Njobeni, A., 2006. The cultivation of *Gracilaria* spp. in an integrated aquaculture system, for the production of abalone feed and the bioremediation of aquaculture effluent. M.Sc. thesis, University of Cape Town, South Africa.
- Nydahl, F. 1976. On the optimum concentrations for reduction of nitrate to nitrite by cadmium. Talanta. **23**: 349 – 357.
- Oakes, F.R.; Fields, R.C., 1996. Infestations of *Haliotis rufescens* shells by a sabellid polychaete. Aquaculture. **140** : 139 – 143.
- Olafsen, J.A., 2001. Interactions between fish larvae and bacteria in marine aquaculture. Aquaculture **200**: 223-247.
- Owen, B. ; Disaivo, L.H. ; Ebert, E.E.; Fonck, E. 1984. Culture of the California red abalone *Haliotis rufescens* Swanson in Chile. Veliger. **27**: 101 – 105.
- Parker, R., 2002. Aquaculture Science. Second Edition. Delmar Publishers, United States of America, 621pp.
- Peck, L.S.; Culley, M.B.; Helm, M.M., 1987. A laboratory energy budget for the ormer *Haliotis tuberculata* L. J. Exp. Mar. Bio. And Eco. **106**(2): 103 - 123.

- Pillay, T.V.R., 1992. Aquaculture and the Environment. Fishing News Books, Oxford, 189pp.
- Pillay, T.V.R., 1994. Aquaculture development: Progress and Prospects. Fishing News Books Publishers, Great Britain, 182pp.
- Pitcher, C.G.; Franco, J.M.; Doucette, G.; Powell, C.; Mouton, A., 2002. Paralytic shellfish poisoning in the abalone *Haliotis midae* on the west coast of South Africa. *J. Shellfish Res.* 20, 895–904.
- Pitcher, G.C., 1998. Harmful Algal Blooms of the Benguela Current. Sea Fisheries Research Institute, Cape Town. 20 pp.
- Potgieter, M., 2005. A comparison of suspended particle size and sediment loading produced by artificial and seaweed diets in integrated flow-trough and re-circulating aquaculture systems on a commercial South African abalone farm. M.Sc. thesis, University of Cape Town, South Africa.
- Preece, M.A.; Mladenov, P.V., 1999. Growth and mortality of the New Zealand abalone *Haliotis iris* Martyn 1784 cultured in offshore structures and fed artificial diets. *Aquaculture Res.* 30: 865 - 877.
- Prince, J.D.; Shepherd, S.A., 1992. Australian abalone fisheries and their management. In: Shepherd SA, Tegner MJ, Guzman del Proo SA (eds) *Abalone of the World. Biology, Fishery and Culture*. Fishing News Books, London. pp. 407 - 426.
- Randall, D., 1991. The impact of variations in water pH on fish. In: Brune, D.E.; Tomasso, J.R. (eds) *Aquaculture and water Quality*. World Aquaculture Society, Baton Rouge, LA, pp 90-104.

- Rawson Jr., M.V.; Chen, C.; Ji, R.; Zhu, M.; Wang, D.; Wang, L.; Yarish, C.; Sullivan, J.B.; Chopin, T.; Carmona, R., 2001. Integration of fed and extractive aquaculture. Paper Collection of International Symposium on Marine Fishery and Aquatic Products Processing Technology, 11– 13 Sept., Rongcheng, China. pp. 263–278.
- Rawson Jr., M.V.; Chen, C.; Ji, R.; Zhu, M.; Wang, D.; Wang, L.; Yarish, C.; Sullivan, J.B.; Chopin, T.; Carmona, R., 2002. Understanding the interaction of extractive and fed aquaculture using ecosystem modeling. In: Stickney, R.R.; McVey, J.P. (Eds.), Responsible Marine Aquaculture. CABI Publishing, Oxon, pp. 263 – 296.
- Reay, P.J., 1979. Aquaculture. Edward Arnold, London, 60pp.
- Reddy-Lopata, K.; Auerswald, L.; Cook, P., 2006. Ammonia toxicity and its effect on the growth of the South African abalone *Haliotis midae* (Linnaeus). Aquaculture. **261**(2): 678 - 687.
- Rengpipat, S.; Rukpratanporn, S.; Piyatiratitivorakul, S.; Menasaveta, P., 2000. Immunity enhancement in black tiger shrimp (*Penaeus monodon*) by a probiotic bacterium (*Bacillus* S11). Aquaculture **191**: 271-288.
- Richards, D.V.; Davis, G.E., 1993. Early warnings of modern population collapse in black abalone *Haliotis cracherodii*, Leach, 1814, at the California Channel islands. J. Shellfish Res. **12**(2): 189 - 194.
- Ridler, N.; Wowchuk, M.; Robinson, B.; Barrington, K.; Chopin, T.; Robinson, S.; Page, F.; Reid, G.; Haya K., 2007. Integrated multi-trophic aquaculture (IMTA): a potential strategic choice for farmers. Aquaculture Economics and Management **11**. pp 99 - 110.

- Robertson-Andersson, D.V., 2003. The cultivation of *Ulva lactuca* (Chlorophyta) in an integrated system, for the production of abalone feed and the bioremediation of Aquaculture effluent. MSc. thesis, University of Cape Town, South Africa.
- Robertson-Andersson, D.V., 2007. Biological and economical feasibility studies of using seaweeds *ulva lactuca* (Chlorophyta) in Abalone farming PHD thesis, University of Cape Town, South Africa. Pages 231.
- Robertson-Andersson, D.V.; Leitao, D.; Bolton, J.J.; Anderson, R.J.; Njobeni, A.; Ruck, K., 2006. Can kelp extract (KELPAK™) be useful in seaweed mariculture? *J. Appl. Phycol.* **18**: 315 – 321.
- Robertson-Andersson, D.V.; Potgieter, M.; Hansen, J.; Bolton, J.J.; Troell, M.; Anderson, R.J.; Halling, C.; Probyn, T., 2008. Integrated seaweed cultivation on an abalone farm in South Africa. *J. Appl. Phycol.* DOI 10.1007/s10811-007-9239-7.
- Rothman, M.D.; Anderson, R.J.; Smit, A.J., 2006. The effects of harvesting of the South African Kelp (*Ecklonia maxima*) on kelp population structure, growth rate and recruitment. *J. Appl. Phycol.* **18**: 335 - 341.
- Ruck, K.R.; Cook, P.A., 1998. Sabellid infestations in the shells of South African molluscs: implications for abalone mariculture. *J. Shellfish Res.* **17**: 693 - 699.
- Russo, R.C., 1985. Ammonia, nitrite and nitrate. In Rand, G.M.; Petrocelli, S.R. (Eds.), *Fundamentals of Aquatic Toxicology*. Hemisphere Publishing, Washington, pp. 455 - 471.
- Russo, R.C.; Thurston, R.V., 1991. Toxicity of ammonia, nitrite and nitrate to fishes. In: Brune, D., Tomasso, J. (Eds.), *Aquaculture and Water Quality*. World Aquaculture Society, Baton Rouge, AL, pp. 58 – 89.

- Rychly, I., 1980. Nitrogen balance in trout. II. Nitrogen excretion and retention after feeding diets with varying protein and carbohydrate levels. *Aquaculture* **20**: 343-350.
- Sahoo, D.; Tang, X.; Yarish, C., 2002. Porphyra—the economic seaweed as a new experimental system. *Curr. Sci.* **83**, 1313 – 1316.
- Sales, J.; Britz, P.J., 2001. Review: Research on abalone (*Haliotis midae* L.) cultivation in SA. *Aquaculture Res.* **32**: 863 - 874.
- Sales, J.; Janssens, G.P.J., 2004. Use of feed ingredients in artificial diets for abalone: a brief update. *Nutrition Abstracts and Reviews Series.* **74**(7): 13N - 21N.
- Samsukal, P., 2004. A preliminary study of effluent water quality in land-based abalone farms in South Africa. M.Sc. Thesis. Norwegian College of Fisheries Science, University of Tromsø, Norway. pp 67.
- SANCOR. 1996. Abalone (perlemoen) resource about to collapse. SANCOR newsletter: May-Aug. 8 – 11.
- SATT. 2006. South African tide table, published by the hydrographer South African navy Cape Town. pp 28.
- Schils, T.; De Clerck, O; Leliaert, F.; Bolton, J.J.; Coppejans, E.C., 2001. The change in macroalgal assemblages through the Saldanha Bay/ Langebaan lagoon ecosystem (South Africa). *Bot. Marina.* **44**: 295 - 307.
- Sebert, L.; Barthelemy, L.; Peyraud, C., 1984. Oxygen toxicity in trout at two seasons. *Comparative Biochemistry and Physiology A* **78**: 719-722.
- Selvamani, M.J.P.; Degnan, S.M.; Degnan, B.M., 2001. Microsatellite genotyping of individual abalone larvae: Parentage assignment in Aquaculture. *Mar. Biotech.* **3**: 478 - 485.

- Shacklock, P.F.; Croft, G.B., 1981. Effect of grazers on *Chondrus crispus* in culture. *Aquaculture* **22**: pp 331 – 342.
- Shannon, L.V., 1985. The Benguela ecosystem. 1 Evolution of the Benguela, physical features and processes. In: *Oceanography and Marine Biology. An annual review.* 23. Barnes, M. (Ed.) Aberdeen University Press. pp 105 - 182.
- Shpigel, M.; Neori, A., 1996. The integrated culture of seaweed, abalone, fish and clams in modular intensive land-based systems: I. Proportions of size and projected revenues. *Aquacultural Engineering*. **15**:(5): pp 313 – 326.
- Simons, R., 1990. Abalone farming: How should the stock be fed? In: Cook P, Britz PJ (eds) *Perlemoen farming in South Africa. Proceedings of a workshop organized by the Mariculture Association of South Africa.* pp 33 - 42.
- Simpson, B.J.A.; Cook, P.A., 1998. Rotation diets: a method of improving growth of cultured abalone using natural algal diets. *J. Shellfish Res.* **17**: 635 - 640.
- Smit, A.J., 1997. Optimising *Gracilaria verrucosa* production in a laboratory based culture system. MSc thesis, University of Port Elizabeth, Port Elizabeth, South Africa.
- Smit, A.J.; Fourie, A.M.; Robertson, B.L.; Du Preez, D.R. 2003. Control of the herbivorous isopod *Paridotea reticulata* in *Gracilaria gracilis* tank cultures. *Aquaculture*. **217**(1): 385 – 393.
- Spotte, S., 1979. *Fish and Invertebrate culture: Water management in closed systems* (2nd Ed.). John Wiley & Sons, Inc., United States of America, 179pp.
- Steinbeck, J.R.; Groff, J.M.; Friedman, C.S.; McDowell T.; Hedrick R.P., 1992. Investigations into a mortality among populations of the California black abalone, *Haliotis cracherodii*, on the central coast of California, USA. In: Shepard, S. A.;

- Tegner M. J.; Guzman del Proo S. A. (Eds.), Abalone of the World: Biology, Fisheries and Culture. Proceedings of the 1st International Abalone Symposium. Fishing News Books, Cambridge. pp. 203 - 213.
- Steinberg, J., 2005. The illicit abalone trade in South Africa. ISS Paper 105. 13pp.
- Steyn, P.P. 2000. A comparative study of the production and suitability of two *Ulva* species as abalone fodder in a commercial mariculture system. MSc Thesis. University of Port Elizabeth. South Africa. 92pp.
- Swann, L-D., 1992. A basic overview of Aquaculture: History, Water quality, Types of aquaculture and Production methods. Technical Bulletin Series **102**: 1-10.
- Sylvester, J.R., 1975. Biological considerations on the use of thermal effluents for finfish aquaculture. *Aquaculture* **6**: 1-10.
- Tacon, A.G.J., 2005. State of information on salmon aquaculture feed and the environment. Report prepared for the WWF US Initiated Salmon Aquaculture Dialogue. Available online at www.worldwildlife.org/cgi/dialogues/salmon.cfm.
- Tahil, A.S.; Juinio-Menez, M.A., 1999. Natural diet, feeding periodicity and functional response to food density of the abalone, *Haliotis asinina* L., (Gastropoda). *Aquaculture Res.* **30**: 95 - 107.
- Tarr, R., 1987. Biology and Management of South African perlemoen. *Underwater*. pp. 41 - 42.
- Tarr, R.J.Q., 1989. Abalone. In: Payne, A.I.L.; Crawford, R.J.M. (Eds.). *Oceans of Life off Southern Africa*. Vlaeberg Publishers. Cape Town. pp. 62 – 69.

- Tarr, R.J.Q., 1992. The abalone fishery of South Africa. In: Shepherd, S.A.; Tegner, M.J.; Guzman del Proo, S.A., (Eds.). *Abalone of the World. Biology, Fishery and Culture.* Fishing News Books, London. pp. 438 - 447.
- Tarr, R.J.Q.; Williams, P.V.G.; Mackenzie, A.J., 1996. Abalone, sea urchin and rock lobster: A possible ecological shift that may affect traditional fisheries. *S. A. J. Mar. Sci.* **17**: 317 - 323.
- The South African experience. In: Mshigeni, K.E.; Bolton, J.J.; Critchley, A.T.; Kiangi, G.(Eds), *Proceedings of the First International Workshop on Sustainable Seaweed Resource Development in Sub-Saharan Africa.* Published by K. Mshigen, Windhoek , Namibiai. pp. 85 - 97.
- Troell, M.; Halling, C.; Neori, A.; Chopin, T.; Buschmann, A.H.; Kautsky, N.; Yarish, C., 2003. Integrated mariculture: asking the right questions. *Aquaculture.* **226**: 69 - 90.
- Troell, M.; Kautsky, N.; Folke, C., 1999a. Applicability of integrated coastal aquaculture systems. *Ocean & Coast. Manag.* **42**(1): 63 – 69.
- Troell, M.; Robertson-Andersson, D.V.; Anderson, R.J.; Bolton, J.J.; Maneveldt, G.; Halling, C.; Probyn, T., 2006. Abalone farming in South Africa: An overview with perspectives on kelp resources, abalone feed, potential for on-farm seaweed production and socio-economic importance. *Aquaculture.* **257**: 266 - 281.
- Troell, M.; Rönnback, P.; Halling, C.; Kautsky, N.; Buschmann, A., 1999b. Ecological engineering in aquaculture: use of seaweeds for removing nutrients from intensive mariculture. *J. Appl. Phycol.* **11**: 89 – 97.

- Tronchin, E.; Anderson, R.J.; Bolton, J.J., 2003. A socio-ecological investigation of the *Gelidium* harvesting industry of the Eastern Cape Province. Proceedings of the 17th International Seaweed Symposium. Oxford University Press. pp. 115 - 121.
- Van Blaricom, G.R.; Ruediger, J.L.; Friedman, C.S.; Woodard, D.D.; Hedrick, R.P., 1993. Discovery of withering syndrome among black abalone *Haliotis cracherodii* Leach, 1814, populations at San Nicolas Island, California. J. Shellfish Res. **12**: 185 - 188.
- Viana, M.T.; López, L.M.; García-Esquivel, Z.; Mendez, E., 1996. The usage of silage made from fish and abalone viscera as an ingredient in abalone feed. Aquaculture. **140**: 87 - 98.
- White, H., 1995. Evaluation of the effect of four anaesthetics on *Haliotis midae* and their suitability for application in commercial abalone culture. MSc. thesis, Rhodes University, South Africa.
- Wickins, J.F., 1976. The tolerance of warmwater prawns to recirculated water. Aquaculture **9**, 19 - 37.
- Wikfors, G.H.; Ohno, M., 2001. Impact of algal research in aquaculture. J. Phycol. **37**: 968.
- Williams, M.J.; Bell, J.D.; Gupta, M.V.; Dey, M.; Ahmed, M.; Prein, M.; Child, S.; Gardiner, P.R., 2000. Responsible aquaculture can aid food problems. Nature. pp. 406, 673.
- Wood, A.D., 1993. Aspects of the Biology and Ecology of the South African abalone *Haliotis midae* Linnaeus, 1758 (Mollusca: Gastropoda) along the Eastern Cape and Ciskei Coast. MSc. thesis, Rhodes University, South Africa.

- Wood, A.D.; Buxton, C.D., 1996. Aspects of the biology of the abalone *Haliotis midae* (Linne, 1758) on the east coast of South Africa. I. Feeding biology. SA. J. Mar. Sci. **17**: 61 - 68.
- Wuenschel, M.J.; Werner, R.G.; Hoss, D.E., 2004. Effect of body size, temperature, and salinity on the routine metabolism of larval and juvenile spotted seatrout. J. Fish Biol. **64**: 1088-1102.
- Zar, J. H., 1999. Biostatistical Analysis. 4th Ed. Prentice-Hall. New Jersey. 663 pp.
- Zemke-White, W.L.; Ohno, M., 1999. World seaweed utilization: an end-of-century summary. J. App. Phycol. **11**: 369 – 76.
- Zhu, W.; Mai, K.; Wu, G., 2002. Thiamin requirements of juvenile abalone, *Haliotis discus hannai* Ino. Aquaculture **207**: 331 – 343.

Appendix

Table 1: ANCOVA treatment weight

H0: $\beta = \beta = \beta$ HA: all the β are not equal

	$\sum x^2$	$\sum xy$	$\sum y^2$	n	b	SS	DF
Abfeed 3	80999.82	190702	611803.2	90	2.354351	162823.7	88
Abfeed 4	80999.82	186052.4	578171.5	90	2.296949	150818.6	88
Abfeed 5	80999.82	186410.4	585903.2	90	2.301368	156904.1	88
"pooled" regression "common"						470546.4	264
regression	242999.5	563164.9	1775878		2.317556	470711.7	266
"Total regression"	756647.5	1780292	5624899	270	2.352869	1436105	268
		F	0.046367				
		$F_{0.05(1),2,264}$	3.029985				

I do not reject H0 as $F < 3.03$ ($p > 0.05$)

Table 2: ANCOVA treatment length

H0: $\beta = \beta = \beta$ HA: all the β are not equal

	$\sum x^2$	$\sum xy$	$\sum y^2$	n	b	SS	DF
Abfeed 3	80999.82	2976974	1.48E+08	90	36.75285	38854031	88
Abfeed 4	80999.82	2870877	1.38E+08	90	35.443	35784384	88
Abfeed 5	80999.82	2863050	1.39E+08	90	35.34638	38002577	88
"pooled" regression "common"						1.13E+08	264
regression	242999.5	8710901	4.25E+08		35.84741	1.13E+08	266
"Total regression"	756647.5	27537224	1.35E+09	270	36.39373	3.44E+08	268
		F	0.117169				
		$F_{0.05(1),2,264}$	3.03				

I do not reject H0 as $F < 3.03$ ($p > 0.05$)

Table 3: ANCOVA January weight

H0: $\beta = \beta = \beta$ HA: all the β are not equal

	$\sum x^2$	$\sum xy$	$\sum y^2$	n	b	SS	DF
Abfeed 3	71783.47	51951.46	53254.11	30	0.723725	15655.57	28
Abfeed 4	71783.47	51968.77	54242.56	30	0.723966	16618.95	28
Abfeed 5	71783.47	53883.72	58982.41	30	0.750642	18535.01	28
"pooled" regression						50809.53	84
"common" regression	215350.4	157803.9	166479.1		0.732778	50843.89	86
		F	0.028408				
		$F_{0.05(1),2,84}$	3.105157				

I do not reject H0 as $F < 3.11$ ($p > 0.05$)

Table 4: ANCOVA February weight

H0: $\beta = \beta = \beta$ HA: all the β are not equal

	$\sum x^2$	$\sum xy$	$\sum y^2$	n	b	SS	DF
Abfeed 3	71783.47	56121.64	83355.23	30	0.781819	39478.29	28
Abfeed 4	71783.47	53661.38	56497.42	30	0.747545	16383.12	28
Abfeed 5	71783.47	53024.03	55632.19	30	0.738666	16465.13	28
"pooled" regression						72326.54	84
"common" regression	215350.4	162807	195484.8		0.75601	72401.09	86
		F	0.043291				
		$F_{0.05(1),2,84}$	3.105157				

I do not reject H0 as $F < 3.11$ ($p > 0.05$)

Table 5: ANCOVA March weight

H0: $\beta = \beta = \beta$ HA: all the β are not equal

	$\sum x^2$	$\sum xy$	$\sum y^2$	n	b	SS	DF
Abfeed 3	71783.47	51854.66	68818.96	30	0.722376	31360.39	28
Abfeed 4	71783.47	50518.14	49771.87	30	0.703757	14219.37	28
Abfeed 5	71783.47	49383.46	48193.77	30	0.68795	14220.41	28
"pooled" regression						59800.17	84
"common" regression	215350.4	151756.3	166784.6		0.704695	59842.8	86
		F	0.029942				
		$F_{0.05(1),2,84}$	3.105157				

I do not reject H0 as $F < 3.11$ ($p > 0.05$)

Table 6: ANCOVA January length

H0: $\beta = \beta = \beta$ HA: all the β are not equal

	$\sum x^2$	$\sum xy$	$\sum y^2$	n	b	SS	DF
Abfeed 3	71783.47	810449	12775686	30	11.29019	3625562	28
Abfeed 4	71783.47	805855.8	13080852	30	11.2262	4034151	28
Abfeed 5	71783.47	827331.4	14067509	30	11.52538	4532203	28
"pooled" regression "common" regression	215350.4	2443636	39924047		11.34726	12191916	84

F 0.012275

$F_{0.05(1),2,84}$ 3.105157

I do not reject H0 as $F < 3.11$ ($p > 0.05$)

Table 7: ANCOVA February length

H0: $\beta = \beta = \beta$ HA: all the β are not equal

	$\sum x^2$	$\sum xy$	$\sum y^2$	n	b	SS	DF
Abfeed 3	71783.47	894893.9	21084151	30	12.46657	9927889	28
Abfeed 4	71783.47	839668.4	13687101	30	11.69724	3865299	28
Abfeed 5	71783.47	826308	13370362	30	11.51112	3858633	28
"pooled" regression "common" regression	215350.4	2560870	48141614		11.89164	17651821	84

F 0.087643

$F_{0.05(1),2,84}$ 3.105157

I do not reject H0 as $F < 3.11$ ($p > 0.05$)

Table 8: ANCOVA March length

H0: $\beta = \beta = \beta$ HA: all the β are not equal

	$\sum x^2$	$\sum xy$	$\sum y^2$	n	b	SS	DF
Abfeed 3	71783.47	792718.2	16065971	30	11.04319	7311836	28
Abfeed 4	71783.47	764152.4	11446886	30	10.64524	3312299	28
Abfeed 5	71783.47	745004.1	11261436	30	10.37849	3529418	28
"pooled" regression "common" regression	215350.4	2301875	38774293		10.68897	14169616	84

F 0.047668

$F_{0.05(1),2,84}$ 3.105157

I do not reject H0 as $F < 3.11$ ($p > 0.05$)

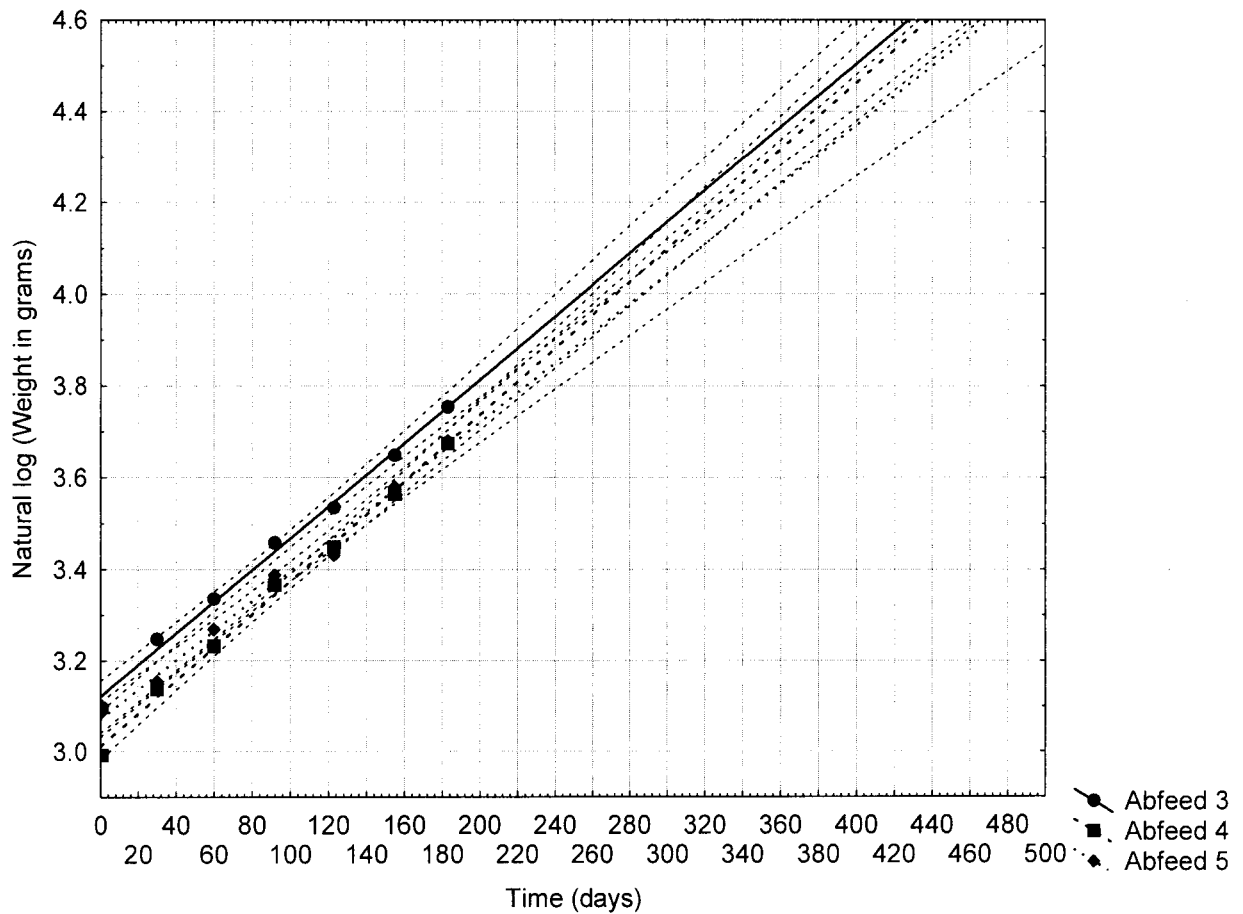


Figure 1: Estimated time to harvest (100 grams) for the three treatments calculated from the natural log of the weight in grams

Abfeed 3[®]: $y = 3.1217 + 0.0035*x$; $r = 0.9970$, $p < 0.001$; $r^2 = 0.9941$, 0.95 Confidence interval.

Abfeed 4[®]: $y = 3.0113 + 0.0036*x$; $r = 0.9980$, $p < 0.001$; $r^2 = 0.9959$, 0.95 Confidence interval.

Abfeed 5[®]: $y = 3.0712 + 0.0032*x$; $r = 0.9955$, $p < 0.001$; $r^2 = 0.9910$, 0.95 Confidence interval.

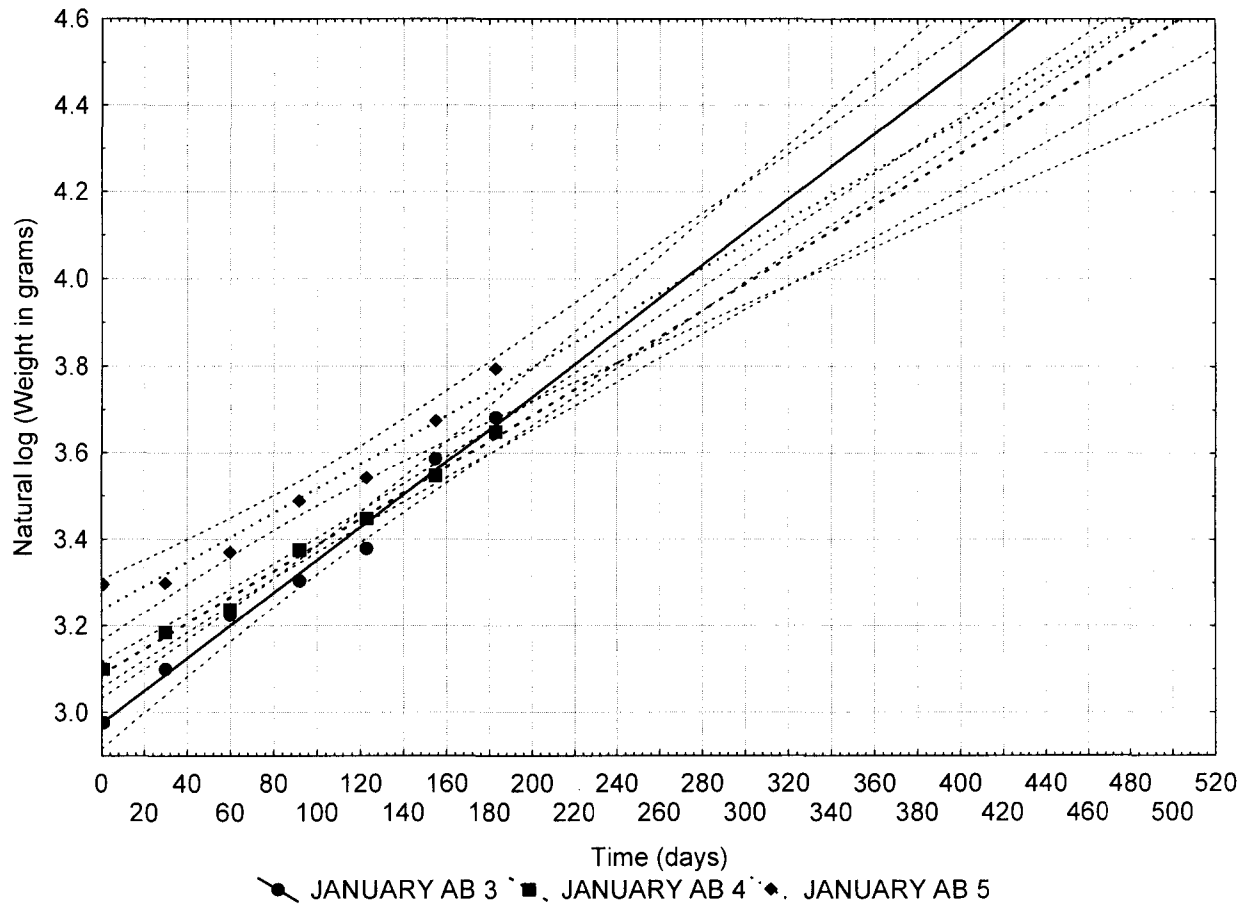


Figure 2: Estimated time to harvest (100 grams) for the January spawned cohorts calculated from the natural log of the weight in grams

JANUARY AB[®] 3: $y = 2.9739 + 0.0038x$; $r = 0.9926$, $p = 0.00001$; $r^2 = 0.9853$, 0.95 Confidence interval.

JANUARY AB[®] 4: $y = 3.0859 + 0.003x$; $r = 0.9970$, $p < 0.001$; $r^2 = 0.9939$, 0.95 Confidence interval.

JANUARY AB[®] 5: $y = 3.2356 + 0.0028x$; $r = 0.9808$, $p = 0.00010$; $r^2 = 0.9619$, 0.95 Confidence interval.

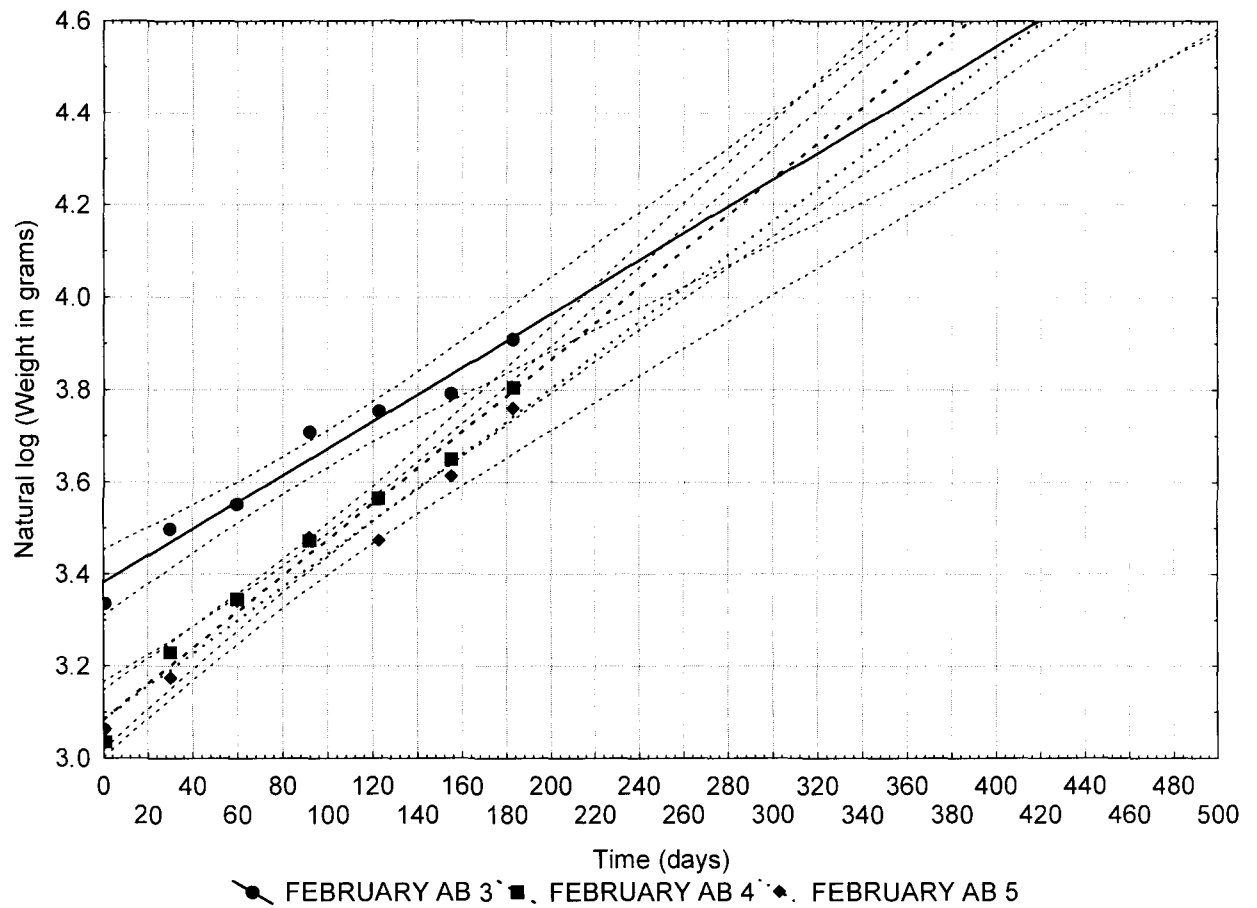


Figure 3: Estimated time to harvest (100 grams) for the February spawned cohort calculated from the natural log of the weight in grams

FEBRUARY AB[®] 3: $y = 3.3816 + 0.0029*x$; $r = 0.9817$, $p = 0.00009$; $r^2 = 0.9637$, 0.95
 Confidence interval.
 FEBRUARY AB[®] 4: $y = 3.083 + 0.0039*x$; $r = 0.9917$, $p = 0.00001$; $r^2 = 0.9835$, 0.95
 Confidence interval.
 FEBRUARY AB[®] 5: $y = 3.0833 + 0.0036*x$; $r = 0.9846$, $p = 0.00006$; $r^2 = 0.9694$, 0.95
 Confidence interval.

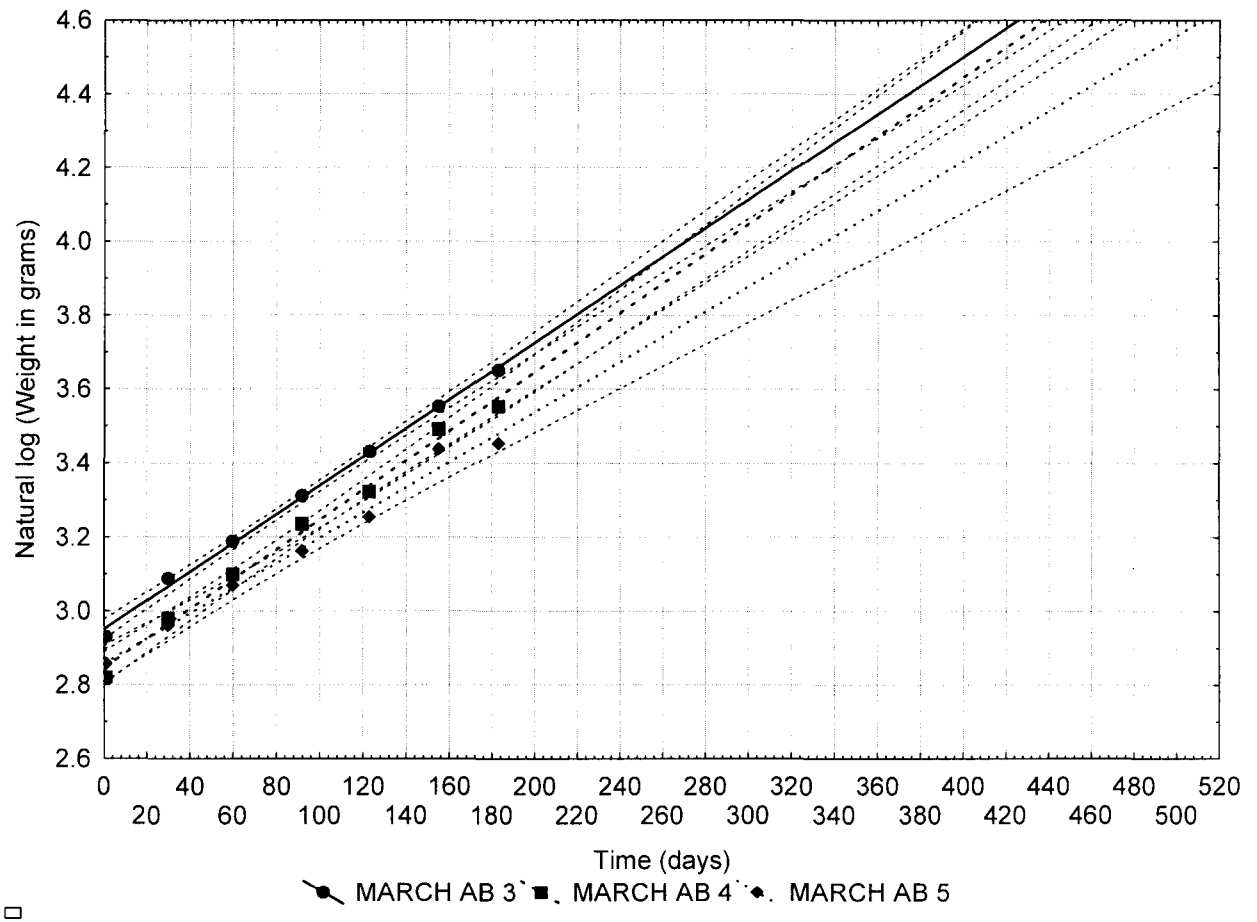


Figure 4: Estimated time to harvest (100 grams) for the March spawned cohort calculated from the natural log of the weight in grams

MARCH AB[®] 3: $y = 2.9507 + 0.0039*x$; $r = 0.9985$, $p < 0.001$; $r^2 = 0.9970$, 0.95
Confidence interval.

MARCH AB[®] 4: $y = 2.8462 + 0.004*x$; $r = 0.9963$, $p < 0.001$; $r^2 = 0.9926$, 0.95
Confidence interval.

MARCH AB[®] 5: $y = 2.8572 + 0.0034*x$; $r = 0.9935$, $p = 0.00001$; $r^2 = 0.9870$, 0.95
Confidence interval.

Table 9: ANCOVA Treatment weight

H0: $\beta = \beta$

HA: $\beta \neq \beta$

	$\sum x^2$	$\sum xy$	$\sum y^2$	n	b	SS	DF
FTU	80999.82	190702.012	611803.2	90	2.354350994	162823.7	88
RECIRC	80999.82	189746.102	610847.1	90	2.342549615	166357.4	88
			$(s^2_{y.x})_p$	1870.347			
			$Sb_1 - b_2$	0.214899			
			t	0.054916			
			v	176			
			$t_{0.05(2),176}$	1.973534			

Do not reject H0, $t < t_{0.05(2),176}$ ($p > 0.05$)

Table 10: ANCOVA Treatment length

H0: $\beta = \beta$

HA: $\beta \neq \beta$

	$\sum x^2$	$\sum xy$	$\sum y^2$	n	b	SS	DF
FTU	80999.82	2976974	1.48E+08	90	36.75284519	38854031	88
RECIRC	80999.82	2963933	1.48E+08	90	36.59184912	40035542	88
			$(s^2_{y.x})_p$	448236.2			
			$Sb_1 - b_2$	3.326798			
			t	0.048394			
			v	176			
			$t_{0.05(2),176}$	1.973534			

Do not reject H0, $t < t_{0.05(2),176}$ ($p > 0.05$)

Table 11: ANCOVA January spawned cohort weight

H0: $\beta = \beta$

HA: $\beta \neq \beta$

	$\sum x^2$	$\sum xy$	$\sum y^2$	n	b	SS	DF
FTU	71783.47	51951.459	53254.11	30	0.723724581	15655.57	28
RECIRC	71783.47	51597.897	53292.42	30	0.718799178	16203.9	28
			$(s^2_{y,x})_p$	568.919			
			$Sb_1 - b_2$	0.125901			
			t	0.039121			
			v	56			
			$t_{0.05(2),56}$	2.003241			

Do not reject H0, $t < t_{0.05(2),56}$ ($p > 0.05$)

Table 12: ANCOVA February spawned cohort weight

H0: $\beta = \beta$

HA: $\beta \neq \beta$

	$\sum x^2$	$\sum xy$	$\sum y^2$	n	b	SS	DF
FTU	71783.47	56121.644	63954.21	30	0.781818527	20077.27	28
RECIRC	71783.47	55311.633	61936.31	30	0.770534443	19316.79	28
			$(s^2_{y,x})_p$	703.4654			
			$Sb_1 - b_2$	0.139999			
			t	0.080601			
			v	56			
			$t_{0.05(2),56}$	2.003241			

Do not reject H0, $t < t_{0.05(2),56}$ ($p > 0.05$)

Table 13: ANCOVA March spawned cohort weight

H0: $\beta = \beta$

HA: $\beta \neq \beta$

	$\sum x^2$	$\sum xy$	$\sum y^2$	n	b	SS	DF
FTU	71783.47	51854.663	52842.39	30	0.722376136	15383.82	28
RECIRC	71783.47	52073.082	54358.95	30	0.725418878	16584.15	28
			$(s^2_{y,x})_p$	570.8567			
			$Sb_1 - b_2$	0.126115			
			t	0.02413			
			v	56			
			$t_{0.05(2),56}$	2.003241			

Do not reject H0, $t < t_{0.05(2),56}$ ($p > 0.05$)

Table 14: ANCOVA January spawned cohort length

H0: $\beta = \beta$

HA: $\beta \neq \beta$

	$\sum x^2$	$\sum xy$	$\sum y^2$	n	b	SS	DF
FTU	71783.47	810449	12775686	30	11.29019041	3625562	28
RECIRC	71783.47	799699.2	12776167	30	11.1404367	3867169	28
			$(s^2_{y.x})_p$	133798.8			
			$Sb_1 - b_2$	1.930762			
			t	0.077562			
			v	56			
			$t_{0.05(2),56}$	2.003241			

Do not reject H0, $t < t_{0.05(2),56}$ ($p > 0.05$)

Table 15: ANCOVA February spawned cohort length

H0: $\beta = \beta$

HA: $\beta \neq \beta$

	$\sum x^2$	$\sum xy$	$\sum y^2$	n	b	SS	DF
FTU	71783.47	894893.9	16179264	30	12.46657466	5023002	28
RECIRC	71783.47	877868.9	15522171	30	12.2294025	4786359	28
			$(s^2_{y.x})_p$	175167.2			
			$Sb_1 - b_2$	2.20917			
			t	0.107358			
			v	56			
			$t_{0.05(2),56}$	2.003241			

Do not reject H0, $t < t_{0.05(2),56}$ ($p > 0.05$)

Table 16: ANCOVA March spawned cohort length

H0: $\beta = \beta$

HA: $\beta \neq \beta$

	$\sum x^2$	$\sum xy$	$\sum y^2$	n	b	SS	DF
FTU	71783.47	792718.2	12336662	30	11.04318673	3582527	28
RECIRC	71783.47	806812.7	12968602	30	11.23953445	3900402	28
			$(s^2_{y.x})_p$	133623.7			
			$Sb_1 - b_2$	1.929499			
			t	0.10176			
			v	56			
			$t_{0.05(2),56}$	2.003241			

Do not reject H0, $t < t_{0.05(2),56}$ ($p > 0.05$)

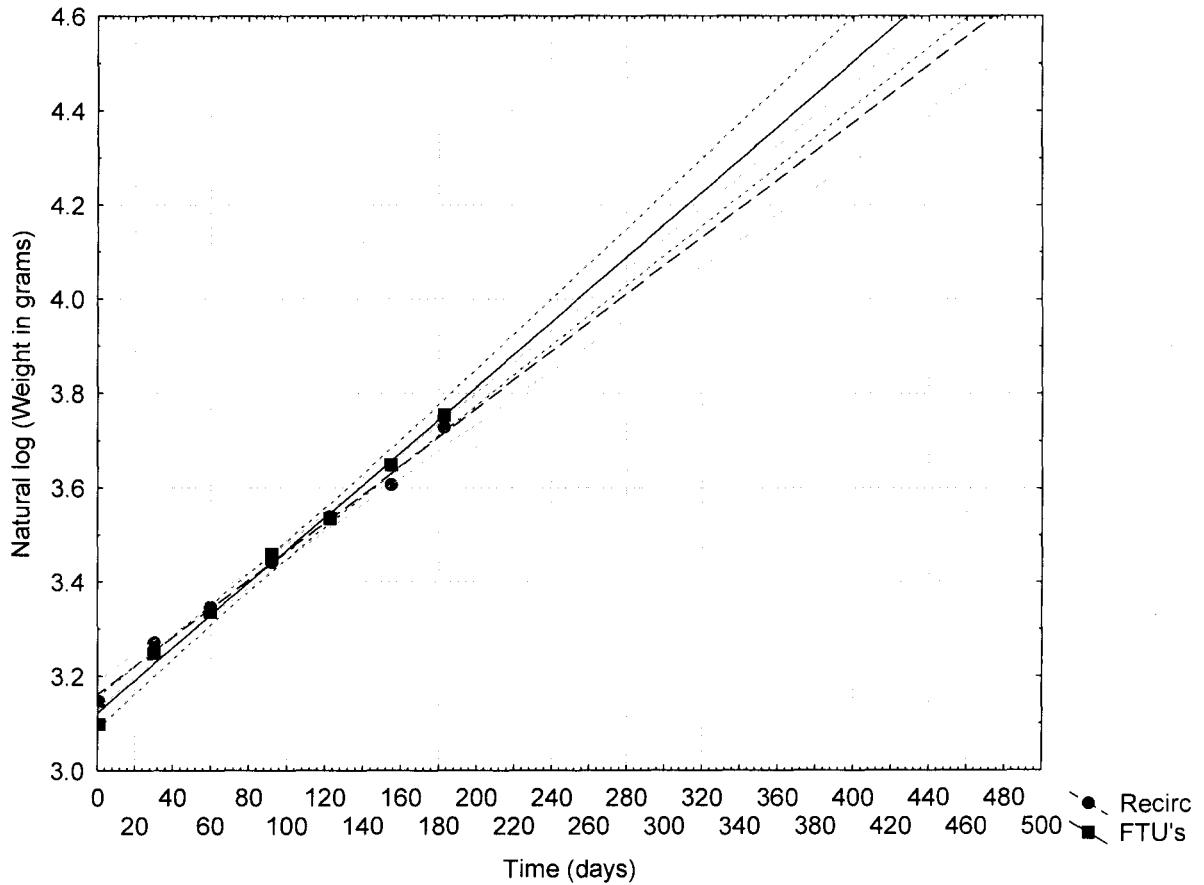


Figure 5: Estimated time to harvest (100 grams) for abalone in the 50 % Recirculation system and the FTUs, calculated from the natural log of the weight in grams

Recirc: $y = 3.1606 + 0.003*x$; $r = 0.9971$, $p < 0.001$; $r^2 = 0.9942$, 0.95 Confidence interval.

FTUs: $y = 3.1217 + 0.0035*x$; $r = 0.9970$, $p < 0.001$; $r^2 = 0.9941$, 0.95 Confidence interval.

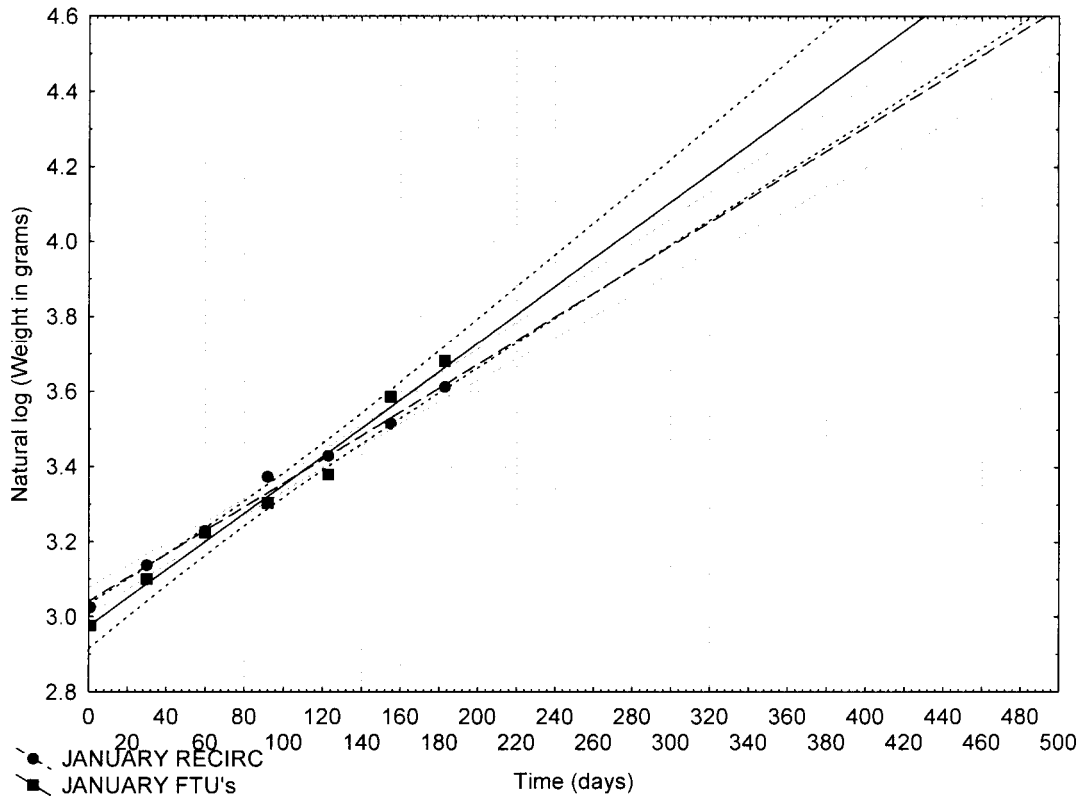


Figure 6: Estimated time to harvest for abalone in the January spawned cohorts in the 50 % Recirculation system and the FTUs

JANUARY RECIRC: $y = 3.0404 + 0.0032*x$; $r = 0.9955$, $p < 0.001$; $r^2 = 0.9910$, 0.95 Confidence interval.

JANUARY FTU's: $y = 2.9739 + 0.0038*x$; $r = 0.9926$, $p = 0.00001$; $r^2 = 0.9853$, 0.95 Confidence interval.

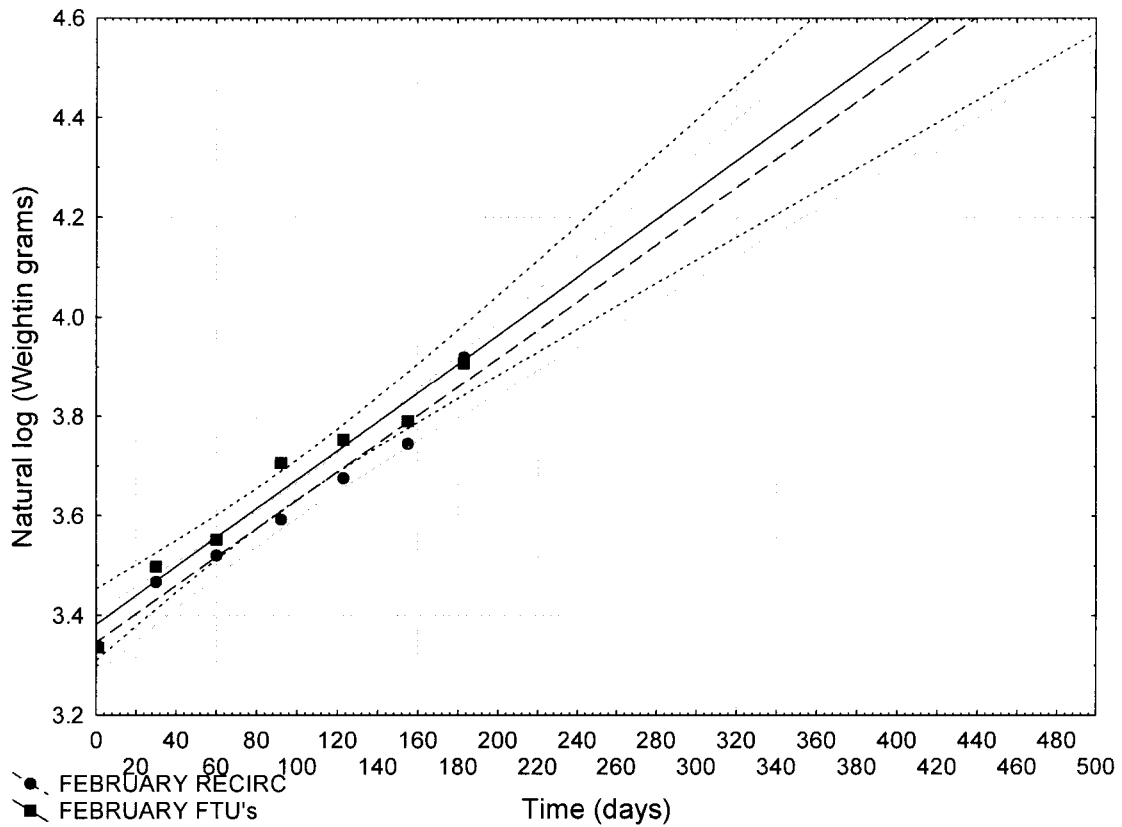


Figure 7: Estimated time to harvest for abalone in the February spawned cohorts in the 50 % Recirculation system and the FTUs

FEBRUARY RECIRC: $y = 3.3456 + 0.0029*x$; $r = 0.9848$, $p = 0.00005$; $r^2 = 0.9699$, 0.95 Confidence interval.

FEBRUARY FTUs: $y = 3.3816 + 0.0029*x$; $r = 0.9817$, $p = 0.00009$; $r^2 = 0.9637$, 0.95 Confidence interval.

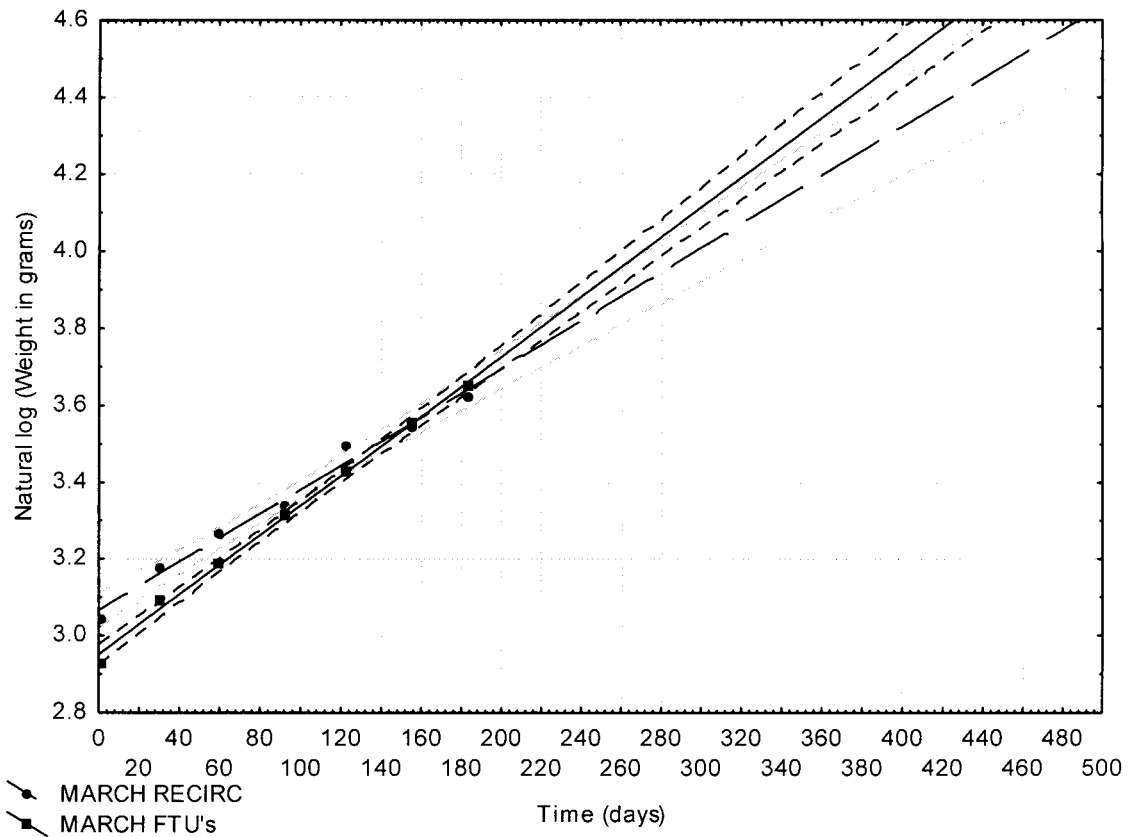


Figure 8: Estimated time to harvest for abalone in the March spawned cohorts in the 50 % Recirculation system and the FTUs

MARCH RECIRC: $y = 3.0659 + 0.0031*x$; $r = 0.9938$, $p = 0.00001$; $r^2 = 0.9876$, 0.95 Confidence interval.

MARCH FTUs: $y = 2.9507 + 0.0039*x$; $r = 0.9985$, $p < 0.001$; $r^2 = 0.9970$, 0.95 Confidence interval.