THE CULTIVATION OF *ULVA LACTUCA* (CHLOROPHYTA) IN AN INTEGRATED AQUACULTURE SYSTEM, FOR THE PRODUCTION OF ABALONE FEED AND THE BIOREMEDIATION OF AQUACULTURE EFFLUENT

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DECLARATION

I declare that this thesis is my own, unaided work and has not been submitted in this or any form to another university. Where use has been made of the research of others, it has been duly acknowledged in the text. Experimental work discussed in this thesis was carried out under the supervision of Associate Prof. J. J. Bolton of the Department of Botany, Dr. R. J. Anderson of the seaweed unit, Marine and Coastal Management and Dr. T. Probyn Marine and Coastal Management. University of Cape Town.

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August 2003

We learn more by looking for the answer to a question and not finding it.

Than we do from learning the answer itself.

Lloyd Alexander

To my family

Thanks for all your love support and encouragement, without which,

this would have been impossible

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The following aspects of this thesis have been presented:

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(ii) Robertson-Andersson, D. V. 2004. Nutritional content of seaweeds and seasonality. AFASA Project Review Conference. Hermanus.

(iii) Robertson-Andersson, D. V.; Bolton, J. J.; Anderson, R. J. & Probyn, T. 2003. You are what you eat: *Ulva* cultivation in aquaculture effluent. University of the Western Cape Seminar Series. Cape Town.

(iv) Robertson-Andersson, D. V.; Bolton, J. J.; Anderson, R. J. & Probyn, T. 2003. *Ulva* cultivation in aquaculture effluent or you are what you eat. Zoology departmental seminars. University of Cape Town.

(v) Robertson-Andersson, D. V.; Bolton, J. J.; Anderson, R. J. & Probyn, T. 2003. *Ulva* cultivation in aquaculture effluent. AFASA Project Review Conference. Stellenbosch.

(vi) Robertson-Andersson, D. V.; Bolton, J. J.; Anderson, R. J. & Probyn, T. 2003. Cultivation of *Ulva* in abalone aquaculture effluent in South Africa. University of Stockholm lecture series. Stockholm. Sweden. 2003.

(vii) Bolton, J. J.; Robertson-Andersson, D. V.;Letio, D.; Njobeni, A.; Anderson, R. J. & Probyn, T. The effects of Kelpak ® on seaweed growth. 2003. 19th Congress of the Phycological Society of Southern Africa, Port Elizabeth.

(viii) Maneveldt, G.W.; Bolton, J. J.; Ruck, K.; Naidoo K.; A. Njobeni & Robertson-Andersson D. 2002. Integrated mariculture systems: Economic potential without fear of loss of biological diversity. Pacem in Maribus 2002. CapeTown, South Africa, December.

(ix) Robertson-Andersson, D. V.; Njobeni, A.; Bolton, J. J.; Anderson, R. J. & Probyn, T. 2002. Experimental growth of two seaweeds (*Gracilaria* and *Ulva*) on a west coast abalone farm. 6TH Conference of the Aquaculture Association of Southern Africa. Stellenbosch. Pp 16.

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(xi) Robertson-Andersson, D. V.; Njobeni, A.; Bolton, J. J.; Anderson, R. J. & Probyn, T. 2002. Pilot commercial scale, cultivation of *Ulva* and *Gracilaria* on an abalone farm at Danger Point (Cape South Coast). Southern African Marine Science Symposium. Swakopmund, Namibia. Pp87.

(xii) Njobeni, A.; Robertson-Andersson, D. V.; Bolton, J. J.; Anderson, R. J. & Probyn, T. 2002. Experimental growth of two seaweeds, *Ulva* (Chlorophyta) and *Gracilaria* (Rodophyta) in sea water and farm effluent. Southern African Marine Science Symposium. Swakopmund, Namibia. Pp75.

(xiii) Robertson-Andersson, D. V.; Bolton, J. J. & Anderson, R. J. 2002. Tank Based cultivation of *Ulva lactuca* (Ulvacaceae, Chlorophyta) on two western Cape abalone farms. 18th Congress of the Phycological Society of Southern Africa, Cape Town. 18:pp28.

(xiv) Robertson-Andersson, D. V.; Bolton, J. J. & Anderson, R. J. 2001. The development and testing of an integrated abalone/seaweed aquaculture system, using *Ulva lactuca* (Chlorophyta) for the production of abalone feed and the bioremediation of abalone waste. 2nd SANCOR Student workshop in the Western/Northern Cape. University of the Western Cape.

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ABSTRACT

ABSTRACT

Significant effort has been put into the development of cost-effective abalone cultivation systems in South Africa, but the limited availability of suitable seaweed for abalone food is an obstacle to future development. The aim of this study was to investigate whether land-based integrated aquaculture (tank cultivation) and seaweed culture using *Ulva lactuca* in aquaculture effluent was feasible. This study was carried out at two abalone farms: Danger Point (I & J) (140 km east of Cape Town) and Jacobsbaai (JSP) (120 km north of Cape Town, South Africa). Both farms want to supplement the abalone feed with *Ulva* and investigate its potential for recirculation. *Ulva* is one of the simplest seaweeds to cultivate as it grows vegetatively. It would have a further benefit in its capacity to absorb nutrients and thus improve water quality of the aquaculture effluent.

Results show that abalone effluent medium alone is insufficient for seaweed cultivation. Turbot effluent media has far more nutrients for seaweed but turbidity due to incomplete turbot feed pellet assimilation could be a problem. The most effective cultivation media on both farms is a fertilized effluent growth medium.

This study established that water exchange rates are important in assuring an optimum nutrient supply for the seaweed. At high water exchange rates (20 Volume Exchanges (VE).d $^{-1}$), Specific Growth Rate (SGR) in turbot and seawater treatments were not significantly different despite a significant difference in water nutrient concentration. Maximum nutrient removal occurs at both 12 and 20 volume exchanges per day, using a stocking density of 3 kgm^{-2} on both farms. Approximately 70 % of the ammonium is removed during the day and 60 % at night at JSP in both turbot and abalone treatments, while at I & J in the abalone treatments, 90 % and 80 % of the ammonium is removed during the day and night respectively. The diel fluctuation in dissolved oxygen is above critical levels (6 mg.l) for abalone respiration at night, thus indicating that direct recirculation is possible.

The seaweeds grown at a high water exchange rate at JSP were all phosphate limited except in winter, when background phosphate concentrations increased. Thus, fertilizers like Maxiphos can benefit the alga, especially if the phosphate ratio were to be increased in summer.

Maintaining a pH below 9 is important in maintaining seaweed health and should become an integral part of the farm management protocol.

Myrionema strangulans is an epiphyte newly recorded for South Africa during this project and has potentially devastating effects for culture of *Ulva*. Pulse fertilization of culture tanks combined with seasonal shading (late September to early February) using 20 % shade cloth controls epiphytic and fouling algal growth, particularly *Myrionema*. Shading also improves thallus condition, increases tissue nitrogen and decreases pH. Shading with a 50 % shade cloth however, has a significant reduction on uptake of ammonium and phosphate by the seaweed as well as decreasing the SGR and resulting in a species dominace switch from *Ulva lactuca* to *Ulva capensis*.

There is a decrease in SGR when scaling up tank sizes, but this decrease can be optimized by cultivating the alga in pulse fertilized effluent water.

Growing *Ulva* in effluent media increases its tissue nitrogen and thus protein content, increasing it above levels found in nature (average protein content in turbot = 49.8 %) and improving it as a source of protein for cultured abalone. A consistent relationship between tissue nitrogen and thallus colour was determined and can be used by mariculture farmers to assess the nutrient quality of *Ulva* as a food source for abalone which has important benefits for *Ulva* aquaculture.

On the I & J farm the chosen stocking density (2 $kg.m^{-2}$) produced maximum SGR and yields. At JSP the chosen stocking density (2 $kg.m^{-2}$) was too high and a stocking density of 1 $kg.m^{-2}$ would have optimized SGR. Seasonal effects on stocking density were not investigated.

Addition of Kelpak ® concentration (commercial kelp extract) of 1: 2 500 pulse fed once a week, increases SGR. This study has shown that Kelpak® in addition to fertilizer may have commercial potential in the seaweed mariculture industry.

These results confirm that *Ulva* is exceptionally suitable for intensive culture in different types of nutrient loaded water, and that its cultivation on an abalone farm could have significant economic benefits. For example a 50 ton abalone farm feeding protein enriched *Ulva* could decrease the production time from 5 years to 3.6 year which equates to a savings of between R 800 000 - R1 300 000.

CHAPTER 1

INTRODUCTION

INTRODUCTION

Aquaculture is the term used for the growing of aquatic flora and fauna in marine, brackish or fresh water (Swift, 1985). Mariculture refers to the growing of aquatic flora and fauna in marine or brackish water. In the past aquaculturists tended to concentrate on one species, however more interest is being shown in integrated aquaculture also known as polyculture (co-cultivation of species in effluent waters) (Ajisaka & Chiang, 1993; Shpigel *et al.* 1993 and Neori & Shpigel, 1999). In this type of farming (land-based integrated aquaculture) fish, shellfish, crustaceans or macro-algae are no longer cultivated in isolation, but rather serve as functional components within the farming system. One aspect of polyculture refers to the utilization of effluent water from culture tanks (such as abalone, fish and prawns) or effluent from faecal production for the additional culture of organisms capable of utilizing low levels of dissolved or particulate organic matter (such as seaweeds and filter feeders). Typical land-based mariculture production has moved away from monocultures and seaweeds are being used as biofilters for effluent water from other farmed animal species. Seaweeds act as biofilters by removing excess dissolved inorganic nitrogen (DIN) and phosphates while simultaneously oxygenating the cultivation medium (Neori *et al.* 1996; Wildman, 1999). This process of improving water quality, with approximately the same nutrient status and temperature as the source waters (though dependant on the culture system used), sometimes has the crucial benefit of extending water residence times within a system, which results in an overall increase in productivity or reduction in running costs. Most importantly, the use of seaweeds as biofilters lessens the negative impact of nutrient loading on the external environment, a factor that is becoming increasingly economically significant as companies are being forced to carry their external polluting costs (Vandermeulen & Gordin, 1990).

Ulva species, commonly known as "sea lettuce", have been used as biofilters for marine fishpond effluents (Cohen & Neori, 1991; Neori *et al.* 1991; Neori *et al.* 1996). The alga is very efficient at removing nitrogenous compounds from wastewater, and the effluents in return support the growth of the alga (Ryther *et al.* 1975;; Cohen & Neori, 1991; Neori *et al.* 1991; Jimenez Del Rio *et al.* 1996;

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Neori *et al.* 1996; Shpigel *et al.* 1997; Goldberg *et al.* 1998). Goldberg *et al.* (1998) recorded a removal of 95 % of the ammonium contained in fish farm effluent in which *Ulva rigida* was grown. The algae which are grown in these nutrient enriched waters are also very high in protein content and could be used for fish aquaculture, such as the catfish *Plotosus* sp. or *Chrysichthys* sp and carp *Chiloscyllium* sp. (Boyd. 1990) or abalone *Haliotis midae* Linn (Simpson & Cook, 1998; Steyn, 2000).

Before integrated aquaculture was used for the purpose of nutrient removal, several other methods were used. These include microbial oxidation, (Jimenez Del Rio *et al.* 1996) or flushing (Vandermeulen & Gordin, 1990). Of these methods, microbial oxidation by means of sludge techniques requires long retention times (Jimenez Del Rio *et al.* 1996). Flushing is also impractical because of the expenses involved (manpower, pumping electricity), especially in cases where enclosed ponds or culture tanks are used (Vandermeulen & Gordin, 1990). Current techniques for the removal of effluent particulate matter (PM) in aquaculture systems involve mechanical removal by sedimentation and micro-sieves. Sedimentation, however, has proved to be very inefficient (Shpigel *et al.* 1997), while micro-sieves are more efficient but expensive and require frequent maintenance (Shpigel *et al.* 1997). Thus, a method that would be most viable would be one that can absorb DIN and PM from the system at minimal costs, while allowing biofiltration of high volumes of water in short time periods. This can be achieved by using seaweeds and suspension feeders as biofilters, which can be grown as an additional crop (Shpigel *et al.* 1997). Bivalves improve the water quality by removing particles and phytoplankton through filter feeding and by increasing particle sedimentation through repacking into pseudofaeces (Shpigel *et al.* 1997). Both processes reduce turbidity. At high densities, bivalves have been shown to be the main factor controlling seston concentration in natural waters (Shpigel *et al.* 1997 Parker *et al.* 1999 and Grosholz, 2002). Seaweeds improve water quality by removing DIN and other nutrients from the water (Cohen & Neori, 1991; Neori *et al.* 1991; Jimenez Del Rio *et al.* 1996; Neori *et al.* 1996; Shpigel *et al.* 1997; Goldberg *et al.* 1998). Benefits of this include reducing the potential for local eutrophication,

reducing potentially toxic byproducts (NH_3 & NO_2) under conditions of recirculation as well as the production of an additional crop.

In South Africa, mariculture is still in its infancy, with the predominant product in the temperate regions being the South African abalone *H. midae*. At present, there are 16 rights to farm abalone which have been awarded by Marine and Coastal Management (M&CM) with a further two under construction (Bennett, 2002). Twelve of these farms are in the Western Cape Province (Stuttaford, 1997; Bennett, 2002). These farms have the luxury of access to large stocks of kelp (*Ecklonia maxima* and *Laminaria pallida*), which provide an easily accessible source of abalone fodder. Of the 12 farms that comprise the Abalone Farmers Association of Southern Africa (AFASA), 4 use kelp exclusively as fodder (Bennett, 2002). The development of artificial feed (ABFEED®) for abalone has resulted in many of the 7 of the AFASA member farms on the west coast feeding the abalone a mixture of kelp and artificial feed (Bennett, 2002). The drawback of artificial feed, is that it gives the abalone a very light coloured shell and meat, which is undesirable in the Far Eastern markets and thus reduces the price of the abalone considerably. A mixed diet of algae however, results in the meat having a red to brown colour, which is the ideal condition for export.

In South Africa there are only two integrated (animal and seaweed) mariculture farms, namely: Marine Growers (Pty.) Ltd. in Port Elizabeth and Wild Coast Abalone in Haga Haga near East London (Bennett, 2002). Here *Gracilaria gracilis* and *Ulva rigida* are cultivated in tanks alongside abalone *Haliotis midae* (Hampson, 1998; Steyn 2000). These farms do not have access to the large kelp beds that the farms on the west coast have. With the water being warmer on the east coast due to the influence of the warm Agulhas current, the average temperatures in the grow out tanks on these farms are warmer than those on the west coast. While this can lead to an increase in the growth rate of the abalone, water temperatures higher than 18° C result in the artificial feed fermenting in the stomachs of the abalone, and cause high abalone mortalities (Steyn 2000). The cultivated seaweeds therefore provide the abalone with a

source of nutrition, while simultaneously acting as a biofilter for abalone effluent water (Hampson, 1998).

By integrating seaweeds with abalone culture, a number of features increase the ecological sustainability of the aquaculture system:

- The use of the same water for the seaweeds and the abalone cultures reduces seawater requirements by half (when compared to two separate systems) which will in turn decrease pumping costs.
- Biofiltration and recycling of the abalone nutrient excretions by seaweeds reduces both the nutrient input requirements and the overall impact of the aquaculture operation.
- The use of biofilter grown seaweeds reduces the need for the destructive harvesting of natural seaweed beds and encourages "good farm management" (having an alternative food source if natural seaweed stocks are compromised.)
- The chemical composition of the cultured seaweeds and hence their nutritional value to the algivores is controllable.
- Recent analysis by Simpson and Cook (1997: 1998), indicated that growth rates of the South African abalone *H. midae* were significantly higher when cultivated with mixed algal diets than when cultivated with single species diets. Thus, *Ulva* could serve to supplement the current, predominantly kelp (*Ecklonia maxima* Papenfuss) based diet of the abalone.
- The west coast of South Africa is often subject to toxic algal blooms (Pitcher, 1998). The threat of shellfish poisoning caused by these blooms is considerably reduced with increased water residence times, particularly if the farm can be isolated from an external seawater source for the period in which the bloom is toxic. The biofiltering function of seaweeds during recirculation could be crucial.

The use of polyculture is being implemented in mariculture operations around South Africa. However, each farm is unique according to its environment and operational management procedures. In order to optimize production, a

comprehensive understanding of the immediate interacting physical and biological variables occurring on the farms is essential.

The principal objective of this study was to investigate the prospect of incorporating *Ulva* sp. cultivation in an existing abalone *H. midae* and turbot *Confulmus maximus* culture system, on a west coast mariculture farm (Jacobs Bay Sea Products) and an abalone farm (Irvin and Johnson – Abalone culture division, Danger Point) on the Cape south coast. The two farms were chosen to identify any differences in growth rates and biochemical composition of the alga when grown under two differing environmental and operational conditions (south coast and west coast).

Growth trials were run over a year to accommodate seasonal differences and under four different growth regimes to evaluate different nutrient supply options/strategies. The aims of this study were:

- To investigate seasonal changes in growth rates, yields and chemical composition of *Ulva* when cultured in different growth media.
- To investigate optimum stocking densities for the different growth media.
- To evaluate differences in growth rates and growth conditions between a west coast and south coast farm.
- To evaluate the effect of tank size on Specific Growth Rates (SGR).
- To investigate uptake rates of ammonium, phosphate, nitrate and nitrite and the relationship with *Ulva* stocking.
- To investigate the influence of fertilizer and commercially produced liquid kelp concentrate (KELPAK ©) on the growth rates and tissue nitrogen and phosphorus content of *Ulva*.
- To investigate the effect of shading on SGR, nutrient uptake and tissue nitrogen in *Ulva*.
- To provide management guidelines for the farmers for optimum *Ulva* growth and biofiltering capacity.

The taxonomy and general biology of *Ulva* and various techniques relevant to its cultivation as well as biological, environmental and physical factors controlling growth are discussed in the following chapter.

CHAPTER 2

LITERATURE REVIEW

LITERATURE REVIEW

2.1 INTRODUCTION

This literature review is divided into four sections:

- 2.2. General overview of *Ulva*
- 2.3 Cultivation methods
- 2.4 Key Biological and Environmental Parameters in land-based tank cultivation of *Ulva*
- 2.5 Abalone mariculture.

2.2 General overview of *Ulva*

2.2.1 Taxonomy

Hoek *et al* (1995), provided a recent review of the Chlorophyta including molecular, morphological and ultra-structural evidence. Earlier work on the phylogenetic relationships in the green algae had largely been based on ultra-structural studies of cytokinesis, the flagellar apparatus and mitosis (e.g. Stewart & Mattox, 1978; Mattox & Stewart, 1984; O' Kelly & Floyd, 1984 and Zechmann *et al.* 1990). Stewart & Mattox (1978) erected the class Ulvophyceae, which encompassed *Ulva* and other related genera. This class (sensu Zechmann *et al.* 1990 & Hoek *et al.* 1995) was circumscribed to included only two orders: the Ulotrichales (which Hoek *et al.* 1995, referred to as the Codiolales) and the Ulvales. Debate surrounding members of these two orders has been ongoing. Papenfuss (1960) and Tanner (1981) noted that elevation of Ulvaceae to ordinal rank by Blackman & Tansley (1902) was influenced by the discovery that the life history of some species of Ulvales was very different in that they possessed an isomorphic alteration of generations when compared to the Ulotrichales life history. Hoek *et al.* (1995) listed characteristics that distinguish the Ulotrichales from the Ulvales (See Table 1.1).

Table 2.1: Distinguishing characteristics of the Ulotrichales and the Ulvales (From Hoek *et al.* 1995).

ULOTRICHALES	ULVALES
Monostromatic	Distromatic
Haplontic life history	Diplohaplontic life history
Heteromophic	Isomorphic
Isogamous or Anisogamous	Anisogamous
Zoids with scales	No scales
Possess a codiolum phase	

Womersley, 1984; Bold & Wynne, 1985; Silva *et al.* 1996; Stegenga *et al.* 1997, have all placed the family Ulvaceae within the order Ulvales. In addition to *Ulva* other genera in the Ulvales are *Blidingia* Kylin, *Percursaria* Bory, *Chloropelta, Ulvaria* Ruprecht and *Letterstedtia*. Hoek *et al.* (1995) included *Acrochaete* within the Ulvales, but this was not supported by Stegenga *et al.* 1997.

Many authors recognize the use of a single family (Joska, 1992, Stegenga *et al.* 1997). Others (Wynne & Kraft, 1981; Bold & Wynne, 1985 & Silva *et al.* 1996) distinguish additional families within the Ulvales. Some authors completely omit the family category (Hoek *et al.* 1995 & Lee, 1999).

2.2.2 Biogeography, ecology and morphology

World wide the order Ulvales contains about 24 genera and 175 species (Hoek *et al.*1995). It is a widespread genus and has an amphiequatorial distribution pattern. Most of the species in the genus are found in near-shore marine and estuarine waters, upper to mid-intertidal (eulittoral, mideulittoral and supralittoral zones), and in some locations may be found in the subtidal zone. The fronds are not situated at the same level throughout the year. In colder months in temperate regions, the algae grow in wide bands while in the warmer months, they grow in a narrower band lower in the intertidal (South & Whittick, 1987; Sze, 1993; Lobban & Harrison, 1997; Lee, 1999). They are normally epilithic,

but some species such as *U. rhacodes* may be epiphytic (Stegenga *et al.* 1997). They are often densest in dynamic environments, e.g. in areas where rocks or boulders are frequently covered or uncovered by shifting sands in the intertidal zone. In estuarine habitats or still protected environments, they can create a dense, continuous green cover not only on solid substrata, but also over the sediments in salt marshes. They proliferate on reefs where nutrients are high, wave-shearing forces are low and herbivory is reduced. They are often present where there is an input source of pollution in fresh water laden with organic nutrients (South & Whittick, 1987; Sze, 1993; Lobban & Harrison, 1997; Lee, 1999).

Ulva are referred to as "opportunistic" or pioneer species that rapidly colonize bare substrata. Several morphological (large Surface Area to Volume (SA:V) ratio) and physiological traits (prolific and continuous reproduction; rapid growth, high nutrient uptake rates and high biomass production in low light) make *Ulva* well suited as a pioneer, but *Ulva* is out-competed by species that are more rugged, more resistant to herbivory and have better abilities to store nutrients (South & Whittick, 1987; Sze, 1993; Lobban & Harrison, 1997; Lee, 1999).

Due to the difficulties in the identification of members of this group, many species names have been misapplied (Silva *et al.* 1996) and this has resulted in artificial ranges for many of the species. For instance the records of *U. lactuca* Linnaeus, also called the Sea Lettuce, shows this species to have a wide distribution from the Bering Sea to Chile; in California from Humbolt to San Diego. As Stegenga *et al.* (1997) suggest, most records are probably not correct. At present, there is no conclusive evidence that the South African *U. lactuca* is genetically identical to the original European *U. lactuca* (sensu Stegenga *et al.* 1997).

Ulva species are membranous green algae and are two cell layers thick (distromatic). This morphology is only induced in the presence of certain bacteria. When *Ulva* species are cultivated aseptically in synthetic media an amorphous mass of loosely organized cells, resembling a pincushion (Provasoli & Pintner, 1980). When the bacteria are reintroduced to the cells, the alga

regains its characteristic morphology. The bacteria that cause this activity in *U. pertusa* Kjellman, were classified into six groups, namely: *Flavobacterium, Vibrio, Pseudomonas, Deleya, Esherichia* and gram-positive cocci (Kong & Chan, 1979 & Nakanishi *et al.* 1996).

Ulva species are parenchymatous: cell division may occur anywhere on the thallus but always in a plane perpendicular to the thallus surface. *Ulva* does not differentiate into tissue layers or show much specialization among cells. The arrangement of cells in surface view varies depending on what part of the blade is being examined and this arrangement is used to distinguish between species. The cells themselves are quadrate to slightly elongate anticlinally (perpendicular to the surface), depending on the species. The cell walls are fibrillar and made up of cellulose. The cells store energy as starch. Every cell in *Ulva* species contains photosynthetic pigments, including the reproductive cells. The cells contain a parietal chloroplast and one or more pyrenoids, this varies from species to species although there is a high degree of overlap. The chloroplasts are large and cup-shaped. All cells are also capable of reproduction, and there are no distinct reproductive structures. *Ulva* cells contain no plamodesmata, and thalli are essentially little more than complex colonies (South & Whittick, 1987; Sze, 1993; Hoek *et al.* 1995; Lobban & Harrison, 1997; Lee, 1999).

The alga attaches to substrata by means of a holdfast composed of rhizoids. The rhizoids are produced by the extension of proximal cells, which grow down between the two cell layers then outward to form the holdfast. A single rhizoid cell can generate a completely new alga, and blade cells of *U. mutabilis* can form new algae with a different morphology (vesicular thalli one cell thick). *Ulva* species are annual or pseudo-perennial in that the holdfast portions are perennial and grow new blades each spring (South & Whittick, 1987; Sze, 1993; Hoek *et al.* 1995; Lobban & Harrison, 1997; Lee, 1999). True floating plants have been observed, as is the case with *U. lactuca* from Simons Town Harbour in Cape Town (pers. obs.).

The shapes of the *Ulva* thalli are varied, and can be circular to oval to long and narrow, ranging in size from microscopic to 65 cm. The thalli range in thickness

from 38 – 209 µm (Stegenga *et al.* 1997). The sheet-like morphology results in a high Surface Area: Volume (SA:V) ratio (South & Whittick, 1987; Sze, 1993; Hoek *et al.* 1995; Lobban & Harrison, 1997; Lee, 1999). However, due to the large cell size and distromatic nature of this alga, internal self-shading is minimized, except in turf forms. The photosynthetic capability of thin, sheet-like forms has shown to be relatively high when compared to thicker forms (Littler, 1980; Littler & Littler, 1980). Littler & Littler, (1983) found sheet and filamentous forms to have the highest productivity of the macro algal functional form groups, with a value of 5.06 mg $C.g^{-1}.h^{-1}$. The price of this is: increased desiccation risk, possible wave damage and increased biomass loss to grazers. They conclude that the sheet-like functional form group incurs up to 42 % loss in biomass from grazing, but the high productivity of these forms seems to compensate for such losses (Littler & Littler, 1983).

Although the filamentous and sheet-like functional forms are delicate and seem very prone to damage, these forms are common in the intertidal zone. A piece of *Ulva* can be stretched more than 35 % of its original length before it breaks, thus presenting very little resistance to water movement. In strong currents or high wave action, these forms align themselves completely with the water flow and lie flat on the substrate surface where turbulence and current velocity is lower, thereby reducing damage by water motion (Norton *et al.* 1980, 1982). *Ulva* species can also produce allelopathic compounds, which can depress the growth of *Gracilaria* beyond simple competition for light and nutrients (Svirski *et al.* 1993; Pedersen *et al.* 1995 & Friedlander *et al.* 1996). Thus, *Ulva* is often considered a pest species in the cultivation of other seaweeds such as *Gracilaria*.

2.2.3 Life-history

The life history of *Ulva* is composed of two isomorphic generations: the diploid sporophyte and the haploid gametophyte. Both haploid and diploid phases are multicellular and vegetative. Distinguishing between generations is very

difficult, with the naked eye. Algae with this type of life history are described as isomorphic and diplohaplontic (Phillips, 1990).

In a typical life history (See Figure 2.1), cells of the diploid sporophyte (*h*) (2n) produce spores (n) in sporangia (*i*) through meiosis and release quadriflagellate haploid zoospores (*j, j'*). The sporangia result from normal peripheral somatic cells becoming reproductive. The zoospores later develop into the haploid thallus (*a, a'*). When conditions for sexual reproduction occur, such as a change in nutrient availability or water temperature, cells in the haploid gametophytes that are unisexual produce biflagellate gametes by mitosis (*b, b'*). Upon release, these gametes (*c, c'*) are positively phototactic and will swim toward the water's surface. This may be advantageous for increasing dispersal, for access to more light for photosynthesis, or for helping mix the gametes so that fusion can occur with gametes of the proper mating type. Upon fusion of gametes and nuclei of the opposite mating strain (*d*), the resulting diploid zygote (*e*) now becomes negatively phototactic and becomes attached to the substrate then grows into the sporophytic phase (*h*) (Tanner, 1981). During the development of both the diploid and haploid vegetative stages, there is a filamentous intermediate stage known as a germling (*k, k', l, l'* haploid and *f, g* diploid). Cells toward the bottom of the germling will form structures called rhizoids that are used for attachment to the substrate.

Propagule release may result in total loss of biomass or simply the sloughing of the marginal portions of the thallus. Thalli that have just released gametes (or spores) will often be noticeable to the naked eye by whitening around the edges or by clear spots. During sporulation, the periphery of the plant disintegrates to produce numerous propagules. The cause of sporulation is not yet well defined. Mohsen *et al.* (1974), found that nitrogen concentration caused sporulation in *U. fasciata.* Specifically, low nitrogen concentrations lead to enhanced gamete formation, while high nitrogen concentrations lead to vegetative growth and asexual reproduction. A study by Niesenbaum (1988) found that propagule release normally occurs during the warmer months of the year, or when the alga is cultivated at high temperatures in laboratory experiments.

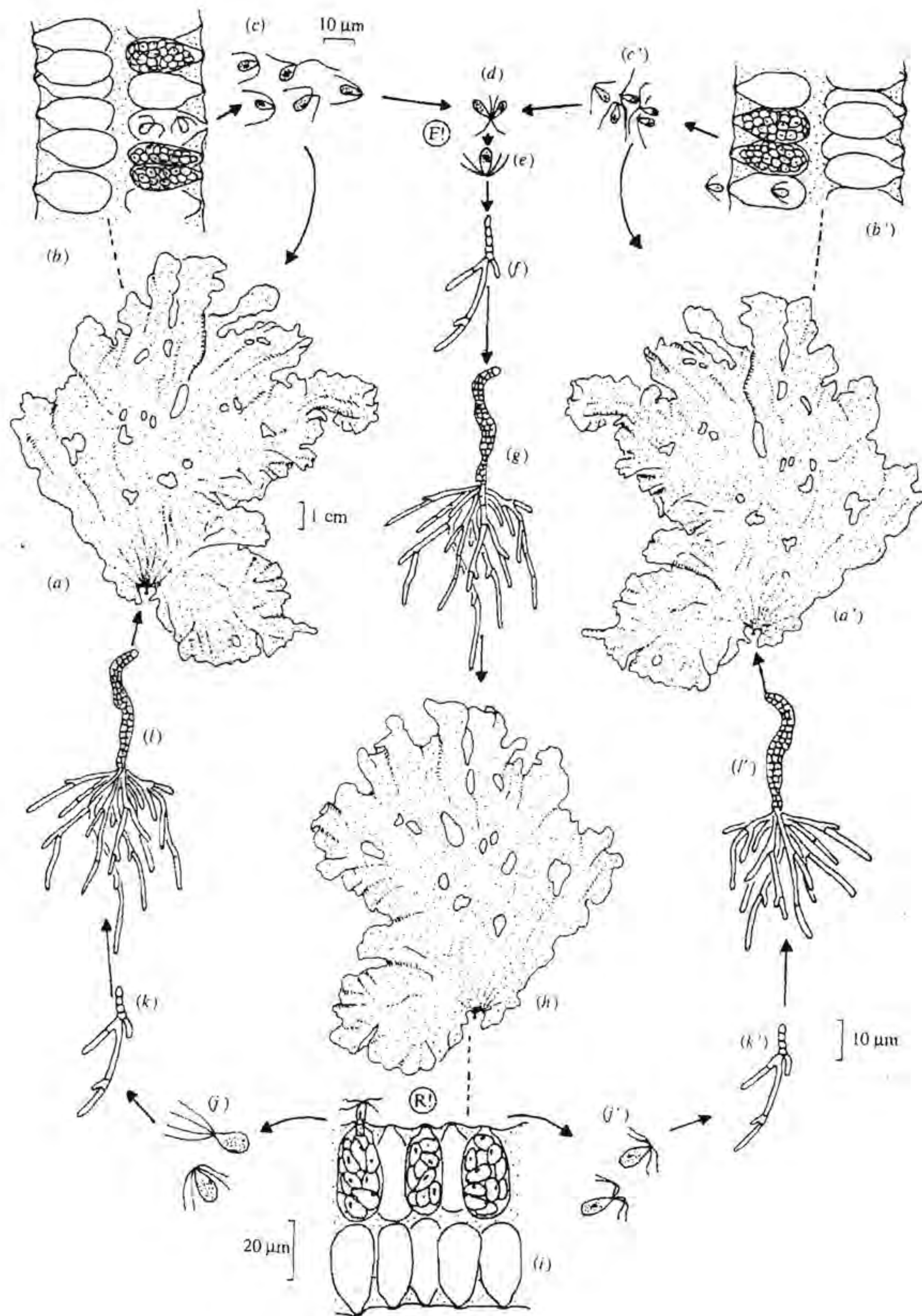

FIGURE 2.1: Life history of *Ulva*.

Source: Hoek *et. al.* (1995).

Propagule release in *U. lobata* in Monterey Bay (as well as the rest of the Pacific Coast of North America) is controlled by the lunar cycle (DeBusk *et al.* 1986). It releases its gametes during a series of spring tides, whereas *U. pertusa* in Japan releases its gametes during neap tides. In both cases, the release appears to be related to the amount of moonlight, and not the associated tidal movement. The release of spores follows the gametes a few days later. This periodicity of gamete formation and release helps insure genetic exchange within the population. Each plant is a different sex and therefore it cannot self-fertilize.

Oza and Roa (1977) suggested that the production of spores depended on the culture media used. A recent study by Stratmann *et al.* (1996) on *U. mutabilis*, found that the blade cells produce regulatory factors, which they excrete into their cell walls and into the environment, causing the alga to remain in a vegetative state. As the thallus matures, the production regulatory factors decrease below a threshold, triggering gametogenesis.

Some species of *Ulva* are able to release spores daily. In some *Ulva* species, between 20 and 60% of their overall biomass can be allocated monthly to reproduction, depending on the season (Niesenbaum, 1988). Sometimes the number of gametes or spores released can actually discolour the water and significantly increase the chlorophyll α concentration in the water column. Because of this, it has been hypothesized that marine macrophytes such as *Ulva* species might contribute to the phytoplankton as food for filter feeders in addition to their traditional role in detritus-based food chains (Niesenbaum, 1988).

High rates of photosynthesis give reproductive *Ulva* cells the selective advantage of being able to subsidize high respiratory costs associated with motility, as well as the ability to grow rapidly upon attachment to the substrate (Niesenbaum, 1988). Reproductive cells of *U. fasciata* have similar photosynthetic rates to adult vegetative cells. Even low rates of photosynthesis in *Ulva*'s spores and gametes can extend their viability and dispersal range.

Consequently, this contributes to their success over algae with shorter-lived or physiologically less competitive reproductive cells.

Parthenogenesis is common in members of the genus *Ulva*. Gametes develop into parthenosporophytes, with a small percentage (1 – 2 %) developing into gametophytes of the same mating type (Tanner, 1981). Apart from reproduction by means of swarmers, *Ulva* may also propagate by means of vegetative fragmentation, with some cells being able to slough off in stressful situations and form new algae. In the laboratory, blade cells of *U. mutabilis* resumed totipotency and blade fragments regenerated into adult algae (Bonneau, 1978).

Sterile strains of *Ulva* have been isolated in *U. pertusa* (Migita, 1985). Non-spore producing algae that propagate through vegetative fragmentation are potentially good cultivars to replace the spore producing strains, which lead to a loss of biomass.

2.2.4 Commercial and ecological value

Ulva lactuca has been used worldwide in the food industries in Peru, Scotland, Chile, Philippines, Jamaica, Hong Kong, and Taiwan (Lee, 1999). *Ulva* can be eaten in salads or used in soups. *U. lactuca* is made of 3 – 27 % protein, 50 % sugar and starch, less than 1 % fat, and 11 % water when dried. It is useful as roughage in the human digestive system (White & Keleshian, 1994). It is very high in iron, as well as in iodine, aluminium, manganese and nickel (See Table 2.2).

In addition, *U. lactuca* has been used in Japan, Europe, and North America as a fertilizer (DeBusk *et al.* 1986), in the cosmetic and pharmaceutical industries, as a food supplement for poultry and bovines, for extraction of colouring matter, and for energy production. It has been used to treat burns and has also been recognized as an extremely valuable ecological tool as a biofilter and a bioindicator (White & Keleshian, 1994).

TABLE 2.2: Nutritional analysis of *U. lactuca*.
Source: White & Keleshian, 1994.

Protein	15-20 %
Fat	0.6-1 %
Carbohydrates	42-46 %
Vitamin A	4286 I.U.
Vitamin C	100-200 ppm
Vitamin B3	98 ppm
Vitamin B12	6 ppm
Calcium	7300 ppm
Iodine	240 ppm
Iron	870-1370 ppm
Magnesium	2.8 %
Manganese	347 ppm
Sodium	1.1 %
Potassium	0.7 %

Since the 1970's, scientists have been looking at *U. lactuca* and *U. expansa* as biological indicators of the environment in which they live (Burrows, 1971; Levine & Wilce, 1977). *U. lactuca* possesses many traits which make it an ideal candidate for bioaccumulators and bioindicators.

A bioaccumulator is a biological organism that accumulates pollutants. *Ulva lactuca* has several traits that make it an ideal bioaccumulator (Ho, 1990):

- The species is widely distributed ranging from the poles to the equator and from coastal waters to estuarine river mouths.
- It is a very hardy species and can withstand levels of pollution that are stressful to most organisms.
- It cannot move when unfavorable conditions arise, thus the alga is exposed to the full effects of a possible pollutant in one area over the entire period of time that the pollutant is present, or that the study is being done.
- The distromatic thallus means that all of the cells in the thallus are in contact with the surrounding environment. Tissue samples of the entire thallus are then more meaningful than if the alga were able to shunt pollutants to certain areas or tissue layers in the thallus.

- *U. lactuca* is a macroalga, thus large tissue samples can be taken making analysis more precise.

Even better than simply testing the water itself, the advantage of using a bioindicator is that the levels of nutrients in the tissues result from long-term integration and accumulation from the surrounding water. A sample of water would only show the relative concentrations at the time the sample was taken (Ho, 1990).

Ulva species can be used as bioindicators of metal and pesticide contamination. *U. lactuca*, for example, is a good indicator of Mn, Fe, Cu, Zn and Pb contaminations (Ho, 1990). Metals inhibit reproduction of *Ulva* by interfering with the ability of male and female gametes to find one another via pheromones (Ho, 1990). Cadmium has been found to reduce growth in *U. lactuca* by inducing a loss of pigments and thus decreasing the rate of photosynthesis (Ho, 1990).

Biodetectors are characterized by having different developmental and growth patterns based on differing environmental conditions. *Ulva thalli* show differential growth not only according to environmental and chemical factors but also to what area of the thallus is being tested and whether or not the thallus was reproductive (Ho, 1990). The zoospore swarmers, when they are taken from a small section of an *Ulva* thallus are all genetically identical and about the same age but they develop differently according to environmental conditions. Studies have focused on germling length as a function of some environmental factor. This enables scientists to compare standards determined in the laboratory to germling development in the field. By using these comparisons, scientists have a way of determining environmental conditions by studying biological development of *Ulva* in the field (Ho, 1990).

U. lactuca is efficient in removing ammonium from the water and thus is suitable as a biofilter. Cohen & Neori (1991); Shpigel & Neori, (1996); Shpigel *et al.* (1997) and Neori *et al.* (1998), studied the possible utilization of *U. lactuca* as a natural filter for fish pond effluents from a fish mariculture farm in Israel. As this farm is situated close to an oligotrophic gulf, there is a possibility of

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

Ulva species are not widely considered to be of great economic importance. The amounts harvested from wild populations are low and it is generally not cultivated as part of mariculture operations for human consumption although cultivation for fodder is increasing. In some parts of the world *Ulva* can be harvested from lagoons and embayments where high densities occur (so called "green tides") (Fredericksen, 1987; De Casabianca & Posada, 1998). However, in these cases, the quality of the product cannot be controlled effectively and the amount and timing of the harvest is linked to weather conditions and is thus very difficult to predict.

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The high specific growth rate and the ability of *Ulva* to grow in eutrophic conditions as well as its nutritional content have made it a good candidate for animal feed and for use as a biofilter (DeBusk *et al.* 1986; Shpigel & Neori, 1996; Neori *et al.* 1998;).

2.3 Cultivation methods

Ulva has traits that make it an excellent candidate for cultivation. It is extremely fast growing, can utilise waste nutrients and can out-compete most species of epiphytic algae. The biggest drawback to its cultivation on a commercial scale is its low economic value. Thus, in order to take advantage of *Ulva's* fast growth rate and its ability to take up nutrients, large volumes must be produced economically and reliably. Cultivation of *Ulva* produces a crop that is more

reliable in terms of volume and quality of the product and the growth can be controlled to a certain extent. Various studies have looked at *Ulva* cultivation on a semi-commercial scale for bioremediation of effluent water. Cultivation systems can be divided up into open water cultivation and land-based cultivation.

The selection of the best technique depends on the species of algae used, the conditions at the cultivation site and labour costs. Each method has its advantages and disadvantages.

2.3.1 Open Water

Open water cultivation is the oldest form of aquaculture, the history of which can be traced back to the Japanese in 2000 BC, as well as the Romans who farmed oysters in 100 BC (Iversen, 1968).

Unlike cultivation in tanks and ponds, to be grown in the sea the macroalga has to be stationary. The seaweed is fixed in place and the water circulates round it to supply nutrients and remove excreted metabolites. Several techniques are used to keep seaweeds in place. One of the most common is to attach the seaweed thalli individually to a substrate or to inoculate the substrate with spores or zygotes. Two methods of out-planting using ropes are utilized. In the first, vegetative thalli, are tied to or inserted within a rope, which is the more common method. In the second, reproductive material, which is used as a source of spores which are settled onto the surface of a rope.

Alternatively, thalli can be planted directly into the seafloor sediments.

Open water cultivation can be either by :bottom stocking/ bottom culture, where the plants are anchored to the sea floor or suspended cultivation (rope farming and attachment to nets, floating rafts and cages) where the thalli are buoyed at varying depths in the water column (Critchley, 1993).

In open water systems, water does not need to be pumped in from the ocean as the organisms are grown in the sea, lagoons or estuaries. No land has to be purchased and thus open ocean farming costs less than land based farming.

This system requires less management than artificial systems and less time is spent monitoring the growth of the cultured organisms. The disadvantage of this type of system is that the farmer has less control over the environmental conditions, resulting in variability in growth performance and the quality of the product.

Open water systems as a whole have both the benefit and drawback of being exposed to the natural environment. Temperature, salinity and pH may be fairly constant, depending on the farm location. Seawater is also in constant motion, distributing fresh nutrients and disposing of waste products. In addition, most areas where this type of cultivation occurs are restricted to areas where wave action and tidal currents are minimal.

2.3.1.1 Bottom culture

The principle idea behind bottom culture or bottom stocking is to duplicate the natural field conditions of the vegetative thalli in soft sediments. There are several methods of bottom cultivation.
A common method is to transfer vegetative thalli, which are attached to small stones and shells, to areas where existing densities are low (Oliveira *et al.* 2000). Other methods include placing vegetative thalli in the sediment in the subtidal or planting them directly in the intertidal (Oliveira *et al.* 2000). Other methods include placing the vegetative thalli on wooden sticks or poles as a substrate and then inserting these into the sand (Oliveira *et al.* 2000). This is usually done in subtidal areas and from a boat in order to stabilize the thalli in soft sediments. Another method includes placing a 10 mm nylon mesh over rocks to keep unattached thalli in place (Oliveira *et al.* 2000).

These are very labour-intensive methods and are only effective in areas where the seaweeds are growing naturally and the cultivation is only required to increase the local density. The seaweed stocking density in bottom culture techniques is usually a function of water clarity and depends on water depth. The largest problem with this type of culture is that the plants remain at a fixed

depth in the water column, which means that light intensity fluctuates with tidal oscillations.

A disadvantage of this type of cultivation is that the survival of the crop is not necessarily ensured and thalli may die if the planting site is environmentally different to that from which the plants originated. Survival of plants during transport from one environment to another is also not ensured and thalli may also tear lose from their substratum during harvest or storms. Epiphytism of the crop, the presence of herbivores and fine sediments accumulating on the thalli can slow growth and controlling these problem is difficult (Oliveira *et al.* 2000).

2.3.1.2 Suspended cultivation

Suspended cultivation includes those methods where the seaweed is attached to lines, nets, floating rafts or enclosed in a cage and the system held above the sea bottom. Numerous methods have been developed. Most try to maximize the light and water flow around the thallus, while avoiding benthic grazers. Although more expensive and difficult to operate, suspended cultivation overcomes the problems of inadequate substrate for direct planting and local depth variations, as the plants are kept at a constant and ideal depth to optimize light supply (although this depends on whether the method used is floating with the tide or anchored to the bottom such as on sticks). However, the seaweeds tend to be more susceptible to fouling by undesirable algae and animals, labour costs are higher and there is more conflict with other users of the water space. Other problems encountered in this type of cultivation include grazing by herbivores such as fish, sea urchins and molluscs and sediment accumulation (Critchley, 1993). The problems of epiphytes and grazers often make sustained production over long periods difficult (Santelices & Doty, 1989). This method is also risky in areas subjected to strong currents and waves.

2.3.1.2.1 Raft culture

Raft culture is used to grow a number of species such as *Undaria, Macrocystis, Laminaria, Gracilaria, Porphyra, Ulva* and *Enteromorpha* (Santelices, 1999). An

advantage of raft culture is that the seaweed is kept in its optimum light zone. Generally, two types of rafts are used: subsurface rafts (those that "float" below the water surface) or floating rafts (those that float above the water surface) (Oliveira *et al.* 2000). Transparency of the water column is an important limiting factor to the growth of seaweeds on ropes or rafts, because too much sunlight can be detrimental to crop growth in surface waters, while at depth too little irradiance will cause dying and bleaching of the thalli (Critchley, 1993). The advantage of using floating rather than subsurface rafts is that tidal level changes do not have an influence on the amount of light reaching the thalli.

2.3.1.2.2 Semi suspended cultivation

In this type of cultivation, poles are driven into the sediment and rope or netting is suspended between the poles so that the nets are parallel to the water surface. An advantage of this type of cultivation is that if the nets are attached in such a way that the algae are exposed to air for a few hours at low tides (if the area is tidal), epiphytes and fungi can be controlled. This method is recommended for the cultivation of *Ulva* (Santelices, 1999). With low fixed rafts however, light levels can be limiting during high tide and therefore this method works best in areas where the water is very clear.
A variation of this method is to have a diver suspend a rope with macroscopic thalli attached, stretched under tension between stakes buried in the sediment so that the thalli are suspended just above the sea floor (Oliveira *et al.* 2000). The stocking density in this method is a function of the water clarity.

2.3.1.2.3 Basket culture

This system of cultivation is used for the cultivation of kelps in the Far East. A variation of this technique is being tested for *Ulva lactuca* in South Africa. The alga are kept in baskets or net bags, which are strung together to form rafts. A porous cylinder containing fertilizer is placed in the basket. This helps to enriched the seawater in the immediate vicinity of the alga with additional

nutrients. The fertilizer containers are replaced periodically (Bardach *et al.* 1972 & H. Otto (pers. comm.))

2.3.1.2.4 Long lines

This type of cultivation has also been used for *Ulva* species. Long lines are constructed by suspending natural or synthetic ropes from glass or plastic buoys. The ropes are strung perpendicular to prevailing currents or tidal streams, with the seaweed inserted into the ropes and the ropes are secured to the bottom. This technique is especially useful in areas with rough water circulation or regular storm events, where heavy wave action would damage the more rigid traditional rafts. The algae are harvested using small boats.

2.3.2. Semi-closed systems or land-based aquaculture

These systems involve the pumping of seawater from the ocean or estuaries into a specifically designed system on land. Land based facilities reduce many of the obstacles related to predation, poaching, weather condition and regulations connected with offshore mariculture (Shpigel & Neori, 1996). In land-based facilities the nutrition of the cultured organisms is manageable, allowing consistent quality of the product. In addition, incoming water can be screened for pathogens, pollutants, poisons and harmful algal species (Shpigel & Neori, 1996). Finally, effluents from land-based systems are treatable, allowing the choice between water recycling or release back to the sea, once the required standards have been met. This is significant, since the quantity of nutrients released from an aquaculture operation often limits the licensing of aquaculture productions (Shpigel & Neori, 1996).

The principal advantage of land-based cultivation is that overall control is possible through integration of all system components. Light is probably the only non-controllable variable and the aim is therefore to devise a system in which all other variables are controllable (Bidwell *et al.* 1985). Land-based

cultivation systems include pond and tank cultivation, r aceways, spray cultivation and very-high-light-intensity systems.

There are many drawbacks with this form of cultivation, including but not limited to, pumping of water, piping, aeration, temperature fluctuations, space, infrastructure, high capital costs, tanks, filtering of seawater, epiphytes, and labour costs etc.

2.3.2.1 Pond cultivation

Pond cultivation can be divided into intensive and non-intensive cultivation systems. Non-intensive systems generally include ponds which are earthen and uncovered and are always without an artificial agitation system (such as aeration pipes), while intensive cultivation ponds are made of concrete and have a water agitation system (Friedlander & Levy, 1995).

Non-intensive pond cultivation has been practiced in the East for centuries. Intensive cultivation of free-floating seaweeds including *Ulva* has developed mainly in the last two decades, first in the U.S. A. (Hanisak and Ryther, 1984) and in Canada (Bidwell *et al.* 1985) and later in other countries. The advantages of this method are its high potential yield, the possibility to control and mechanize its major operations and the possibility to use the seaweed ponds as biofilters for fish-pond and other effluents. The disadvantages are the high costs involved, which are related to the energy required to pump seawater and to aerate the ponds (Friedlander & Levy, 1995). Another problem that has been identified in these systems is that of epiphyte development on the thalli of the cultured algae. Subsequent studies have revealed that with a system of polyculture, in which several economic species are cultured in the same pond at the same time, epiphytic species on the cultured algae may be controlled via selective grazing of the other cultured organisms (Critchley, 1993; Oliveira *et al.* 2000).

Non-intensive pond cultivation occurs in natural lagoons, or ponds that are little more than holes in the ground, with a few being above the ground with embankments constructed to retain the water. The ponds can be of any shape or size, but most are rectangular, so that little space is wasted between ponds. The depth of the pond may vary according to the species requirements. In very large ponds, wind causes the thalli to pile up in some areas and growth rates are reduced. The main problems of pond cultivation are the burial of thalli in oxygen poor sediments, large fluctuations in temperature and salinity in shallow ponds, excessive growth of epiphytes and grazers and low water motion (Boyd, 1990, 1998 and Oliveira *et al.* 2000).

With artificial ponds, the highest costs could be associated with excavating the pond, pumping seawater (if there is not enough tidal variation), fertilizing, or labour for the farming and subsequent processing (e.g. drying and sorting). The costs of these activities varies from place to place (Boyd, 1990, 1998).

2.3.2.2 Tank cultivation

Tanks are usually built from fiberglass, treated wood, concrete or PVC plastic, with capacities ranging from a few hundred litres to thousands of cubic meters (Oliveira *et al.* 2000). Tank cultivation can be controlled so that high production rates can be obtained throughout the year, irrespective of the climatic and seasonal conditions. The efficiency of these systems depends on the input of various types of energy, such as compressed air for aeration, carbon dioxide and pumping of water. Because of the high costs involved in setting up and maintaining such an operation, this is the most expensive form of cultivation and is limited to situations where capital required for set up can be recovered (Hanisak & Ryther, 1984; Critchley, 1993; Oliveira *et al.* 2000).

The shapes of the tanks are designed to facilitate water movement when aerated and can be either U-shaped (flat at the bottom) or V-shaped (See Figure 2.2). Tanks are then usually aerated by means of a perforated PVC pipe, which is secured to the bottom of the tank.

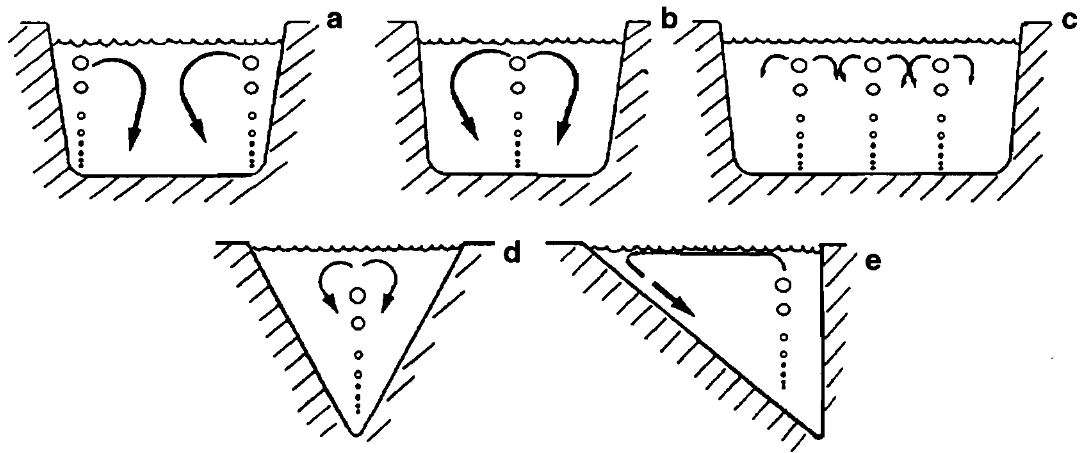

FIGURE 2.2: U (a - c) – and V (d; e) – shaped tank configuration with aeration to aid the circulation of seaweed (Critchley, 1993; Vandermeulen, 1989)

Experiments conducted worldwide have indicated several important factors in the tank cultivation of *Ulva*. These include: water exchange rate, aeration, temperature, light intensity, pH, carbon dioxide concentration, nutrient supply, the presence of epiphytes and marine herbivores and stocking density. These factors are discussed in section 2.4.

2.3.2.3 Raceways, spray cultivation and very-high-light-intensity systems

Other land-based systems that have been tested in *Ulva* cultivation include raceways, spray cultivation and very-high-light-intensity systems.

Raceways are similar to tanks except that they are much longer than they are wide. They are generally shallow allowing good light penetration and rapid water movement through the system. The rate at which the water is moved through the system affects the production of the seaweed. The rate is determined by the species, water temperature, stocking density and fertilizing strategy. One problem with raceways is that the concentration of nutrients

decreases with increasing distance away from the inlets (Shpigel *et al.* 1997). This means that seaweeds grown close to the inlets will receive more nutrients than those grown further away. Compared to tanks which have a uniform removal efficiency, a raceway has a decreasing removal efficiency over its length but higher total removal efficiency. Raceways have also been used effectively in polyculture operations with bivalve cultivation (Lapointe *et al.* 1976; Ryther *et al.* 1978; Shpigel *et al.* 1997).

A common variation on traditional raceways is the "double-ended D" raceway. In these systems, water doesn't leave the system after a single pass, but rather remains in the raceway for a longer period of time. This increases the water retention time and thus the rate at which nutrients can be removed by the algae. The difference between a circular raceway and a circular pond is that raceways are shallower and water movement is faster. Raceways are often painted white to increase the amount of light reflected to the cultures.

In spray cultivation, the thalli are placed onto nets, which are then suspended over a box that collects the sprayed water before it is returned to a reservoir. Problems with spray cultivation are that thin thalli form clumps on the nets resulting in self-shading of the thalli (Steyn, pers. comm.). Nitrogen may become deficient because of the inability of the seaweed to take up nutrients, due to the lack of a sufficient diffusion medium (seawater), it is also thought that this lack of a diffusion medium prevents gaseous exchange to due insufficient replacement of the boundary layer (Lignell *et al.* 1987).

Very-high-light-intensity systems have been shown to increase algal production. Under these conditions, submerged light sources are used in addition to very powerful overhead light sources. The numbers of epiphytes are reduced using filters and diatoms numbers are kept low due to vigorous aeration causing the seaweeds to rub against each other. An obvious disadvantage of this system is the high cost involved, making such a cultivation system economically non-viable in commercial scale systems (Lignell *et al.* 1987).

2.3.3 Land–based integrated aquaculture

Aquaculture, like any other industry, has the potential to generate products that can act as pollutants if they are released into the natural marine environment (Odum, 1974).
Pollutants from aquaculture include organic materials resulting from excess secondary production or from inefficient supplemental nutrient enrichment. The concept of ecological sustainability in aquaculture refers to the maximization of internal feedback (e.g. recycling) within a culture system. This minimizes the inputs and the wasted outputs of resources, such as nutrients, water and energy in effluent water (Chopin & Yarish, 1998; Neori *et al.* 1998 and Chopin *et al.* 2001).

A common practice and one that has found favour among land-based aquaculture operations during the last 2 – 3 decades is to integrate seaweed farming into a polyculture operation where seaweeds are cultured in the effluent water of abalone (Shpigel & Neori, 1996; Neori *et al.* 1998), prawns (Chiang, 1981), oysters (Shpigel *et al.* 1997), clams (Shpigel & Neori, 1996) or fish (Shpigel & Neori, 1996; Troell *et al.* 1997; Chopin & Yarish, 1998; and Chopin *et al.* 1999a, b, c, d). As the algae are capable of utilizing low levels of DIN and DON, the final effluent from the algal tanks can be returned to the sea, estuary or river with approximately the same nutrient status and temperature as the resource waters (Vandermeulen & Gordin, 1990; Cohen & Neori, 1991; Shpigel & Neori, 1996; Shpigel *et al.* 1997; Chopin & Yarish, 1998; Chopin *et al.* 1999a, b, c, d and Troell *et al.* 1999a,b).

Most seaweeds are commercially less valuable than cultured marine animals. When used as fodder for cultured marine invertebrates, such as sea urchins and abalone, the low-value seaweeds are metabolized into highly valued commodities (Shpigel & Neori, 1996; Neori *et al.* 1998).

Inorganic Nitrogen such as ammonia (a major metabolite produced by fish and abalone, which is toxic at very low concentrations) is a major effluent in

aquaculture, and when added to pristine coastal areas causes eutrophication (Cohen & Neori, 1991; Neori *et al.* 1998 and Troell *et al.* 1999a,b). In integrated polyculture, the seaweeds play an important ecological role, by introducing to nutrient loaded water new energy in the form of organic carbon via photosynthesis. The algae re-assimilates inorganic nitrogen back to utilizable algal protein, making eutrophication beneficial by containing it within the culture system and steering it to desirable organisms (Troell *et al.* 1997 and 1999a,b).

2.4 Key Biological and Environmental parameters in land-based, tank cultivation of *Ulva*

Factors that influence the growth and chemical composition of *Ulva* (and other cultured seaweeds) in tank cultivation are: temperature, light, salinity, water flow, aeration, nutrient and carbon supply, pH, grazer density, epiphyte density and stocking density (Duke *et al.* 1986; Duke *et al.* 1989; Friedlander *et al.* 1990; Lüning, 1990 & Critchley, 1993). These factors operate together to affect the ultimate growth rate, physiological processes, chemical composition (e.g. nitrogen and protein content) and overall quality of the *Ulva* species being cultivated. Consequently, manipulation of one factor could alter one or more of the others. For example under conditions of low water flow, the system may not only be nutrient (carbon or nitrogen) limited, but productivity may also be influenced by temperature. Carbon supply can be improved by the addition of carbon dioxide gas (an expensive option) or by increasing the amount of seawater flowing through the system (Lapointe *et al.* 1976). Another example is that pigment content of *Ulva* sp. increases with decreasing light, while pigment content at a given light level is controlled by DIN availability (Rosenberg & Ramus, 1982a). Responses to one environmental variable can affect the alga's ability to deal with other environmental variables. For example, *U. fasciata* grown at 7 – 19 % of incident light flux (I_o) has a narrowed range of response to variations in nutrients and temperature, when compared to plants grown at I_o (Lapointe & Tenore, 1981 and Duke *et al.* 1986).

2.4.1 Aeration

Experiments conducted by Hanisak & Ryther (1984) indicated that for unattached populations, water motion plays a vital role in keeping thalli from sinking, thus maintaining them in circulation in the water column and bringing them to the surface into the light for photosynthesis. Niesch & Knutson (1977) showed that culture tank mixing or agitation was also necessary for high seaweed yields. DeBusk *et al.* (1986), found that without aeration the seaweed was buoyed to the water surface by photosynthetic oxygen bubble formation. Conditions of high temperature, dessication and high light intensity caused the death and decay of the plants. The same study found that the highest yields in *U. lactuca* cultivation occurred with continuous (24hr) aeration, while lowest yields occurred in non-aerated tanks. The decline in algal yields with decreasing aeration time was not linear.

Aeration results in rotation, which in turn causes abrasion between the algae and the tank walls and so serves as a mechanism for epiphyte control, in that opportunistic algae are unable to adhere to the tank walls or the algae (Hanisak & Ryther, 1984).

Aeration stimulates growth by breaking down diffusive boundary layers at the surface of the thalli that would otherwise slow the uptake of nutrients and inorganic carbon (Hanisak & Ryther, 1984). Also, increased movement results in increased exposure to light (Parker, 1981; Hanisak & Ryther, 1984; DeBusk *et al.* 1986 & Vandermeulen & Gordin, 1990). Blakeslee (1984) (cited by DeBusk *et al.* 1986) demonstrated that aeration supplies little carbon (via CO_2 from the air) into culture tank waters to simulate growth.

DeBusk *et al.* (1986) showed that it is possible to aerate for 12 hours per day and still maintain growth rates. This is a substantial cost saving for a big mariculture operation, if the aeration is spread over the daylight hours.

Aeration can also increase oxygen concentrations, resulting in increased oxidative metabolism. To neutralize this it is possible to use CO_2 for aeration, but this in turn may increase running costs.

2.4.2 Growth rates and Seasonality

Seaweeds show cycles in their growth and reproduction that can be correlated with seasonal fluctuations in light intensity, temperature and nutrients i.e. the primary ecological factors (Lüning & Dieck, 1989). Not only do environmental factors interact with each other, they also affect growth rate and nutrient uptake differently. Light and temperature affect growth rate more than does nitrogen uptake, while DIN affects nitrogen uptake more than it does growth rate (Duke *et al.* 1986). Nitrogen tends to accumulate when light levels or temperatures are low or DIN is high. Stored nitrogen is depleted by growth when light or temperatures increase or nitrogen is limiting (Duke *et al.* 1986).
Light and temperature were shown to control growth in *U. rigida* (Riccardi & Solidoro, 1996), although nutrient limited growth is also well documented for both phosphate and nitrogen compounds (Altamirano *et al.* 2000a).

Altamirano *et al.* (2000a) found that seasonal changes in relative growth rates (RGR) of *U. olivescens* Dangeard, under natural conditions, were explained mainly by variations in nitrate/nitrite (NO_3^- and NO_2^-) concentrations (70 % of the variation), photosynthetically active radiation (PAR) (15 %), UVB radiation (5 %) and seawater temperature (4 %).

2.4.3 Light

Light is the driving force in a cultivation system and is the one uncontrollable factor in outdoor cultivation systems (Bidwell *et al.* 1985). Light is seldom a limiting factor in suspended or land based algal cultivation techniques (Bidwell *et al.* 1985; Duke *et al.* 1986).

Physiological responses to variations in light include: changes in light harvesting pigment concentrations, pigment ratios, and activities of Calvin Cycle enzymes, particularly ribulose biphosphate carboxylase-oxygenase (RuBPCase). Chlorophyll *a* ratios typically increase with decreasing light, while RuBPCase varies positively with light and DIN (Duke *et al.* 1986). N-limited seaweeds growing at high light levels accumulate carbohydrates (Rosenberg & Ramus, 1982b), but have relatively low RuBPCase activity.

Thallus structure also affects seaweed's responses to light. Not all light absorbed by algal thalli is absorbed by photosynthetic pigments only, some light is reflected or back scattered: this is referred to as the optical density of the thalli. *Ulva* shows differential photosynthetic responses to light quality at different depths and because it contains a high concentration of pigments and is therefore able to absorb almost all wavelengths of light, it does better in shallow waters. Beer *et al.* 2000, found that photosynthetic pigments are responsible for most of the light absorption in *Ulva* thalli, and that reflection from the thallus surfaces is very low, especially if the light is provided perpendicular to the thallus surface.

An important factor to remember when considering the light regime in tank culture systems is the tank depth. Light intensities will be higher in shallow tanks and together with temperature, may become too high in summer. The converse is also true: during winter and in tanks that are too deep, light may become a limiting factor. Shallow depths usually ensure that the system has a relatively large SA/V (surface area/volume) ratio. In general, the greater the SA/V ratio the greater the predicted productivities (Khailov and Silkin 1986, cited by McLachlan, 1991). For a cultivation system to operate at maximum efficiency, all the biomass in the system must be exposed to light. It follows then that the stocking density must essentially remain constant, therefore material must be removed from the cultivation system at regular intervals, i.e. weekly or daily, depending on the Specific Growth Rate (SGR) of the seaweed (McLachlan, 1991).

Ulva is also able to photo acclimatise within days to lower light levels and can maintain growth rates even if total irradiance is reduced slightly (by a factor

such as self shading at high density (Vandermeulen & Gordin, 1990; Altamirano *et al.* 2000b)

Hydrogen peroxide (H_2O_2), like other forms of active oxygen, can cause substantial damage to seaweeds through the destruction of lipids, proteins and nucleic acids (Collén & Pedersén, 1996). H_2O_2 production by *Ulva*, at high light intensities is a mean of energy dissipation (Collén & Pedersén, 1996). The toxicity of H_2O_2 to seaweeds is reduced by diffusion and enzymatically by peroxidative scavenging. Since H_2O_2 can diffuse readily through biological membranes and cell walls out into the water and as *Ulva* is only two cell layers thick, diffusion is rapid, *Ulva* therefore has a limited ability to break down H_2O_2 (Collén & Pedersén, 1996).
In general, *Ulva* shows a remarkable ability to cope with a changing light climate, such as that which occurs in the intertidal zone.

2.4.4 Nutrients and carbon supply

Of all the environmental variables manipulated in algal cultivations, nutrient concentrations are the most decisive in optimizing growth rates. A range of macro- and micronutrients as well as various trace elements are necessary for algal growth (see De Boer, 1981). Most of these are present in concentrations adequate for intensive cultivation, except nitrogen, phosphorus and carbon, which can often be limiting, resulting in decreased growth rates and ultimately productivity. It has been proven that the addition of inorganic nutrients (N & P) to nutrient depleted water can significantly increase the growth rate of *Ulva* species (Björnsäter & Wheeler, 1990;DeBusk *et al.* 1986).

2.4.4.1 Nitrogen

Nitrogen is one of the resources limiting seaweed growth in the natural environment and seaweed growth rates tend to parallel nitrogen supply (Duke *et al.* 1989). Nitrogen uptake capacity of seaweeds (V_{max}) is a direct function of SA/V (Rosenberg & Ramus, 1982b) whereas nitrogen storage capacity varies

approximately inversely with SA/V (Rosenberg & Ramus, 1989; Duke *et. al.* 1989). DIN is available to the algae in three forms: ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-). Of these, nitrate is the most abundant in seawater, followed by ammonium, while nitrite is present in very dilute concentrations (De Boer, 1981).

Seaweeds differ in their capacities for using different sources of nitrogen. Nitrate first has to be reduced by the algae before it can be utilized in the cells. For this reason, ammonium uptake is better in some species as it can be used immediately in the plant metabolism and can be taken up both actively and passively (DeBusk *et al.* 1986). Neither light intensity differences nor temperature fluctuations affect the uptake of ammonium in *Ulva* sp. (DeBusk *et al.* 1986; Duke *et al.* 1986b; Duke *et al.* 1989; Vandermeulen & Gordin, 1990), nor does changing the stocking density (Cohen & Neori, 1991). Ammonium uptake is regulated by tissue nitrogen and DIN (Duke *et al.* 1989).

2.4.4.2 Phosphorus

Orthophosphate ions, primarily HPO_4^2 are the primary source of P available to marine algae, at concentrations of 1 – 3 µM (De Boer, 1981). This resource may be exhausted at high seaweed densities making P concentration a limiting factor in seaweed growth.

2.4.4.3 Carbon

Carbon is available to seaweeds in the form of dissolved CO_2, bicarbonate (H_2CO_3) or as carbonate ions (HCO_3^- and CO_3^{2-}) (De Boer, 1981). At natural pH values of seawater (7.8 – 8.2) and salinity of 35 $^0/_{00}$, HCO_3^- constitutes 90 % of the available dissolved inorganic carbon (DIC) in seawater (Kremer, 1981). Only CO_2 penetrates cell membranes easily, but it is only present at a concentration of approximately 10 µM (approximately the same concentration as air, but the diffusion rate is 10^4 times slower) (Kremer, 1981). *Ulva* does not utilize CO_3^{2-} in photosynthesis, even though it is present in high concentrations

at normal pH in seawater (Maberly, 1992, cited by Björk *et al.* 1993). There are three mechanisms by which algae can acquire inorganic carbon:

1) Diffusive uptake of CO_2
2) CO_2 uptake dependant on a dehydration of HCO_3^- catalyzed by an external carbonic anhydrase (CA_{ext})
3) Direct transport of HCO_3^- into the cell (Johnston, 1991, cited by Björk *et al.* 1993).

In nature, adaptation to differing concentrations of CO_2 by *Ulva* is important for plants in a constantly changing environment such as the intertidal zone. In the splash zone agitation provides a constant supply of dissolved CO_2, the role of CA_{ext} is minimal and activity is not induced. However, for plants in stagnant waters such as rock pools, where pH can become very high, the ability to use HCO_3^- becomes important. *Ulva* uses bicarbonate (HCO_3^-) as a primary carbon source (Vandermeulen & Gordin, 1990), although *Ulva* does have a limited ability to use organic-carbon sources, such as glucose, acetate and leucine, if they are available (Markager & Sand-Jensen, 1990).
Björk *et al.* 1993, found that *U. reticulata* Forskaal, *U. pulchra* Jaasund and *U. rigida* all depend on CA_{ext} for photosynthetic HCO_3^- utilization. Drechsler & Beer, 1991 (cited by Björk *et al.* 1993) showed that *U. lactuca* displayed direct uptake of HCO_3^- in the absence of CA_{ext}.

2.4.4.4 Fatty acids

The fatty acid content (% of total fatty acids (FA)) of algae is known to be enhanced by manipulating algal culture conditions such as temperature, light, salinity or nutrient composition of the medium (Floreto & Teshima, 1998). Lipids in seaweeds make up < 4 % of seaweed dry weight (Floreto & Teshima, 1998). Despite these low levels, there are indications that herbivores have essential fatty acid requirements (Floreto & Teshima, 1998). Floreto *et al.* (1993, 1994) reported increased proportions of polyunsaturated FA's in *U. pertusa* when cultured at low temperature, low light intensity and high salinity. High light intensities increased the quantitative levels of most saturated FA's and FA

content was directly correlated to growth rate. Floreto & Teshima (1998), found that holding *Ulva* under conditions of low salinity increased the fatty acid content prior to feeding.

2.4.5 pH

When photosynthesis occurs in sea water and some of the dissolved CO_2 is removed, HCO_3^- ions will react with free H^+ ions to replace it according to Equation 2.1:

$$HCO_3^- + H^+ \rightarrow CO_2 + H_20 \quad \text{.................................(2.1)}$$

This reaction will reduce the H^+ ion concentration, so that the pH will rise. This rise is offset by the simultaneous dissociation of other HCO_3^- ions (Equation 2.2), which liberate sufficient H^+ ions to balance the loss in the first reaction:

$$HCO_3^- \rightarrow H^+ + CO_3^- + H_20 \quad \text{.................................(2.2)}$$

The CO_2 fixed in photosynthesis is not entirely replaced from the bicarbonate pool because of the reduction in the overall concentration of the dissolved organic carbon. This results in a shift in the balance of the components in the system and a slight rise in pH. Large changes in total CO_2 concentration are required to cause small changes in pH outside the normal range of sea water. It is vitally important that pH in an algal culture system is maintained at a level near that of normal seawater (pH 7.5 - 8.5) (Kremer, 1981 and Lobban & Harrison, 1997).

Monitoring pH to assess plant health (photosynthetic rate) could be a valuable tool in flow through waste systems as healthy plants can alter pH levels in the tanks.

2.4.6 Salinity

Salinity changes are important to marine algae in several ways. Salinity levels outside an individual species tolerance level may result in osmotic stress, unfavourable ionic balances, or a shortage of essential metabolites.

Salinity stress often occurs in tidal pools as they become extremely saline under hot, dry conditions and tend toward fresh water under rainy conditions. *Ulva*, which has adapted to this environment, has a high salinity tolerance ranging from as little as 3 $^0/_{00}$ to as much as 115 $^0/_{00}$ (Loban & Harrison, 1997). *Ulva* plants are able to regulate the amounts of dissolved internal salts, keeping their internal osmotic pressures somewhat higher than the surrounding medium. This process prevents loss of water to the surrounding saline environment allowing them to maintain a constant turgidity (Loban & Harrison, 1997).

2.4.7 Stocking density

For a cultivation system to operate at maximum efficiency, light absorption must be optimal. If the plant material is too concentrated, plants compete for resources, predominantly light, whereas, if densities are too low resources are wasted and systems run sub-optimally. Biomass must therefore remain the same and not be left unchecked (McLachlan, 1991).
Optimal stocking densities depend on a number of factors, including the morphology of the thallus. For maximum production efficiencies, all of the biomass in the cultivation system must be exposed to light. If the stocking density is too high, the thalli will shade themselves and thus production will decrease (the so called self shading effect). Similarly, when the stocking density is too low, thalli will receive too much light and energy will be wasted (McLachlan, 1991).

Physical constraints and tank dimensions (surface area and depth) are also relevant in determining optimal stocking densities. When stocking densities get too high (> 6kg.m^2) (DeBusk *et al.* 1986a,b), aeration in the tanks is insufficient to circulate the alga properly. This leads to bleaching of the thalli that are left on the surface and results in a loss of biomass (pers. observ.). To overcome this problem frequent harvesting is necessary at high stocking densities. Neish & Knutson (1978), have shown that there is an optimum stocking density (or density range) for which maximum seaweed yields are attained.

Lapointe and Tenore (1981) have established that the yields of *U. fasciata* generally increased with increased light, irrespective of nitrogen loading. This was also true of *U. pertusa* (Altamirano *et al.* 2000a).

2.4.8 Temperature

Temperature is an important determinant of plant production. Its effect on respiration and dark reaction photosynthesis is well known. While the optimum temperature of a species varies, the general pattern is usually an increase in growth rate with temperature, to a maximum growth rate that is near the end of the tolerated temperature range (Loban & Harrison, 1997). However, temperature is a variable that is not easily manipulated in cultivation systems due to the high cost associated with cooling and heating water. An understanding of the effects of temperature on the growth of *Ulva* can be useful in predicting seasonal productivity fluctuations.

2.4.9 Water flow rates and water quality

Algae derive all of their metabolic requirements from the water around them. The pH, nutrient concentration and availability of CO_2 in the water all affect growth rates. *Ulva* cultivated in tanks will rapidly use the available nutrients and CO_2 and sustained high yields are possible only if the seawater is constantly renewed or additional nutrients are added. Seawater supply, its subsequent removal from the tanks and efficient circulation have important consequences for cultivation. A good supply of seawater ensures a supply of nutrients and CO_2 and ensures even distribution in the culture medium, it removes waste materials and diminishes the effect of the boundary layer (the layer of water that doesn't move on a seaweed thallus), thus improving uptake and excretion rates and achieving some degree of temperature control (Bidwell *et al.* 1985).

2.5 Epiphytes

Diseases and pests are major problems in the mariculture industry. This is due to the artificial nature of the cultivation. Large scale monocultures provide ideal conditions for the spread of diseases and contaminants. The growth of the organisms under unnatural conditions also renders them more susceptible to attack. The three main problems are: I) competition for space, light or nutrients in the habitat; II) attack by pathogens such as bacteria and viruses; III) growth of epiphytes and endophytes. Epiphytic growths are the largest problem and constraint in commercial seaweed culture (Wheeler *et al.* 1981; Fletcher, 1995).

Epiphytic algae reduce yields in cultivation by competing for light, nutrients (including CO_2) and by increasing mechanical drag on the host plants. They may also damage the host thallus due to the penetration of rhizoids and the production of detrimental allelochemicals. Non-epiphytic fouling algae, including benthic algae such as those which grow on the sides of the tanks; unattached algae contaminating the system, such as dense populations of unicellular algae or other macro algae also compete for light, space and nutrients.

Epiphytes bloom when the cultivation environment is more suited to the epiphyte than to the cultured species. Although epiphytes can become a serious problem in some places and situations, especially tank cultivation, there is little experimental information on the interaction of the hosts and various types of epiphytes (Fletcher, 1995).

Most of the concern with epiphytes is centered on the decline in productivity of the cultured species. However, if *Ulva* is cultivated for abalone feed, the impact of epiphyte colonization is not as critical, provided that the epiphytic species is palatable to the abalone. Simpson and Cook (1998) have demonstrated that abalone prefer a diet of mixed seaweeds. Although abalone prefer a single species diet of *Gracilaria* to *Ulva*, when these plants are combined with *Ecklonia*, the preference swaps to the *Ulva*/*Ecklonia* mixture. If epiphytes are

present on the cultured *Ulva* it may be prudent to first present them to the abalone, if they are not eaten then effective controlling mechanisms can be initiated.

2.5.1 Effects of epiphytes and fouling algae on *Ulva* cultivation

Epiphytes are seen as opportunistic because of their tolerance of a wide range of environmental conditions such as temperature and irradiance levels (Fletcher, 1995). Particularly important is their competitive response to high nutrient levels. When epiphytes remove nutrients and inorganic carbon from the water column they can significantly reduce production of the cultured species (Fletcher, 1995). Another important effect of epiphytes is that they increase load and drag on the target seaweed, weakening them and making them much more vulnerable to breakage. Such loses can also reduce biomass production from seaweed farms (Fletcher, 1995). Other adverse affects include increased competition for light. This applies especially in non-aerated cultivation system, where the contaminants obstruct water circulation resulting in the formation of dense patches of seaweed. Such dense patches are self-shading and can lead to anoxic conditions, which cause further damage (Fletcher, 1995). Tissue damage to the host, resulting from the penetration of rhizoids of the epiphytes can be exacerbated if attempts at hand removal are made. Other possible adverse effects include the release of exudates by the epiphytes that may interfere with or reduce the growth of the host (Fletcher, 1995).

It is thought that the production of H_2O_2 exudates by *Ulva* is a means of epiphyte reduction (Collén & Pedersén, 1996). Vandermeulen & Gordin (1990) and Neori *et al.* (1991), have both shown that *U. lactuca* can grow free of epiphytes for long periods of time. This may be due to the production of H_2O_2. H_2O_2 has been used to treat the brown alga *Laminaria japonica* in commercial cultivations affected by a disease caused by *Alteromonas* sp (Collén & Pedersén, 1996). 6.5 µM H_2O_2 is also known to affect the germination of some

pathogenic fungi, while a concentration of 0.5 μM slowed the respiration of *Nereis diversicolor* (Polychaeta) (Collén & Pedersén, 1996).

2.5.2 Methods of control

Methods of epiphyte and fouling algal control can be:

(1) Physical
(2) Chemical or
(3) Biological

2.5.2.1 Physical methods

The principal method for controlling epiphytes in cultivation is to physically remove them from the host species. This method is labour intensive and can be uneconomical, depending on the degree of fouling and size of the cultivation operation (Ugarte & Santelices, 1992). Other methods that are also labour intensive require removing the cultivated algae from the tanks and then cleaning the tanks with a high pressure water hose to remove the fouling algal growth.

Manipulation of environmental conditions in favour of the host species is often the most effective method of control. Particular success has been obtained by controlling the irradiance level and quality of light (Friedlander, 1991; 1992 and Santelices & Ugarte, 1992).

Other physical methods include monitoring the epiphyte and fouling development and constant care in the form of (a) cleaning and sterilizing the tanks, (b) filtering the water supply and (c) screening cultivation material for the presence of epiphytic organisms, prior to their addition in the cultivation system and (d) drying the seaweeds for short periods (Fletcher, 1995). Short term emergence or dipping the host plants in fresh water for a short period have been shown to be an effective control against epiphytes (Smit *et al.*, in press).
Maintaining an optimal density of host plants may prevent epiphytes from colonizing by allowing self cleaning to occur via thallus movement (Lignell *et al.*

1987). This can also occur if sufficient aeration is provided, causing thalli to rub against each other.

Friedlander & Ben-Amotz (1991) and Friedlander (1992) state that a decrease in UV irradiation in the range of 290 – 400 nm can significantly increase epiphyte biomass, presumably due to removing the damaging effect of UV light on the activity of pigments.

2.5.2.2 Chemical methods

Preventative chemical methods have largely involved the use of sodium hypochlorite solution to pre-treat the seawater, tanks and equipment used. Following this treatment, the chlorine is neutralized and the seaweed is then placed into the sterilized tanks (Ugarte & Santelices, 1992). Copper chloride has been used to treat epiphytic *Enteromorpha* and *Ectocarpus*, but at the risk of harming or contaminating the host (Hampson, 1998).

Manipulation of the growth medium pH helps keep epiphyte biomass down, especially at high pH levels.

In tanks, "pulse feeding" (manipulating the supply of nutrients, particularly nitrogen) can effectively control epiphytes. *Ulva* can take up nitrogen far in excess of that required for growth (termed 'luxury' uptake) and it can grow on this store for up to 10 days (Fujita, 1985). Thus, it is possible to fertilize *Ulva* at intervals to reduce the frequency of epiphytes. High ammonium levels (> 0.5 $mmol.L^{-1}$) can be toxic to epiphytes (Friedlander, 1992). Thus, a pulse feed of nutrients can be particularly successful in reducing epiphytic load when used in conjunction with other methods of control.

2.5.2.3 Biological methods

Grazers can selectively control epiphytes in seaweed culture. Especially small invertebrates or "mesoherbivores" (isopods, amphipods, caprellids, gastropods

and opisthobranchs) and fish that occur amongst host plants and eat the epiphytes (Brawley & Fei, 1987). The use of a commercial grazer is particularly attractive in view of its commercial value. However, care must be taken to monitor grazer density to ensure that it is only the epiphytes that are being eaten and not the host seaweed or that damage is not occurring to the host.

High numbers of mesoherbivores could eat significant amounts of the cultivated seaweeds, not only the epiphytes and thus their density needs to be monitored (Anderson *et al.* 1998 and Smit, in press). A fresh water treatment of the seaweeds helps in reducing populations of these grazers (Smit & Bolton, 1999; Smit, in press & pers. Obs.).

2.6 Diseases

The increasing use of macroalgae for commercial purposes requires that more attention should be directed at their pathology and susceptibility to infection. Asian farming of brown, red and green seaweeds has shown that all are susceptible to disease (Craigie & Correa, 1996; Correa & Sánchez, 1996). The diseased state is recognized as an adverse or abnormal state resulting in unfavourable changes in any parameter such as SGR (Specific Graoth Rate), appearance and economic value (Craigie & Correa, 1996). Conditions such as overcrowding of thalli, nutrient or temperature stress, pathogenic organisms and pests such as grazing animals, produce signs of disease. Biotic pathogens known to infect macroalgae include other algae, bacteria, fungi, mycoplasma-like organisms and viruses (Craigie & Correa, 1996). Research on diseases has to follow Koch's postulate (a procedure by which a presumed disease pathogen is clearly demonstrated to be the cause of the disease) (Craigie & Correa, 1996; Richardson, 1998):

Understanding infectious disease mechanisms in cultivated species is crucial for management in mass cultivations and monocultures, as artificial populations of genetically homogeneous plants at high densities, provide an ideal environment for pathogens to become epidemics.

2.7 Abalone mariculture

Abalone are gastropod molluscs (marine snails), all belonging to the genus *Haliotis* (Hahn, 1989). Abalone are a sought after delicacy in the Far East due to the subtly flavoured meat. Throughout their global distribution, only 10 of the approximately 100 species of abalone are commercially and recreationally exploited, as most of the others are too small or rare to be of interest to the abalone farmer or fisherman (Fallu, 1991). Of the ten species that are currently utilized most come from temperate waters (Fallu, 1991). The continuing demand for abalone is the cause of overexploitation of wild stocks throughout the world. Abalone mariculture was pioneered in Japan, through the development of reseeding programs. Hatchery-reared abalone were reseeded into depleted wild abalone populations and are now harvested sustainably by local fishermen (Shaw, 1982). In addition to reseeding programmes, abalone culture has provided new products for the Far East seafood markets. Abalone are exported live from several countries as "cocktail" abalone and command prices in excess of those paid for canned, dried or frozen abalone (Simpson, 1994). Abalone are presently cultured in Japan (*H. gigantea* and *H. discus hanni*), Taiwan (*H. diversicolor supertexta*), California (*H. rufescens, H. fulgens* and *H. corrugata*), Australia *(H. ruber*), New Zealand (*H. iris*), China (*H. discus hannai*) and South Africa (*H. midae*) (Lyon, 1995). *H. midae* is highly sought after in the Far East (Simpson, 1994).

Six species of *Haliotis* have been recorded on the South African coast (Muller, 1986). *Haliotis. midae* is the largest of the South African species and is the only one which is commercially exploited. A number of attributes of *Haliotis midae* make this species as well as other Haliotids amenable for aquaculture. Some of these features are: prolific gamete production, therefore requiring minimal numbers of adult brood stock; the planktonic larval stage is non-feeding, as it carries a vital supply of yolk material; the algal food supplied to juveniles during feeding is relatively non-fouling and the animals survive well even under relatively crowded conditions (Tarr, 1990).

Haliotis. midae is distributed from St Helena Bay in the Western Cape to Port St Johns on the Transkei coast (Muller, 1986). The harvesting of *H. midae* is most productive in the South Western Cape. The abalone are found between the low tide mark and approximately 25m depth, but mostly at depths of 2 – 10 m in *Ecklonia maxima* beds (Tarr, 1992). Juvenile abalone are found in the subtidal zone under small rocks and sea urchins (Tarr, 1989; SANCOR, 1996; Tarr *et al.* 1996 and Day, 1998).

2.7.1 Fisheries and aquaculture

The South African abalone has a current (2003) export price of $ 30 - 35 per kg live weight (Bennett, 2002 and K. Ruck JSP. pers. comm. 2003) and after shipping and freight charges the value is $ 25 per kg for abalone in the 100 – 150 g size class. This makes the South African abalone a highly sought after resource, placing tremendous pressure on the wild abalone stocks, whose restricted distribution makes them very vulnerable to exploitation by commercial divers, recreational divers and poachers. A high market price and the decline of wild stocks the world over has provided an incentive for the culture of this species.

Abalone culture can be broken down into several components according to the culture process. These components include:

- Conditioning of sexually mature male and female abalone to ensure a constant supply of ripe specimens for spawning
- Spawning inducement
- Fertilization and hatching
- Rearing/management of the pelagic larval stages
- Settling and first feeding
- Management of juvenile abalone through the micro-algae feeding stage
- Weaning on to macrophytes
- Grow-out

The only two components of abalone aquaculture that need to be considered in this study are abalone stress due to water quality and nutrition of the grow-out abalone.

2.7.2 Stress

Disease may be defined as any process that impairs the normal physiological function of an organism (Lindgren, 2000). It may be caused by stress or infection by a pathogen (a disease-producing micro-organism or substance, e.g. virus, bacterium, fungus, protozoan or even a metazoan) (Lindgren, 2000). As a general rule when pathogens are large enough to be seen with the naked eye they are called parasites (Lindgren, 2000).
Infectious disease occurs when a micro-organism comes to live in, or on, a host and damages the host's health. Usually, a host in perfect health is resistant to infection and the pathogen requires a stressed host to allow it to gain a foothold. Some virulent pathogens can infect a relatively healthy host (Lindgren, 2000).

The single most important factor affecting an abalone is water quality. Water is not only a source of essential substances such as oxygen, but is a waste disposal system as well. So poor water quality may contain substances that are toxic to abalone or may not contain sufficient quantities of essential substances (Boyd, 1998). Usually, the life processes of animals degrade water quality (Boyd, 1998). Toxic substances are produced and excreted in the form of faeces and urine and at the same time metabolic processes use up oxygen. In natural waters, physical processes quickly remove or dilute wastes and other kinds of life process change them into less harmful forms. On a farm this does not happen and thus water quality must be strictly controlled (Boyd, 1998).

One of the most toxic waste substances is ammonia. Ammonia-nitrogen results from the end product of protein catabolism in most aquatic poikilotherms (Boyd, 1998). High animal stocking densities can lead to a build up of this metabolite to potentially toxic levels. Ammonia toxicity is caused by high ammonia concentrations in the blood resulting from an inability of the animal to excrete

ammonia, or from the uptake of ammonia from the water at the surface membranes, particularly the gills. Ammonia-nitrogen occurs in two forms, an un-ionized form (NH_3) which is toxic, and an ionized form (NH_4^+) which is non toxic (Lyon, 1995). Un-ionised ammonia, because of its lack of charge and low solubility is more toxic, as it can readily diffuse across gill membranes. The ionized form of ammonia occurs as a larger, charged, hydrated molecule that cannot easily pass through the hydrophobic micropores of the gill membrane, rendering it less toxic (Lyon, 1995). The equilibrium between the two forms of ammonia-nitrogen is determined by the pH and temperature of the water. Consequently, ammonia-nitrogen is more toxic in seawater, which is a better buffer and has a higher pH than freshwater. The proportion of un-ionized ammonia-nitrogen present in solution also increases as temperature increases and decreases as the ionic strength of water increases (Lyon, 1995). The temperature effect is due to increased hydrolysis of ammonium ions (NH_4^+) at higher temperatures (Lyon, 1995). In intensive aquaculture systems the primary factor limiting a build up of ammonia is the rate of supply of water. Knowledge of ammonia production levels and tolerance level of abalone to ammonia facilitates the management of flow rates so that optimal water quality conditions can be maintained at maximum stocking densities (Fallu, 1991).

In nutrient-rich sludge saturated with water, such as at the bottom of a pile of rotting seaweed, bacterial action quickly uses up all the available oxygen and anaerobic bacteria processes begin. One product of the anaerobic breakdown of waste material is hydrogen sulphide (H_2S), which is very toxic (less than 1 part per million is toxic to abalone) (Fallu, 1991).

Abalone require oxygen levels greater than 3 – 4 parts per million (Fallu, 1991). In a farm, abalone may have to compete with algae for oxygen. In the day, algae in the tank will produce oxygen, but at night they will consume it. During the spat phase and the final grow out phase where algae are an important food source for the abalone, dark conditions can create a localized shortage of oxygen close to the algae. Decomposition of waste products will also remove oxygen from the water. It is also possible to have too much oxygen, and if the

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2.7.3 Abalone nutrition

Abalone are generally herbivores and feed mostly on seaweed (Barkai & Griffiths, 1986). Adult abalone eat between 10 – 30 % of their body weight per day (Hahn, 1989). The provision of a suitable diet and the subsequent increased growth rate is important in the success of abalone aquaculture (Capinpin, 1996). However, because of problems associated with the use of seaweed as feed for abalone, like the unreliability of the supply of these seaweeds due to over-exploitation of natural stocks, intensive abalone culture is becoming increasingly reliant upon formulated diets (Hahn, 1989; Britz 1995). Dry, pelleted abalone feeds are currently being used in Japan, China, Australia, New Zealand and South Africa. In formulating these diets, it is important however, to know the response of abalone to various nutrients in order to be able to produce an effective low-cost diet.

Abalone require a balanced diet of lipid and essential and non-essential amino acids (Mai *et al.* 1995a). Protein is an essential but expensive component in the artificial feed diet of abalone. The optimum protein level in a casein-based diet was determined to be 20-30% by Uki & Watanabe (1992). Britz (1995) found that the growth rate of *Haliotis midae* increased with an increase in protein content from 27 to 47% whereas the protein efficiency ratio (PER) was negatively correlated with protein level. Brett (1979) and Britz (1996) also reported that a dietary protein level higher than 20-30% may be required to achieve maximum growth rate (G_{max}). Research has shown that a *ca.* 30 – 40 % protein level in the diet of abalone is most suitable for both large and small size classes (Hahn, 1989; Mai *et al.* 1995; Britz & Hecht, 1997).

The amount of artificial dry feed ingested by abalone appears to be governed by their metabolic rate, as consumption in *Haliotis discus hannai* (Hann, 1989) and *Haliotis midae* (Britz & Hecht, 1997) has been shown to be a predictable function of body size and temperature.

To achieve maximum growth, the rate of protein assimilation by abalone must be maximized, which implies that formulated feeds should contain sufficient protein. This shows therefore that the expensive protein fraction should be optimally utilized for growth rather than be used for energy by the abalone. This results in increased production costs if abalone are to be cultured intensively using formulated feeds.

Lipids in the diet are important as a source of concentrated energy, essential fatty acids and some other non-fat nutrients (Mai *et al.* 1995a).

Carbohydrate is another important energy source for abalone. Britz and Hecht (1997) found maximum growth rates in *H. midae* at dietary carbohydrate levels of 33 – 58 %.

The high price of formulated feeds in South Africa has resulted in the increased use of fresh seaweeds as an alternative feed. These include the brown algae (kelps), *Ecklonia* and *Laminaria*, the green alga *Ulva* and the red alga, *Gracilaria*. Other seaweeds have also been tried but the growth rate of abalone that were given these algae were not promising. This includes the red seaweed *Plocamium corallorhiza* (Simpson & Cook, 1997:1998).

CHAPTER 3

FARM DESCRIPTION AND TANK DESIGN

University of Cape Town

3.1 INTRODUCTION

Jacobs Bay (Jacobsbaai) Sea Products Abalone Farm (JSP) in Jacobs Bay and Irvin & Johnson (I & J) Cape Cultured Abalone Farm on Danger Point near Gansbaai were the aquaculture facilities used in this investigation. The farms have slightly different methods of culturing abalone. The tank sizes and types used to culture the seaweeds on each farm are different, and the fact that the farms are separated by greater than 250 km and are therefore subject to different weather and sea conditions makes comparisons between the two farms difficult. It was decided to treat the I & J farm as a pilot scale commercial operation and thus run the experiment on the farm as if we were cultivating for commercial reasons, while the *Ulva* was grown on an experimental scale at JSP with the majority of culture–related experiments occurring on this farm. The results from the JSP farm tanks were then compared to the results obtained in the commercial scale system used at I & J.

The aim of this chapter is to provide an overview of the farms, tanks and water sources as well as and the experimental design on each farm.

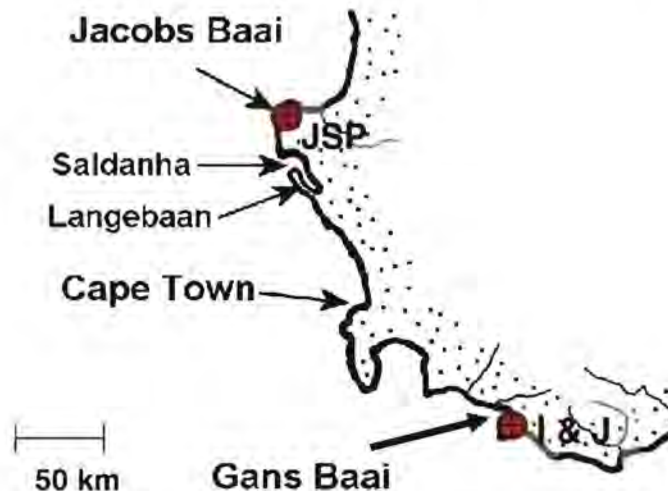

FIGURE 3.1: Map showing places mentioned in text.

3.2 JSP

Jacobs Bay is located along the West coast of South Africa approximately 120 km north of Cape Town (Figure 3.1). The farm Jacobs Bay Sea Products Pty., is a land-based intensive mariculture operation of ± 11 ha situated on the point of Jacobs Bay. The farm cultivates mainly abalone (*H.* midae) and turbot (*Scopthalmus maximus*). They also cultivate oysters (*Crassostrea gigas*) and previously cultivated *Gracilaria gracilis* on an experimental scale. The farm has an abalone stock of approximately 2.4 million abalone (± 76.8 tons), which range from spats to 6-year-old animals. 77 tons of fresh harvested kelp (*Ecklonia maxima* and *Laminaria pallida*) are used each month as abalone feed. The abalone eat approximately 5 – 7 % of their body weight in kelp per day.

The farm has been in operation since 1994. In 2002 small scale *Gracilaria* cultivation was tried in both existing dams and oyster raceways. The *Gracilaria* was obtained from Saldanha Bay. The cultivation project failed due to sediments settling on the thalli and smothering the algae. This was partially due to insufficient water flow in both the raceways and the dam. The algae that were placed in the dam also disappeared after a two week period. This was thought to be due to the isopod *Paridotea reticulata* which consumed the *Gracilaria* (Miller, 2001).
The farm wishes to cultivate algae, primarily to absorb nutrients in waste water in case the farm has to re-circulate its water during a harmful algal bloom event (HAB's). HAB's are common on the west cost and have been known to cause large abalone mortalities (Matthews & Pitcher 1996; Pitcher, 1998). HAB's can be costly for the farms especially if the HAB tide event consists of the dinoflagellate *Alexandrium*, where in which case abalone that were ready for live sale had to be canned as they are unsellable in the live form. Even though abalone are not filter feeders it was found that they still carry the toxins in the viscera and on the skin epithelium, especially the epipodial fringe. At JSP, the abalone had to be canned for a year and a half following a red tide event and this resulted in a 30 % revenue loss for the farm (K. Ruck pers. comm.). The residues produced by HAB's can also slow the growth rate of the animals. The

second reason to cultivate algae is as an alternative food supply. This is because in winter, westerly storms make kelp harvesting in the area difficult and dangerous and thus the farm has to get its kelp from another area, which is costly. Thirdly, the farm has a limited water intake ability. The cost of laying new pipes to increase the water supply to the farm is significant and if the farm were able to recirculate its water supply, they could increase the amount of water available on the farm and thus increase the farm's abalone carrying capacity.

Figure 3.2 is a scheme of the layout of JSP Pty (Morgan, 2000). There are four primary components to the farm:

- The settling and holding dams (a, b, and c),
- The abalone tanks (d),
- The turbot tanks (e) and
- The inactive oyster raceways (f).

Seawater is pumped directly into the top settling reservoir (a) at a rate of 1 200 – 1 300 $m^3\ hr^{-1}$, and from here it is gravity fed to either the turbot tanks (e) or the bottom holding dams (b and c). Water can be pumped directly into the holding dams where it is heated by solar radiation (in summer). This is done to combat the low water temperatures experienced in summer when upwelling of cold water occurs off the west coast. Low water temperatures (< 11°C) slow the growth rate of the abalone (K. Ruck & H. Otto pers com.). The water turnover rate for the top-settling reservoir is 5.6 volumes d^{-1} and 4.5 volumes d^{-1} for the bottom 2 dams. From the dams, the water is pumped into the mixing tank (g) where it can be distributed to the abalone tanks, turbot tanks, oyster raceways or returned back to the dams. The water is not filtered for particles before being distributed from the dam to the tanks. However, some settling does occur in the dam. Effluent water is channeled up to the sump (h) where the particles are settled out, and the remaining wastewater is returned to the sea.

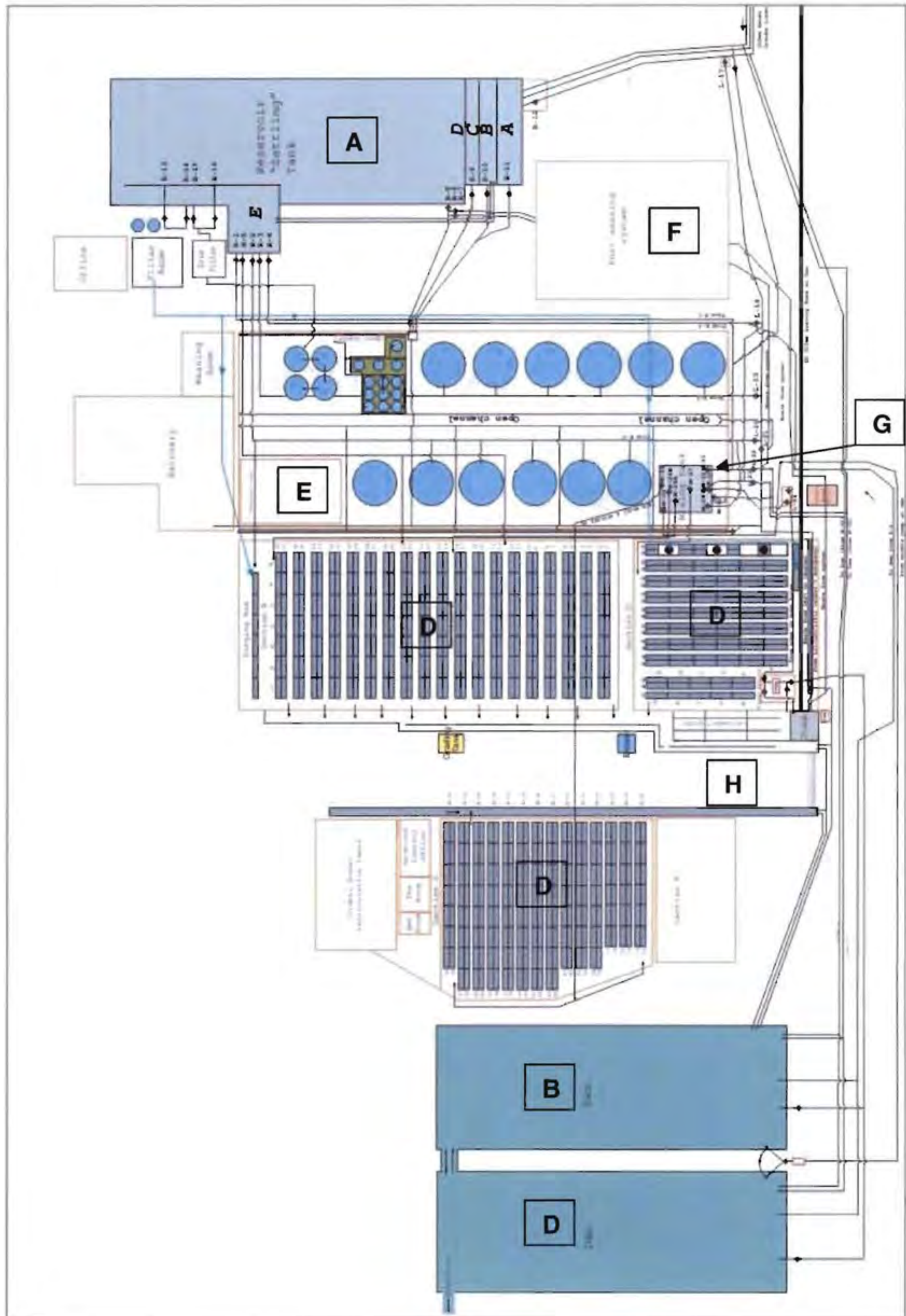

FIGURE 3.2: Scheme of the layout of JSP Pty.

3.3 I & J

Gansbaai is located along the south coast of South Africa approximately 140 km east of Cape Town (Figure 3.1). The farm I & J Cape Abalone Mariculture Pty, Ltd., is a land-based intensive mariculture operation situated at Danger Point. The farm is situated on 5 ha of land and cultivates primarily abalone (*H. midae*). Ko b (*Argyrosomus japonicus*), the west coast rock lobster (*Jasus lalandii*) and small quantities of *Gracilaria* and *Ulva* are cultivated on an experimental scale. The abalone stock consists of spat and approximately 3.6 million grow-out abalone ($\pm$ 125 M tons), which range from 1- to 6-year-old animals. The farm uses about 7 M tons of kelp a day, 5 days a week. Thus about 1 800 M tons of kelp (*Ecklonia maxima*), per year, is required as abalone feed.

As with JSP the farm has been in operation since 1994, and for the last four years (since 1998) they have been cultivating both *Gracilaria* and *Ulva*. The *Gracilaria gracilis* (Iyer, 2001) was obtained from both Saldanha Bay, RSA and Luderitz, Namibia, while the *Ulva* was obtained from Gansbaai harbor. The *Gracilaria* cultivation occurs in the same type of tanks as the *Ulva.* They have also attempted to grow *Gracilaria* in the re-circulation dam, but with little success. The farm needs to cultivate the *Gracilaria* as they intend to set up a raft mariculture operation in Gansbaai harbor and need a parent stock.

In current farm operations, both seaweeds are stocked at an initial density of 2 $kg.m^{-2}$. Both are fed nutrient supplements once weekly, to compensate for the low and variable water turnover rates ($\pm$ 4 volume exchanges per day). The fertilizer used is a combination of Maxiphos ® (a commercial phosphorus fertilizer) and ammonium sulfate. *Gracilaria* is fertilized with a ratio of 10 parts Maxiphos to 1 part ammonium sulfate, while for *Ulva* the ratio is 6:1. During fertilization, the water supply to the *Ulva* tanks is turned off at 08h30 and 100 g of the 6:1 mix is added to each tank. The water supply to these tanks is turned on again at 16h30. The water supply to the *Gracilaria* tanks to be fertilized is turned off at 16h30, and the tanks are dosed with 100 g of 10:1 mix per tank.

This pulse feeding serves two purposes. Firstly it helps to combat epiphytic growth (Ryther *et al.* 1981;Friedlander & Ben-Amotz, 1990; Friedlander, 1991) and secondly it allows for maximum absorption of the nutrients by the algae. The water supply to the tank is then turned on again at 08h30, the following morning. The difference in the fertilization regiemes is due to luxuary uptake of nutrients by *Gracilaria* at night (Friedlander & Ben-Amotz, 1990; Friedlander, 1991). The algae are harvested monthly. The harvested material is then fed to the grow-out abalone bigger than 50 mm, where it lasts for 2 days before being completely eaten.

The water temperature range on the farm is 15 to 18 ° C. This varies seasonally, and the mean is about 16.5 ° C. With partial water re-circulation the water temperature on the farm can be increased by 1 ° C.

Figure 3.3 is a scheme of the layout of the I & J farm. There are four primary components to the farm:

- The pump house,
- The header tank,
- The abalone tanks and seaweed tanks and
- The re-circulation dam .

Seawater is drawn from the sea into a pump house and then pumped directly into the top header tanks at a rate of 1 200 m^3. hr^{-1}. From there it is gravity fed to either the abalone tanks or the seaweed tanks. The water is not filtered before entering the tanks. The water turnover rate for the header tank is 5 - 6 volumes d^{-1}. Under normal farm conditions, effluent water is returned directly to the sea. When the farm recirculates its water, effluent water is channeled to the re-circulation dam. Before going into the dam the water is drawn over a conveyor filter which removes about 85 % of the water-borne faeces. The recirculation dam holds approximately 2 500 m^3 of water, and here some of the particulates are settled out due to sediment traps (a series of low walls in the dam which slow water motion and allow for sedimentation of particles). From the dam, about 1 000 to 1 500 m^3 hr^{-1} is re-circulated around the farm before returning to the dam from where excess wastewater is returned to the sea.

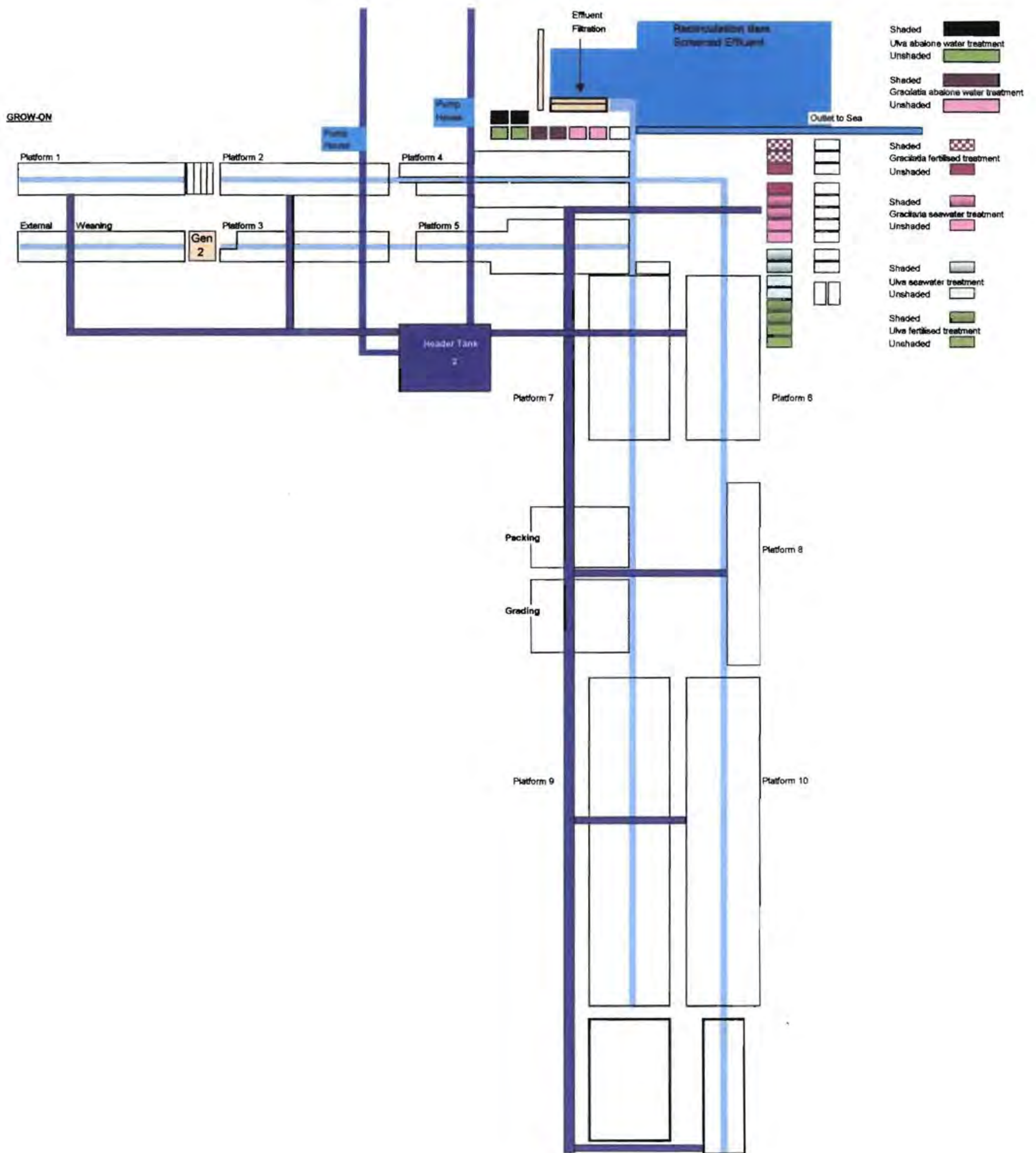

FIGURE 3.3: Scheme of the layout of the I & J farm

The re-circulation consists of 50 % fresh seawater and 50 % wastewater. During a period of HAB (which can last between 2 – 7 days), the farm re-circulates water to the brood stock and the spats on a fast cycle, while a slower re-circulation occurs for the grow-out abalone.

3.4 Tank design

There are many designs of tanks for the mass culture of marine macrophytic algae. These range from small tanks (i.e. with tens of liters volume), to intermediate and large sized tanks (200 – 800 liter volume). These tanks typically employ some form of air bubbling to circulate thalli within the tank (so called "tumble culture" effect) (Vandermeulen, 1989). Early designs for tumble culture tanks frequently employed a sloping bottom with a single air pipe placed along the bottom of the deep end. Water left the system via a simple vertical standpipe drain (Vandermeulen, 1989). Agitation can also be achieved by using paddle wheels. These tanks can be made of concrete or plastic material. Tanks may be rectangular or have 'U' or 'V' shaped-bottom (see Figure 1.2). High levels of production have been achieved for various *Ulva* species in small culture vessels, but with each scale-up, production (m^{-2} of surface area) declined markedly (Hanisak and Ryther, 1984). However, for large-scale production, rectangular tanks are the easiest to build and the most economical according to Neish (1979) and Neish and Knutson (1979), who grew the carrageenan-producing seaweed *Chondrus crispus* on a large scale.

Ulva has a very flexible thallus, which means that it rapidly clogs drains and will wrap itself around any irregularity on the tank surface including freestanding air or drain pipes (Vandermeulen, 1989). Thus, a modified tank design was needed to overcome these problems.
The design we based our tanks on was a modification of the tank used by Vandermeulen (1989). For this project, we used three different sizes of tanks.

3.4.1 Small tanks at JSP

The smallest tanks were made of a speckled, light grey polyethylene. The colour was chosen to minimize reflection within the tanks and to reduce heat absorption. Tank volume was approximately 100 ℓ with inside bottom dimensions of 0.60 m X 0.40 m X 0.40 m, which is slightly smaller than described by Vandermeulen (1989). However, the water level was kept about 5 – 10 cm below the rim of the tank after immersion of the seaweed, hence the actual water volume was about 96 ℓ. The majority of experiments were conducted in these small tanks. Figure 3.4 shows a longitudinal view of the tank.

FIGURE 3.4: Longitudinal view of small tanks

Smooth water circulation within the tank depends on a stream of bubbles rising up evenly along the entire length of the pipes to form an air curtain. Several designs of pipe placements were tested in order to achieve this smooth flow. Designs ranged from a single pipe running down the inside edge of the tank or in the middle of the tank. The most successful design was a U – shaped pipe system that was blocked off on either end of the U. This system was a modification of a rectangular system that had been tried earlier. However, it was noted that there was insufficient backpressure on the ends furthest away

from the air inlet. This caused the formation of dead circulation spaces in the corners of the tank where the algae would collect and bunch up. In order to create the backpressure needed the ends of the U were blocked off, however the dead air spaces still existed. After discussion with staff at the Two Oceans Aquarium in Cape Town (P. Lotter & S. Chater, pers. com.) about the technique that they use to keep the jellyfish circulating in the display, it was decided to drill 2 mm diameter holes at 90 mm spacing along each leg. To compensate for the decreased air pressure towards the ends of the legs, 2 intermediate holes were drilled between the last two holes. In this way we achieved a relatively even quantity of bubbles to form the air curtain. This differs from the uniform spacing of air holes suggested by Vandermeulen (1989).

Air was supplied to the pipes via a Howard & Donkin channel blower and not by a compressor (thus the air was at atmospheric pressure when delivered to the tanks). Air is delivered from the blower to the tanks in a 100 mm PVC pipe (2 mm thick). A 20 mm tapping was made and air was delivered to the 'U' tubes via a flexible 20 mm hose and a rigid 50 cm X 20 mm pipe. The aeration system was made of a 'U' shaped frame comprised of two 54.4 cm, 20 mm pipes closed at the terminal ends by a stopper. These were joined with 20 mm elbows to two 14.5 cm X 20 mm pipes, which were then joined by a 20 mm "T". PVC solvent was used to permanently connect these pipes and prevent air leakage. To ensure that the pipe frames stayed in position at the bottom, they were cemented to the base of the tanks using silicone sealer. This was important because it was noted in a preliminary study that when either of the two arms of the frame lifted there was reduction in air supply from the arm located in deeper water. Hence, it was essential that both arms were cemented to the bottom of the tank to ensure a uniform supply of air.

Tests performed with unattached *Ulva lactuca* collected at Simons Town harbour, demonstrated that dead circulation spaces were absent, and that the *Ulva* circulated from bottom to top as well as from side to side in an alternating pattern that was repeated approximately every 15 seconds.

Water from the main water system was fed into the tank through a 20 mm PVC pipe. The water was supplied directly to the tanks from above, with the supplying pipe facing downward. A 32 mm drain hole was drilled for the output pipe. This hole was 8 cm from the top of the tank on the end of the tank opposite to the air supply. Through this hole a running nipple 10 cm X 32 mm was threaded. It was kept in place by two 32 mm diameter nuts on either side of the tank. To ensure that no thalli were washed out through the output pipe, a 20 cm diameter common kitchen colander was placed over the outlet hole and was held in place using silicone and two cable ties. This acted as an effective sieve. An earlier outlet system that consisted of two pipes, one of which was inside the tank and had many 4 mm diameter holes drilled into it failed, due to the fact that in the tumble culture the thalli kept breaking up into smaller pieces and blocking the outlet. In addition, the thalli would become entwined around the pipe and ultimately desiccated.

3.4.2 Medium tanks at JSP

In order to obtain sufficient material for experiments it was decided to use a slightly larger tank for growth of a parent stock. These tanks were supplied with fresh seawater and the algae in these tanks were used as a baseline to compare growth rates in the smaller experimental tanks. These tanks were made of light grey, heavy duty polyethylene, which had been UV stabilized. The tanks had been manufactured 20 % thicker than standard (9 mm), to prevent the walls of the tanks from bulging outwards when filled with water (Figure 3.5). They held approximately 356 ℓ, but after immersion of the seaweed, the water level was kept 5 – 10 cm below the rim of the tank, hence the actual water volume was 350 ℓ. The bottom inside tank dimensions were 960 mm X 630 mm X 590 mm. There was no ribbing on the outside surface of the tank and the tank walls sloped outward. The tank walls were drilled through allowing the attachment of pipes and connections.

The aeration frame was identical to those in the small tanks except that the 'U' shaped frame comprised of two 75.2 cm X 20 mm pipes. Water was fed into

the tank the same way as the small tanks and the outlet system was identical except that the colander diameter was 30 cm and the outlet hole was drilled 14 cm from the top of the tank

FIGURE 3.5: Longitudinal view of medium tanks

Large tanks at I & J

I & J has been cultivating *Gracilaria* and *Ulva* at their farm for 4 years. They have tanks designed specifically for this purpose. These were the largest tanks used in this study and they are probably the size of tanks that would be used if the alga is to be grown on a commercial scale.

FIGURE 3.6: Oblique view of the large tanks

The tanks were approximately 5 m X 1m surface area and 0.6 m deep with an outlet 17cm from the top (Figure 3.6). They were made of white PVC lining (to facilitate easy cleaning and to reduce light absorption) supported on a wooden frame. The PVC lining was rounded on the bottom. Earlier tests had shown that triangular tanks tore with the pressure of the water in them. The tanks were aerated by a 30 mm PVC pipe that ran along the bottom, in the centre of the tank. Holes (3 mm) were spaced evenly every 250 mm along the pipe. There was a noticeable decrease in backpressure at the end of the pipe, thus, dead circulation spaces occurred at the ends of the pipe. The circulation pattern was such that the seaweed moved from bottom to top approximately every 15 seconds. The air supply to each tank was controlled by a valve, as with JSP the air was supplied by a Howard & Donkin channel blower.

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(b) Fluctuations in phosphate and ammonium due to the diurnal variability in abalone feeding and excretion

The third water source (termed "turbot") originating from the turbot culture tanks, is collected in a separate sump, from where it gravity fed into a third series of seaweed tanks.

3.5.2 Water Sources at I & J

Four types of growth media were employed at this farm. The first source (termed "seawater I & J"), is pumped directly from the adjacent surf zone area (by means of a sub-surface filter system). From the pump house, it is pumped into a reservoir before being distributed to the abalone and seaweed tanks via separate pipelines. Nutrient concentrations of the filtered seawater are those of the natural coastal waters.

The second water source (termed "fertilized I & J") is seawater that has been fertilized once weekly. The fertilizer consisted of Maxiphos ® (a commercially available fertilizer) and ammonium sulphate in the ratio of 1 part ammonium sulphate to 6 parts Maxiphos ®. The N: P ratio for Maxiphos ® in mg N/P per g DW of Maxiphos ® is 80,56: 55,15 which is a ratio of 1.46: 1 (Std. Dev $\pm$ 0.02 from own measurements using methods for tissue analysis (see Section 4.7)). The concentration of ammonium and phosphate in the water after the addition of fertilizer is 574.73 µM ammonium to 280.41 µM Phospate: a ratio of 2.05: 1 (Std. Dev $\pm$ 0.02 from own measurements using methods for water analysis (see Section4.4)).
The water to the tanks to be fertilized is turned off at 08h30. The tanks are then fertilized with 100 g of Maxiphos ® per tank. The water is turned on again at 16h30.

The third water source (termed "abalone, I & J") initially flows through the abalone culture tanks and is collected in the recirculation dam at night, before being pumped into a third series of seaweed tanks. During normal farm

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operations at I & J all the waste water is channeled out of the farm during the day, and at night the waste water is channelled into the re-circulation dam where particles then settle out. Under normal conditions the effluent growth media for the seaweed tank is pumped from the dam continuously resulting in a lowered water level in the dam during the day. Abalone excretion at night therefore provides the only additional nutrient source in the inflow to the tanks.

The fourth source of water (termed "recirculated, I & J ") is waste water from the farm which is taken over a conveyor filter (which removes 84 % of water-borne faeces), then pumped into the recirculation dam, where suspended matter settles out and then is pumped into the same series of seaweed tanks as the effluent media. Under conditions of re-circulation, the waste water is channeled into the dam, implying that the effluent growth media will experience peaks and troughs in nutrient concentration which would not be evident under normal farm operating conditions. The nutrient composition of the effluent water is therefore determined by nutrients introduced to the abalone and commercial seaweed tanks, including:

(a) cleaning of the abalone culture tanks (resulting in an ammonium pulse),

(b) fertilizer pulsing of the seaweed tanks (i.e. increased phosphate and ammonium concentration in the effluent water,

(c) fluctuations in phosphate and ammonium due to the diurnal variability in abalone feeding and excretion.

This water source was only used for the duration of Experiment 2 (Chapter 10), as the conveyer belt system is only used when the farm recirculates.

3.6. Shading

In early summer of 2001 it was noticed that there was a decrease in SGR of *Ulva* at both farms. This decrease was more pronounced at the I & J farm. In order to investigate if shading of the tanks would prevent this decrease in growth, half of the tanks in all the treatments on both farms were shaded. The shade cloth used was that which was available at the two farms.
On the JSP farm an 80 % shade cloth covered half the tanks from November 2001 to January 2002. From January to May 2002 a 20 % shade cloth was used to shade the tanks, this was done as it was noted that SGR did not improve with heavy shading.
On the I & J farm a 50 % shade cloth was used from November 2001 to June 2002 to cover half of the tanks.

3.7 Experimental design

With the variation in tank sizes and number on the farms the experimental set up was different for the two farms.

The experimental set up at JSP was 16 small tanks and 4 medium tanks. The medium tanks were all fed unfiltered seawater at 20 volume exchanges per day. The small tanks were divided up into three treatments. 4 small tanks were also fed unfiltered seawater, 6 tanks were then fed abalone effluent water and the remaining 6 tanks were fed turbot effluent water. Due to a pump burning out in the abalone effluent sump, the abalone effluent treatment only ran for a month in 2001 (November) and then continuously from February 2002. All the small tanks received water flow rates of 20 volume exchanges per day for the duration of the experiment.

The experimental set up at I & J was 12 tanks with 3 sets of four tanks each fed on a different type of water supply. A series of 8 tanks were fed fresh unfiltered seawater at approximately the same flushing rate as the rest of the tanks on the farm (i.e. 4 volume exchanges d^{-1}). Four of these tanks were pulse fertilizer fed.

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This was later changed to 12 volume exchanges d^{-1} in March 2002, when it was discovered that 4 volume exchanges d^{-1} was insufficient to maintain the culture population in summer. The remaining 4 tanks were fed unfiltered abalone effluent water drawn from the inlet supplying the recirculation dam. The inlet rate was 12 volume exchanges d^{-1}. The conditions (e.g., fertilization regime, sunlight, flow rate) in all sets of tanks remained the same, the only variable being the nature of the water supply.

The water supply rate at the farm is fixed by the limited pumping capacity. The proportion of this supply available for the growth trials was limited by operational demands. Thus, it was not possible to experiment with exchange rates higher than 12 volume exchanges d^{-1}.

CHAPTER 4

METHODS CHAPTER:
A LIST OF EXPERIMENTS PERFORMED AND METHODS USED IN THIS STUDY

4.1 INTRODUCTION

This Chapter gives a brief description of the experiments performed at both farms. In addition, as there is a large overlap in the methods used in the various Chapters it was decided to have a separate Methods Chapter to allow for quick referral for the various methods used in the individual chapters.

4.2 Experiments at JSP

4.2.1 SGR, yields, moisture content, tissue nutrients, epiphytes, species dominance and meso-herbivores

From the 7th August 2001 to the 10th of October 2002, samples were taken on a biweekly basis to record: seasonal changes in RGR, yield, tissue moisture content, nitrogen and phosphorus levels as well as species dominance and meso-herbivore abundance in the small and medium tanks supplied with turbot effluent, abalone effluent and unfiltered seawater. Measurements taken in the tanks included daily pH, temperature and dissolved oxygen.

4.2.2 Water exchange rates

A short water exchange experiment was run from the 2nd – 3rd of October 2001 in the small and medium seawater tanks. Half of the tanks had their flow rates decreased to 4 volume exchanges per day, while the other half remained at 20 volume exchanges per day.

4.2.3 KELPAK® and fertilizer experiments

Three separate experiments were run. The first was run from the 17th of May to 14th of June 2002, had 16 small tanks receiving turbot effluent only and were subjected to 4 different concentrations of KELPAK ® (a commercial kelp extract). The four medium and small seawater tanks were used as controls. The second run of the experiment from 28th of June to 30 July 2002, was also

run using turbot effluent, however the 16 tanks were subject to different combinations of fertilizer and KELPAK ®, with the seawater tanks again being used as the controls. The third run of the experiment was from the 31st of July to 27th of August 2002, with 16 tanks receiving various combinations of abalone effluent, KELPAK ® and fertilizer. The seawater tanks were again used as a control.

4.2.4 Stocking density

Three stocking density experiments were run in seawater, abalone and turbot effluent from the 28th of August to the 9th of October 2002.

4.2.5 Nutrient uptake experiments

From the 9th – 13th of October 2002 two water nutrient analysis experiments were performed. The first experiment investigated uptake in abalone and turbot effluent at a set stocking density (Experiment 3) and the second measured uptake at various stocking densities (Experiment 4).

4.3 Experiments at I & J

4.3.1 RGR, yields, moisture content, tissue nutrients, epiphytes, species dominance and meso-herbivores

From June 2001 to November 2002 a seasonal growth experiment was conducted in the large tanks and using the following culture media:

Fertilized seawater

Abalone effluent

Unfiltered seawater.

Measurements taken included: monthly measurements of RGR and yield as well as samples for analysis of moisture content and tissue nutrients. Tanks and algae were inspected to determine fouling by other organisms and samples

were identified. Daily measurements included temperature, pH and dissolved oxygen.

4.3.2 Nutrient uptake experiments

Two water nutrient uptake experiments were performed, the first (Experiment 1) from the 25th of February to the 27th of February 2002 looked at uptake rates in shaded and unshaded tanks at a set stocking density and with variable water exchange rates in seawater, fertilized seawater and abalone effluent water. The second experiment (Experiment 2) performed was from the 15th August to the 17th of August 2002. This experiment looked at uptake rates with all tanks receiving the same flow rates but varying stocking densities. The water used were seawater, fertilized seawater and recirculated abalone effluent.

4.3.3 Stocking density

Three stocking density experiments were tested in seawater, fertilized seawater and abalone effluent from the 14th of August to 16th of November 2002.

4.4 Methods

4.4.1 Water Nutrient Analysis

Water nutrient analyses were performed in Chapters 6 and 10 using the methods described below.

Four nutrient uptake experiments were conducted to provide preliminary quantification of the biofiltering capacity of *Ulva* sp. at both farms.

Ammonium and phosphate samples were taken in triplicate and analysed as such. Nitrate and nitrite were analyzed from a single sample. Nutrients tested for in each of the experiments were:

(a) ammonium

(b) phosphate
(c) nitrate
(d) nitrite

The exception was Experiment 1, in which nitrate and nitrite were not tested. Samples were taken from the inlet pipe before the water entered the tanks and from the outlet pipe after the water exited the tanks. The samples were syringe filtered on site and then frozen.

4.4.1.1 Ammonium Analysis

Ammonium concentration was determined using the method described by Grasshoff *et al.* (1976), scaled down to a sample volume of 5 ml and reagent additions of 0.2 ml.

A 5 ml aliquot of filtered, defrosted water sample was placed in a test tube, to which was added 0.2 ml of phenol, citrate and trione solution with mixing between additions. The sample was covered with aluminium foil and left for a minimum of 8 hours after which it was read at 640 nm on a Cecil 1000 UV-visible spectrophotometer (Cecil). The values obtained were compared with a standard curve obtained by analyzing a series of standards in the same way as the samples. The standard series was made up by serial dilution of ammonium stock solution. 1 ml of stock solution was diluted to 100 ml with ammonium-free water. By taking 0, 1, 4, 8, 15 & 25 ml of diluted stock and making these up to 25 ml with ammonium-free water, a standard series of 0, 4, 16, 32, 60 & 100 µM NH_4 was obtained. A blank of 0.002 was subtracted from the sample reading for contamination from NH_4 in reagents.

Phenol solution was made up by dissolving 13.58 g of colourless phenol (C_6H_5OH) and 0.1435 g disodium nitroprusside dihydrate ($Na_2Fe(CN)_5NO.2H_2O$) made up to 500 ml with MilliQ water (filtered water).

Citrate solution was made up by dissolving 34.28 g citrate dihydrogen in 100 ml of MilliQ water. To this 1 µmol NaOH (0.04 g) was added. The mixture was boiled while stirring on a heater-stirrer. The solution was then cooled and made up to 100 ml.

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Trione solution was made up by dissolving 0.1833 g Trione (110 mg Cl) in 100 ml of NaOH reagent and made up fresh every week.

The NaOH (0.36M), used in the reagents was made up by dissolving 14.25 g NaOH in 1 ℓ of MilliQ water.

The ammonium standard stock solution (10 µmol N/ml) was made up by dissolving 0.2675 g NH_4Cl in 500 ml of MilliQ water. (The standard was dried at 100 °C). To this a few drops of chloroform were added. All reagents were stored in amber glass bottles in the refrigerator to prevent light and heat breakdown.

4.4.4.2 Phosphate analysis

Phosphate (PO_4^{3-}) concentration was determined using the method described by Grasshoff *et al.* (1976), with a slight modification in that samples and reagent amounts were reduced by a factor of 10.

A 5 ml aliquot of filtered, defrosted sample was placed in a test tube and 0.1 ml of ascorbic acid and mixed reagent, mixing between additions, were added in that order. The sample was left for a minimum of 30 minutes after which it was read at 880 nm on a Cecil Spectrophotometer. The values obtained were compared with a standard curve obtained by analyzing a series of standards in the same way as the samples. The standard series was made up by serial dilution of phosphate stock solution. 1 ml of stock solution was diluted to 100 ml with MilliQ water. By taking 0, 1, 4 & 8 ml of diluted stock and making these up to 25 ml with MilliQ water. A standard series of 0, 4, 16 & 32 µM PO_4 was obtained.

Ascorbic Acid solution was made up by dissolving 10 g of ascorbic acid ($C_6H_8O_6$) in 50 cm^3 MilliQ water. To this was added 50 cm^3 sulphuric acid, a fresh solution was made up weekly.

The mixed reagent was made up by dissolving 12.5 g ammonium heptamolybdate tetrahydrate, $(NH_4)_6MO_7O_{24}.4H_2O$, in 125 ml of MilliQ water. A separate solution of 0.5 g potassium antimony tartrate, $K(SbO)C_4H_4O_6$, (with or with out 0.5 H_2O), in 20 ml of MilliQ water was also prepared. The molybdate solution was added to 350 ml sulphuric acid with continuous stirring. The

tartrate solution was then added and well mixed and was made up fresh every week.

The sulphuric acid reagent (4.5M), was prepared by adding 250 ml concentrated acid (ç = 1.84 g.ml) to 750 ml MiliQ.

The phosphate standard stock solution (10 μmol PO_4/ml), was made up by dissolving 136.1 mg potassium dihydrogen phosphate KH_2PO_4 in 100 ml MilliQ water to which 0.2 ml sulphuric acid had been added (the standard was dried at 110 °C and then stored in a desiccator). All reagents were stored in amber glass bottles in the refrigerator.

4.4.4.3 Nitrate analysis

Nitrate (NO_3^-) concentration was determined using the copper-cadmium method described by Nydahl (1976).

About 17 g coarse cadmium powder (0.5 – 1 mm fraction) was washed with 2 M HCL and rinsed 5 times with MilliQ water. Approximately 40 ml of 0.08M Copper sulphate ($CuSO_4.H_2O$) was added and stirred with a glass rod until the colour of the solution changed from blue to clear. The cadmium powder was then rinsed with MilliQ water to remove all the precipitated copper or copper oxide, until the supernatant was clear and the copperised cadmium powder was silver grey in colour. The cadmium was then stored in a container under MilliQ water. A cadmium powder column was made by placing the cadmium in a glass tube (5 mm Φ) column filled with MilliQ water to ensure that no air was present in the cadmium. The tube was connected to a peristaltic pump to provide a constant flow rate.

0.1 ml of Tris Buffer (pH 8) was added to a test tube and then filled with the sample/standard. The column was first flushed with MilliQ water and then activated before use with 60 μmol/ℓ NO_3 in seawater. The column was then flushed with 8 – 9 ml of sample/standard at 170 ml/hr flow rate. This flush was discarded while the column was still running and 5 ml was collected for analysis. The pump was paused and a new sample/standard was set up. The sample order was 3 standards (60,60 & 20 μM), then 10 samples then 1 standard then 10 samples etc. and finally ending with three blanks.

Colour determination used 5 ml of sample with 0.1 ml of sulfanilamide. After 5 minutes 0.1 ml of NED was added and the test tube agitated to mix the reagents. Colour was developed for 15 minutes after which samples were read at 540 nm on a Cecil spectrophotometer.

The copper sulphate solution comprised of 10 g copper sulphate pentahydrate dissolved in 1 ℓ of MilliQ water.

A 1 M Tris buffer (pH 8.0) stock solution was diluted 1:100 with sample for use.

Sulphanilamide (2.5 g) was added to 50 ml 2M HCl and was diluted to 250 ml using MilliQ water.

N-(1-Naphthyl)ethlenediamine dihydrochloride (NED) (0.250 g) was dissolved in MilliQ water and diluted to 250 ml. The solution was stored in an amber glass bottle in a refrigerator. Small portions were withdrawn and brought to room temperature before use.

The standard series was made up by serial dilution of Nitrate stock solution. 1 ml of stock solution was diluted to 100 ml with low NO_3water. By taking 0, 1, 4, 8 & 15 ml of diluted stock and making these up to 25 ml with low NO_3 water, a standard series of 0, 4, 16, 32 & 60 μM NO_3 was obtained.

A (10 μmol NO_3/ml) standard solution, was made up by dissolving 1.011 g potassium nitrate KNO_3 in 1 ℓ MilliQ water. All reagents were stored in amber glass bottles in the refrigerator.

4.4.4.4 Nitrite analysis

Nitrite (NO_2^-) concentration was determined using the method described by Nydahl (1976).

5 ml of sample was used and the colour determination was the same as for Nitrate. The standard series was made up by serial dilution of Nitrite standard stock solution. 1 ml of stock solution was diluted to 100 ml with MilliQ water. By taking 0, 1, 4, 8 & 15 ml of diluted stock and making these up to 25 ml with MilliQ water, a standard series of 0, 4, 16, 32 & 60 μM NO_2 was obtained.

The nitrite standard solution of sodium nitrite (10 μmol NO_3/ml), was made up by dissolving 0.690 g anhydrous sodium nitrite in 1 ℓ of MilliQ water. (The standard was dried at 100 °C for 1 hour). The standard solution had a few

drops of chloroform added to it as a preservative and then was stored in an amber glass bottle in the refrigerator.

4.4.4.5 Calculations

Uptake rates were calculated from water nutrient concentrations using the formula obtained from Grasshoff *et al.* (1976):

Fertilized treatment no flow:

$$V = \frac{(C_i - C_t).Vol}{t.w} \qquad 4.1$$

Where V is the nitrogen uptake rate in $\mu mol.g^{-1}.h^{-1}$(DW), C_i, is the concentration in the tank (µmol), C_t, is the concentration leaving the tank(µmol), Vol is the volume of the tank (ℓ), t, is the time between samples (hours) and w, is the dry weight (grams) of the seaweed. The dry weight was calculated using wet to dry weight ratios (see Eqn. 4.7).

All treatments with flow:

$$V = \frac{(C_{in} X C_{out}) X \text{ flow rate}}{w} \qquad 4.2$$

Where V is the nitrogen uptake rate in $\mu mol.g^{-1}.h^{-1}$(DW), C_{in}, is the incoming concentration (µmol), C_{out}, is the outgoing concentration (µmol), flow rate and includes the volume of the tank (ℓ^{-1}) and the exchange rate ($\ell.h^{-1}$) and w, is the dry weight (grams) of the seaweed. C_{in} for the fertilized treatments was calculated from the difference between the residual concentration decrease (i.e. theoretical decrease) in the empty tank and the tank with seaweed over the same time period. The dry weight was calculated using wet to dry weight ratios obtained for each tank (see Eqn. 4.7).

It was decided to treat the fertilizer experiments as perturbation experiments. One problem with this method is that uptake of ammonium and nitrate varies with time when they are limiting (Probyn & Chapman, 1982; Lobban & Harrison, 1997). Under conditions of nitrogen limitation intracellular nitrogen pools may

be low, and the initial uptake over the first 10 – 60 minutes represent a pool filling phase (Fujita *et al.* 1988). As the pools fill the decrease in uptake may be due to feedback inhibition (Lobban & Harrison, 1997). This uptake rate does not represent the true transmembrane transport that is free from feedback inhibition. Thus, the perturbation method is not recommended for the calculation/estimation of V_{max} and K_s because of the past nutritional history of the thallus is changing with time. Nevertheless, this method is useful in determining the assimilation rate, the rate at which ammonium is incorporated into amino acids and proteins. This rate has been termed V_i, the internally controlled uptake rate (Lobban & Harrison, 1997). For this reason uptake rates directly after fertilizer was added (i.e. surge uptake) could not be fitted to the Michaelis-Menten relationship and thus were discarded (Pedersen, 1994). The equation used is:

$$V_i = V_{max} \cdot \frac{S}{K_m + S} \qquad 4.3$$

Where V_i is nitrogen uptake rate (µmol N. g DW^{-1}. h^{-1}), S is substrate concentration (µM), V_{max} is the maximum uptake rate and K_m is the half saturation constant for uptake (Lobban *et al.* 1985).

The predicted rate of dilution of ammonium and phosphate in the fertilized tanks was calculated from :

$$C_f = C_o e^{-rt} \qquad 4.4$$

Where C_f is the final fertilizer concentration (µM), C_o is the initial fertilizer concentration (µM), r is the rate constant (min^{-1}) and t is time in hours since start of experiment.

4.5 Ammonia

As ammonia is toxic to abalone, it is important to know how much was removed/produced by the seaweeds in the different treatments. Using the ammonium, temperature and pH values from the water Experiments 1 and 3 (Chapter 6) and Experiments 2 and 4 (Chapter 10), the Table of fraction of un-ionized ammonia from Boyd (1990) and Emmerson *et al.* (1990) was used to calculated the fraction of ammonia that was present in the water for four periods

(08:00; 12:00; 20:00 and 08:00) in the water in Experiments 1 – 4 (Chapters 6 and 10).

For the fertilized treatments at I & J in Experiments 1 and 2, these time periods were before fertilizer was added and approximately 24 hours after the fertilizer was added. As the water in these tanks was turned off (to allow the fertilizer to be assimilated), the water would not be part of a recirculation system and ammonia values during this period were disregarded. Ammonia values were only important when the waste water became part of the system (i.e. when the water to the tanks was turned on).

4.6 Growth rates and yields

The growth rates and yields referred to in Chapter 8 and 11 were calculated as laid out below.

4.6.1 Growth rate experiments

Specific Growth Rate of the biomass harvested expressed as daily fresh biomass increase (SGR = $g.wwt.d^{-1}$) was determined according to Evans (1972) and calculated as:

$$SGR = [\ln(W_t / W_0)] / (t_t - t_0) \quad \ldots 4.5$$

Where W_0 and W_t are initial and final wet weights (wwt) in grams and t_0 and t_t are initial and final times in days respectively. The SGR values were averaged and were calculated from all harvests. (Duke et al. 1986)

4.6.2 Yield experiments

The yield ($Y = g\ wwt\ m^{-2}\ d^{-1}$) was calculated as follows:

$$Y = [(W_t - W_0) / t] / SA \quad \ldots 4.6$$

Where W_0 and W_t are initial and final wet weights (wwt) in grams and t is time in days and SA is the surface area of the tank in m^2 (Evans, 1972).

4.7 Tissue analysis

The tissue analyses of the *Ulva* referred to in Chapters 9 and 11 were performed according to the following methods.

Samples were taken at every harvest to record dry to wet weight ratios and for biochemical analysis. After each weighing, the seaweed samples collected were washed in distilled water, and visible epiphytes and epifauna were removed. After washing, the samples were spun in a salad spinner for 1 minute, weighed on an OHAUS electronic balance to 2 decimal places, oven dried (70 °C, 72 hours) and then reweighed, to determine dry to wet weight ratios. The dried seaweed was then ground to a fine powder using a mechanical grinder with a maximum mesh size of 1 mm. The powder was stored in sealed glass jars in a desiccator at room temperature. Silica gel crystals in the desiccator were replaced with dry granules every two weeks or more often as the crystal colour changed. The material was kept under these conditions until biochemical analyses could be completed.

4.7.1 Total Nitrogen (N) micro - Kjeldahl technique

Total nitrogen was determined using the micro-Kjeldahl technique (Solorzano, 1969). Dried seaweed samples were milled with a Wiley mill using a 0.5 mm mesh. 0.05 g of material was placed in 35 cm long tubes with 4 ml 3.4 % (w/v) salicylic acid in 13.5 M sulphuric acid and a pellet of selenium. The samples were digested at room temperature for 2 h, 200 °C for 1 h, 270 °C for 1 h and 370 °C for 1 h or until clear. The digest was made up to 50 ml by repeatedly washing acid from the tubes with small amounts of MilliQ water into volumetric flasks.

Sub samples of 50:1 were added to 1 ml of 0.12 % (w/v) in 0.5 M phosphate buffer (pH 7.7), 1.25:1 phenolic nitroprusside and 400:1 alkaline hypochloride. The sample was covered with aluminium foil and left for a minimum of one hour after which it was read at 640 nm on a GBC UV-visible spectrophotometer. The

values obtained were compared with a standard curve obtained by analyzing a series of standards in the same way as the samples. The standard series was made up by serial dilution of ammonia stock solution. 1 ml of stock solution was diluted to 100 ml with ammonia-free water. By taking 0, 0.1, 0.2, 0.5; 0.8 & 1 ml of diluted stock and making these up to 25 ml with ammonia-free water, a standard series of 0, 1, 2, 5, 8 & 10 μM NH_4 was obtained.

EDTA 0.12 % (w/v) (disodium form no Fe) was made by dissolving 0.12 g EDTA in 100 ml MilliQ water water. Phenol solution was made up by dissolving 10 g of colourless phenol (C_6H_5OH) in 100 cm^3 95 % ethanol.

Alkaline phosphate buffer was made by dissolving 26.84 g $Na_2HPO_4.12H_2O$ and 20.65 g NaOH in 1 ℓ of MilliQ water water.

Commercial bleach was diluted to make a 3.5 % solution of sodium hypochlorite.

0.25 g sodium nitroprusside dihydrate ($NaFe(CN)_5NO.2H_2O$) was mixed with 50 ml MilliQ water water was mixed.

Phenolic nitroprusside was made by mixing in a 1:1 (60 ml) phenol and nitroprusside immediately before use.

Alkaline hypochlorite was made by mixing in a 4:1 (120 ml) alkaline phosphate (0.075 M) and (30 ml) sodium hypochlorite.

The ammonia standard stock solution (10 μmol N/ml), was made up by dissolving 0.2675 g NH_4Cl in 500 ml of MilliQ water. (The standard was dried at 100 °C). To this a few drops of chloroform were added. The sample was stored in an amber glass bottle in the fridge.

The advantage of this protocol is the double use of phosphate buffer: before adding phenolic nitroprusside and after it. It allows a linear standard curve, while measuring NH_4^+ in very acid samples (1 – 3 M H_2SO_4). Neutralization of a sample before adding phenolic nitroprusside is essential for this method because high acidity sharply decreases absorbance of coloured solutions. Thus, fluctuation of acidity of a sample solution, which always takes place during digestion of the seaweed sample, greatly influence readings.

4.7.2. Phosphate analysis (Triacid digestion)

Phosphate (PO_4^{3-}) concentration was determined using the method described by Murphy & Riley (1962).

Dried seaweed samples were milled with a Wiley mill using a 0.5 mm mesh. 0.1 g of material was placed in 35 cm long tubes. The samples were pre-digested by adding 1 ml of concentrated nitric acid to each tube and heating from 150 °C – 180 °C until the samples were almost dry. Three blanks were included. The tubes were then allowed to cool. Working in the fume hood, 1 ml of triacid mix (HNO_3:$HClO_4$:H_2SO_4) was added to each tube. The tubes were then further digested for 60 minutes at 180 °C until the solution went white to yellowish. The tubes were cooled then filled with 25 ml of MilliQ water.

5 ml of sample was then pipetted from the digest solution into 50 ml volumetric flasks. 25 ml of MilliQ water was added to each flask, to dilute the acidity of the sample. 8 ml of Murphy and Riley reagent was added to the flasks which were then mixed. The sample was then made up to 50 ml with MilliQ water and mixed by inverting. The sample was left for a minimum of one hour after which it was read at 882 nm on a GBC UV-visible spectrophotometer. The values obtained were compared with a standard curve obtained by analyzing a series of standards in the same way as the samples. The standard series was made up by serial dilution of phosphate stock solution. 1 ml of stock solution was diluted to 100 ml with MilliQ water. By taking 0, 1, 2; 5; 7.5 & 30 ml of diluted stock and making these up to 50 ml with MilliQ water, a standard series of 0, 2, 4; 10; 15 & 30 µM PO_4 was obtained.

The triacid mix was made to the required volume with the ratio 10 HNO_3:1 $HClO_4$:1 H2SO_4.

The Murphy and Riley reagent was made using the following components and added in the order listed, with mixing after each addition:

250 ml sulphuric acid with 150 ml Ascorbic Acid with 75 ml Ammonium molybdate and finally 25 ml antimony potassium tartrate.

The sulphuric acid (2.5M) was made up by adding 140 ml concentrated acid (ç = 1.84 gcm^{-3}) to 860 ml MiliQ.

Ascorbic acid solution was made up by dissolving 2.64 g of ascorbic acid ($C_6H_8O_6$) in 500 cm^3 MilliQ water.

Ammonium molybdate was made up by dissolving 20 g ammonium heptamolybdate tetrahydrate, $(NH_4)_6MO_7O_{24}.4H_2O$, in 500 ml of MilliQ water.

Antimony potassium tartrate was made up with 0.5486 g potassium antimony tartrate, $K(SbO)C_4H_4O_6$, (with or with out 0.5 H_2O), dissolved in 200 ml of MilliQ water.

The phosphate standard stock solution (2 μmol PO_4/ml), was made up by dissolving 0.44394 g Potassium dihydrogen phosphate KH_2PO_4 in 1 ℓ of MilliQ water, 10 ml of this was then diluted to 500 ml MilliQ water. The sample was stored in an amber glass bottle in the fridge.

4.7.3 Moisture content

The seaweed was washed, spun in a salad spinner for 1 minute, weighed, then placed in a drying oven (70 °C: 72hr) until all the water was removed. The samples were allowed to cool to room temperature, then reweighed.

Percentage moisture was calculated as follows:

$$\% \text{ Moisture} = [(WW - DW) / WW] * 100 \quad \text{..................4.7}$$

Where WW and DW are wet and dry weights respectively.

4.7.4 Seaweed colour

The samples used in the analysis of the relationship between thallus colour and nitrogen content where collected at the same time as the samples taken for biochemical analysis. When the samples were brought to the lab they were washed with distilled water placed on white board and the colour quantified visually under fluorescent light according to the Pantene colour printers guide using matt colors.

4.7.5 Protein content

The protein content was determined by multiplying the N concentration obtained from the micro-Kjeldahl technique by a factor of 6.25, based on the protein N content of 0.16 $g.g^{-1}$ from methods described by Fleurence *et al.* 1995.

CHAPTER 5

SPECIES MORPHOLOGY AND DOMINANCE CHANGES

5.1 INTRODUCTION

In order to successfully cultivate seaweed a farm needs to have a parent stock of that seaweed. This is because repeat cultivation of the seaweed in tumble culture causes thinning of the thalli due to either the faster growth rates or the tumble culture itself. If seaweed material was continually restocked from harvested material, thallus degradation would occur resulting in a loss of material as the thallus would break into small pieces which would leave the cultivation system. If the seaweeds are to be utilized as fodder, larger thalli are preferential.

Both farms were set up with parent stocks for the following reasons:

1. Farms should be able to maintain a healthy stock of seaweed and not have to harvest natural populations every time they want to set up new cultivation. This was especially important for this series of experiments, where it was important to use the same material if possible. It is assumed that clean seawater would provide fewer problems with respect to epiphytes and contaminants: Marine Growers in Port Elizabeth use this means of culture and it has been fairly successful (Steyn, 2000).
2. By using culture conditions that are optimal for maintenance of healthy growth (based on personal observations and published information) seasonal trends in growth could be observed. These trends would serve as a control data set for comparison of other treatments.
3. To provide material for experimental tanks if the harvest was insufficient to restock the tanks
4. If the harvested material was heavily contaminated by epiphytes the material was replaced from the parent stock.
5. The growth rates in these tanks were used as a baseline (control) to compare growth rates obtained in the other tanks
6. At JSP the parent stock was used to investigate differences in scaling up tank sizes.

There is substantial debate surrounding the classification of members of the genus *Ulva* (see Wynne & Kraft, 1981; Womersley, 1984; Bold & Wynne, 1985;

Joska, 1992; Hoek *et al.* 1995; Silva *et al.* 1996; Stegenga *et al.* 1997; Lee, 1999 and Hayden *et al.* 2003). Identification of *Ulva* species is also difficult with many having varying cell size characters due to age (Bliding, 1968; Coat *et al.* 1998), ploidy level (Coat *et al.* 1998), season (Titlyanov *et al.* 1975; Phillips 1988) and wave exposure (Tanner, 1986, Phillips, 1988). It is possible that species growing in a more sheltered habitat compared to tumble culture could have different cellular characteristics. For this reason samples were routinely analyzed for cellular characteristics.

The tanks at I & J and JSP were initially stocked with non-reproductive thalli of what was thought to be *U. lactuca* from Simon's Town. Almost 500 kg of free-floating material was collected. Random analysis of the collected material prior to initiating the experiments showed there to be 5 species present in the initial stocking material for both farms. The species were:

Ulva lactuca

Ulva capensis

Ulva fasciata

Ulva rhacodes

Ulva rigida

Due to the number of species occurring in the tanks, changes in species dominance were investigated, to see if *U. lactuca* remained the dominant species in culture or if there were seasonal effects of dominance.

The aim of this part of the study were to:

- Identify species persisting in the culture
- Investigate species dominance changes
- Investigate the suitability of maintaining a parent stock.

5.2 METHODS

Because of the number of species making up the parent stock, at each harvest random samples were collected, identified and pressed.

Thallus texture, colour, and dentation were noted on the specimens collected. The shape and size of the blade, as well as the holdfast morphology were noted.

Anatomical characteristics are very important in distinguishing between different species of this genus. The material was examined in section and surface view under magnification up to 400 X. Sections for examination came from the holdfast, mid thallus and apical regions of the specimens.
The microscopic characters used for identification were: cell size and shape (in cross section and surface view of at least five randomly chosen cells), thallus (lamina) thickness, cell arrangement in surface view, and number of pyrenoids per cell. For each plant, several parts of the thallus were examined. These were the upper (marginal), mid and the basal (just above the holdfast or the rhizoidal section) region. Cross sections of *U. lactuca* holdfasts could not be made, as the seaweed in free-floating culture had no holdfast region. Other algae although not growing attached had a region of the holdfast that was thicker than the rest of the blade and this was treated as the holdfast.
Blade thickness and cell dimensions were measured with an ocular micrometer at 400 X magnification. When determining cell size, the cells in the mid thallus region were used.

Specimens once identified were pressed and dried. Permanent slides were prepared using 50% corn syrup (Karo® corn syrup, 1% Aniline blue, 3% 1N HCl, 50% 46% H_2O) and Fast green.
Identifications of local material were made using Joska (1992) and Stegenga *et al.* (1997). Where necessary, additional references such as Bliding (1963, 1968), Koeman and Hoek (1981, 1984) Hoeksema and Hoek (1983), Womersley (1984), Phillips (1988), Burrows (1991), and Adams (1994) were

consulted. Kandjengo (2002), summarised this information in a table which has been shortened resulting in Table 5.1.

5.2.1 Species dominance

As mentioned in the beginning of this chapter, the algae in the tanks were not all one species of *Ulva*. On the JSP farm an 80 % shade cloth shaded the tanks from November 2001 to January 2002. From January to May 2002 a 20 % shade cloth was used to shade the tanks. On the I & J farm a 50 % shade cloth was used from November 2001 to June 2002. Samples were taken from shaded and unshaded tanks and the percentage wet mass that each species occupied in the sample was noted.

Table 5.1: Modified table used for species identification by Kandjengo (2002) based on morphological, anatomical and cytological characters (T/S = Transverse Section and S/V = Surface View).

Species name	Thallus morphology	Cell shape (T/S)	Cell shape (S/V)	Cell size (T/S) µm	Cell size (S/V) µm	Thallus thickness (µm)	Cell arrangement	No. of Pyrenoids
U. capensis	Dentate (double) or not, porous, wrinkled around perforations, undulate, thick lamina, (un)branched, tough basally, ovate, dull and rough, lanceolate, dark patches	Rectangular (rounded angular), slender – bullet, spindle shaped, squarish-roundish,	Rectangular-rounded, polygonal or irregular, bean shaped and well paired	(8-17) X (27-67)	(4-17) X (8-23)	72 – 209	Curved rows in part	1 – 4 (- 5)
U. fasciata	Undulate, pale rigid midrib, strap shaped, ribbony, tufted at base, branched to the base, usually epiphytic on *Gracilaria*	Rectangular-rounded angular, cylindrical, conical shaped, slender,	Rounded-rectangular, Irregular-polygonal, squarish	(6-25) X (15-46)	(6-15) X (8-21)	57 – 140	None	1 – 2 – (3 - 4)
U. lactuca	Porous, thin, undulate, (un)branched, floating unattached thalli form flat sheets, rough, tougher basally	Rounded to rectangular	Rectangular, rounded-polygonal	(8-15) X (15-25)	(6-15) X (10-21)	38 – 86	Short rows in part	1 – 2 (- 3)
U. rhacodes	Tufty, dentate, epiphytic	Rounded						1
U. rigida	Thick, consistency firm, tufty at the base, incised, entire margins, smooth and shiny, flat sheet, perforated,	Rectangular (rounded angular) – slender	Rectangular-rounded, polygonal, irregular, bean shaped well paired	(6-27) X (21-53)	(6-21) X (10-29)	57 – 247	Short rows to none	1 – 3 (- 4)
Ulva sp	Undulate, wrinkled around perforations	Rectangular – rounded	Polygonal, round-squared	(13-19) X (21-27)	(10-17) X (13-21)	53 – 61	In rows	(1) - 3

5.3 RESULTS

5.3.1 Species identification

There were two problematic species to identify in this study namely *U. capenis* and *U. lactuca*. Both species appeared to have two morphs associated with each of them that differed from available taxonomic descriptions.

Plate 5.1: *U. lactuca* morph A.

U. lactuca morph A, had a very varied thallus size, with specimens up to 55 cm long and 35 cm wide being found at both farms (Plate 5.1). Thallus size was often larger in the unshaded tanks receiving a higher water exchange rate. The alga has a firm consistency but is thinner (25 – 35 μm) than other species of *Ulva,* and could be differentiated with a high degree of certainty by feel alone.

Colour of the thalli varied depending on the culture media, flow rates and shading regime. The thalli are irregular in shape and can be perforated or entire. There are two possible explanations for the perforated appearance. One is due to grazers such as *Paridotea reticulata* or a side effect of tumble culture, where the algae were often found wrapped around themselves and the holes were in the center of the thallus. Thus, the holes could have been caused by the alga abrading itself while being tumbled. Some plants had a thicker central section. This usually occurred when the alga wound around on itself, and the center was often darker in colour and sometimes had rhizoidal outgrowths.

Plate 5.2: *U. lactuca* morph B.

U. lactuca morph B, was collected from both farms, although it was more common at the I & J farm (Plate 5.2). It closely resembled *U. lactuca* with similar cell shape, however, cell dimensions were larger and the thallus differed by being consistently wrinkled at perforations, the number of pyrenoids (1 – 4)

(in *U. lactuca* morph A the number rarely exceeded 2). The chloroplasts tended to be in the centre of the cells. The specimen was also much thicker (above 53 µm) than *U. lactuca* morph A (25 - 35 µm) but the same thickness as *U. capensis* (Stegenga *et al.* 1997), but cell shape and size were not consistent with those reported for *U. capensis.* In comparison with other *Ulva* species, this specimen exhibited cells that, in transverse section, were cubed to rounded and the thalli had no dentation. Dr Herre Stegenga was unable to identify this species on its microscopic characteristics and for this reason it was decided to include it in Kandjengo's 2002 molecular systematic study of the *Ulva* genus. Internal Transcribed Spacer (ITS) molecular analysis showed it to belong to the group containing *U. rigida* and *U. capensis* (Kandjengo, 2002).

Plate 5.3: *U. capensis* morph A.

The cell shape character and thallus dentation (both microscopic and visible with the naked eye), were used to separate *U. capensis*, which had spindle or bullet shaped cells in transverse section, especially in the basal region. *U. capensis* had much bigger cells than *U. lactuca* (up 67 µm high). The thallus

lamina of the two *capensis* species was also generally much thicker (up to 247 µm).

The characteristic dentation of the thallus was missing in *U. capensis*, morph B. In addition, the cells tended to be bullet to rectangular shaped. One constant feature was the presence of air bubbles in the holdfast region of the thallus. This has not been observed in other *Ulva* sp previously (H. Stegenga pers. comm.), but they are similar to the bubbles mentioned by Steffensen, (1976) except the cells were not rhizoidal. The bubbles tended to cause thallus separation, increasing identification difficulties by causing it to resemble exceptionally large *Enteromorpha*. The thallus thickness was the same as *U. capensis* Morph A.

Most of the other species identifications agreed with those of Stegenga *et al.* (1997) as both *U. fasciata* and *U. rhacodes* were epiphytic and were only found growing attached to *Gracilaria* stalks that were blown over from adjacent tanks. Both species occurred in very low densities. *U. rigida* was also found in low densities in the tanks and was relatively easy to identify as it had a definite holdfast section.

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5.3.2 Species dominance

On the JSP farm the tanks were shaded by an 80 % shade cloth in November 2001 to January 2002 after the *Ulva* population crashed at I & J in November 2001 due to a severe *Myrionema strangulans* epiphyte epidemic. Shortly after the 80 % shade cloth was applied (Figure 5.1, bar 1), a change in species dominance was observed, from *U. lactuca* to *U. capensis* (both morphs). The change in species dominance was also accompanied by a reduction in the SGR in the shaded tanks (see Chapter 8). As a result of the species switch, it was decided to change the shade cloth from 80 % to 20 % (bar 2, Figure 5.1), in January. From January to April, a slow recovery of the proportion of *U. lactuca* was observed. When the shade cloth was removed in April (Bar 3, Figure 5.1), the change in dominance was much more rapid. In winter, there was

approximately 5 % more *U. capensis* present in the unshaded tanks than at any other time in the year, and the presence of *U. lactuca* also decreased.

The same changes in species dominance occurred at I & J where a 50 % shade cloth was used, but the change was much more gradual, and was also accompanied by a decrease in SGR (see Chapter 8).

Shading at I & J was applied In November 2001, and a complete change in species dominance occurred shortly afterwards in January. The shade cloth was removed in May 2002 and a slow change of species dominance was observed. Due to a *Myrionema strangulans* infection in the shaded tanks in April, most of the material was replaced with fresh material from other tanks, thus ending the change in observable species dominance as the experiment was essentially halted.

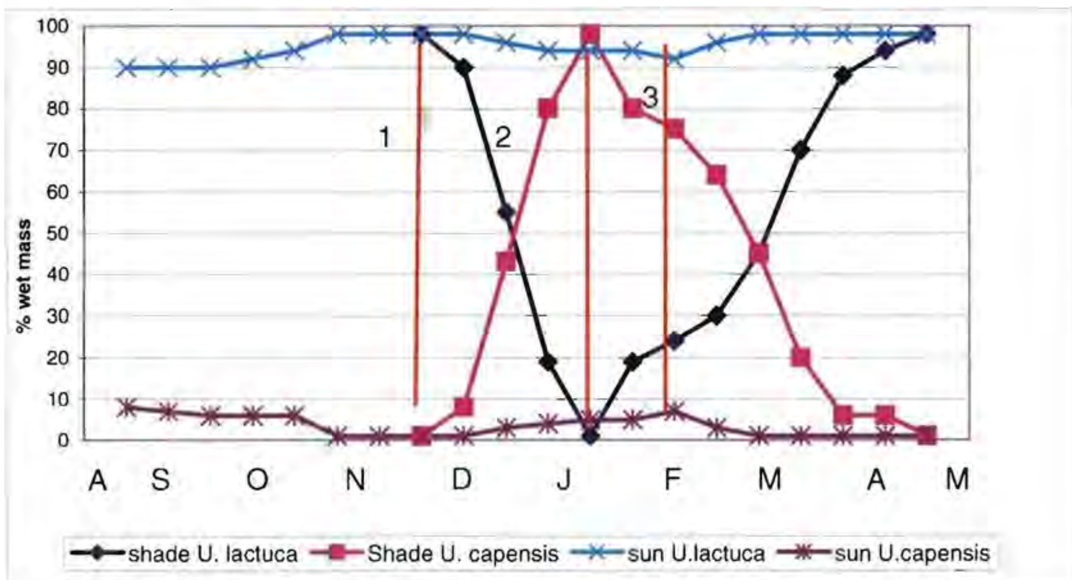

FIGURE 5.1: Change in species dominance between *U. lactuca* and *U. capensis* and other *Ulva* species (U. other) in shaded and unshaded tanks at JSP from August 2001 to May 2002. From the left Bar 1 illustrates where shading was started (80%), Bar 2 illustrates where shade cloth changed (20 %), Bar 3 illustrates where shading ended.

5.3 DISCUSSION

The genus *Ulva* is known for its taxonomic difficulties (Papenfuss, 1960; Bliding, 1963, 1968; Steffensen, 1976; Koeman and Hoek, 1981; Hoeksema and Hoek, 1983; Tanner, 1986; and Joska, 1992), despite it's cosmopolitan distribution and the fact that it was one of the first four algal genera to be described in Linnaeus' *Species Plantarum* (Papenfuss, 1960). Most of the taxonomic problems have been associated with the morphological variation that is widespread in the genus, which remains difficult even with fresh material (Kapraun, 1970; Steffensen, 1976; Bonneau, 1977; Mshigeni and Kajumulo, 1979). Many authors agree that it is unrealistic to base the taxonomy of *Ulva* and related genera on herbarium material (Bliding 1963, 1968; Koeman and Hoek, 1980).

Morphological differences between the species are often small and difficult to detect. Morphological and cytological characteristics of *Ulva* sp. are known to vary with season, salinity, wave energy, latitude and geographical location, even within a single population at a given time (Titlyanov, *et al.* 1976; Steffensen, 1976; Reed and Russell 1978; Tanner, 1986; Phillips, 1984, 1988; Woolcott & King, 1999). Several studies have found wave energy was a factor that affected the size and morphology of *U. californica* (Tanner, 1986); *U. fasciata* (Mshigeni and Kajumulo, 1979) and *U. fenestrata* (Titlyanov *et al.*, 1975). This was demonstrated in the present study by the difficulty in identifying tumble culture *Ulva* sp., as well as the finding of different morphological variations of what are currently the same species.

Taxonomic keys are quantitative, each showing a graded series with overlap between species (Bliding, 1963). This results in taxonomists disagreeing on some species (Bliding, 1963, 1968). Iidentification difficulty is increased by morphological plasticity, particularly when the algae are grown outside of their natural environment such as occurs in tumble culture.

The switch of species dominance from U. *lactuca to U. capensis* when grown under a lower light level, suggests that *U. capensis* is better adapted to low light conditions than *U. lactuca*. This would make sense as *U. lactuca* is a free

floating form and thus is not constrained by depth whereas *U. capensis*, which normally is found attached, would be under the influence of tidal variations and therefore depth and would receive less light at higher tides or greater depth.

This hypothesis is further supported by the findings of Kandjengo (2000), that compared the SGR of *U. lactuca* and *U. capensis* grown under different shading regimes. Using his data with permission, statistical testing showed that *U. capensis* grew significantly better under low light conditions compared to *U. lactuca*, while *U. lactuca* grew significantly better than *U. capensis* under high light conditions (ANOVA; $n = 39$; $p < 0.5$; LSD post-hoc test, $p < 0.05$). I tested this hypothesis using a high growth intensity system described by Smit (in press) and found the same results. This suggests that *U. lactuca* is specialized to grow in a high light environment while *U. capensis* is specialized to grow in a low light environment. The preferred light intensity environment of each species may be an ecological niche strategy applied by each species to avoid competition.

5.4 CONCLUSION

The change in cultivation methods (i.e. tumble culture vs. free-floating) and the associated morphological changes were demonstrated in this project and these differences need to be noted for future work done on these species in tumble culture.

The dominance changes exhibited by the algae also need to be investigated and an ecological niche strategy for *U. capensis* and *U. lactuca* needs to be investigated.

U. lactuca was the preferred species in this study as it grew unattached and could take advantage of high light situations. *U. capensis* might be preferable in an indoor culture system, where there is a lower light intensity.

CHAPTER 6

NUTRIENT CONCENTRATIONS IN DIFFERENT CULTURE MEDIA ON THE FARMS AND UPTAKE IN SHADED AND UNSHADED TANKS

6.1 INTRODUCTION

Intensive aquaculture, where large amounts of feed are provided is essentially an "aquatic livestock" rearing operation. Large scale aquaculture generates large amounts of effluent water. Two pollutants of particular concern are nitrogen (particularly ammonia and nitrate) and phosphorous. Cohen & Neori (1991); Shpigel & Neori, (1996); Shpigel *et al.* (1997) and Neori *et al.* (1998) have shown that *U. lactuca* is very efficient at removing these two contaminants form aquaculture effluent. Compared to intensive aquaculture with other high value species (e.g. shrimps and many marine fin-fish species), abalone cultivation has less potential for damaging oceans and coastal resources (Naylor et al., 2002). The main explanation is that abalone are herbivorous (Barkai and Griffiths, 1986) and lower in the trophic food chain, and hence the farming does not depend on fishmeal and fish oil inputs.
Water quality has been identified as the single most important factor in the culture of any aquatic organism (Boyd, 1990). DeBusk *et al.* (1986), identified the nutrient content of the growth medium as one of the most important factors in the tank cultivation of algal species. Waste effluents from the abalone farms can today theoretically be treated with both chemical and biological filters removing the dissolved nutrients. However, as these water treatment systems are both complex and costly integration of seaweed farming could provide a more viable and profitable alternative. By growing seaweed in effluent water they reduce the dissolved nutrients from the abalone farm, and at the same time additional crops are produced. This alternative make it possible to re-circulate both water and nutrients (by feeding the abalone the cultivated seaweed).

Experiments conducted on the effect of water exchange rates of seawater on the SGR of several species of *Ulva*, indicate that higher production rates are obtained in systems that have high water exchange rates (20 volumes d^{-1}) compared to systems that had low water exchange rates (4 volumes d^{-1}) (DeBusk *et al.* 1986). Parker (1981) also found that ammonium uptake and SGR

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increased with increasing current speed, due to the rapid replacement of nutrients in the culture system, as nutrient-depleted seawater is constantly renewed. A continual high water exchange rate is not always possible in tank culture operations (due to high pumping costs), so that artificial nutrient enrichment of the cultures is often common practice. It is well documented that the addition of inorganic ammonium and phosphate which can be limiting nutrients to nutrient depleted water significantly increases the growth rates of *Ulva* species (Björnsäter & Wheeler, 1990; DeBusk *et al.* 1986).

An alternative to artificial enrichment (in the form of fertilizer) of the seaweed cultures, is to utilize effluent seawater from animal grow-out tanks in a polyculture operation. Effluent water from primary culture tanks (e.g. fish, abalone or prawns) has been used to culture additional organisms that are capable of utilizing low levels of DIN/P (such as seaweed) (Chiang, 1981; Vandermeulen & Gordin, 1990; Cohen & Neori, 1991; Buschmann, 1996; Petrel & Alie, 1996; Shpigel & Neori, 1996; Shpigel *et al.* 1997; Neori *et al.* 1998; Troell *et al.* 1997; Chopin & Yarish, 1998; and Chopin *et al.* 1999a, b, c, d; Troell *et al.* 1999; Chopin *et al.* 2001). An advantage of such a polyculture system is that overall economic efficiency is improved, because nutrients available in the effluent reduce the need for supplementation. Polyculture involving seaweed can provide an environmentally sound operation: seaweeds act as a biofilter, removing excess nutrients from the water before it is pumped back into the river, estuary, or coastal waters from which it originated (Neori *et al.* 1998).

There are differences in the two farms locations and cultivation methods and nutrient concentration in what I have termed the same water source are likely to vary. In order to test this, two experiments were initiated at each farm. Ammonium, ammonia, nitrate, nitrite, and phosphorous values for the water sources were compared. Differences in nutrient uptake rates between shaded and unshaded tanks at I & J were investigated as well as Michaelis-Menten kinetic parameters of ammonium uptake, where the rate of enzymatic catalysis is

related to the concentration of the substrate (DeBoer, 1981). Nutrient uptake rates in each of the growth media at a set stocking density were also investigated.

6.3 METHODS

6.3.1 Incoming water sources

Incoming water samples were taken from 3 tanks in each treatment both at I & J and at JSP. The water sources were obtained over all 4 experimental periods and consisted of the following water sources and exchange rates:

Experiment 1: I & J, February 2002

Abalone effluent 12 $VE.d^{-1}$ (Volume exchanges per day)

Fertilized seawater 4 $VE.d^{-1}$

Unfiltered seawater 4 $VE.d^{-1}$

Experiment 2: I & J, August 2002

Recirculated Abalone effluent 12 $VE.d^{-1}$

Fertilized seawater 12 $VE.d^{-1}$

Unfiltered seawater 12 $VE.d^{-1}$

Experiment 3 & 4: JSP, October 2002

Abalone effluent 20 $VE.d^{-1}$

Turbot effluent 20 $VE.d^{-1}$

Unfiltered seawater 20 $VE.d^{-1}$

Temperature, pH and dissolved oxygen measurements were recorded in each tank at the same time (see Chapter 7). Water sampling and storage was described in Chapter 4, Section 4.4.

6.3.2 Shaded vs. unshaded uptake

Set stocking density, varying flow rates, shading with 50 % shade cloth

The uptake experiment comparing shaded and unshaded tanks was done during experiment 1 at I & J using a set stocking density of 2 $kg.m^{-2}$ per tank with two shaded and two unshaded treatments in each culture type (seawater, fertilized & abalone) and 2 replicates for each treatment. The water exchange rate to the sea water and fertilized treatments was set at 4 volume exchanges per day while the water supply to the abalone treatment was set at 12 water volume exchanges as the water to the other tanks was gravity fed, while the water to the abalone tanks was pumped from the recirculation dam. At the same time that water samples were taken, temperature, pH and dissolved oxygen measurements were recorded in each tank (see Chapter 7, Section 7.3). Water sampling began at 9:00 am on the 26th of February 2002 after fertilizer was added to the fertilized treatment, and ended 56 hours later. During the first 12 hours of sampling, water to the fertilized treatment was turned off. To compare the uptake of the fertilized treatment against the background concentrations normally present in sea water, as well as the dilution rate of the fertilizer, without the seaweed uptake influence, a control tank was set up. This tank had fertilizer added to it and was kept under the same conditions as the fertilized treatment, except that it had no seaweed in it.

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Samples were taken (as per Chapter 4, Section 4.4) at two-hour intervals for the first 12 hours and then four-hour intervals for the next 24 hours and then at six-hour intervals for the remainder in each of the tanks.

6.3.3 Statistics

Student T-tests were used to determine significant differences between nutrient uptake rates for shaded and unshaded treatments in each media. ANOVAs using Statistica V6, were done to compare differences in uptake rates between

treatments for ammonia and phosphate only. LSD post hoc test was used to differentiate between the significant differences.

6.4 RESULTS

6.4.1 Nutrient concentrations at JSP

Turbot effluent (Figure 6.1) had a significantly higher ammonium ($p < 0.05$) and nitrite ($p < 0.05$) concentrations than either abalone effluent or seawater. Turbot effluent also had the highest phosphate concentration range (8.2 – 4.6 µM) although this was not significant (Figure 6.1). The abalone effluent ammonium concentration was also significantly higher than seawater ($p < 0.05$).

6.4.2 Nutrient concentrations at I & J

The residual ammonium concentration at 4 $VE.d^{-1}$ and 12 $VE.d^{-1}$ is significantly higher 36 and 26 hours after fertilizer had been added, respectively ($p < 0.5$) compared to the other treatments (Figure 6.2). The concentration of ammonium decreases exponentially after the water is turned back on. The rate of dilution is given by the equations: $y = 574.73 * exp^{-0.050448*t}$ ($R = 0.87$) at 4 $VE.d^{-1}$ and $y = 573.92 * exp^{-0.166847*t}$ ($R = 0.99$) at 12 $VE.d^{-1}$. Although the fit for the curves are good (shown by R values) deviations can be explained by variable additions of nutrients from the seawater. The rate of dilution is faster at 12 $VE.d^{-1}$, and is due to the water exchange rates increasing three-fold.

The recirculated abalone effluent had nutrient pulses during the day (11h00 to 13h00) which were significantly different abalone effluent water at the same time (t-test, $p < 0.05$). At all other times in the experiments, there is no significant difference between recirculated abalone effluent and abalone effluent ammonium concentrations, thus indicating that there are more nutrients available in recirculated abalone effluent, during the day.

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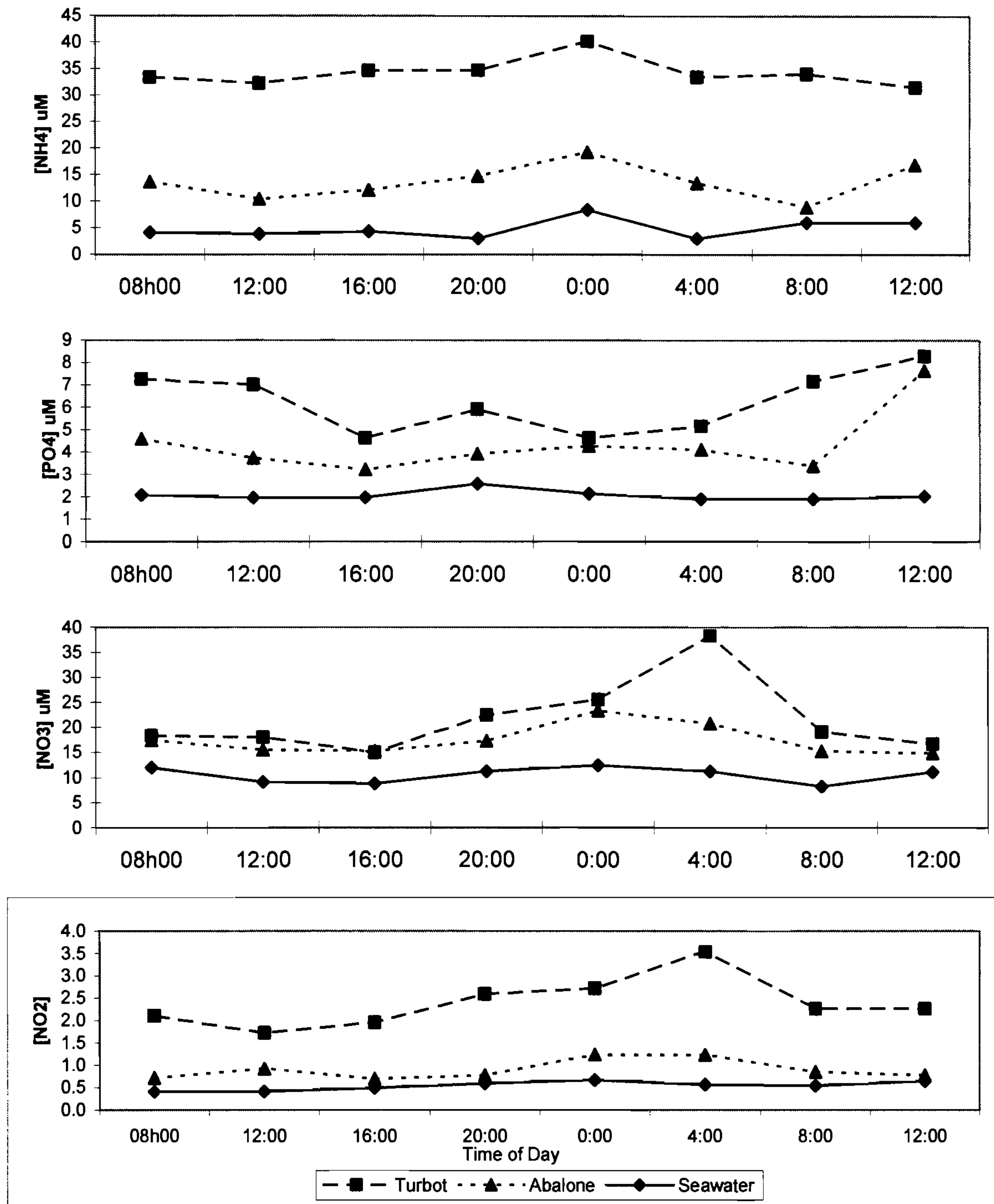


FIGURE 6.1: Incoming nutrient concentrations of three water types at JSP. Std. deviations are all less than 0.1, n = 3.

There are "feeding peaks" (increases in the concentration of measured nutrients perhaps as a direct result of abalone feeding) in the recirculated abalone effluent at 20h00 and 02h00 and at 19h00 and 02h00 in abalone effluent respectively. It is during these feeding peaks that there is a significant difference in ammonium concentration in the recirculated and abalone effluent compared to the seawater treatments (ANOVA; $p < 0.05$).

In the fertilized tanks the decrease in phosphate concentration is slower than ammonium and decreases exponentially after the water is turned back on. The rate of dilution is given by the equation: $y = 290.41 * \exp^{-0.030787*t}$ ($R = 0.82$) at 4 $VE.d^{-1}$ and $y = 274.73 * \exp^{-0.117616*t}$ ($R = 0.96$) at 12 $VE.d^{-1}$. Residual phosphate concentrations in the fertilized seawater were significantly higher than all other treatments 36 and 26 hours after the addition of fertilizer at 4 and 12 $VE.d^{-1}$ respectively (ANOVA, $p < 0.05$). Incoming PO_4 concentrations in the recirculated abalone tanks were significantly different compared to seawater, at the feeding peaks at 20h00 and 02h00 (ANOVA, $p < 0.05$ in both cases). There is no significant difference in the daytime phosphate concentrations between recirculated abalone effluent and abalone effluent.

The fertilizer nitrate concentration dilutes exponentially according to the following equation: $y = 9.282 * \exp^{-0.108655*t}$ ($R = 0.99$) up until 23:00 on the first night. The second slight peaks shown correspond to peaks in the seawater treatment, indicating that these elevated values are as a result of higher nitrate concentrations in the seawater being pumped into the farm.

There is no significant difference between incoming nitrate concentrations in all three treatments 12 hours after the fertilizer has been added (ANOVA, $p < 0.05$) (Figure 6.2).

The fertilizer treatment contains very little nitrite (Figure 6.2).

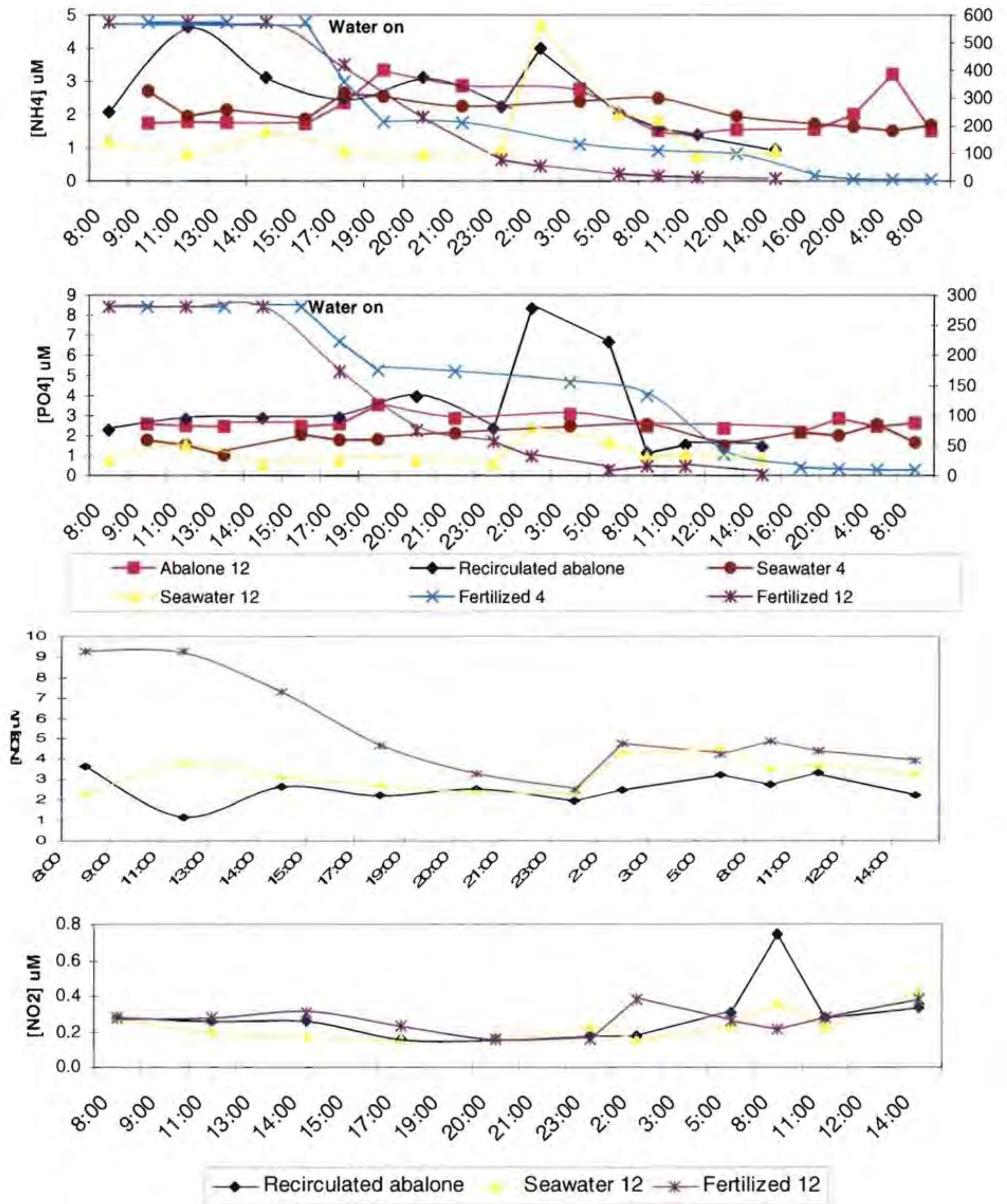


FIGURE 6.2: Incoming nutrient concentrations of culture media at I & J at 4 and 12 V.E.d^{-1}. Std deviations are all less than 0.1, y axis on left hand side indicates fertilizer concentrations. n = 3.

6.4.3 Shaded vs unshaded uptake at I & J

When running the statistics for fertilizer uptake it was decided to ignore the values obtained for surge uptake and use only those for external and internally controlled uptake (See Figure 6.4 for details). The range was given rather than the standard deviation to show the effect of pulse additions on the values. Ammonium uptake was significantly higher in the unshaded tanks compared to the tanks shaded with a 50 % shade cloth in all three growth media (t- test, $n = 12$; Abalone effluent:, $t = 2.83$, $p = 0.01$; Sea water: $t = 6.43$, $p = 0.001$; Fertilizer: $n = 10$, $t = 5.71$, $p = 0.004$) (Figure 6.3).

Phosphate uptake in the unshaded tanks was also significantly higher compared to the shaded tanks in all three growth media (t- test, $n = 12$: Abalone effluent:, $t = 4.51$, $p = 0.06$; Sea water: $t = 3.68$, $p = 0.01$; Fertilizer: $n = 10$, $t = 5.83$, $p = 0.004$).

Table 6.1 is a tabular form of Figure 6.4. Figure 6.4 shows the uptake rates for phosphate calculated from the fertilized treatments treated as a perturbation experiment. The most important point to note from Figure 6.4 is the three defined regions. These regions were described by Pedersen (1994). Many authors (Fujita, 1985; Pedersen, 1994) have stated that at high substrate concentrations and /or with N-limited algae, Michaelis-Menten plots are often inadequate in describing nitrogen uptake. Thus, we have followed Pedersen (1994) protocol and Michaelis-Menten kinetics are only given for the region that is externally controlled uptake.

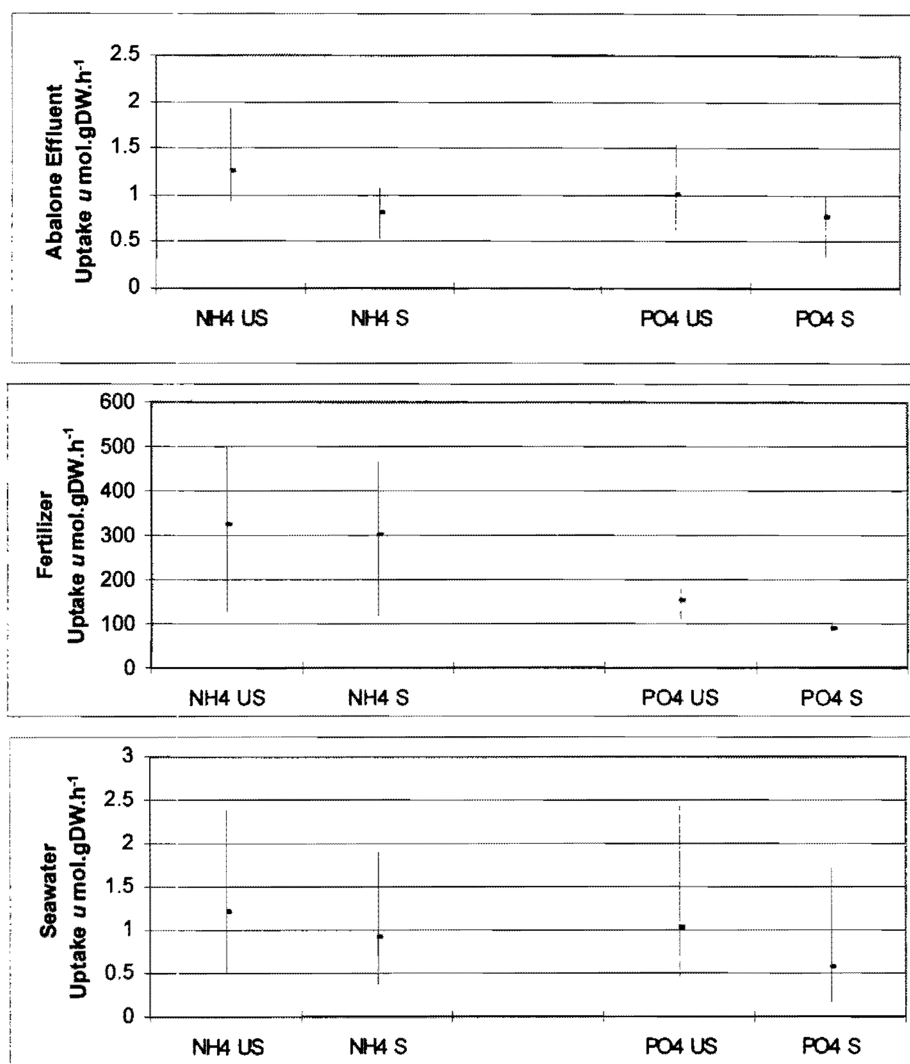


Figure 6.3: Uptake rate of ammonium and phosphate in shaded (s) and unshaded (us) tanks in three growth media at I & J during daylight hours. Range and mean values are shown, n = 12.

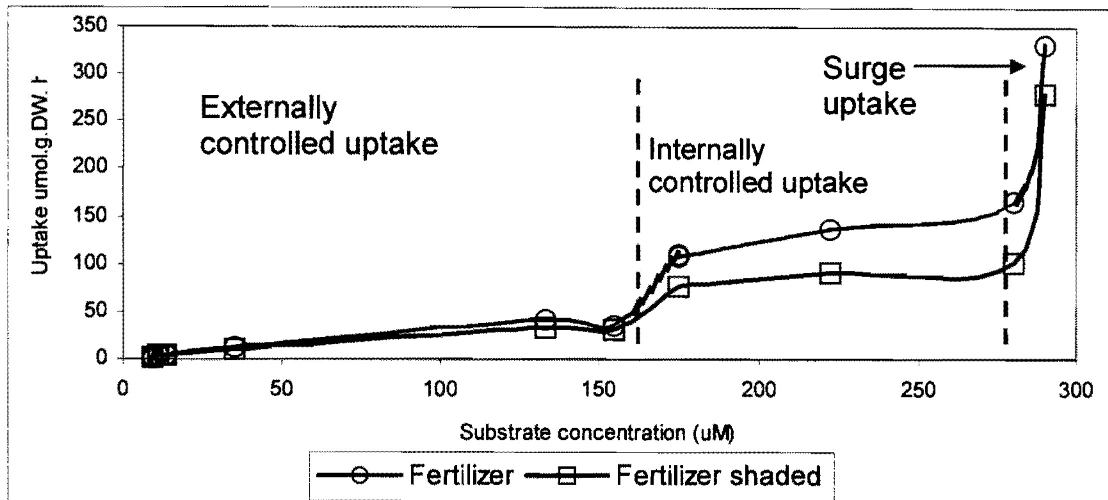


Figure 6.4: Perturbation uptake of phosphate in the fertilized tanks showing shaded and unshaded treatments. The three regions defined on the graph are according to Pedersen (1994).

Table 6.1: Surge uptake rates, average internally controlled uptake rates with standard deviations and Vmax, Km and R values for the Michaelis-Menten regression line (externally controlled uptake portion), for phosphate and ammonium in Experiment 1.

Nutrient	Shading	Uptake rates µmol.g.DW^{-1}.h^{-1}				
		Surge uptake	**Internally controlled uptake**	Vmax	Km (µM)	R value
NH_4	Unshaded	1718.35 ± 8.87	377.3 ± 1.45	420.94	172.80	0.99
	Shaded	1446.20 ± 4.48	350.25 ± 3.98	417.77	160.29	0.99
PO_4	Unshaded	330.39 ± 7.13	137.53 ± 6.23	300.52	234.26	0.98
	Shaded	136.83 ± 4.31	92.09 ± 3.21	142.43	104.90	0.97

6.5 Discussion

One of the most interesting results from this series of experiment was that JSP, which had less abalone and lower stocking densities compared to I & J, had ammonium and nitrate concentrations that were approximately 3 times higher than I & J, in abalone effluent. This study's values for ammonium, phosphate, nitrate and nitrite at I & J, both under normal farm operating conditions and under recirculation, were all lower than JSP. this is due to the total volume of water passing through I & J being larger than JSP and this results in dilution o.

Nitrite concentrations (Figure 6.1) in the turbot were higher than the fertilized treatment at I & J (Figure 6.2), indicating that turbot effluent is a good source of nitrite. This could lead to a possible management concern under conditions of recirculation using turbot effluent because nitrite above certain levels can be toxic to fish and crustaceans (Boyd, 1990). As the turbot are fed at 08h00, 12h00 and 16h00, the increase in nitrate is not as a result of food being added. It could however, be related to the breakdown of the food or the residence time of the food in the fish.

The high ammonium and phosphate concentrations in the recirculated abalone effluent during the day can be attributed to the daily cleaning of the abalone grow-out tanks, which results in a sudden pulse of waste products in the water. It is important to note that the abalone effluent water comprised waste from all the seaweed tanks, including the *Gracilaria* tanks, which also had a pulse fertilization although the ratio of Maxiphos to ammonium sulphate was different (10:1). The ammonium in the abalone effluent remained constant through out the day due to the fact that there are no additions in the day to the water in the dam.

The peak in ammonium and phosphate concentrations in the seawater (4 $VE.d^{-1}$) at 02h00 is likely due to variability in the source waters (ocean).

The peaks in nitrite concentrations in abalone effluent at night are problematic as abalone do not excrete nitrate. Thus, the increased activity by the abalones during this period could have released both increased nitrite and nitrate into the effluent water.

Table 6.3 compares results obtained in this study with figures from the literature on the ammonium uptake kinetics of the genus *Ulva.* Most of the V_{max} values are for continuous flows, although Cohen & Neori (1991), obtained ammonium V_{max} rates in pulse treatments that were four times higher than those of continuous flow treatments.
The V_{max} values that we obtained for ammonium uptake for experiments 1 are slightly higher than the largest value obtained by Cohen & Neori (1991), with starved *U. lactuca* under pulse conditions. The conditions under which the algae were grown by Cohen & Neori (1991), were very similar to our experiment and the values are comparable. The values obtained from experiment 2 compare well with values given by Cohen & Neori (1991) and with the starved *U. lactuca* of Fujita (1985).

Vandermeulen & Gordin (1990) and DeBusk *et al.* (1986) found that there is a rapid initial uptake of ammonium by *Ulva* during pulsed additions. This rapid nutrient uptake is typical of N-starved or N-depleted seaweeds, which can take up large quantities of ammonium in a short time. Unlike the continuous values, however, pulse rates cannot be sustained for more than a few hours and are thus less important when considering these algae for the application in effluent treatment.

Probyn & Chapman (1982); Fujita (1985); Fujita *et al.* (1988) and Pedersen (1994), showed that the uptake of ammonium by *U. lactuca* and other seaweeds had three distinct phases which are not described by the Michaelis-Menton equation. At high substrate concentrations, there is surge uptake. This surge uptake rate is directly related to the duration of prior nitrogen starvation and is

inversely related to the tissue nitrogen content (Rosenberg *et al.* 1984). Surge uptake tends to be a function of increasing substrate concentration (Pedersen, 1994). This would explain why our surge uptake rates were so high.

TABLE 6.3: Kinetic parameters of ammonium uptake by *Ulva* species

Species	Vmax μmol g^{-1} h^{-1}	Km μM	Vmax/Km	N-starved/ sufficient	Reference
U. curvata	250[P]	14	17.9		Rosenberg & Ramus (1982b) *
U. lactuca	138[P]	41	3.5	Sufficient	Fujita (1985) *
U. lactuca	252[P]	15	17.1	Starved	Fujita (1985) *
U. curvata			9.2		Rosenberg & Ramus (1982b) *
U. curvata	[P; C]		3.9 – 27.5	Both	Duke et al. (1989b) **
U. curvata			9.2		Rosenberg & Ramus (1984) *
U. lactuca	50[C]	5.2	9.5	Sufficient	Cohen & Neori (1991) **
U. lactuca	100[C]	27.5		Sufficient	Cohen & Neori (1991) **
U. lactuca	390[P]			Starved	Cohen & Neori (1991) **
U. lactuca	211[P]	20	10.6	Starved	Pedersen (1994)*
Ulva sp.	146	14.4	10.1		Campbell (1999) **
U. lactuca	420[P]	172.8	2.43	Starved	This study Exp. 1**
U. lactuca	274[P]	160.1	1.71	Starved	This study Exp. 2 **

* Under laboratory conditions
** Under field conditions
c Continuous effluent treatment
P Inorganic nutrient pulse

Surge uptake can take place across a wide range of substrate concentrations (from as little as 7 μM) (Pedersen, 1994). This ability for surge uptake at low nitrate and nitrite concentrations confounded the calculation of Michaelis-Menten kinetics. Internally controlled uptake and externally controlled uptake phases are much slower than surge uptake and occurred at lower concentrations (Pedersen, 1994).

Fujita (1985) and Fujita *et al.* (1988), showed that ammonium surge uptake rates increased with increasing nitrogen starvation. This could explain the high surge uptake rates that were obtained in the fertilized treatment in Experiment 1. The low exchange rate (4 volume exchanges. d^{-1}) used in this treatment most likely resulted in some degree of nutrient limitation. By comparison, in Experiment 2 where the water exchange rate was tripled (12 $V.d^{-1}$), the uptake rates were lower (See Chapter 10). Parker (1981), found that increasing the current flow can compensate for N limitation to some extent provided light is non-limiting.

Habig *et al.* (1984) and DeBusk *et al.* (1986), reported that N-starved *U. lactuca* thalli contained high levels of soluble carbohydrate. It is thought that energy obtained from these carbohydrate reserves is used to support rapid nitrogen uptake in the dark. This ability of N-starved *U. lactuca* to quickly absorb and store nitrogen in excess of current demands (luxury consumption) means that a continuous supply in the medium is not necessary for algal growth, but that the alga's requirement can be met by periodic fertilization. This has further benefits for epiphyte control (Ryther *et al.*1981). However, Neori *et al.* (1991) found that *U. lactuca* grown in continuous fish effluent had a higher yield (by up to 38 %), higher growth rate, and higher percentage carbon compared to thalli grown under a pulsed nutrient supply.

Wallentinus (1984), found that ammonium uptake rates by *Ulva* sp. were greater than phosphate uptake rates which were greater than nitrate uptake rates and that these uptake rates were higher than most other species of algae tested. Such high uptake rates have been attributed to the large surface area to volume ratio of *Ulva* (Littler & Littler, 1981; Wallentinus, 1984; Ramus & Venable, 1987 and Duke *et al.* 1989b). The order of uptake rates is consistent with the data from experiments 1 and 2.

Harlin *et al.* (1978) found that ammonium uptake by *U.lactuca* halved when the temperature was increased from 15 to 20 °C. It is possible that the temperature

increase from 15 – 20 °C, observed at I & J from morning to afternoon (See Chapter 7), while the tanks were being fertilized, could have resulted in the uptake rate of the alga being lowered. However, Duke *et al.* (1989b), found that temperature contributed very little to the variation in uptake rates. They also found that uptake rates at 5 °C were similar to those at 29 °C. Our results parallel those of Duke *et al.* 1989b.

This study showed that applying a 50 % shade cloth had a significant reduction on uptake by the seaweeds at I & J. At JSP, where an 80 % shade cloth was used initially, this reduction in uptake would have been even greater and therefore could account for the lower growth rates obtained when using an 80 % shade cloth (See Chapter 8). It is possible that shading with a 20 % shade cloth would not have a significant impact on uptake although this would need to be tested.

Nitrite is toxic to fish if it reaches levels of 7 µM, due to methemoglobinemia. This is when the nitrite reacts with hemoglobin to form methemoglobin (Boyd, 1990; 1998). Its toxicity to abalone has not been established. However, the nitrite concentrations in the turbot effluent are well below this figure but may be cause for concern if used in a recirculation system. A means of reducing nitrite levels is to treat the water with sodium chloride or calcium chloride to reduce the molar ratio of nitrite to chloride (i.e. increasing the salinity) (Boyd, 1990;1998).

6.6 Conclusions

The positive correlation between nutrient availability in seaweed culture media and the specific growth of *Ulva* has been well documented (Duke *et al.* 1989). In tank cultivation, nutrients in the growth media become depleted over time and artificial enrichment of the growth media is often necessary to sustain production rates. However, the amount of nutrients added to a seaweed culture system needs to be carefully monitored as insufficient amounts will have no effect on production, while over fertilization wastes fertilizer and money, and may cause other problems such as excessive epiphyte growth as well as affecting growth rates and photosynthesis of cultivated *Ulva.*

There are two peaks in abalone feeding one at 20h00 and another at either 00h00 or 04h00. It is only during these brief periods that nutrient concentrations are elevated above background levels. Turbot water has far more nutrients for seaweed but turbidity due to incomplete pellet assimilation could be a problem. This is because light penetration (as measured by secchi disk) is reduced, thus inhibiting photosynthesis.
The uptake rates that occur in the fertilized treatments indicated that abalone effluent medium on its own is insufficient for seaweed cultivation. Thus the most effective cultivation medium on both farms would be a fertilized effluent growth medium, as this supplies sufficient nutrients for growth as well as an additional pulse, which would help to control epiphytes.

The shading was primarily applied to control *Myrionema strangulans* an epiphyte which decimated the culture material (see Chapter 8). The differences in uptake between shaded and unshaded material were done not to see how shading affects uptake but rather by how much and whether or not tanks could be shaded if recirculation was to be implemented. As shading with a 50 % shade cloth had a significant reduction in uptake efficiency by *Ulva*, the shade cloth was too dense.

CHAPTER 7

DISSOLVED OXYGEN, pH, TEMPERATURE AND WATER VOLUME EXCHANGE RATES IN THE EXPERIMENTAL SYSTEMS

7.1 INTRODUCTION

The locations of the two farms result in differences in their seawater micro-environments. The I & J farm, located on the Western Agulhas Bank is subject to intermittent wind-driven coastal upwelling, particularly northward of prominent capes (including Danger Point). The region is also strongly influenced by warm water intrusions of the Agulhas current water in the summer, particularly over the outer shelf (Boyd *et al.*, 1985 and Probyn *et al.* 1994).

The JSP farm is under the influence of the cold Benguela current and is located close to a major coastal upwelling cell at Cape Columbine and is subject to regular cycles of upwelling in the summer (Hutchings & Andrews, 1980; Chapman and Shannon, 1985; Mitchell-Innes & Walker, 1991; Largier & Boyd, 2001). Upwelling takes place when cold central Atlantic water is brought up from depths of greater than 200 m to the surface by Ekman forcing. The outcome of this is that summer average water temperatures at the farm intake are lower than in winter. To counteract this, the farm has two large, shallow dams that are lined with black polyurethane. The water is gravity fed into these dams where it is heated by solar radiation before being pumped into the abalone tanks. Due to the lag time from pumping, the abalone receive a higher water temperature at night when they are feeding compared to the incoming seawater temperature. This difference in seawater temperature could have influences on SGR of abalone and *Ulva* and for this reason temperature, salinity and dissolved oxygen measurements were taken daily on both farms.

Water exchange rates at I & J were lower at the start of the project, presumably contributing to the lower seaweed growth rates measured there compared with JSP (See Chapter 8 for details). There were also more carbon and nutrient stress related problems in the fertilized and seawater tanks which were receiving four water volume exchanges per day when compared to the abalone effluent tanks which were receiving twelve water exchanges per day. The low growth rates were also accompanied by a paler thallus colour and breakup of the thalli. It was likely that the algae in the low flow tanks were stressed in

some manner. Another likely explanation could be carbon or nutrient limitation (not light limitation, as shading had little to no effect on SGR) due to low rates of water replenishment. To test this, a set of water volume exchange rate experiments were initiated.

Lyon (1985), found that exposure to 1.08 $mg.l^{-1}$ ammonia at a temperature of $\pm$ 16 °C and a pH of 8.19 $\pm$ 0.03, for 96 hours was toxic to *H. midae*. This toxicity is independent of pH but increases with increasing temperatures. If levels remained below 0.88 $mg.l^{-1}$ there was no effect on the abalone. Acute threshold (threshold beyond which growth and survival are adversely affected) is 0.88 – 1.08 for abalone and 0.11 $mg.l^{-1}$ for turbot at 16 °C and 34 $^0/_{00}$ for 42 days (Alderson, 1979 cited by Lyon, 1985). The ammonia level for abalone toxic was therefore taken as above 0.88$mg.l^{-1}$.

The aim of this part of the study was to investigate seasonal and daily differences in physico-chemical variables (Temperature, pH, dissolved oxygen and ammonia toxicity) in the different types of growth media on the two farms. Values were compared:

- Between shaded and unshaded tanks,
- At different exchange rates.
- The amount of ammonia produced at a set stocking density was also calculated, with regard to potential toxicity problems.

7.2 MATERIALS AND METHODS

7.2.1 Environmental parameters

In situ measurements (water samplings) were carried out at both farms every working day during the course of this study (June 2001 – October 2002). Temperature (± 0.1 °C), salinity (ppt) and dissolved oxygen (± 0.01 mg L^{-1}) and pH were measured with electronic probes (Cyberscan).

7.2.2 JSP

Due to the scale of this project, seasonal environmental conditions were only recorded in a limited number of water sources and in a limited number of tanks. Temporal changes in physico-chemical conditions were recorded when experiments were performed on the farm.

Two continuous temperature data loggers recorded temperature in the reservoir, where seawater is held before being distributed on the farm and in the abalone raceways. pH, temperature and dissolved oxygen in unshaded tanks were recorded by farm staff every working day. These measurements were only recorded in the small and medium seawater tanks. Readings were initially taken at 10h00, though this changed to 14h00 after February 16th 2002. In December, these measurements were expanded to include the shaded tanks.

On October 2nd 2001, a water exchange rate experiment was performed at JSP. All seawater tanks were harvested on October 1st to a stocking density of 2 $kg.m^{-2}$. Water exchange rates of 4 and 20 volume exchanges per day were used. Two small slow-flow (4 volume exchanges per day) seawater tanks and two small fast-flow (20 volume exchanges per day) tanks were used for this experiment. This was repeated for the medium tanks as well. The tanks were left overnight to stabilize at these flow rates and measurements were started at 08:00 the following day.

Temperature recorders were placed in both a small slow- and fast-flow tank as well as a medium slow-flow. These recorded tank temperature at 15 minute intervals. Temperature and pH were recorded in all the tanks on the hour from 08:00 to 15:00.

Physico-chemical variables were sampled on the 10th of October 2002, during the water experiment 3 (Chapter 6, Experiment 3).

7.2.3 I & J

Physico-chemical measurements were taken at 08h00 and 15h00 every working day from June 2001 to October 2002 at the pump house inlet.

On the 25th of February 2002, while the first of the nutrient uptake experiments (Experiment 1, Chapter 6) was being performed (at set stocking densities and varying water exchange rates and with half the tanks shaded with a 50 % shade cloth) environmental measurements (pH, temperature and DO) were taken, with additional measurements being taken every hour on the first day and every two hours at night. Thereafter, they were taken when water samples were taken.

University of Cape Town

7.3 RESULTS

7.3.1 Annual environmental variability at I & J

Figures 7.1 to 7.3 show pH, temperature and dissolved oxygen of the incoming seawater to the tanks. The extreme pH range for the incoming water was 4.7 – 9.9, with the mean pH varying between 6 – 9.1. There appears to be a seasonal trend in the pH with the winter and spring months having a much higher pH than the summer and autumn months (Figure 7.1).

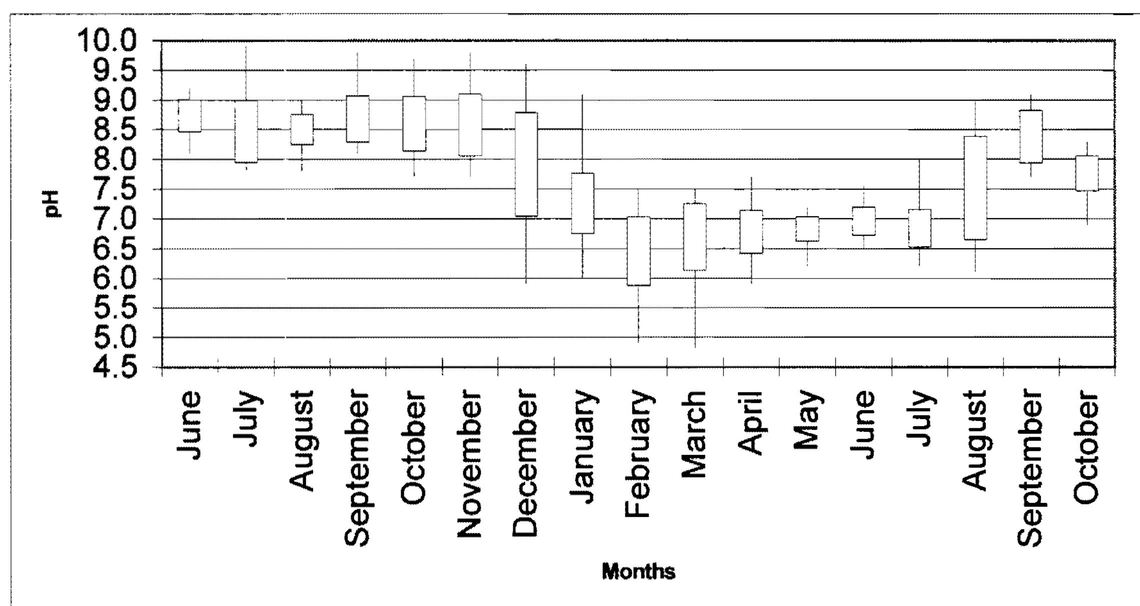


FIGURE 7.1: Mean pH of the incoming seawater from June 2001 to October 2002 at I & J. The whiskers give maximum and minimum values, while the box shows the standard deviation from the mean (n= 30).

Water temperatures increased from spring to summer reaching a maximum in January (Figure 7.2). The extreme range of temperatures on the farm was 10.2 – 24.2 °C. Mean summer temperature was 18 °C, but showed considerable variability due to seasonal upwelling events. Mean winter temperature was 14.5 °C.

Dissolved oxygen values of the incoming water showed little or no seasonal variation (7.41 – 8.3 $mg.l^{-1}$) (Figure 7.3). Salinity on the farm reached a maximum of 39 $^0/_{00}$ and a minimum of 29.7 $^0/_{00}$ with an average of 33.72 $^0/_{00}$.

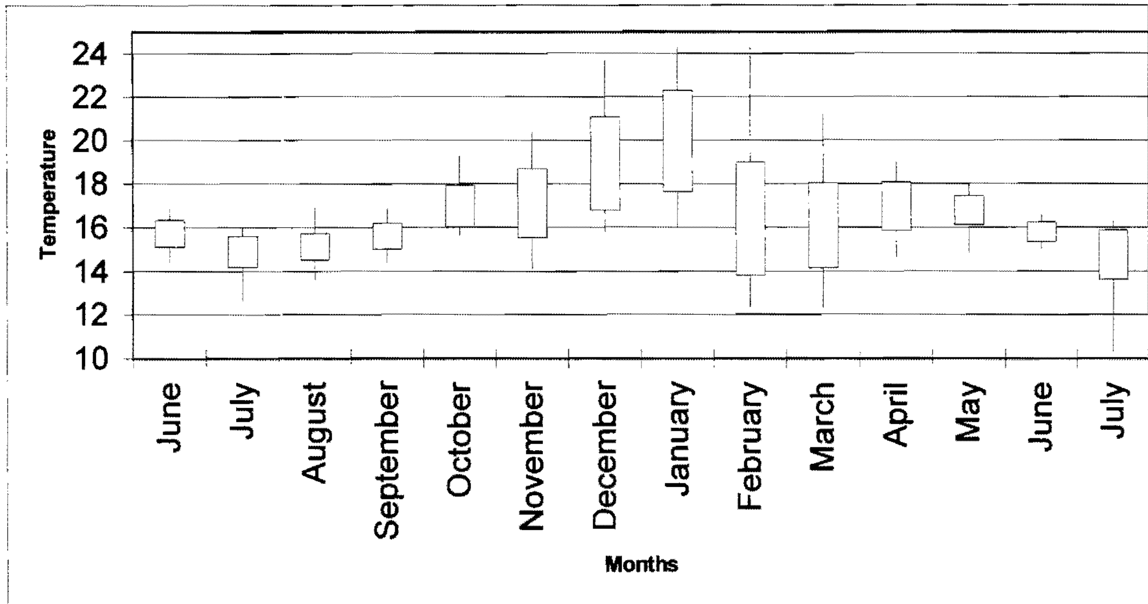


FIGURE 7.2: Monthly average temperature of the incoming seawater from June 2001 to October 2002 at I & J. The whiskers give maximum and minimum values, while the box shows the standard deviation from the mean (n = 30).

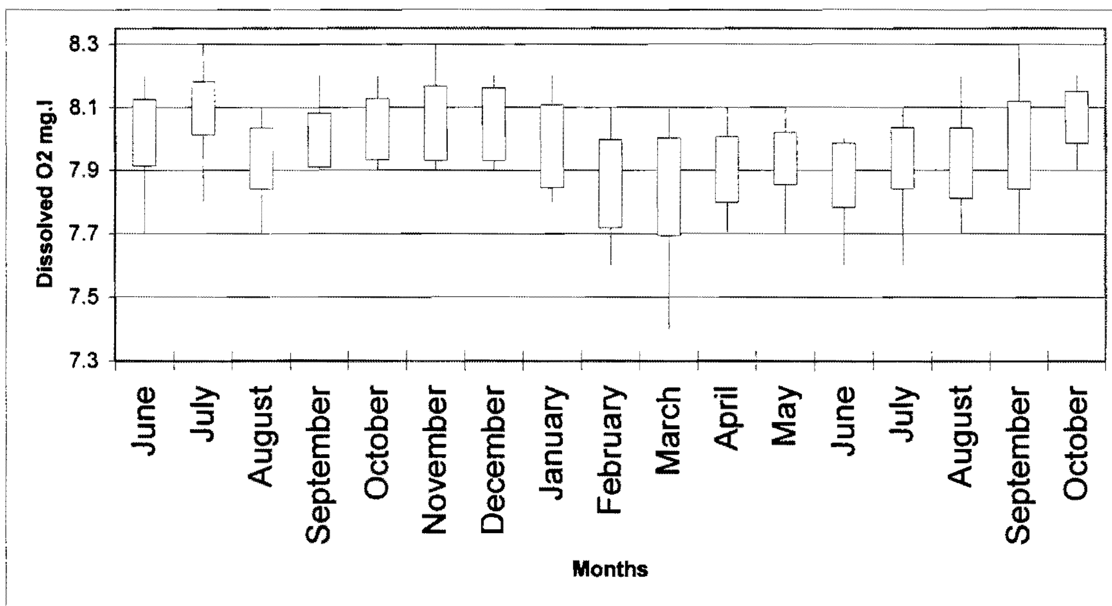


FIGURE 7.3: Monthly dissolved oxygen values of the incoming seawater from June 2001 to October 2002 at I & J. The whiskers give maximum and minimum values, while the box shows the standard deviation from the mean (n = 30).

7.3.1.1 Experiment 1

Varying flow rates (4 & 12 VE.d^{-1}), set stocking densities and shading at I & J

The first detailed measurements of pH were taken by the farm on the 21st of February 2002. They show that the pH in fertilized and seawater tanks reached 9, while the pH in the abalone tanks remained at around 8 (Figure 7.4).
The second measurements were taken on the 26th of February 2002 during the first nutrient uptake experiment, when the fertilizer experiment tanks were fertilized and the water to these tanks was turned off. This explains the high pH (in excess of 9.5 after 11:00) in these tanks. pH in the seawater tanks remained above 9 until 21:00, while the abalone tanks remained below pH 9 (Figure 7.4). Shaded abalone effluent tanks had a lower pH than all the other treatments from 14h00 to 19h00. The last set of measurements was on 27th of February 2002, when all the tanks were receiving water. The unshaded seawater and fertilized seawater tanks had the highest pH.

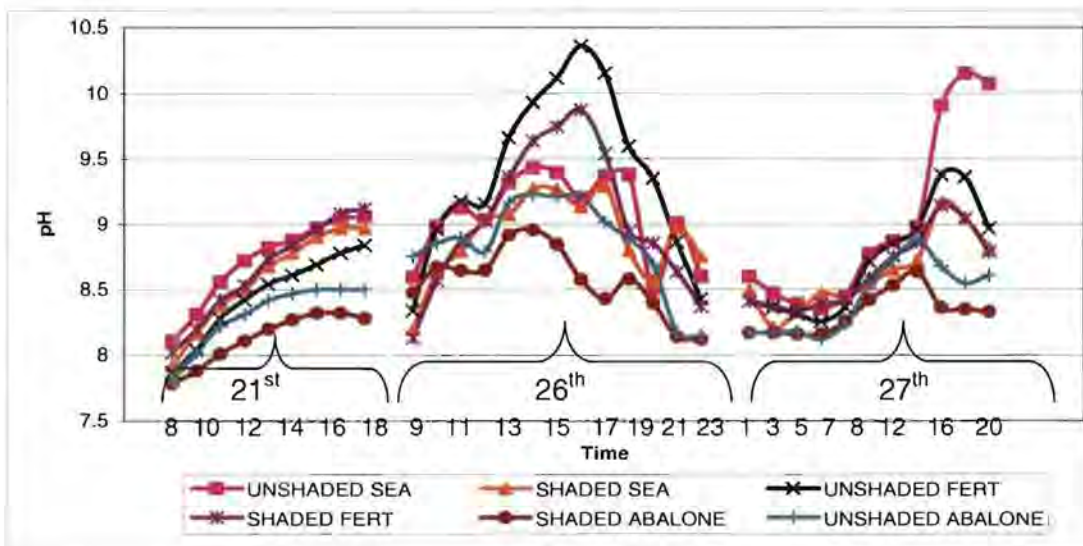


FIGURE 7.4: Mean pH in all the tanks on the 21, 26 – 27th of February 2002 at I & J. The 21st was a hot day with pH measurements taken by the farm. The 26th was a hot day during which the fertilized treatment received no flow. The 27th was a cool day with all the tanks receiving water. The fertilized tanks and seawater tanks received 4 volume exchanges per day while the abalone tanks received 12 (n = 2).

The temperature in the tanks from the 26th – 27th of February 2001 was higher in the fertilized tanks with no flow, than the tanks receiving water (Figure 7.5.). When all the tanks are receiving water, the unshaded seawater tanks have the highest temperature. The abalone tanks, which have a higher flow rate than the seawater and fertilized tanks, have a lower mean temperature than the other treatments.

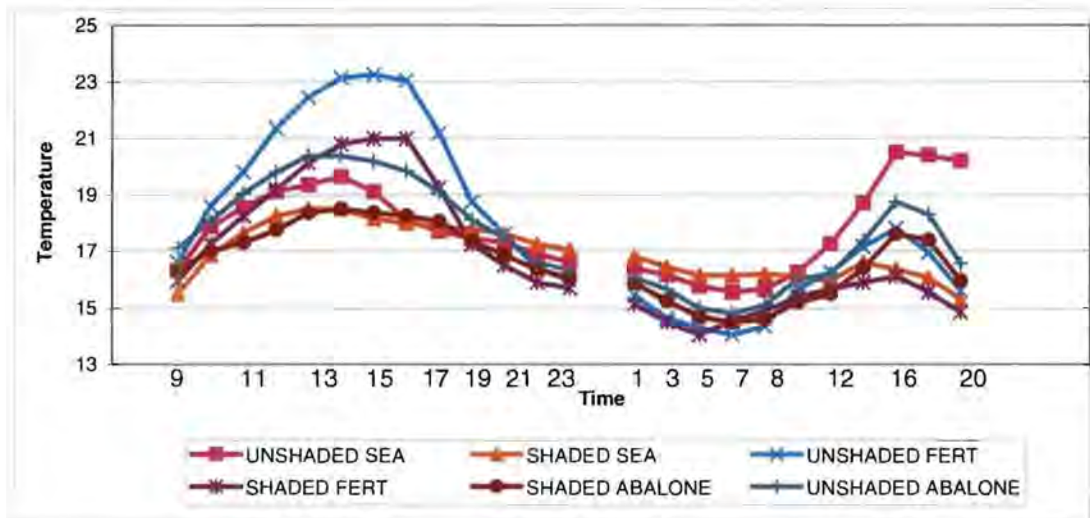


FIGURE 7.5: Temperature in all the tanks on the 26 – 27th of February 2002 at I & J (n = 2).

7.3.2 Annual environmental Variability at JSP

7.3.2.1 Incoming sea water

The salinity ranged, on this farm from a minimum of 29 $^{0}/_{00}$ to a maximum of 40 $^{0}/_{00}$ with a mean of 34 $^{0}/_{00}$.

Figures 7.6 and 7.7 show the average temperature of water coming into the sea water tanks (and turbot effluent tanks) and the abalone effluent respectively. Summer temperatures of the incoming water in the abalone and turbot tanks is approximately 4 ° C warmer on average than water received by the seawater tanks. The temperature range of incoming abalone water is 10.3 – 26.3 °C

(Figure 7.6) while the range for incoming seawater is 9.8 – 19.2 °C (Figure 7.7). Variability increased in summer in both water types, due to regular upwelling events. The graphs indicate that in summer maximum water temperatures are higher and minimum temperatures lower than in winter.

When Figures 7.6 and 7.7 are compared to Figure 7.2, there is clear difference in the water temperature of the incoming seawater on both farms. In both summer and winter on the I & J farm, the incoming abalone effluent water temperature is 2 – 3 °C warmer than the incoming seawater. The water that is fed to the abalone at JSP has the same temperature range as the I & J incoming seawater. This implies that the solar heating of the water by the dams is very effective.

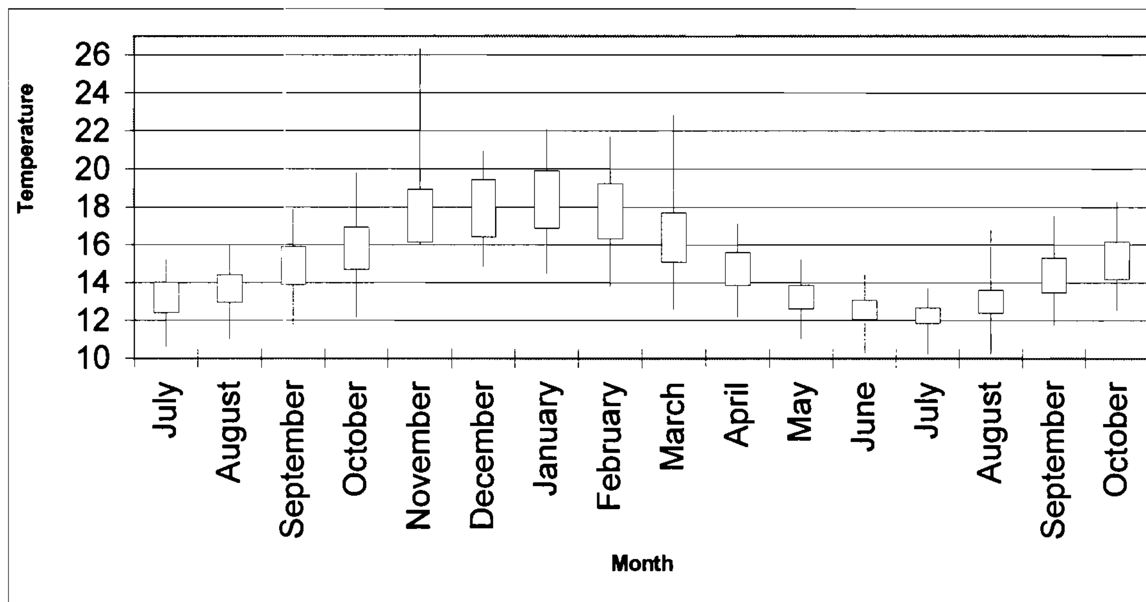


FIGURE 7.6: Average temperatures of the incoming abalone water averaged from 15 minute intervals from July 2001 to October 2002 at JSP. The whiskers show the range and the box the standard deviation from the mean (n = 30).

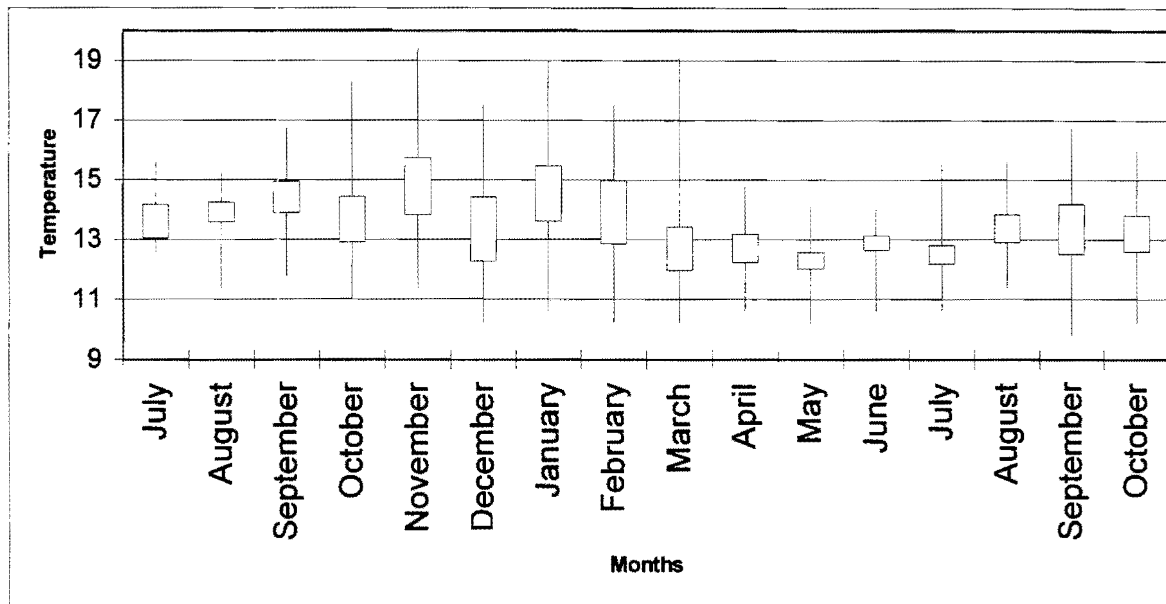


FIGURE 7.7: Average temperatures of the incoming seawater averaged from 15 minute intervals from July 2001 to October 2002 at JSP. The whiskers show the range and the box the standard deviation from the mean (n = 30).

7.3.2.2 Sea water conditions at JSP

Figures 7.8 and 7.9 show the average temperature over a 24-hour period for incoming seawater and abalone water, for summer and winter. In winter, there is no significant difference in temperature between the two incoming water types (ANOVA, $n = 3007$, $p > 0.05$). In summer, however, the abalone wastewater is significantly warmer than seawater (ANOVA, $n = 3007$, $p < 0.05$;).

The graph also shows that highest temperatures in the incoming abalone system are between 19:00 – 20:00 hours. This is due to the long residence time in the dam, which is about 8 hours (K. Ruck, pers. com). This peak in temperature also corresponds to the first feeding peak by the abalone (see Chapter 6).

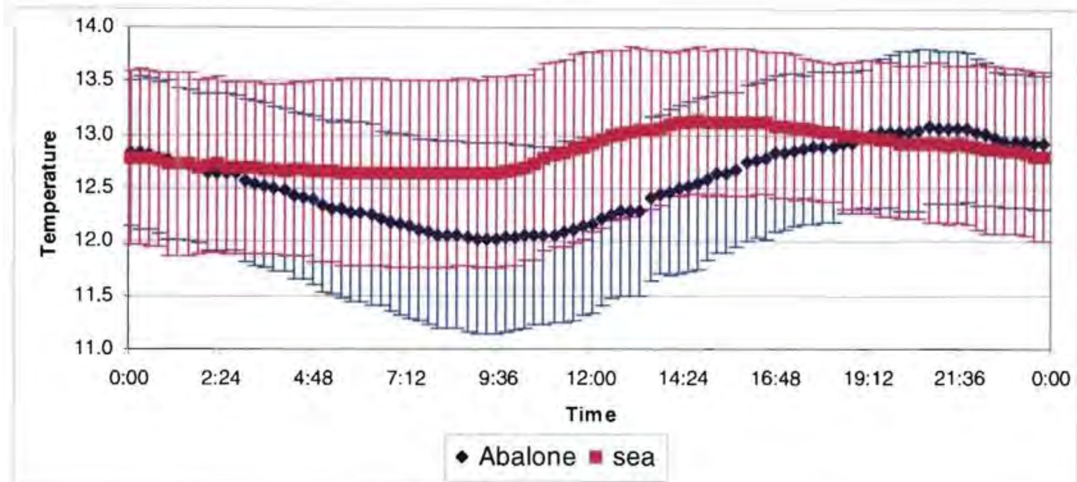


FIGURE 7.8: Average temperatures of the incoming seawater and abalone water in winter over a 24 hour period averaged from1st – 30th June 2002 at JSP. Points show mean temperature, while whiskers give range (n = 30).

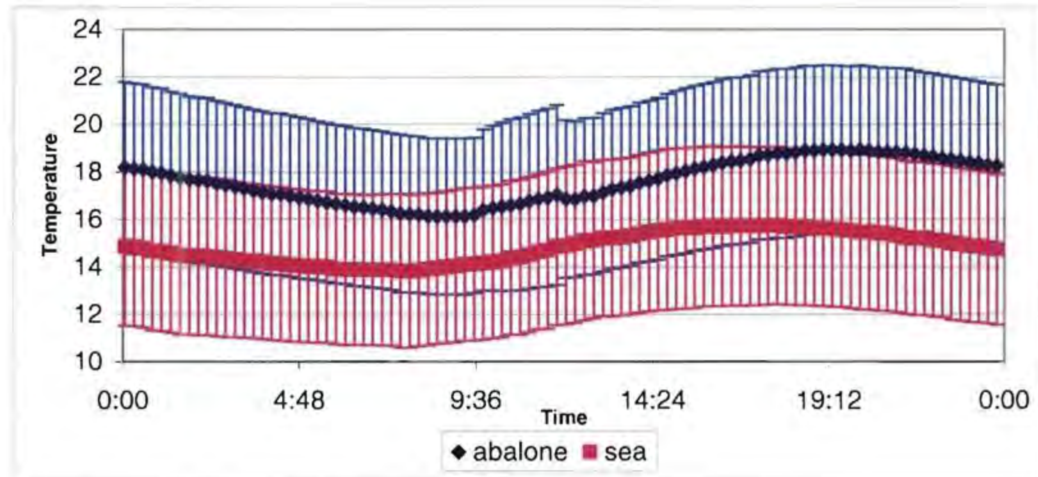


FIGURE 7.9: Average temperatures of the incoming seawater and abalone water in summer over a 24 hour period averaged from 1st – 30th November 2002 at JSP. Points show mean temperature, while whiskers give range (n = 30).

The maximum temperature measured in the small sea water tanks both in summer and winter tanks occurred at about 14:00 (Figures 7.10 and 7.11), and was approximately 5 °C warmer than the incoming seawater (Figures 7.8 and 7.9) in both summer and winter. The range of temperatures experienced in the

tanks (Figures 7.10 and 7.11) was also greater than the range from the incoming seawater (Figures 7.8 and 7.9).

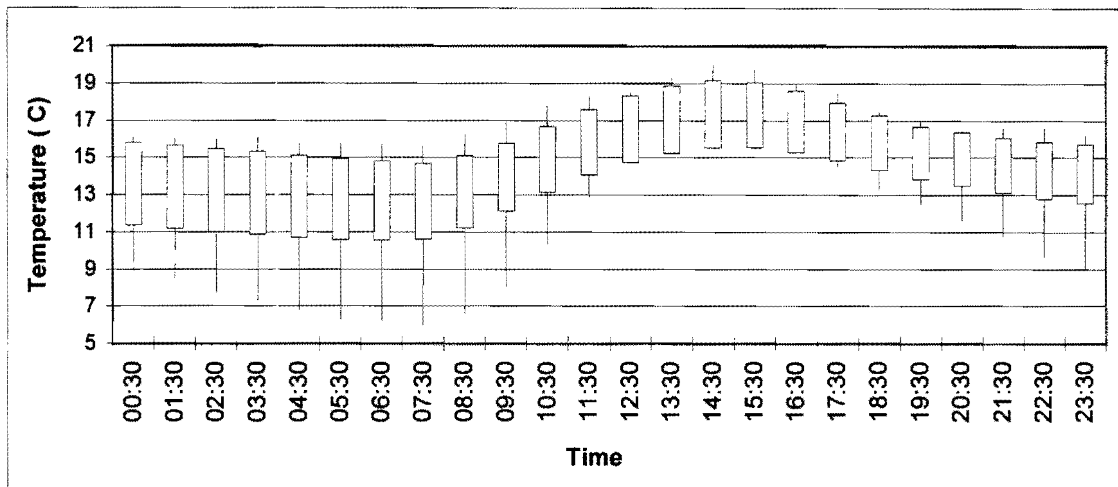


FIGURE 7.10: Minimum, maximum and standard deviation from the mean as recorded by a temperature data logger taken hourly for the period 30th June to 13th July 2002 in small sea water tanks at JSP (Winter) (n = 14).

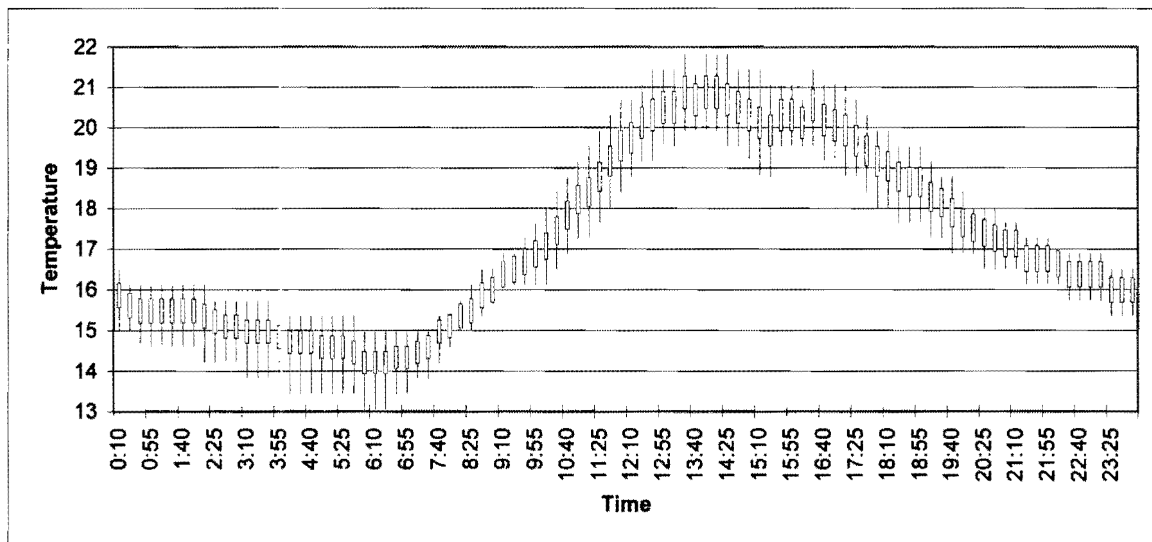


FIGURE 7.11: Minimum, maximum and the standard deviation from the mean as recorded by a temperature data logger taken every 15 minutes average over a 24 hours, for the period 2nd – 5th November 2001 in small seawater tanks at JSP (Summer) (n = 5).

The average temperature in the small seawater tanks in winter was 14.6 °C, while the lowest temperature recorded in the tanks in winter was 6.0 °C and the maximum temperature recorded in the tanks was 20.0 °C. In summer the average temperature was 16.9 °C, while the maximum recorded temperature was 22.1 °C and the minimum was 12.6 °C. The lowest temperature in winter was recorded during an intense winter cold front.

Figure 7.14 show the monthly average temperature values for the seawater tanks (small and medium) at a set stocking density (2 $kg.m^{-2}$) and set flow rate (20 $VE.d^{-1}$). From September 2001 to February 2002, the measurements were all taken at 10:00, after this date they were taken at 14:00. By comparing Figures 7.8 and 7.9, with Figure 7.14, it is evident that this change in monitoring time resulted in a slightly higher temperature being recorded. This temperature difference is not significant. The figures also give values for shaded tanks. The tanks were shaded in December 2001 with an 80 % shaded cloth and in February 2001 this was changed to a 20 % shade cloth. Again, none of the variables showed any significant change as a result of difference in shading.

In summer, there was a slight increase in pH in both small and medium tanks (Figure 7.12). The pH was lower in both tank sizes that were shaded by both an 80 % and 20 % shade cloth, with no significant difference between the two shade levels. There was no significant difference in pH between the small and medium tank sizes in either the shaded or the unshaded tanks. The average pH range for the farm in the seawater tanks is 7.1 – 9.1, which is lower than measured at the I & J farm.

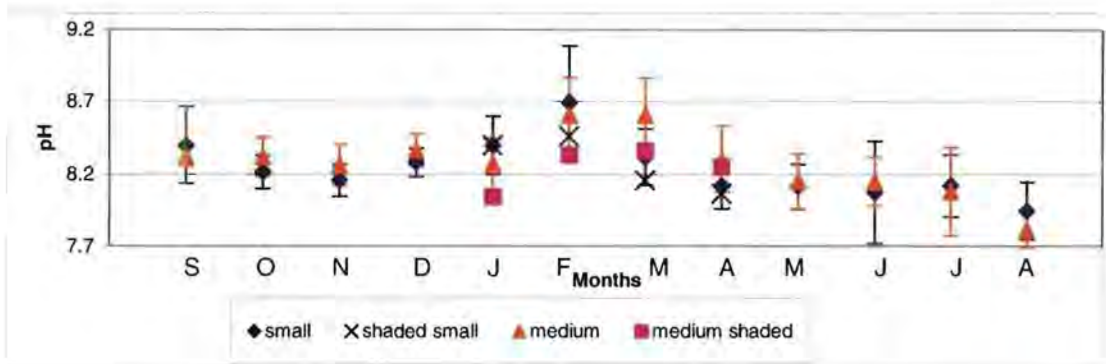


FIGURE 7.12: Monthly average pH for the period September 2001 to August 2002 at JSP in small and medium seawater tanks at a set stocking density (2 $kg.m^{-2}$) and set flow rate (20 $VE.d^{-1}$). Whiskers represent standard deviations from the mean (n = 30).

Shading reduced the concentration of dissolved oxygen in the seawater tanks (Figure 7.13), although not significantly. There was also no significant difference in dissolved concentration values between small and medium tanks. There was no clear seasonal pattern in these values, although the range was slightly larger in summer than winter. The dissolved oxygen range at JSP was slightly more variable than I & J.

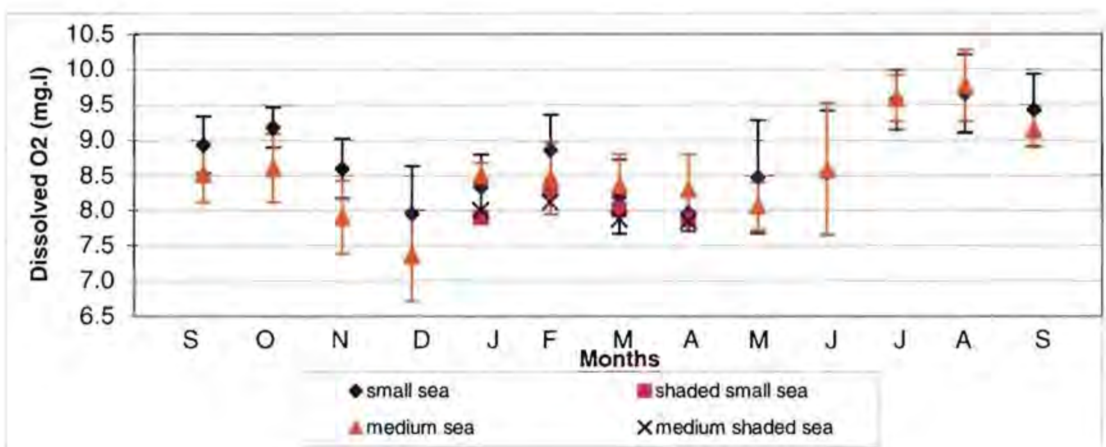


FIGURE 7.13: Dissolved oxygen ($mg.\ell^{-1}$) in the small and medium seawater treatments at a set stocking density (2 $kg.m^{-2}$) and set flow rate (20 $VE.d^{-1}$) for the period September 2001 to September 2002 at JSP with standard deviations from the monthly mean (n = 30).

There was no significant difference in temperature (Figure 7.14), between small and medium tanks or shaded and unshaded tanks.

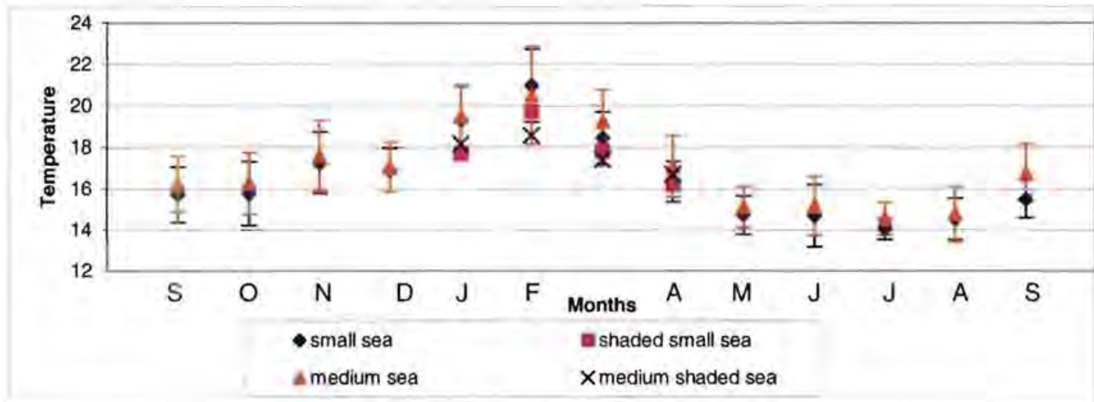


FIGURE 7.14: Temperatures in the small and medium seawater tanks with monthly means and the standard deviation from the mean for the period September 2001 to September 2002 at JSP (n = 30).

7.2.2.3 Experiment 3

Set flow rates (20 VE.d^{-1}), set stocking densities and no shading at JSP

The small and medium seawater tanks had a higher pH (Figure 7.15), though not significantly higher than the effluent tanks, and the pH rose above 9, which means that carbon could become limiting in these tanks. The pH showed a diurnal pattern in these tanks, rising to a maximum between middle and late afternoon and then decreasing in the evening. This diurnal pattern of pH is due to the diurnal nature of photosynthetic activity.

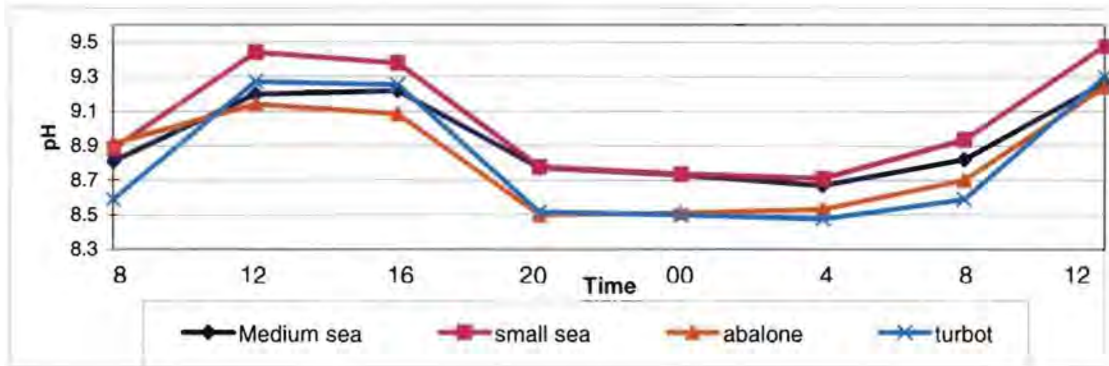


FIGURE 7.15: pH in all the tanks at a set stocking density of 2 kg.m^{-2}. From 08h00 10th October – 12h00 11th October 2002 (n = 3).

Dissolved oxygen concentration was maximal in the effluent tanks at midday and decreased during the afternoon and evening (Figure 7.16), presumably due to respiration by the seaweeds and a decrease in photosynthesis. The peak and trough in the effluent media were significantly different from the seawater media (ANOVA; n = 23; $p < 0.05$), possibly indicating that more photosynthesis is occurring in the effluent tanks during the day due to higher nutrient concentrations, while during the night there is great uptake of nutrients in the effluent tanks which results in a greater oxygen demand.

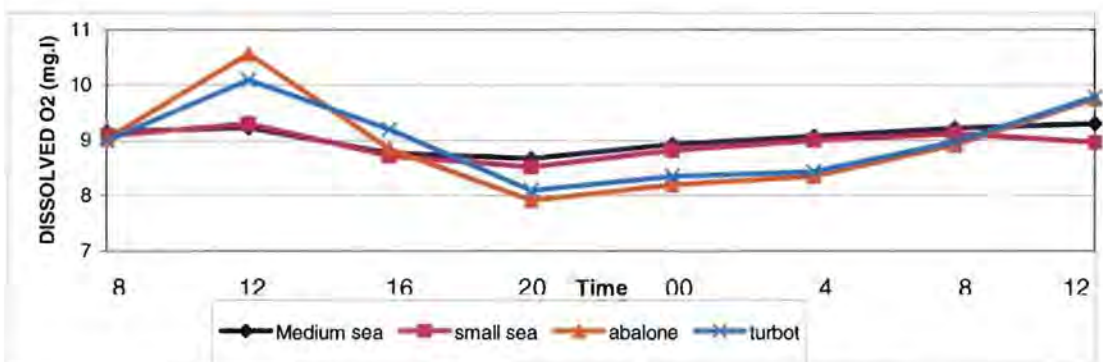


FIGURE 7.16: Dissolved oxygen (mg.ℓ$^{-1}$) for all the tanks at a set stocking density of 2 kg.m^{-2}. From 08h00 10th – 12h00 11th October 2002 at JSP (n = 3).

Temperatures in the abalone effluent media in the evening and late at night were significantly higher than in the other culture media (ANOVA; n = 23; p <

0.05), by almost 2.5 °C (Figure 7.17). There was no significant difference between the small and medium seawater tanks or the turbot effluent tanks. All three treatments (small & medium sea and turbot) showed a diurnal pattern in temperature.

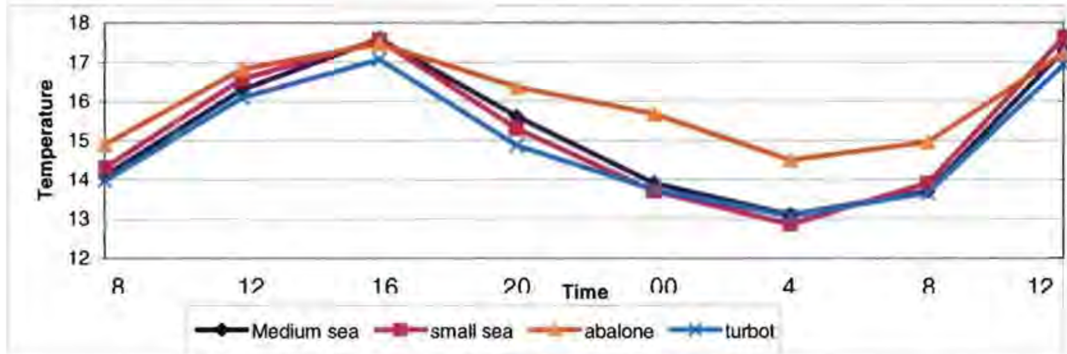


FIGURE 7.17: Temperature in all the tanks at a set stocking density of 2 kg. m^{-2}. From 08h00 10th – 12h00 11th October 2002 at JSP (n = 3).

7.3.3 Water exchange rates vs. physico-chemical variables at JSP

Both the small and medium seawater tanks which had low water exchanges showed an increase in pH over the day and this increase was more pronounced than measured under faster flow in both small and medium tanks (Figure 7.18). There is a significant difference in pH after 12:00, between slow flow and fast flow tanks, with the slow flow tanks experiencing a higher pH than the fast flow tanks (Figure 7.18) (ANOVA; n = 3; $p < 0.05$ for small tanks and ANOVA; n = 3; $p < 0.05$ for large tanks). In addition, the pH rose above 9, indicating that some form of carbon limitation might be occurring.

After 12:00, temperatures in tanks with 4 volume exchanges. d^{-1} rose significantly higher than in tanks with 20 volume exchanges. d^{-1} (Figure 7.19) (ANOVA; n = 11; $p < 0.05$ for small tanks and ANOVA; n = 7; $p < 0.05$ for large tanks).

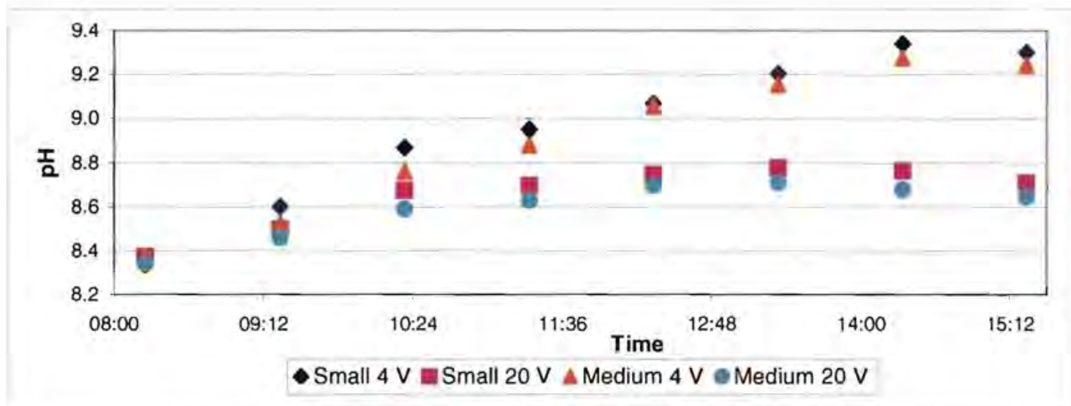


FIGURE 7.18: pH increases on October 2nd 2001 for small and medium seawater tanks at 4 and 20 volume exchanges per day.

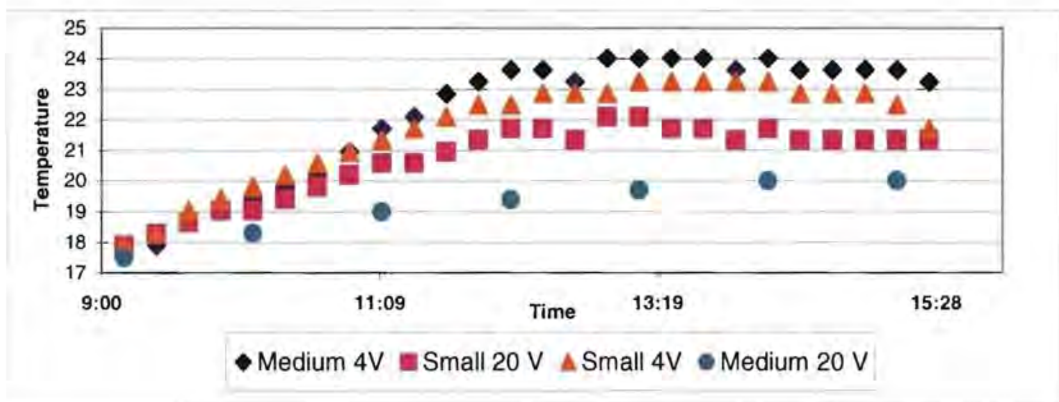


FIGURE 7.19: Temperature on October 2nd 2001 for small and medium seawater tanks at 4 and 20 volume exchanges per day recorded from the three data recorders.

7.3.3 Ammonia Toxicity

Ammonia levels calculated using data from Experiment 1 (I & J), indicate that 4 volume exchanges in both the seawater and fertilized treatments are too low to be considered for recirculation as ammonia remained at toxic levels until midnight in the case of seawater, while in the abalone treatment, which was receiving a higher water exchange rate, the levels of ammonia were not toxic

(Table 7.1). The effect of adding fertilizer into the tanks was felt 36 hours after the tanks have been fertilized at this low flow rate, with values remaining toxic.

TABLE 7.1: Ammonia values (µM) for four periods calculated using data from Experiment 1 at I & J. Values with an asterisk indicate those that are toxic to abalone according to Lyon (1985).

Treatment	Stocking density	Flow rate	Shading	Ammonia concentrations			
				08:00	12:00	20:00	00:00
Sea	2	4	Yes	0.83	2.02*	3.64*	3.44*
Sea	2	4	No	1.38	2.00*	7.04*	0.47
Fertilized	2	4	Yes	4.26*	13.70*	16.72*	0.49
Fertilized	2	4	No	4.90*	10.11*	23.40*	0.58
Abalone	2	12	Yes	0.65	1.39	0.54	0.42
Abalone	2	12	No	0.75	2.83*	1.40	0.70

Turbot effluent had very high levels of ammonia (Table 7.2), compared to seawater and abalone effluent at JSP. Ammonia levels were higher on the JSP farm than I & J, though this may be a function of flow rates.

TABLE 7.2: Ammonia values (µM) for four periods calculated using data from Experiment 3 at set flow rate (20 VE.d^{-1}) at JSP. Values with an asterisk indicate those that are toxic to abalone according to Lyon, (1985).

Treatment	Stocking Density	Ammonia concentrations			
		08:00	12:00	20:00	00:00
Medium Sea	2	0.56	0.49	0.12	0.09
Small Sea	2	0.21	0.66	0.13	0.11
Turbot	2	1.51*	2.94*	1.86*	2.08*
Abalone	2	0.46	0.81	0.22	0.22

7.4 DISCUSSION

There is a high variability in the temperature of the small tanks in winter. This occurs as the tanks have a high surface to volume ratio and a low specific heat capacity. Since the tanks were not insulated, temperatures fluctuate quite readily in response to cold air temperatures and solar radiation, despite the tanks having a high water exchange rate.

The minimum temperature in the small seawater tanks in winter (5.9 °C) is at the low end of the growth range for *Ulva* growth. Duke *et al.*, (1986) grew *U. curvata* in a temperature range of 5 – 26 °C, however they reported a ten fold difference in growth rates between 5 and 20 °C. The maximum temperature for growth of *U. curvata* was around 20 °C as SGR decreased when temperatures were higher than 20 °C (Duke *et al.* 1989). De Casabianca & Posada (1998) found that *U. rigida* grew in Thau lagoon between temperatures of 10 and 24 °C. They found that maximal growth occurred between temperatures of 12 and 23 °C and above and below these temperatures growth was restricted. Thus, at both farms the average seawater and effluent water temperatures in which the alga are grown fit in well with optimum temperature ranges for maximal growth rates of other *Ulva* species.

Incoming seawater temperatures at I & J were higher than at JSP and also showed less variability. The JSP farm policy of solar-heating the water before pumping it to the abalone tanks has the benefit of providing warmer water during abalone feeding. Abalone feeding and growth rates are elevated in warmer water, provided that is does not exceed a optimum value. The second benefit is that it raises the temperatures so that they are comparable with the I & J Farm.

The salinity range on both farms is optimal for the cultivation of *Ulva* (30 – 40 $^{0}/_{00}$) (De Casabianca & Posada, 1998). DeBusk *et. al.* (1986) grew *U. lactuca* in salinities ranging from 18 – 35 $^{0}/_{00}$. While De Casabianca & Posada (1998) found that *U. rigida* grew in salinities of 29 to 42 $^{0}/_{00}$. Floreto & Teshima (1998)

found that *U. pertusa* had highest SGR in 35 $^{0}/_{00}$ while algae grown in 10 $^{0}/_{00}$ were chlorotic, moribund and had a very high moisture content (± 86% compared to 77 % in those exposed to other salinities).

Photosynthesis stops at night, but respiration processes continue to use oxygen. This pattern of daytime production and continuous use of oxygen leads to diel fluctuations of dissolved oxygen concentrations (Boyd, 1990; 1998). Maximum concentrations of dissolved oxygen occur during the afternoon, when temperature is highest and oxygen solubility is lowest and this illustrates the overriding effect of oxygen production by photosynthesis. Minimum concentrations of oxygen occur at or just after sunrise due to maximum period of respiration by the seaweeds. This diel pattern was seen on both farms during the short experiments (1 - 4).

Lower pH in both shaded and unshaded abalone effluent treatment tanks compared to fertilized seawater and filtered sea water treatments can be attributed to higher flow rates (12 volume exchanges per day) in the abalone effluent treatment tanks. The shading decreased the photosynthesis which resulted in a higher CO_2 concentration in the water, and thus a lower pH. The higher pH in the unshaded seawater and fertilized tanks at 12 $VE.d^{-1}$, can be attributed to their low water exchange rates (4 volumes. d^{-1}). The unshaded seawater tank's pH was higher than the other tanks, including the unshaded fertilized tanks. This indicates that the algae in these tanks were severely stressed because at high pH's CO_2 becomes limiting. As the unshaded fertilizer tanks are not as stressed (indicated by the lower pH) it is assumed that the stress is due to carbon and nutrient limitation caused by the low water exchange rates. The lower pH in both the unshaded and shaded abalone effluent treatments indicate that high water exchanges (12 volume exchanges per day) are beneficial compared to the lower water exchanges (4 volume exchanges per day) received by the seawater and abalone treatments.

The combination of high pH and high temperatures in the seawater and fertilized tanks when receiving no flow, indicates that the algae in these tanks

are experiencing some form of stress: either carbon or nutrient limitation. This was visible in that the thalli were also bleached and almost yellow in colour.
In addition the high pH values in samples from the water volume exchange experiment indicate that at low water exchange rates (4 volume exchanges per day) the seaweed may become carbon limited, as evidenced by the increase in pH. This occurs in addition to nitrogen and phosphate limitation at low flow rates (Chapter 4), thus accounting for the low growth rates (Chapter 6) and thallus colour change (Chapter 7).

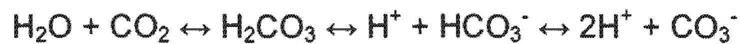
An increase in temperature and pH causes the concentration of ammonia to increase, and the solubility of oxygen to decrease (T = 11.6 °C; Oxygen saturation = 6.219 mℓ/ℓ while at T = 15.5 °C; Oxygen saturation = 5.736 mℓ/ℓ at the same temperatures and a pH of 8.4, % ammonia = 2.88 and 6.93 respectively (Boyd, 1990)). If seaweeds were used in a recirculation system, the oxygen consumption by the seaweeds at night would become important because if the seaweeds consume too much oxygen, then the animals that receive the water could die of asphyxiation. A figure of 6 mg.ℓ is the ideal oxygen concentration for abalone (K. Ruck and H. Otto pers. comm.). Dissolved oxygen in Experiments 1 – 4 remained above this figure, even with the diel variation with the lowest value being 6.5 mg.ℓ at 4 $kg.m^{-2}$ stocking density. This means that incorporation of the seaweeds into a direct recirculation system is feasible as long as the stocking density is maintained.

Ryther *et al.* (1981), found that when internal N-reserves are depleted, *Gracilaria* started to lose its dark reddish-brown color, and became pale straw yellow coloured and stopped growing. The loss of colour was due to pigments being metabolised as a source of protein (Lapointe, 1981; Bird *et al.* 1982). Red seaweeds have phycobilins that are used as nitrogen reserves. Green seaweeds do not have phycobilins. In *Ulva* however, the chlorophyll a molecule contains nitrogen (protein), which could be metabolized, as shown by Duke *et al.* (1989a,b) who correlated chlorophyll a with tissue nitrogen. Because of the high water residence time in the I & J culture tanks, tissue nitrogen consumption by the seaweeds may be an explanation of the bleaching effect experienced by

the *Ulva* (Chapter 8 and 9) as the fertilized seawater tanks did not experience this bleaching.

During daylight, seaweeds remove carbon dioxide from the water for use in photosynthesis, but at the same time they are releasing CO_2 into the water via respiration (Boyd, 1990; 1998). This diel cycle of pH resulting from CO_2 removal and evolution is clearly illustrated in Figures 5.18 and 5.21.

The equilibrium reaction for CO_2 is illustrated below:



$$H_2O + CO_2 \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^-$$

Reactions shift to the right as pH rises and left as it falls.

During the day plants usually remove CO_2, via photosynthesis, from the water faster than it can be replaced by respiration, which results in a rise in pH.

Free CO_2 will in turn decrease further in response to rising pH. As CO_2 is removed, carbonate accumulates and hydrolyses resulting in a pH increase (Boyd, 1990; 1998). As pH rises towards 9, free CO_2 becomes unavailable to algae, which then lose the ability to photosynthesize (Kremper, 1981) and absorb NO_3 (Turpin, 1991). pH 9 is about the pKa value (50 % distribution of carbon species) for the deprotonation of HCO_3 to CO_3: essentially when zero CO_2 is available in the water. Certain species of *Ulva* however, are known to be users of HCO_3 in photosynthesis e.g.: *U. lactuca* (Markager & Sand-Jensen, 1990; Vandermeulen & Gordin, 1990; Drechsler & Beer, 1991 cited by Björk *et al.* 1993); *U. reticulata, U. pulchra, U. rigida* (Björk *et al.* 1993). Collèn *et al.* (1995), noted that bicarbonate is as effective as a carbon source as CO_2.

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At the normal seawater pH of approximately 8.2, virtually all (> 95 %) of the inorganic carbon is present as bicarbonate (HCO_3^-). Free aqueous CO_2 tends to equilibrate with the gas phase (atmosphere). As temperature and salinity increase the partitioning of CO_2 between aqueous solution and gaseous phase, favours the gas phase.

Either low CO_2 concentrations or high pH or a combination of both can limit *Ulva* growth. Kremper (1981), noted that if the pH is controlled (by adding HCl but not by adding inorganic carbon), the growth rate remained minimal.

Consequently, in cultures with limited water exchange rates, it is necessary to control pH and supplement inorganic carbon. Experiments done by DeBusk *et al.* (1986) found that reduced carbon availability was the limiting factor in *U. lactuca* growth at low water exchanges. Low water exchanges and correspondingly low seaweed growth on the I & J farm support this finding (See Chapter 6).

Neori *et al.* (1991) found that pH levels measured in the afternoon in clean seawater were lower than those with algae fed with abalone effluent water, sometimes by over a whole pH unit. This was not the case in the systems tested here, although if the abalone stocking densities were increased, such a variation is possible.

Higher flow rates also promote higher growth rates through the provision of more inorganic C to the thalli per unit time, providing required macro- or micronutrients, moderating temperatures, or flushing out toxic metabolites (DeBusk *et al.* 1986). However, high growth rates obtained in high flow tanks would also lead to a low cellular N content via a biomass "dilution" of stored nitrogen reserves or rapid flushing of nutrients in a pulse fed system (DeBusk *et al.* 1986).

In a recirculation system, pH and temperature are two of the most important variables. The pH, temperature and salinity determine the amount of ammonia that is in the non-ionic (toxic) form. The ratio between ionised ammonium (non-toxic) and non-ionized (toxic) ammonia can change rapidly depending on the pH. Higher pH implies more ammonia is in the toxic form, while the lower pH implies less ammonia is in the toxic form. Temperature and salinity have a similar role in this relationship (Boyd, 1990; 1998).

It was found during the course of this study that *Ulva* tanks were 1 – 2 °C warmer than *Gracilaria* tanks under the same conditions. The reason for this may be that the *Ulva* growth form and pigment complex may be more effective at absorbing incoming radiation and thus increasing the temperature.

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It was also found that at the same stocking densities *Ulva* tank levels were a pH unit higher than *Gracilaria* tanks (Njobeni, 2003). A possible reason for this is that the algae were producing Hydrogen peroxide (H_2O_2). *U. rigida* is known to produce and excrete hydrogen peroxide (H_2O_2) in light (Collén *et al.* 1995). H_2O_2 excretion increases exponentially with increasing photon radiance (Collén & Pedersén, 1996) suggesting that H_2O_2 acts as a sink for energy. Since H_2O_2 can diffuse readily through biological membranes and cell walls out into the water and as *Ulva* is only two cell layers thick, diffusion is rapid and this can in turn raise pH of the culture media (Collén & Pedersén, 1996).

7.5 CONCLUSION

A monitoring schedule for pH in seaweed tanks should become an integral part of farm management. This is because pH is a very good indicator of seaweed health and pH greater than 9 may be detrimental to seaweed health. The consequences of prolonged periods of high pH are decreased growth rates and decreased pigment content (Lapointe, 1981; Bird *et al.* 1982 & DeBusk *et al.* 1986). In terms of farm management, this means decreased yields and poor quality feeds.

pH can be used as a reliable index of culture medium carbon speciation in a tank and should be maintained at normal seawater range. If the pH rises above 9, carbon supply can be improved by increasing the flow rate of water into the cultivation system or through the addition of HCO_3^- or CO_2 or carbonate salts. Increasing the flow rate is often the more costly option and consequently it may be more practical to provide carbon through CO_2 enriched aeration. Alternatively the tanks can be shaded with shade cloth as shading decreases photosynthesis, which in turn reduces pH.

It is also important to note that the diel fluctuation in DO is above critical levels for abalone respiration at night ($6mg.l^{-1}$) (Lyon, 1995), thus indicating that direct recirculation of water from seaweed cultivation is possible.

CHAPTER 8

SEASONAL VARIATION IN GROWTH RATES, YIELDS AND EPIPHYTE CONTAMINATION IN DIFFERENT WATER TREATMENTS ON BOTH FARMS

8.1 INTRODUCTION

One might expect that growth of *Ulva* sp. and other species in South Africa would be better in the summer months with a longer day length of 15 hrs (Astronomical Applications) while decreasing in winter due to a shorter day length of 11hrs (Astronomical Applications). Other studies have also found a seasonal growth in *Ulva* (Rosenberg & Ramus, 1982a; DeBusk *et al.* 1986; Israel *et al.* 1993; Fillit, 1995; De Casabianca & Posada, 1998 & Altamirano *et al.* 2000a). Photosynthetic performance and therefore growth has also been shown to be influenced by temperature (especially low temperatures) and would also show seasonal variation (Henely & Ramus, 1989 & Fillit, 1995).

The I & J farm at Danger Point has limited access to kelp which is the main feed of cultured abalone. Recently warm water events in summer have caused the quality of the kelp to deteriorate and have decreased the size of harvestable beds (H. Otto, and N. Loubser pers. comm.). In addition to this, several severe winter storms made kelp harvesting difficult and thus the farm has had to switch to a kelp and Abfeed ® diet for the abalone. This has been an undesirable move as the quality of the abalone meat deteriorates and thus the price per kilo drops. Access to a seaweed resource of known yield and nutritional values has quantifiable value to the farm management. By growing the seaweeds in the farm's effluent media they decrease the amount of pumping required and also have an option for recirculation.

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The aim of this part of the study were:

- To investigate seasonal growth rates and yields in the different types of growth media on the two farms in shaded and unshaded tanks and to compare this to a base line growth in seawater.
- To investigate differences in scaling up of tank sizes
- To investigate seasonal changes in epiphyte production.

8.2 MATERIALS AND METHODS

8.2.1 Seasonal production

Seasonal production of *Ulva* was examined from August 2001 to October 2002 at both farms in all treatments. The seaweed material was harvested from each tank in net bags, then left to air dry for 15 minutes. The wet weight was then determined. Following weighing, the seaweeds were harvested back to initial stocking densities. This occurred at monthly intervals at I & J and at bi-weekly intervals at JSP. All the tanks were scrubbed to remove fouling epiphytic algae from the sides and bottom of the tanks, after *Ulva* culture had been temporarily removed.

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8.2.2 Epiphytes, endophytes and mesoherbivores

It was difficult to quantify epiphyte biomass, due to epiphytes being a variety of species. A modification of the Braun-Blanquet (BB) cover-abundance scale was developed to compare fouling and epiphyte biomass, rather than measuring weights and ratios.
The scale consisted of three parts:

Tank wall contamination

Fouling algae as a percentage of total algae

Presence of epiphytic algae as a percentage of area covered on *Ulva* thalli.

This scale was further divided to include the occurrence of *Myrionema strangulans* as an exclusive epiphyte

The range of the BB values given as percentage cover is as follows:
0 % = 1; 1 - 10 % = 2; 11 - 25 % = 3; 26 - 50 % = 4; > 50 % = 5.

The reason for the modification was that as we were assessing a 3 – dimensional volume for percentage cover and thus were unable to use quadrats, thus the assessment was based on visual estimates. In addition, comparisons between mass and cover of diatoms to macro algae would result

in inaccuracies. This is because dry weight to wet weight of diatoms and macroalgae and animals is highly variable.

Whenever epifauna were removed from the samples for biochemical analysis they were collected and preserved in 10 % formaldehyde in seawater, until they could be identified. During harvests, estimates were made of the densities and notes made of the species of epifauna that occurred in the tanks.

8.2.3 Statistics

One way ANOVA's were used to determine significant differences in SGR between the different treatments.

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8.3 RESULTS

8.3.1 Seasonal production on both farms

8.3.1.1 SGR at JSP

SGR at JSP showed a seasonal trend with the highest SGR in summer (Figure 8.1). The decline in the seawater tanks in December and January are difficult to explain as there is great variability within the tanks. Maximum SGR in the small seawater treatment at JSP was higher that of I & J (12 vs. 8 $\%.day^{-1}$). However, comparisons between the two farms must be treated with caution, because of the differences in tank sizes and flow rates.

Generally, SGR was lower in the medium seawater tanks than in the small seawater tanks. This difference became significant in the winter months (ANOVA; $n = 7$; $p < 0.05$ for 5 out of 6 cases).

Because of problems experienced with the pump supplying water to the abalone treatment on this farm there were only 3 short data periods. However, SGR appears to be comparable to levels obtained in other treatments.
There was no significant difference between the turbot or abalone effluent and the small or medium seawater treatments.

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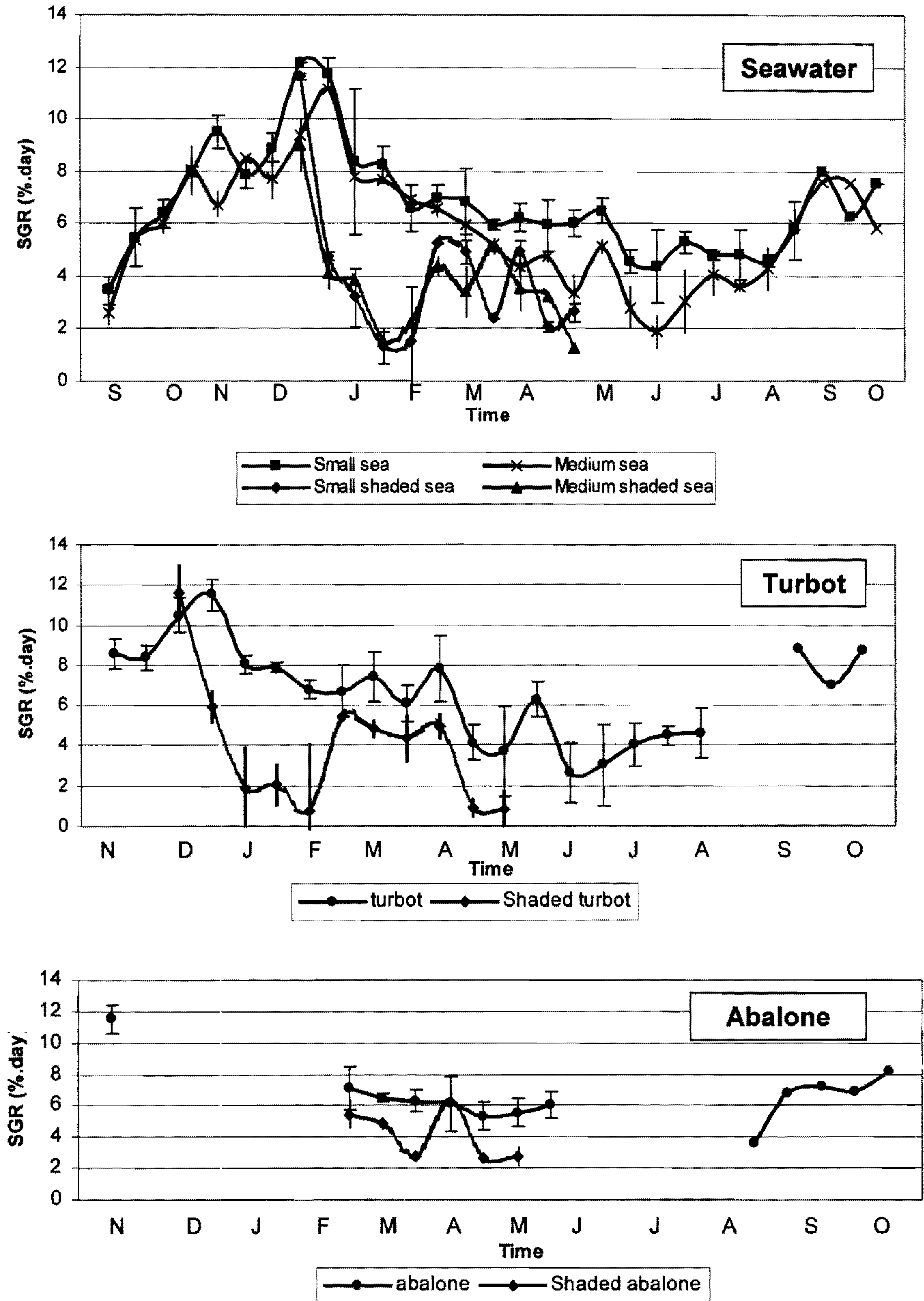


FIGURE 8.1: Seasonal SGR in the three treatments at JSP from September 2001 to October 2002, with standard deviations (n = 3).

8.3.1.2 Yield

Yields at JSP showed a seasonal trend (Table 8.1), and were highest in summer in both the turbot effluent and small seawater growth media tanks. Yields were lowest in the seawater and turbot effluent growth media in winter. Yields for the abalone effluent were similar per tank at both farms, while yields from the seawater and turbot tanks were higher at the JSP farm.

Table 8.1: Average yields kg.wwt.m^{-2}.d^{-1} with standard deviation in brackets, obtained in unshaded tanks in each treatment by season at the JSP farm.

TREATMENT	SPRING	SUMMER	AUTUMN	WINTER
Small Seawater	0.40 (± 0.31)	0.58 (± 0.01)	0.29 (± 0.37)	0.20 (± 0.64)
Medium seawater	0.39 (± 0.12)	0.55 (± 0.21)	0.25 (± 0.11)	0.10 (± 0.45)
Turbot	0.43 (± 0.15)	0.57 (± 0.35)	0.31 (± 0.37)	0.18 (± 0.50)
Abalone	0.29 (± 0.01)		0.28 (± 0.05)	

8.3.1.3 Shading

Shading was applied at JSP in November 2001, as SGR in the unshaded tanks leveled off and began decreasing. Initially tanks were shaded with an 80 % shade cloth. Shading by 80 % had a dramatic effect on the SGR of all three treatments and in general, SGR was halved in shaded treatments (8 %.d^{-1} vs. 4 %.d^{-1} in unshaded and shaded treatments respectively) (Figure 8.1). There was a significant difference in the SGR of turbot effluent and small and medium seawater treatments shaded with the 80 % shade cloth (ANOVA; $n = 19$; $p < 0.5$ in 4 out of 4 cases). As occurred at I & J, shading reduced tank epiphytes but led to a species dominance switch which may have been linked to the lower SGR in the shaded tanks. As SGR did not improve but decreased, the 80 % shade cloth was changed to a 20 % shade cloth in February 2002. This improved the SGR of the shaded tanks, but shaded tanks had a lower (but not significantly so) SGR than unshaded tanks. Also since shading was only

applied towards the end of November 2001 and SGR in the unshaded tanks was already decreasing, the shade cloth may have been applied too late.

8.3.1.4 SGR at I & J

The base line growth shown by the unshaded seawater treatments, gives a SGR of 3.5 $\%.d^{-1}$ in summer and 2 $\%.d^{-1}$ in winter (Figure 8.2). The seasonal trend is obscured by a severe *Myrionema strangulans* infection in November 2001 (point 1, Figure 8.2), and it is likely that SGR could be higher in summer and show similar seasonal patterns as were obtained at JSP. In February 2002, when the water exchange rates where altered from 4 to 12 volume exchanges per day, there was no significant difference in SGR at the two water exchange rates.

All three unshaded treatments showed a seasonal trend in SGR with highest growth occurring in summer (January – February 2001). Maximum SGR was higher in abalone (8 $\%.d^{-1}$) followed by the fertilized treatment (4.5 $\%.d^{-1}$) and then the seawater treatment (3.5 $\%.d^{-1}$), the significant difference between the treatments at this point being likely to be due to the difference in the water exchange rates (ANOVA; $n = 5$; $p < 0.5$;).

All three treatments were affected by a *Myrionema* infection in November 2001. The only treatment that survived the infection was the fertilized treatment, possibly indicating that fertilization can control *Myrionema* infections. The entire experiment was replaced in November 2001 due to the *Myrionema* infection.

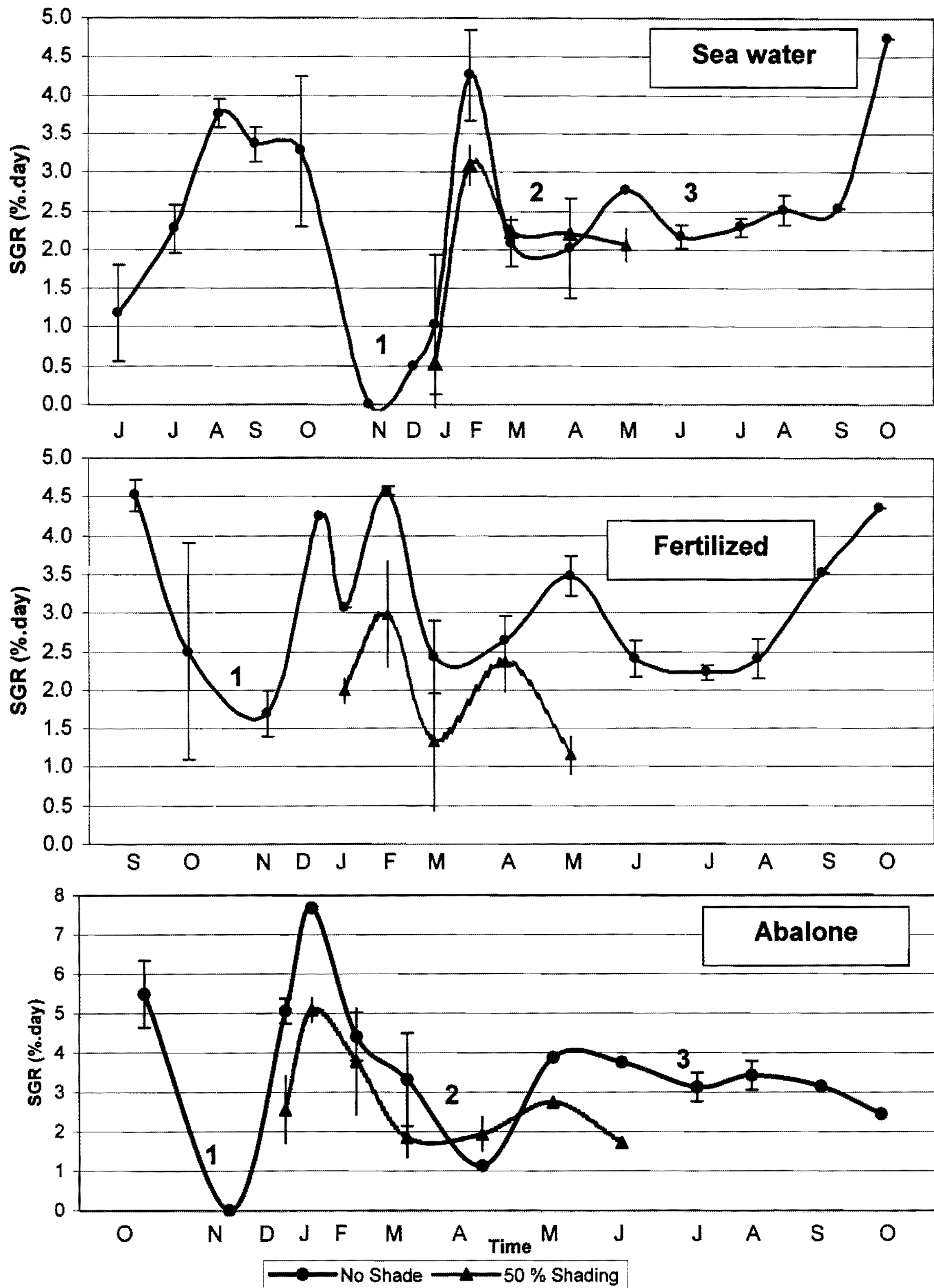


FIGURE 8.2: Seasonal SGR in the three treatments at I & J from June 2001 to November 2002, with standard deviations. The number 1 illustrates first bad *Myrionema* infection (greater than 50 % of thallus covered), 2 = second etc.

Points 1, 2 and 3 (Figure 8.2) show periods in the seasonal SGR of the abalone and seawater treatments, where SGR decreased due to *Myrionema* infections. At point 2, in the abalone and seawater treatment the shaded tanks had a higher SGR, thus indicating that shading could to some extent prevent the decrease in SGR caused by the *Myrionema* infection. The *Myrionema* infections affected the spring SGR of the abalone treatment depressing it to below levels obtained the previous spring. There were no significant differences in SGR between the fertilized and seawater treatments.

8.3.1.5 Yield at I & J

Yields showed a seasonal trend (Table 8.2), with highest yields occurring in summer in the abalone effluent and fertilized growth media tanks. Yields were lowest in the seawater growth medium.

Table 8.2: Average yields kg.wwt.$m^{-2}.d^{-1}$ at the I & J farm with standard deviation in brackets, obtained in each unshaded treatment for each season.

TREATMENT	SPRING	SUMMER	AUTUMN	WINTER
Seawater	0.1 (± 0.01)	0.05 (± 0.05)	0.07 (± 0.07)	0.06 (± 0.06)
Fertilized	0.16 (± 0.01)	0.26 (± 0.03)	0.12 (± 0.05)	0.08 (± 0.05)
Abalone	0.17 (± 0.05)	0.35 (± 0.01)	0.10 (± 0.05)	0.12 (± 0.05)

8.3.1.6. Shading at I & J

Shading on the whole was not beneficial to the treatments (Figure 8.2) and there was a significant difference between shaded and unshaded tanks in all treatments in March (ANOVA; $n = 11$; $p < 0.05$;). In the fertilized treatment, shaded tanks had significantly lower SGR for the majority of the experiment (ANOVA; $n = 4$; $p < 0.05$ for 3 out of 5 cases).

Shading did, however, significantly reduce tank epiphytes (ANOVA; $n = 11$; $p < 0.05$ in 4 out of 5 cases) and generally improved the condition of the *Ulva* thalli. Shading also slightly increased tissue nitrogen concentrations (Chapter 9) and

helped to control *Myrionema* outbreaks. As SGR did not improve with shading the 50 % shade cloth used in this experiment may have been too dark. Also shading was only applied after the November 2001 SGR crash and this means that it could have been applied too late. Shading should have been removed in February 2002 as SGR in the shaded tanks decreased after this point.

One untested effect of the shading is the effect due to the change in species dominance (Chapter 6). It is possible that *U. capensis* has a slower SGR than *U. lactuca*. This could also account for the difference in SGR between the shaded and unshaded treatments.

8.3.2 Epiphytes, endophytes and mesoherbivores

The density of tank fouling algae increased in early spring and peaked in the summer months at both farms (data not presented). Fouling was more prevalent in the unshaded effluent media treatments at I & J and JSP, and in the darker medium tanks at JSP. Summer density of tank fouling algae were significantly higher than in winter at both farms (ANOVA; $n = 20$; $p < 0.5$ in 4 out of 4 cases for JSP and ANOVA; $n = 12$; $p < 0.5$ in 5 out of 6 cases for I & J) (See Plate 8.1). There was also a significant difference between tank-fouling algal density in shaded and unshaded tanks (ANOVA; $n = 20$; $p < 0.5$ in 6 out of 9 cases for JSP and ANOVA; $n = 12$; $p < 0.5$ in 5 out of 6 cases for I & J) with the unshaded tanks having more fouling algal cover. There was no significant difference in tank fouling cover at JSP if the shade cloth used was 80 % or 20 %.

Epiphyte densities in the unshaded tanks in summer were significantly higher in the tanks using effluent media especially the turbot tanks at JSP and the abalone effluent and sea water tanks at I & J (ANOVA; $n = 20$; $p < 0.5$ in 9 out of 11 cases for JSP and ANOVA; $n = 12$; $p < 0.5$ in 3 out of 4 cases for I & J). Except for *Myrionema* there was no seasonality shown in any of the other epiphytic algae.

There was a wide variety of fauna and flora found associated with the tanks (Table 8.3). On the I & J farm, densities of the keyhole limpet (*Dendrofissurella scutellum*) on the tank walls and bottom, increased in early spring to summer.

Several limpets (*Scutellastra granularis*) were found on the tank walls and were grazers of diatomaceous algae and larger algae such as *Ulva Intestinalis* sp. The limpets cleared 25 cm^2 areas of tank walls of tank fouling algae (Plate 8.2). This could be a useful management tool for controlling tank-fouling algae and needs to be further investigated.

There were a variety of meso-herbivores (5 – 55 mm) in the tanks. Meso-herbivore densities were also higher in the effluent media tanks at both farms. The tanks at I & J also supported higher densities of meso-herbivores, possibly because they were larger and offered more refugia.

PLATE 8.1: Summer epiphyte density on tank walls at I & J.

TABLE 8.3: Fauna and flora found in the tanks at each farm.

Species		I & J	JSP
	FAUNA		
Black mussel	*Mytilus galloprovincialis*	yes	Yes
Horned Isopod	*Deto echinata*	yes	Yes
Reticulate kelp isopod	*Paridotea reticulata*	yes	Yes
	Paridotea ungulata	yes	
	Paridotea fucicola	yes	
Unidentified orange arthropod		Yes	Yes
Polychaeta/ Worms sp.		Yes	Yes
Mussel worm	*Pseudonereis variegata*	Yes	
Comb toothed nereid	*Platynereis dumerilii*	Yes	
Three-antennae worm	*Lysidice natalensis*	Yes	
Strawberry anemone	*Corynactis annulata*	Yes	Yes
Plum anemone	*Actinia equina*	Yes	Yes
Granular limpet	*Scutellastra granularis.*	Yes	Yes
Gastropods small black		Yes	Yes
Saddle-shaped key hole limpet	*Dendrofissurella scutellum*	Yes	Yes
Cape rock crab	*Plagusia chabrus*	Yes	Yes
Big eyed amphipod	*Paramoera capensis*	Yes	Yes
Golden sea cucumber	*Thyone aurea*	Yes	Yes
Orange Sponge sp		Yes	Yes
Hitchhiker amphipods	*Jassa falcata*	Yes	
Bearded limpet	*Scutellastra barbara*	Yes	
Goats eye limpet	*Cymbula oculus*	Yes	
Conical key hole limpet	*Diodora parviforata*	Yes	
Tulip chiton	*Chiton tulipa*	Yes	Yes
Dwarf chiton	*Ischnochiton oniscus*	Yes	Yes
Spiked Back isopod	*Parisocladus perforatus*	Yes	Yes
Button isopod	*Sphaeramene polytylotos*	Yes	Yes
Roll tailed isopod	*Dynamenella huttoni*	Yes	Yes
Pocket amphipod	*Amaryllis macrophthalma*	Yes	Yes
Ornate amphipod	*Cyproidea ornata*	Yes	Yes
Ridgeback amphipod	*Ochlesis lenticulosus*	Yes	Yes
Nesting amphipod	*Cymadusa filosa*	Yes	Yes
	FLORA		
	Cladophora capensis	Yes	Yes
	Codium stephensiae	Yes	Yes
Diatoms		Yes	Yes
	Ceramium diaphanum	Yes	Yes
	Gracilaria gracilis	Yes	Yes
	Ulva intestinalis	Yes	Yes
	Aeodes orbitosa	Yes	Yes
	Cladophora capensis	Yes	Yes
	Porphyra sp.	Yes	Yes
	Myrionema strangulans	Yes	Yes
	Colaconema nemalionalis	Yes	Yes
	Ectocarpus siliculosus	Yes	Yes
	Polysiphonia sp.	Yes	Yes
chain forming diatoms		Yes	Yes

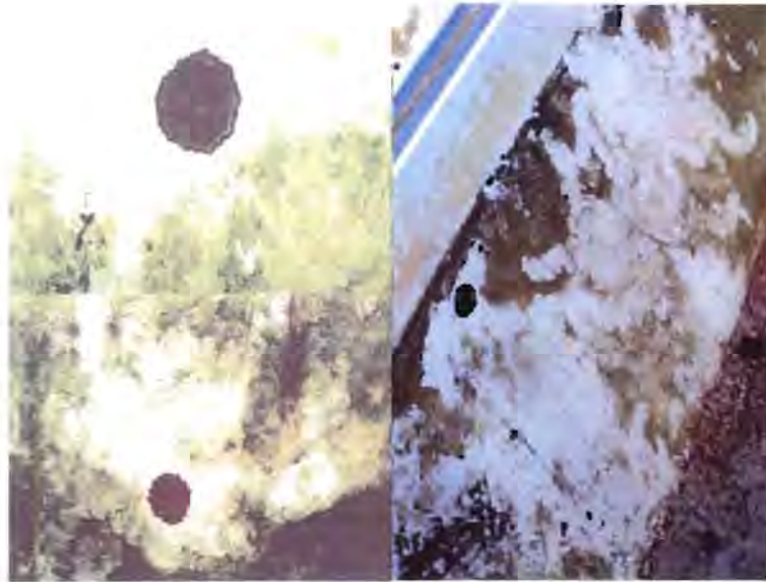


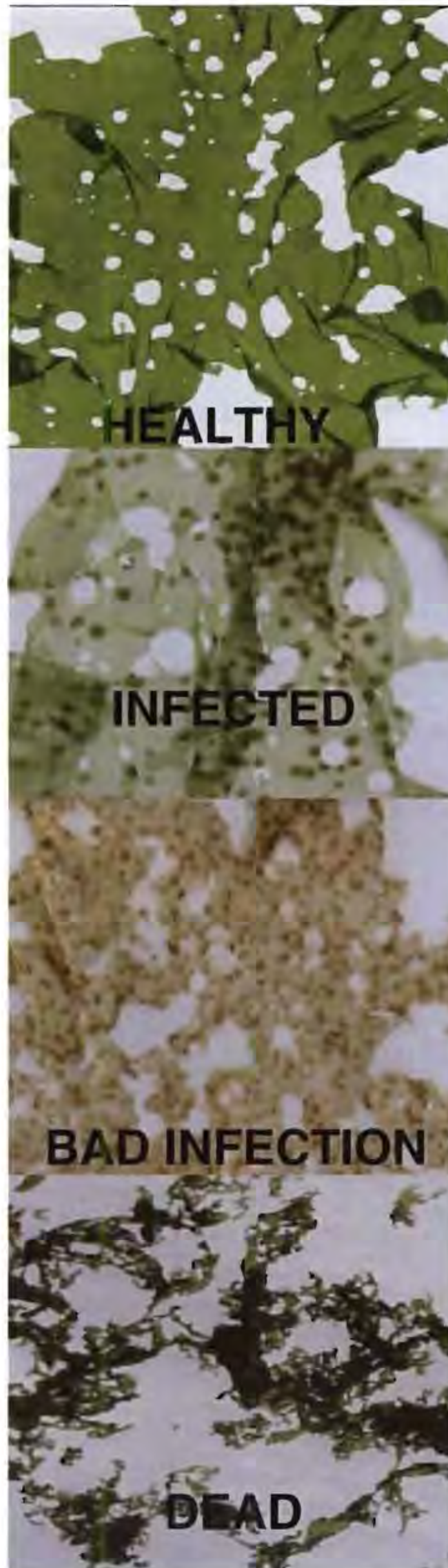
PLATE 8.2: *Scutellastra granularis* grazing, cleaning large areas of tank wall surface.

8.3.2.1 *Myrionema strangulans*

Myrionema. strangulans occurs in groups of between 5 and 10 spots on the holdfast section of *Ulva* thalli in the United Kingdom (Fletcher, 1987). It occurs naturally in wild populations of *U. lactuca*, in False Bay. Here it persists as brown spots, with up to 10 occurring on the holdfast section (pers. obs.). The spots are brown regular discs, 1 – 3 mm in diameter (Fletcher, 1987). It was first recorded as occurring at I & J in October 2001, when thalli in tanks were covered in brown spots. It was identified by Dr. Herre Stegenga, and is the first record of this species in South African waters.

M. strangulans appears to persist on *Ulva* throughout the year. There is a slight seasonal pattern in infection rates, in that infestations of *Ulva* in culture were first noted in late spring and by late summer culture populations were decimated. Infestation occurred throughout autumn, but the effects of the infection were not as severe and appeared worse in abalone culture effluent. Re-infection also occurred more frequently in abalone effluent culture medium (Figure 8.2).

4 stages of infection were observed:



Stage 1: Healthy

A "healthy" *Ulva* thallus, usually dark green in colour, with 5 – 10 brown spots on the holdfast region (slightly thicker part of the thallus with intertial rhizoids).

Stage 2: Infected

Typical effects on U*lva* thalli are that the spots increase in number and move over the entire thallus surface. The *Ulva* thallus becomes yellow in colour and becomes thinner.

Stage 3: Bad Infection

Spots increase in number and move over entire thallus and on both sides. Thallus becomes very light in colour and very fragile. Ice-Ice like symptoms appear.

Stage 4: Dead

Due to some unknown process (as *M. strangulans* is an epiphyte with no endophytic filaments (Fletcher, 1987) the thallus breaks up into pieces of between 1 – 3 cm diameter or smaller. Once this has happened the culture populations never recover.

Fertilization and shading appeared to control outbreaks in the fertilized treatment. Although *Myrionema* was present on *Ulva* thalli at JSP, there were no outbreaks and this may be attributed to the higher water exchange rate on this farm.

Marine Growers in Port Elizabeth have also had outbreaks of *M. strangulans*. W. De Wet (seaweed manager at Marine Growers) is of the opinion that the *Myrionema* alga shades the *Ulva* thallus in the area that it grows on. This then leads to a weakening of the *Ulva* thallus and a pinprick hole is formed. This perforation in the thallus could be an entry point for a secondary infection. If there are large amounts of *M. strangulans* spots on the thallus the holes eventually join and the thallus disintegrates. Usually this infection only occurs when the algae are stressed and thallus lightening has begun.

Infestation is particularly prevalent in culture tanks that are carbon limited due to low water exchange rates or in tanks that have a high pH. Pulse fertilization and improved water exchange rates (12 – 20 volume exchanges per day) appear to control or minimize infections. However repeat infection occurs in tanks that are receiving recirculated water.

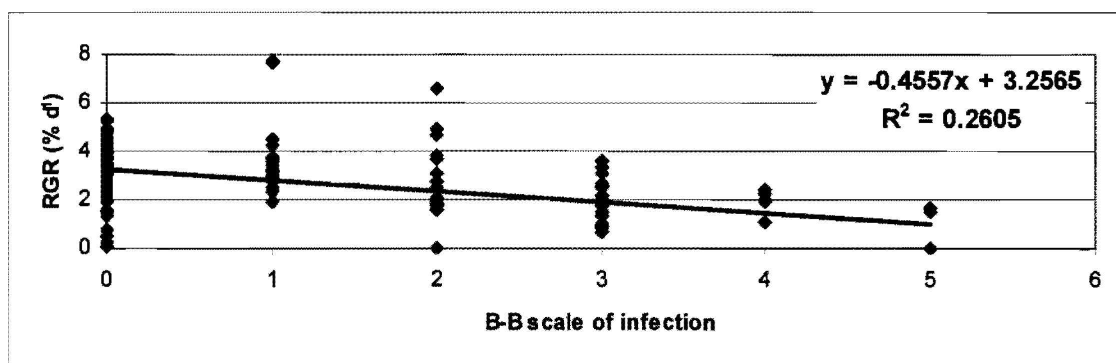


FIGURE 8.3: Relationship between SGR of *Ulva* at I & J and B – B scale of *M. strangulans* infection. Trend line equation and r^2 value are indicated.

There is a significant negative correlation between SGR and B – B scale of infection (Figure 8.3) (R value = 0.510, $P < 0.0005$). This means that severe infections lower the SGR of *Ulva*.

As this is a new record in South Africa, identification is the first step in prevention of infections. *M strangulans* disc colour can vary (according to other descriptions) from light greenish brown to medium brown (Fletcher, 1987; Womersley, 1987). The author has only observed dark brown spots. It is discoid in shape and is epiphytic on *Ulva,* according to international literature, including species previously *Enteromorpha* (Fletcher, 1987; Womersley, 1987). It consists of a monostromatic basal layer with adjacent subdichotomous radiating filaments. The older cells have short attachment pegs that adhere to the host surface (Fletcher, 1987; Womersley, 1987). The marginal cells are more elongate and the inner cells are between 8 – 14 µm long, 5 – 8 µm broad and 4 – 8 µm high (Fletcher, 1987; Womersley, 1987). Erect assimilatory filaments form a shallow, even dome (Fletcher, 1987; Womersley, 1987). It is listed as being widespread in temperate seas.

8.4 DISCUSSION

SGR shows a seasonal trend and is highest in summer. Maximum SGR at I & J was lower than at JSP (12 %.d^{-1} in small seawater at JSP vs 8 %.d^{-1} in abalone effluent at I & J) although this difference can be attributed to differences in tank sizes and flow rates. SGR observed in this study were lower than those obtained in the literature, but in most cases, smaller tank sizes were used and this study has shown that there is a decrease in SGR when scaling up tank sizes (Figure 8.1). Growth rates for *U. fenestrata* Postels & Ruprecht under experimental conditions were 16 % wwt.day^{-1} (Björnsäter & Wheeler, 1990), this is lower than those obtained for *U. curvata*, 52 % wwt.day^{-1} which was a laboratory short term study (Duke *et al.* 1989); *U. lactuca*, 18.6 % wwt.day^{-1} (Neori *et al.* 1991) and *U. fasciata*, 36 % wwt.day^{-1} (Lapointe & Tenore, 1981). The low growth rate for *U. fenestrata* may be attributed to the lower water temperature used (13 °C) compared to other experiments (Björnsäter & Wheeler, 1990). The SGR obtained by Neori *et al.* (1991) are also low and could also be due to the scaling up of the tanks sizes as the other studies were in experimental small scale setups. It is possible to increase SGR by using a raceway system (Mata & Santos, 2003). This type of system may be better for intensive culture and for recirculation purposes as water residence time is increased and thus nutrient removal efficiency could be increased (Mata & Santos, 2003).

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Water exchange rates of 4 volume exchanges a day are too low for the cultivation of *Ulva* as seen by the low growth rates and low tissue nitrogen content (Chapter 9). Water exchange rates of 12 volume exchanges per day are adequate for the cultivation of *Ulva*, while rates of 20 are good. A possible disadvantage of 20 volume exchange rates per day (such as those at JSP) is that uptake efficiency is decreased, and that SGR is compromised as there was no significant difference in SGR between the turbot and seawater treatments at JSP. However K. Ruck commented that the stocking density of turbot on JSP is lower than used in farms elsewhere, including the stocking density used by Neori *et al.* (1991). Thus the nutrient flux at JSP is likely to be lower, leading to a lower SGR than Neori *et al.* (1991) obtained.

The yields obtained from the quasi-commercial scale study at I & J were low (compared to JSP and the literature), with a maximum summer monthly biomass of 291 kg.wwt and maximum winter monthly biomass of 135 kg.wwt. This may be due to recurring *M. strangulans* infestation and the low water exchange rates at the I & J farm.
The higher summer biomass at JSP (111 kg.wwt summer and 51 kg.wwt in winter from a smaller surface area) from both the seawater and turbot treatments may be a function of both the higher water exchange rates and the higher nutrient concentrations in the turbot effluent tanks.

The decrease in SGR between the small and medium tanks at JSP could be due to either the scaling up of tank sizes or the slightly darker colour of the medium tanks.

There was no significant difference in SGR at JSP between the small seawater, turbot or abalone effluent treatments, despite the fact that both the turbot and abalone effluent culture media had higher nutrient concentrations, while at I & J there were significant differences in SGR between the different treatments. This indicates that at high water exchange rates (20 volume exchanges.d^{-1}), excess nutrients in the water have no benefit on SGR, while at lower water exchange rates (4 and 12 volume exchanges.d^{-1}) excess nutrients in the culture medium can have significant effects on SGR.

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Non-infectious diseases have been overlooked in discussions on algal pathology. A very common symptom reported from algae maintained at high densities, under laboratory conditions and in culture systems, is bleaching accompanied by fragmentation of the thallus. Generally known as Ice-Ice, the etiology was undetermined until recent experiments indicated that the symptoms were caused by strong oxidant substances, produced by the alga itself in response to physiological stress (Correa, 1996). Symptoms like Ice-Ice occurred at stage 3 infection of *M. strangulans*, suggesting that a secondary infection was occurring after the *M. strangulans* outbreak. As SGR is negatively influenced by increasing density of *M. strangulans* infestations it is important that cultivators recognize the alga and monitor infection density. The

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Smit *et al.* 2003), but if crop production is low or the density of grazers is high the effect of their grazing can be substantial.

The herbivorous isopod *Paridotea reticulata* can establish itself in *Gracilaria* cultures (± 616 isopods per 100 g.wwt of seaweed) and can reach numbers of up to 3000 individuals per 100 g.wwt. Each isopod can consume about 0.05 gDW of *Gracilaria* per day, at these densities it is possible that 7.8 % of the total seaweed culture can be consumed every day (Anderson *et al.* 1998 and Smit *et al* 2003). Anderson *et al.* (1998) showed that meso-herbivore abundance was correlated to seasons of high epiphyte biomass, and in our study the increase in the occurrence of the keyhole limpets in summer when tank epiphyte density was greatest is in agreement with this finding. Migration of meso-herbivours to new areas when food becomes limiting is not an option in tank cultivation and thus the beneficial relationship that exists between the grazer, the host alga and the epiphyte would no longer occur. In addition, higher water temperatures in the tanks would improve breeding and feeding rates of the meso-herbivores and reduce mortality rates (due to the absence of predators). These factors could lead to an outbreak of meso-herbivores that would quickly decimate the cultured alga.

Although meso-herbivores could theoretically be used to control epiphytes in tank systems, management of such a system is difficult. Thus for practical purposes meso-herbivores must be considered a pest in tank cultivation of seaweeds, and management of grazer levels in tanks is critical, even though low grazer densities could be beneficial to *Ulva* cultivation. Options for control include arthropod poison and predators such as *Clinus* sp., but both of these options have their drawbacks (Smit *et al.* 2003). A much simpler method of meso-herbivore and epiphyte control (Smit & Bolton, 1999) is to treat the seaweed in a fresh water bath. Smit *et al.* (2003) suggest that a fresh water treatment of 4 minutes can remove 50 % of the isopods. This method was used in this study and was relatively successful. For large harvests, however it is necessary to replace the water often as repeated washing in the same water resulted in a brine which was not as effective (Smit *et al.* 2003 and this study).

The possible use of limpet grazers to control tank fouling algae needs to be looked into. If limpets can be used to control tank-fouling algae, then costs

incurred from cleaning the tanks in terms of manpower and chemicals used can be reduced. Questions that need to be answered include:

- What density of limpets can the tanks support?
- How big is the area which the limpets graze?
- Are they dietary specialists i.e. eat only one type of fouling algae or would they consume a range?
- Do different year classes of limpets have different diets?

The effects of summer outbreaks of keyhole limpets need to be investigated to see if they consume significant amounts of viable algal material or if they are only consuming the dead and rotting algal material on the bottom of the tanks and, if so, do they have an important bio-filtering role?.

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8.5 CONCLUSIONS

These results confirm the exceptional suitability of *Ulva* for intensive culture in different types of nutrient loaded water as already shown by a variety of authors (Guist and Humm 1976; Langton *et al.* 1977 and Neori *et al.*, 1991).

In order to prevent *M. strangulans* infections the tanks need to be fertilized regularly. When *M. strangulans* outbreaks occur, tanks should be dosed with a second fertilizer treatment, as the fertilization treatment at I & J was the only treatment that survived the November infestation (Figure 8.2). Or ideally high flow rates as occurred at JSP.

Tanks should also be shaded with a 20 % shade cloth from late September to early February to reduce the chance of *M. strangulans* outbreaks occurring (this Chapter) and to reduce epiphyte loading (this Chapter) and improve thalli condition and tissue nitrogen content (Chapter 9).

There is a decrease in SGR when scaling up tank sizes, but this decrease can be optimized by cultivating the alga in pulse fertilized effluent water (Chapter 11).

Water exchange rates are important in assuring optimum nutrient supply. An exchange rate of 12 is adequate, a rate of 4 such as we had at I & J is too low and when using 20 at JSP, no difference in SGR was experienced between the Turbot and seawater treatments even though there was a large difference in nutrient concentrations (Chapter 6).

The role of meso-herbivores needs to be established.

CHAPTER 9

COMPARISONS OF THE SEASONAL NUTRITIONAL COMPOSITION OF *ULVA* GROWN IN DIFFERENT TREATMENTS

9.1 INTRODUCTION

One of the main aims of this project was to assess the suitability of *Ulva* as a food source for the abalone. For this reason it is important to determine the nutritional content of the cultivated seaweeds. Since seasonality has been reported in algal biochemistry for *U. lactuca*, *U. rigida* and a number of other macro-algal species (Fillit, 1995; Flores-Moya *et al.* 1995; Sfriso, 1995), we need to compare the nitrogen content of samples taken from all the harvests in each of the treatments over the entire period of the experiment.

Proteins are nutritional components of particular importance to abalone. Although the seaweeds consumed by abalone generally contain less than 20 % protein, (Nisizawa *et al.* 1987) abalone have been shown to be capable of digesting high levels of dietary protein (20 – 50 %) in concentrated form (Hahn, 1989; Uki & Watanabe, 1992; Britz, 1995 and Mai *et al.* 1995a,b). *H. discus hannai* attains maximum weight gain when fed a diet with 23.3 – 35.6 % protein while *H. tuberculata* has its maximum weight gain at 22.3 – 32.2 % (Hahn, 1989; Britz, 1995 and Mai *et al.* 1995a,b).

Size class also affects the protein requirements in *H. midae* where 44 % protein level in the diet resulted in maximum growth in the larger abalone, while the smaller abalone showed the best growth at 34 % protein (Britz & Hecht, 1997; Shipton, 1999). Both studies indicated that a lipid content of 12 % and higher produced lower growth rates than diets with only 6 and 10 % lipid content, especially in the smaller size classes.

With this in mind, it was particularly important to consider the protein content of the *Ulva* species cultivated at on the farms. Nitrogen and phosphorus content were determined for comparative purposes. Nitrogen content was specifically measured in order to find out whether the algae were obtaining sufficient nitrogen from the seawater or increasing N content in more concentrated media i.e. effluent water and fertilizer treatments.

9.2 MATERIALS AND METHODS

The same samples that were taken to record dry to wet weight ratios and for biochemical analysis were used. The method of collection and treatment of the samples is the same as described in Chapter 4, Sections 4.6 and 4.7.

9.2.1 Statistics

one way ANOVA's using STATISTICA v6 were used to determine significant differences SGR between the different treatments. PRIMER v5 was used to run an ANOSIM between tissue nitrogen and SGR.

9.3 RESULTS

9.3.1 Nitrogen values at JSP

In June and November 2001, the high water exchange rate at JSP resulted in the tissue nitrogen values of the cultivated stock being higher than the wild stock (Figure 9.1). The plants treated with turbot effluent had a significantly higher tissue nitrogen than those from any other treatments from November 2001 to March 2002 (ANOVA, $n = 19$, $p < 0.05$). Generally, the plants in the small seawater tanks had a lower tissue nitrogen than those in the the medium tanks. In general, there was a slight increasing trend in tissue nitrogen with time for all the treatments. Shading in the treatments had no significant effect on tissue nitrogen. Shaded tanks had elevated tissue nitrogen values, but this difference was not significant.

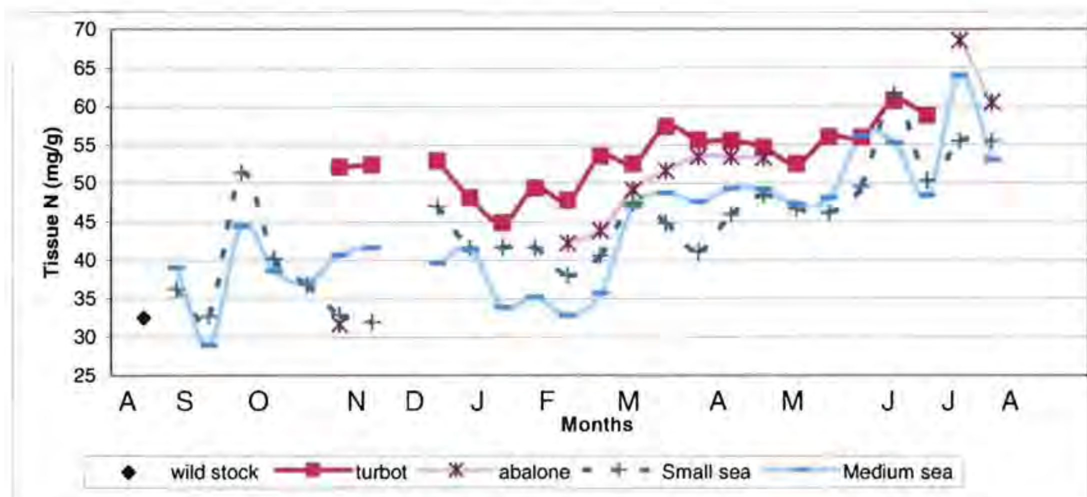


FIGURE 9.1: Tissue nitrogen content, from August 2001 to August 2002 for the seawater, turbot and abalone treatments at JSP ($n = 3$).

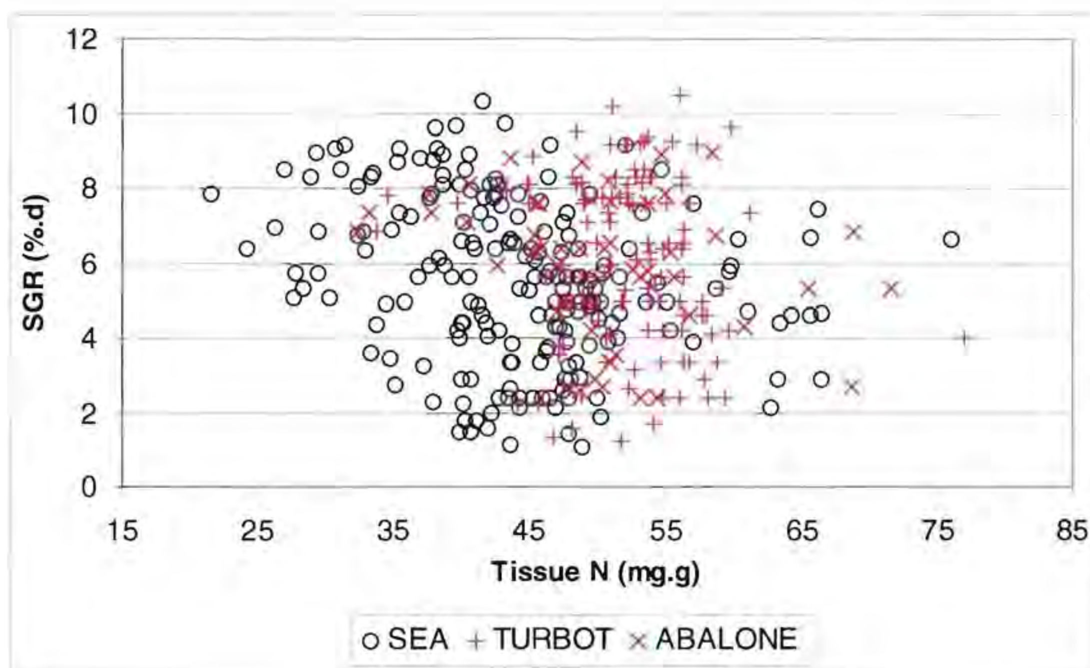


FIGURE 9.2: Relationship between tissue nitrogen and SGR, from August 2001 to August 2002 for the seawater, turbot and abalone treatments at JSP (n = 3).

The biggest difference between Figure 9.2 (which shows the relationship between tissue nitrogen and SGR in the three treatments for the entire study period at JSP) and Figure 9.5 is that the tissue nitrogen and SGR are higher at JSP (tissue N = 47.3 $\pm$ 8.6; SGR = 6 $\pm$ 4) than at I & J (tissue N = 26.6 $\pm$ 15.4; SGR = 3 $\pm$ 2.5).

A relationship exists between SGR and tissue nitrogen content (Figure 9.2) where plants in the seawater treatment forms 3 separate groupings that are significantly different from one another (ANOSIM, 1-way; < 25 mg N, R = 0.909; p = 0.01; 25 – 50 mg N, R = 1.000; p = 0.02; > 50 mg N; R = 0.514, p = 0.01 respectively), with the separation between groups 1and 2, occurring at the same point as I & J (Figure 9.6) (around 25 – 35 mg N per gram) and group 3 at a higher level of tissue nitrogen (> 50 mg N per gram). The plants in the abalone effluent treatment formed the same three groups as those in the seawater (ANOSIM, 1-way; < 25 mg N, R = 0.789; p = 0.01; 25 – 50 mg N, R = 0.93; p = 0.02; > 50 mg N; R = 0.324, p = 0.01 respectively). The turbot treatments formed two groupings with the separation the same as in I & J

(ANOSIM, 1-way; 25 – 50 mg N, R = 0.623; p = 0.01; > 50 mg N; R = 0.772; p = 0.01). The groupings for plants in the turbot effluent treatment indicate that there are three separate groups of SGR and tissue N. The switch over to the third group occurs around 50 – 55 mg N per gram in the tissue.

If outliers are removed from the data set using 95 % confidence limits a straight line regression can be placed through the data. For the plants in the turbot effluent treatment the straight line value of x = 55 $\pm$ 5 mg N per g. tissue. This indicates that the plants in the turbot effluent treatment had an average tissue nitrogen content of 55 mg per g. This translates to a protein content of 36.6 % (Chapter 4, Section 4.7.5). Including outliers the regression line through the plants in the turbot effluent is y = - 0.101x + 11.355, (r = 0.186; n = 144; p = 0.025), indicating a slightly nitrogen-limited growth (Duke *et al.* 1986a,b; 1989). By removing outliers plants in the abalone and seawater treatments, plants in the seawater treatment were estimated to have an average tissue nitrogen of 45 $\pm$ 7 mg N per g (protein content of 30.05 %) and plants in the abalone effleutn treatment hasdan average tissue nitrogen of 50 $\pm$ 7 mg N per g (protein content of 33.35 %). This indicates that the higher the nutrient concentration there is in the water (From Chapter 6) the more tissue nitrogen the *Ulva* is able to accumulate.

The seawater data were separated into seasons and using Duke *et al.* (1986a,b; 1989) theory of the relationship between growth rate and tissue nitrogen (i.e. relationship between SGR and tissue nitrogen is an increasing exponential trend which then levels off, and the influence of temperature and N limitation on these two variables), regression lines were drawn through data grouped into seasons. From August to November, the seaweed has temperature-limited growth, from October to January, there is severe nitrogen limited growth, from February to May, there is slight nitrogen limited growth and from June to August there is temperature limited growth. This indicates that although there is no obvious seasonal trend in tissue nitrogen in Figure 9.1, the decrease in tissue nitrogen from October to January, followed by a slight rise from February to May is a seasonal trend of tissue nitrogen limited growth, followed by nitrogen sufficiency. The reason that the trend is difficult to interpret

is that ideally the study needed to run over 18 months, as there could be a lag phase between seasonal switches from one year to the next. This seasonal increase in tissue nitrogen content is linked to increases in nitrogen in the incoming seawater and is supported by data from Hutchings & Andrews (1980); Chapman and Shannon (1985) and Mitchell-Innes & Walker (1991).

There was no significant difference in shading in any of the treatments with either a 20 % or an 80 % shade cloth.

9.3.2 Phosphate values at JSP

Tissue phosphate levels at JSP remained low (Figure 9.3) for the majority of the year and only increased in early winter, thus indicating a slight seasonal trend in tissue phosphate with an increase in incoming water in early autumn. This trend was also shown at I & J (Figure 9.6), but in late autumn/early winter. There was no significant difference in tissue P in the plant in all the treatments. There was also no significant difference in shading either at 80 % or 20 % shade cloth.

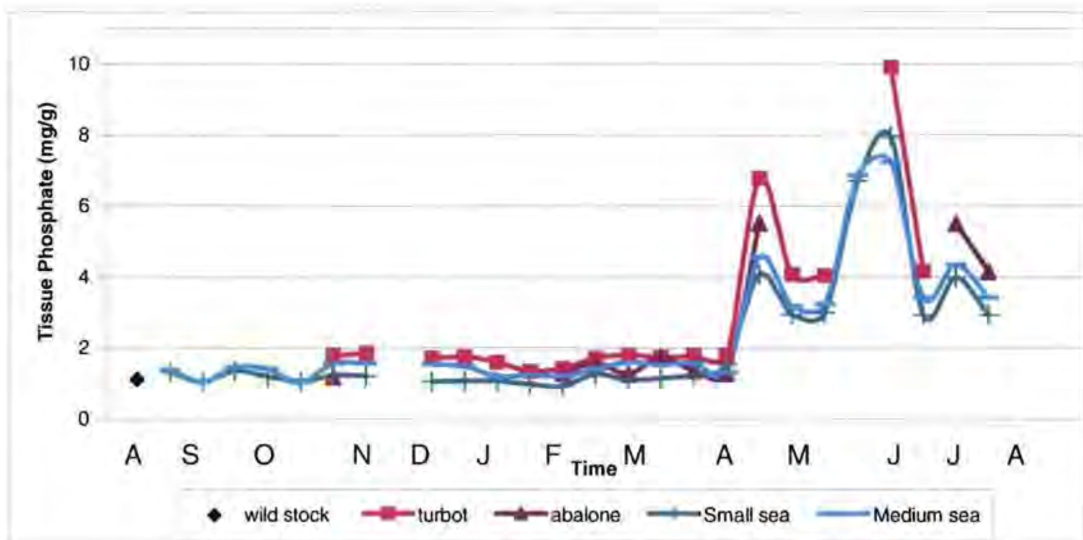


FIGURE 9.3: Levels of tissue P, from August 2001 to August 2002 for the seawater, turbot and abalone treatments at JSP (n = 3).

9.3.3 Nitrogen values at I & J

From July 2001 to November 2002, when there was a *M. strangulans* infestation, tissue nitrogen decreased in all treatments (Figure 9.4) as well as in the abalone effluent water treatment in April and the abalone and seawater treatments in July 2002. There was a significant correlation between the *M strangulans* infection density greater than 2 and tissue nitrogen ($y = -15.728x + 81.829$, $r^2 = 0.6714$, $n = 41$; $p < 0.005$). The correlation was not good at high infection densities (i.e. B-B scale 5) and this is probably due to the *M. strangulans* contributing towards tissue nitrogen.

Water volume exchange rates have an influence on tissue nitrogen in that in December, the abalone treatment had a significantly higher tissue nitrogen than the other treatments (ANOVA, $n = 5$, $p < 0.05$). In February when the water volume exchange rates were increased in the fertilized and seawater treatments, tissue nitrogen also increased. However because tissue nitrogen increased in the abalone treatment as well, there must be another reason for the increase.

Towards the end of the measurement period tissue nitrogen from plants in the fertilized treatment was significantly higher than those from the abalone and seawater treatments, thus indicating that fertilization can increase tissue nitrogen in winter (ANOVA, $n = 11$, $p < 0.05$;). There were no significant differences in tissue nitrogen (mg/g) in the shaded and unshaded tanks for all treatments.

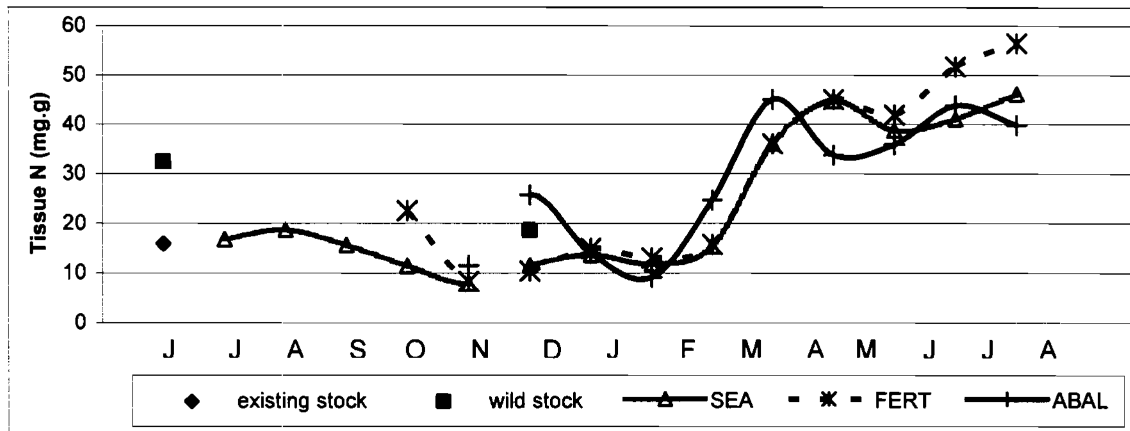


FIGURE 9.4: Tissue nitrogen, from June 2001 to August 2002 for the seawater, fertilized and abalone treatments at I & J (n = 3).

The plants in the seawater, abalone and fertilized treatments at I & J separate and form two distinct groups that are significantly different from one another in their tissue nitrogen concentration (ANOSIM, 1-way; $R = 0.938$; $p < 0.01$ for seawater; ANOSIM, 1-way; $R = 0.772$; $p < 0.01$ for abalone and $R = 0.949$; $p < 0.01$ for fertilized) (Figure 9.5). This grouping separates out roughly into the treatments that received 4 volume exchanges per day on the left (Group 1) and 12 volume exchanges per day on the right (Group 2) with the plants in the abalone treatment separating out into the same groups but having tissue nitrogen values from spring and summer in group 1 and autumn and winter tissue nitrogen values in group 2.

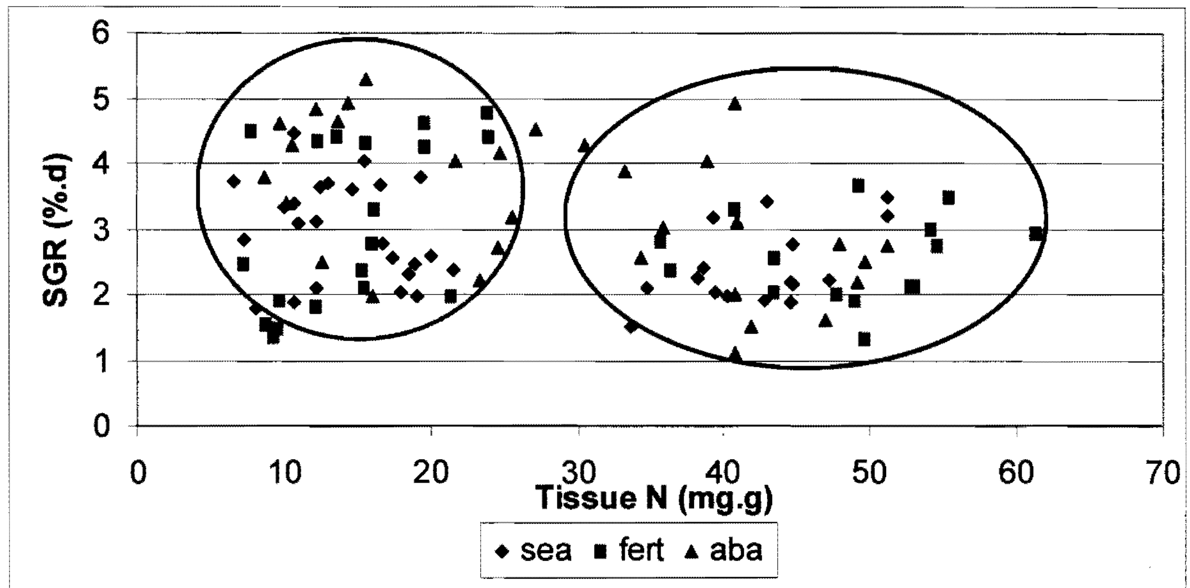
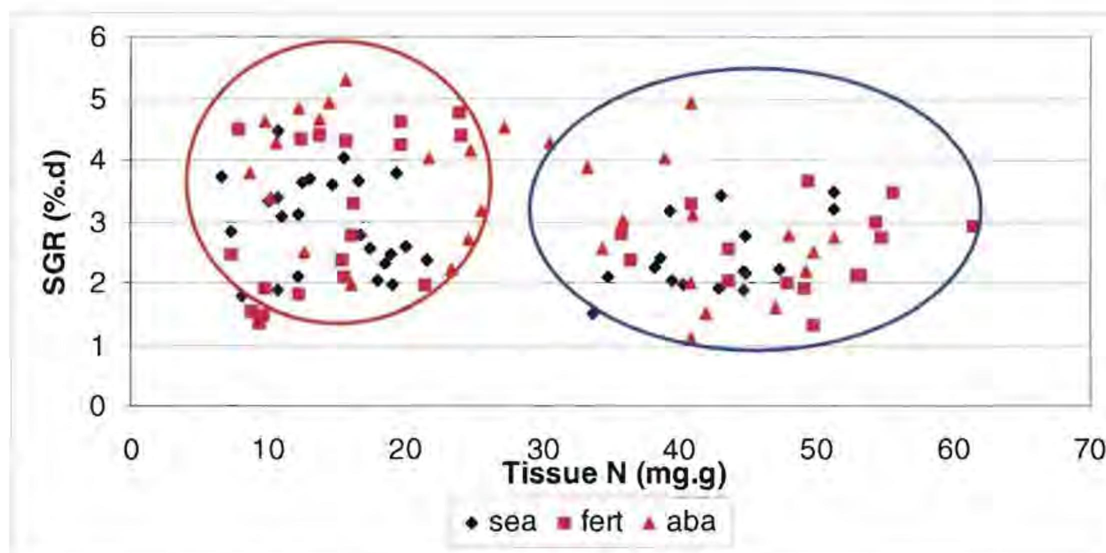


FIGURE 9.5: Relationship between tissue nitrogen and SGR, from June 2001 to August 2002 for the seawater, fertilized and abalone treatments at I & J. The circle on the left denotes Phase 1, while the circle on the right denotes Phase 2 as well as ANOSIM significance groupings (See text) (n = 3).

9.3.4 Phosphate values at I & J

Only tissue P values for plants from the unshaded treatments at I & J are shown (Figure 9.6), there was no significant difference in tissue phosphate between any of the treatments. In November when the tanks were infested with *M. strangulans* and the culture was replaced, tissue phosphate in the wild stock was very high and was significantly different from any other measurement (ANOVA, n = 11, $p < 0.05$) and may be due to the fact that the replacement algae was obtained from a harbor.

From April to May after the water flow rates were increased from 4 to 12 volume exchanges per day the tissue P decreased. However, as it decreased in the plants in the abalone treatment, which was running on 12 volume exchanges for the duration of the experiment, this decrease in tissue phosphate was related to both water exchange rates being changed and a seasonal effect. Unfortunately as with tissue nitrogen the period over which this was observed is too short to get an accurate idea of seasonal tissue phosphate levels. Tissue



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phosphate between plants in shaded and unshaded treatments was not significantly different in any of the treatments.

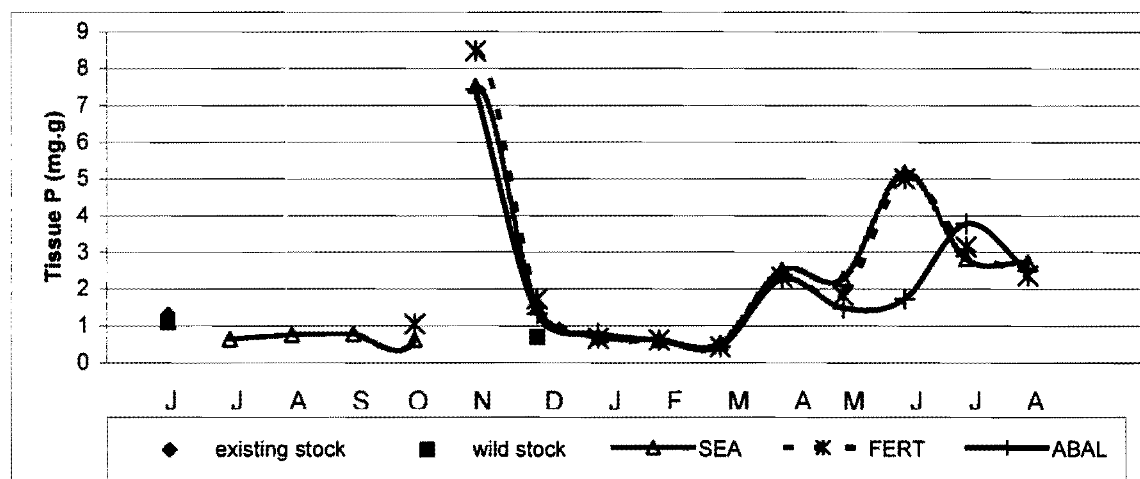

FIGURE 9.6: Ratio of tissue P, from June 2001 to August 2002 for the unshaded seawater, fertilized and abalone treatments at I & J.

9.3.5 Nitrogen to phosphate ratios

Work done by Björnsäter & Wheeler, (1990), showed that there is a relationship which exists between tissue N and P for *Ulva* in the North East Pacific. This relationship was used to determine N or P limitation in our cultivated seaweeds. Figure 9.7 shows that in late winter (July 2001) and early spring 2001 (September 2001) at I & J, the plants in the treatments were nutrient sufficient. When there was a large *M. strangulans* infestation all plants became nitrogen limited. The algae that were used to replace the experiment were also nitrogen limited. When the water exchange rates were increased in February 2002, in the fertilized and seawater treatments, the algae became phosphate limited at the higher water exchange rate, however plants in the abalone treatment which was always running at a higher water exchange rate showed more phosphate limitation than the other treatments, thus there must be a background phosphate limitation around this period. Tissue nitrogen remained high and phosphate values gradually increased due to the seasonal increase in autumn (March – April 2002) (Hutchings & Andrews, 1980; Chapman and Shannon,

1985 and Mitchell-Innes & Walker, 1991) causing the alga to be nutrient sufficient in winter (June 2002).

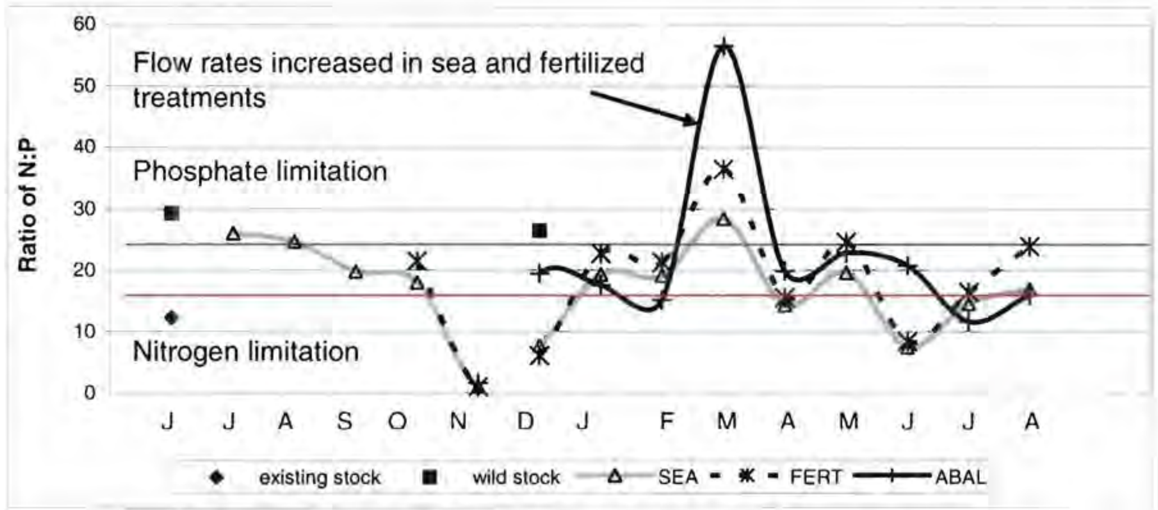

FIGURE 9.7: Ratio of tissue N to P at I & J, from June 2001 to August 2002 for the seawater, fertilized and abalone treatments. Area above top line indicates phosphate limitation, while area below bottom line indicates nitrogen limitation and area between lines indicates nutrient sufficiency according to Björnsäter & Wheeler, 1990. Standard deviations are not shown for increased clarity but were all less than 0.45 (n = 3).

Until early winter (March 2002), all treatments for JSP were phosphate deficient (Figure 9.8), after which there was a sudden switch from being phosphate deficient to nitrogen deficient. This graph shows quite clearly that at high water exchange rates and corresponding high SGR there was a phosphate deficiency being experienced by the algae.

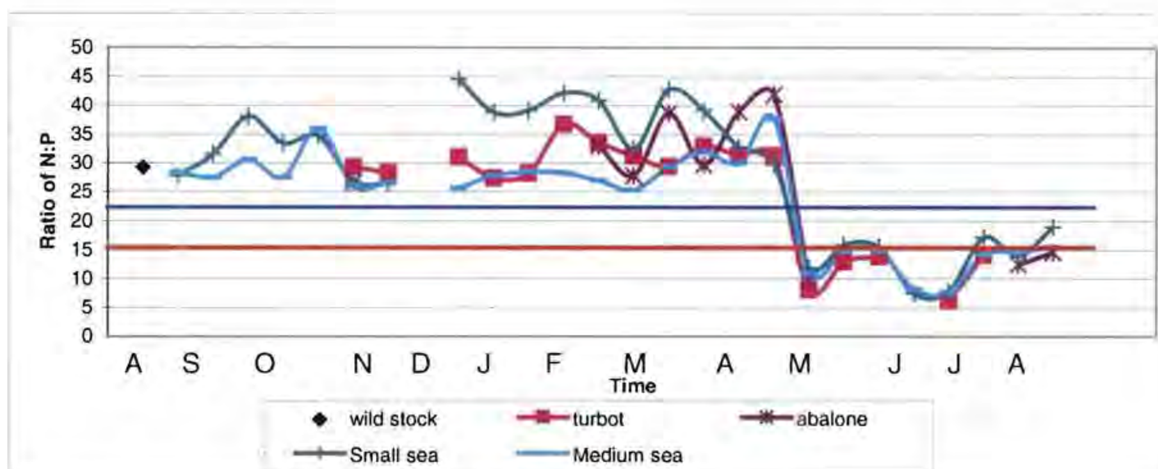

FIGURE 9.8: Ratio of tissue N to P at JSP, for the study period over which all treatments were running. Area above blue line indicates P limitation, while area below red line indicates N limitation and area between lines indicates nutrient sufficiency according to Björnsäter & Wheeler, 1990.

9.3.6 Nitrogen and thallus colour

Figure 9.5 shows a clear transition in tissue nitrogen values between two groups 1 and 2. Accompanying this transition was a change in tissue colour. In Figure 9.9 tissue nitrogen is plotted against tissue colour from all farms and in all treatments. Although the colour reproduction is not perfect, the broad relationship between thallus colour and nitrogen content is clear to see, with darker colour indicating more nitrogen rich material than paler colours (Figure 9.9). The transition between green-yellows and green appears to occur between 25 – 35 mg N per g tissue and is indicated by bars labeled with Pantone ® matt colours 585u and 583u. Laboratory experimentation would be useful to check the validity of these results.

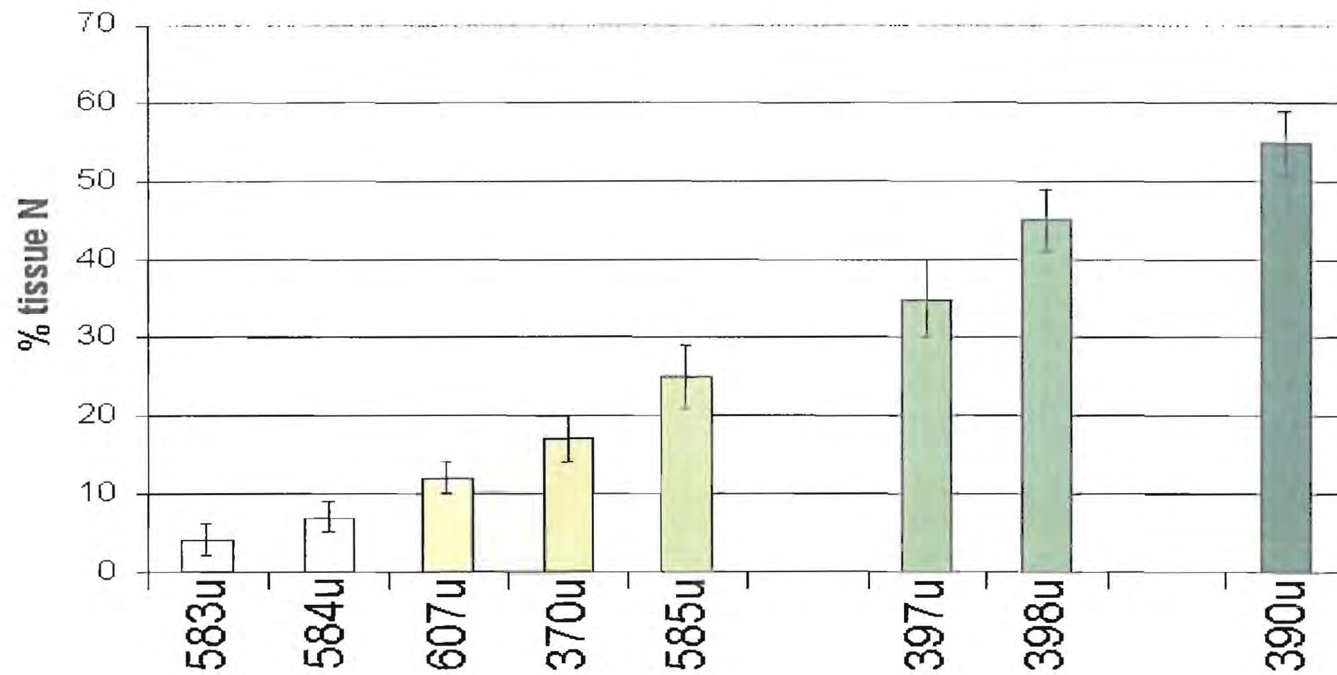

FIGURE 9.9: Relationship between tissue nitrogen and thallus colour (shown by Pantone ® matt colour labels).

9.3.7. Water content at JSP

There was no seasonal difference in tissue water content at JSP (Figure 9.10). There was also no significant difference between tissue water in shaded and unshaded treatments or between treatments although the effluent media treatments had a lower average tissue moisture. Average tissue moisture contents for the treatments were as follows: small seawater (86.32 % $\pm$ 1.97); medium seawater (86.29 % $\pm$ 1.74); turbot (84.42 % $\pm$ 2.09) and abalone effluent (84.99 % $\pm$ 2.60).

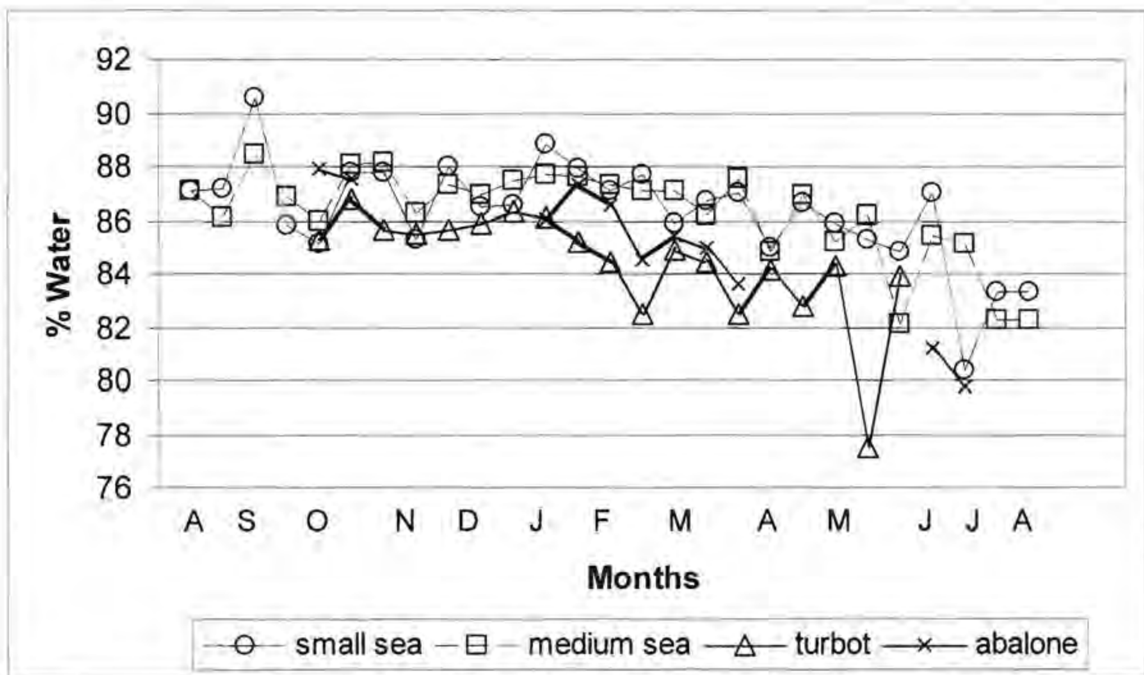

FIGURE 9.10: Percentage tissue water content at JSP from August 2001 to August 2002 in the small seawater, medium seawater, turbot and abalone effluent unshaded treatments.

9.3.8 Water content at I & J

There seems to be a seasonal trend in tissue water content in all treatments at I & J, with high water content from October 2001 to February 2002 (late spring to late summer) and decreasing in March 2002 to August 2003 (autumn to winter) (Figure 9.11). There was no significant difference between tissue moisture in

shaded and unshaded treatments or between any of the treatments. In March 2002, the decrease in tissue moisture does not seem to be linked to an increase in flow rates as the abalone treatment decrease proportionality. Average tissue moisture contents for the treatments were as follows: seawater (86.48 % $\pm$ 2.98); Fertilized seawater (86.33 % $\pm$ 2.71) and abalone effluent (86.48 % $\pm$ 1.99).

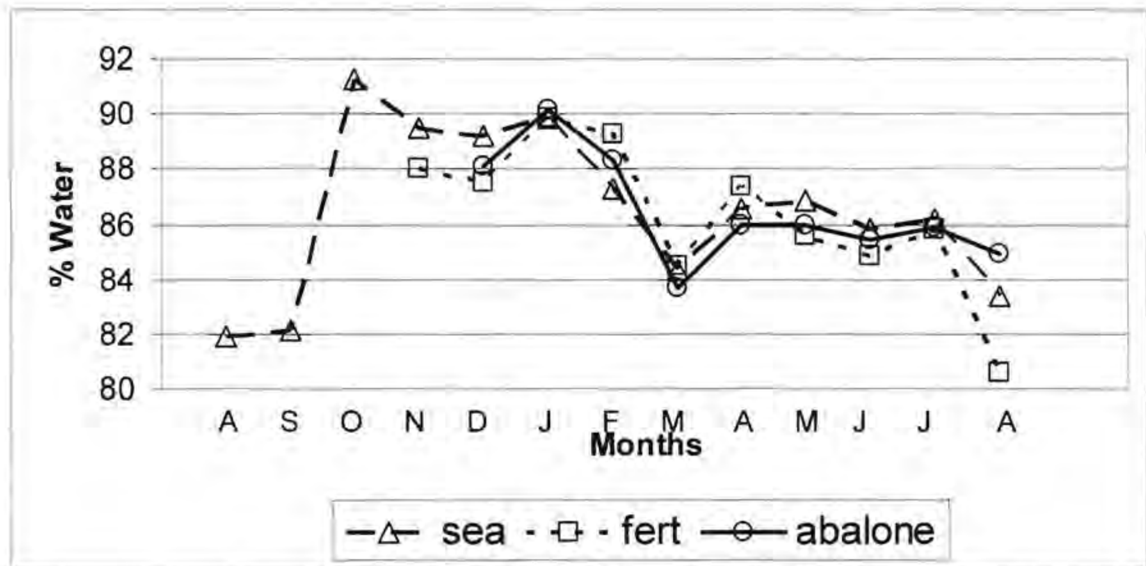

FIGURE 9.11: Percentage tissue water content at I & J from August 2001 to August 2002 in seawater, fertilized and abalone effluent unshaded treatments.

9.4 DISCUSSION

Our results suggest that there are three groups of tissue nitrogen levels associated with high SGR, low tissue N, medium SGR and medium tissue N and low SGR and high tissue N, with distinct switch over points between these states at 25 – 35 mg N and > 50 mg N respectively. The switch over between the states is also accompanied by a physical change in thallus colour. This implies that the change between the states is a physiological one. This has important implications for aquaculture, as this illustrates that there can be low tissue nitrogen values associated with high SGR, thus although yields may be good, nutritive value will be low. As the abalone treatment also shows a significant difference between the groups containing a low tissue nitrogen and a high tissue nitrogen, this illustrates that even though the water exchange rates were high, this is not related to water exchange but nitrogen limitation.
The data agreed with previous reports (see Rosenberg & Chapman, 1984; Duke *et al.* 1986a,b; 1987 & 1989), which found the relationship between SGR and tissue nitrogen to be an increasing exponential trend which then levels off. This relationship was found to be true with the growth experiments in Chapter 8.

Past studies with *Gracilaria* have found that levels of pigment proteins are often closely correlated with N content (Lapointe & Ryther, 1979). A pigment protein such as phycoerythrin is largely responsible for determining the red colour of the thalli, changes in the concentrations of these pigments according to N availability, cause lightening or darkening of seaweed colour.
In *Ulva* the main pigment proteins are chlorophylls. Changes in the concentration of chlorophylls would lead to changes in thallus colour. While this has been shown clearly for *Gracilaria* (Lapointe & Ryther, 1979 and Wilson, 1999), this author was unable to find literature relating to this relationship for *Ulva,* although chlorosis has been well documented (Turpin, 1991; Floreto & Teshima, 1998). This occurs when the ph rises above 9, (and free CO_2 becomes limiting), when NO_3 absorption would become inhibited and leads to intracellular N-limited conditions which in turn would cause chlorosis (yellowing

of the thalli due to pigment destruction) and an accumulation of carbohydrates (Turpin, 1991; Floreto & Teshima, 1998).

To our knowledge no one has tried to quantify the relationship for *Ulva* as has been done here. The results suggest that seaweed colour can be used effectively to assess the nitrogen status of cultivated *U. lactuca*. Naturally, there is some variation in colour for a given N content, but this would be taken into account by analysis of a large number of samples. As the accumulation of pigment occurs directly in response to the availability of N in excess of that required for growth, then green would indicate nitrogen replete material and yellow-green would indicate nitrogen starved material.

The relationship between growth rate and internal nutrient concentration of seaweeds generally indicates a threshold response (Björnsäter & Wheeler, 1990). Björnsäter & Wheeler (1990), showed that growth rates in *U. fenestrata* decrease faster under P-limitation than during N-limitation. This is because nitrogen pools in algae, which consist mainly of chlorophyll-protein complexes and other soluble organic nitrogen, are depleted at a slower rate than the phosphorus pools, which probably consist of polyphosphates (PoP) (Rosenberg & Ramus, 1982a). Eppley (1962), showed that *Ulva* can hydrolyse PoP. Although this relationship has not yet been determined for *U. lactuca* it has been assumed that the values obtained for *U. fenestrata* are similar to those likely to occur in *U. lactuca*.

Björnsäter & Wheeler, 1990 showed that *U. fenestrata* regulated N or P uptake in order to maintain a balanced internal N: P ratio. They showed that N: P ratios can be used to determine the nutritional status of the alga. A N: P tissue composition of 16 – 24 reflects nutrient sufficiency, whereas N:P < 16 reflects N-limitation and N:P > 24 reflects P-limitation in this species. The changes in the ratio occur because photosynthesis of algae under nitrogen-limited conditions can result in the accumulation of non-nitrogenous organic compounds (carbohydrates and lipids) depending on the species. As no studies had been done on the South African *U. lactuca* I assumed that the values obtained for *U. fenestrata* would be similar and thus used its threshold values.

The protein content of the *Ulva* grown in the systems at JSP compares favorably with the 44 % reported by Goldberg *et al.* (1998) in their recirculation system. It also falls within the protein range found to be most beneficial to abalone (36 – 44 % protein) (Britz, 1995). The tissue nitrogen analysis performed in this Chapter indicates that by growing the seaweeds in nutrient rich media one can increase the average tissue nitrogen content of the seaweeds above that which is generally found. *Ulva* in the wild typically has a protein content of 3.7 – 24 % (Smith and Young, 1954, Nisizawa *et al.* 1987; Simpson, 1994; Castro-Gonzales et al., 1996; Simpson & Cook, 1998; Wilkinson, 2001 and Wong and Cheung 2001). Average tissue protein values obtained for this study at JSP were: turbot 36.6 %, Abalone 33.35 % and seawater 30.03 %. This is much higher than wild harvested *Ulva* and has positive effects on abalone growth when used as a food source (Naidoo, in preparation).
It must be said that the conversion factor used although commonly accepted, includes N not in the form of protein but intracellular reserve pools of N as well. (Fleurence *et al.* 1995). Therefore the micro-Kjeldahl method used would tend to overestimate the actual protein content.

The fact that there was no significant difference in tissue P at I & J between the fertilized treatments indicates that the Maxiphos ® ratio used in the fertilizer may have been too low. *Gracilaria* in the same study had a higher tissue phosphate than *Ulva* (Njobeni in preperation) and this is probably due to the differences in the phosphate ratios in the fertilizer added (i.e. 6:1 for *Ulva* and 10:1 for *Gracilaria*). The fertilized treatment had higher tissue nitrogen than the other treatments, indicating that fertilizing could increase tissue nitrogen when the algae are stressed. At high water exchange rates on both farms there appeared to be some form of P limitation occurring. It is also possible that altering the ratio of N:P in the fertilizer could have some benefits with higher N in winter and a higher P in summer.

Hutchings & Andrews (1980); Chapman and Shannon (1985) and Mitchell-Innes & Walker (1991) have shown an increase in the phosphate concentrations of seawater in winter off the west coast, while Boyd *et al.* (1985) show that there is

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suggests that the alga has a greater uptake and storage capacity of dissolved inorganic nutrients (De Boer, 1981).

9.5 CONCLUSIONS

The colour relationship shown in Figure 9.9 could be used by mariculture farmers to assess the nutrient value of *Ulva* as a food source for abalone and has important benefits for *Ulva* aquaculture. More laboratory work needs to be done to find the exact switch over point as it is represented by two colours in the graph (Figure 7.7). More laboratory work also needs to be done to confirm the three stable states of tissue nitrogen and SGR.

Growing the seaweeds in effluent media increases their tissue nitrogen content and thus protein above levels found in the wild, improving them as a source of protein for cultured abalone.

The seaweeds grown at a high water exchange rate at JSP were all phosphate limited except for the winter period when background phosphate concentrations increased. This indicated that using a fertilizer such as Maxiphos could benefit the alga, especially if the phosphate ratio were to be increased in summer.

CHAPTER 10

EFFECTS OF STOCKING DENSITY OF SEAWEED ON UPTAKE RATES, PHYSICO-CHEMICAL VARIABLES, TOXIC AMMONIA, SGR AND YIELDS

10.1 INTRODUCTION

In a commercial operation, the main objective is to maximize yields and quality with the minimum amount of effort and cost. High yields are therefore not always achieved under conditions that are optimum for high relative growth rates. As yield = stocking density X SGR. The mass of algae in a culture vessel affects the relative growth rates in a number of ways, the most important being competition for resources. In a system where nutrients are not a limiting factor, such as in an aquaculture system which is either fertilized or uses nutrient enriched wastewater, light is more often the important limiting factor. This is because as stocking density increases, there is a corresponding increase in light attenuation in the culture due to self-shading, which leads to reduced growth (Lapointe & Tenore, 1981, Israel *et al.* 1993). Therefore, in most cases lower stocking densities would increase the specific growth rate but not necessarily the yield.

A number of authors have suggested that stocking density is culture system and species specific as the physical constraints (e.g. tank surface area and depth) of the culture system vary (Neish & Knutson, 1978;. DeBusk *et al.* 1986a and McLachlan, 1991). When stocking densities get too high (> 6 $kg.m^2$), aeration in the tanks is insufficient to circulate the alga properly. This leads to bleaching of the thalli that are left on the surface and results in a loss of biomass (pers. observ.). To overcome this problem frequent harvesting is necessary at high stocking densities. Neish & Knutson (1978), have shown that there is an optimum stocking density (or density range) for which maximal seaweed yields are attained.

One of the most important physico-chemical variables in the cultivation of aquatic animals is ammonia. Even very low levels of ammonia are harmful to many aquatic organisms (Boyd, 1990). If the seaweeds are to be used in a recirculation system due to their capacity for ammonium uptake, it becomes important to know ammonia concentrations at elevated temperatures and pH of different stocking densities of seaweeds.

The aims of this part of the study were:

- To investigate the SGR and yields of *Ulva* grown at different stocking densities in different culture media on both farms.
- To investigate nutrient uptake rates in each of the growth media at varying stocking densities.
- To investigate temporal differences in physcio-chemical variables in the different types of growth media at varying stocking densities

10.2 Methods

10.2.1 Stocking density experiment at both farms

Each set of treatment tanks were stocked with material from existing *Ulva* cultures on the farms.

Three treatments were used for this experiment. Each treatment consisted of a set of four tanks that were stocked with seaweed that had been air dried for 15 minutes and made up to stocking densities of 1; 2; 3 and 4 $kg.m^{-2}$ with JSP having additional stocking densities of 0.5 and 5 $kg.m^{-2}$ in the turbot and abalone tanks. The treatments were seawater, fertilized seawater and abalone effluent at I & J and small and medium seawater, turbot effluent and abalone effluent water at JSP. The water flow rates were set at 12 and 20 volume exchanges per day on the I & J and JSP farms respectively. The experiment was run three times at I & J from the 14th of August to the 16th of November 2002, for approximately a month each. At JSP the experiment was run three times (between the 27th of August to the 9th of October 2002) for approximately two weeks each.

On each sampling date, the seaweed was removed from the tanks, left to air dry for 15 minutes and then the wet weight was recorded. Following weighing and maintenance the material was then reintroduced to the tanks at the required stocking density. Samples were taken to record dry to wet weight ratios.

10.2.2 Nutrient uptake experiments

Two experiments looked at water nutrient uptake rates at varying stocking densities. Experiment 2 at I & J and Experiment 4 at JSP were set up to investigate uptake rates at varying stocking densities at a set water exchange rate.

10.2.2.1 Experiment 2, I & J

For the second experiment at I & J, stocking densities were varied between 1, 2, 3 & 4 kg m^2 per tank in each culture type (seawater, fertilized & recircultaed abalone) with all the tanks unshaded. There were no replicates in the tanks, but the whole experiment was repeated three times. The water exchange rate to all the treatments was set at 12 water volumes exchanges per day. At the same time that water samples were taken, temperature, pH and dissolved oxygen measurements were recorded in each tank. Water sampling began at 8:00 am on the 15th of August 2002, 30 minutes before fertilizer was added to the fertilized treatment and ended 36 hours later, samples were taken at 3 hour intervals. During the first 12 hours of sampling, water to the fertilized treatment was turned off. The water samples were obtained in the same as described in Chapter 4, Section 4.4.

10.2.2.2 Experiment 4, JSP

The experiment at JSP was at various stocking densities of 1, 2, 3 & 4 kg m^2 per tank in each culture type (seawater, turbot & abalone) with all the tanks unshaded. There were no replicates, but the experiment was repeated three times. This experiment was only run in the abalone and turbot effluent tanks. The water supply rate to all the treatments was 20 water volume exchanges per day. Temperature, pH and dissolved oxygen measurements were recorded in each tank at the same time (This Chapter). Water sampling began at 8:00 AM on the 10th of October 2002 and ended 36 hours later.

Samples were taken at 4 hour intervals. Water samples were collected and stored in the same manner as described in Chapter 4, Section 4.4.

10.2.3 Data analysis

At the end of each experiment SGR and yield were determined using the formulae in Chapter 4, Section 4.6. Nutrient uptake was measured using the methods described in Chapter 4, sections 4.5, and the concentration of ammonia was calculated from this data using the methods of in Chapter 4, section 4.6.

10.2.4 Statistical analysis

For the stocking density experiments SGR and yield were statistically analyzed using ANOVA's using Statistica V6 but treating each run of the experiment as a replicate. LSD post hoc test was used to differentiate between the significant differences.

10.3 Results

10.3.1 Stocking density at JSP

In all three runs of this experiment and in all three treatments the SGR decreased logarithmically as a function of stocking density (Table 10.1 & Figure 10.1). There was no significant difference between SGR and stocking density between culture media. Maximum SGR varied from 8 – 15 % $.d^{-1}$ obtained from stocking at a density of 0.5 $kg.m^{-2}$. The minimum SGR obtained was 0.89 – 3.62 % d^{-1}, from a stocking density of 5 $kg.m^{-2}$.

There was a significant difference in stocking densities and SGR within treatments (ANOVA, df = 74; $p < 0.01$), with 0.5 $kg.m^{-2}$ having apparently higher SGR although it was not significantly different from 1 $kg.m^{-2}$. Growth rates at these two stocking densities were significantly higher than all other

stocking densities that were tested ($p < 0.01$). There was no significant difference in SGR at 4 and 5 $kg.m^{-2}$, but 3 $kg.m^{-2}$ was significantly higher than 4 and 5 $kg.m^{-2}$ ($p < 0.05$).

Although seasonality changes in optimum stocking density were not tested, the experiment was run from late spring to early summer. At each run of the experiment, the growth rates increased slightly but the general trend remained the same.

Yields for both seawater and abalone treatments at 0.5 $kg.m^{-2}$ stocking density were lower (0.29 – 0.6 kg $wwt.m^{-2}.d^{-1}$) than at 1 $kg.m^{-2}$ (0.25 – 0.8 kg $wwt.m^{-2}.d^{-1}$) (Figure 10.2), despite the higher SGR (Figure 10.1). Yield declined steadily from 1 – 3 $kg.m^{-2}$ after which it leveled off, Figure 10.2 suggests that there is an optimum stocking density of 1.5 $kg.m^{-2}$. There was no significant difference between the different treatments at different stocking densities. There was no significant difference in yield between 0.5 and 1 $kg.m^{-2}$, however there was a significant difference between 1 $kg.m^{-2}$ and all other stocking densities with 1 $kg.m^{-2}$ being significantly higher ($p < 0.01$).

Table 10.1: Logarithmic equations relating SGR as a function of stocking density at JSP.

Treatment	Equation	R value
Turbot	SGR = - 4.1756 Ln(stocking density) + 8.9541	0.86
Abalone	SGR = - 4.2317 Ln(stocking density) + 8.7348	0.87
Small seawater	SGR = - 5.1367 Ln(stocking density) + 9.7938	0.95
Medium seawater	SGR = - 4.9163 Ln(stocking density) + 9.0339	0.91

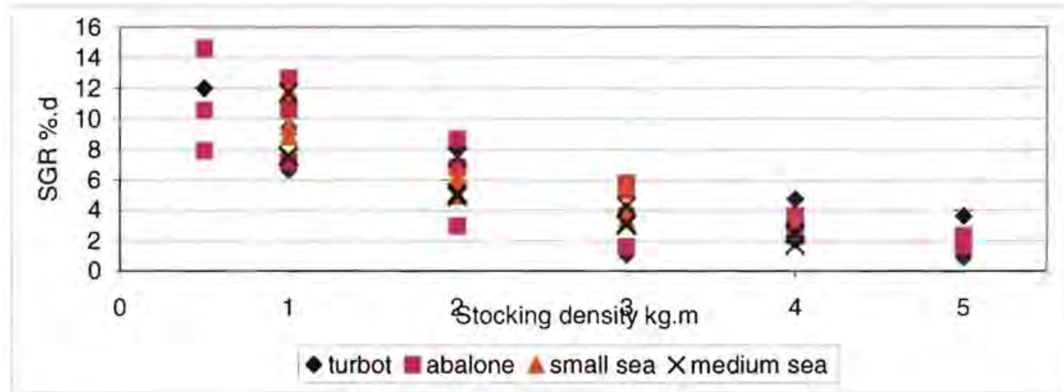

Figure 10.1: The comparison between SGR and stocking density in the various culture media at JSP (n= 3).

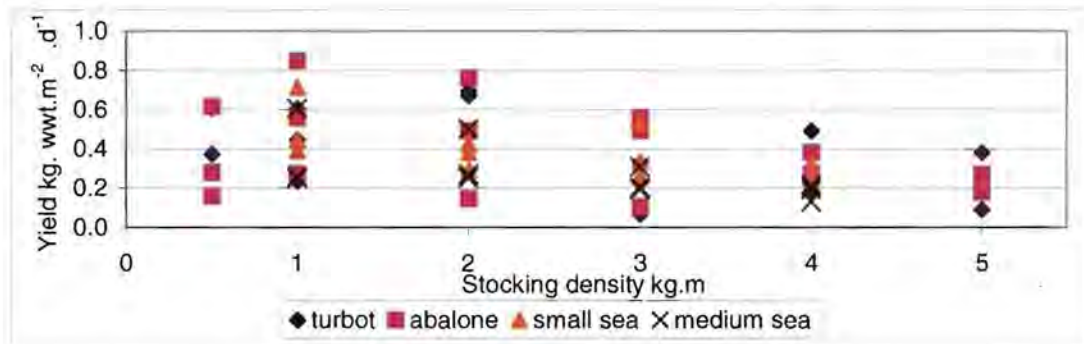

Figure 10.2: The comparison between yield and stocking density in the various culture media at JSP (n = 3).

10.3.2 Stocking density at I & J

SGR decreased logarithmically as a function of stocking density (Figure 10.3 and Table 10.2). There was no significant difference in SGR and stocking density between culture media. Maximum SGR was 6.2 % $.d^{-1}$ obtained from stocking at a density of 1 $kg.m^{-2}$. The minimum SGR obtained was 1.9 % d^{-1}, from a stocking density of 4 $kg.m^{-2}$.

There was a significant difference in stocking densities and SGR within treatments (ANOVA, df = 36; $p < 0.01$), with 1 $kg.m^{-2}$ having the best SGR as well as being significantly different from all other stocking densities. 2 $kg.m^{-2}$

was also significantly different from 3 and 4 kg.m^{-2} (LSD post-hoc test, $p < 0.01$).

Table 10.2: Logarithmic equations relating SGR as a function of stocking density at I & J.

Treatment	Equation	R value
Fertilizer	SGR = - 2.26704 Ln(stocking density) + 4.7579	0.91
Abalone	SGR = - 2.2403 Ln(stocking density) + 5.5032	0.944
Seawater	SGR = - 2.776 Ln(stocking density) + 5.4886	0.901

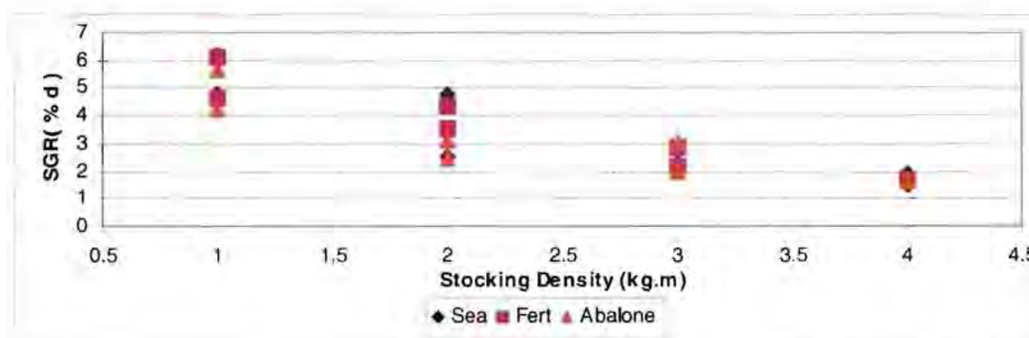

Figure 10.3: The comparison between SGR and stocking density in the various culture media at I & J (n = 3).

Yield at 1 kg.m^{-2} stocking density was less (0.07 – 0.15 kg wwt.m^{-2}.d^{-1}) than at 2 kg.m^{-2} (0.151 – 0.27 kg wwt.m^{-2}.d^{-1}) the yield decreased from 2 kg.m^{-2} and leveled off at 4 kg.m^{-2} (Figure 10.4). There was no significant difference between the yields from different stocking densities in different treatments but there were significant differences within treatments (ANOVA, df = 36, $p < 0.01$). 2 kg.m^{-2} gave a significantly higher yield than any other stocking density on this farm.

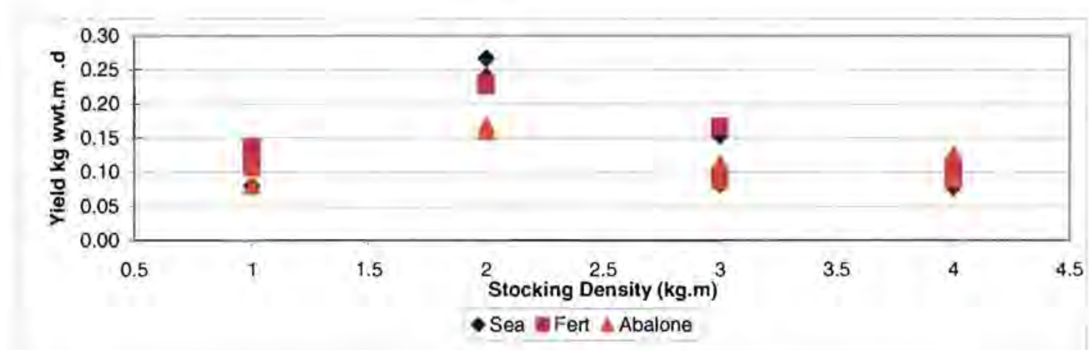

Figure 10.4: The comparison between yield and stocking density in the various culture media at I & J (n = 3).

10.3.3 Uptake, physico-chemical variables and ammonia at JSP

Due to logistical problems it was impossible to have replicates and because of the large volume of data, only graphs showing ammonium uptake (*u*M) and uptake $g.DW^{-1}.h^{-1}$ are shown.

Maximum ammonium removal from the turbot and abalone tanks was at a stocking density of 3 $kg.m^{-2}$ (Figure 10.5), while the uptake ($g.DW^{-1}.h^{-1}$) was highest using a stocking density of 1 $kg.m^{-2}$ (Figure 10.6). At the 3$kg.m^{-2}$ stocking density approximately 80 - 88 % of the ammonium was removed during the day and 60 % at night.

The most phosphate was removed at a stocking density of 3 $kg.m^{-2}$, while the uptake per $g.DW^{-1}.h^{-1}$ was highest using a stocking density of 1 $kg.m^{-2}$. Phosphate removal ranged from 9 - 82 %, with a stocking density of 3 $kg.m^{-2}$ removing approximately 58 - 63 % of the phosphate during the day and 39 - 41 % occurring at night.

Nitrite showed a trend of increasing values to a peak at 00h00, with a corresponding increase in uptake. The peak experienced at night corresponds

to the feeding peaks at I & J, and strongly suggests nitrite excretion by the abalone at night. The most nitrite was also removed at a stocking density 3 $kg.m^{-2}$ in both the turbot and abalone tanks, with approximately 76 % being removed during the day and 59 % removal occurring at night. The uptake per $g.DW^{-1}.h^{-1}$ was highest using a stocking density of 3 $kg.m^{-2}$ in the turbot and 2 $kg.m^{-2}$ in the abalone tanks.

Nitrate values in turbot water, at 00h00, showed a massive increase, with a corresponding increase in uptake rates. The most nitrate was removed at a stocking density 3 $kg.m^{-2}$ in both the turbot and abalone tanks, with approximately 73 % being removed during the day and 66 % at night. The uptake per $g.DW^{-1}.h^{-1}$ was higher using a stocking density of 1 $kg.m^{-2}$ in both the turbot tanks and abalone tanks. There was a double feeding peak evident in the incoming water in the abalone treatment (20h00 and 00h00).

Total percentage removal of nitrate and nitrite was lower in the turbot treatments compared to abalone. This despite the incoming concentrations in the turbot treatments being higher than in abalone (See Chapter 6).

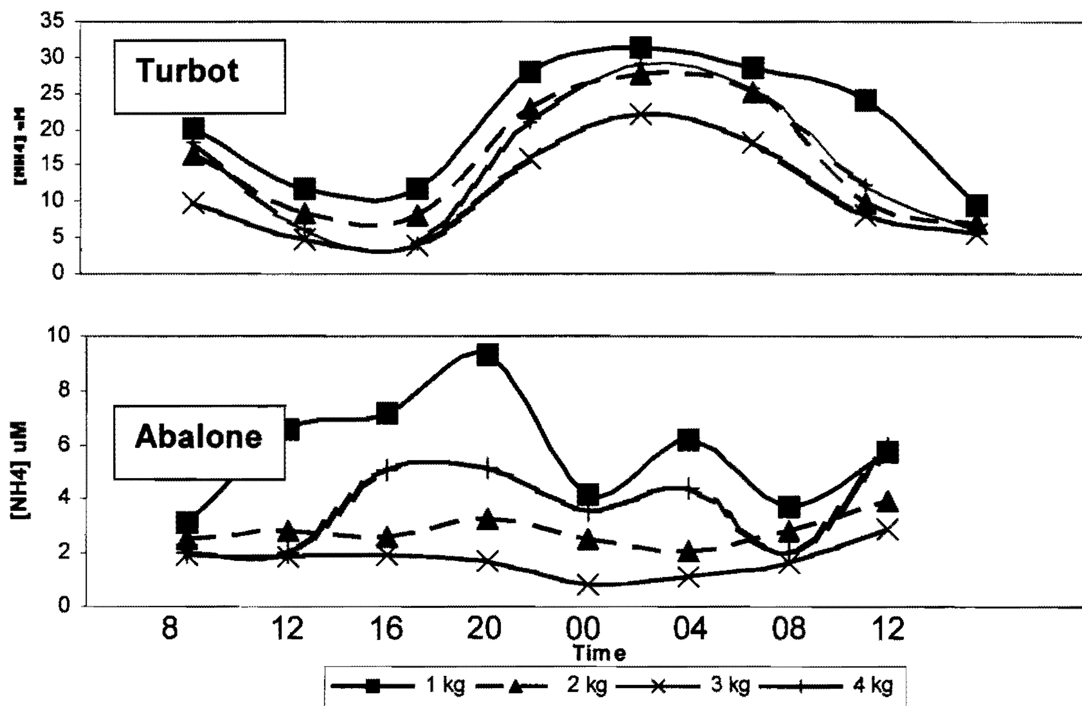

FIGURE 10.5: Ammonium concentrations (*u*M) in Experiment 4. Water exchange rates are 20 $V.E.d^{-1}$.

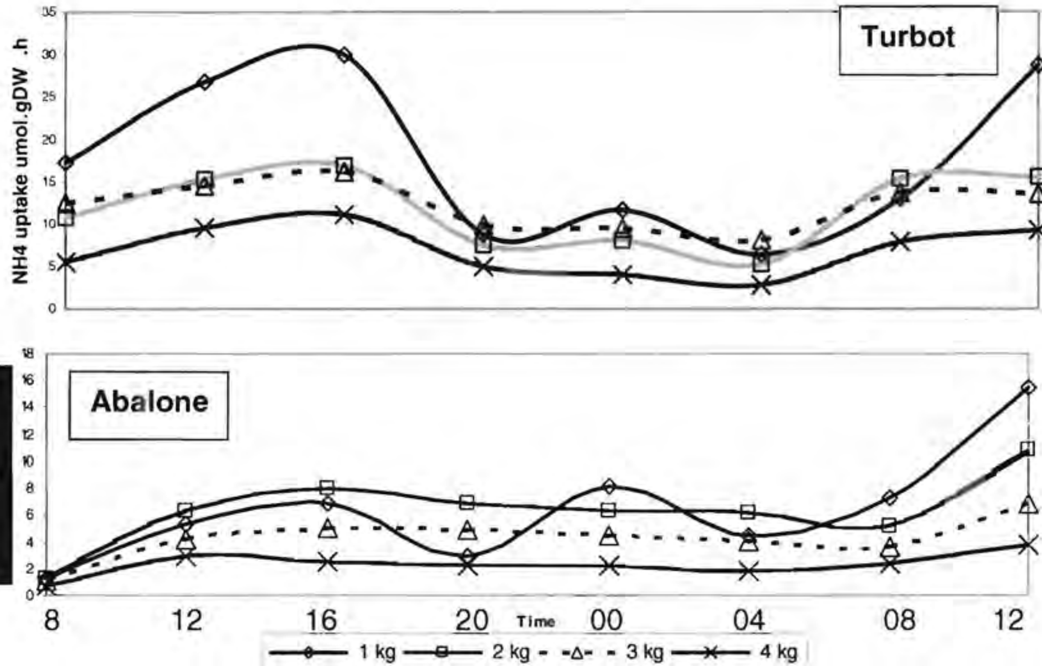

FIGURE 10.6: Ammonium uptake in (μmol.gDW^{-1}.h^{-1}) in Experiment 4 at different stocking densities. Water exchange rates were 20 V.E.d^{-1}.

There was no difference between abalone and turbot tanks with regards to physico-chemical variables, thus only graphs for the abalone tanks are shown. The pH was higher in the more densely stocked tanks in both treatments. The pH showed a diurnal pattern in all the tanks, with highest pH values corresponding to the time of day when photosynthesis was at a maximum (Figure 10.7).

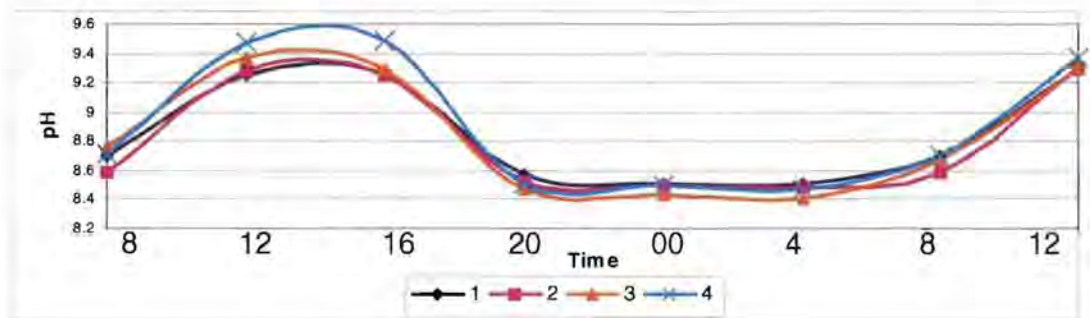

FIGURE 10.7: pH in the abalone tanks with varying stocking densities (kg.m^{-2}) at JSP (n = 1).

Temperature in the tanks also showed a diurnal pattern with the temperature range being 4 °C in both systems (Figure 10.8). The turbot effluent had a slightly higher temperature and this could be due to the higher turbidity, which would increase solar absorption.

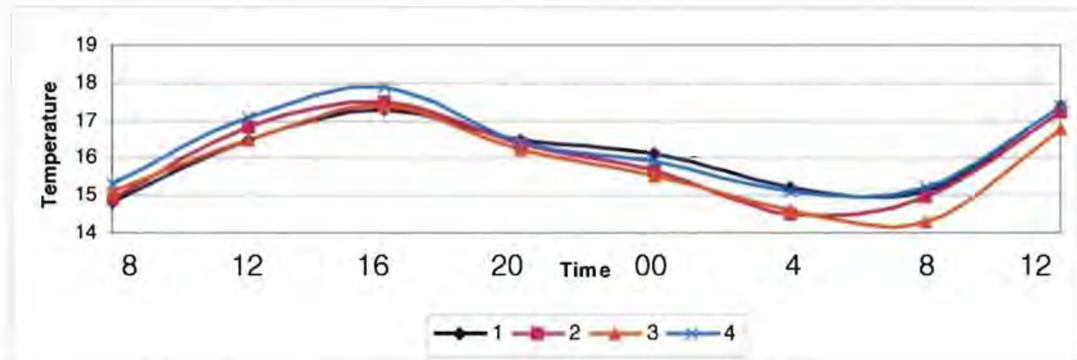

FIGURE 10.8: Temperature, in the abalone tanks with varying stocking densities ($kg.m^{-2}$) (n = 1).

There was also a diurnal pattern in dissolved oxygen (Figure 10.9) which is positively correlated with pH. A plot of pH vs. dissolved oxygen for abalone and turbot gives highly significant correlation coefficients shown in Table 10.3.

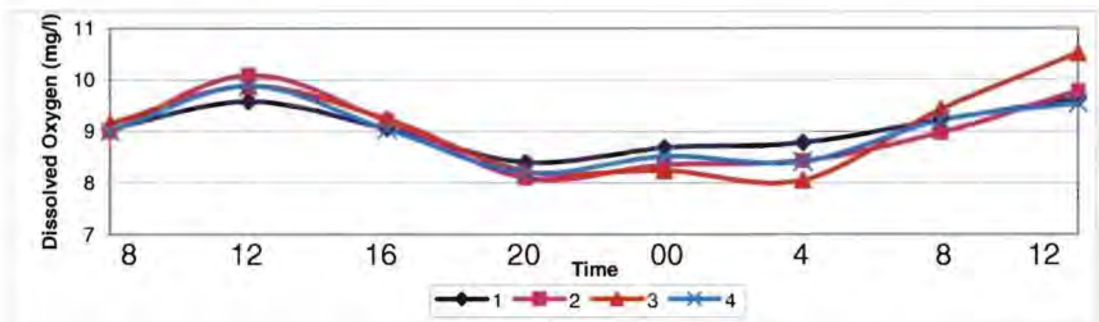

FIGURE 10.9: Dissolved oxygen, in the abalone tanks with varying stocking densities ($kg.m^{-2}$) (n = 1).

TABLE 10.3: Correlation coefficients for pH vs. dissolved oxygen for Experiment 4 at JSP.

Treatment	Stocking density	Equation	R^2 value	P value
Abalone	1	Y = 2.0477x - 9.2291	0.791	0.001
	2	Y = 2.4055x - 12.292	0.7201	0.001
	3	Y = 2.0766x - 9.3628	0.637	0.005
	4	Y = 1.0371x - 0.595	0.6607	0.001
Turbot	1	Y = 0.658x + 2.8932	0.6377	0.005
	2	Y = 0.4823x + 4.4785	0.7597	0.001
	3	Y = 0.4101x + 5.1086	0.7417	0.001
	4	Y = 0.6442x + 3.1202	0.6568	0.001

TABLE 10.4: Ammonia values (µM) for four periods during Experiments 3 and 4 at set flow rate (20 VE.d^{-1}) at JSP. Values with an asterix indicate those that are toxic to abalone, calculated from Lyon (1985).

Treatment	Stocking	Ammonia concentrations			
	Density	08:00	12:00	20:00	00:00
Medium Sea	2	0.56	0.49	0.12	0.09
Small Sea	2	0.21	0.66	0.13	0.11
Turbot	1	2.31*	4.18*	2.74*	2.36*
Turbot	2	1.51*	2.94*	1.86*	2.08*
Turbot	3	1.34	1.91*	1.29	1.23
Turbot	4	2.09*	2.85*	1.71*	2.20*
Abalone	1	0.67	0.73	0.87	0.29
Abalone	2	0.46	0.81	0.22	0.22
Abalone	3	0.24	0.38	0.12	0.10
Abalone	4	0.43	0.90	0.45	0.38

Ammonia concentration reached levels that were toxic to abalone in the turbot effluent, except in the 3 kg.m^{-2} stocking density which was only toxic at midday (Table 10.4).

10.6.4 Uptake, physico-chemical variables and ammonia at I & J

Results from Experiment 2 at I & J were analyzed Because of the large volume of data, only graphs showing ammonium uptake (uM) and uptake $g.DW^{-1}.h^{-1}$ are shown.

Following the input of fertilizer in the tank uptake rates for ammonium, phosphate and nitrite increased dramatically and then declined in concert with decreasing nutrient concentration (Figure 10.10 for ammonium). This suggests that high nutrient concentrations are required for surge uptake, consistent with the findings of Pedersen (1994).

For all nutrients tested maximal nutrient removal in all treatments occurred at a stocking density of 15 kg seaweed material per tank (stocking density of 3 $kg.m^{-2}$) while nutrient uptake per gram of seaweed was highest in the tanks stocked with 5 kg of seaweed material (stocking density of 1 $kg.m^{-2}$).

Ammonium uptake in the fertilized treatment peaked at 1454 $\mu mol.gDW^{-1}.h^{-1}$ (Figure 10.11). Uptake in the abalone treatment was slightly higher than in seawater except at the first feeding peak at 20h00. The range for abalone and seawater was 3.2 – 0.5 and 2.4 – 0.07 $\mu mol.gDW^{-1}.h^{-1}$ respectively. Uptake per gram of tissue is related to ambient nutrient concentration and is highest in all treatments in the tanks stocked with 5 kg of seaweed material, which corresponds to a stocking density of 1 $kg.m^{-2}$. There did not appear to be any diurnal change in uptake rates. Ammonium removal at 3 $kg.m^{-2}$ stocking density in all the treatments ranged from 88 – 92 % during the day to 77 – 85 % during the night.

Phosphate uptake in the fertilized treatment peaked at 1041 $\mu mol.gDW^{-1}.h^{-1}$. Uptake in the abalone treatment was slightly higher than in seawater especially during the feeding peaks. The range for abalone and seawater was 5.2 – 0.02 and 0.94 – 0.03 $\mu mol.gDW^{-1}.h^{-1}$ respectively. Phosphate removal

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at 3 $kg.m^{-2}$ stocking density, ranged between 73 – 97 % in the day and 71 – 84 % at night.

Nitrite uptake per gram of seaweed was very variable, and there was a general trend of maximal uptake per gram of seaweed at 1 $kg.m^{-2}$. The uptake range for all three treatments varies between 0.18 – 0.00 $\mu mol.gDW^{-1}.h^{-1}$. A stocking density of 3 $kg.m^{-2}$ removed the most nitrite in all the treatments. Nitrite removal at 3 $kg.m^{-2}$ stocking density ranged from 84– 89 % during the day and 82 – 86 % at night in all treatments.

Surge uptake of nitrate in the fertilized treatment peaked at 8.69 $\mu mol.gDW^{-1}.h^{-1}$. The recirculated tanks started with a relatively high uptake in all treatments, this could be due to night time feeding activity causing higher concentrations of nitrate in the water leading to high uptake, as the high levels are repeated at 08:00 the following morning. The system was run 12 hours in advance of the start of the experiment to allow the seaweed to acclimatize to the new conditions. The subsequent decrease after the high initial values was due to a lag in pumping as the seawater also had low nitrate values at 08:00. Other increases in nitrate during the day occurred due to activities such as tank cleaning. The uptake range for all three treatments varied between 8.7 – 0.39 $\mu mol.gDW^{-1}.h^{-1}$. As with other nutrients uptake normalized to seaweed biomass is highest in tanks with a stocking density 1 $kg.m^{-2}$. Nitrate removal at 3 $kg.m^{-2}$ stocking density ranged from 89 – 100 %, while night time removal ranged from 91 – 92 %.

There was a large difference between surge, internally and externally controlled uptake (Table 10.5). Ammonium and phosphate surge uptake for 1 $kg.m^{-2}$ was greater than at the higher stocking densities. Nitrate surge uptake was highest at 3 $kg.m^{-2}$. The Vmax values are larger than the internally controlled uptake values, due to the fact that this is a non ideal way to explain Michaelis-Menten kinetics as this was not a true perturbation type experiment.

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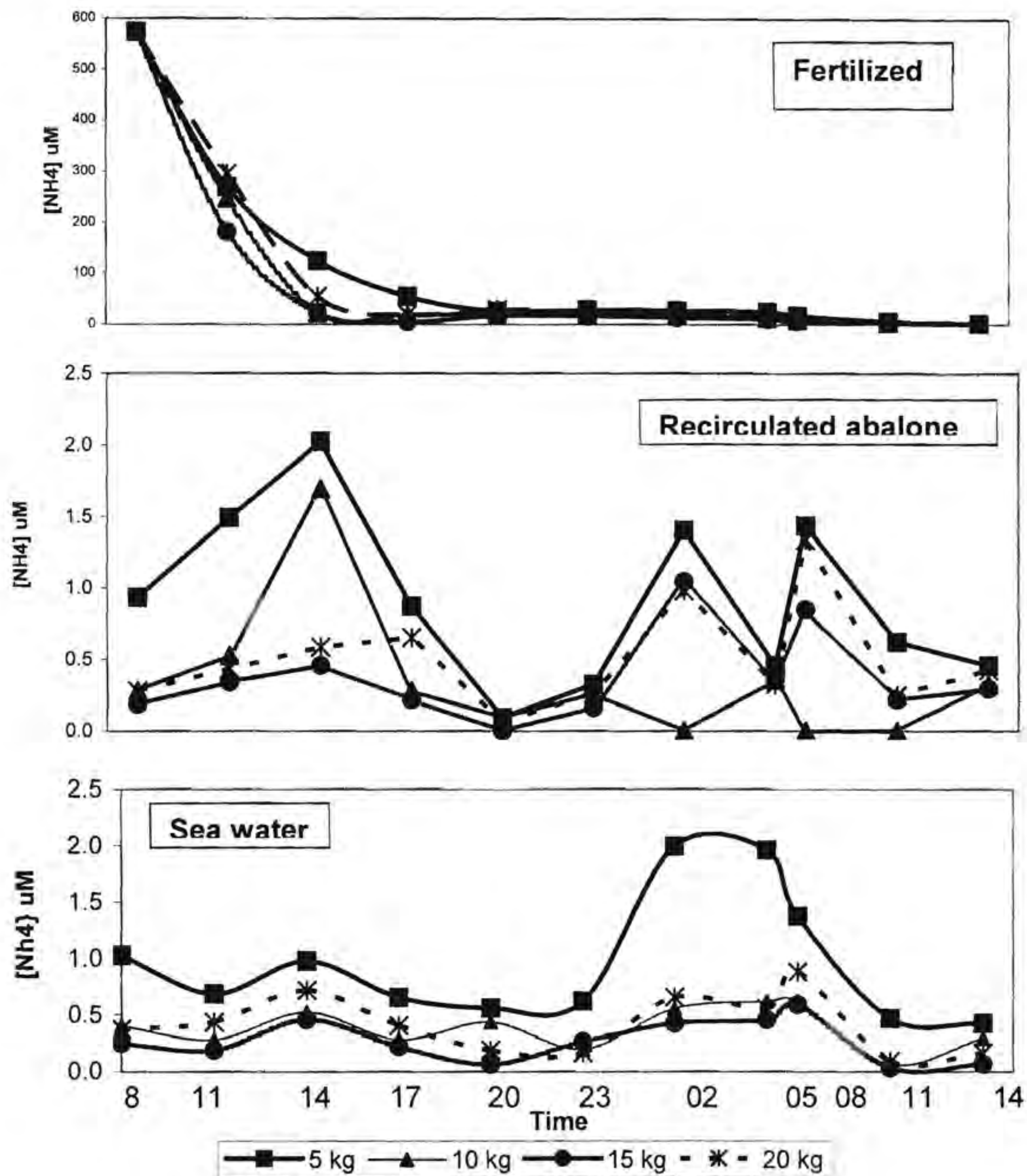

FIGURE 10.10: Ammonium concentrations (NH_4 μM) in Experiment 2. Water exchange rates for all treatments are 12 V.E.d^{-1}. n = 1

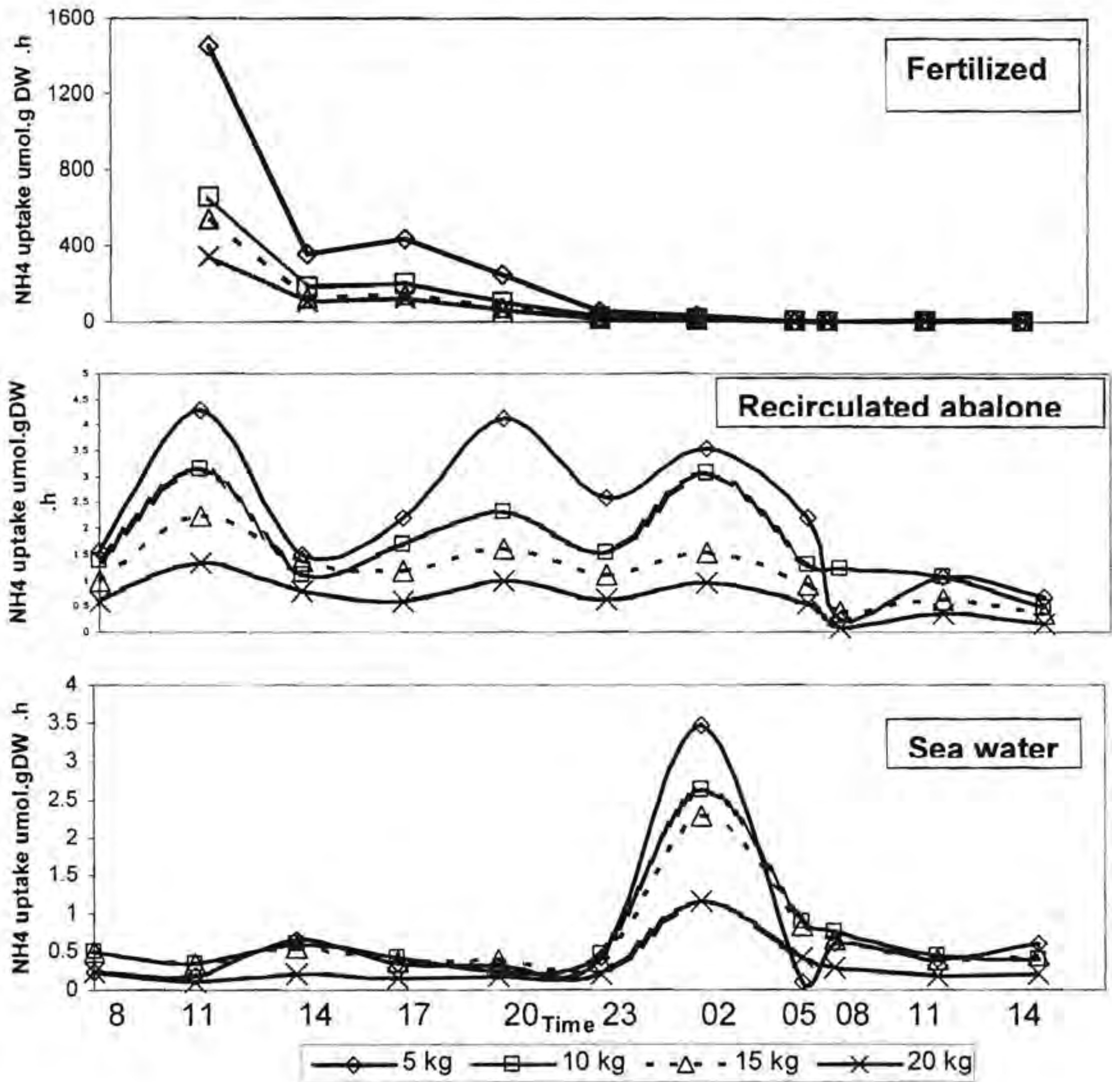

FIGURE 10.11: Ammonium uptake (µmol.gDW^{-1}.h^{-1}) in Experiment 2 at different stocking densities. Water exchange rates for all treatments are 12 V.E.d^{-1}. n = 1

Table 10.5: Surge uptake rates, the average internally controlled uptake rates with standard deviations and the Vmax and Km values and the R value for the Michaelis-Menten regression line, for all nutrients measured at different stocking densities in Experiment 2.

Nutrient	Stocking density ($kg.m^{-2}$)	Uptake rates ($\mu mol.gDW^{-1}.h^{-1}$)				
		Surge uptake	Internally controlled uptake	Vmax ($\mu mol.g^{-1}.h^{-1}$)	Km (μM)	R value
NH_4	1	1454.52 ± 15.35	356.78 ± 16.38	434.20	356.41	0.88757
	2	655.27 ± 5.53	183.99 ± 8.68	274.70	160.13	0.90207
	3	540.22 ± 4.18	126.49 ± 5.22	383.91	134.35	0.94646
	4	343.86 ± 3.34	106.97 ± 2.26	341.27	106.65	0.92399
PO_4	1	1041.42 ± 17.76	185.23 ± 19.55	241.20	206.00	0.96560
	2	479.60 ± 9.65	78.77 ± 8.72	258.11	120.80	0.97902
	3	334.18 ± 3.45	59.87 ± 1.34	380.61	124.67	0.97526
	4	288.76 ± 4.55	49.58 ± 1.02	294.15	89.92	0.96250
NO_3	1	6.47	0.83	Michaelis-Menten curves could not be fitted		
	2	8.21	0.62			
	3	8.68	0.80			
	4	6.71	0.54			
NO_2	1	6.47	0.55			
	2	8.21	0.62			
	3	8.68	0.80			
	4	6.71	0.54			

Figures 10.12 – 10.14 show the physico-chemical conditions in the abalone tanks. Only the graphs for the abalone treatment are shown, as the results were similar for the seawater and fertilized treatments.

The pH (Figure 10.12) in all treatments followed the same trend with 4 $kg.m^{-2}$ having the highest pH and 1 $kg.m^{-2}$ having the lowest pH during the day. In the fertilized treatments the pH at all stocking densities rose above 10, while the water was turned off. The pH remained high (above 9) in the 4 $kg.m^{-2}$ treatment until after 20h00.

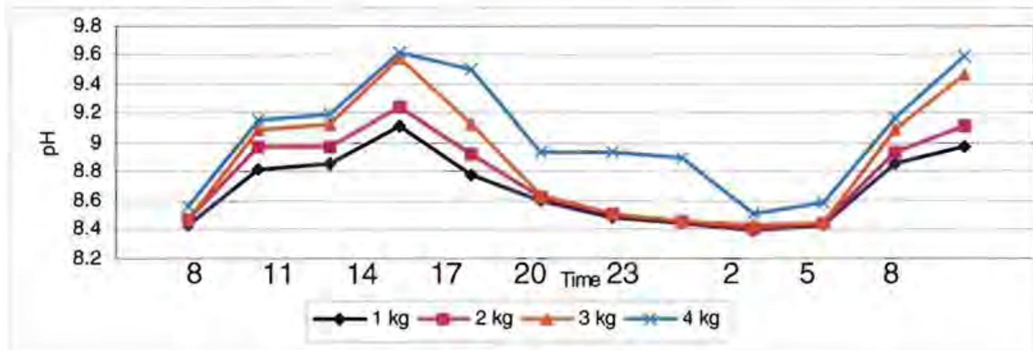

FIGURE 10.12: pH in abalone tanks on the 26 – 27th of February 2002 at varying stocking densities ($kg.m^2$) at I & J.

Temperature (Figure 10.13) and dissolved oxygen (Figure 10.14) followed the same pattern as pH, with the 4 $kg.m^{-2}$ stocking density having a higher temperature than the 1 $kg.m^{-2}$ during the day in all the treatments.

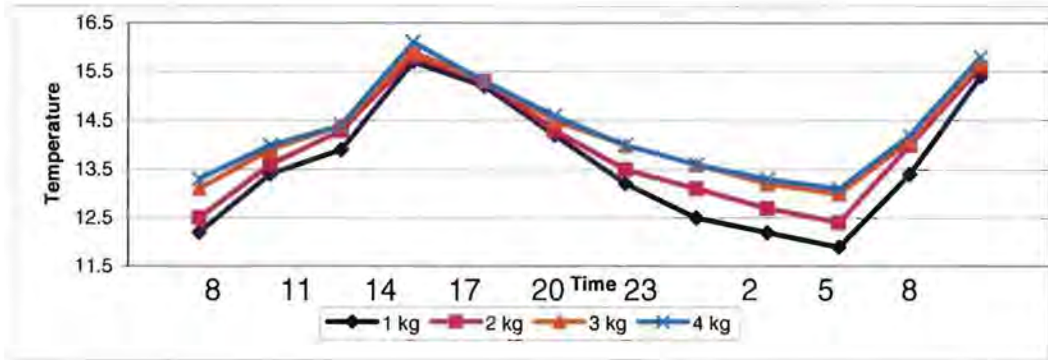

FIGURE 10.13: Temperature (° C) in abalone tanks on the 26 – 27th of February 2002 at varying stocking densities ($kg.m^2$) at I & J.

pH and dissolved oxygen also were closely correlated to each other ($P < 0.05$) in the seawater, fertilized and abalone treatment for all stocking densities.

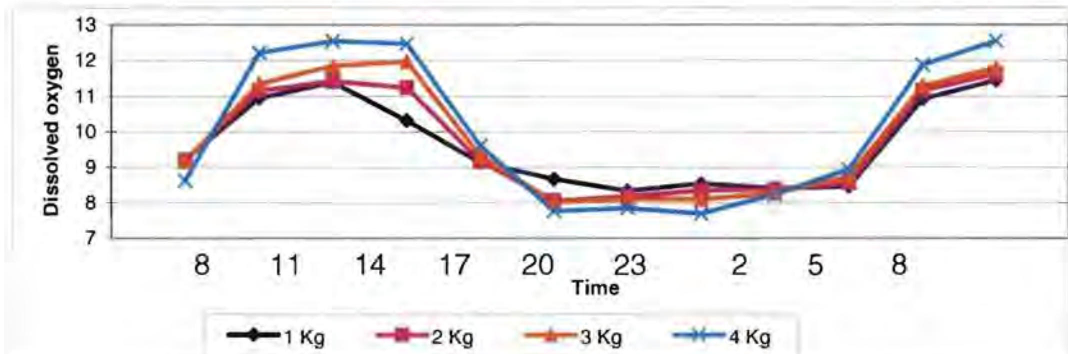

FIGURE 10.14: Dissolved oxygen ($mg.l^{-1}$) in abalone tanks on the 26 – 27th of February 2002 at varying stocking densities ($kg.m^2$) at I & J.

In Experiment 2, only the fertilized treatment had toxic values of ammonia at the higher water exchanges (Table 10.6). In all treatments at I & J, a stocking density of 3 $kg.m^{-2}$, had the lowest ammonia values. Although the fertilizer is diluted much faster in experiment two these results suggest that, if fertilizer is to be added to seaweed in a recirculation system the water from these tanks must be isolated from the rest of the farm for 36 hours after the fertilizer has been added.

As with the water experiments from I & J, the stocking density that produced the least frequent toxic concentration of ammonia was 3 kg.m^{-2}.

TABLE 10.6: Ammonia values (µM) for four periods during Experiment 2 at I & J. Values with an asterisk indicate those that are toxic to abalone calculated from Lyon (1985).

Treatment	Stocking density	Flow rate	Shading	Ammonia concentrations			
				08:00	12:00	20:00	00:00
Sea	1	12	No	0.11	0.21	0.14	0.11
Sea	2	12	No	0.05	0.08	0.11	0.03
Sea	3	12	No	0.03	0.07	0.02	0.04
Sea	4	12	No	0.04	0.15	0.05	0.03
Fertilized	1	12	No	2.81*	4.77*	1.46	0.32
Fertilized	2	12	No	1.52*	7.68*	0.97	0.18
Fertilized	3	12	No	1.11	2.89*	0.65	0.07
Fertilized	4	12	No	1.58*	4.85*	0.75	0.15
Abalone	1	12	No	0.05	0.25	0.01	0.02
Abalone	2	12	No	0.02	0.11	0.01	0.02
Abalone	3	12	No	0.01	0.08	0	0.01
Abalone	4	12	No	0.02	0.12	0.01	0.04

10.7 DISCUSSION

SGR at I & J were almost half those obtained at JSP at the same stocking densities. Several authors have suggested that the optimum stocking density is system and species specific (Hanisak & Ryther, 1984). Our data confirms this, with an optimum stocking density for SGR of 2 – 3 kg.m^{-2} at I & J and 1 – 2 kg.m^{-2} at JSP. It is possible that the amount of nutrient available in the growth media affects the stocking density. The turbot effluent at JSP had more nutrients available than either seawater or abalone effluent (Chapter 6). The high nutrient content of turbot effluent appears to support a larger stocking density of 2 kg.m^{-2} than the other treatments (1 kg.m^{-2}) (Figure 10.2).

The optimum stocking densities for both these farms seem to fit in the range described in the literature. *U. fasciata* Delile, has an optimum stocking density of 0.8 kg m^{-2} (Lapointe & Tenore, 1981). DeBusk *et al* (1986), reported that *U. lactuca* had an optimum stocking density of 0.8 kg m^{-2}, while Ryther *et al.* (1981, cited by Neori *et al.* 1991) obtained an optimum of 1 kg m^{-2} as did Cohen & Neori (1991) and Neori *et al.* (1991). All these studies used small tanks that were similar in volume to the tanks at JSP. Thus, the values of 1 kg.m^{-2} for the optimum stocking density at JSP corresponds well to the literature. A comparison between the two farms indicates a linear relationship between tank size and optimum stocking density.

DeBusk *et al* (1986), compared the reported stocking density of *Ulva* with that of other genera. They found that the density optima for other genera were 2 – 4 times higher than those reported for *Ulva*. Under natural conditions, *Ulva* species are considered among the most productive seaweeds (Littler & Arnold, 1982), with fast growth rates. However, because yield is a function of both standing crop and SGR, the low standing crop optimum means that the specific growth rate must be extremely high if yields of *Ulva* species in cultivation are to approach those of other macroalgae. Neori *et al.* (1991), found that increasing the stocking density of *U. lactuca* above the optimum 1

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kg m^{-2} decreased the growth rate and yield. This result is consistent with what we found at JSP.

The one other important point, which this experiment has not answered, is whether or not stocking density changes with season. Work done by Steyn (2000), showed that both yields and SGR's were higher at 2 kg.m^{-2} stocking density in summer, but in winter a stocking density of 1.5 kg.m^{-2} gave higher yields for both *U. rigida* and *U. fasciata*. Steyn (2000) used similar tanks sizes to our experiment although the water temperatures are much higher for Steyns study (the farm he was working on is on the East coast of South Africa).

A possible explanation for the seasonal change in stocking density is that there is more light available in summer than in winter. By increasing the stocking density, one will reduce the amount of light that is available at the bottom of the tanks. Vandermeulen and Gordin (1990), reported a reduction in photon flux density at the bottom of their tanks of 89 % and 100 % with 1 kg m^{-2} and 4 kg m^{-2}, respectively. This means that there is less light available for photosynthesis and that self-shading of the algae is occurring, which will reduce SGR.

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Maximum nutrient removal at both 12 and 20 volume exchanges per day occurs when stocking density is 3 kg.m^{-2} on both farms. Approximately 85 % of the ammonium is removed during the day and 70 % at night at JSP in both turbot and abalone treatments, at this stocking density. At I & J in the abalone treatments, 90 % and 80 % of the ammonium is removed during the day and night respectively. These results compare favorably with the 90 % removal of fish effluent in Israel (Goldberg *et al.* 1995). The removal efficiency is better at the slower flow rate and is consistent with what Manta & Santos (2003) found. Thus 3 kg.m^{-2} would be the best stocking density to use if I & J needed to recirculate. This contrasts with biomass-normalized uptake rates (g.DW^{-1}.h^{-1}) which are highest at 1 kg at I & J and 0.5 kg stocking density at JSP. Thus, the maximum uptake per alga is occurring at these stocking densities but not maximum nutrient removal, which is the aim behind recirculation.

With regards to temperature and DO, the 4 kg.m^{-2} stocking density had a higher temperature than the 1 kg.m^{-2} during the day, as the higher biomass absorbs more radiant energy and dissipates it as heat. Higher stocking densities resulted in greater extremes of diurnal oxygen concentration due to photosynthesis and respiration.

When considering a stocking density to use in a recirculation system, one must avoid a situation where algal metabolism increases either of these two variables beyond certain critical limits. A stocking density of 1 kg.m^{-2} resulted in the lowest temperature and pH values. However, this low density removed the least amount of ammonium from the tanks, even though it had the highest uptake per gDW^{-1}.h^{-1}. Maximum ammonium and ammonia removal occurred at a stocking density of 3 kg.m^{-2}. The temperature and pH values at this density were not significantly higher from a stocking density of 1 kg.m^{-2}. At both farms and in all treatments a stocking density of 4 kg.m^{-2} had both the highest temperature and pH. Thus, when considering which stocking density to use, pH and temperature are minimal considerations, particularly if recirculation is the primary goal.

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Increasing stocking density does not lead to nutrient limitation if the algae are grown at high ammonium concentrations. Cohen & Neori (1991), found that by increasing the stocking density you can get a corresponding increase in the amount of nitrogen taken up from the water over the range of 1 – 6 kg.m^{-2}. In our stocking density experiments at I & J, Vmax increased to a stocking density of 3 kg.m^{-2} and then decreased in ammonium and phosphate. This suggests that for these systems the optimum stocking density to maximize uptake is 3 kg.m^{-2}. The difference between our study and Cohen & Neori (1991) is tank design.

At higher stocking densities the amount or the frequency of fertilizer that is placed in the tanks needs to be increased.

10.8 CONCLUSION

On the I & J farm the stocking density that was chosen for the other studies (Chapters 8, and 11) (2 $kg.m^{-2}$) was correct to obtain maximum SGR and yields while at JSP our results in these experiments indicate that our chosen stocking density was too high. However, if there is a seasonal effect on stocking density as found by Steyn, (2000) then perhaps our 2 $kg.m^{-2}$ for summer on the farm is sufficient. This needs further investigation.

In our experiments the stocking density chosen for our experiment appears to be appropriate for recirculation purposes. No extremes of temperature or pH occurred when the seaweed is stocked at 2 $kg.m^{-2}$.

Furthermore the best stocking density for recirculation purposes lies between 2 - 3 $kg.m^{-2}$. This stocking density is slightly lower than the ideal 3 $kg.m^{-2}$ (based on uptake) for nutrient removal, and toxic ammonia levels, as the higher stocking density maximizes nutrient removal, while a lower stocking density of 2 $kg.m^{-2}$ results in less of a temperature or pH increase.

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CHAPTER 11

THE EFFECT OF KELPAK ® ADDITIONS AND FERTILIZER ON SGR AND TISSUE N AND P IN DIFFERENT WATER TREATMENTS

11.1 INTRODUCTION

The use of marine algae as manures and fertilizers for crops dates back centuries. In the twelfth century, kelps (Phaeophyta), were used as manure on the coastal lands of Europe (Crouch, 1990; Lee, 1999). Much more recently, the use of seaweed extracts as a foliar spray on agricultural plants is increasing, even though the literature on seaweed extracts is contradictory. Documented studies (Featonby-Smith & van Staden, 1983, 1987; Crouch, 1990) have reported that seaweed extracts can improve the growth rates and yields of plants, as well as prevent pests and improve the overall quality of the product. Other studies however, claim that some extracts have no effect at all (Verkleij, 1992).

It has been well documented that algae show alterations in growth in response to light quantity and quality and nutrient availability. This thesis has investigated seasonal growth response to different nutrient regimes. Although seaweeds are viewed as a more primitive group than higher plants many studies have looked at the effect of applying plant growth hormones (PGH), such as auxins and gibberellins to seaweeds (reviewed in Lobban & Harrison, 1997).

PGH are specialized chemical substances that are the main internal factors controlling growth and development in algae (Lobban & Harrison, 1997). PGH are produced in one part of the alga and transported to others, where they are effective in very small amounts. Depending on the target tissue a PGH may have different effects. Bradley (1991), states that they regulate the rate at which individual parts of the plant grow, integrate those parts to form the whole organism, control reproduction and also allow mature plants to respond to changes in the environment.

Commercial liquid algae fertilizers which contain PGH, have also been used in tanks to grow *Gracilaria* in Molokai, Hawaii (Glenn *et al.* 1996). A preliminary study done by Leitao, (2001) using Kelpak® extract on *Gracilaria* tips suggested

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that concentrations of 1:1000 and 1:500 significantly increased apical growth as well as possibly increasing branching.

If plants benefit from foliar sprays the question I attempt to answer is, would a seaweed extract increase the growth rates of tank-cultivated algae and at what concentration? In Chapter 6, I discussed the benefits of fertilizer additions to nutrient depleted waters, but are there any benefits in adding fertilizer to effluent waters? BjörnsẆer & Wheeler, (1990) and DeBusk *et al.* (1986) showed that additions of fertilizer to nutrient depleted water significantly increased SGR of *Ulva* sp. Ryther *et al.* 1975; Vandermeulen & Gordin, 1990; Cohen & Neori, 1991; Neori *et al.* 1991; Jimenez Del Rio *et al.* 1996; Neori *et al.* 1996; Shpigel *et al.* 1997; Goldberg *et al.* 1998 have all shown that *Ulva* sp. can be grown in effluent water (abalone, fish and human) and that SGR's are significantly higher than when grown in seawater.

On the I & J farm, where *G. gracilis* was being grown in abalone effluent with pulse additions of fertilizer, the colour of the seaweed is a very dark red. Studies done by Lapointe & Ryther, (1979), correlated pigment levels with N content, thus health could be measured by the seaweed colour, this was also shown for *U. lactuca* in Chapter 8. This prompted the question, "Do additions of fertilizer to effluent water benefit the seaweed?" Kelpak® is a plant growth stimulator due to its hormonal content and not its nutrient content (Feantonby-Smith & van Staden, 1983; 1987), thus would additions of Kelpak® and fertilizer have more benefit if they were combined than if they were added separately? What would the effect of these treatments be on tissue N and P composition of the alga?

At I & J, the water exchange rate varied from 4 – 12 volume exchanges per day. This water exchange has been shown to be too low to provide sufficient nutrients to sustain growth in *Ulva* without nutrient supplementation (Steyn, 2000 & Chapter 8). Previous work has shown that a water turnover rate of 12 volume changes per day is sufficient to sustain growth in *Ulva* cultivation (DeBusk *et al.* 1986 & Del Rio *et al.* 1996). However, a crucial issue in exchange rate considerations is the amount of nutrient loading reaching the

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thallus surface, thus, volume flow data need to be supported by concentration data.

The aims of this part of the study were to:

- investigate whether a seaweed extract would increase the growth rates of tank-cultivated algae
- identify the best concentration of seaweed extract
- investigate benefits in adding fertilizer to effluent waters
- investigate if additions of Kelpak® and fertilizer have more benefit if they were combined than if they were added separately.
- Investigate the effect of these treatments on tissue N and P composition of the alga

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11.2 MATERIALS AND METHODS

11.2.1 Kelpak® concentration and fertilizer experiment at JSP

11.2.1.1 Seaweed extract

Kelpak ® was the commercially available seaweed extract used in this experiment. It is manufactured by Kelp Products (Pty) Ltd. in Simons Town, South Africa. It is made using epiphyte-free fronds and stipes of the brown alga *Ecklonia maxima* (Osbeck) Papenfuss, using a cold cell burst process (J. Nell & N. Christie pers. com.). The process involves the use of pressure on macerated fresh material to compress the cells in the absence of air or water, followed by a sudden release of pressure. This results in the cell walls rupturing and releasing their contents. The seaweed is progressively reduced in particle size, and the particles pass from an extremely high pressure to a low pressure chamber where they disintegrate, resulting in the liquid extract. This process excludes the use of heat, chemicals or dehydration that could effect some organic components of the concentrate (Verkleij, 1992). The N: P ratio in mg N/P per g DW of Kelpak ® is 55.98: 49.15 ($\pm$ 0.01; n = 6) which is a ratio of 1.14: 1 (using methods in Chapter 4, Section 4.7).

11.2.1.2 Fertilizer

The fertilizer used was a combination of Maxiphos ® and ammonium sulfate. The fertilization regime used was the same as that used by the I & J farm. The water to all the tanks including the seawater controls was turned off at 12:00 on a Monday and the fertilizer added. The water was turned on again at 8:00 on the following day. The fertilizer N:P ratios and concentrations are given in Chapter 4.

11.2.1.3 Effluent media

This experiment was run on the JSP Farm in the small tanks. With the farm design we were able to place 12 tanks under each effluent source. The two effluents originated from the turbot and abalone out-growing tanks.

11.2.1.4 Experimental design

Three different experiments were run in order to investigate the effects of fertilizer in effluent culture media. The first experiment was run to determine the correct Kelpak® concentration to use in conjunction with fertilizer. The second and third experiments were run to test the effect of fertilizer and Kelpak® concentration (as determined from the first experiment) in stand alone additions or combined in both turbot and abalone effluent culture media. All tanks were run with 20 water volume exchanges per day.

11.2.1.4.1 Kelpak® concentration

To find the right concentration of Kelpak®, we set up the tank system at JSP to run 12 tanks on turbot effluent. We then had two controls, a seawater control consisting of four tanks and a turbot control of three tanks. The tanks were set up in the following manner:

- 4 small seawater control tanks
- 3 small turbot effluent control tanks
- 3 series of three turbot effluent tanks with the following Kelpak® concentrations (1:500; 1:2 500; and 1:5 000).

All treatments were run in triplicate. The experiment was run for two weeks under normal farm conditions after which the algae were harvested then restocked and the experiment repeated. Run one was from the 17th – 31st May 2002, run two was from the 31st of May to 14th of June 2002. The decision as to which Kelpak® concentration to use in the following two experiments was based on SGR of the *Ulva*. .

11.2.1.4.2 Turbot

The first run of the turbot experiment was from the 28th of June to the 16th of July 2002 and the second run was from the 16th – 30th of July 2002, using a Kelpak ® concentration of 1:2 500, as ascertained as optimal in Section 11.2.2.4.1. The tanks were set up in the following manner:

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- 4 small seawater control tanks
- 3 small turbot effluent control tanks
- 3 small turbot effluent tanks with fertilizer and a 1:2 500 concentration of Kelpak®
- 3 small turbot effluent tanks with just fertilizer
- 3 small turbot effluent tanks with just Kelpak® at 1:2 500 concentration

11.2.1.4.2 Abalone

The abalone tanks were set up in the same manner as the turbot tanks, with exactly the same experimental design. The first run of the abalone experiment was from the 30th of July to the 13th of August 2002 and the second run was from the 13th – 27th of August 2002.

11.2.1.5 Nutrient analysis

At each harvest, samples were taken for wet to dry weight analysis and tissue N and P content according to the methods described in Chapter 4, Sections 4.6 and 4.7.

11.2.1.6 Statistical analysis

The effect of various Kelpak® treatments on the SGR were analyzed using ANOVA, the single factorial analysis of variance ($p = 0.05$) statistical package in STATISTICA. The least significance difference (LSD) test or planned comparison test was conducted on the 95 % level, to distinguish significantly different results following the ANOVA test.

11.3 RESULTS

11.3.1 Kelpak® Concentration in turbot effluent

An important finding from these experiments was that SGR was always better on the second run when compared to the first. This implies that the alga needs time to acclimatize to the experimental conditions before the effects can be monitored.

SGR in 1:5 000 Kelpak ® concentration remained unchanged compared to the turbot control (Figure 11.1). The SGR in the 1:500 Kelpak ® concentration had the lowest SGR compared to both the seawater and turbot control and thus had an inhibitory effect. The Kelpak® concentration that gave the best SGR was 1:2 500. ANOVA's done on the results show that there was a significant difference in SGR between the treatments (ANOVA, df = 20; $p < 0.01$). Growth at the concentration 1:2 500 was significantly higher than all others, while the seawater and 1:500 concentrate concentration were significantly lower than all other treatments (LSD post-hoc test, $p < 0.01$ in all cases).

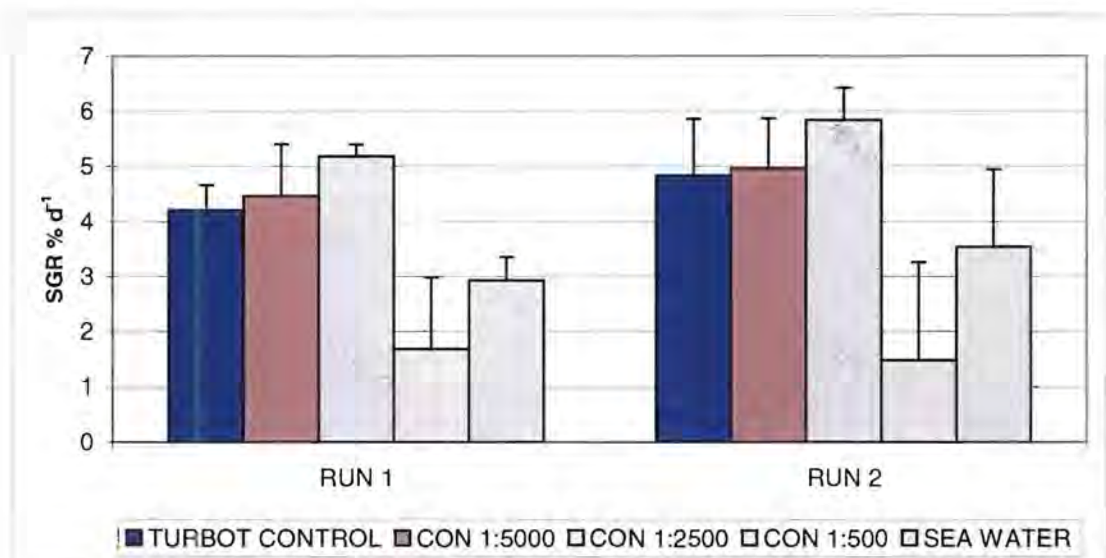

Figure 11.1: The effect of various Kelpak ® concentrations (CON) in turbot effluent on SGR (% d^{-1}) on *Ulva lactuca* at JSP, from 17th May to 14th June 2002. Bars show standard deviations (n = 3).

Tissue nitrogen values were similar in both runs of the experiment (Figure 11.2), unlike SGR. The seawater treatment had significantly lower tissue nitrogen levels when compared to all other treatments (ANOVA; df = 20; $p < 0.01$; LSD post-hoc test, $p < 0.01$).

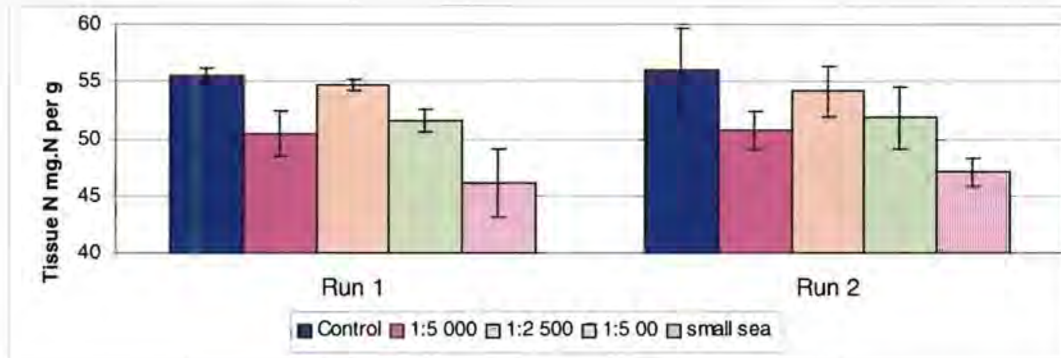

Figure 11.2: The effect of various Kelpak ® concentrations in turbot effluent on tissue N mg N per g *Ulva lactuca* at JSP. Bars show standard deviations (n = 3).

Tissue P increased slightly in the second run of the experiment (Figure 11.3). The seawater treatment had significantly lower tissue phosphate compared to other treatments (ANOVA; df = 20; $p < 0.01$; LSD post-hoc test, $p < 0.01$). There was no significant difference between any of the other treatments in both runs.

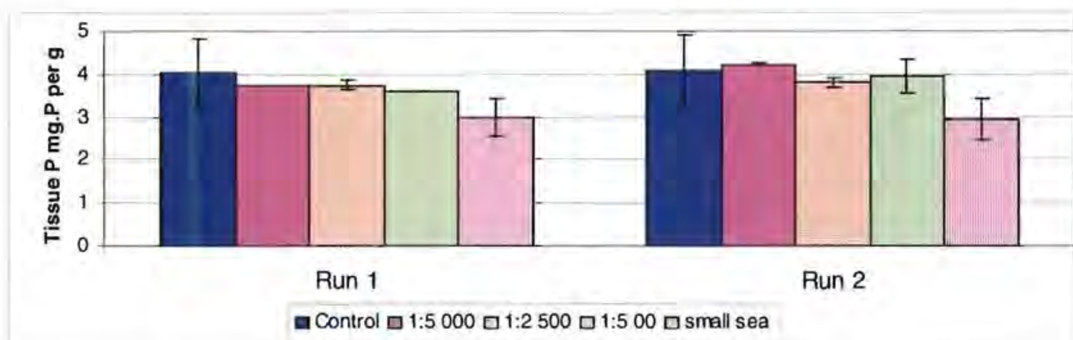

Figure 11.3: The effect of various Kelpak® concentrations in turbot effluent on tissue P mg P .g *Ulva lactuca* at JSP. Bars show standard deviations (n = 3).

11.3.2 Turbot + Kelpak ® +Fertilizer Combination

The second run of the experiment gave higher SGR than the first (Figure 11.4). The seawater control had the lowest SGR, while the Turbot effluent with Kelpak® and fertilizer had the highest SGR. In all cases the addition of either fertilizer or Kelpak ® or a combination of both, increased the SGR above the turbot control and the seawater control. ANOVA's performed on the results showed that there was a significant difference between treatments (ANOVA, df = 20; $p < 0.01$). The turbot and seawater controls were significantly lower than the other treatments and the Kelpak ®, fertilizer and turbot effluent media was significantly higher than all other treatments except the turbot and fertilizer treatment (LSD post-hoc test, $p < 0.01$).

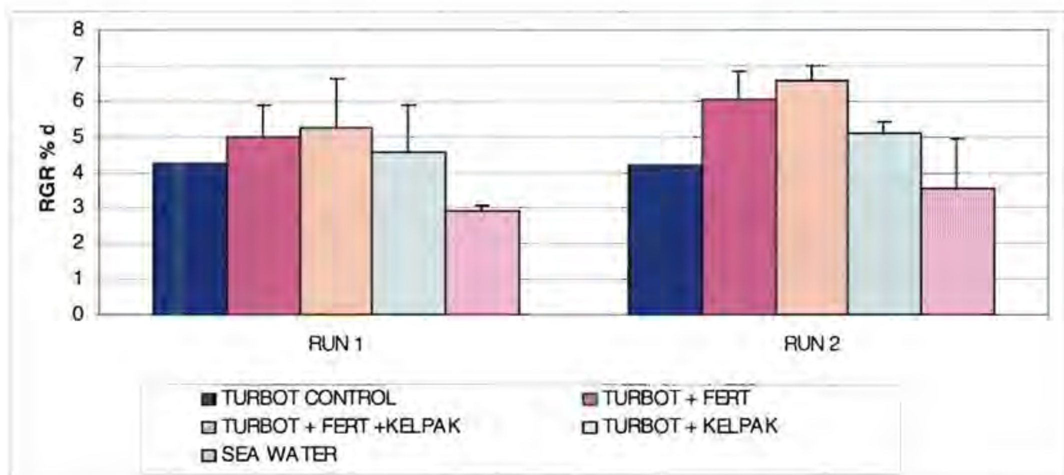

Figure 11.4: The effect of additions of Kelpak ® and fertilizer in stand alone or combined in turbot effluent on SGR (% d^{-1}) on *Ulva lactuca* at JSP, from 28th June to 30th July 2002. Bars show standard deviations (n = 3).

Tissue nitrogen (Figure 11.5), also increased in all treatments on the second run. The seawater tissue N was significantly different from all other treatments (ANOVA; df = 20; $p < 0.01$; LSD post-hoc test, $p < 0.01$). The Kelpak ® and fertilizer treatment tissue N was also significantly different from the turbot control and the Kelpak ® stand-alone addition.

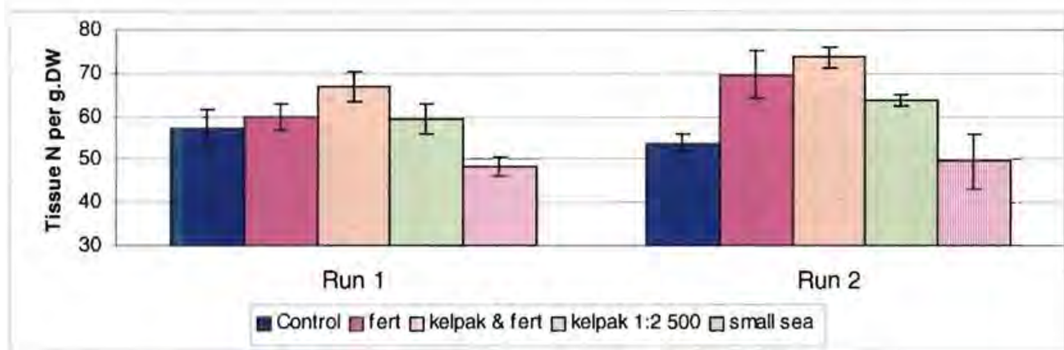

Figure 11.5: The effect of various combinations of Kelpak® and fertilizer in turbot effluent on tissue N mg N per g *Ulva lactuca* at JSP. Bars show standard deviations (n = 3).

Tissue P (Figure 11.6), decreased on the second run and seawater was the only treatment that was significantly different to the others in both runs. The Kelpak ® stand alone treatment in Run 2 had a slightly higher tissue P than the other treatments but was not significantly different.

Tissue N:P ratios were much lower than in the Kelpak ® concentration experiment, in the range of 6.7 – 11.9 with seawater again having the highest ratio.

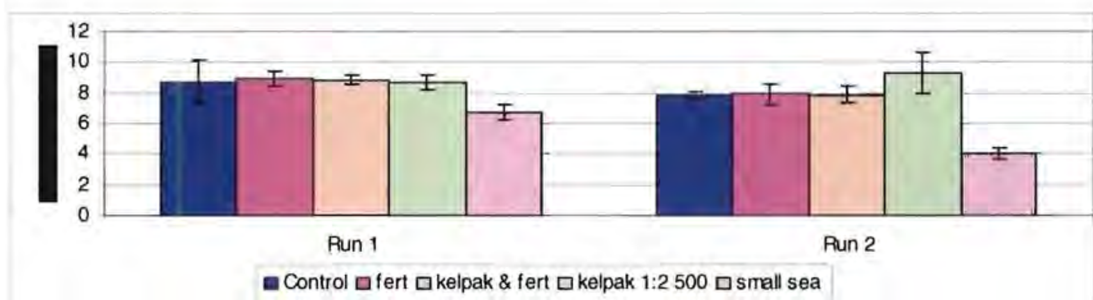

Figure 11.6: The effect of various combinations of Kelpak ® and fertilizer in turbot effluent on tissue P mg P per g *Ulva lactuca* at JSP. Bars show standard deviations (n = 3).

11.3.3 Abalone + Kelpak ® + Fertilizer Combination

The results with the abalone effluent experiment are similar to those obtained for the turbot effluent experiment. Again, the second run had higher SGR than the first run (Figure 11.7). As with the turbot experiments additions of Kelpak ® and/or fertilizer increased the SGR above that of the Abalone and seawater controls. The ANOVA showed that there were significant differences between the treatments (ANOVA, df = 20; $p < 0.01$), with the abalone effluent, Kelpak ® and fertilizer having a significantly higher SGR than all other treatments (LSD post-hoc test, $p < 0.01$) except, the abalone and fertilizer treatment. Unlike the turbot experiments, there was no significant difference between the abalone and seawater control.

Tissue nitrogen (Figure 11.8), decreased in the second run. The treatments that had the highest SGR also had the lowest tissue N content per gram, which was different from the turbot experiment. There were no significant differences between treatments.

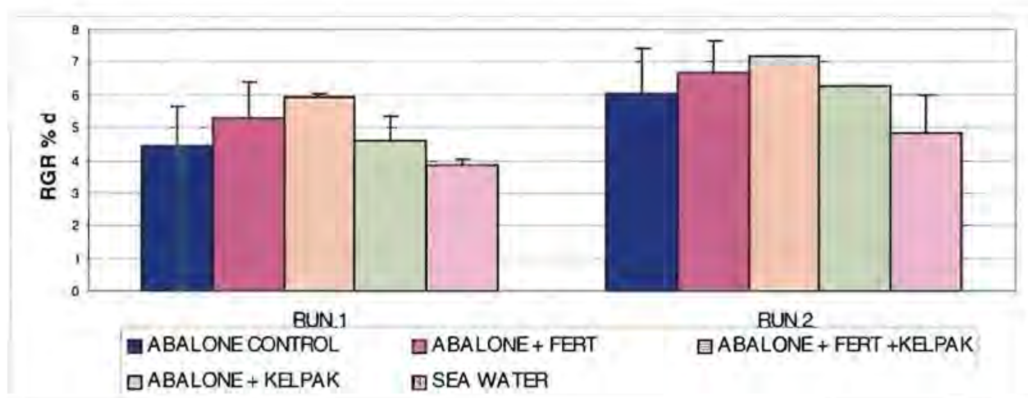

Figure 11.7: The effect of additions of Kelpak® and fertilizer in stand alone or combined in abalone effluent on SGR (% d^{-1}) on *Ulva lactuca* at JSP, from 30th July to 27th August 2002. Bars show standard deviations (n = 3).

The ratio of N:P for this experiment was higher than that for the turbot runs. Run 1 had values of 9.9 – 12.5. While run 2 the values increase to a range of 15.5 – 20.3, with the seawater treatment having the highest ratio in both runs.

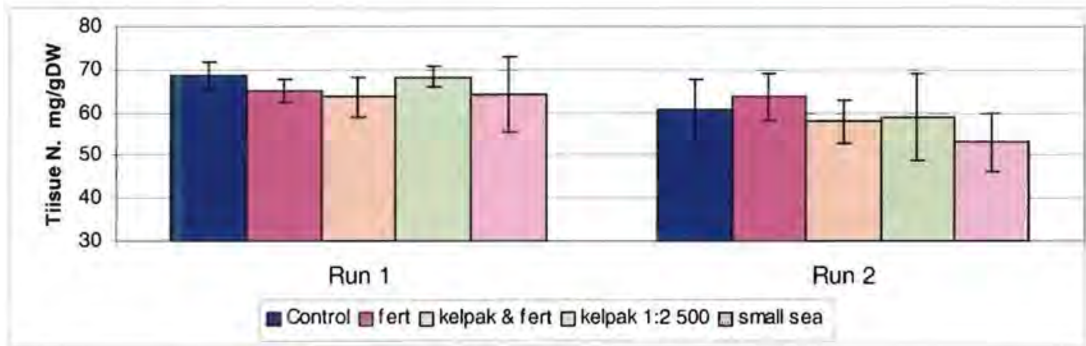

Figure 11.8: The effect of various combinations of Kelpak ® and fertilizer in abalone effluent on tissue N (mg N per g) *Ulva lactuca* at JSP. Bars show standard deviations (n = 3).

Tissue P also decreased on the second run (Figure 11.9). Seawater had a significantly lower tissue P than the other treatments (ANOVA; df = 20; $p < 0.01$; LSD post-hoc test, $p < 0.01$).

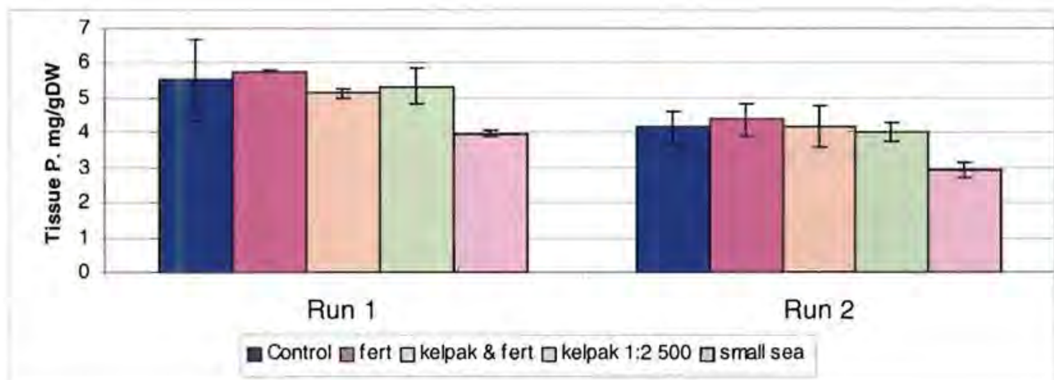

Figure 11.9: The effect of various combinations of Kelpak ® and fertilizer in abalone effluent on tissue (P mg P per g) *Ulva lactuca* at JSP. Bars show standard deviations (n = 3).

11.4 DISCUSSION

To our knowledge, this is the first study of the effect of seaweed concentrate on seaweed growth. This despite the beneficial effect of seaweed concentrate on the growth and yield of field crops which has been documented for the past 30 years. In land based agriculture and horticulture, dilute extracts of seaweed are applied to promote growth, prevent pests and diseases and improve the quality of the product (Finnie & van Staden, 1985).

Finnie & van Staden, (1985) demonstrated that the concentration ratio of *Ecklonia maxima* kelp extract is an important factor in controlling its efficiency. In tomato plants, strong concentrations (1:100 seaweed extract: water) were found to have an inhibitory effect upon root growth, whereas weak concentrations (1:600) were stimulatory. This could be due to growth inhibiting substances in the concentrate. The Kelpak ® concentrations used in this study fall within the range commonly used in land plant studies.

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Treatment with 1: 5 000 Kelpak ® concentration did not significantly increase SGR of the seaweed. This result is inconsistent with Leitao (2000), but consistent with Beckett & van Staden (1990), who showed that the growth of wheat was not stimulated by very low concentrations (1: 1000 retail product) of Kelpak® compared to the controls. The 1:2 500 Kelpak® concentration caused the highest SGR increase compared to the control. This is consistent with reports that Kelpak® at a concentration of 1:2 500 applied regularly, improved the total biomass of *Beta vulgaris* and *Phaseolus vulgaris* (Crouch, 1990) the SGR of *Gracilaria gracilis* (Leitao, 2001) and the root growth of cucumber plants (Nelson & van Staden, 1984).

A pronounced inhibitory effect was observed using a 1: 500 concentration. This effect was also found by Leitao (2001). Finnie & van Staden (1985) also reported inhibition of tomato roots at this concentration.

Our experiments in abalone and turbot water indicate that additions of both fertilizer and Kelpak ® (1:2 500 concentration) significantly increase SGR above that of effluent or seawater controls. They also indicate that in both abalone and turbot effluent a combination of Kelpak ® (1:2 500 concentration) and fertilizer significantly increased SGR over all the treatments. A possible reason for this is that the Kelpak ® is supplying PGH's and the fertilizer is providing additional nutrients that are not present in the effluent media.

Tissue N and P values decreased on the second runs, where SGR increased. This relationship follows that described by Rosenberg & Chapman, 1984; Duke *et al.* 1986; 1987 & 1989 and Chapter 9, with an increase in SGR causing a decrease in tissue nitrogen. This is due to the faster SGR requiring more nutrients.

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11.5 CONCLUSIONS

The results of the Kelpak ® investigation confirm previous findings on the effect of seaweed extract applications on certain field crops (Feantonby-Smith & van Staden, 1983). The reasons for the increase in SGR are not understood, but it is thought that the hormonal content, particularly cytokinin, plays an important role (Feantonby-Smith & van Staden, 1983; 1987). Other studies have looked at the effects of additions of cytokinin (Blunden & Wildgoose, 1977) and synthetic cytokinin (Finnie & van Staden, 1985) on SGR and have come to the same conclusions.

The highest SGR was obtained using a concentration of 1: 2 500 of Kelpak ®. The weakest concentration used did not change SGR (1: 5000) and the highest concentration used is likely to inhibit SGR (1:500). This study has shown that Kelpak ® used in addition to fertilizer may have commercial potential in the seaweed mariculture industry.

Even though SGR of seaweeds have been shown to be higher in effluent water when compared to seawater, they can be increased by fertilizing the effluent medium with a pulse fertilization.

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CHAPTER 12

ECONOMICS

12.1 INTRODUCTION

This project has been concerned with the cultivation of *Ulva* in a commercial environment and as such the economics of the project are important. The big question the farm managers want to know is: "is this economically viable?"

This study formed part of a much larger project which included a feed aspect done by K Naidoo (in preparation) at JSP. One aspect of her project looked at the difference when feeding a rotation diet of 3 weeks kelp and 1 week *Ulva* and *Gracilaria* vs. a kelp only diet to abalone, and measuring the increase in weight and length over a 9 month period using the different diets. The abalone were grown on a commercial farm and thus the growth rates are commercial not experimental. The algae for the mixed seaweed diet was obtained from the excess *Gracilaria and Ulva* tanks in this study. In Chapter 9, we saw that the protein content of the *Ulva* had increased to levels above those found in the wild. This meant that the abalone receiving the mixed rotation diet were in effect obtaining a much higher protein diet when compared to the natural seaweed diet. This accounted for significant increase in K. Naidoo growth data for abalone receiving the mixed rotation diet.

The aim of this part of the study was to:

- calculate how much the increase in growth rates in abalone being fed a high protein would equate to in monetary terms
- work out how many seaweed tanks would be required to supply a 50 ton abalone farm with enough seaweed to maintain this diet.
- Work out a direct saving due to requiring less kelp.

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12.2 METHODS

Using the growth rates obtained from (Naidoo, in preparation) of a kelp only diet vs. a 3 week rotation diet, growth rates were calculated using growth models that agreed with the data.

The cost of an abalone was determined using the current export price and calculating the cost to produce backwards, knowing that the average time to export is 5 years on both farms.

12.2.1 Theoretical abalone growth rate increase

Using Naidoo's data and a growth curve calculation program called Simply Growth ®, the growth curves in Figure 12.1 were calculated. Tarr, 1995 calculated wild abalone growth curves using the von Bertalanffy growth curve equation and thus one of the theoretical curves was a von Bertalanffy growth curve. The wild abalone growth curve from Tarr (1995) was compared with our theoretical curves. Due to the exponential nature of the data two exponential curves were calculated using the lowest and highest increases in growth rates from Naidoo data.

12.2.2 Economics

To calculate what this weight gain means in monetary terms to an abalone farmer we have to calculate the average cost of a 100 g abalone. The weight used is 100 g as this is the standard export weight. The standard export price is $30 per kg live weight (Bennett 2001).

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12.3 RESULTS

12.3.1 Growth rates

The abalone growth data from the two diets (Table 12.1) clearly shows a higher growth rate using the rotation diet.

Table 12.1: Average differences in length (mm) and weight (g) of abalone when fed different diets over a 9 month period (data from Naidoo, in preparation, used with permission).

Variable	Diet	Initial	Final	% Increase	Monthly increase
Weight (g)	Kelp only	7.60	22.81	15.21	0.056 $g.d^{-1}$
	Rotation	7.83	27.78	19.95	0.073 $g.d^{-1}$
Length (mm)	Kelp only	34.45	49.24	14.79	0.055 $mm.d^{-1}$
	Rotation	34.74	52.59	17.85	0.06 $mm.d^{-1}$

A potential problem with Naidoo data is that her length and weight increases measured at 3 monthly intervals were exponential. This caused problems when trying to fit growth curves to the data as most curves assume that the rate of growth of an organism declines with an increase in size (Simply Growth, 2003). In order to combat this a number of different growth curves were calculated (Figure 12.1 and Table 12.2).

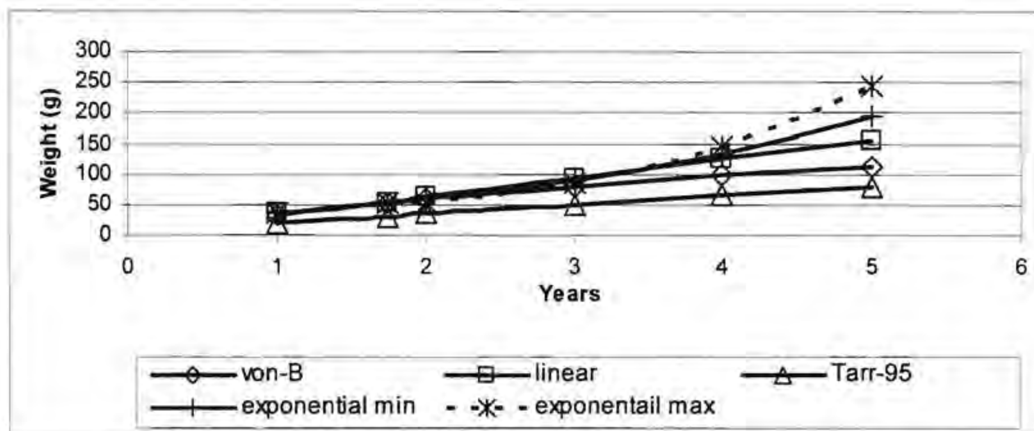

Figure 12.1: Theoretical growth curves of abalone fed the effluent enriched rotation seaweed diet vs. wild abalone von Bertalanffy growth from Tarr, (1995). Theoretical curves include von Bertalanffy growth Equation, Linear and two exponential curves. Equations and values shown in Table 12.2 (data from Tarr (1995) and Naidoo (in preparation).

Table 12.2: Growth curve parameters for *H. midae* derived from a maximum likelihood analysis of growth increment data. (Data from Tarr, 1995 and Naidoo, in preparation).

Name	K	L_∞	Equation	R^2
Von Bertallanffy (Tarr, 1995)	0.092	210.32	$dl / dt = K (L_\infty - l)$	0.732
Linear			$Y = 31.206 x$	0.896
Von Bertallanffy Naidoo, in preparation	0.227	163.29	$dl / dt = K (L_\infty - l)$	0.899
Exponential min			$Y = 24.727 e^{0.4147x}$	0.997
Exponential max			$Y = 20.612 e^{0.4945x}$	0.997

12.3.2 Economics

Cultured abalone are sold at around 5 years of age when their weight is approximately 100 g. The wild growth curve from Tarr (1995) illustrates this clearly and closely resembles I & J and JSP abalone growth rates. All of the theoretical curves calculated indicate that it takes approximately 3.3 – 3.6 years to reach 100 g if the abalone were fed the effluent enriched seaweed rotation diet.

The cost per kilo of live culture abalone varies from $ 30 – 35 per kilo. Using the lower cost price and calculating backwards we obtain the following:

Cost: $30 per kg X R 8 = R 240 (@R8 = 1US$)
10 (100g) abalone in 1 kg
cost per abalone = R 24
less profit and freight
= R 15 per abalone

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Using the two exponential growth curves (both the minimum (49 % increase in growth in a year) and maximum (68 % increase in growth in a year) and calculating the increase in weight from Naidoo (in preparation) figures we get the following figures:

Increase in SGR: 49 – 68 % per year
Normal Growth to 100g = 5 years
Accelerated growth = 3.3 – 3.6 years

At a cost of R 15 per abalone after 5 years if we can accelerate the growth by 1.7 – 1.4 years then we can save money on production. The saving on production very simply is R 1.50 or R 2.60 depending on the decrease in production time. This gives a new cost of R 12.40 – R 13.50 per 100g abalone, which is a saving of 10 – 17 %.

For a 50 ton abalone farm (that is a farm that sells 50 tons of 100g abalone in a year) this equates to a potential saving of:

Average farm (50 tons @ 100 g per abalone)
= 500 000 100g abalone
X R 1.50 or R 2.60
EQUALS a saving of between R 800 000 – R 1.3 million

This figure is actually an underestimate as we are not taking into account the increase in production from having a faster turn around time.

For a farm to cultivate enough seaweed we need to look at how much seaweed a 50 ton farm uses. Generally the food conversion ratio for abalone fed kelp is 10:1 (10 kg of kelp feed produces 1 kg of abalone) (based on I &J and JSP Kelp harvest and abalone biomass records). This means that a 50 ton farm requires 500 tons of seaweed in a year. Using a 4 week rotational diet as mentioned above the farm requires 125 tons of cultivated seaweed in a year. From this study we can cultivate approximately 7.2 tons from 12 tanks (those used on the I & J farm). To get a yield of 125 tons of seaweed the farm would require approximately 204 tanks. This would require an area of approximately 1 071 m^2, which is slightly less than the surface area covered by 1 Olympic swimming pool (50 X 25 m). The tanks used at I & J are expensive compared to other cultivation methods such as raceways. It has been suggested that productivity in a raceway system would be better, cheaper and a space saving option (Neori *et al.* 1998).

A ton of seaweed at January 2004 prices was R800 per ton. The farm using the above tank system could supply 125 tons of seaweed which means that the farm would require 125 tons less kelp. This equates to a direct saving of R 100 000.

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12.4. DISCUSSION

The monthly increases both in length and weight using the rotational diet (Naidoo in preparation) out rank the values for the best natural abalone diet obtained by Simpson & Cook (1998), in an experimental system.

A potential benefit of feeding P enriched seaweed is that shell growth can be increased. The I & J, JSP and HIK abalone farms all reported maximum growth rates in winter months (pers. com.) and it is possible that this is due to increased tissue P in the wild harvested seaweeds due to increases in the phosphate of the water (see Chapters 7 and 9).
Since the cultured abalones are sold live and by weight, shell weight is an important component in the weight of the abalone. Abalone can experience a 15 – 20 % weight loss due to fluid loss during export (Vosloo, 2003) and farms can lose up to 15 % of the purchase price of the abalone (Vosloo, 2003). If farmers can increase the shell component of the weight of the sold abalone which is normally around 35. 3 % (Vosloo, 2003) then the weight loss due to fluid loss can be minimized.

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12.5. CONCLUSIONS

There are both direct and indirect economic benefits to be obtained by an abalone farm when cultivating seaweed with a high protein content and feeding it as a rotation diet. Other benefits include the using the seaweeds as a biofilter and cleaning farm effluents, as well as reducing the amount of kelp required to feed the abalone.

By cultivating seaweeds on a farm the N and P contents of the seaweeds can be increased above the levels in the wild, thus improving the natural seaweed diet and making management of feed for the abalone easier.

CHAPTER 13

CONCLUSIONS, BEST MANAGEMENT PRACTICES (BMP's)

13.1 CONCLUSIONS

The *in situ* cultivation of seaweeds for abalone fodder is not a widespread practice in South Africa, with only 3 farms participating (Bennett, 2001). With the future expansion of the abalone industry (Bennett, 2001), kelp resources are going to be placed under increasing pressure for harvest, in fact the maximum sustainable yield of some kelp beds was reached in 2002 (Anderson, 2003). The increase in Harmful Algal Blooms (HAB's) (Pitcher, 1998) mean that at times farms may have to be separated from the sea and move to recirculation for short periods. Both farms that we worked on are also limited by pumping capacity and high associated costs of pumping. Recirculation within the farm is a way to increase capacity while still maintaining current pumping costs.

This project was designed to answer two main questions relating to *Ulva* cultivation on an abalone farm, depending on whether the seaweeds were to be grown as an additional food source or for bioremediation. The first aim related to *Ulva* as a food source for abalone and questions such as seasonal growth rates and yields and food quality needed to be investigated. The second aim was to investigate whether *Ulva* could be grown in effluent media and what were its biofiltering capabilities are, and how to improve this. This project answered many of these questions.

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There are a number of reasons that *U. lactuca* is a good candidate for cultivation as abalone fodder.

- It grows vegetatively and therefore doesn't go into a reproductive state causing heavy biomass losses through propagule loss.
- It has a high SGR and yields which can be considerably increased with a combination of Fertilizer and Kelpak (1:2 500 dilution).
- It very seldom had problems with epiphytic growth except *M. strangulans*, which can be controlled with a good shading and fertilization regime.

- *Ulva* has high nutrient uptake rates that make it ideal as a biofilter in a polyculture system.
- It has good nutritional value for young abalone and its nutritional value can be increased using fertilizer, Kelpak® and shading in combination or separate and this has positive benefits for abalone SGR.
- It is a relatively low maintenance species to cultivate.

13.2 BEST MANAGEMENT PRACTISES FOR CULTIVATION AS FODDER

There is a seasonal trend in *Ulva* cultivation with an increase in the SGR in summer and a decline in winter (Figure 8.1).
Yields and SGR can be increased further by using pulse fertilization and an effluent culture medium (Chapter 11). Maximum summer yields at I & J were 0.35 $kg.wwt.m^{-2}.d^{-1}$ and in winter were 0.12 $kg.wwt.m^{-2}.d^{-1}$ (Chapter 8, Table 6.2). Theoretically, these yields can be increased by 15.6 % by using a combination of fertilizer and Kelpak ® or increased by 7.2 % using just fertilizer in the abalone effluent (calculated from the experiments in Chapter 11, Figure 11.7). This means that the total yields from the abalone treatments obtained in this experiment (210 $kg.wwt.m^{-2}.month^{-1}$ in summer and 72 $kg.wwt.m^{-2}.month^{-1}$ in winter) could be increased to (724 $kg.wwt.m^{-2}.month^{-1}$ in summer and 248 $kg.wwt.m^{-2}.month^{-1}$ in winter) using a combination of 12 volume exchanges per day of Fertilized abalone effluent and Kelpak® in the 12 I & J tanks used.

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Tissue nitrogen and protein are also increased above levels found in wild harvested *Ulva* (3.7 – 24 % depending on season that was tested: Nisizawa *et al.* 1987; Simpson, 1994; Simpson & Cook 1998 and Wilkinson, 2001) to 30 – 36.6 % protein depending on the effluent media used. This increased range is ideal for abalone, which require 30 – 40 % protein in their diet (Hahn, 1989; Mai *et al.* 1995; Britz & Hecht, 1997). Total protein value of *Ulva* grown in Turbot effluent was 36.6 % (Chapter 9) but can be increased to 49.8 % using both fertilizer and Kelpak ® (calculated from N values in Chapter 11, Figure 11.4). These protein values are consistent with those found by Goldberg *et al.* 1998 who obtained tissue protein levels in *Ulva* of 44 % which was cultivated in turbot

effluent culture. Figure 9.9 (the relationship between thallus colour and tissue nitrogen) could be a useful tool for farm management to determine the feed quality of the *Ulva*.

By changing the ratios of N:P in the fertilizer nutrient limitation could possibly be avoided. Increasing the P ratio could have further benefits in promoting abalone shell growth rates.

Shading of the culture needs to be carried out from September to early February using a 20 % shade cloth. In Chapter 8, shading with both 80 and 50 % shade cloth resulted in lower SGR in unshaded tanks. Shading has three potential benefits: 1) it reduces the biomass of tank fouling algae, 2) it reduces the temperature and pH in the tanks due to a decrease in photosynthesis this reduces the possibility of carbon limitation occurring, 3) It can increase tissue nitrogen content of the thalli.

Yields obtained in this study are lower than those reported in literature by 3 - 5 % day^{-1}, but smaller tanks were used in those studies (Duke *et al.* 1986, 1987,1989; Goldberg *et al.* 1998). There was a clear decrease in yields when scaling up tanks sizes from JSP to I & J, and this could be the reason for the lower yields in our study compared to those in the literature.

Results of Njobeni (in preperation) show that *Gracilaria* has a seasonal growth rate with maximal growth in winter at I & J, this was also seen in farm harvest records (H. Otto pers. Comm.) therefore by growing a combination of *Ulva* and *Gracilaria* one can account for seasonal growth in seaweeds and maintain a constant yield. This is very important when trying to secure a food source for the abalone.

A good management practice would be to rotate the harvest in a quasi-commercial system around a 4-week period and have four sets of tanks that are harvested once every four weeks. This would ensure a constant food source for the farm.

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As *M. strangulans* can be eaten by abalone (Nash *et al.* 1995) there is no problem with it occurring on the *Ulva* thalli. However, with the negative correlation between *M. strangulans* and SGR (Chapter 8) and the accompanying thallus lightening and decreasing protein value of the thalli during a severe infection, this decreases its value as a food source. Thus, dealing with infection needs to be a part of farm management. In spring and summer when outbreaks are more common, shading of the tanks will help, but the most important farm management option is recognition of an outbreak and prevention of spread. If an outbreak is occurring then the tanks should be pulse fertilized, at night so as to reduce the pH and temperature stress on the algae. This was because fertilization reduced the amount of *M. strangulans* on *Ulva* thalli (Chapter 8, Figure 8.2).

At water exchange rates of 4 volume exchanges per day the algae were both carbon and nitrogen limited (Chapters 6, 7, 8 and 10 respectively). Water volume exchange rates of 12 volume exchanges per day appear sufficient to sustain *Ulva* SGR (Chapter 8) and although 20 volume exchanges increased SGR, the associated pumping costs may be too high and the alga receives very little benefit from the additional nutrient availability. Uptake efficiency also decreases with an increase in water exchange rate (Mata & Santos 2003).

At both farms and in all treatments (including the fertilizer treatment) the algae were P limited until early winter. This P limitation could be dealt with by increasing the P ratio in the fertilizer, thus having a ratio of 8 part Maxiphos to 1 part ammonium sulphate. The fertilizer amount could also be reduced to 80 g of fertilizer per 10 kg of seaweed, for the first growth periods and then increased to 120 g for the second growth period, to take into account the change in biomass due to growth. The very high surge uptake in the seaweed in the fertilized treatment is probably due to the high concentration of nutrients and one would still want this uptake to occur, but there is a large amount of nutrient that is not absorbed by the seaweeds and just leaves the system. Thus, reducing the fertilizer amount at the beginning of the growth period would minimize the nutrient loss, while increasing it towards the end of the growth period would take into account the higher biomass and possibly the higher

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nutrient trigger required for surge uptake. Tanks also need to be shaded during fertilization so as to reduce photosynthesis: the lower pH and temperature will minimize the stress that the algae are under during periods of fertilization.
Optimal stocking density appears to increase with an increase in tank size and may also vary with season, increasing in summer and decreasing in winter due to the changes in light availability. However, the most important point to note is that stocking density is system specific and thus needs to be experimented within each system.

13.3 BEST MANAGEMENT PRACTISES FOR CULTIVATION AS A BIOFILTER

The aims behind growing the alga as a biofilter differ to growing it as a food source. The principle aim behind a biofilter is to maximize nutrient uptake in the effluent water, thus additions of nutrients like fertilizer would be detrimental to the system. Benefits from having *Ulva* as a biofilter in a recirculation system include:

- Uptake of nutrients such as ammonium, ammonia, phosphate, nitrate and nitrite.
- Reduction in CO_2 due to photosynthesis and
- Increase in dissolved oxygen during the day
- Increase in the amount of ammonia volatized due to seaweed tank depth and aeration (Boyd, 1990)
- Surplus seaweed can be used as an additional food source for abalone.

Two potential disadvantages are:

- Decrease in dissolved oxygen and an increase in CO_2 production at night due to respiration by the algae
- Elevated temperature and pH caused by photosynthesis during day

The last point however is minor as temperature and pH can be reduced using shading. Depending on the lag during pumping water from the seaweeds to the

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abalone, an increased temperature at night, while the abalone are feeding, would be beneficial.
As yields and tissue nitrogen are not important considerations for a biofilter, vs fodder farm management practices do not need to take these factors into account when cultivating the algae for this purpose.

The shading regime is the same used when cultivating for fodder.

A good management practice would be to rotate the harvest in a quasi-commercial system around a 4-week period and have four sets of tanks that are harvested once every four weeks. This would ensure a constant biomass on the farm that is able to deal with the wastes produced by the farm.

M. strangulans infections and their potential impact on reducing the biofiltering capacity of the *Ulva* means that it must be dealt within management. As with cultivation for fodder, in spring and summer when outbreaks are more common, shading of the tanks will help, but the most important farm management option is recognition of an outbreak and prevention of spread. Again if an out break occurs the tank should be isolated from the system and pulse fertilized.

At water volume exchange rates of 12 volume exchanges per day, *Ulva* takes up to 90 % ($\pm$ 5) of the ammonium during the day and 80 % ($\pm$ 6) at night while at 20 volume exchanges 70 % ($\pm$ 7) of the ammonium is removed during the day and 30 % ($\pm$ 5) at night using a stocking density of 2 kgwwt.m^{-2}. This shows the decreased uptake efficiency at high water exchange rates and the associated pumping costs at high exchange rates are an ineffective option.

If it is possible to remove the water exiting the tanks from the recirculation system then the tanks should be pulse fertilized using the fertilizer regime described above. The tanks would need to be isolated from the recirculation system for 36 hours after fertilization on a 12 volume exchange rate system as this is the time required for the fertilizer to dilute to background values.

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Stocking density for quasi-commercial tanks like I & J's, for optimum uptake of ammonium and decreased production of ammonia is 3 $kg.m^{-2}$. So keeping the system running at between 2 – 3.5 $kg.m^{-2}$ stocking density should result in maximal uptake. Uptake may vary with increasing tank size and season, increasing in summer and decreasing in winter due to the changes in light availability. However, the most important point to note is that stocking density is system specific and thus needs to be experimented with in each system.

13.4 UNANSWERED QUESTIONS

As with any study, many more questions were raised. These questions need further investigation to understand fully the relationships between integrated abalone and seaweed aquaculture especially on this system.

Questions that were raised during this study are:

- Is the change in species dominance between *U. capensis* and *U. lactuca* due to adaptations to different light regimes?
- Does the relationship between tissue nitrogen and thallus colour hold up under laboratory conditions to be able to provide a conclusive guide for farmers?
- Is there any change in isotopes due to the different nutrient sources? Are the seaweeds obtaining the nitrogen from different sources and will this have consequences when used as a biofilter.
- Can limpets be used to control tank fouling algae?
- What do the different meso-herbivores consume and is this amount significant?

Last but not least and perhaps the most important question

- Is it possible to fully integrate seaweed and abalone cultivation in a re-circulating system?

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AND

REFERENCES

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University of Cape Town

References

Adams, N. M. 1994. Seaweeds of New Zealand. Canterbury University Press, Christchurch, New Zealand.

Ajisaka, T. and Chiang, Y. M. 1993. Recent status of *Gracilaria* cultivation in Taiwan. *In:* Chapman, A.R.O., Brown, M.T. and Lahaye, M. (eds.). Fourteenth International Seaweed Symposium. pg 260 – 261.

Altamirano, M.; Flores-Moya, A.; Conde, F. & Figueroa, F. L. 2000a. Growth seasonality, photosynthetic pigments and carbon and nitrogen content in relation to environmental factors: a field study of *Ulva olivascens* (Ulvales, Chlorophyceae). Phycologia. **39** (1): pg 50 – 58.

Altamirano, M.; Flores-Moya, A. & Figueroa, F. L. 2000b. Long-term effects of natural sunlight undervarious ultraviolet radiation conditions on growth and photosynthesis of *Ulva rigida* (Chlorophyceae) cultivated *in Situ.* Bot. Mar. **43**: pg 119 – 126.

Anderson, R.C., Smit, A.J. & Bolton, J.J. 1998. Differential grazing effects by isopods on *Gracilaria gracilis* and epiphytic *Ceramium diaphanum* in suspended raft culture. Aquaculture **169**: pg 99 – 109.

Anderson. R. 2003. Seaweed. In Research highlights 2001 – 2002. Department of Environmental Affairs and Tourism and Marine and Coastal Management. Cape Town. pg 31 – 32.

Andrews, W. R. H. and Hutchings, L. 1980. Upwelling in the Southern Benguela Current. Prog. Oceanog. **9**: pg 1 – 81.

Astronomical Applications, U. S. Naval Observatory. Washington, D. C. http://riemann/mach.usno.navy.mil/AA/

Bardach, J. E.; Ryther, J. H. & McLarney, W. O. 1972. Aquaculture – the farming and husbandry of freshwater and marine organisms. John Wiley & Sons. Inc. pg. 777 – 814.

Barkai, R & Griffiths, C. L. 1986. Diet of the South African abalone *Haliotis midae.* S. Afr. J. Mar. Sci. **4**: pg 37 – 44.

Beach, K. S.; Smith, C. M.; Michael, T. & Shin, H. 1995. Photosynthesis in reproductive unicells of *Ulva fasciata* and *Enteromorpha flexuosa:* implications for ecological success. Mar. Ecol. Prog. Series. **125**: pg 229 – 237.

Beckett, R. P. and Van Staden, J. 1989. The effect of seaweed concentrate on the growth and yield of potassium stressed wheat. Plant and Soil. **116**: pg 20 – 36.

University of Cape Town

Beer, S.;Larsson, C.; Poryan, O. & Axelsson, L. 2000. Photosynthetic rates of *Ulva* (Chlorophyta) measured by pulse amplitude modulated (PAM) fluorometry. Europ. J. Phycol. **35**: pg 69 – 74.

Bekheet, I. A.; Kandil, K. M. & Shaban, N. Z. 1984. Studies on urease extracted from *Ulva lactuca*. Hydrobiologia. **116/117**. pg 580 – 583.

Bennett, T. 2002. Situation report on abalone aquaculture business. AFASA. 6th Conference of the Aquaculture Association of southern Africa. Stellenbosch.

Bidwell, R. G. S.; McLachlan, J. & Lloyd, N. D. H. 1985. Tank cultivation of Irish moss, *Chondrus crispus* Stackhouse. Bot. Mar. **28**: pg 87 – 97.

Bird, K. T.; Habig, C. and Debusk, T. 1982. Nitrogen allocation and storage patterns in *Gracilaria tikvahiae* (Rhodophyta). J. Phycol. **18**: pg 344 – 348.

Björk, M.; Haglund, K.; Ramazanov, Z. & Pedersén, M. 1993. Inducible mechanisms for HCO_3^- utilization and repression of photorespiration in protoplasts and thalli of three species of *Ulva* (Chlorophyta). J. Phycol. **29**: pg 166 – 173.

Björnsäter, B. O. & Wheeler, P. A. 1990. Effect of nitrogen and phosphorus supply on growth and tissue composition of *Ulva fenestrata* and *Enteromorpha intestinalis* (Ulvales, Chlorophyta). J. Phycol. **26**: pg 603 – 611.

Blackman, F. F. & Tansley, A. G. 1902. A revision of the classification of the green algae. New Phytologist. **1**: 17 – 24, pg 133 – 144.

Bliding, C. 1963. A critical survey of European taxa in Ulvales. Part I: *Capsosiphon, Percursaria, Blidingia, Enteromorpha*. Opera Botanica a societate Lundensi. **8**: pg 1 – 160.

Bliding, C. 1968. A critical survey of European taxa in Ulvales. Part II: *Ulva, Ulvaria, Monostroma, Kornmannia*. Botaniska Notiser. **121**: pg 535 – 629.

Blunden, G. & Wildgoose, P. B. 1977. The effects of aqueous seaweed extract on sugar beet. J. of the Science of Food and Agriculture. **28**: pg 121 – 125.

Bold, H. C. & Wynne. M. J. 1985. Introduction to the algae 2nd ed. Prentice Hall, New Jersey. Pg 940.

Bonneau, E. R. 1977. Polymorphic behaviour in *Ulva lactuca* L. (Chlorophyceae) in anexic culture. I. Occurrence of *Enteromorpha* – like plants in haploid clones. J. Phycol. **13**: pg 133 – 140.

Bonneau, E. R. 1978. Asexual Reproductive Capabilities in *Ulva lactuca* L. (Chlorophyceae). Bot. Mar. **21**(2): pg 117 – 121.

University of Cape Town

Boyd, C. E. 1990. Water Quality in ponds for aquaculture. Ala. Agr. Exp. Sta. Auburn Univer. Ala. 462 pg.

Boyd, C. E. 1998. Water Quality for pond aquaculture. Research and Development series. 43. Ala. Agr. Exp. Sta. Res. And Dev. Series. No. **43**. 36 pg.

Boyd, A. J.; Tromp, B. B. S. and Horstman, D. A. 1985. The hydrology off the South African South Western coast between Cape Point and Danger Point in 1975. S. Afr. J. Mar. Sci. **3**: pg 145 – 168.

Bradley, P. M. Plant hormones do have a role in controlling growth and development of algae. J. Phycol. **27**: pg 317 – 321.

Brawley, S. H. & Fei, X. G. 1987. Studies of meisoherbivory in aquaria and an un-barricaded mariculture farm on the Chinese coast . J. Phycol. Vol. **23**: pg 614 – 623

Brett, J.R. 1979. Environmental factors and growth., *in*: Hoar, W.S., Randall, D.J., & Brett, J.R. (eds). Fish Physiology, New York, NY: Academic Press. Vol. **8**. pg. 599–675.

Britz, S. J. & Briggs, W. R. 1976. Circadian rhythms of chloroplast orientation and photosynthetic capacity in *Ulva*. Plant physiology. **58**: pg 22 – 27.

Britz, P. J. 1996. The Nutritional requirements of *Haliotis midae* and development of a practical diet for abalone aquaculture. PhD Thesis. Rhodes University. South Africa. 150 pg.

Britz, P. J. & Hecht, T. 1997. Effect of dietary protein and energy level on growth and body composition of the South African abalone, *Haliotis midae*. Aquaculture. **140**: pg 63 – 73.

Burrows, E. 1971. Assessment of pollution effects by the use of algae. Proc. Roy. Soc. London. (B) **177**: pg 295 - 3O6.

Burrows, E. 1991. Seaweeds of the British Isles: Volume 2. Chlorophyta. London. British museum of Natural history. 238 pg.

Buschmann, A. H. 1996. An introduction to integrated farming and the use of seaweeds as biofilters. Proceedings of the 15th International Seaweed Symposium. **15**: pg 326 – 327.

Campbell, S. J. 1999. Uptake of ammonium by four species of macroalgae in Port Phillip Bay, Victoria, Australia. Mar. Freshwater. Res. **50**. pg 515 – 522.

Capinpin, E. C. & Corre, K. G. 1996. Growth rate of the Philippine abalone, *Haliotis asinine* fed an artificial diet and macroalgae. Aquaculture. **144**: pg 81 – 89.

University of Cape Town

Carter, R. A. 1990. Abalone: Culture methods. In Perlemoen farming in South Africa. Proceedings of a workshop convened by The Mariculture Association of Southern Africa. Ed. P. Cook. The mariculture Association of Southern Africa. Cape Town. pg 7 – 19.

Castro-Gonzales, M.I., Perez-Gil Romo, F., Perez-Estrella, S. and Carillo-Dominguez, S.D. 1996. Chemical composition of the green alga *Ulva lactuca* . Ciencas Marinas **22**: pg 205 – 213.

Chaiang, Y. M. 1981. Cultivation of *Gracilaria* (Rhodophyta, Gigartinales) in Taiwan. Proceedings of the 10th International Seaweed Symposium. **10**: pg 569 – 573.

Chapman, P. and Shannon, L. V. 1985. The Benguela ecosystem part II. Chemistry and related processes. Oceanogr. Mar. Biol. Ann. Rev. **23**: pg 183 – 251.

Chopin, T. and C. Yarish. 1998. Aquaculture does not only mean finfish monoculture...seaweeds must be a significant component for an integrated ecosystem approach. Bull Aquacul. Assoc. Canada. pg 98 – 33.

Chopin, T.; Yarish, C.; Wilkes, R.; Belyea, E.; Lu, S. & Mathieson, A. 1999. Developing *Porphyra/*salmon integrated aquaculture for bioremidiation and diversification of the aquaculture industry. J. Applied Phycol. **11.** pg 463 – 472.

Chopin, T.; Yarish, C.; Belyea, E. & Wilkes, R. 1999. Sustainable integrated fish/nori aquaculture for bioremediation and production of food and biochemicals. J. Phycol. , **35** (3 SUPPL.): 8 – 18.

Chopin, T. C. Yarish R. Wilkes S. Lu and Mathieson A. C.. 1999. Developing Porphyra/salmon aquaculture for bioremediation and diversification of the aquaculture industry. J Applied Phycol. **11**(5): pg 463 – 472.

Chopin, T.; Yarish, C.; & Parsons, G. J. 1999. Seaweeds must be a significant component of aquaculture for an integrated ecosystem approach. Proceedings of the Workshop on Coldwater Seaweed Aquaculture held at Aquaculture Canada '98., Mar, , Bulletin of the Aquaculture Association of Canada.St.Andrews NB. **99** - 1, pg.35 – 37.

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.

Cohen, I. & Neori, A. 1991. *Ulva lactuca* Biofilters for marine Fishpond effluents: I: Ammonium uptake kinetics and nitrogen content. Bot. Mar. **34**: 475 – 482.

University of Cape Town

Collén, J.; Del Rio, M. J.; García-Reina, G. & Pedersén, M. 1995. Photosynthetic production of H_2O_2 by *Ulva rigida* C. Ag. (Chlorophyta). Planta: **196**: pg 225 – 230.

Collén, J. & Pedersén, M. 1996. Production, scavenging and toxicity of H_2O_2 in the green seaweed *Ulva rigida*. Eur. J. Phycol: **31**: pg 265 – 271.

Correa, J. A. 1996. Diseases in seaweeds: an introduction. In Lindstrom, S. C. & Chapman, D. J. (ed.). Proceedings of the 15th International Seaweed Symposium. Kluwer Academic, London: pg 89 – 96.

Correa, J. A. & Sánchez, P. A. 1996. Ecological aspects of algal infectious diseases. In Lindstrom, S. C. & Chapman, D. J. (ed.). Proceedings of the 15th International Seaweed Symposium. Kluwer Academic, London: pg 97 – 106.

Craigie, J. S. & Correa, J. A. 1996. Etiology of infectious diseases in cultivated *Chondrus crispus* (Gigartinales, Rhodophyta). In Lindstrom, S. C. & Chapman, D. J. (ed.). Proceedings of the 15th International Seaweed Symposium. Kluwer Academic, London: pg 97 – 104.

Critchley, A. T. 1993. *Gracilaria* (Rhodophyta, Gracilariales): An economically important agarophyte. In: Ohno, M. & Critchley (eds.), Seaweed cultivation and marine ranching. JICA. pg 89 – 112.

Crouch, I. J. 1990. The effect of seaweed concentrate on plant growth PhD. Thesis. Department of Botany, University of Natal. South Africa.

Day, E. 1998. Ecological interactions between abalone (*Haliotis midae*) juveniles and echinoids (*Parechinus angulosus*) off the southwest coast of South Africa. Ph.D. Thesis. UCT.

De Boer, J. A. 1981. Nutrients. *In*: The biology of seaweeds. Lobban, C. S. & Wynne, M. J. (eds.). Botanical monographs Vol. 17. Blackwell scientific publications. Oxford. Pg 356 – 392.

De Busk, T. A., Blakeslee, M. & Ryther, J. H. 1986. Studies on the outdoor cultivation of *Ulva lactuca* L. Bot. Mar. **29**(5): pg 381 – 386.

De Casabianca, M. –L. & Posada, F. 1998. Effect of environmental parameters on the growth of *Ulva rigida* (Thau Lagoon, France). Bot. Mar. **41**: pg 157 – 165.

Del Campo, E.; Garćia-Reina, G. and Correa, J. A. 1998. Degradative tissue disease in *Ulva rigida* (Chlorophyceae) associated with *Acrochaete geniculata* (Chlorophyceae). J. Phycol. **34**: pg 160 – 166.

Dickinson, C. I. 1963. British Seaweeds. Frome. Londen.

University of Cape Town

Duke, C. S., Lapointe, B. E. & Ramus, J. 1986. Effects of irradiance on growth, RuBPCase activity and chemical composition of *Ulva* species (Chlorophyta). J. Phycol. **22**(3): pg 362 – 370.

Duke, C. S., Litaker, W. & Ramus, J. 1987. Seasonal variation in RuBPCase activity and N allocation in the Chlorophyte seaweeds *Ulva curvata* (Kutz.) and *Codium decorticatum* (Woodw.) Howe. J. Exp. Mar. Biol. Ecol. **112**: pg 145 – 164.

Duke, C. S., Litaker, W. & Ramus, J. 1989a. Effects of the temperature, nitrogen supply and tissue nitrogen on ammonium uptake rates of the Chlorophyte seaweeds *Ulva curvata* and *Codium decorticatum*. J. Phycol. **25**: pg 113 – 120.

Duke, C. S., Litaker, W. & Ramus, J. 1989b. Effect of temperature on nitrogen limited growth rate and chemical composition of *Ulva curvata* (Ulvales, Chlorophyta). Mar. Biol. (Ber). **100**: pg 143 – 150.

Edward Lee. R. 1999. Phycology. 3rd Ed. Cambridge University Press. Cambridge. Pg. 176 – 185, 207 – 233.

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): pg 2379 – 2383.

Eppely, R. W. 1962. Hydrolysis of polyphosphates by *Porphyra* and other seaweeds. Physiol. Plant. **15**: pg 246 – 51.

Evans, G. C. 1972. The quantitative analysis of plant growth. Studies in Ecology. Blackwell Scient. Pupl. Oxford. pg 247 – 254.

Fallu, R. 1991. Abalone farming. Fishing news books. Oxford. 195 Pg.

Featonby-Smith, B. C. & van Staden, J. 1983. The effect of seaweed concentrate on the growth of tomatoes in nematode infested soil. Sci. Hortic. **20**: pg 137 – 146.

Featonby-Smith, B. C. & van Staden, J. 1987. Effects of seaweed concentrate on grain yield in barely. S. Afr. J. Bot. 53: pp 125 – 128.

Fillit, M. 1995. Seasonal changes in photosynthetic capacities and pigment content of *Ulva rigida* in a Mediterranean coastal lagoon. Bot. Mar. **38**: pg 271 – 280.

Finnie, J. F. & van Staden, J. 1985. Effect of seaweed concentrate and applied hormones on *in vitro* cultured tomato roots. J. of Plant Physiology. **120**: pg 215 – 222.

Fletcher, R. L. 1995. Epiphytism and fouling in *Gracilaria* cultivation: an overview. Journal of applied phycology. **7**: pg 325 – 333.

University of Cape Town

Fletcher, R. L. 1987. Seaweeds of the British Isles. Volume 3. Fucophyceae (Phaeophyceae). Part 1. British museum (Natural History). London. pg 112 – 115.

Fleurence, J., Le Coeur, C., Mabeau, S., Maurice, M. & Landrein, A. 1995. Comparison of different extractive procedures for proteins from the edible seaweeds *Ulva rigida* and *Ulva rotundata*. J. Applied Phycol. **7**: pg 577 – 582.

Fleurence, J. 1999. Seaweed proteins: biochemical, nutritional aspects and potential uses. Trends in Food Science & Technology **10**: pg 25 – 28.

Floreto, E. A. T.; Hirata, H.; Ando, S. & Yamasaki, S. 1993. Effects of temperature, light intensity, salinity and source of nitrogen on the growth, total lipid and fatty acid composition of *Ulva pertusa* Kjellman (Chlorophyta). Bot. Mar. **36**: pg 149 – 158.

Floreto, E. A. T.; Hirata, H.; Yamasaki, S. & Castro, S. C. 1994. Influence of light intensity on the fatty acid composition of *Ulva pertusa* Kjellman (Chlorophyta). Bot. Mar. **37**: pg 143 – 149.

Floreto, E. A. T. & Teshima, S. 1998. The fatty acid composition of seaweeds exposed to different levels of light intensity and salinity. *Bot. mar.* **41**: pg. 467 – 481.

Flores-Moya, A,; Fernández, J. A. & Niell, F. X. 1995. Seasonal variations of photosynthetic pigments, total C, N, and P content, and photosynthesis in *Phyllariopsis purpurascens* (Phaeophyta) from the Strait of Gibralter. J. Phycol. **31**: pg 876 – 874.

Friedlander, M. and Ben-Amotz, A. 1991. The effect of out door culture conditions on growth and epiphytes of *Gracilaria conferta*. Aquat. Bot. **39**: pg 315 – 333.

Friedlander, M. 1992. *Gracilaria conferta* and its epiphytes: The effect of culture conditions on growth. Bot. Mar. **35**: pg 423 – 428.

Fredericksen, O. T. 1987. The fight against eutrophication in the inlet of "Odense Fjord" by reaping sea lettuce (*Ulva lactuca*). *In*: (D. Athie and C. C. Cerri, eds.) *the use of Macrophytes in Water pollution control.* Pergamon Press, Exeter, U. K. pg 81 – 87.

Foster, G. L. 1914. Indications regarding the source of combined nitrogen for *Ulva lactuca*. Ann. M. Bot. Gard., **1**: pg 229 – 235.

Friedlander, M.; Galai, N. & Farbstein, H. 1990. A model of seaweed growth in an outdoor culture in Israel. Hydrobiologica. **201/205**: pg 367 – 373.

University of Cape Town

Friedlander, M. & Ben-Amotz, A. 1991. The effect of outdoor culture conditions on growth and epiphytes of *Gracilaria conferta*. Aquatic Botany. **39**: pg 3 – 4.

Friedlander, M. 1992. *Gracilaria conferta* and its epiphytes: The effect of culture conditions on growth. Bot. Mar. **35** : pg 423 – 428.

Friedlander, M. & Levy, I. 1995. Cultivation of *Gracilaria* in outdoor tanks and ponds. J. applied Phycol. **7**: pg 315 – 324.

Friedlander, M.; Gonen, Y.; Kashman, Y. & Beer, S. 1996. *Gracilaria conferta* and its epiphytes:3. Allelopathic inhibition of the red seaweed by *Ulva* cf. *lactuca*. J. applied Phycol. **8**: pg 21 – 25.

Fujita, R. M.; Wheeler, P. A. & Edwards, R. L. 1988. Metabolic regulation of ammonium uptake by *Ulva rigida* (Chlorophyta): a compartmental analysis of the rate limiting step for uptake. J. Phycol. **24**: pg 560 – 566.

Fujita, R. M. 1985. The role of Nitrogen status in regulating transient ammonium uptake and storage by macroalgae. J. of experimental marine biological ecology. **92**: pg 283 – 301.

Glenn, E. P.; Moore, D.; Fitzsimmons, K.; Azevedo, C. 1996. Spore culture of the edible red seaweed, *Gracilaria parvispora* (Rhodophyta). Aquaculture. vol. **142**, no. 1/2; pg. 59 - 74,

Grosholz, E. 2002. Ecological and evolutionary consequences of coastal invasions. *Trends in Ecology and Evolution* **17**(1): pg 22 – 27.

Goldberg, R.; Clark, P.; Wikfors, G. H. and Shpigel, M. 1998. Performance of *Ulva ridgida* as a biofilter in a flow-through mariculture system. J. of Shellfish Research. **17**(1): pg 345 – 355.

Grasshoff, K.; Ehrhardt, M. & Kremling, K. 1976. (eds.) Methods of seawater analysis. 2nd ed. Weinheim. Germany. 419 pg.

Guist, G. G. Jr. & Humm, H. J. 1976. Effects of sewage effluents on the growth of *Ulva lactuca*. Biol. Sci. **4**: pg 267 – 271.

Habig, C.; Debusk, T. A. & Ryther, J. H. 1984. The effect of nitrogen content on methane production by the marine algae *Gracilaria tikvahiae* and *Ulva* sp. Biomass. **4**: pg 239 – 251.

Hahn, K. O. 1989. CRC Handbook of culture of abalone and other marine gastropods. CRC Press. Inc. Boca Raton. pg 3 – 154.

Hampson, S. 1998. Evaluation of effluent water from an abalone mariculture system as a culture medium for two strains of *Gracilaria gracilis*. Unpublished MSc Thesis. University of Port Elizabeth. 155 pg.

University of Cape Town

Hanisak, M. D. & Ryther, J. H. 1984. Cultivation biology of *Gracilaria tikvahiae* in the United States. Hydrobiologica. **116/117**: pg 295 – 298.

Harlin. M. M.; Thorne-Miller, B. and Thursby, G. B. 1978. Ammonium uptake by *Gracilaria* sp. (Florideophyceae) and *Ulva lactuca* (Chlorophyceae) in closed system fish aquaculture. Proceedings of the 9th International Seaweed Symposium. **9**: pg. 285 – 292.

Hayden, H. S.; Blomster, J.; Maggs, C. A.; Silva, P. C.; Stanhope M. and Waaland J. R. 2003. Linnaeus was right all along: *Ulva* and *Enteromorpha* are not distinct genera. European J. of Phycol. **38**: pg 277-294

Hecht, T. 1994. Behavioral thermoregulation of the abalone, *Haliotis midae*, and the implications for intensive culture. Aquaculture. **126**: pg 171 – 181.

Henley, W. J. & Ramus, J. 1989. Photoaccumulation of *Ulva rotundata* (Chlorophyta) under natural irradiance. Marine Biology. **103**: pg 261 – 266.

Ho, Y. B. 1990. *Ulva lactuca* as bioindicator of metal contamination in intertidal waters in Hong Kong. Hydrobiologia. **203**: pg 73 – 81.

Hoek, C. van den, Mann, D. G., Jahns, H. M. 1995. Algae, An introduction to phycology. Cambridge University Press. Cambridge. Pg 390 – 408.

Hoeksema, B. W. and Hoek, C. van den. 1983. The taxonomy of *Ulva* (Chlorophyceae) from the coastal region of Roscoff (Brittany, France) Bot. Mar. **26**: pg 65 – 86.

Israel, A.; Friedlander, M. & Neori, A. 1993. Biomass, yield, photosynthesis and morphological expression of *Ulva lactuca*. Bot. Mar. **38**: pg 297 – 302.

Iversen, E. S. 1968. Farming the edge of the sea. Fishing News Books. London

Jimenez del Rio, M.; Ramazanov, Z. and Garcia-Reina, G. 1996. *Ulva rigida* (Ulvales, Chlorophyta) tank culture as biofilters for dissolved inorganic nitrogen from fishpond effluents. Hydrobiologia. **326/327** pg 61 – 67.

Joska, M. A. P. 1992. Taxonomy of *Ulva* species (Chlorophyta) in the South Western Cape, South Africa. Unpublished MSc Thesis. University of Cape Town. 126 pg.

Kandjengo, L. 2000. The effects of temperature and light on three South African *Ulva* species, and their potential in integrated aquaculture. Honours Thesis. University of Cape Town. South Africa. 31pg.

University of Cape Town

Kandjengo, L. 2002. The Molecular systematics of *Ulva* Linnaeus and *Enteromorpha* Link (Ulvales, Chlorophyta) form the South Western Cape, South Africa. Masters Thesis. University of Cape Town. South Africa. 80 pg.

Kapraun, D. F. 1970. Field and actual studies of *Ulva* and *Enteromorpha* in the vicinity of Port Aransas, Texas. Contributions in marine science. **15**: pg 205 – 285.

Koeman, R. P. T. and Van Den Hoek, C. 1981. The taxonomy of *Ulva* (Chlorophyceae) in the Netherlands. British Phycological Journal. **16**: pg 9 – 53.

Koeman, R. P. T. and Van Den Hoek, C. 1984. The taxonomy of *Enteromorpha* Link, 1820 (Chlorophyceae) in the Netherlands. III. The sections *Flexuosae* and *Clathratae*. Cryptogramie: Algologie **5**: pg 21 – 61.

Kong, M. K. & Chan, K. 1979. A study on the bacterial flora isolated from marine algae. Botanica marina. Berlin. **22**: pg 83 – 97.

Kremer, B. P. 1981. Carbon metabolism. *In*: The biology of seaweeds. Lobban, C. S. & Wynne, M. J. (eds.). Botanical monographs Vol. **17**. Blackwell scientific publications. Oxford. pg 493 – 533.

Langton, R.W.; Haines, K.C.; Lyon, R.E. 1977. *Ammonia-nitrogen production by the bivalve mollusc Tapes japonica* and its recovery by the red seaweed *Hypnea musciformis* in a tropical mariculture system. Helgol. Wiss. Meeresunters, **30**(1-4), pg 201 – 213.

Lapointe, B. E.; Williams, L. D.; Goldman, J. C. & Ryther, J. H. 1976. The mass outdoor culture of macroscopic marine algae. Aquaculture. **8**: pg 9 – 21.

Lapointe, B. E. & Ryther, J. H. 1979. The effects of nitrogen and seawater flow on growth and biochemical composition of *Gracilaria foliifer var. angustissima* in mass outdoor cultures. Bot Mar. **22**:(8) pg 529 – 539.

Lapointe, B. E. & Tenore, K. R. 1981. Experimental outdoor studies with *U. fasciata* Delile, I: Interaction of light and nitrogen on nitrogen uptake, growth and biochemical composition. Journal of experimental marine biology and ecology. **53** (2 – 3): pg 135 – 152.

Lee, E. R. 1999. Phycology. 3rd Ed. Cambridge University Press. Cambridge. Pg. 176 – 185, 207 – 233.

Leito, D. 2001. Effects of commercial seaweed concentrate (Kelpak ©) on growth of *Gracilaria gracilis* (Stackhouse) Steentoft (Rhodophyta, Gigartinales) in laboratory culture. Honours Thesis. University of Cape Town. South Africa. 49pg.

University of Cape Town

Levine, H. & Wilce, R. 1977. *Ulva lactuca* as a bioindicator of coastal water quality. Water Resources Research Centre. University of Massachusetts. Amherst, Massachusetts.

Lignell, A.; Ekman, P. & Pedersén, M. 1987. Cultivation technique for marine seaweeds allowing controlled and optimized conditions in the laboratory and on a piolet scale. Bot. Mar. **30**: pg 417 – 424.

Linnaeus, C. 1753. Species Plantarum. Vol. 2. Stockholm.

Littler, M. M. 1980. Morphological form and photosynthetic performances of marine macroalgae: Tests of a functional/form hypothesis. Bot. Mar. **22**: pg 161 – 165.

Littler, M. M. & Littler, D. S. 1980. The evolution of thallus form and survival strategies in benthic marine macroalgae: field and laboratory tests of a functional-form model. The American naturalist. **116**(1): pg 25 – 44.

Littler, M. M. & Arnold, K. E. 1982. Primary productivity of marine macroalgae functional form groups from South Western North America. J. Phycol. **18**: pg 307 – 311.

Littler, M. M. & Littler, D. S. 1983. Evolutionary strategies in a tropical barrier reef system: functional-form groups of marine macroalgae. J. Phycol. **19**: pg 229 – 237.

Lindgren, E. 2000. The new environmental context for disease transmission – with case studies on climate change and tick-borne encephalitis. PhD thesis, Dept. of Systems Ecology, Stockholm University, Sweden. 112 pg.

Lingnell, A.; Eckman, P. & Pedersén, M. 1987. Cultivation techniques for marine seaweeds allowing controlled and optimized conditions in the laboratory and on a pilot scale. Bot. Mar. **30**: pg 417 – 424.

Lobban, C. S. & Harrrison, P. J. and Duncan, M. J. 1985. The Physiological Ecology of Seaweeds. Cambridge University Press. Cambridge. 242 pg.

Lobban, C. S. & Harrrison, P. J. (eds.) 1997. Seaweed ecology and physiology. Cambridge University Press. Cambridge. 366 pg.

Lünning, K. 1981. Light: Algae, ocean environment, photosynthesis. *In*: The biology of seaweeds. Lobban, C. S. & Wynne, M. J. (eds.). Botanical monographs Vol. **17**. Blackwell scientific publications. Oxford. pg 493 – 533.

Lünning,K. 1990. Seaweeds their environment, biology and ecophysiology. John Wiley & Sons. New York. Pg 207,241

Lünning, K & Dieck, T. 1989. Environmental triggers in algal seasonality. Bot. Mar. **32**: pg 389 – 397.

University of Cape Town

Lünning, K. 1990. Seaweeds: Their environment, biogeography and ecophysiology. John Wiley and sons. Inc. Intersience.. 527 pg

Lyon, R. 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. 85 pg.

Mai, K., Mercer, J. P. & Donlon, J. 1995a. Comparitive studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino. III. Response of abalone to various levels of dietry lipid. Aquaculture. **134**: pg 65 – 80.

Mai, K., Mercer, J. P. & Donlon, J. 1995b. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino. IV. Optimum dietary protein level for growth. Aquaculture. **134**: pg 65 – 80

Markager, S. & Sand-Jenson, K. 1990. Heterotrophic growth of *Ulva lactuca* (Chlorophyceae). J. Phycol. **26**: pg 670 – 673.

Marta, de, L. and Santos, R. 2003. Cultivation of *Ulva rotunda* in raceways using semi-intensive fish pond effluents. (In press.)

Mathews, S. G. and Pitcher, G. C. 1996. Worst recorded marine mortality on the South African coast. In harmful and toxic algal Blooms. Yasumoto, T.; Oshima, Y, and Fukuyo, Y. (eds.) Intergovernmental Oceanographic Commision of UNESCO. pg 89 – 92.

Mattox, K. R. & Stewart, K. D. 1984. Classification of the green algae: A concept based on comparative cytocology. *In* Systematics of the green algae, eds. D. E. G. Irvine & D. M. John. Academic Press. London. Pg 29 – 72.

McLachlan, J. L. 1991. General principles of on shore cultivation of seaweeds: effects of light on production. Hydrobiologica. **221**: pg 125 – 135.

Melkonian, M. 1980. Flagellar apparatus, mating structure and gametic fusion in *Ulva lactuca* (Ulvales, Chlorophyceae). British J. Phycol. **15**: pg 197 – 200.

Migita, S. 1985. The sterile mutant of *Ulva pertusa* Kjellman from Omura Bay. Bulletin of the Faculty of Fisheries, Nagasaki University. **57**: pg 33 – 37.

Miller, D. 2001. Outdoor cultivation of *Gracilaria* in pond and raceway systems at Jacobsbaai Sea Products (Pty) Ltd., South Africa. Honours Thesis. University of Cape Town. South Africa. 38pg.

Mitchell- Innes, B. A. and Walker, D. R. 1991. Short term variability during an anchor station study in the Southern Benguela upwelling system:

University of Cape Town

Phytoplankton production and biomass in relation to species changes. Prog. Oceanog. **28**: pg 65 – 89.

Mohsen, A. F.; Khaleata, A. F.; Hashem, M. A. & Metwalli, A. 1974. Effect of different nitrogen sources on growth, reproduction, amino acid, fat and sugar contents in *Ulva fasciata* Delile. Bot. Mar. **17**: pg 218 – 222.

Morgan, D. 2000. The potential for *Gracilaria* polyculture at Jacobs Bay Sea Products Pty. Honours Thesis. University of Cape Town. South Africa. 60 pg.

Mshigeni, K. E. and Kajumulo, A. A. 1979. Effects of the environemtn on polymorphism in *Ulva fasciata* Delile (Chlorophyta, Ulvaceae). Bot. mar. **22**: pg 145 – 148.

Muller, S. 1986. Taxonomy of the genus *Haliotis* in South Africa. *Trans. Roy. Soc. S. Afr.* **46** Part 1: pg 69 – 77.

Murphy, J & Riley, J. P. 1962. A modified single-solution method for the determination of phosphate in natural waters. Analytical Chim. Acta. **27**: pg 31 – 36.

Nadioo, K. 2004. (in preparation). The testing of various abalone diets on a commercial abalone farm on the west coast of South Africa. Maters Thesis. University of the Western Cape.

Nakanishi, K.; Nishijima, M.; Nishimura, M. Kuwano, K. & Saga, N. 1996. Bacteria that induce morphogenesis in *Ulva pertusa* (Chlorophyta) grown under anoxic conditions. J. of Phycol. **32**: pg 479 – 482.

Nash, W. J.; Sanderson, J. C.; Bridley, J.; Dickson, S. and Hislop, B. 1995. Post larval recruitment of Black lip abalone (*Haliotis rubra*) on artificial collectors in Southern Tasmania. J. Mar. and Fresh water Res. **46**(3): pg 531 – 538.

Neish, I. C. 1976. Culture of algae and seaweeds. FAO. Technical conference on Aquaculture, Kyoto, Japan. FIR: AQ/conf/76/R36.iv. 13 pg.

Neish, I. C. & Knutson, L. B. 1979. The significance of density, suspension and water movement during commercial propagation of macrophyte clones. Proc. Int. Seaweed Symp. **9**: pg 451 – 461.

Nelson, W. R. & van Staden, J. 1984. The effect of seaweed concentrate on growth of nutrient stressed cucumbers. Hort. Science. **19**: pg 81 – 82.

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**: pg 483 – 489.

University of Cape Town

Neori, A. 1996. The type of N supply (ammonia or nitrate) determines the performance of seaweed biofilters integrated with intensive fish culture. Israeli Journal of Aquaculture-Bmidgeh, **48**(1): pg 19 – 27.

Neori, A.; Krom, M.; Ellner, S.; Boyd, C.; Popper, D.; Rabinovitch, R.; Davison, P.; Dvir, O.; Zuber, D.; Ucko, M.; Angel, D.; and Gordin, H. 1996. Seaweed biofilters as regulators of water quality in integrated fish-seaweed culture units. *Aquaculture*, **141**(3-4): pg 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. Aquacultural Engineering. **17**: pg 215 – 239.

Neori, A. and Shpigel, M. 1999. Using algae to treat effluents and feed invertebrates in sustainable integrated mariculture. World Aquaculture, **30**(2) pg 46 - 51.

Niesenbaum, R. A. 1988. The ecology of sporulation by the macroalga *Ulva lactuca* L. (Chlorophyceae). Aquatic botany. **32**(1 – 2): pg 155 – 166.

Nisizawa, K.; Noda, H.; Kikuchi, R. and Watanabe, T. 1987. The main seaweed foods in Japan. Hydrobiologica. **151/152**. pg 5 – 29.

Njobeni, A. 2003. (in preparation) The cultivation of *Gracilaria gracilis* (Rhodophyta) in an integrated aquaculture system, for the production of abalone feed and the bioremediation of aquaculture effluent. Master thesis. University of Cape Town.

Norton, T. A.; Mathieson, A. C. & Neushul, M. 1981. Morphology and environment Chapter 12. *In*: The biology of seaweeds. Lobban, C. S. & Wynne, M. J. (eds.). Botanical monographs Vol. 17. Blackwell scientific publications. Oxford. Pg 421 – 451.

Norton, T. A.; Mathieson, A. C. & Neushul, M. 1982. A review of some aspects of form and function in seaweeds. Botanica Marina. **25**: pg 501 – 510.

Novalek, Inc. 1997. Un-ionized ammonia (NH_3) table. http://www.petsforum.com/novalek/kpd66.htm

Nydahl, F. 1976. On the optimum concentrations for reduction of nitrate to nitrite by cadmium. Talanta. **23**: pg 349 – 357.

Odum, W. E. 1974. Potential effects of aquaculture on inshore coastal waters. In: Oceanography. Contemporary readings in ocean sciences. Oxford University press. Oxford. pg: 334 – 341.

O' Kelly C. J. & Floyd, G. L. 1984. Correlations amoung patterns of sporangial structure and development, Life Histories, and ultrastructural features in

University of Cape Town

the Ulvophyceae. *In* Systematics of the green algae, eds. D. E. G. Irvine & D. M. John. Academic Press. London. Pg 29 – 72.

Oliveira, E. C.; Alveal, K. & Anderson, R. J. 2000. Mariculture of the agar-producing Gracilarioid Red Algae. Reviews in fisheries science. **8**(4): pg 345 – 377.

Oza, R. M. & Sreenivasa Rao, P. 1977. Effect of different culture media on growth and sporulation of laboratory raised germlings of *Ulva fasiata* Delele. Botanica marina. **20** (7): pg 427 – 431.

Papenfuss, G. F. 1960. On the genera of the Ulvales and the status of the order. J. Linn. Soc. (Bot.) **56**, pg 303 – 318.

Parker, H. S. 1981. Influence of relative water motion on the growth, Ammonium uptake and Carbon and Nitrogen composition of *Ulva lactuca* (Chlorophyta). Marine Biology. **63**(3): pg 309 – 318.

Parker, I. M.; SImberherloff, D.; Lonsdale, W. M.; Goodell, K.; Wonham, M.; Kareiva, P. M.; Williamson, M. H.; von Hoile, B.; Moyle, P. B.; Byers, J. F. & Goldwasser, L. 1999. Impact. Towards a framework for understanding the ecological effects of invaders. *Biological invasions.* **1**. pg 3 - 19.

Pedersen, M. 1994. Transient ammonium uptake in the macroalga *Ulva lactuca* (Chlorophyta): Nature, regulation, and the consequences for choice of measuring technique. J. Phycol. **30**. pg 980 – 986.

Pedersen. M.; Cellen, J.; Abrahamsson, K.; Mtolera, M. Semesi, A. Gracia Reina, G. 1995. Non-infectious diseases. Int. Seaweed Symp. **15**: pg 188 (Abstract).

Petrell, R. J. & Alie, S. Y. 1996. Integrated culture of salmonids and seaweeds in open systems. Proceedings of the 15th International Seaweed Symposium. **15**: pg 67 – 73.

Phillips, J. A. 1984. The validity of morphological and characters in distinguishing species of *Ulva* in Southern Australia. In. Systematics of the green algae. Irvine, D. E. G. NS John, D. M. (eds.) Academic Press, London. Pg 353 – 361.

Phillips, J. A. 1988. Field, Anatomical and developmental studies on Southern Australian species of *Ulva* (Ulvaceae, Chlorophyta). Australian Systematic Botany. **1**: Pg 411 – 456.

Phillips, J. A. 1990. Life history studies of *Ulva rigida* C. Ag. And *Ulva stenophylla* S. *et.* G. (Ulvaceae, Chlorophyta) in Southern Australia. Bot. Mar. **33**: pg 79 – 84.

Pitcher, G. C. 1998. Harmful Algal blooms of the Benguela current. Sea Fisheries Research Institute. Cape Town. 20 pg.

University of Cape Town

Probyn, T. A. & Chapman, A. R. O. 1982. Nitrogen uptake characteristics of *Chordaria flagelliformis* (Phaeophyta) in batch mode and continuous mode experiments. Mar. Biol. (Berl.) **71**: pp 129 – 133.

Probyn, T. A.; Mitchell-Innes, B. A.; Brown, P. C.; Hutchings, L. and Carter, R. A. 1994. A review of primary production and related processes on the Agulhas Bank. S. A. J. Sci. **90**: pg 166 – 173.

Provasoli, L. & Printer, I. J. 1980. Bacteria induced polymorphism in an axenic laboratory strain of *Ulva lactuca* (Chlorophyceae). J. Phycol. **16**: pg 196 – 201.

Ramus, J. 1978. Seaweed anatomy and photosynthetic performance: the ecological significance of light guides, heterogenous absorption and multiple scatter. J. Phycol. **19**. pg 352 – 362.

Ramus, J. 1983. Ecological growth stratergies in the seaweeds *Gracilaria foloofera* (Rhodophyceae) and *Ulva* (Chlorophyceae). Ph.D. Thesis, Yale University, 151 pg.

Ramus, J & Venable, M. 1987. Temporal ammonium patchiness and growth rate in *Codium* and *Ulva* (Ulvophyceae. J. Phycol. **23**. pg. 518 – 523.

Reed, R. H. and Russel, G. 1978. Salinity fluctuations and their influence on "bottle brush" morphogenesis in *Enteromorpha intestinalis* (L.) Link. British Phycol. J. **13**: pg 149 – 153.

Riccardi, N. & Solidoro, C. 1996. The influence of environmental variables on *Ulva rigida* C. Ag. Growth and production. Bot. Mar. **39**: pg 27 – 32.

Rosenberg, G. & Ramus, J. 1982a. Ecological growth strategies in the seaweeds *Gracilaria foliifera* (Rhodophyceae) and *Ulva* sp. (Chlorophyceae): Photosynthesis and antenna composition. Mar. Ecological Prog. Series. **8**: pg 233 – 241.

Rosenberg, G. & Ramus, J. 1982b. Ecological growth strategies in the seaweeds *Gracilaria foliifera* (Rhodophyceae) and *Ulva* sp. (Chlorophyceae): soluble nitrogen and reserve carbohydrates. Marine Biol. (Berl). **66**:pg 251 – 259.

Rosenberg, G. & Ramus, J. 1984. Uptake of inorganic nitrogen and seaweed surface area:volume ratios. Aquat. Bot. **19**: pg 65 – 72.

Rosenberg, G.; Probyn, T. A. & Mann, K. H. 1984. Nutrient uptake and growth kinetics in brown seaweeds: response to continuous and single additions of ammonium. J. Exp. Mar. Biol. Ecol. **80**: pg 125 – 146.

Ryther, J. H.; Goldman, J. C.; Gifford, C. E.; Huguenin, J. E.; Wing, A. S.; Clarner, J. P.; Williams, L. D. & Lapointe, B. E. 1975. Physical models of

integrated waste recycling marine polyculture systems. Aquaculture. **5**: pg 163 – 177.

Ryther, J. H.; Deboer, J. A. & Lapointe, B. E. 1978. Cultivation of seaweeds for hydrocolloids, waste treatment and biomass for energy conversion. Proceedings of the 9th international seaweed symposium. **9**: pg 1 –16.

Ryther, J. H.; Corwin, T. A. & Williams, L. D. 1981. Nitrogen uptake and storage by the red algae *Gracilaria tikvahiae* (McLachlan 1979). Aquaculture. **26**: pg 107 – 115.

Sales, J. & Britz, P. 2000. Mineral composition of abalone (*Haliotis midae*) Shells. Report for Marine Products. 16pg.

SANCOR. 1996. Abalone (perlemoen) resource about to collapse. SANCOR newsletter: May – Aug. Pg. 8 – 11.

Santelices, B. & Ugarte, R. 1987. Production of Chilean *Gracilaria*: Problems and Perspectives. Hydrobiologia. **151/152**: 295 – 299.

Santelices, B. 1999. A conceptual framework for marine agronomy. Hydrobiologica. **398/399**. pg 15 – 23.

Santelices, B. & Doty, M. 1989. A review of *Gracilaria* farming. Aquaculture . **78**: pg 98 – 133.

Sfriso, A. 1995. Temporal and spatial responses of Growth of *Ulva ridgida* C. Ag. to environmental and tissue concentrations of nutrients in the lagoon of Venice. Bot. Mar. **38**: pg 557 – 573.

Shaw, W. N. 1982. The culture of molluscs in Japan. III. Abalone culture. Aquaculture. **9**: 43 pg.

Shin, Hyun-Woung and C. M Smith. 1995. Characteristics of pigments in motile cells of *Ulva fasciata*: In vivo absorption, in vivo flourescence emission and HPLC determination. J. Phycol **31**:8 pg.

Shipton, T. A. 1999. The protein requirements of the South African abalone, *Haliotis midae*. PhD Thesis. Rhodes University. South Africa. 150 pg.

Shpigel, M.; Neori, A.; Popper D.M. and Gordin, H. 1993. A proposed model for "clean" land based polyculture of fish, bivalves and seaweeds. Aquaculture, **117:** pg 115 – 128.

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). pg 313 – 326.

Shpigel, M.; Gasith, A. & Kimmel, E. 1997. A biomechanical filter for treating fish-pond effluents. Aquaculture. **152**: pg 103 – 117.

Silva, P. C.; Basson, P. W. & Moe, R. L. 1996. Catalogue of the benthic Marine algae of the Indian Ocean. University of California Press. Berkeley.

Simpson, B. J. A. 1994. An investigation of diet management strategies for the culture of the South African abalone, *Haliotis midae*. MSc. Thesis. University of Cape Town. South Africa. 80pg

Simpson, B. J. A. & Cook, P. A. 1998. Rotation diets: A method of improving growth of cultured abalone using natural algal diets. J. Shell. Res. **17**: pg 635 – 640

Smit, A. J. and Bolton, J. J. 1999. Organismic determinants and their effect on growth and regeneration in *Gracilaria gracilis*. Journal of Applied Phycology **11** (3): pg 293 – 299.

Smit, A. J.; Fourie, A. M.; Robertson, B. L. and Du Preez, D. R. 2003. Control of the herbivorous isopod *Paridotea reticulata* in *Gracilaria gracilis* tank cultures.

Smith, D.G. and Young, E.G. 1954. Amino acids of marine algae. J. Biochem, **217**: pg 845 – 853.

Solorzano, L. 1969. Determination of ammonium in natural waters by the phenol-hypochlorite method. Limnology and Oceanography. **14**: pg 799 – 801.

South, G. R. & Whittick, A. 1987. Introduction to Phycology. Blackwell Scientific. Oxford. 341pg.

Steffensen, D. A. 1976. The effect of nutrient enrichment and temperature on the growth in culture of *Ulva lactuca* L. Aquatic Botany **2**: pg 337 – 351.

Stegenga, H., Bolton, J. J. & Anderson R. J. 1997. Seaweeds of the South African West Coast. Contributions from the Bolus herbarium. Number **18**. 655 pg

Stewart, K. D. & Mattox, K. R. 1978. Structural evolution in the flagellated cells of green algae and land plants. Biosystems. **10**: pg 145 – 152.

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. 92pg.

Stratmann, J.; Paputsoglu, G. & Oertal, W. 1996. Differentiation of *Ulva mutabilis* (Chlorophyta) gametangia and gamete release are controlled by extracellular inhibitors. J. of Phycology. **32** (6): pg 1009 – 1021.

Stuttaford, M. (ed.). 1997. Fishing industry handbook, South Africa, Namibia & Mozambique. Marine information. Stellenbosch.

Svirski, E., Beer, S. and Friedlander, M. 1993. *Gracilaria conferta* and its epiphytes. II. Interrelationships between the red seaweed and *Ulva* cf. *lactuca*. Hydrobiologia **260/261**: pg 391 – 396.

Swift, D. R. 1985. Aquaculture Training Manual. Fishing News Book. London.

Sze. P. 1993. A biology of the algae. 2nd Ed. Wm. C. Brown. Australia. **72** Pg 63 – 64,

Tanner, C. S. 1981. Chlorophyta: Life Histories. *In*: Lobban, C. S. & Wynne, M. J. (eds.), The biology of the seaweeds. Botanical Monographs **17**. Blackwell. Oxford. Pg 218 – 247.

Tanner, C. S. 1986. Investigations of the taxonomy and morphological variation of *Ulva* (Chlorophyta): *Ulva californica* Wille. Phycologia. **25**(4). Pg 510 – 520.

Tarr, R. J. Q. 1989. Abalone. Pp. 62 – 69. In: Payne, A. I. L. & Crawford, R. J. M. (eds.). Oceans of Life off Southern Africa. Vlaeberg Publishers. Cape Town. 380 pg.

Tarr, R. J. Q. 1990. Aspects of the reproductive biology of *Haliotis midae*. In Perlemoen farming in South Africa. Proceedings of a workshop convened by The Mariculture Association of Southern Africa. Ed. P. Cook. The mariculture Association of Southern Africa. Cape Town. pg 2 – 6.

Tarr, R. J. Q. 1993. Stock assessment, and aspects of the biology of the South African abalone *Haliotis midae*. Masters thesis. University of Cape Town. 101 pg.

Tarr, R. J. Q. 1995. Growth and movement of the South African abalone *Haliotis midae*. A reassessment. Mar. Freshwater Res. **46**: pg 583 – 590.

Tarr, R. J. Q., Williams, P. V. G. & Mackenzie, A. J. 1996. Abalone, echinoids and rock lobster: a possible ecological shift may affect traditional fisheries. S. Afr. Mar. Sci. **17**: pg 319 – 323.

Titlyanov, E. A.; Glebova, N. T. and Kotlyarova, L. S. 1975. Seasonal changes in structure of the thalli of *Ulva fenestrata* P. et R. Ekologiya. **9**: pg 36 – 41.

Troell, M.; Halling, C.; Nilsson, A.; Buschmann, A. H.; Kautsky, N. and Kautsky L. 1997. Integrated open sea cultivation of Gracilaria chilensis (Gracilariales, Bangiophyceae) and salmonids for reduced environmental impact and increased economic output. Aquaculture **156**: pg 45 – 61.

Troell, M., Kautsky, N., and Folke, C. 1999. Applicability of integrated coastal aquaculture systems. *Ocean & Coastal Management*, **42**(1): pg 63 – 69.

Troell, M.; Rönnback, P.; Halling, C.; Kautsky, N. & Buschmann, A. 1999. Ecological engineering in aquaculture: use of seaweeds for removing nutrients from intensive mariculture. J. Appl. Phycol: **11**: pg 89 – 97.

Turpin, D. H. 1991. Effects of inorganic N availability on algal photosynthesis and carbon metabolism. J. Phycol. **27**: pg 14 –20.

Uki, N. and Watanabe, T. 1992. Review of the nutritional requirements of abalone (*Haliotis* spp.) and development of more efficient artificial diets. In: Shepherd, S. A.; Tegner, M. J. and Guzmán del Prĉo (Eds.) Abalone of the world, Biology Fisheries and Culture. Fishing News Books, Cambridge. Pg 504 – 517.

Van den Hoek, C., Mann, D. G., Jahns, H. M. 1995. Algae, An introduction to phycology. Cambridge University Press. Cambridge. Pg 390 – 408.

Vandermeulen, H. 1989. A low maintenance tank for the mass culture of seaweed. Aquacultural engineering **8**: pp 67 – 71.

Vandermeulen, H. & Gordin, H. 1990. Ammonium uptake using *Ulva* (Chlorophyta) in intensive fishpond systems: mass culture and treatment of effluent. J. applied Phycol. **2**(4): pg. 363 – 374.

Verkleij, F. N. 1992. Seaweed extracts in agriculture and horticulture: A Review. Biol. Agri. & Horti. **8**: pg. 309 – 324.

Vosloo, A. 2003. Water balance in abalone (*Haliotis midae*) during air exposure – a simulation of the export of live animals. Proceedings from Wet ń wild life. Current trends in zoology and limology in Southern Africa. Cape Town. Abstract only. Pg. 97

Waite, T. & Mitchell, R. 1972. The effect of nutrient fertilization on the benthic alga *Ulva lactuca*. Bot. Mar. **15**: pg 151 – 156.

Wallentinus, I. 1984. Comparisons of nutrient uptake rates for Baltic macro-algae with different thallus morphologies. Mar. Biol. **80**. pg 215 – 225.

Wilkinson, S. 2001. An investigation of protein variation in *Ulva lactuca* and *Ulva rigida* under high nitrogen culture conditions. Botany honours thesis. University of Cape Town. 31 pp.

Woolcott, G. W. and King, R. J. 1999. *Ulva* and *Enteromopha* (Ulvales, Ulvphyceae, Chlorophyta) in Eastern Australia: comparison of morphological features and analysis of nuclear rDNA sequence data. Australian Systematic Botany. **12**: pg 709 – 725.

Wheeler, W. N.; Neushul, M. & Harger, B. W. W. 1981. Development of a coastal marine farm and its associated problems. In Levring, T. (ed.). Proceedings of the 10th international seaweed symposium. Walter de Gruyter, Berlin: 631 – 648.

White, S. & Keleshian. M. 1994. A field guide to economically important seaweeds of Northern New England. University of Maine. http://www.noamkelp.com /technical/handbook.html

Wilson, D. T. 1999. Some aspects of the nitrogen nutrition and growth of *Gracilaria gracilis* grown by suspended cultivation in Saldanha Bay, South Africa. Honours Thesis. University of Cape Town. South Africa. 31pg.

Womersley, H. B. S. 1984. The marine benthic flora of southern Australia. Government Printer, South Africa. 329 pg.

Wong, K.H. and Cheung, P.C.K. 2001. Nutritional evaluation of some subtropical red and green seaweeds Part II. In vitro protein digestibility and amino acid profiles of protein concentrates. Food Chemistry **72**: pg 11-17.

Wynne, M. J. & Kraft, G. T. 1981. Appendix: Classification summary. *In*: Lobban, C. S. & Wynne, M. J. (eds.), The biology of the seaweeds. Botanical Monographs 17. Blackwell. Oxford. Pg 743 – 750.

Zar, J. H. 1999. Biostatistical Analysis. 4th Ed. Prentice-Hall. New Jersey. 663 pg.

Zechman, F. W.; Theriot, R. L.; Zimmer, E. A. & Chapman, R. L. 1990. Phylogeny of the Ulvophyceae (Chlorophyta): cladistic analysis of nuclear encoded rRNA sequence data. J. of Phycol. **26**: pg 700 – 710.