Experimental cultivation of the red seaweed, *Gracilaria gracilis* (Rhodophyta) in land-based tank culture systems on abalone farms in the Western Cape, South Africa

By

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Abstract

The feasibility of growing red seaweed, *Gracilaria gracilis* (Gigartinales, Rhodophyta) in land-based culture systems was investigated in two Western Cape abalone farms i.e. Jacobsbaai Sea Products (JSP) and Irvin & Johnson (I&J). Experiments were conducted using small (82 litres) and medium-sized (283 litres) plastic tanks (JSP) and large size culture tanks (I&J). All the tanks were stocked with at 2 kg m^{-2} supplied with unfiltered seawater (24 and 4 volume exchanges d^{-1}) JSP and I&J, respectively. This study also tested the efficiency of *G. gracilis* as a biofiltering species as well as investigating the effectiveness and influence of nutrient-enriched seawater i.e. turbot and abalone effluent (JSP) and fertilized seawater and abalone effluent (I&J) on the growth of *Gracilaria*.

In seawater (JSP), *Gracilaria* grew better in small tanks, with a RGR and yield of 4.2 $\pm$ 3.56 % d^{-1} and 0.15 $\pm$ 0.14 kg W wt. m^{-2} d^{-1} than in medium-sized tanks i.e. 2.3 $\pm$ 4.15 % d^{-1} and 0.08 $\pm$ 0.11 kg W wt. m^{-2} d^{-1}, RGR and yield, respectively. *Gracilaria* growth followed a seasonal pattern. High yields (0.19 $\pm$ 0.11 kg W wt. m^{-2} d^{-1}) were obtained during spring-summer months (October 2001-early April 2002) and low yields (0.02 $\pm$ 0.07 kg W wt. m^{-2} d^{-1}) were observed during autumn-winter months (late April- August 2002).

Gracilaria exhibited a similar growth pattern in turbot and abalone wastewater. In turbot and abalone culture tanks, higher RGR and yield was recorded during spring-summer months (e.g. 8.2 $\pm$ 2.97 % d^{-1} and 0.32 $\pm$ 0.13 kg W wt. m^{-2} d^{-1}) and (6.8 $\pm$ 1.06 % d^{-1} and 0.258 $\pm$ 0.07 kg W wt. m^{-2} d^{-1}), respectively. Whereas poor RGR and yield was obtained during autumn-winter months (e.g.-3.4 $\pm$ 5.02 % d^{-1} and $-$ 0.036 $\pm$ 0.07 kg W wt m^{-2} d^{-1}) and (e.g. -2.4 $\pm$ 4.61 % d^{-1} and $-$ 0.02 $\pm$ 0.07 kg W wt. m^{-2} d^{-1}), turbot and abalone culture tanks, respectively. In large culture tanks (I&J), *Gracilaria* growth in seawater was poor at the beginning of growth experiments (1.7 $\pm$ 0.84 % d^{-1} and 0.05 $\pm$ 0.03 kg W wt. m^{-2} d^{-1}) probably due to the low turnover rate i.e. 4 volume exchanges d^{-1}. *Gracilaria* growth increased significantly (2.9 $\pm$ 0.45 % d^{-1} and 0.09 $\pm$ 0.03 kg W wt. m^{-2} d^{-1}), p < 0.001 when the turnover rate was increased to 12 volume exchanges d^{-1} RGR and yield, respectively. *Gracilaria* growth did not appear to be following a seasonal pattern. In nutrient-enriched seawater, better RGR (2.4 $\pm$ 0.79 % d^{-1}) and yield (0.08 $\pm$ 0.04 kg W wt. m^{-2} d^{-1}) was

obtained in abalone effluent culture tanks and poor RGR (1.8 ± 1.24 % d^{-1}) and yield (0.05 ± 0.04 kg W wt. m^{-2} d^{-1}) n = 4 and 10, respectively. No seasonal growth pattern was observed in *Gracilaria*.

The chemical analysis of water (culture media) samples showed that *Gracilaria* significantly reduces NH$_4$-N concentration in the culture medium i.e. 77 % and 82 % reduction was observed in small and medium-sized tanks, respectively. However, reduction in nitrate and nitrite concentration was minimal. PO$_4$ concentration was higher in the outgoing water and this could not be explained in the present study. *Gracilaria* showed a preferential uptake for ammonia than for nitrate. *Gracilaria* tissue analysis showed the total-N concentration of *Gracilaria* to be season-dependant with high nutrient concentrations during winter months and lower concentrations during summer months. However, PO$_4$ concentration in *Gracilaria* tissues showed no seasonal preference. From this study it was discovered that *Gracilaria* growth in land-based systems is biological and technical feasible, nutrient-enriched seawater influences biomass production, *Gracilaria* effectively reduces nutrient concentration in culture media and high turnover rates enhance biomass production. Turbot wastewater was found to be a suitable culture medium for *Gracilaria* growth.

CHAPTER I

INTRODUCTION

The red algal genus *Gracilaria* is among the most economically important seaweeds, with a variety of uses. Besides being a source of agar, species of *Gracilaria* have also been used for human consumption (Levring *et. al.*, 1969), food for invertebrate culture (Chiang, 1981), fertilizer (Zaneveld, 1959), medicine (Chapman, 1950), in the tertiary treatment of sewage (Ryther *et al.* 1979), and in biogas production (Hanisak, 1981). Currently in South Africa *Gracilaria* is used either as a major feed or as supplementary feed for abalone. Abalone is a commercially valuable marine gastropod (Oakes and Ponte, 1996). Its culture is often limited by the supply of suitable seaweed (Uki and Watanabe, 1992).

In the past, sufficient quantities of *Gracilaria* were collected from beach-cast, but in Chile, for example, demands led to the over-harvesting and the collapse of some natural populations; Subsequently experiments on different methods of *Gracilaria* farming were conducted (Santelices and Doty, 1989). Commercial cultivation of *Gracilaria* is widespread and is taking place in Chile, China, Taiwan and Namibia (Critchley and Ohno, 1998) while countries like Brazil, Israel, Mexico, the Philippines, South Africa and Venezuela are still engaged in preliminary experiments and are investigating the feasibility of growing *Gracilaria* (Critchley and Ohno, 1998).

In South Africa, a commercial *Gracilaria* industry has been in existence since 1951, based on beach-cast collections of *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine *et* Farnham previously known as *Gracilaria verrucosa* (Hudson) Papenfuss, from Saldanha Bay on the west coast (Anderson *et al.*, 1989). However, in 1974, beach cast collection stopped and the industry collapsed probably because of the changed water circulation in the bay due to the construction of an ore jetty (Anderson *et al.*, 1989). Even though beach cast collections restarted again, from a few remnant plants, the collections have never recovered to previous levels and have collapsed several times. Inconsistencies in collections have made the resource unreliable for export and local agar production (Anderson *et al.*, 1996). Several pilot scale experiments have been undertaken on

Gracilaria mariculture in Saldanha Bay and St Helena Bay areas to develop a stable *Gracilaria* industry in South Africa. The utilization of *G. gracilis* as a mariculture species for raft culture was experimentally investigated in Saldanha Bay, and relatively high average growth rates were obtained for many years (Anderson *et al.*, 1996). However, growth rates in summer were often low. Since Saldanha-Langebaan Bay is the only deep, large embayment in the very exposed west coast of South Africa, it is a site for many and sometimes conflicting human activities. Another promising place for gracilarioid farming is St. Helena Bay and this area showed great potential for mass production of *Gracilaria* using suspended open-water methods. Good experimental yields were obtained during September and October 1997 but lower yields were recorded in January and February 1998. However, the occurrence of a 'black tide' in April 1998 led to the collapse of the experiment as all the plants on the rafts died. This phenomenon is caused by low oxygen levels that result from massive plankton blooms, which, when they decay, lead to the production of hydrogen sulphides in seawater (Matthews and Pitcher, 1996).

There is presently no commercial scale processing of agar-producing red seaweeds in southern Africa. The *Gracilaria* populations have been commercially exploited at Luderitz Bay, Namibia since 1981 (Rotmann, 1987; Critchley *et al.*, 1991). Dried beach-cast *Gracilaria gracilis* from Luderitz Bay, Namibia is sold abroad for the production of agar (Anderson *et al.*, 1989; Critchley *et al.*, 1991; Critchley and Rotmann, 1992; Molloy, 1992). The reliance on collection of beach-cast material has proved to be unstable and unpredictable (Critchley *et al.*, 1991; Molloy, 1992). The prohibition of direct harvesting from the Namibian *Gracilaria* beds coupled with the unreliability of beach-cast material justified the need for cultivation of *Gracilaria* to ensure consistent supply of this seaweed and to meet the ever-increasing demands for agar and its derivatives (Critchley *et al.*, 1991; Molloy and Bolton, 1992; Molloy and Bolton, 1995).

Globally, the *Gracilaria* mariculture industry has developed remarkably rapidly over the past decade in an attempt to stop over-exploitation of wild plants and to produce sufficient quantities to meet the high demands for agarophytes and their products (Hansen *et al.*, 1981). The genus *Gracilaria* showed great potential as a mariculture species because of its ability to give high yields and produce commercially valuable extracts (Lapointe and Ryther, 1978). The need to increase control production led to the testing of *Gracilaria* farming in many countries using various

cultivation methods (Dawes, 1995). These ranged from simple methods like planting of *Gracilaria* on sandy bottoms in protected bays to land-based simple-technology dams and sophisticated tanks (see Section 2.2).

In sea-based facilities, plantations are affected by many natural disturbances like storms and currents that remove portions of *Gracilaria* thalli. *Gracilaria* cultivation studies is not new in southern Africa, however the previous research studies focused mainly on sea cultivation using the suspension method i.e. "floating rope rafts cultivation" in Saldanha-Langebaan and St Helena Bay areas which were inshore-based. Whereas the current study focuses on land-based cultivation of Gracilaria. There is a rapid increase in the number of land-based abalone farms along the south and west coast of South Africa This increase has put much pressure on the kelp, a common brown seaweed species, *Ecklonia maxima* found in abundance (kelp beds) on the west and south-west coast of South Africa, hence the need to investigate the feasibility of incorporating *Gracilaria* in already existing abalone farms.

In South Africa there are basically five factors that make land-based seaweed cultivation of *Gracilaria* for abalone feed important.

➢   The potential over-exploitation of kelp, particularly *Ecklonia maxima* that is used as the primary feed for abalone. Kelp beds are restricted to the southwest and west coast of South Africa and therefore there is pressure on the available kelp beds as the industry is growing rapidly. Artificial feed and the transporting of fresh seaweed material is very expensive, making on-farm cultivation a better alternative (Anderson et al, 2003).

➢   South Africa has few protected areas (bays and lagoons) where mass marine farming can take place in situ, and in some places there is conflicting use of these areas (Smit, 1998, Wakibia *et al.,* 2001).

➢   The occurrence of harmful algal blooms contaminates water causing a lot of mortalities of cultured species in the mariculture industry in the Benguela region and this affects the local industry as well (Pitcher, 1998).

➢   Previous research studies on dietary preference of abalone revealed that abalone grow faster when their diet (kelp, *Ecklonia maxima*) is supplemented with other algae, such as *Gracilaria* (Simpson and Cook, 1998), which is available in South Africa.

➤ The biofiltering capacity and the vegetative form of reproduction of *Gracilaria* make it a suitable candidate for mariculture.

This study was designed to investigate the technical and biological feasibility of growing the local red seaweed, *Gracilaria gracilis* in land-based tank systems, to investigate the effect of natural seawater, fertilized seawater, turbot and abalone effluent on *Gracilaria* growth and finally, to investigate the efficiency of *Gracilaria* in absorbing nutrients from various cultivation media.

CHAPTER 2
LITERATURE REVIEW

2.1   General overview of *Gracilaria*

Gracilaria is among the world's most economically important genera of macroalgae.. *Gracilaria* is the major source of agar that is used as a solidifying agent in various foodstuffs and is also an important medium for the cultivation of microorganisms in the laboratory (Chapman and Chapman, 1980). Gracilarioid algae have also been utilized as human food, mostly in salads and soups (Araski and Araski, 1983), as feed for marine animals such as abalone (Ajisaka and Chiang, 1993), as likely candidates for nutrient removal in waste-water (Fralick *et al.*, 1981) and in biomass production for energy (Ryther *et al.*, 1979; Hanisak and Ryther, 1986; Flowers and Bird, 1990).

Initially, *Gracilaria* was harvested from the beach cast populations and subsequently treated for agar extraction. However, its growing economic importance led to an increase in demand that led to over-harvesting of this resource, and consequently decreased agar production; Experimentation with a variety of methods of *Gracilaria* farming (Santelices and Doty, 1989) led to the development of *Gracilaria* mariculture in tanks, ponds and in the sea. These methods became successful and this was reflected in the increase in *Gracilaria* production, and agar yields (Santelices and Doty, 1989, Dawes, 1995). Among the characteristics that qualify *Gracilaria* as a potential candidate for cultivation are fast growth rates, good agar yield and quality and the relative ease of cultivation (Buschmann *et al.*, 1995). Equally important is that almost all cultivated *Gracilaria* species reproduce solely through fragmentation, and this results in high regenerative capacity (Hurtado-Ponce, 1990; Santelices and Varela, 1995).

2.1.1 Taxonomy

Gracilaria is a genus of red algae (Rhodophyta) that belongs to the order Gracilariales and family Gracilariaceae (Fredericq and Hommersand, 1989b). Even though it is a rhodophyte, it can be dark-brown, red, green or yellowish. *Gracilaria* is an easily identified and recognizable genus but the situation is quite different when it comes to identification at the species level. According to Stegenga *et al*, (1997) key characters are found in the female reproductive system that has a

supporting cell with two celled carpogonial filament and one or two sterile filaments. Despite much research, the identification of many species remains controversial. Although subject to criticism, use of gross morphology in combination with vegetative and reproductive anatomical details is still largely used in species identification, (e.g. Yamamoto, 1978; Abbott, 1985a). This system of identifying *Gracilaria* is reported to be adequate for limited geographical areas, on condition that the author has a good knowledge of most species in question, and that the description is based on adequate sampling in space and time. Many authors have relied largely on anatomical detail including the presence and position or absence of structures in the cystocarp known variously as connecting tubes, or filaments or absorbing filaments (Abbott 1985d) or traversing filaments (Kraft 1977b) or tubular nutritive cells (Fredericq and Hommersand 1989b). Dawson (1949) established the genus *Gracilariopsis* and he based his findings mainly on the absence of the 'nutritive filaments' that connect the gonimoblasts and the pericarp. According to Papenfuss (1966) filaments were lacking in the cystocarps of *Gracilaria verrucosa* (Hudson) Papenfuss, the type for the genus *Gracilaria*, and he synonymised *Gracilariopsis* with *Gracilaria*. This was generally accepted until 1989, when Fredericq and Hommersand (1989b) 'resurrected' the genus *Gracilariopsis*. The diagnostic characters used to distinguish *Gracilariopsis* from other genera in the family are:

(i)   Cystocarpic cavity not completely filled by gonimoblast.

(ii)   Absence of tubular nutritive filaments in cystocarp.

(iii)   Superficial spermatangia (Fredericq and Hommersand 1989a).

Gracialaria and *Gracilariopsis* find support as different entities in data from nucleotide sequences of 18s ribosomal RNA genes (Bird *et al.*, 1990), but this is not the case in the pattern of genome organization (Dutcher *et al.*, 1990). Attempted crossing seemed to be an important tool to recognize morphological populations and to separate cryptic species. The discovery by Bird and McLachlan (1982) and Plastino and Oliveira (1988b) was that geographically separated populations are interfertile and that no hybridization takes place in these species. Plastino and Oliveira (1990) revealed that there are genetic inhibitions between populations of plants regarded as *Gracilaria verrucosa* even though this is subject to misidentifications. Generally, the genus *Gracilaria* is easily recognized but identification at the species level remains problematic, particularly because some species are widely distributed (see section 2.1.2). On the west coast of South Africa and in Namibia, the common member of the Gracilariaceae is a species originally

known as *Gracilaria confervoides* (L.) Greville (Anderson *et al.,* 1989), then subsequently as *Gracilaria verrucosa* (Hudson) Papenfuss and at present as *Gracilaria gracilis* (Stackhouse) Steentoft.

2.1.2 Distribution and habitat of *Gracilaria*

The genus *Gracilaria* is widely distributed geographically with most species reportedly found in the warm-water areas of the northern hemisphere, with fewer species (e.g. *Gracilaria chilensis* and *Gracilaria gracilis*) extending into temperate waters (Oliveira and Plastino, 1994). The general distribution of *Gracilaria* species in the world extends from Argentina to Canada (Oliveira, 1984) and from South Africa (Papenfuss, 1940) to Norway (Rueness et al., 1987) in the Atlantic: from New Zealand (Nelson, 1987) and Australia (May, 1948) to northern Japan (Yamamoto, 1978), and from southern Chile (Romo *et al.,* 1979) to British Columbia (Saunders and Lindsay, 1979) in the Pacific; it is common in the Mediterranean (Gargiulo *et al.,* 1985) and widespread in the Indian Ocean (Umamaheswara Rao, 1972). *Gracilaria* is also found in the Antarctic Peninsula, and is abundant in temperate waters (Wiencke and Clayton, 2002). Most of the world's production of *Gracilaria* comes from Chile, Malaysia, Thailand, New Zealand, Philippines, Indonesia, China, Taiwan and southern Africa. *Gracilaria* can tolerate extremely high salinities i.e. up to 60 ppt (Yokoya and Oliveira, 1992a) and even freshwater (Macchiavello, 1994). The genus *Gracilaria* lives in a wide range of temperatures, i.e. some up to 35° C (Yokoya and Oliveira, 1992a,b) and some down to freezing, surviving such low temperature conditions for a few months (Titlyanov et al., 1995). Certain species have been found to survive burial by sand for a few months and these plants can be found attached either to calcareous tubeworms, mussel byssal threads or even attached to other algae and marine angiosperms (Santelices and Doty, 1989). With regard to their vertical distribution, *Gracilaria* species are found in intertidal and subtidal areas, but stringy 'gracilarioids' are most common in wave-sheltered, generally non-carbonate, sandy to muddy sediment, where there are unstable substrata. Generally, the most economically important *Gracilaria* beds usually occur as free-living, monospecific stands on mud or sand (McLachlan and Bird, 1986; Kautsky, 1989) and in Chile, commercial *Gracilaria* stands are usually infertile (Santelices and Ugarte, 1990), e.g. the population of *Gracilaria chilensis* (Bird *et al*, 1986) on muddy substrata in Bahia la Herradura and Bahia Tongoy (Santelices *et al*, 1984). In South Africa *G. gracilis* grows well in the shallow Langebaan Lagoon that forms part of the Saldanha Bay

complex (Isaac, 1956, as *G. confervoides* (Stackhouse) Greville, Simons, 1977; Christie, 1981; Rotmann, 1990). Anderson *et al,* (1989) and Rotmann (1990) reported commercial collections of the seaweed from Saldanha Bay, which in three years prior to 1974 were over 1000 t (dry) per annum. Fox and Stephens (1942) discovered *Gracilaria* at Langebaan Lagoon that grew on sandy or mixed substrata and reported large beach casts during winter storms.

In Namibia, *Gracilaria gracilis* is common at Luderitz, which is situated on the southwestern Atlantic coast of the Namib Desert. The common species growing here was originally known as *Gracilaria verrucosa* (Simons, 1976), an identification confirmed by Bird *et al.,* (1994), but has now been shown to be *G. gracilis* (Steentoft *et al.,* 1995; Bird and Kain, 1995; Iyer, 2002). The beach-cast *Gracilaria* has been collected since 1950 (Isaac, 1964). The fresh *Gracilaria* material is processed for agar production and prepared for the export market by drying it (Rotmann, 1985, 1987; Critchley *et al.,* 1991). No fertile material was ever found at this site (Molloy and Bolton, 1992).

2.1.3 Life-history
Members of the Gracilariaceae can generally be described as macroscopic algae from about 0.1 to about 5 m long, in many shades of red and brown-yellowish, with a branched, cylindrical to flattened solid thallus of pseudoparenchymatous, filamentous construction. Green forms are genetically determined and have been described for a few species (van der Meer and Bird, 1977; Kursar *et al.,* 1983; Plastino *et al.,* 1999).
The *Gracilaria* life history follows the basic pattern known as "Polysiphonia-type" (Ogata *et. al.,* 1972; Bird *et. al.,* 1977; McLachlan and Edelstein, 1977; Oliveira and Plastino, 1984; Rueness *et al.,* 1987; Plastino *et al.,* 1999).
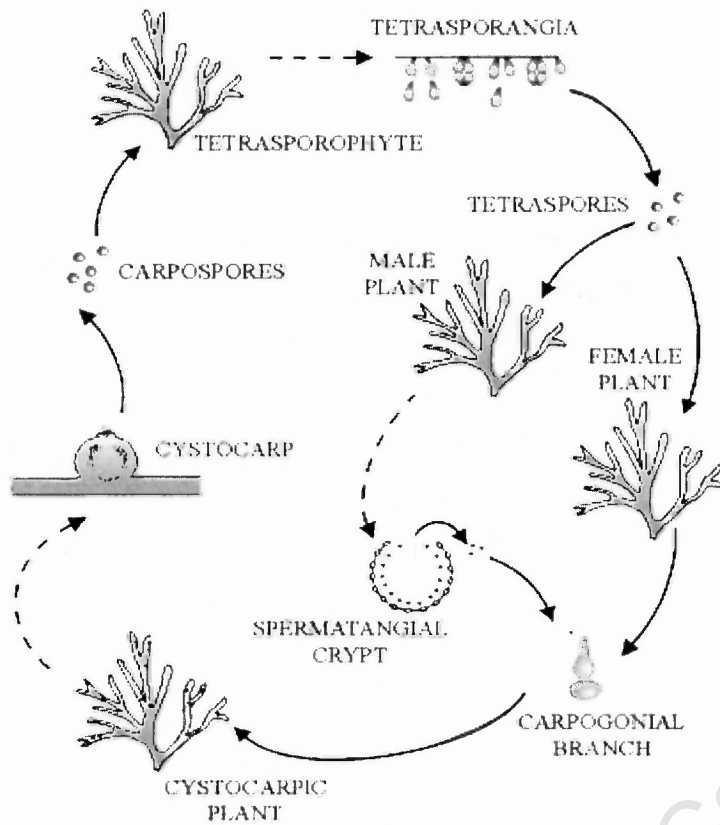
Figure 2.1: Polysiphonia-type life history of a gracilarioid alga (after Oliveira and Plastino, 1994).

2.2 *Gracilaria* cultivation methods

2.2.1 In the sea

This form of growth involves the cultivation of seaweeds in the sea (usually in protected bays or estuaries where there is little or no control over environmental factors. This type of seaweed cultivation can be divided into at least two different methods, bottom culture and suspended culture.

2.2.1.1 Bottom culture

Two different methods of bottom cultivation of *Gracilaria* are used, the first based on the use of non-sexual parts of the algae, and the second using plants grown from spores.

In *Gracilaria* cultivation there is no perfect method or technique; every method that is employed has its advantages and disadvantages. The bottom rope system has been a success with *Eucheuma* and *Kappaphycus* in the Philippines (Doty, 1979), Indonesia (Istini *et. al.,* 1998), and Tanzania (Mshingeni, 1998) but for *Gracilaria* cultivation, the floating rope system is a better method. The

selection of the best technique depends on the species of gracilarioid algae to be cultivated, on conditions at the cultivation site and on labour costs. Many planting techniques are used to attach *Gracilaria* to the bottom, but only two were often used in commercial scale farming in Chile (Santelices and Ugarte, 1987). The first of these, known as the direct method, involves direct insertion of bundles of *Gracilaria* thalli into the sandy bottom using different forked tools. This method is used mainly in intertidal areas. The second method, known as the plastic tube method, involves the fixing of bundles of *Gracilaria* thalli to plastic tubes (1 m long, 0.1 mm thick and 40 mm in diameter) filled with sand which then attaches the thalli on the sea bottom (Buschmann *et al* 1995). This method is more common in subtidal areas. Later, these plastic anchors tend to break up but this usually happens when the thalli have grown enough to withstand sea-bottom conditions and maintain position (Santelices and Ugarte, 1987). Other methods include attaching *Gracilaria* to rocks with rubber bands so as to make the thalli stable in soft sediments. Earlier studies have shown that only a few species of *Gracilaria* can tolerate direct planting in the sediment. *Gracilaria chilensis* is adapted to this method in that it can tolerate some degree of burial (Santelices and Doty, 1989).

Alternatively, *Gracilaria* can be grown by the inoculation of spores onto ropes, a bottom culture method known as rope seeding. This method begins in indoor tanks on an inoculation chamber, which contains the fertile thalli supplied with compressed air to keep the sporelings healthy until they reach the desired size and are ready to be transplanted to the field. This method has been commercially successful only in Chile (Alveal, *et. al.,* 1997). According to Li *et al.,* (1984) sea bottom cultivation of *Gracilaria sjoestedtii* produced 3 t ha^{-1} y^{-1} d. wt (see Table 2.1), while *Gracilaria* spp. in suspended cultivation produced 21 t ha^{-1} y^{-1} d. wt (Pizarro and Barrales, 1986) see Table 2.1.

Table 2.1: **Productivity of _Gracilaria_ cultivated under different methods in different regions (after Oliveira _et al._, 2000)**

Method	Species	Productivity ($t\ ha^{-1}\ y^{-1}$ d.wt.)	Reference
Tank	_Gracilaria_	25	Edding et al., 1987
	G. tikvahiae	80-91 (120)	Hanisak and Ryther, 1984
		73	Buschmann et. al., 1996
Pond	_G. confervoides_	10	Shang, 1976
	G. verrucosa	40	Chiang, 1981
	G. tenusipitata	20-25	Haglund and Pedersen, 1993
	G. verrucosa	24	Rotmann, 1987
	G. tenuistipitata	1.3-3.0	Ohno et al., 1997
Sea: Bottom	_G. sjoestedtii_	3	Li et al., 1984
	Gracilaria	21	Pizarro and Barrales, 1986
	Gracilaria	24.6-30.8	Martinez et al., 1990
Sea: Suspended	_G. verrucosa_	5.5	Li et al., 1984
	G. sjoestedtii		
	G. gracilis	45	Dawes, 1995
	G. gracilis	40	Anderson et al., 1996a
	G. chilensis	34	Troell et al., 1997

2.2.1.2   Suspended culture

This method of *Gracilaria* cultivation includes two techniques. The first technique involves the use of vegetative thalli or cuttings being inserted within, or tied to a rope. The second one involves the use of reproductive material as a supplier of spores that are then settled on ropes or lines. This can be done in two ways, either under natural conditions by placing new lines in dense wild crops (Smith *et al.*, 1984; Smith, 1992; Doty, 1986) or by putting spore lines in nursery tanks on land (Doty, 1986; Doty and Fisher, 1987; Jayasuriya, 1993). Another way of growing *Gracilaria* using this method is by using bamboo for flotation and this has been tried in places like Saint Lucia (Smith, 1990) and Venezuela (Dawes, 1995) but this method failed in countries like Brazil because the bamboo rots quickly.

In Namibia, the feasibility of growing *Gracilaria* using floating rope systems in the Luderitz lagoon was investigated. Wire hooks are used to insert *Gracilaria* thalli sideways (Racca *et al.*, 1993), through a superope (a tubular mesh stocking) that is placed horizontally about 1.0 to 0.5 m below the surface and remains in suspension throughout the experiment (Dawes, 1995). As the "superope" is pulled tight, the tufts of thalli are trapped in the middle leaving both ends free in the water. The Luderitz system covers about 5 ha of water, but even though it was producing about 45 t dry wt ha^{-1} y^{-1} in 1995 (Dawes, 1995), yields are now lower (Oliveira *et a.,l* 2000). In Luderitz, cultivated *Gracilaria* produces a higher agar yield than beach-cast from the wild population (Dawes, 1995). Water in Luderitz Bay is usually cold i.e. 13^{0}C and rich in nutrients. *Gracilaria* grows much better in summer probably due to strong winds that move the water surface and keep the seaweed free of sediments and epiphytes. The major problem is that tidal currents sometimes damage the system ropes and anchors.

In South Africa, Anderson *et al* (1996a) tested the performance of the rope system in Saldanha Bay. Average growth rates of about 5 % d^{-1} were recorded over several years where commercial stocking rates and methods were used, and a potential commercial yield approximately 26 t dry wt. ha^{-1} y^{-1} was estimated. The major problem in Saldanha Bay seemed to be the very low nitrogen levels in the surface waters in summer, during periods when strong thermal stratification is formed in the water column. *Gracilaria* grew well in Winter and Spring seasons but growth was highly unstable and sometimes poor in summer. Similar *Gracilaria* experiments were carried out on rafts

in St Helena Bay, which is the only other site on the west coast of South Africa that is sheltered enough from the swell for seaweed farming. The coastal upwelling increases the N-levels in the surface water during most of summer, and production is usually better than in Saldanha Bay (Anderson, *et al.*, 2003). The Namibian *G. gracilis* commercial farm yields 27-37 t dry wt ha^{-1} y^{-1} (Rotmann, pers. comm.). In South Africa, Anderson *et al.*, (1996) estimated 40 t dry wt ha^{-1} y^{-1} for Saldanha *G. gracilis*, from a small-scale experimental raft and Wakibia *et al.*, (2001) reported net productivity ranging from 18 to 127 t dry wt ha^{-1} y^{-1} from St Helena Bay. But Lapointe and Ryther (1978) reported a very high production (127 t dry wt ha^{-1} y^{-2}) for *G. tikvahiae*, and pointed out that small-scale experimental data only shows the potential productivity of the seaweed but such results cannot be extrapolated to a large-scale commercial operation. Li *et al.*, (1984) estimated production of 5.5 t ha^{-1} y^{-1} d. wt. for *G. verrucosa* and *G. sjoestedtii*; Dawes (1995) estimated 45 t ha^{-1} y^{-1} d. wt. for *G. gracilis*; Anderson *et al.*, (1996a) estimated production of 40 t ha^{-1} y^{-1} d. wt. for *G. gracilis*; Troell *et al.*, (1997) estimated a production of 34 t ha^{-1} y^{-1} d. wt. for *G. chilensis*.

2.2.2 Land-based aquaculture

Usually only two methods are used for the cultivation of *Gracilaria* in land-based systems (pond and tank culture systems). Pond cultivation is regarded as the cheaper and simpler method, while tank cultivation is considered to be the most expensive method of cultivating seaweeds because the operation is far more intensive (labour and capital).

2.2.2.1   Pond cultivation

Ponds can be either artificial excavations in the ground or natural lagoons that usually vary in length from a few metres to several hundred metres and depth from a few centimeters to more than a metre. This method of *Gracilaria* farming has been practiced in a number of countries (Oliveira and Alveal, 1990; Santelices and Doty, 1989; Critchley and Ohno, 1998), but commercial success has so far only been achieved in Taiwan, southern China, and Vietnam (Ohno *et al.*, 1997). Ponds are usually 0.7- 1.0 ha in area and 60-70 cm deep, with ranges in temperature and salinity of 15-30°C and 10-20 ppt. respectively (Santelices and Doty, 1989). Sheltered areas that cannot be affected by strong prevailing winds make suitable areas for pond cultivation of *Gracilaria*, and these should be situated in close proximity to sources of both freshwater and seawater. Although pond cultivation requires less control (semi-controlled system), certain factors need to be

considered, like the size of the pond, the water pH, light, temperature, water depth, nutrient addition, water exchange, epiphytes and grazers. The size of the pond is also important and it should not be too big because the thalli tend to be blown into concentrated areas in large ponds. Ponds should be shallow to ensure sufficient and uniform light absorption by thalli. Water exchange rate is crucial in pond cultivation to maintain salinity and mineral nutrient supplies for algal growth. To improve the quality of *Gracilaria,* the addition of nutrients in the form of fertilizers is highly recommended. Optimisation of culture conditions in ponds is very difficult to achieve because of the large size of the pond. Yields from ponds are generally low compared with tanks. For example, Chiang (1981) reported 16 to 43 t fresh wt. ha^{-1} y^{-1} in Taiwan and Ohno *et al.* (1997) reported similar yield (assuming about 5% dry weight) of 1.5 to 3 t dry wt. ha^{-1} y^{-1} in Vietnam.

One of the major problems associated with pond cultivation of *Gracilaria* is the development of accompanying algae and epiphytes. These have to be removed periodically by hand. However, in some countries biological methods of minimizing the epiphyte problem are used (see section 2.6). Pond cultivation is regarded as the simple and cheap method of *Gracilaria* cultivation. However, artificial ponds can prove to be expensive because of the high costs associated with excavating the pond, pumping of seawater, fertilization, labour for farming and subsequent processing. A pilot scale project is necessary to assess performance before embarking on a commercial operation.

In South Africa, particularly on the semi-desert west coast, a reduction in the scale of diamond mining has created possible opportunities for *Gracilaria* pond cultivation. In the search for alluvial diamonds, numerous shallow excavations were dug on the shore, and in some places there is even pumping equipment that was used for washing gravel (Oliveira *et al.,* (2000). In order to improve and maintain employment levels in the area, the co-cultivation of *Gracilaria* with animals such as abalone is being investigated (Oliveira *et al.,* 2000). Slight modifications on these excavations can turn these to suitable *Gracilaria* cultivation sites. Ponds are easy and simple to operate and with some degree of environmental and biotic parameter control, they can make potential *Gracilaria* cultivation sites (Oliveira *et al.,* 2000). The following authors report successful pond cultivation and the first one, Shang (1976) reported production of 10 t ha^{-1} y^{-1} d. wt. for *G. confervoides*; the second one, Chiang (1981) reported production of 40 t ha^{-1} y^{-1} d. wt. for *G. verrucosa;* Haglund

and Pedersen (1993) reported production of 20-25 t ha^{-1} y^{-1} d. wt. for *G. tenuistipitata*; Rotmann (1987) reported production of 24 t ha^{-1} y^{-1} d. wt. for *G. verrucosa*; Ohno *et al.* (1997) reported production of 1.3-3.0 t ha^{-1} y^{-1} d. wt. for *G. tenuistipitata.*

Gracilaria tank cultivation is referred to as an intensive cultivation system since it requires high energy inputs and can be controlled to a much larger extent than previously used methods. Of all the techniques used for the production of *Gracilaria*, tank cultivation provides the greatest productivity per unit area, and so far its production is unmatched by any of the other methods. Besides high potential yield, the tank method enables the farmer to optimize and control most environmental and biotic parameters. Where *Gracilaria* is cultivated together with an animal such as fish, the fish effluent from the farm is also treatable, therefore allowing a choice between internal re-circulation of water or release back to the sea (Shpigel and Neori, 1996). Another important advantage is that there is no extra pumping of water needed since *Gracilaria* can be cultured using abalone or fish effluent, therefore receiving nutrient-rich water. In this set up *Gracilaria* serves two functions, that of being a potential biofilter species purifying the nutrient-rich abalone effluent and being used as potential nutritious supplementary feed for abalone. Tanks have been used in several countries, mostly on an experimental level (Oliveira and Alveal, 1990; Critchley, 1993; Martinez and Buschmann, 1996; Bird and Ryther, 1990; Friedlander and Levy, 1995; Troell *et al*, 1999). Tank size, depth, shape, and colour are also of considerable importance in tank cultivation. To ensure a high productivity, tanks should be of large but manageable size, and be light in colour to minimize light absorption by the tank itself. Edding *et al.* (1987) reported *Gracilaria* production of 25 t ha^{-1} y^{-1} d. wt.; Hanisak and Ryther (1984) reported production of 80-91 t ha^{-1} y^{-1} d. wt. for *G. tikvahiae*; Buschmann *et al.* (1996) reported production of 73 t ha^{-1} y^{-1} d. wt. for *G. chilensis*.

2.3 Abalone nutrition

Abalone is an herbivore that feeds mostly on seaweed (McShane and Smith, 1988). Because of problems associated with the use of seaweed as feed for abalone (the unreliability of the supply of these seaweeds due to over-exploitation of natural stocks), abalone farming is becoming more and more dependant upon formulated artificial diets (Hahn, 1989; Anonymous, 1991; Britz *et al.,*

1994). These artificial abalone feeds are now used in Japan, China, Australia, New Zealand and South Africa. This requires careful monitoring of abalone response to the various nutrients contained in the diet and this is to ensure the production of low-cost and yet effective abalone diet. Protein is the most essential but expensive component in the diet of abalone. Uki *et al* (1985a) monitored abalone growth using diets containing various proteins, including casein, soya bean meal, rye grass concentrate, egg albumin, whole egg and fishmeal. They found casein to be the most suitable protein for inclusion in artificial diets. The optimum protein level in a casein-based diet was found to be in the range of 20-30% by Uki *et al* (1986). Britz (1996) discovered that there was a positive correlation between growth rates of *Haliotis midae* with protein content from 27 to 47% whereas the protein efficiency ratio (PER) decreased with an increase in protein level (negatively correlated). In his report, Britz (1996) suggested that a dietary protein level higher than 20-30% might be required to achieve maximum growth rate. The artificial dry feed consumption by abalone seems to be controlled by their metabolic rate, since consumption in *Haliotis discus hanna* (Hahn, 1989) and *Haliotis midae* (Britz et. al., 1997) has been found to be a function of body size and temperature. To obtain optimal growth, the rate of protein deposition by abalone must be maximized, which means that formulated feeds should contain enough protein. This shows that the expensive protein fraction should be used effectively (for growth rather than for energy). This leads to increased production costs if abalone is to be cultured intensively using formulated feeds. In South Africa, because of high costs associated with using formulated feed, fresh seaweeds are used alternatively as abalone feed. These include the kelp, *Ecklonia,* green algae, *Ulva* and red algae, *Gracilaria.* Kelp, being the abundant species, particularly in the southwest and west coast of South Africa, is being used as the primary feed for abalone. In the past, dietary research on abalone concentrated on single-species diets. But research about the natural diet of the South African abalone, *Haliotis midae* has shown that abalone feed on a variety of algae usually with at least two species being found in the gut content at any time (Barkai and Griffiths, 1986).

2.4   Integrated Aquaculture

Aquaculture has grown fast over the past decade and is still growing in view of its importance in producing protein food for the ever-increasing world population. According to Edwards (1997) consumption of cultured fish has doubled over the past three decades. However, the rapid increase in aquaculture has caused significant environmental problems (marine pollution and environmental

degradation) that are unavoidable by-products of intensification of marine aquaculture systems (ICLARM 1993, FAO/NACA 1995, Stewart 1997). Large monoculture systems usually contribute in the degradation of species quality and this result in a progressive decline in species resistance to pathogenic infections (Liao 1992). It is shown in previous research studies that integrated culture systems promoted growth of both algae and animals (Chiang 1980; Chang and Wang 1985; Shan and Wang 1985; Tian *et al.,* 1987; Trono 1989; Wei 1990; Qian *et al.,* 1996).

Many seaweed species are usually of less economic importance than the cultured marine animals (Shpigel and Neori, 1996). This makes seaweed monoculture in land-based systems generally non-viable at commercial levels. Pumping of seawater has been found to be the single most expensive factor in this process. The release of nutrient-rich water from animal monoculture systems raises environmental and economic concerns because usually fish excrete into the water 70–80 % of their ingested protein N, 80 % of it in dissolved forms (Porter et al., 1987). This eutrophication problem, coupled with the high expenses of running a monoculture system justified the start of integrated culture systems that would combine the cultivation of seaweeds with abalone as well as to other fish culture systems. Integration proved to be a success not only for production in mariculture systems but for the coastal environment because a considerable reduction in mariculture effluent by seaweed biofilters was observed in several systems (Ryther *et al.,* 1975; Gordin, 1983; McDonald, 1987; Vandermeulen and Gordin, 1990; Cohen and Neori, 1991; Buschmann *et al.,* 1994).

2.5   Key Biological and Environmental parameters in *Gracilaria* cultivation

Environmental factors play a critical role in the growth, reproduction and distribution of marine macroalgae (Gessner, 1970; Gessner and Schramm, 1971; Luning, 1981; Lobban and Harrison, 1994). Understanding how these factors affect growth and production of *Gracilaria* is of great importance in the management and cultivation of these marine macroalgae. Land-based culture systems allow for the manipulation of some of these factors to desired levels, but other factors are very difficult to control even in land-based culture systems (e.g. light and temperature). Interrelationships among these environmental factors remain poorly understood (Kautsky, 1989).

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2.5.1 Temperature

Temperature plays an important and a significant role in controlling the growth rate of *Gracilaria* species (Friedlander *et. al.,* 1987; Laing *et. al.,* 1989; Molloy, 1992; Anderson *et. al.,* 1996; Molloy and Bolton, 1996). To improve the biomass production of *Gracilaria* it is necessary to optimize photosynthesis, with temperature and light being the most crucial factors in this process. Temperature and light availability are usually determined by geographical factors, both decreasing with an increase in distance from the equator. The major limiting factor in temperate climates is usually the short growing season and the low winter temperatures. Temperate species usually grow better at higher temperatures (Fortes and Luning, 1980) and often exhibit a seasonal shift in heat tolerance with a peak in summer (Luning, 1984). According to McLachlan and Bird, (1984) for each species, optimal growth often takes place within a narrow temperature range. Optimal temperatures for *G. tikvahiae* in Florida were in the 24–30 ^{0}C range (Hanisak, 1987).

On the west coast of South Africa, (in Saldanha Bay and Langebaan Lagoon), the mean monthly temperatures range from 13^{0}C to 18^{0}C (Bolton 1986; Grindley 1976), with a highest recorded temperature of 23.9^{0}C in Langebaan Lagoon (Day 1959). Temperature for growth is also higher than for most temperate species of *Gracilaria* tested, which usually grow faster in the 15–20 ^{0}C range (McLachlan and Bird 1984). According to Engledow and Bolton (1992), South African *Gracilaria* also grows best at 15-20 ^{0}C.

2.5.2 Light

Gracilaria is a widely distributed genus with a broad range of optimal irradiance levels for growth (Kim, 1970; Bird *et. al.,* 1979; Friedlander *et. al.,* 1987; Lignell, 1988; Friedlander, 1991; Engledow and Bolton, 1992; Haglund, 1992). Light, just like temperature, significantly influences the growth of *Gracilaria*, e.g. Beer and Levy (1983); Edding *et al.,* (1987); Friedlander *et al.* (1987); Friedlander (1991); Engledow and Bolton (1992); Molloy and Bolton (1996). Irradiance levels between 50 μmol m^{-2}s^{-1} and more than 1450 μmol m^{-2}s^{-1} have been shown to induce maximum growth of various *Gracilaria* species (Kim 1970; Bird *et al.* 1979; Friedlander *et al.* 1987; Lignell *et al.* 1987; Friedlander 1991; Engledow and Bolton 1992; Haglund 1992). The effect of irradiance on growth is through its effect on photosynthesis (Macler and Zupan 1991). Extremely high irradiance levels can cause loss of colour in *Gracilaria* spp. Light, just like

temperature, can potentially be controlled in tank systems, but it is easier to lower than to raise light levels, by using shade-cloth to cover the material during periods of extremely high irradiance. Beer and Levy (1983), working on *Gracilaria* sp. from Israel, found that the photosynthetic pigments, chlorophyll a and phycoerythrin were negatively correlated with irradiance levels. The ratio of chlorophyll to phycoerythrin increases with increasing irradiance levels, (the thalli grown at low irradiance levels appeared reddish while thalli grown at higher irradiance levels were greenish in colour). The density of algae per unit area also affects *Gracilaria* growth (by increasing self-shading), and can be manipulated to optimize growth in tank systems.

2.5.3 Salinity

Generally, *Gracilaria* species are euryhaline (Trono and Azanza-Corrales, 1981; Lapointe *et. al.,* 1984; Penman and Mathieson, 1985; Bird and McLachlan, 1986; Lignell, 1988; Xin, 1989; de Castro *et. al.*, 1991; Engledow and Bolton, 1992; Haglund and Pedersen, 1992). In some areas of the intertidal zone, estuaries and rock pools, seaweeds usually experience regular changes in salinity (Koch and Lawrence 1987), but most species have a wide tolerance range for salinity with maximum growth occurring down to, and sometimes below 10‰ (Kain and Norton 1990). Bird and McLachlan (1986) found that the maximal growth of many *Gracilaria* species is in the 15 ‰-38 ‰ range. Salinity affects both the growth rate and the plant morphology (Yokoya and Oliveira, 1992). At low salinity, bleaching of the thalli occurred; for *G. aculeata* bleaching occurred in segments grown at 5 %, 15%, 25% and 30% while for *G. gracilis* the change of colour occurred in segments grown at 5% and 15% i.e. whole segments or regions of segments changed from red to green-white (Wilson and Critchley 1997). Similar results were recorded for numerous *Gracilaria* species i.e. necrotic zones appeared, especially in apical tissues, and thalli even became bleached (Bird and McLachlan, 1986). Rhenzi *et al.* (1989) also reported necrosis of apical segments of *G. tenuistipitata* when grown at 3‰. According to Kock and Lawrence (1987) the amount of chlorophyll a and phycoerythrin declined with the exposure of *G. verrucosa* to low salinities (10%). Many *Gracilaria* spp. exhibit wide salinity tolerances (5‰-60‰) and optimal salinities for various *Gracilaria* species are very variable, occurring in the 10-35% range (Chiang, 1981; Trono and Azanza-Corrales, 1981; Rueness and Tananger, 1984; Penniman and Mathieson, 1985; Bird and McLachlan, 1986; Penniman *et al.,* 1986; Lignell, 1988; Rhenzi *et al.,* 1989, Tra, 1989, Xin, 1989, Yu and Pedersen, 1990; Haglund, 1992). The South African species, *Gracilaria gracilis* (as

G. verrucosa) was shown to be euryhaline with sustained growth from 9 – 45 ‰ salinity range. Optimal growth was obtained around the salinity of full seawater with reduced but reasonable growth in lower and higher salinities (Engledow and Bolton, 1992).

2.5.4 Inorganic carbon and pH

The supply of inorganic carbon (usually CO_2) is critical in maintaining high growth rate in cultured seaweeds (DeBusk and Ryther, 1984). Declining CO_2 levels are indicated by a rise in pH in the seawater and thus pH is a convenient measure of CO_2 availability in cultivation tanks. Inorganic carbon can be supplied as CO_2, compensating for low water exchange. This was observed in an experiment with *G. conferta* (using CO_2 addition) achieved by using a controlled pH 8 with low water exchanges (2 volume exchanges d^{-1}); the results showed the same weekly growth rate as a non-controlled pH treatment with high water exchanges (24 volume exchanges d^{-1}), but results with low water exchanges and no CO_2 addition or pH control gave significantly lower growth rates. However, in the same study, controlled pH (7.5-8.0) treatments compensated for low water exchange only under summer conditions (Friedlander and Ben-Amotz, 1991). Studies with *G. tikvahiae* confirmed that pH control by CO_2 addition could be used as an alternative to high water exchange, but that HCl is not useful in this regard without increased water exchange (DeBusk and Ryther, 1984; Bird, 1990; Schramm, 1991). The optimal pH for high growth rates tends to be species specific, with some species showing a maximum growth rate at pH 12 and some at pH 7-9. However, the most suitable pH for maximum growth rate is usually in the pH range 7.0-9.0, close to that of normal seawater.

2.5.5 Water exchange

Water exchange is essential in *Gracilaria* tank cultivation, to supply nutrients and CO_2. When culture systems are maintained at high water exchange rates a certain degree of temperature and salinity control is also achieved. Through the supply of CO_2 in the water, pH in the culture systems is also controlled. Even though the pumping of water has proved to be the single most expensive factor in *Gracilaria* tank cultivation, when these seaweeds are incorporated into abalone cultures, no additional water is needed since seaweeds can be grown using abalone water (Friedlander and Levy, 1995). Therefore integration with abalone makes *Gracilaria* cultivation in tank systems both

technically and economically feasible. High water exchange rates of 20 to 30 volumes per day are suggested for maximal growth rate of *Gracilaria* in seawater (Santelices and Doty, 1989).

2.5.6 Aeration

A certain degree of water movement is necessary in the culture tanks if the desired quantity and health of *Gracilaria* are to be obtained in land-based systems. It is therefore essential that the seaweeds are kept moving in the tanks to expose them to light as well as to improve nutrient and gas exchange. Without water movement the *Gracilaria* thalli may be subject to self-shading, leading to unequal light and temperature exposure and this can have severe detrimental effects on photosynthesis. Oxygen levels may also increase, and metabolites may build up in the medium. In order for the water-movement system to function properly there should be control of the biomass in the culture system and this can be done by frequently harvesting the seaweed back to an initial stocking biomass. There are basically two ways of keeping seaweeds moving in culture systems, and both methods have been shown to be effective. These include the paddle-wheel method and the more commonly used method of compressed air released from a perforated pipe placed along the bottom of the tank (Friedlander and Levy, 1995). The latter moves the thalli in a circular motion so as to enable uniform light absorption (Mathieson, 1982). Practicing periodic aeration may reduce aeration costs. Ryther *et al.,* (1983), showed that there was no difference in growth of the thalli when there was only 15 min h^{-1} aeration for a total of 6 h d^{-1} compared to continuous aeration (24 h d^{-1}).

2.5.7 Nutrients

One of the important requirements for tank cultivation of *Gracilaria* is the provision of nutrients, particularly nitrogen, phosphorus and carbon, which can often be limiting. The nutrient status of the medium needs to be monitored regularly. *Gracilaria* tends to lose colour under low nutrient conditions. In many culture systems the crop is pulse-fed with nitrogen and phosphorus. Generally, nitrogen and phosphorus are regarded as growth-limiting nutrients under optimal seaweed density (Guist *et. al.,* 1982). Nutrient addition for *Gracilaria* growth may also increase the development of epiphytes. The amount of N uptake by *Gracilaria* is usually more than that required for growth, and has been termed 'luxury' uptake (Bird *et. al.,* 1982). The *Gracilaria* has a potential to store excess N for long periods of time (Fujita, 1985), mainly as free amino acids, chlorophyll-a and

phycoerythrin (Bird *et al.*, 1982). Because the common epiphytes are not able to store nutrients for as long as *Gracilaria*, pulse feeding gives it a temporary competitive advantage over the epiphytes when compared to continuous feeding (Ryther *et. al.*, 1981). Night pulse fertilization had the same effect on *Gracilaria* growth as day pulse fertilization, also giving a competitive advantage over epiphytes (Bidwell *et al.*, 1985; Hanisak, 1987). Pulse feeding, therefore is an effective way to counteract the undesirable occurrence of epiphytes in *Gracilaria* cultivation. Friedlander and Levy (1995) suggested the maintenance of N: P ratio at 10:1. But seaweeds differ in their capacities for utilizing different sources of nitrogen. *Gracilaria* showed a marked preference for NH_4+ in many reports (Deboer, 1978; Delia and Deboer, 1978 and Ryther *et. al.*, 1981) demonstrated that *Gracilaria gracilis* is more efficient at absorbing NH_4+ at low ambient nitrogen concentrations. Ammonium is usually the most preferred N source for pulse doses, as both N uptake rates and plant growth rate are higher when compared with nitrate at short pulse duration (Ryther *et al.*, 1981). A good understanding of the nutrient requirements and uptake capability of *Gracilaria* species is needed when maximizing the productivity in land-based mariculture systems.

2.5.8 Stocking biomass

Density is an important factor in the culture of *Gracilaria* in outdoor tanks and if it is not kept under control it can have detrimental effects on the growth of the seaweed. Sufficient knowledge of the species to be cultivated is critical in seaweed tank cultivation. The optimal stocking density that yields maximum growth rate for *Gracilaria* species may be species-specific. Usually *Gracilaria* grown under low stocking densities gives higher growth rate than the one grown under high stocking densities. However, the latter gives better yields and that is what is important in commercial farms (Lapointe and Ryther, 1978). According to Lapointe and Ryther (1978), maximal yields are obtained at a higher density i.e. 2 to 4 kg m^{-2} while maximal growth are obtained at a low density i.e. 0.4 kg m^{-2}. Season can influence the stocking biomass as well. Ugarte and Santelices (1992) recommended low stocking biomass i.e. 4 kg m^{-2} in autumn, winter and spring and 8 kg m^{-2} in summer and the latter produced the greatest growth of *Gracilaria* in their culture systems in Chile. In order to keep maximal yield and to prevent self-shading due to the growth of seaweed, harvesting of stock at regular intervals i.e. weekly is recommended (Friedlander and Levy, 1995). An increase in the stocking density during the summer months is

possible because of the high temperatures and irradiance during this season. When stocking density is too high, self-shading and competition for nutrients will limit growth.

2.5.9 Epiphytes

One of the main problems in the cultivation of *Gracilaria* is the development of unwanted epiphytes and accompanying algae. Epiphytes are plants or animals that grow on a plant and accompanying algae refers to any other unwanted plant material that develops in the tank (either loose or on the tank walls). Epiphytes are mainly thread or sheet- like with a high surface area/volume ratio (Littler and Littler, 1980). The most common epiphytes associated with *Gracilaria* cultivation include *Ulva* spp., *Enteromorpha* spp., *Ceramium sp.*, *Cladophora* spp., *Rhizoclonium* spp. and *Ectocarpus* spp. (Lapointe and Ryther, 1978; Chiang, 1981; Friedlander *et al.*, 1987; Anderson *et al.*, 1998). Regardless of the cultivation method used, epiphytes have caused problems. The success of *Enteromorpha* and *Ulva* as epiphytes is probably a result of their wide geographical distribution and wide range of tolerance of temperature and irradiance levels (Enright, 1979). *Ulva* species are well suited for an epiphytic mode of life and respond well to enhanced supplies of nitrogen, for which they have rapid uptake rates (Fujita, 1985) and they have high growth rates (Enright, 1979).

2.5.9.1   Effects of epiphytes in *Gracilaria* cultivation

Epiphytic algae are opportunistic and are reported to show high rates of nitrogen uptake and photosynthesis, resulting in their high growth rates (e.g. in *Ulva lactuca*; Svirski et al., 1993). The level of the damage to the crop seaweed is determined by the degree of contamination, and there are reports of epiphytes reaching 60-70% of the harvested weight of *Gracilaria* plants in some parts of the world (Cancino *et al.*, 1987; Buschmann and Gomez, 1993). If these epiphytic algae are not controlled, they reduce yields in cultivation by competing for light and nutrients and increasing the mechanical drag on the host plant, thus leading to poor *Gracilaria* yields and quality. *Ulva* is an important epiphyte both in sea and in cultivated gracilarioid populations (Buschmann and Gomez, 1993), where it can minimize light that reaches *Gracilaria* by 50% or more (Pizarro and Santelices, 1993). *Ulva* also affects *Gracilaria* growth by its ability to produce allelopathic compounds that lead to retarded growth of the seaweed beyond simple competition for light and nutrients (Friedlander and Gonen, 1996). Svirski et al. (1993) reported similar results,

where the growth inhibition of *Gracilaria* spp. when cultured in the presence of *Ulva lactuca*, was found not to be due to shading or nutrient depletion, but appeared to be caused either by competition for inorganic carbon or some kind of allelopathy. In Britain epiphytism was a serious threat to *Gracilaria* cultivation as a result of both epiphytic biomass that led to shading and drag, and the amount of silt accumulating on the seaweed (Jones, 1959a). In China there was negative correlation between epiphytism and growth rate of *Gracilaria* (Li *et al.*, 1984), and the floating *Ulva* that settled on the growth areas resulted in the shading of *Gracilaria* material in Chile (Pizarro and Barrelas, 1986).

Epiphytes also compete for dissolved gases. This usually occurs in closed non-aerated cultivation systems (e.g. large dams), where the contaminants prevent water movement resulting in heterogeneous distribution of the host plants (Hanisak, 1987). Self-shading usually result from this and can lead to severe damage to the *Gracilaria* (Hanisak, 1987).

2.5.9.2   Methods of control

Generally, three methods have been tried in attempts to control, prevent, remove or reduce epiphytes in *Gracilaria* cultivation.

2.5.9.2.1 Physical method of controlling epiphytes

These involve the physical removal of epiphytes from the host species. There are also other ways of removing these epiphytes, particularly diatoms, from the host species, like the use of water jets, which is an effective way of washing of host material after harvest. For the epiphytes with penetrating rhizoidal filaments hand removal is required.

This method of epiphyte removal is more time-consuming, more expensive, and often impractical on a commercial scale and can cause damage to the primary species (Ugarte and Santelices, 1992). Preventive measures are usually the best practical way of controlling epiphytes in large-scale culture systems. These include screening and filtering the in-coming seawater to remove contaminating organisms (Guiry and Ottway, 1981; Edding *et al.,* 1987; Pickering *et al.,* 1993), and regular renewal of seawater (Hanisak, 1987; Pickering *et al.,* 1993). Also, surfaces of the tanks need to be cleaned regularly (Edding *et al.,* 1987). The removal of epiphytes by hand from the

stock material before the tanks are stocked can minimize the problem (Santelices and Doty, 1989). This means checking the stock material regularly.

Another important way of preventing epiphyte problems is by manipulating some of the environmental conditions, for example light. In many studies, epiphyte problems in *Gracilaria* cultivation seem to be enhanced by high irradiance levels, so that lowering of irradiance levels that can favour the growth of *Gracilaria* while eliminating the epiphytes. Light can be lowered either by screening the tanks with a shade net (Guiry and Ottway, 1981; Hansen, 1984; Rueness, and Frederiksen, 1989), or the use of fixed raft techniques (Ren *et al.,* 1984; Lipkin, 1995). Varying the depth of the cultivation substrata (Wheeler *et al.,* 1981) has been found useful. Ugarte and Santelices (1992) found that covering the tanks with black plastic for 48 hours excluded *Ectocarpus* and reduced *Enteromorpha* contamination by 80%. Friedlander (1991, 1992) reported that a green cover reduced the wavelengths of light available to the green epiphytes more than those available to the red algae, thus favouring *Gracilaria* over green algae.

2.5.9.2.2   Chemical method of controlling epiphytes

The development of epiphytes and their competition with the host plants usually take place in high nutrient conditions (Yoneshigue-Braga and Baeta Neves, 1981; Fujita, 1985; Fujita and Godman, 1985). The continued addition of nitrogen for *Gracilaria* growth enhancement also encourages the development of epiphytes. Careful management and manipulation of nutrients, particularly nitrogen, can prevent this problem. *Gracilaria* has an ability to absorb nitrogen far in excess of that needed for its growth, this is termed 'luxury' uptake, and the nitrogen is stored in proteins and used during the following 7-14 days as it is slowly released, whereas most epiphytes cannot store N and require a constant supply (Lapointe and Duke, 1984). *Gracilaria* also stores nitrogen in free amino acids (Lignell and Pedersen, 1987). Pulse feeding of nutrients is therefore a better option in *Gracilaria* cultivation, since it gives these algae a temporary advantage over the competing epiphytes (Ryther *et al.,* 1981). Night pulse fertilization also gives *Gracilaria* a similar competitive advantage over epiphytes (Bidwell *et al.,* 1985; Hanisak, 1987).

In controlled land-based environments (e.g. tanks or small ponds) copper chloride has been used to treat epiphytic *Enteromorpha* (Haglund and Pedersen, 1993) and *Ectocarpus* (Van Heerden *et al.,*

1997), but this may damage the host as well. Other toxins, such as sodium hypochlorite have been found to be damaging on both epiphytes and *Gracilaria* (Ugarte and Santelices, 1992).

2.5.9.2.3   Biological methods of controlling epiphytes

The most economically feasible method of controlling epiphytes in large-scale cultivation of *Gracilaria* is the use of biological methods. Epiphyte control is achieved by introducing the natural grazers of these problematic species (Shacklock and Doyle, 1983) or milkfish (Chiang, 1981). These grazers have a potential and are effective in minimizing epiphyte contamination, both in natural and cultivated *Gracilaria* populations (Shacklock and Doyle, 1983). A study in Saldanha Bay, South Africa, showed that cultivated *Gracilaria* could be heavily infested with *Ceramium diaphanum*. Another important grazer is the isopod, *Paridotea reticulata* which occurs naturally in the environment, selectively feed on *Ceramuim,* and can reduce epiphyte loading considerably if it is not so abundant (Anderson *et al.,* 1998).

2.6   *Gracilaria* growth rate

Gracilaria growth rate in land-based systems is a function of the combination of various factors including temperature, light, salinity, pH, nutrients, stock density, seawater supply and other factors. To obtain the desired high levels of growth rate in *Gracilaria* cultures, there should be continuous monitoring of these factors. Variations in one of these factors can lead to poor growth rate because of the interdependence of these factors. However, the under-supply of some of these factors can be compensated for by other factors. Controlled pH (7.5-8.0) treatments compensated for low water exchange under summer conditions but not in autumn and spring for *G. conferta* (Friedlander and Ben-Amotz, 1991). This compensation was also shown for *Chondrus crispus* (Bidwell *et al.* 1985) and *G. tikvahiae* (Ryther *et al.,* 1981).

There seems to be a positive relationship between growth rate and temperature. In Great Britain *G. gracilis* was found by Jones (1959a) to grow fastest (2.6% d^{-1}) during summer when temperature and light quantity were maximal, however, bleaching was also found in *G. gracilis* grown in full daylight near the surface (Jones 1959b), which was explained as an effect of breakdown of the photolabile phycoerythrin. In northern Chile, *Gracilaria* sp. attached to substratum by sand filled tubes or stones also exhibited a maximum growth rate in summer (Pizarro and Barrales, 1986). In

China *G. gracilis* grew maximally in summer with an optimum temperature of 12-20°C (Li *et al.,* 1984).

Light is one of the growth-limiting factors and poor control of it affects photosynthesis and thus growth (Macler and Zupan, 1991). Molloy (1992) reported that growth rate of *Gracilaria gracilis* in Luderitz Bay was controlled primarily by light availability. A wide range of optimal irradiance levels for growth has been reported (e.g. Kim, 1970; Bird *et al.,* 1979; Friedlander *et al.,* 1987; Lignell, 1988; Friedlander, 1991; Engeldow and Bolton, 1992; Haglund, 1992).

Chapter 3

3.1 Study Sites

This study was conducted at two Western Cape abalone farms in order to assess the potential for seaweed cultivation in this region, which has a large number of abalone farms (about 10 at present). Two established abalone farms, Irvin & Johnson (I&J) in Gansbaai and Jacobsbaai Sea Products (JSP) in St Helena Bay were used as experiment sites. The I&J farm is situated approximately 140 km east of Cape Town, while the JSP farm is situated approximately 120 km north of Cape Town. Some of the reasons that made these two farms suitable for this study were: both farms agreed to be part of this collaborative work and were eager to incorporate seaweed in their abalone culture systems; their geographical location, I&J farm is situated on the south-west coast of South Africa while JSP farm is situated on the west coast of South Africa, exposed to the strong upwelling process; I&J farm offered an ideal place and culture units suitable for commercial scale experiments, while JSP farm offered an ideal space to set up a pilot study to test the feasibility, both biologically and technically of growing *Gracilaria* at high turnover rates.|

3.2 *Gracilaria* culture tanks at JSP and I&J farms

A variety of tanks exist for the mass culture of marine macroalgae. These range from small tanks (i.e. 50 litres volume), intermediate-sized tanks (i.e. 300 litres) to large tanks (i.e. ranging from 700 litres to over 1000 litres volume). The latter range is the most commonly used size in tank cultivation of *Gracilaria*. These tanks can be made of concrete or plastic material, with water aeration being supplied in the form of compressed air via the perforated PVC pipes that are placed in the bottom of these tanks. However, air supply to *Gracilaria* culture tanks can also be achieved by using paddle wheels. High levels of production have been achieved for various gracilarioids in small culture vessels, but with each scale-up attempt, production tends to decline (Hanisak and Ryther, 1984). 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. In this study we used small and medium-

sized plastic tanks and large plastic tanks with wooden frames. Air was supplied from perforated PVC pipes that were placed in the bottom of these tanks (see section 3.3.1.2 and 3.3.2.2).

The farms were visited once (I&J) or twice (JSP) every month and otherwise the experiments were maintained by the farm staff. Controllable culture conditions were kept similar in both farms. At JSP farm, the experiments were done on an "experimental scale" using small and medium sized tanks (Figure 3.1 and 3.2), while at I&J farm, the experiments were done on a 'pilot commercial scale' using large tanks that are normally used for abalone cultivation (Figure 3.3).

Figure 3.1: The culture system set-up of small and medium-sized tanks with two seawater supply tanks at JSP farm.
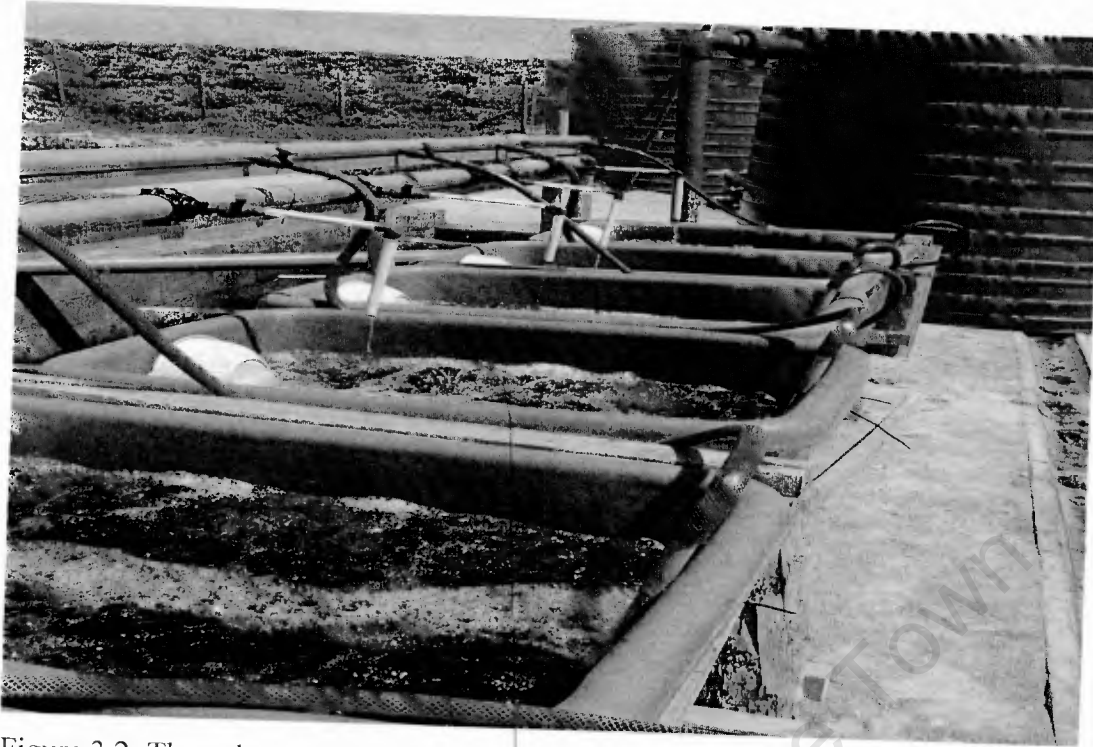
Figure 3.2: The culture system setup of medium-sized tanks with two seawater supply tanks at JSP farm.

Figure 3.3: The culture system setup of large *Gracilaria* tanks at I&J farm.

3.3. Farm background

3.3.1 Jacobsbaai Sea Products (JSP)

Jacobsbaai Sea Products Pty is a land-based intensive mariculture operation situated on the point of Jacobsbaai. The farm has an abalone (*Haliotis midae*) stock of approximately 2.7 million individuals (about 76.8 tons), which ranges from spats to 6 year-old animals. About 40 tons of kelp (*Ecklonia maxima*) is used each month as abalone feed. Abalone eats approximately 1.3-1.4% of its body weight in kelp per day (K. Ruck personal communication). Through the daily pumping in of seawater, harmful algal blooms periodically threaten JSP farm, just like other abalone farms in the Western Cape. Before the start of this study, the farm had no system in place to address the problem of the inability to recirculate water when harmful algal blooms occur. The farm was also not self-reliant in terms of abalone feed and dependant only on kelp, *E. maxima*, that was harvested daily from a nearby shore and the farm consists of four primary components: the settling and holding dams, the abalone tanks, the turbot (fish) tanks and the oyster raceways. The farm had previously grown oysters in raceway channels that are about 50 m long and approximately 0.5 m deep, but these are presently not functional and oysters are being grown in small cages in the two big holding dams.

Seawater is pumped directly into the top settling reservoir dam and from there it is gravity-fed to either the turbot tanks or into holding tanks. Water can be pumped directly into the holding dams where it is heated by solar radiation (in summer). This is done to increase the water temperatures experienced in summer due to upwelling off the west coast to the levels desired for abalone growth. Low water temperatures slow the growth rate of the abalone. The water turnover rate for the top-settling reservoir dam is 5.6-volumes d^{-1} and 4.5-volumes d^{-1} for the bottom two dams. From the dams, the water is pumped into a mixing tank where it can be distributed to the abalone tanks, turbot tanks, and oyster raceways or returned back to the dams. Effluent water is channeled down to the sump where the particulate are settled down, and the remaining wastewater is returned to the sea.

3.3.1.1 Tank design (small and medium-sized tanks)

Small and medium-sized tanks were used at JSP. The small plastic tanks were rectangular in shape, light grey in colour with an effective volume of 82 litres. The inside dimensions were, 0.60 m X 0.38 m X 0.36 m and the walls of the tanks were two mm thick. The medium-sized tanks were also rectangular in shape, grey in colour and their volume was about 283 litres. The inside dimensions were 0.92 m X 0.55 m X 0.56 m and they were approximately 10 mm thick. These tanks were used mainly to grow the parent stock material. All the controllable factors such as water flow rate and stocking density were kept similar in both small and medium tanks for the entire growth period.

Air was supplied via a 20 mm diameter and 2 mm thick PVC pipe. The pipes were perforated by drilling 2 mm holes at 90 mm intervals except the last three holes, which were drilled closer (i.e. 4.5 mm apart) along the length of the pipe. The reason for this difference was that I noticed that air supply was not uniform along the entire length of the bottom pipes when the holes were all drilled at equal 90 mm intervals. The first few holes were producing air at a higher pressure than the last few holes hence the need to change spacing towards the end of each pipe. The aeration system was made of a 'U' shaped frame comprising two 54.3 cm long, 20 mm diameter pipes closed on one end by a 20 mm stopper and each pipe joined to a 14.4 cm long, 20 mm diameter pipe by a 20 mm 'tie' and the whole system was connected to the main air supply 63 mm diameter pipe. PVC solvent was used to permanently join the pipes to avoid air leakage. To ensure that the pipes would not float and would remain attached, I cemented the frames to the base of the tanks using silicone glue.

Water was supplied using pipes of the same material as the aeration system but these were about 32 mm in diameter. Water was supplied directly from the main pipe via a small tap from which an 'L' piece of 20 mm diameter PVC pipe was joined to direct water down and into the tank. The output system consisted of a 20 cm diameter colander that was placed over the outlet hole drilled at about 8 cm from the rim. The colander was held in place using silicone and two small cable ties and prevented *Gracilaria* from washing out of the tank. A 10 cm running nipple was connected through the hole with nuts and gaskets to tighten it. Another 20 mm diameter PVC pipe was joined from the nipple, channeling the output water to the sump. A similar output system was designed in

medium-sized tanks except that here outlet hole was drilled at about 30 cm from the rim. On 2 November 2001, another set of small tanks was set up to complete the three-treatment research experiment. These small tanks were the same as the small tanks used in the first phase (seawater growth experiment). Six of these were used to grow *Gracilaria* using abalone wastewater only as culture medium while the other six were used for *Gracilaria* growth using turbot wastewater only.

3.3.1.2 Inoculation of culture units

Gracilaria gracilis material was collected in Saldanha Bay by SCUBA divers at a depth of approximately three metres. The material was first cleaned by removing epiphytes and weighed to the required stocking density of 2 kg m^{-2} (0.5 kg and 1kg for small and medium-sized tanks respectively). The material was then placed in culture tanks with running seawater (first phase), turbot and abalone wastewater (second phase) and air supply as described in see section 3.3.1.2. The first phase of the experiments was started on 5 August 2001 using only seawater as growth medium. Only eight tanks (four small and four medium-size) were used in the first phase. The culture units had to be slightly modified due to technical problems observed and few changes were made to make them suitable for our experiments (see section 3.3.1.2). Another two sets of small tanks (abalone and turbot wastewater) were introduced on 2 November 2001 (see section 3.3.1.2). However, two weeks after the introduction of the two wastewater experiments, technical problems with the abalone wastewater experiment led to the reduction of treatments to two (normal seawater and turbot wastewater). The six culture units that were formerly supplied with abalone wastewater were now supplied with turbot wastewater and I then had eight (four small and four medium-sized) seawater tanks and 12 turbot wastewater tanks. Culture conditions were uniform in both treatments and the culture tanks were monitored daily by the farm personnel. In November 2001 *Gracilaria* thalli started to lose the dark-brown to reddish pigmentation (bleaching) and this occurred probably due to an increase in irradiance levels and with increasing temperatures in summer. The shading (50% light reduction) of material began early in December 2001 where half of the tanks in both treatments i.e. seawater (small and medium-sized tanks) and turbot treatment were shaded. The shading continued until autumn (April 2002).

3.3.2 I&J Farm

This abalone farm is situated at Danger Point near Gans Bay, approximately 140 km east of Cape Town. This is a well-established farm that produces *Haliotis midae*. One of the challenges facing the farm is the periodic occurrence of harmful algal blooms as well as the lack of an on-farm biological water purifying system to improve the quality of the wastewater that is released to the surrounding environment by the farm. The farm is not able to recirculate water when harmful algal blooms occur. So far the only reliable and cheap feed for abalone is kelp. Kelp is abundant on the southwest and west coasts of South Africa where it forms extensive kelp beds. Seawater is pumped from the sea to a reservoir dam first and then it is gravity-fed to both the hatchery unit and abalone tanks. This water is usually at temperatures that are suitable for abalone growth in the shallow bay from which it is pumped. From the abalone tanks the effluent water is channeled to another big dam on the lower end of the farm. The wastewater goes through a series of sieves using mesh fitted in the channel and in the end it goes through a conveyor-belt filter where tiny suspended particles that managed to go through the mesh are removed from the water. The water then is channeled into the dam from which it is discharged to the sea. When water is to be partially recirculated it can be pumped from this dam and mixed with the normal seawater from the upper (primary) holding dam, then go into the culture system.

In an attempt to maximize the yield of abalone through seaweed mass production as well as achieve some degree of water purification, seaweed tanks were prepared and stocked for the growth of *Gracilaria*.

3.3.2.1 Tank design (large tanks)

These were the largest tanks used in this study and they are probably the size of tanks that would be used if the algae were to be grown on a commercial scale. They were approximately 5 m X 1 m surface area and 0.60 m deep (3000 litres). They were made of wooden frames with white PVC lining inside. The tanks were aerated by a 30 mm PVC pipe placed along the bottom in the middle of the tank. This pipe was perforated with 3 mm diameter holes drilled at 9 cm intervals. Initially, four tanks were used and these were supplied with unfiltered seawater.

3.3.2.2 Inoculation of culture units

The stocking *Gracilaria* material was collected in Saldanha Bay using the same technique as in section 3.3.1.2. Before placing *Gracilaria* in culture tanks, the material was cleaned of epiphytes and weighed to the required stocking density (2 kg m^{-2}). The first experiment (seawater) was started on 12 June 2001 using only four tanks and another two sets of physically identical tanks were introduced on 19 August 2001 and 18 September 2001, fertilized seawater and abalone wastewater culture tanks, respectively (section 3.3.2.2). Water flow rates to the various treatments were, unfortunately, not identical at the beginning of the experiment due to pumping limitations on the farm. All tanks had the same turnover rate (4-5 volume exchanges d^{-1}), except the abalone effluent tanks, which received 12-volume exchanges d^{-1} throughout the 12-month experiment. At the end of February 2002 extra pumps were available and turnover rates in the other two treatments (seawater and fertilized seawater tanks) were increased to 12-volume exchanges d^{-1} and from that date all the three treatments were receiving equal turnover rates.

3.3.2.3 Supply of fertilizer

On the two additional set of tanks, the first set (three tanks) was supplied with seawater fertilized with Maxiphos, and the second set (four tanks) with abalone wastewater. Maxiphos is a commercial granular fertilizer with ammonia to phosphate ratio 1:6 and 105 g phosphate kg^{-1} of fertilizer. Fertilizer was applied once a week at 100g maximum per tank and water flow was turned off for about 12 hours to allow for the absorption of nutrients. Bleaching of material was also observed on this farm as well and similar procedure was followed as described in section 3.3.1.2.

Chapter 4

Gracilaria growth in tanks using seawater and measurement of environmental factors

4.1 Introduction

Several cultivation methods have been used previously to grow *Gracilaria* and are being used today in many countries. These include the land-based systems (tanks and dams) and open ocean cultivation system. Out of all the techniques and methods that have been used for the production of *Gracilaria*, tank cultivation gives by far the better productivity per unit area. The success of this method may be attributed to the fact that nutrition of the cultured organisms can be managed; the in-flowing water can be screened for pathogens, pollutants, poisons and red-tide organisms (Shpigel and Neori, 1996). However, this method of cultivation requires high-energy input and capital investment. Seawater contains relatively low nutrient concentrations (especially nitrogen and phosphorus). When animals such as abalone and fish are grown, they are sensitive to low dissolved oxygen levels, and thus high water turnover rates are essential for successful farming of the species. Successful tank cultivation of *Gracilaria* also depends on the optimization and manipulation of various environmental factors and the most important of these are temperature and light which are difficult to control in commercial land-based systems, but are known to have a limiting effect on the growth of *Gracilaria*. Other important environmental factors include CO_2 levels, salinity and dissolved oxygen (Kautsky, 1989). As a result, under extreme temperature or light conditions, proper precautionary measures are necessary to prevent the detrimental effects associated with such conditions i.e. the screening of culture tanks during summer to reduce light penetration and prevent the bleaching of *Gracilaria* material. Sufficient knowledge about factors limiting growth and production of algae is of great importance and plays a crucial role in *Gracilaria* in tanks cultivation. This experimental study seeks to investigate the feasibility of growing *Gracilaria* using normal seawater under controlled conditions. The other objective is to investigate the effect of environmental parameters in *Gracilaria* growth as well as their interactions.

Temperature plays an important role in *Gracilaria* growth regulation (Friedlander *et al.,* 1987; Laing *et al.,* 1989; Molloy 1992; Anderson *et al.,* 1996; Molloy and Bolton 1996). Low temperatures coupled with short growing season are regarded as the main limiting factors in

temperate climates. In many temperate species, there is a clear relationship between the experimentally determined temperatures limiting growth and survival and the extreme summer and/or winter temperatures at their geographical boundaries (Hoek, 1979; Luning, 1984). Optimal growth in temperate species is usually obtained at higher temperatures. (Fortes and Luning, 1980), and they show a seasonal shift in heat tolerance with peaks during summer (Luning, 1984).

Inorganic carbon is one of the main limiting growth factors in *Gracilaria* tank cultivation and its availability depends on the water pH. Lapointe and Ryther (1979) observed that, as the rate of water exchange was reduced the pH in culture tanks of *Gracilaria tikvahiae* increased to ca 9.0 and concluded that reduction in yields may be attributed to increased pH at low turnover rates. This is because at high pH ($\geq$ 9.0), free CO_2 is almost completely depleted and a significant decrease in photosynthesis of *Gracilaria* occurs (Blinks, 1963; Ryther and DeBusk, 1982). Therefore the pH level in culture tanks can be used as an indicator of CO_2 availability. There are generally two ways to increase CO_2 levels, either via the direct addition of CO_2 or by increasing the water turnover rate. The latter method is the most commonly used in land-based farms, but the cost of pumping water is regarded as the single most expensive operation cost for any land-based seaweed cultivation system (Huguenin, 1976).

The measurement of dissolved oxygen (DO) in culture tanks is also crucial in mariculture systems since many cultured primary species (abalone and fish) are very sensitive to oxygen levels in the water hence high turnover are essential in land-based systems. This is particularly important in land-based farms that need to practice internal re-circulation of water. The availability of DO also depends on temperature as well as on the amount of suspended particles.

4.2 Materials and Methods

4.2.1 JSP Farm

4.2.1.1 Harvesting and restocking

The farm was visited every two weeks to harvest material, clean tanks and record growth data. Epiphytes and fouling contaminants were also identified and removed during these visits. On our arrival at the farm, we first checked the overall operation of the system in case there were some irregularities, then identified and recorded the presence of epiphytes and seaweed contaminants. *Gracilaria* was harvested from the tanks by collecting the material by hand into net bags, draining excess water by hanging the bags until dripping ceased (taking about 4 minutes) and weighing it using spring balance scales (1 kg and 5 kg). Small plastic bags were used to collect samples from each individual tank harvest. These were later used in the laboratory for wet weight to dry weight conversions, tissue analysis and for the preparation of herbarium specimens. After harvesting the tanks were emptied, cleaned and re-supplied with water and air. Before the tanks were restocked with the standard initial stocking weight (2 kg m^{-2}), the stocking material was treated with fresh water for about three minutes so to reduce grazers and epiphytes. All the data were recorded for later analysis.

4.2.1.2 Growth rate and yield of *Gracilaria*

Relative growth rate (RGR) was determined from biomass increase of the material every two weeks and this was done using the formula of Evans (1972) to calculate relative growth rate (as % d^{-1}): $R = In ((W_t/W_0)) t * 100$, where

R = relative growth rate,

W_0 = initial biomass,

W_t = final biomass and

t = growth period in days.

A mean dry/wet weight coefficient of 0.1 was obtained. This was obtained by using the following formula:

D wt/W wt * 100

D wt = dry weight

W wt = wet weight

4.2.1.3 Environmental factors

4.2.1.3.1 Temperature

Usually, tank water temperature was recorded at about 10 cm below surface once every day (12h00) except on weekends. However, I conducted a two-day experiment on 1 to 2 November 2001 where temperature measurements were recorded (about 10cm below surface) at hourly intervals using a thermometer from 08h00-14h00 . The turnover rate in half of the culture tanks was reduced the previous day, i.e. 1 November 2001 (two small and two medium-sized tanks), from 24 volume exchanges d^{-1} to 4 volume exchanges d^{-1}. This was done to check if water residence time in tanks had an effect on water temperature. .

4.2.1.3.2 pH

An intensive pH measurement experiment was run at JSP farm on 1 and 2 November 2001 using small and medium-sized seawater tanks. This experiment was done at the same time as the temperature experiment and the procedure followed was the same.

Generally, there are two ways of CO_2 replenishment in tank culture systems: CO_2 enrichment by directly adding carbon dioxide into the tanks or by increasing the water turnover rates. Poor growth rates were recorded at I&J farm and I thought that was probably due to low turnover rates (4-5 volume exchanges d^{-1}) and CO_2 depletion on the farm. I thus conducted this experiment at JSP to investigate if CO_2 is depleted at low turnover rates and to check if CO_2 could be replenished sufficiently by increasing the turnover rate. This was done by reducing the turnover rate (from 24 to 4 volume exchanges d^{-1}) in half the tanks 12 hours before the experiment and measuring pH levels both in tanks receiving low turnover rate and those that were receiving high turnover rate. On the 02 November 2001, I measured pH at hourly intervals from 08h00 to 14h00 in all the tanks using a pH meter (Cyberscan, pH 300 series waterproof) and recorded the data. After the experiment the turnover rates in the tanks with low flow rate were increased to the normal 24-volume exchanges d^{-1}.

4.2.1.3.3 Dissolved oxygen

Dissolved oxygen was measured using an oxygen meter (Cyberscan, DO 300 series waterproof), at about 10 cm below surface at the same time as temperature and pH measurements were made. Frequent measurements of dissolved oxygen were also done to draw a daily DO profile during the two-day experiment on 1 and 2 November 2001.

4.2.2 I&J

4.2.2.1 Harvesting

This farm was visited once every month for harvesting, sampling, growth measurements and cleaning of tanks. The procedure followed was the same as described in section 4.2.1.1.

4.2.2.2 Growth rate and yield of *Gracilaria*

The tanks were harvested following the same procedure as described (see section 4.2.1.2). The procedure for growth rate measurements, sampling and processing of *Gracilaria* material in the laboratory was the same as described in section 4.2.1.2.

4.2.2.3 Environmental factors

4.2.2.3.1 Temperature

Water temperature was recorded twice every day (08h00 and 16h00) in all treatments using a thermometer. Measurements were taken at approximately 10 cm below water surface.

4.2.2.3.2 pH

Water pH measurements were taken simultaneously with temperature measurements i.e. at 08h00 in the morning and at 16h00 in the afternoon. The measurements were taken at a depth of about 10 cm below water surface using a pH-meter.

4.2.2.3.3 Dissolved oxygen

Dissolved oxygen data was recorded at the same time (08h00 and 16h00) as the pH and temperature data using oxygen-meter (at about 10 cm below surface).

4.3 Data and statistical analysis

All statistical analyses were done using Windows Statistica 6. Data sets were tested for normality using Kolmogorov-Smirnov and Lilliefors test. To test for significant of differences we used the probability level $p < 0.05$. Student t-test was used to compare two data sets (e.g. growth in summer and growth in winter, growth in shaded and growth in unshaded, growth in small and growth in medium-sized tanks). Analysis of variance was used to compare three or more data sets and treatments as well as to test for possible interactions. Tukey Honest Significance Difference (HSD) test was used as the post hoc test for comparisons of treatment means.

4.4 Results

4.4.1 JSP

4.4.1.1 Growth rate and yield of *Gracilaria* at JSP

At JSP, spring-summer (October 2001-April 2002) RGR and yield were relatively high, with 6.6-11.2 % d^{-1} and 5.6-8.7 % d^{-1} range, small and medium-sized tanks, respectively (Figure 4.1 and Figure 4.2). These high levels of RGR and yield were obtained during the period when water temperature and water pH were at their peak and during the time when day length and irradiance levels were highest. In small tanks, RGR reached its peak (11.2 % d^{-1}) in February 2002 that coincides with the period when both water temperature and water pH was at their highest i.e. 21.9 ^{0}C and 8.8, respectively (Figure 4.3 and Figure 4.4). In winter at JSP, RGR in all tanks fell to very low values and in several tanks yields were almost zero, or fell below zero indicating extremely poor growth conditions. Unshaded tanks (small and medium-sized) gave higher RGR and yield, than shaded tanks (Figure 4.1 and 4.2). Shading of *Gracilaria* material significantly reduced the RGR and yield in both small and medium-sized tanks (Table 4.3). There was about 63 % reduction in *Gracilaria* growth rate due to shading (Table 4.3). No significant interactions were observed between tank size and season and between tank size and shading.
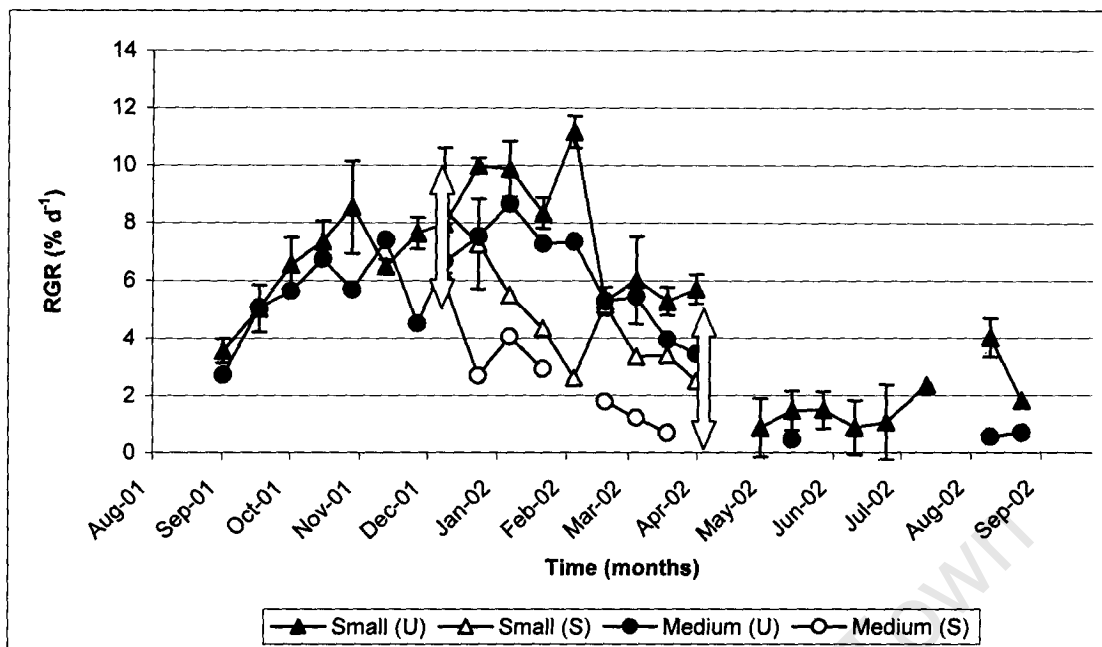
Figure 4.1: Average RGR of *Gracilaria* in small and medium seawater culture tanks over 12 months growth period at JSP farm. All values are means ± S D.

⇕ Denotes the start and the end of shading of thalli. SW- seawater, U- unshaded, S- shaded.
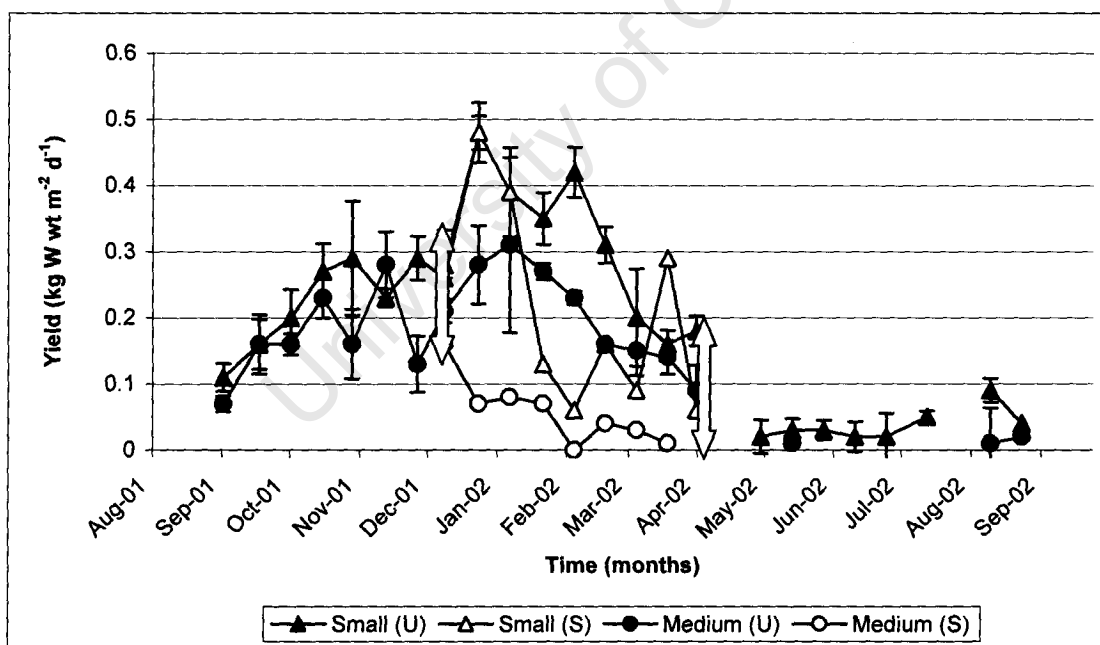
Figure 4.2: Average yield of *Gracilaria* in small and medium seawater culture tanks over 12 months growth period at JSP farm. All values are means ± S D.

⇕ Denotes the start and the end of shading of thalli. SW- seawater, U- unshaded, S- shaded.

Table 4.1: Mean RGR and yield of *Gracilaria* at JSP farm in autumn/winter and spring/summer.

Variable	Autumn-Winter (n = 76)	Spring-Summer (n = 128)	P-value
RGR (% d^{-1})	0.50 ± 2.92	5.51 ± 2.57	0.00
Yield (kg W wt. m^{-2} d^{-1})	0.00 ± 0.05	0.19 ± 0.11	0.00

Table 4.2: The effect of tank size on RGR and yield of *Gracilaria* at JSP farm.

Variable	Small tanks (n = 102)	Medium tanks (n = 102)	P-value
RGR (% d^{-1})	4.22 ± 3.56	2.32 ± 4.15	0.00
Yield (kg W wt. m^{-2} d^{-1})	0.15 ± 0.14	0.08 ± 0.11	0.00

Table 4.3: The effect of shading on RGR and yield of *Gracilaria* at JSP farm.

Variable	Unshaded (n = 12)	Shaded (n = 12)	P-value
RGR (% d^{-1})	4.97 ± 1.33	1.84 ± 1.83	0.00
Yield (kg W wt. m^{-2} d^{-1})	0.15 ± 0.05	0.08 ± 0.11	0.04

4.4.1.2 Environmental factors at JSP

4.4.1.2.1 Temperature

The monthly average water temperature in the culture tanks ranged between 14.2^{0}C in winter to 21.9 ^{0}C in summer. Water temperature was low at the start of the experiment in early spring (September 2001) but it increased early in November 2001 reaching its peak (21.9 ^{0}C) in February 2002 (Figure 4.3). The average temperature dropped in March 2002 and continued to decrease, reaching the lowest mark (14.2 $\pm$ 0.67 ^{0}C and 14.6 $\pm$ 0.73 ^{0}C) in July 2002, small and medium-sized tanks, respectively. There was no significant difference in temperature between small and medium-sized tanks (p = 0.10), n = 13. However, an increase in temperature positively correlated with RGR and yield of *Gracilaria*.
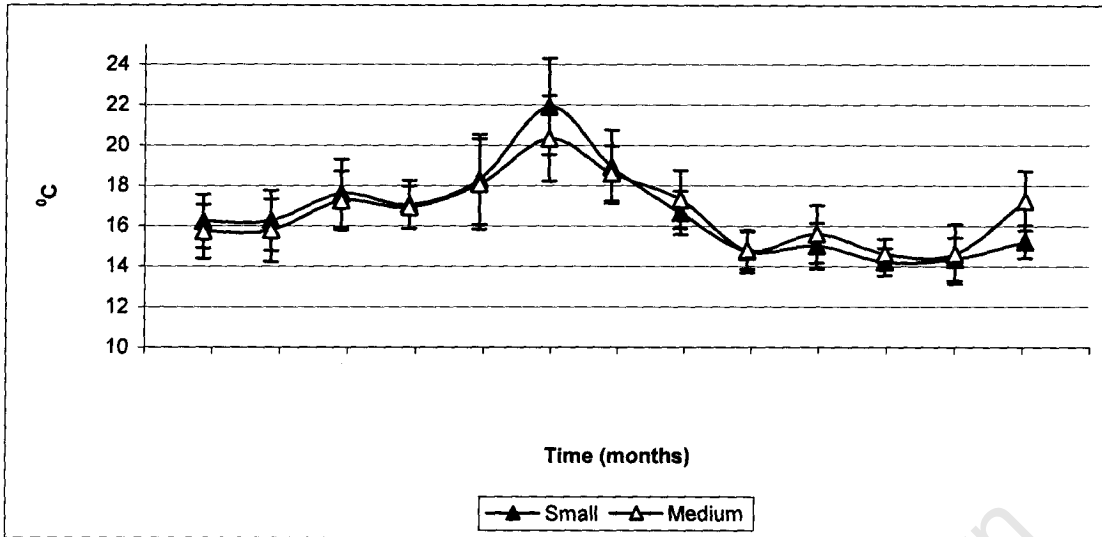
Figure 4.3: Mean monthly water temperature in small and medium-sized seawater culture tanks at JSP. All values are means ± SD.

4.2.1.2.2 pH

Average monthly pH level remained below the critical limit ca 9.0 ranged between 7.89 ± 0.16 and 8.8 ± 0.23. The highest mean monthly pH was obtained in summer (February 2002) and the lowest in winter (August 2002) (Figure 4.4). The pH level, like temperature had the highest levels recorded in summer (October 2001-early April 2002) and lower levels in winter (late April-June 2002). Both the highest and the lowest pH levels were obtained in small tanks, but no significant difference was observed between small and medium-sized tanks.
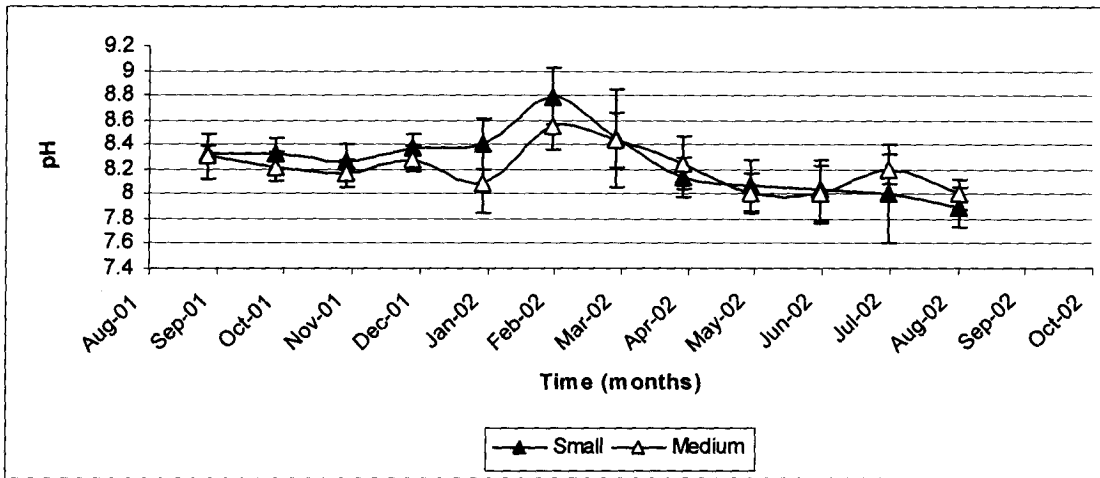
Figure 4.4: Mean monthly water pH in small and medium-sized seawater culture tanks at JSP. All values are means ± SD.

4.4.1.2.3 Dissolved oxygen

The amount of dissolved oxygen in the culture tanks was in the ranged of 7.36 - 9.61 ± mg l^{-1} and 7.95 - 9.45 mg l^{-1}, in small and medium-sized tanks, respectively. The highest DO concentration was obtained in December 2001 and the lowest DO concentration was obtained in August 2002, for small and medium-sized tanks, respectively (Figure 4.5). No significant difference in DO concentration was observed between small and medium-sized tanks. There was no obvious seasonal pattern in DO.
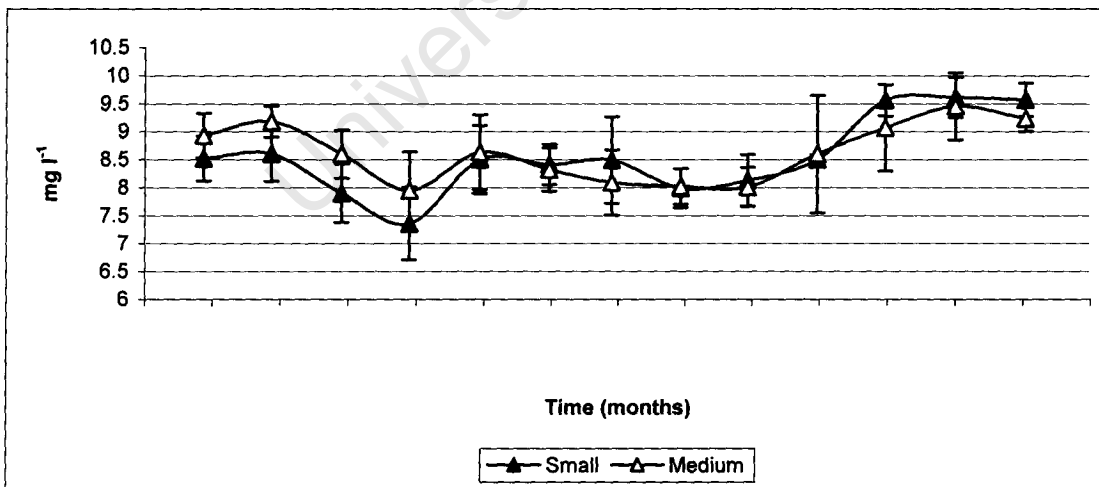
Figure 4.5: Mean monthly water DO in small and medium-sized seawater culture tanks at JSP. All values are means ± SD.

4.4.2 I&J

4.4.2.1 Growth rate and yield of *Gracilaria*

Lower RGR and yield was obtained during summer (Table 4.5). RGR reached its peak (3.19 % d^{-1}) in April 2002 during the high flow rate period and the lowest recorded RGR (1.2 % d^{-1}) was obtained in October during the low flow rate period (see Figure 4.6). An increase in the water turnover rate (4 to 12 volume exchanges d^{-1}) significantly improved *Gracilaria* growth. RGR and yield during high flow rate nearly doubled compared to that obtained during low flow rate (Table 4.4). No significant seasonal difference was observed in RGR and yield during both low and high turnover rates.

Shading caused little reduction in RGR and yield during low water turnover rate period. However, during high water turnover rate considerably higher (but not significant) RGR and yield was obtained in unshaded tanks than in shaded tanks (Table 4.6). No significant interaction was observed between flow rate and season.
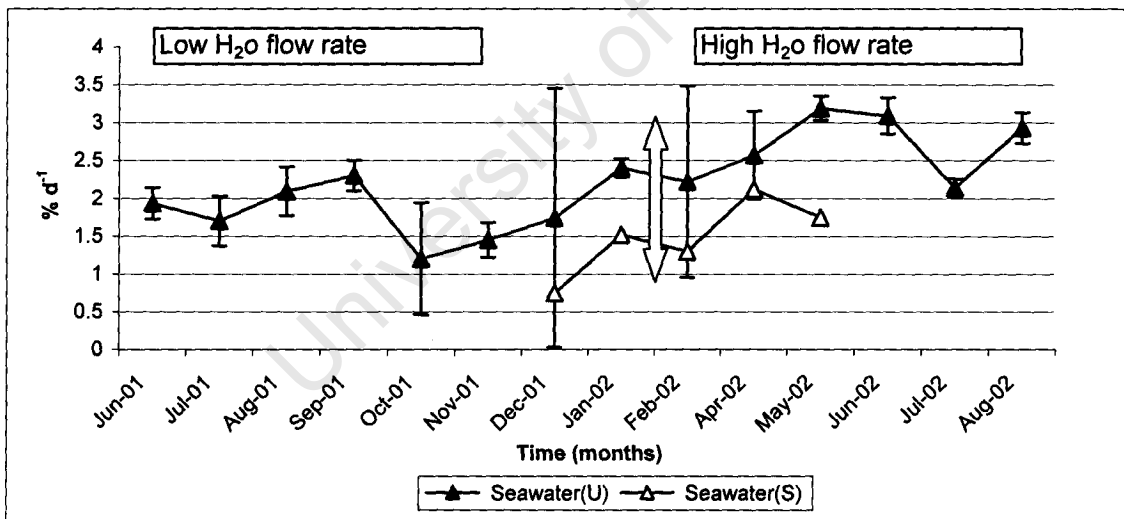
Figure 4.6: Average RGR of *Gracilaria* in large seawater culture tanks at I&J farm. All values are means ± S D.

⇕   Denotes the change (increase) in water flow rate form 4 to 12 volume exchanges d^{-1}.
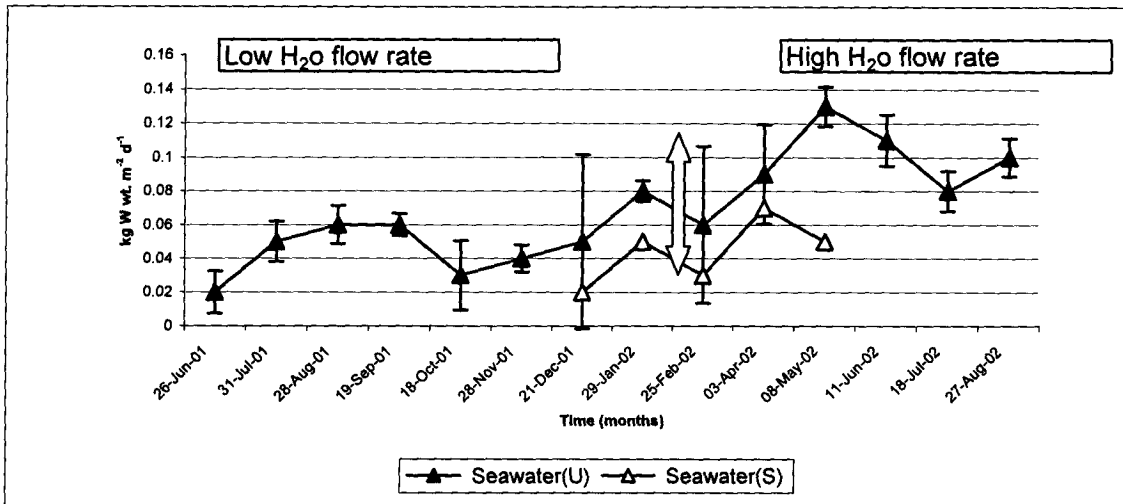
Figure 4.7: Average yield of *Gracilaria* in large seawater culture tanks at I&J farm. All values are means $\pm$ S D.

⇕ Denotes the change (increase) in water flow rate form 4 to 12 volume exchanges d^{-1}.

Table 4.4: The effect of water exchange rate on RGR and yield of *Gracilaria* in seawater culture tanks at I&J farm. Values are means $\pm$ S.D.

Variable	Low (4 vol. exch. d^{-1}) (n = 30)	High (12 vol. exch. d^{-1}) (n= 16)	P-value
RGR (% d^{-1})	1.66 $\pm$ 0.84	2.86 $\pm$ 0.45	0.00
Yield (kg W wt. m^{-2} d^{-1})	0.05 $\pm$ 0.03	0.09 $\pm$ 0.03	0.00

Table 4.5: Seasonal differences in mean RGR and yield of *Gracilaria* at low and high flow rate in seawater at I&J abalone farm. All values are mean $\pm$ S. D.

Variable	Autumn-Winter	Spring-Summer	P-value
Low flow rate	(n = 12)	(n = 18)	
RGR (% d^{-1})	1.40 $\pm$ 0.87	1.83 $\pm$ 0.79	0.18
Yield (kg W wt. m^{-2} d^{-1})	0.04 $\pm$ 0.03	0.05 $\pm$ 0.03	0.62
High flow rate	(n = 14)	(n = 2)	
RGR (% d^{-1})	2.90 $\pm$ 0.44	2.57 $\pm$ 0.58	0.35
	(n = 14)	(n = 2)	
Yield (kg W wt. m^{-2} d^{-1})	0.10 $\pm$ 0.03	0.09 $\pm$ 0.03	0.81

Table 4.6 The effect of shading in mean RGR and yield of *Gracilaria* at low and high flow rate in seawater at I&J abalone farm. All values are mean ± S. D.

Variable	Unshaded	Shaded	P-value
Low flow rate (n =6)			
RGR (% d^{-1})	2.15 ± 1.01	1.21 ± 0.77	0.10
Yield (kg W wt. m^{-2} d^{-1})	0.06 ± 0.04	0.03 ± 0.03	0.13
High flow rate (n = 4)			
RGR (% d^{-1})	2.88 ± 0.50	1.93 ± 0.60	0.05
Yield (kg W wt. m^{-2} d^{-1})	0.11 ± 0.03	0.06 ± 0.03	0.05

4.4.2.2 Environmental factors at I&J

4.4.2.2.1 Temperature

Mean monthly water temperature in culture tanks ranged from 14.9-20.0 ^{0}C (Figure 4.8). The temperature was higher in summer than in winter. The highest mean monthly temperature was in January 2002 and the lowest temperature was in July 2001. In culture tanks, the monthly mean temperature was significantly higher in the afternoon (16h00) than in the morning (08h00) p = 0.02, n = 4. The average temperature was 15.7 ± 0.67 ^{0}C and 17.51 ± 0.89 ^{0}C, morning and afternoon data recordings, respectively.
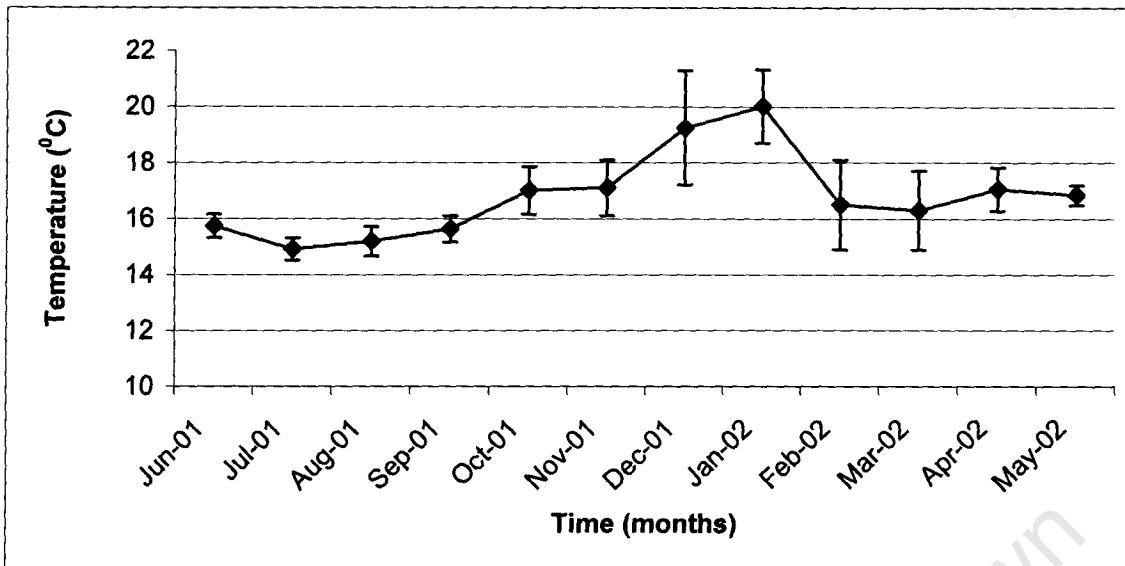
Figure 4.8: Mean monthly water temperature in large seawater culture tanks at I&J farm. All values are means $\pm$ SD.

4.4.2.2.2 Water pH at I&J

Tank water pH levels ranged from 7.85-8.10 (Figure 4.9). The highest pH was recorded in July and December 2001 and the lowest pH was recorded in May 2002. The pH levels were significantly higher in the afternoon ($p = < 0.01$, $n = 4$) than in the morning. The average pH level was 8.13 ± 0.09 (morning) and 8.54 ± 0.05 (afternoon).

pH level

8.3
8.2
8.1
8
7.9
7.8
7.7
7.6
7.5
7.4

Jun-01 Jul-01 Aug-01 Sep-01 Oct-01 Nov-01 Dec-01 Jan-02 Feb-02 Mar-02 Apr-02 May-02

Time (months)
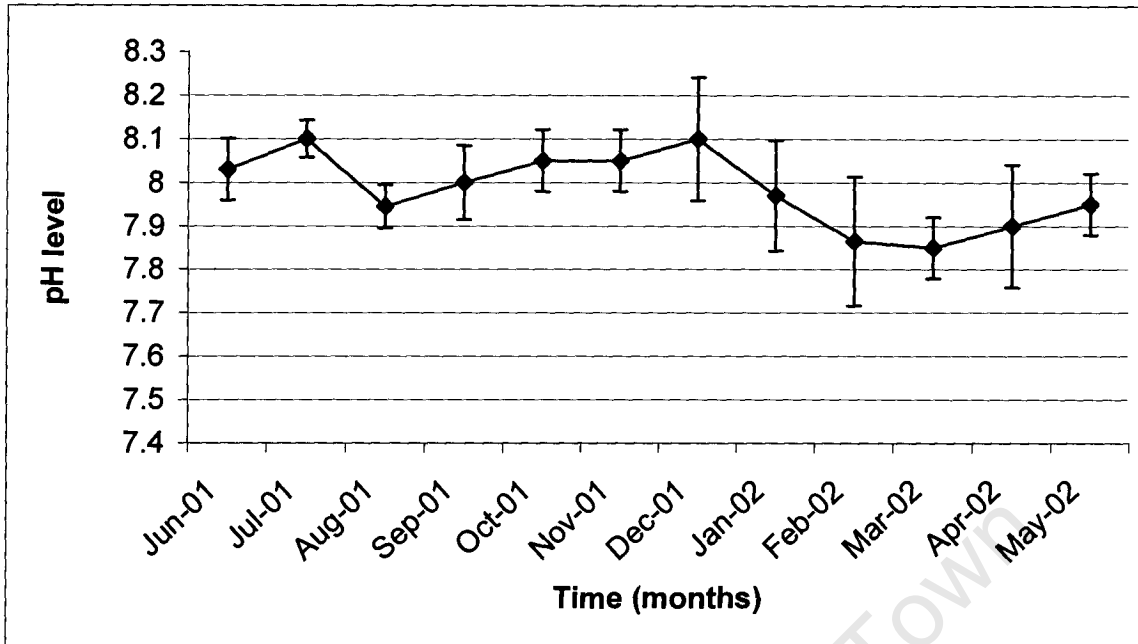
Figure 4.9: Mean monthly water pH in large seawater culture tanks at I&J farm. All values are means $\pm$ SD.

4.4.2.2.3   Dissolved oxygen

DO concentration ranged from 6.7-8.75 mg l^{-1} (Figure 4.10). The highest DO was obtained in October 2001 and the lowest DO was obtained in March 2002. No significant difference was observed in DO levels between the morning and afternoon measurements ($p = 0.58$), n = 4.
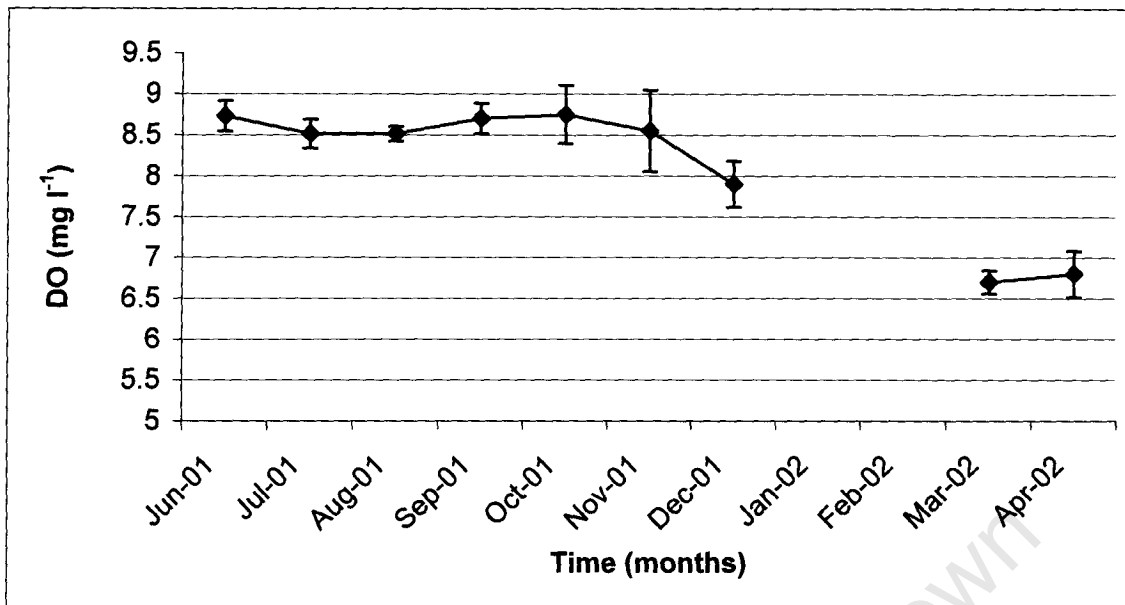
Figure 4.10: Mean monthly water DO in large seawater culture tanks at I&J farm. All values are means ± SD.

4.5 Discussion

The results of this study demonstrated the technical feasibility of cultivating the South African *Gracilaria gracilis* in tanks on land-based fish farms using seawater as a growth medium. However, at JSP, RGR in both tanks fell to very low values in winter, and in several weeks yields were almost zero, or fell below zero indicating extremely poor growth conditions. It is doubtful that lower temperatures had a causative effect to the collapse of the experiment due to the fact that even though temperature declined during winter it did not fall below 10 ^{0}C, which is within the range for reasonably good growth of this species as shown in laboratory studies (Engeldow and Bolton, 1992). It is likely that low growth in winter was caused by low light levels (shorter day-length and weaker sunlight). At JSP, Spring-Summer RGR and yield were relatively high, during the period (November 2001-February 2002) when water temperatures were at their highest, and day length and irradiance levels would be highest. A similar positive relationship between growth rate and temperature was observed with *Gracilaria tenuistipitata* (Haglund and Pedersen, 1993). Summer maxima in RGR and yields have been recorded in numerous studies of *Gracilaria* tank cultivation. Jones (1959a) reported fastest *G. gracilis* growth rates during the period when temperature and light intensity were high (summer). In a study in Chile, *Gracilaria* sp. growth in

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open-circuit tanks was also highest in spring and early summer. However, the production dropped during summer and was lower during winter (Edding *et al,* 1987). Results from the present study agree with findings from the Namibian study with RGR between 2.9 – 11 % d^{-1} (Rebello *et. al.,* 1996). However, the Namibian experiment did not collapse in winter.

The effect of tank size was demonstrated by the significantly higher RGR and yield in smaller tanks at JSP farm and this was observed throughout the experiment. Several studies have shown a marked effect of tank size on *Gracilaria* growth. For example Oliveira *et al,* (2000) found the use of small tanks to be a better option of increasing the biomass because of the features such as resistance to epiphytism, faster growth. Hanisak and Ryther (1984) observed that high levels of production have been achieved in small culture units and each scale-up led to considerable decline in production of various gracilarioids. Although shading was used to try and improve the apparent condition (health) and the growth of *Gracilaria gracilis* in summer, it significantly reduced growth and yields. It is thus clear that physical appearance (including colour) can be a misleading indicator of growth potential (and growth rate). An improvement in appearance coincided with lower growth. Part of the explanation for this must lie in the relationship between colour (pigment content), ambient N in the medium and growth. Shading (reducing light) would have slowed RGR and allowed the plants to store more N as phycoeythrin. Lapointe and Ryther (1979) reported this shading effect with *Gracilaria tikvahiae*. However, low phycoerythrin levels (and hence a greenish rather than reddish colour) are not necessary an indicator of low growth. A constant, but low supply of N may be enough to sustain a high growth rate. These results show that shading of tanks in summer is unnecessary.

Temperature remained favourable for growth with mean monthly range of 13 – 21 ^{0}C. Mean monthly pH (measured at 16h00 in the afternoon) did not exceed 8.8 $\pm$ 0.23 suggesting that CO_2 would seldom, if ever, have become limiting i.e. a pH of 9.0 is cited by Blinks, (1963), Ryther and Debusk (1982) as indicating possible CO_2 limitation.

Temperature, pH and nutrients at JSP were maintained through the water flow rate of 24 volume exchanges d^{-1}. Light (irradiance) was the one factor critical for *Gracilaria* growth that was not measured. It is likely that low light levels and short day lengths were the single most important

reason for low winter growth at JSP. This farm lies in a winter rainfall area where overall irradiance is strongly seasonal.

At I&J farm, in large "commercial scale" tanks there were no seasonal differences in RGR or yield. However, RGR was consistently lower than in the smaller tanks at JSP, with an average RGR of 6 % d^{-1} and 2.5 % d^{-1}, small and large tanks, respectively. Monthly means remained under 4 % d^{-1}, indicating relatively poor growth conditions. At I&J farm, the low flow rate of 4 volume exchanges d^{-1} caused a significant reduction in RGR and yield, and it is possible that even 12 volume exchanges d^{-1} were insufficient for optimal growth. There was better growth in winter at I&J farm than at JSP farm and this is probably due to the infestation of *Gracilaria* with epiphytes and the breaking of thalli that led to the sudden fall in production during winter months at JSP farm. Water exchange may account for better performance at JSP. Water exchange rate has been widely used in the cultivation of *Gracilaria* to improve culture conditions and yield better production. High water turnover rates (20 – 30 volume exchanges d^{-1}) are mainly used as an alternative way of supplying nutrients and CO_2 in culture media (Santelices and Doty, 1989, Friedlander and Levy, 1995). Water temperature and pH are also indirectly regulated through an increased turnover rate.

Chapter 5

Gracilaria growth in nutrient-enriched seawater

5.1 Introduction

Aquaculture is playing a significant role in the production of fish products worldwide, but fish farms produce large amounts of wastes (including dissolved inorganic phosphorus and nitrogen). The nutrient-rich wastewater released from the farms often causes eutrophication and sometimes toxic algal blooms. The incorporation of seaweeds into aquaculture farms cannot only supply cheap feed but can also improve the wastewater quality. High biofiltering capacity of *Gracilaria* and its vegetative reproduction make it a suitable candidate for integrated aquaculture. A study conducted by Troell *et al.,* (1997) showed that *Gracilaria* grown on open sea rope rafts near (10 m) fish cages had up to 40% higher growth rate (specific growth rate 7% d^{-1}) compared to those growth at 150 m and 1km distance from the fish cages. According to Buschmann *et al.,* (1996) *Gracilaria* is able to remove 50% of dissolved ammonium (the nutrient that increases most in fish effluents) in winter and can remove as much as 90-95% in spring. Tank systems are the most productive among all the tried *Gracilaria* cultivation techniques, but they are expensive to operate because they require high-energy input and capital investment. Normal seawater may not contain sufficient nutrients to grow *Gracilaria* on a commercial scale and to ensure high and sustainable production; the nutrient enrichment (especially nitrogen and phosphorus) of culture tanks is necessary. There are two ways of ensuring that there is enough nutrient supply in *Gracilaria* culture tanks, the first one is to have a high seawater exchange rate (20-30 volume exchanges d^{-1}) and the second is to have direct nutrient replenishment (addition of fertilizer or use of wastewater). The former is regarded as the single most expensive factor in tank cultivation systems (Huguenin, 1976). The latter is the most cost-effective way of culturing species since it requires lower turnover rates thus reducing the operational costs in the farm.

The main objective of these experiments was to investigate the effect of using nutrient-enriched seawater to grow *Gracilaria* with a view to minimizing water flow rate while maintaining high production. This study was also conducted to investigate the possibility of practising internal re-circulation of water and nutrient recycling in the farm.

5.2 Materials and Methods

5.2.1 JSP

5.2.1.1 Harvesting

The wastewater culture tanks were visited and harvested simultaneously with seawater tanks, following the same procedure, and the processing of material was also similar to that described in section 4.2.1.1.

Growth rate determination and formulae used were the same as described in section 4.2.1.2.

5.2.2 I&J

5.2.2.1 Harvesting

The harvesting and the processing of thalli was the same as described in section 4.2.1.1.

Growth rate determination was the same as described in section 4.2.1.2.

The statistical analysis used was the same as described in section 4.3.

5.3 Data and statistical analysis

The data were analysed using the same method as described in section 4.3.

5.4 Results

5.4.1 JSP

5.4.1.1 Growth rate and yield of *Gracilaria*

There was little data from the abalone treatment (March 2002- April 2002) and RGR ranged between 6.5 and 7.6 % d^{-1}. In the turbot treatment, higher RGR and yield was obtained in summer (November 2001-early April 2002) and negative RGR and yield was obtained in winter (late April-May 2002), Figure 5.1 and figure 5.2. Turbot wastewater RGR was at its peak early in November, declining afterwards and remaining lower but consistent until the fall in production in winter (Figure 5.1 and figure 5.2). Therefore, there were no winter measurements and no statistical analysis was made. Higher RGR and yield was obtained in unshaded tanks and lower RGR and yield was obtained in shaded tanks, (see Figure 5.1 and 5.2). Shading of *Gracilaria* material significantly reduced RGR and yield (see Table 5.1). No significant interaction was observed between treatment (turbot and abalone effluent) and shading.
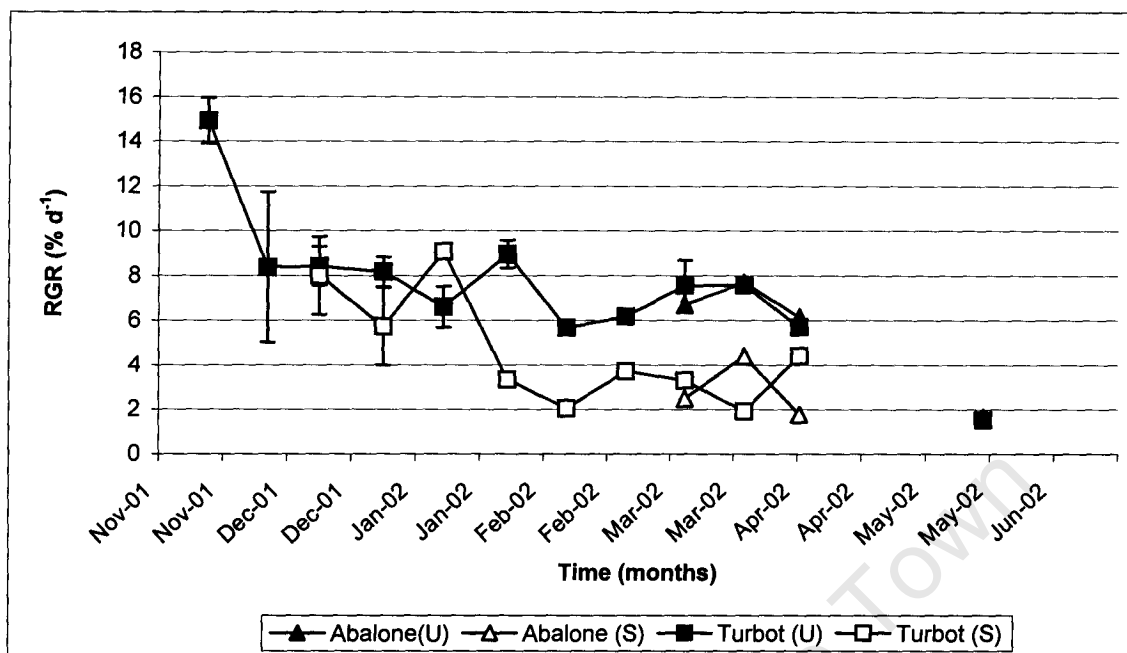
Figure 5.1: Average RGR of *Gracilaria* in turbot and abalone wastewater culture tanks at JSP. All values are means ± SD. U- unshaded, S- shaded.
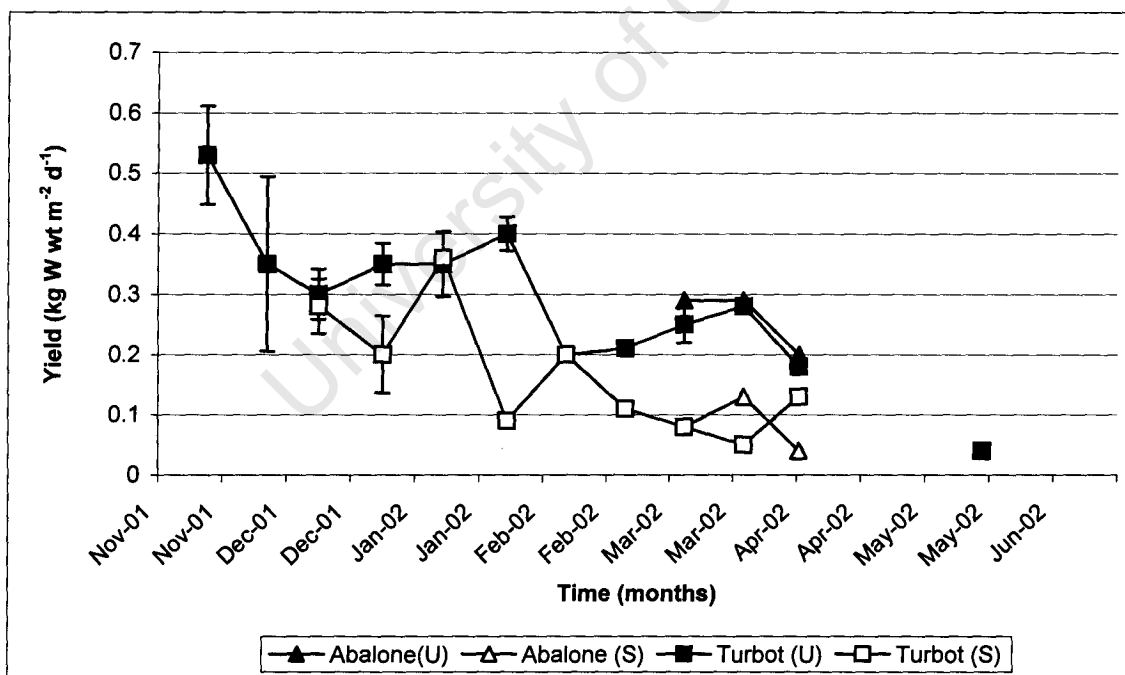
Figure 5.2: Average yield of *Gracilaria* in turbot and abalone wastewater culture tanks at JSP. All values are means ± SD. U- unshaded, S- shaded.

Table 5.1: Shows the difference in RGR and yield between unshaded and shaded *Gracilaria* in turbot and abalone wastewater tanks at JSP farm. All values are means ± SD.

Treatment	Unshaded	Shaded	Pvalue
Turbot			
RGR	7.1 ± 2.22 % d^{-1}	4.9 ± 2.92 % d^{-1}	< 0.001
	N=45	N=45	
Yield	0.29 ± 0.12 kg W wt. m^{-2} d^{-1}	0.16 ± 0.13 kg W wt. m^{-2} d^{-1}	< 0.001
Abalone			
RGR	6.8 ± 1.06 % d^{-1}	2.9 ± 1.67 % d^{-1}	< 0.001
	N= 9	N= 9	
Yield	0.26 ± 0.07 kg W wt. m^{-2} d^{-1}	0.08 ± 0.06 kg W wt. m^{-2} d^{-1}	< 0.001

5.4.2 I&J

5.4.2.1 Growth rate and yield of *Gracilaria*

The RGR in fertilized seawater tanks *Gracilaria* reached its peak in May 2002 and the lowest RGR was recorded in July 2002 whereas in abalone tanks the highest RGR was recorded in December 2001 and the lowest RGR in February 2002 (Figure 5.3). In fertilized seawater, highest and lowest yields were recorded in May 2002 and July 2002 while in abalone water, the highest and lowest yields were recorded in December 2001 and February 2002, respectively. (Figure 5.4). There was no significant difference in RGR and yield of *Gracilaria* between the low water flow rate (4 volume exchanges d^{-1}) and high water flow rate (12 volume exchanges d^{-1}) in fertilized seawater tanks (Table 5.2). On average, higher RGR and yield was obtained during low water flow rate (September 2001-February 2002) and lower RGR and yield was obtained during high water flow rate (March-August 2002) (Table 5.2). There was reduction in RGR and yield during the shading of *Gracilaria* material during both high and low flow rate in fertilized seawater tanks, but it was not significant (Table 5.3). During high flow rate, higher RGR and yield was obtained in unshaded tanks and lower RGR and yield was obtained in shaded tanks. In contrast, during low flow rate high RGR and yield was obtained in shaded tanks and lower RGR and yield was obtained in unshaded tanks (Table 5.3). There was no significant seasonal difference in RGR and yield of *Gracilaria* in abalone wastewater. Higher RGR and yield was obtained during winter (late April-May 2002) and lower RGR and yield was obtained during summer (October 2001- April 2002)

(Table 5.4). There was no significant reduction in RGR and yield during shading of *Gracilaria* material but higher RGR and yield was obtained in unshaded tanks than in shaded tanks (Table 5.5).
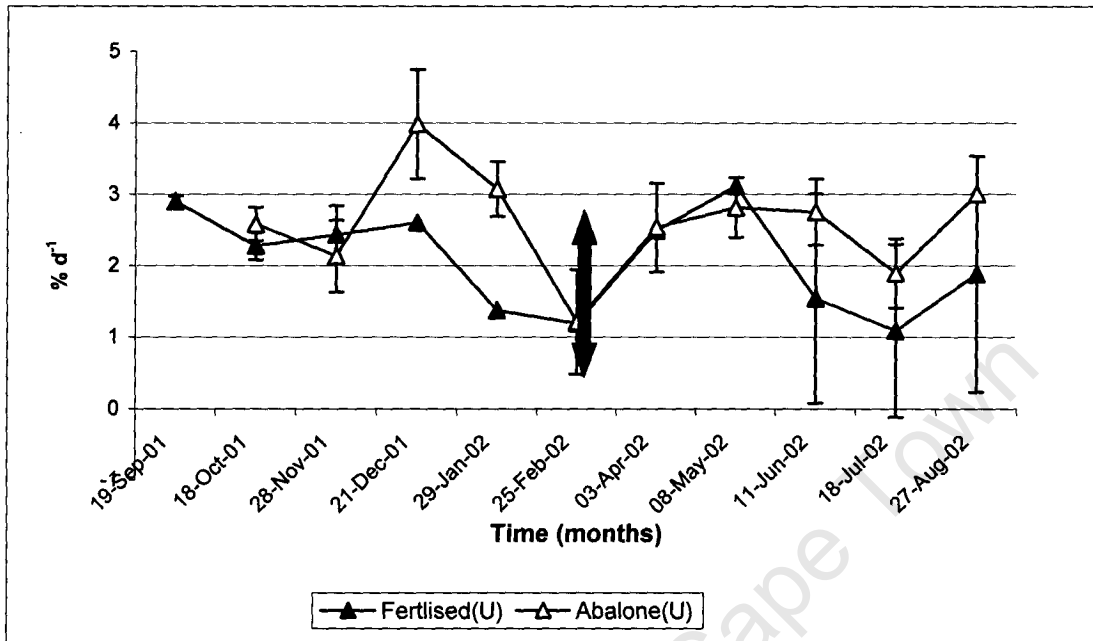
Figure 5.3: Average RGR of *Gracilaria* in large fertilized seawater and abalone wastewater culture tanks at I&J farm. All values are means $\pm$ SD, U- unshaded.

⬍ Denotes the start of an increase in water exchange rate.
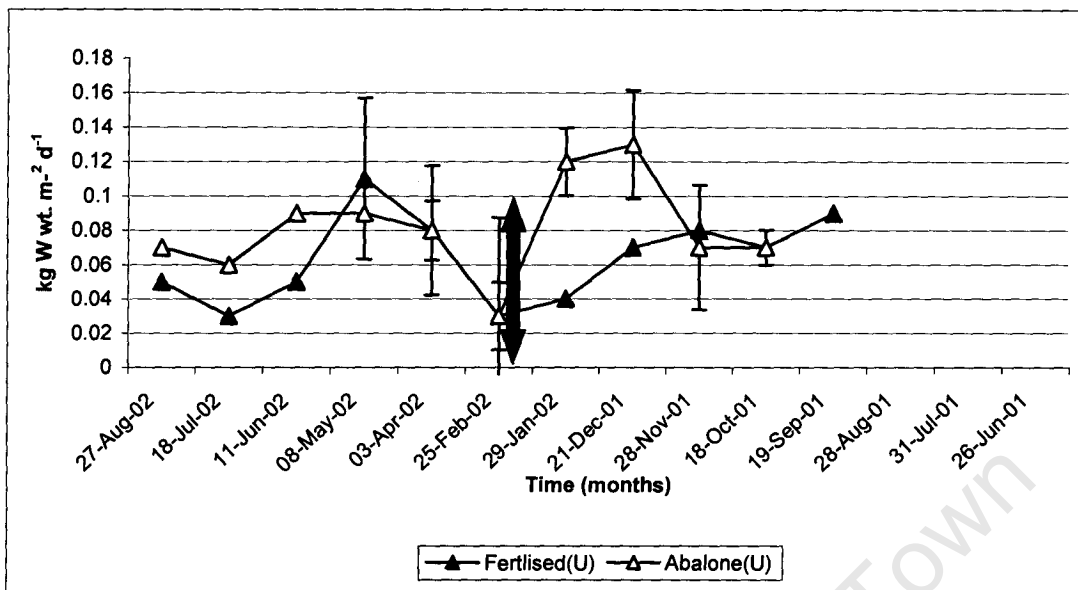
Figure 5.4: Average yield of *Gracilaria* in large fertilized seawater and abalone wastewater culture tanks at I&J farm. All values are means $\pm$ SD, U- unshaded.

⬍ Denotes the start of an increase in water exchange rate.

Table 5.2: Shows the difference in RGR and yield of *Gracilaria* grown with fertilized seawater at low and high turnover rate at I&J farm. All values are means $\pm$ SD.

Treatment	Low Flow rate	High Flow rate	Pvalue
RGR (% d^{-1})	2.4 $\pm$ 0.67	1.8 $\pm$ 1.25	0.17
Yield (kg W wt. m^{-2} d^{-1})	0.70 $\pm$ 0.02	0.05 $\pm$ 0.04	0.22

Table 5.3: The effect of shading on the RGR and yield of *Gracilaria* in fertilized seawater tanks during both low and high turnover rate at I&J farm. All values are means $\pm$ SD.

Flow rate	Unshaded	Shaded	Pvalue
Low Flow rate			
RGR (% d^{-1})	1.8 $\pm$ 0.84	2.3 $\pm$ 1.10	0.48
Yield (kg W wt. m^{-2} d^{-1})	0.05 $\pm$ 0.02	0.07 $\pm$ 0.04	0.41
High Flow rate			
RGR (% d^{-1})	2.8 $\pm$ 0.44	1.6 $\pm$ 0.66	0.01
Yield (kg W wt. m^{-2} d^{-1})	0.10 $\pm$ 0.02	0.05 $\pm$ 0.02	0.01

Table 5.4: The seasonal effect on the RGR and yield of *Gracilaria* in abalone wastewater at I&J farm. All values are means $\pm$ SD.

Variable	Spring-Summer	Autumn-Winter	Pvalue
RGR	2.4 ± 0.79 % d^{-1}	2.6 ± 0.63 % d^{-1}	0.44
Yield	0.08 ± 0.04 kg W wt. m^{-2} d^{-1}	0.08 ± 0.02 kg W wt. m^{-2} d^{-1}	0.85

Table 5.5: The shading effect on the RGR and yield of *Gracilaria* in abalone wastewater tanks at I&J farm. All values are means $\pm$ SD.

Variable	Unshaded	Shaded	Pvalue
RGR	2.7 ± 0.97 % d^{-1}	2.5 ± 0.93 % d^{-1}	0.65
Yield	0.09 ± 0.04 kg W wt. m^{-2} d^{-1}	0.07 ± 0.04 kg W wt. m^{-2} d^{-1}	0.46

5.5 Discussion

This study demonstrated the biological and technical feasibility of growing *Gracilaria* in tank culture systems using nutrient-enriched water as growth medium. However, no clear comparison could be made between the two farms because of different culture conditions and culture media used i.e.tank size; flow rate, abalone and fertilized seawater (I&J) abalone and turbot water (JSP). There seems to be no clear seasonal growth pattern for *Gracilaria* in nutrient-enriched seawater, with a similar average RGR in summer as in winter months (Figure 5.1 and figure 5.3). This shows that optimal water temperature alone is not enough for maximum production of *Gracilaria*. This is in contrast to what is observed in seawater experiments in the current study where (Figure 4.1) there is a clear seasonal growth pattern with higher RGR during summer when there is a peak in temperature and pH and low RGR in winter when water temperature levels are lower. Data for *Gracilaria* growth between turbot and abalone effluent were available only for three-month data (March 2002- May 2002) and there is no difference in the growth rate of *Gracilaria* between the two growth media. Bleaching of thalli observed in both farms during early summer is consistent with observations by Ryther *et al.* (1981) that *Gracilaria* begins to lose the dark reddish-brown colour when internal nutrient reserves are declining, turning into a pale straw-yellow colour. However, *Gracilaria* continues to grow as it had been observed in a study by Ryther *et al.* (1981). At JSP farm growth experiments were affected by the 'white tip' disease early in winter and that

resulted in the collapse of the experiments. Shading has an adverse effect on growth in small and medium-sized tanks.

Unlike in seawater culture experiments, increased water turnover rate had no growth enhancing effect on *Gracilaria* grown in fertilized seawater. This is probably due to inadequate conditions that may have affected the photosynthesis process during winter, where the average RGR in both fertilized and abalone wastewater tanks suddenly dropped to less than 2 % d^{-1}. This study demonstrates the importance of understanding the interactions of factors that increase production as observed by Santelices (1999), including interactions between environmental factors i.e. water temperature and water flow rate. From this current study, experimental growth of *Gracilaria gracilis* in large culture tanks proved to be sustainable and no restocking was necessary. This is probably due to minimal infestation of culture tanks with fouling species and epiphytes compared to the culture tanks at Jacobsbaai Sea Products. However, with extremely high irradiance levels during summer months, *Gracilaria* in abalone culture tanks becomes threatened by the bleaching of thalli with material losing the dark-red colour and turning yellowish to straw colour. No bleaching was observed in fertilized culture tanks. It appears, from the present study that shading is necessary at low turnover rates but not at higher turnover rates in fertilized seawater. Shading of abalone culture tanks has no effect on *Gracilaria* growth.

Chapter 6

Nutrient analysis of *Gracilaria* grown in seawater and in nutrient-enriched media

6.1 Introduction

According to Hanisak (1979), when the total-N content of the thallus drops below a critical level, growth rates are reduced in most macroalgae species. High yields of *Gracilaria* are usually produced when there is fast growth rate at high stocking densities but this is usually achieved by adding considerable amounts of nitrogen (Smit *et al.,* 1997). Tank cultivation is the most productive system for *Gracilaria* cultivation. However, one of the main problems associated with outdoor cultivation of *Gracilaria* is the growth of competing epiphytes. Ryther (1977) reported that epiphyte growth is probably the single major problem and constraint on commercial scale seaweed cultivation, especially in the tropics and subtropics. Wheeler *et. al.* (1981) reported that the control of epiphytes and the provision of nutrients are two major problems in macroalgal farming. Epiphytes and fouling algae consist of members of the three main macroalgal groups (Chlorophyta, Rhodophyta and Phaeophyceae) and are usually described as filamentous and weedy. Because of the small amounts of nutrients essential for mass production of *Gracilaria* in normal seawater (particularly nitrogen and phosphorus) it has become usual to enrich the normal seawater with fertilizers or to use wastewater from animal aquaculture to grow *Gracilaria*. The addition of nutrients is essential for high production of *Gracilaria* but this also stimulates the growth of phytoplankton and epiphytes (Oliveira *et al.,* 2000). Several techniques have been used in an attempt to reduce and/ or eliminate epiphyte contamination. These include biological, chemical and physical methods (see section 2.5.9.2). Epiphyte problems can be reduced by regular management and monitoring of nutrient supplies, especially nitrogen (Friedlander, 1991; Friedlander and Ben-Amotz, 1991; Pickering *et al.,* 1993). Another way of minimizing epiphyte contamination is the treatment of the seaweed material in freshwater (3-4 minutes) before re-stocking of culture tanks (R. Anderson, personal communication). The main objective of this experiment was to investigate the effectiveness of nutrient-enriched media in enhancing *Gracilaria gracilis* growth in culture tanks. At JSP two nutrient-enriched culture media was used, additional

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to the normal seawater (e.g. abalone and turbot effluent) whereas at I&J, *Gracilaria gracilis* were cultured with normal seawater, abalone effluent and fertilized seawater.

6.2 Materials and Methods
6.2.1 JSP and I&J
6.2.1.1 Water sampling and analysis

A two-day water sampling and analysis experiment was conducted at JSP farm from the 9-11 October 2002 in small tanks. Water samples were taken from two points in each individual tank (incoming water and outgoing water). Glass bottles (100 ml) with lids were used to collect water samples at four-hour intervals from the tanks. Each water sample taken from the inlet and outlet pipes of the culture tanks was poured into four smaller bottles for water nutrient quantification (e.g. NH_4^+-N, PO_4, NO_3^--N and NO_2^--N). Water samples were filtered immediately and about 20 ml sample was then transferred to each of the four small glass bottles before they were stored in a freezer. After the two-day experiment, samples were taken out of the freezer to a cooler box and covered with ice to keep them frozen while being transported from the farm to the Marine and Coastal Management laboratory for nutrient analysis.

At I&J farm, a three-day experiment was conducted from the 26-28 February 2002. This was done by taking water samples at four-hour intervals during the day and at two-hour intervals at night. Water samples were collected at two points from each individual tank (incoming and outgoing water), using three labelled bottles from each point. In the laboratory, the water was immediately filtered and then stored in a freezer. After the three-day experiment the samples were transferred to a cooler box and covered with ice to keep them frozen while being transported to the Marine and Coastal Management laboratory for chemical analysis. The nutrient (NH_4-N, PO_4, NO_3-N and NO_2-N) determination was done using the following methods, determination of ammonia (Koroleff, 1983), determination of phophorus (Koroleff, 1983), determination of nitrate and nitrite (Strickland and Parsons, 1968) a modification of Morris and Riley (1963). No water chemical analysis was made at I&J.

6.2.1.2 Tissue collection and analysis (JSP and I&J)

During each harvest trip, i.e. at two-week intervals, the same procedure, as described in section 4.2.1.1 was followed. In the laboratory each sample was rinsed with distilled water then weighed. The individual samples were then labelled and transferred into foil trays and oven-dried at 70 ^{0}C for 72 hours. The dried material was then re-weighed and the dry weight recorded. The dry samples were ground to fine powder using a milling machine. The fine powder material was kept in small bottles and stored in a desiccator for chemical analysis. At I&J farm, a similar collection and analysis procedure was followed as described above except that on this farm harvesting and restocking of tanks was done once per month.

6.2.1.3 Total-N tissue analysis (JSP and I&J farm)

The tissue analysis of dried *Gracilaria* samples was done using the Kjeldahl digestion method for total N determination. The digestion of the material was done as follows. About 0.1g aliquots of the dry finely ground material (approximately 0.1mm mesh) was measured out into long, thick-walled boiling tubes marked with permanent ink. I added 1 mL-distilled water to each sample tube and prepared the standards and these had to go through the complete digestion process along with the samples. About 1 mg N mL^{-1} of stock solution was added to boiling tubes to give the standards in the range of 0.5-2.5 mg N per tube. Appropriate amounts of distilled water were added to standards that were less than 1 mg N mL^{-1} to make the final volume in the tubes 1 mL. Three distilled water blanks, with 1 mL of distilled water per tube were included. Then working in the fume hood, 3 mL of acidified salicylic acid was added to each tube of samples, standards and blanks. A spatula tip of sodium thiosulphate crystals to each tube and 1-selenium Kjeldahl tablet were added. All the tubes were placed in a cold digestion block and were heated at 150 ^{0}C overnight to drive off water. The material was digested further using the following time and temperature sequence:

220 ^{0}C for 1 hour, 250 ^{0}C for 1 hour, 280 ^{0}C for I hour, 300 ^{0}C and 350 ^{0}C for 2-4 hours until digest is clear. The material was then cooled (it can be heated, if that is necessary) to 150 ^{0}C and about 5-10 mL of distilled water was added carefully. Each digest was made to 50 mL by adding 20 mL of distilled water and this was mixed thoroughly.

Reagents used:

(i)   Standard N stock solution (1 mg N mL^{-1}): 4.7170 g (NH$_4$)$_2$ SO$_4$ in 1L distilled water.

(ii)   Acidified salicylic acid (34 g salicylic acid L^{-1}): 34 g salicylic acid per litre of H$_2$SO$_4$.

The colour determination of kjeldahl digestion solution:

Appropriate aliquots (0.5 mL) of digest solution were pipetted into thin-walled boiling tubes. About 25 mL (0.12% w/v) EDTA-Na$_2$ was added to each tube and mixed and then 2 mL of Reagent A was added and mixed. Then about 3.5 mL of Reagent B was added and mixed as well and the tubes were filled up to 50 mL with distilled water. The tubes were mixed thoroughly by sealing with parafilm and inverting, and left for one hour for the blue colour to develop.

The blanks were used to zero the machine and the absorbency was read at 635 nm.

The tissue analysis for phosphate concentration of dried *Gracilaria* material was done using Triacid digestion method for total P determination. About 0.1 g of aliquots was weighed out into long, thick-walled test tubes marked with permanent ink. The samples were pre-digested by adding 1 mL of concentrated nitric acid to each tube and heating on a digestion block at 150-180 ^{0}C until the samples were almost dry (this took between 10-60 minutes and we had to keep a careful watch). Three blanks tubes were included (nitric acid only). 1 mL of triacid mix (HNO$_3$: HClO$_4$: H$_2$SO$_4$) was added to each tube in a fume hood. The material was digested for another hour at 180 ^{0}C until white perchloric fumes had dissipated and the solution was white to yellowish and viscous. The tubes were not allowed to dry out. The tubes were cooled and carefully diluted to a total volume of 25 mL, mixed thoroughly and white precipitate allowed to settle.

The colour determination of phosphate was conducted using the Murphy and Riley colorimetric determination of phosphate. We started by pipetting aliquots of digest solution (within the range of 0.2-10 mL) into 50 mL volumetric flasks using 5 mL. I prepared the standards in the range of 2-30 μg P. I added appropriate amounts of stock solution (2μg P mL^{-1}) to 50 mL volumetric flasks (i.e. 1 mL of 2μg P mL^{-1} stock solution for 2μg P standard, 2 mL for 4 μg P standard, 15 mL for 30 μg P standard etc). 25 mL of distilled water was added to each volumetric flask (samples and blanks) to dilute the acidity of sample. This was followed by the addition of 8 mL of Murphy and Riley reagent to each flask, and this was mixed thoroughly. To make up to 50 mL, distilled water was added and mixed well by inverting. The flasks were left for about an hour to allow the blue colour

to develop (colour is stable for 40 minutes to 3 hours). The blanks were used to zero the machine and the absorbencies were read at 882 nm. After each harvest *Gracilaria* samples were taken to the laboratory and the same procedure, both for the processing and tissue analysis was followed as described (see section 6.2.1.2).

6.2.2 Daily profile of environmental factors

6.2.2.1 JSP

6.2.2.1.1 Temperature, pH and dissolved oxygen

This experiment was done over a two-day period. Water temperature, pH and dissolved oxygen measurements were taken every two hours at about 10 cm below the surface using a digital thermometer, pH meter and oxygen meter, respectively.

6.2.2.2. I&J

6.2.2.2.1 Temperature, pH and dissolved oxygen

A three-day experiment was undertaken on this farm to take the water samples for nutrient analysis as well as to get a daily (24-hour) profile of environmental factors, i.e. temperature, pH and DO. Water temperature, pH and dissolved oxygen measurements were taken over a three-day period at four-hour intervals during the day and at two-hour intervals at night using a digital thermometer, pH meter and oxygen meter, respectively.

6.3 Data and statistical analysis

All statistical analyses were done using Windows Statistica 6. A Student t-test was used to test for the significance of difference in means in all treatments. One-way Anova was used to test for the significance of differences in means between the three treatments. Tukey Honest Significance Difference (HSD) test was used as post hoc test for the significance of the treatment means. To test for the significance of difference we used the probability level $p = 0.05$. The normality tests were done using Kolmogorov-Smirnov and Lilliefors test for normality.

6.4 Results

6.4.1 JSP

6.4.1.1 Water analysis

There was a highly significant reduction in NH_4-N concentration in the outgoing water in seawater culture tanks. There was a slight, though significant, increase in PO_4 in the outgoing water (Table 6.1). The other nutrients (NO_2-N and NO_3-N) were also significantly reduced (Table 6.1). There was a significant difference in nutrient concentration between the incoming and outgoing water in small seawater tanks (Table 6.1). However, PO_4 concentration was higher in the outgoing water in small seawater tanks (Table 6.1). There was a significant reduction of dissolved nutrients in both turbot and abalone wastewater tanks with the exception of nitrate (Table 6.1).

Table 6.1: Nutrient concentration of three different culture media in small culture tanks at JSP farm.

Nutrient	Incoming water	Outgoing water	Pvalue
Seawater			
NH4	5.99 μ M l^{-1}	1.36 μ M l^{-1}	< 0.001
PO4	2.06 μ M l^{-1}	2.34 μ M l^{-1}	< 0.001
NO2	0.54 μ M l^{-1}	0.13 μ M l^{-1}	< 0.001
NO3	16.91 μ M l^{-1}	10.25 μ M l^{-1}	< 0.001
Abalone effluent			
NH4	10.39 μ M l-1	5.76 μ M l-1	0.01
PO4	4.21 μ M l-1	2.27 μ M l-1	< 0.001
NO2	0.90 μ M l-1	0.40 μ M l-1	< 0.001
NO3	10.78 μ M l-1	9.74 μ M l-1	0.40
Turbot effluent			
NH4	34.37 μ M l-1	25.33 μ M l-1	0.01
PO4	6.26 μ M l-1	3.98 μ M l-1	< 0.001
NO2	2.39 μ M l-1	1.32 μ M l-1	< 0.001
NO3	21.63 μ M l-1	20.30 μ M l-1	0.66

6.4.1.2 Tissue analysis

No significant difference in total-N and PO_4 tissue content was observed between small and medium-sized tanks (Table 6.2). There was a significant seasonal difference in total-N tissue content and PO_4 concentration and higher nutrient concentrations were obtained during winter and

lower nutrient concentrations in summer in both small and medium-sized tanks (Table 6.3). In small tanks, PO_4 tissue content was lower and unstable during summer months i.e. October 2001-early April 2002 and slightly higher in winter i.e. September 2001, late April -early August 2002 (Figure 6.3). In medium-sized tanks, a higher PO_4 tissue content was obtained in winter and a lower PO_4 tissue content was obtained in summer (Figure 6.4).

In turbot effluent tanks, tissue concentration of total N also followed a seasonal pattern, with higher levels during winter and lower levels during summer months (Figure 6.5). No sufficient data was obtained in abalone culture tanks due to technical problems in the farm. *Gracilaria* growth in abalone effluent only occured over a three month period (Figure 6.6 and Figure 6.8). Higher total tissue N and P content was obtained during winter (late April 2002-August 2002) and low total tissue N and P content was obtained in summer (October 2001-early April 2002) in turbot effluent treatment (Figure 6.5 and Figure 6.7). There was a significant seasonal difference in *Gracilaria* total tissue N and P content in turbot wastewater but only the total tissue N had a significant seasonal difference in abalone wastewater (Table 6.4). There was a significant difference in total tissue N content between seawater and turbot wastewater (Table 6.5). However, no significant difference in *Gracilaria* total tissue N content was observed between seawater and abalone wastewater during summer (early November 2001, March 2002-early April 2002) (Table 6.5). There was no significant difference in *Gracilaria* total tissue N content between turbot and abalone wastewater (Table 6.5).

There was no significant difference in PO_4 tissue concentration between seawater and abalone wastewater (Table 6.5).

Table 6.2: The effect of tank size (small and medium) on total-N and PO_4 tissue concentration of *Gracilaria* cultured in seawater at JSP farm.
All values are mean $\pm$ S. D.

Variable	Small tanks	Medium tanks	P-value
Total-N			0.84
Mean (mg N g^{-1})	41.39 ± 14.66	40.95 ± 13.70	
	(n = 85)	(n = 83)	
PO_4			
Mean (mg P g^{-1})	299.43 ± 67.88	311.62 ± 63.75	0.27
	(n = 74)	(n = 71)	

Table 6.3: The seasonal effect in total-N and PO_4 tissue concentration of *Gracilaria* in seawater at JSP farm. All values are means $\pm$ S. D.

Variable	Spring-Summer	Autumn-Winter	P-value
Total-N			
Small tanks			
Mean (mg N g^{-1})	41.07 ± 7.73	65.10 ± 18.00	< 0.001
Medium tanks	41.11 ± 7.14	64.73 ± 17.41	< 0.001
Mean (mg N g^{-1})			
PO_4			
Small tanks			
Mean (mg P g^{-1})	277.98 ± 68.97	339.03 ± 44.69	< 0.001
Medium tanks	289.36 ± 58.14	350.09 ± 54.79	<0.001
Mean (mg P g^{-1})			

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Table 6.4: The seasonal effect in total-N and PO$_4$ tissue concentration of *Gracilaria* in turbot and abalone culture tanks at JSP farm. All values are means ± S. D.

Variable	Spring-Summer	Autumn-Winter	P-value
Turbot			
(NH$_4$-N)			
Mean (mg N g^{-1})	41.06 ± 6.59	65.59 ± 17.18	< 0.001
PO$_4$			
Mean (mg P g^{-1})	347.40 ± 70. 49	373.90 ± 51.06	0.02
Abalone			
(NH$_4$-N)			
Mean (mg N g^{-1})	40.60 ± 5.34	50.84 ± 10.38	< 0.001
PO$_4$			
Mean (mg P g^{-1})	344.02 ± 76.12	342.36 ± 52.17	0.94

Table 6.5: Shows the P-values associated with total tissue N and P tissue concentrations in different treatments used on the growth of *Gracilaria* at JSP farm.

Variable	Seawater	Turbot	Abalone
NH4			
Seawater		**0.00**	0.14
Turbot	**0.00**		0.19
Abalone	0.14	0.19	
PO4			
Seawater		**0.00**	0.13
Turbot	**0.00**		0.33
Abalone	0.13	0.33	

Figure 6.1: Shows the total-N concentration in tissue of *Gracilaria* over a 12-month growth period in small seawater tanks at JSP farm. All values are means ± SD.

Figure 6.2: Shows the total-N concentration in tissue of *Gracilaria* over a 12-month growth period in medium-sized seawater tanks at JSP farm. All values are means ± SD.

Figure 6.3: Shows the PO_4 concentration of *Gracilaria* tissue over an eleven-month growth period in small seawater tanks at JSP farm. All values are means $\pm$ SD.

Figure 6.4: Shows the PO_4 concentration in tissue of *Gracilaria* over an eleven-month growth period in medium-sized seawater tanks at JSP farm. All values are means $\pm$ SD.
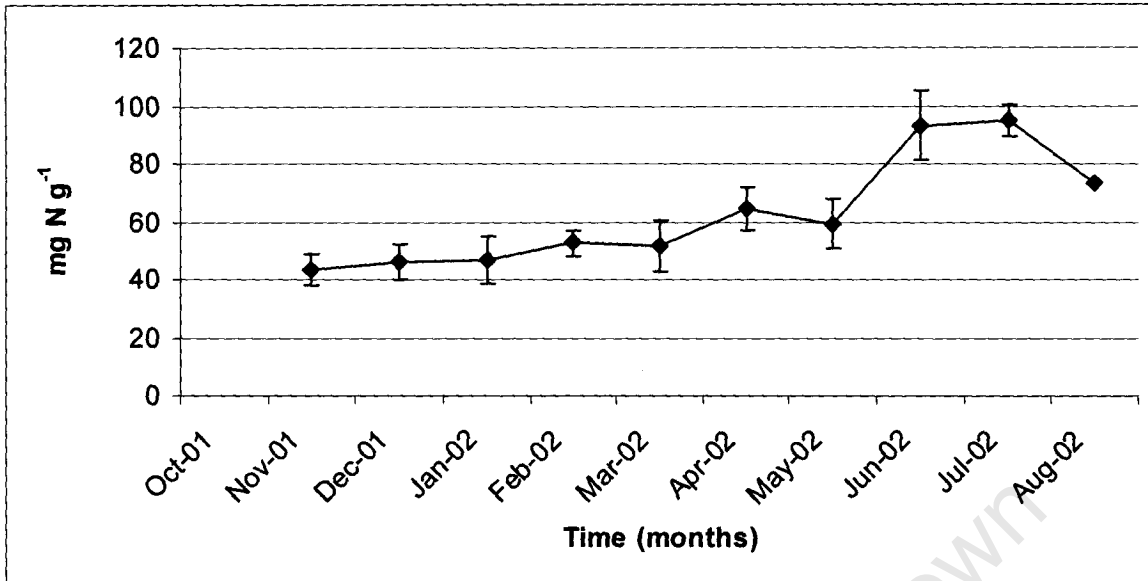
Figure 6.5: Shows the total-N concentration of *Gracilaria* tissue over a 10-month growth period in turbot tanks at JSP farm. All values are means ± SD.
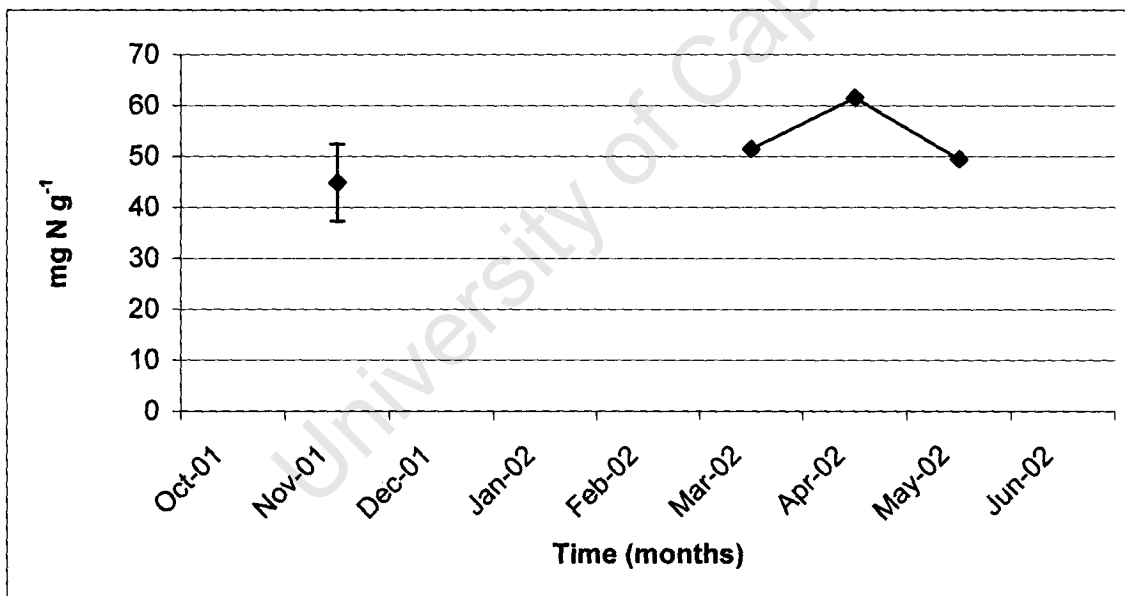
Figure 6.6: Shows the total-N concentration of *Gracilaria* tissue over a 3- month growth period in abalone tanks at JSP farm. All values are means ± SD.

Figure 6.7: Shows the PO_4 concentration of *Gracilaria* tissue over a 9-month growth period in turbot tanks at JSP farm. All values are means $\pm$ SD.
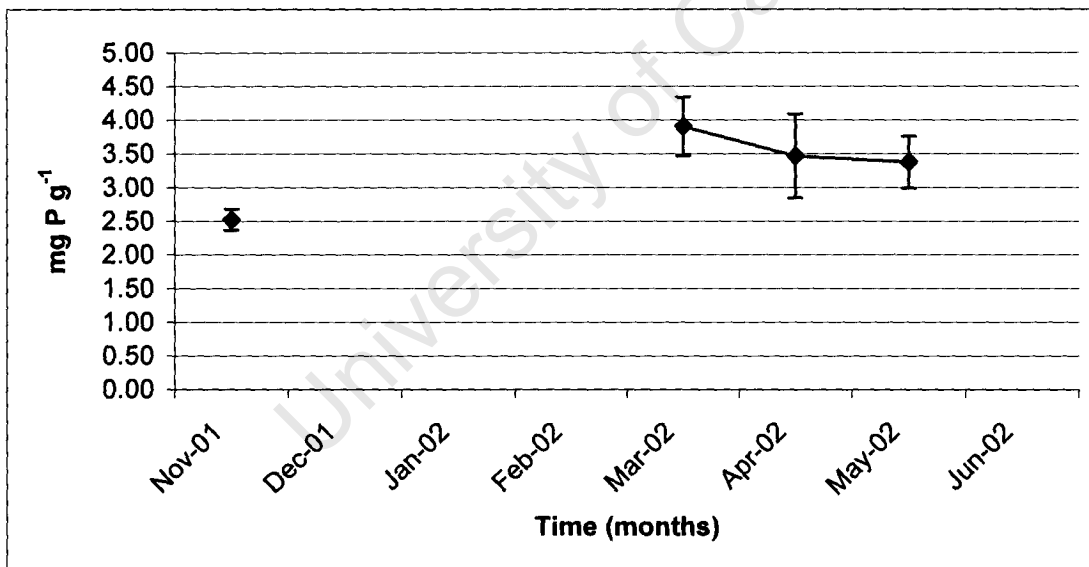
Figure 6.8: Shows the PO_4 concentration of *Gracilaria* tissue over a 3-month growth period in abalone tanks at JSP farm. All values are means $\pm$ SD.

6.4.2 I&J

6.4.2.1 Water analysis

No analysis was made.

6.4.2.2 Tissue analysis

In seawater culture tanks, the total-N and PO4 concentration in *Gracilaria* tissue followed a seasonal pattern; it was higher during autumn-winter months and lower during spring-summer months (Figure 6.9 and Figure 6.10). Tissue nutrient concentration followed a seasonal pattern with higher concentrations during winter months and lower nutrient concentration during summer months. However, the significance of the difference between spring-summer months and autumn-winter months could not be tested because of different water exchange rates in culture tanks during summer and winter months (4 and 12 volume exchanges d^{-1}).

In fertilized seawater and abalone effluent culture tanks, *Gracilaria* tissue N concentration followed a seasonal pattern and higher concentrations of total tissue N and P were obtained during winter months and lower concentrations were obtained during summer months. However, the difference in tissue nutrient concentration between winter and summer months was generally higher in total N and was minimal in total P, both in fertilized seawater and in abalone wastewater *Gracilaria* (Figure 6.11- 6.14).

There was a significant difference in total tissue N concentration between seawater and fertilized seawater and between fertilized seawater and abalone wastewater (Table 6.6). Fertilized seawater had a slightly higher concentration than both seawater and abalone wasterwater (Figure 6.9, 6.11, 6.12). No significant difference was observed in total tissue N concentration between seawater and abalone wastewater (Table 6.6).

There was a significant difference in PO$_4$ tissue concentration between seawater and fertilized seawater and between seawater and abalone wastewater (Table 6.7). Fertilized seawater and abalone wastewater had higher PO$_4$ concnetrations than seawater treatment (Figure 6.10, 6.13, 6.14). No significant difference in PO$_4$ tissue concentration was observed between fertilized seawater and abalone wastewater (Table 6.7).
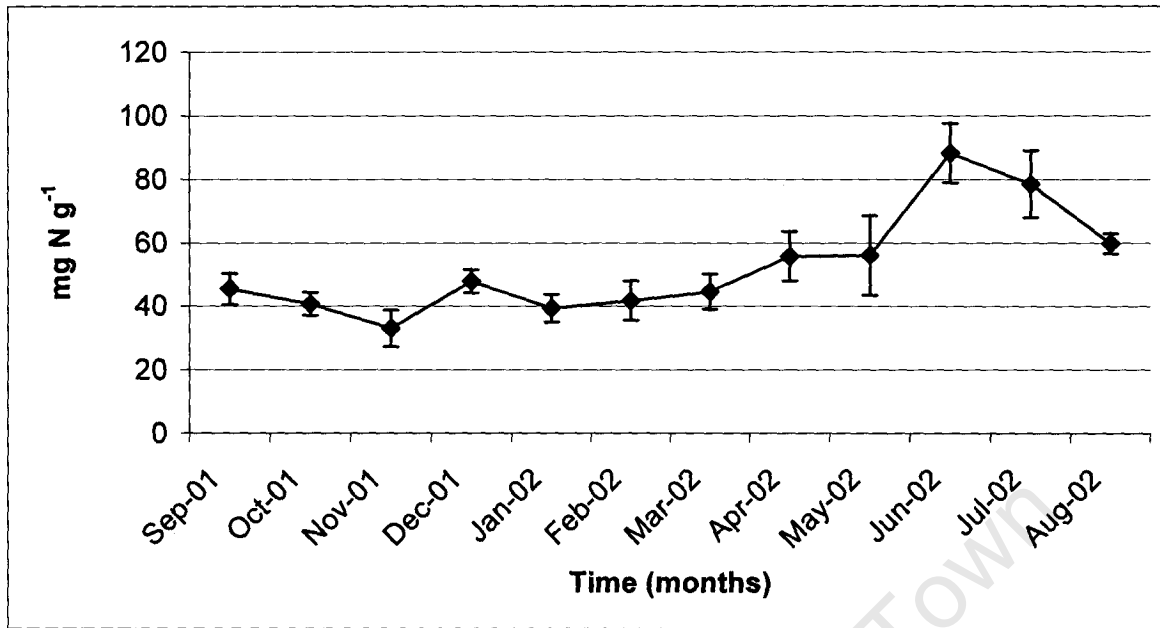
75
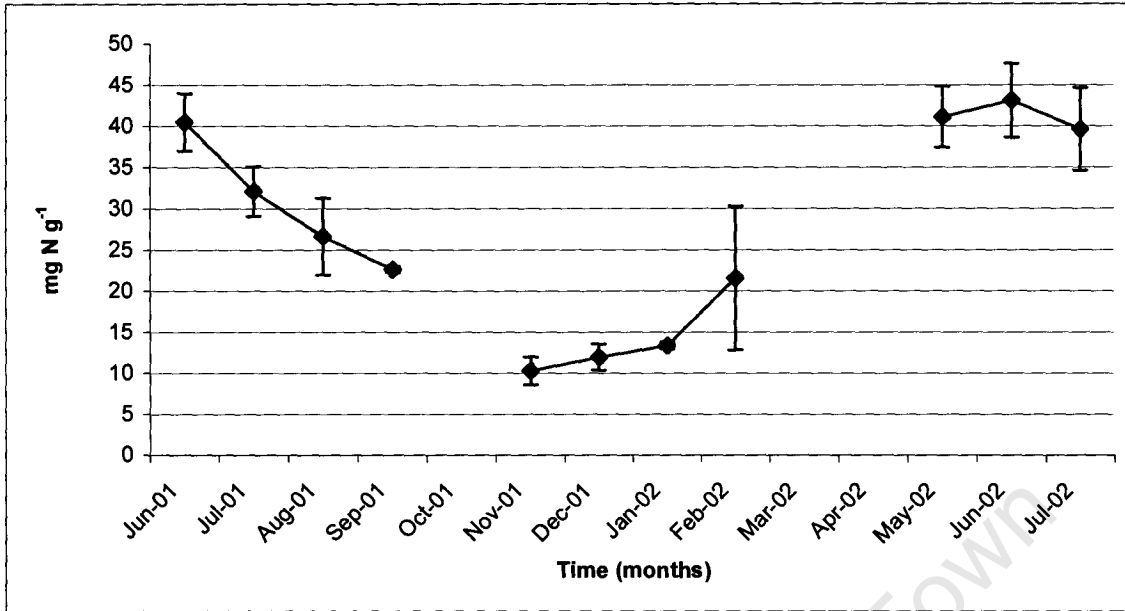
Figure 6.9: Shows the total-N concentration in tissue of *Gracilaria* over a 14-month growth period in large seawater tanks at I&J farm. All values are means $\pm$ SD.
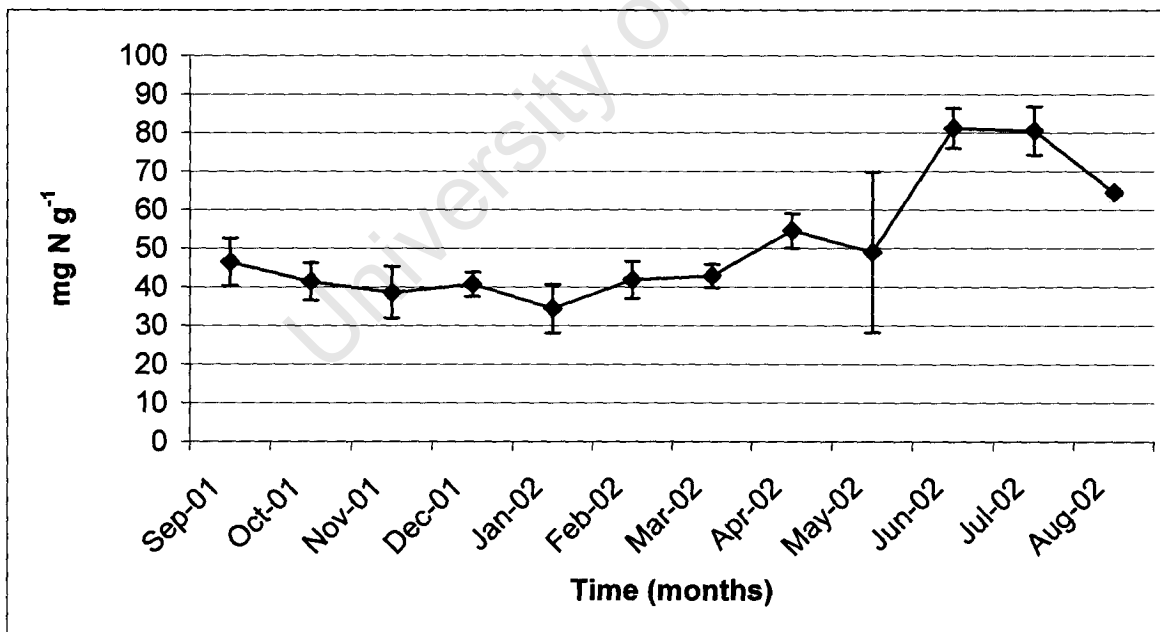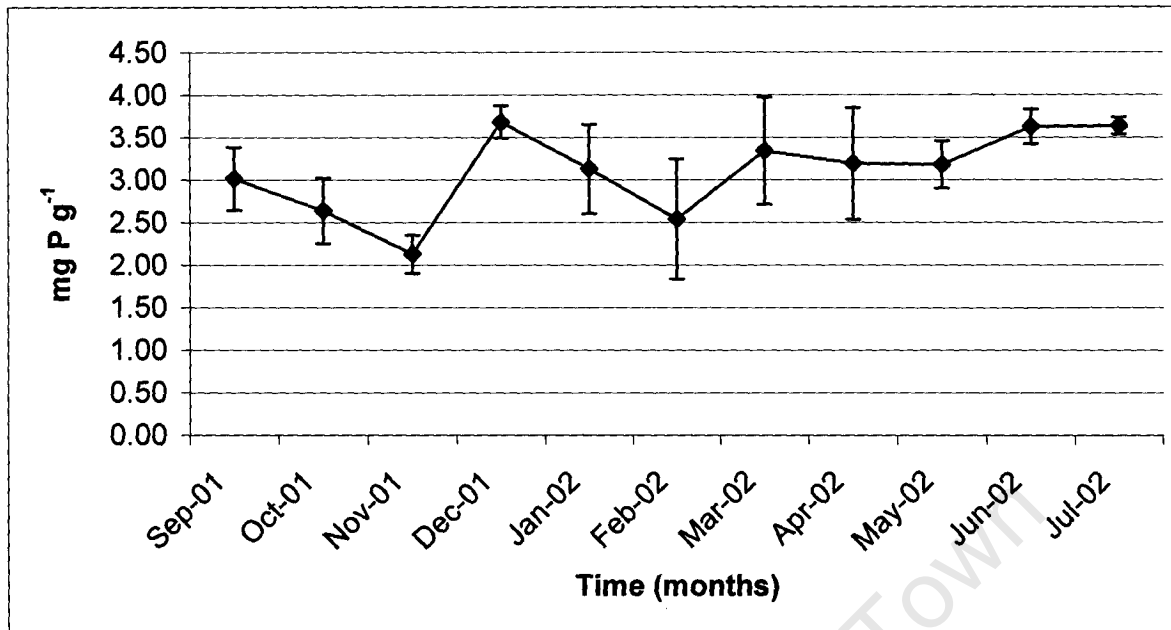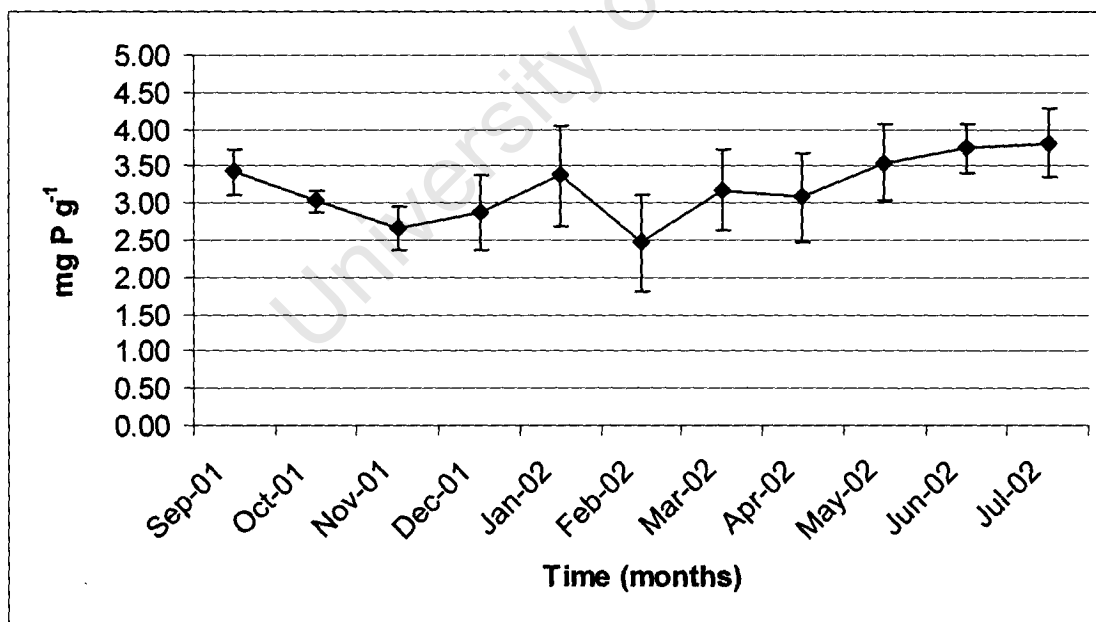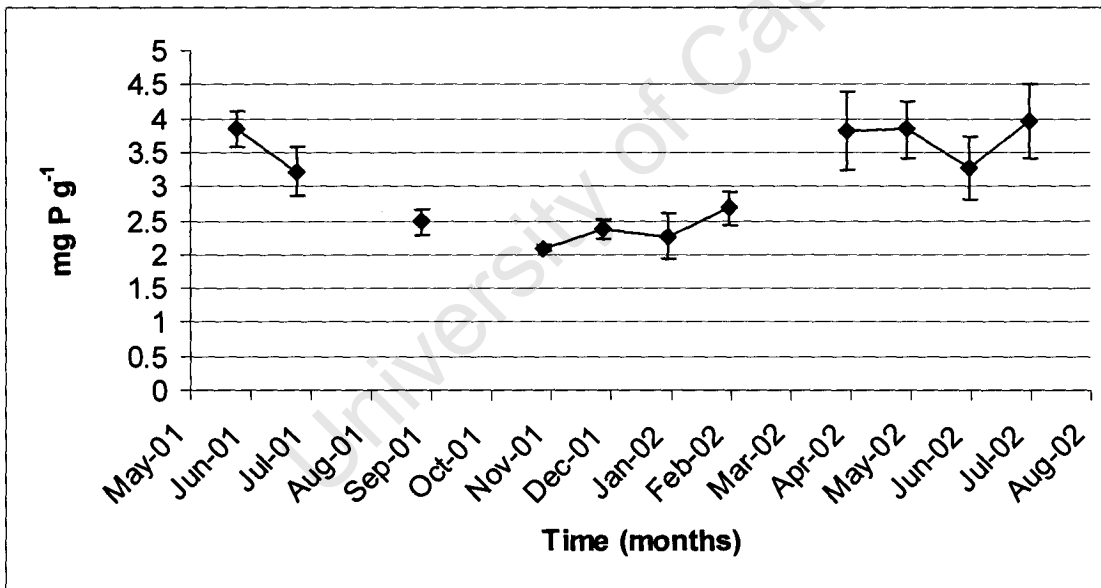

Figure 6.10: Shows the PO_4 concentration in tissue of *Gracilaria* over a 14-month growth period in large seawater tanks at I&J farm. All values are means $\pm$ SD.
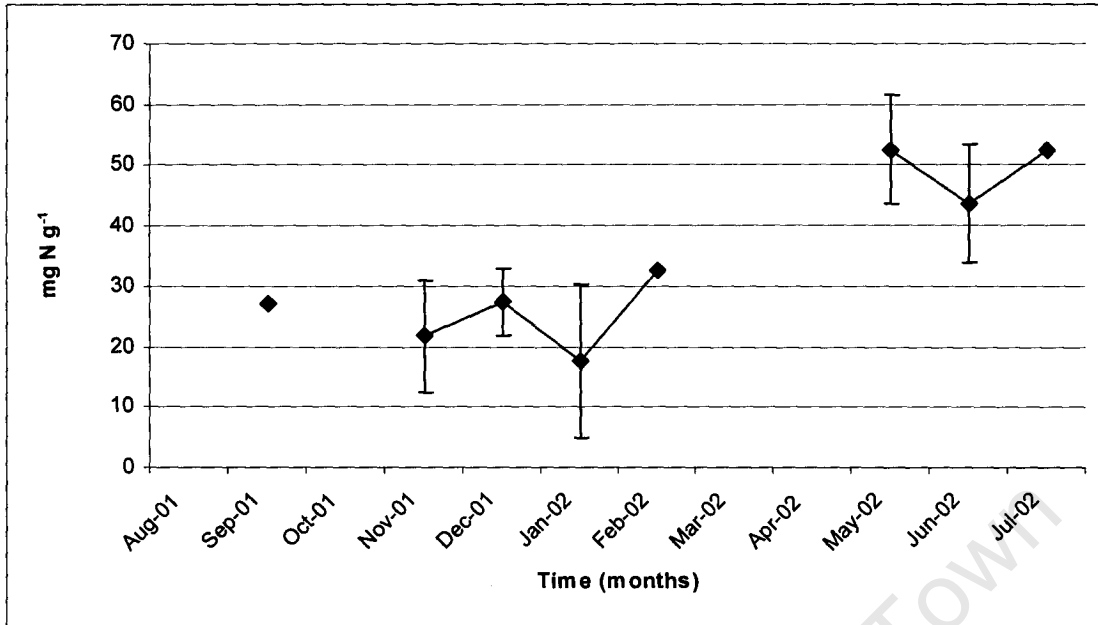
Figure 6.11: Shows the total-N concentration in tissue of *Gracilaria* over 10-months growth period in large fertilized seawater tanks at I&J farm. All values are means ± SD.
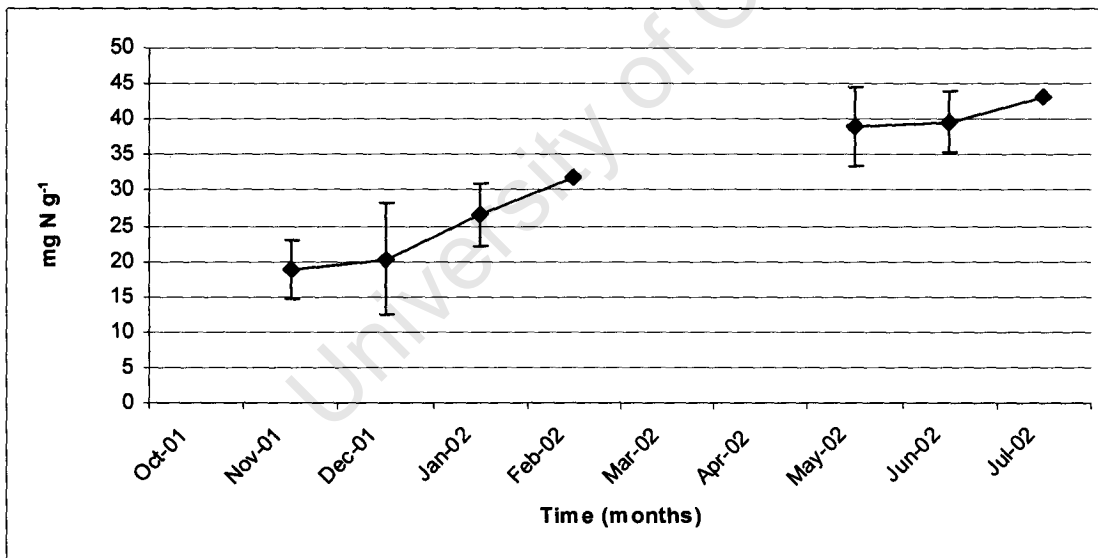
Figure 6.12: Shows the total-N concentration in tissue of *Gracilaria* over 9-months growth period in large abalone tanks at I&J farm. All values are means ± SD.
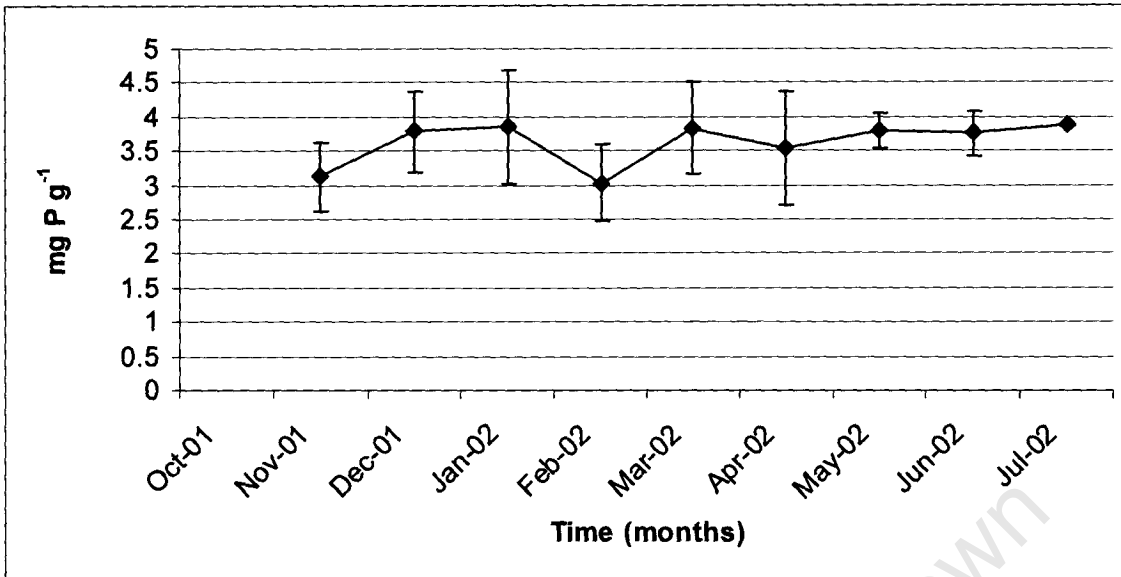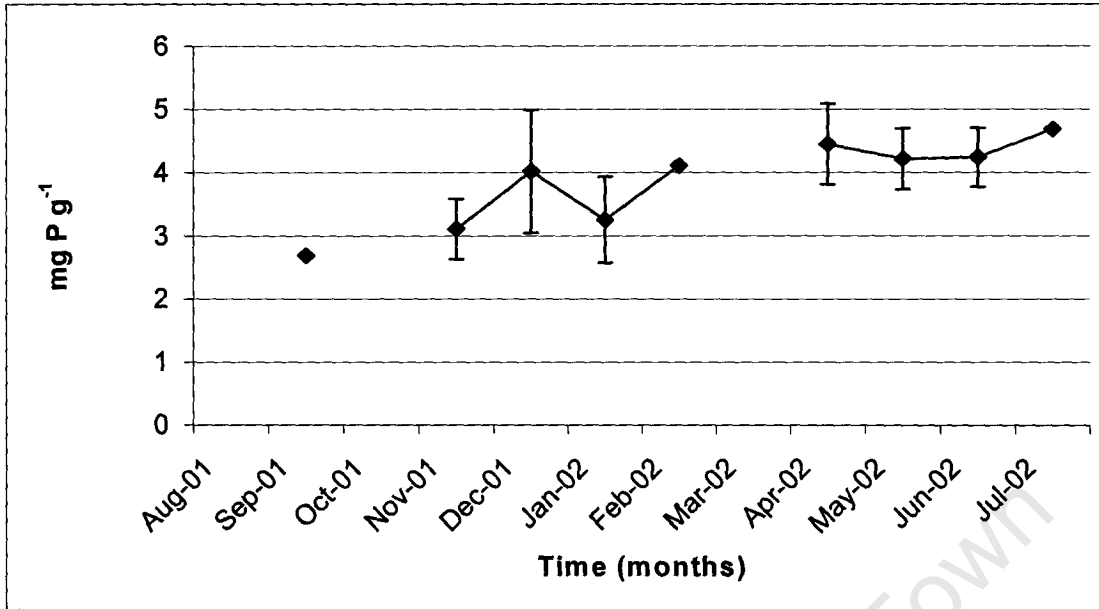
Figure 6.13: Shows the PO_4 concentration in tissue of *Gracilaria* over 10-months growth period in large fertilized seawater tanks at I&J farm. All values are means $\pm$ SD.
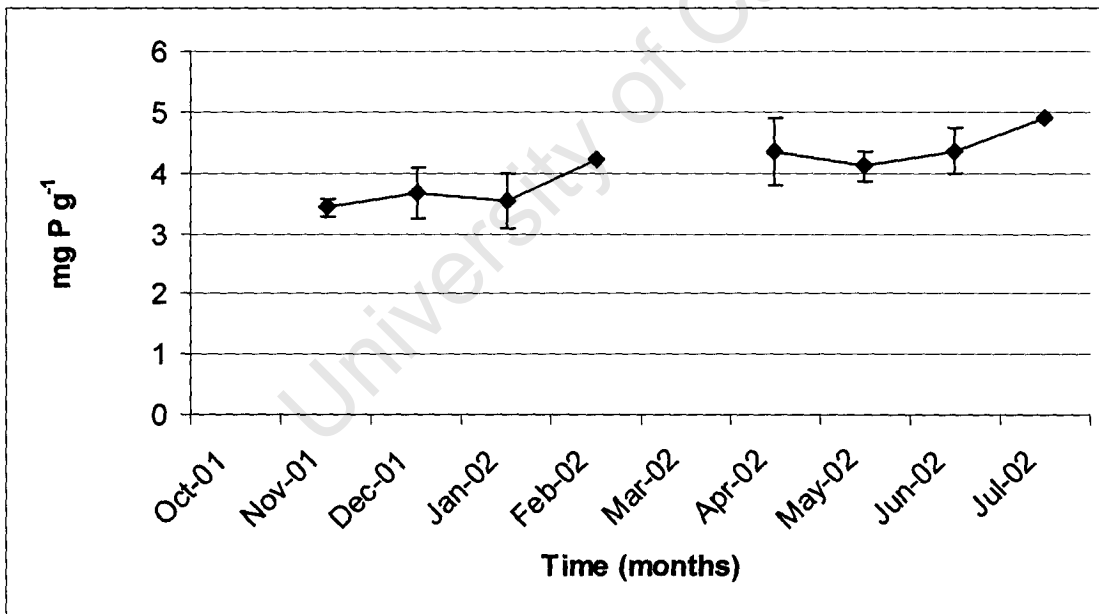
Figure 6.14: Shows the PO_4 concentration in tissue of *Gracilaria* over 9-months growth period in large abalone seawater tanks at I&J farm. All values are means $\pm$ SD.

Table 6.6: P values associated with tissue total-N concentration in different *Gracilaria* culture media at I&J farm.

Treatment	Seawater	Fertilised seawater	Abalone effluent
Seawater		0.02	0.96
Fertilised seawater	0.02		0.01
Abalone effluent	0.96	0.01	

Table 6.7: P values associated with tissue PO_4 concentration in different *Gracilaria* culture media at I&J farm.

Treatment	Seawater	Fertilised seawater	Abalone effluent
Seawater		0.01	< 0.01
Fertilised seawater	0.01		0.99
Abalone effluent	< 0.01	0.99	

6.4.3 Day profile of environmental factors

6.4.3.1 JSP

6.4.3.1.1 Temperature

Fluctuation in temperature in tank water over the two-day experiment ranged from 12.9-17.6 ^{0}C. High temperature (17.6 ^{0}C) was recorded at two different times (16h00 and 12h00) and in two different treatments (seawater and abalone water tanks), day one and day two, respectively (Figure 6.15). Low water temperature (12.9 ^{0}C) was recorded in seawater tanks at 04h00 on day two.
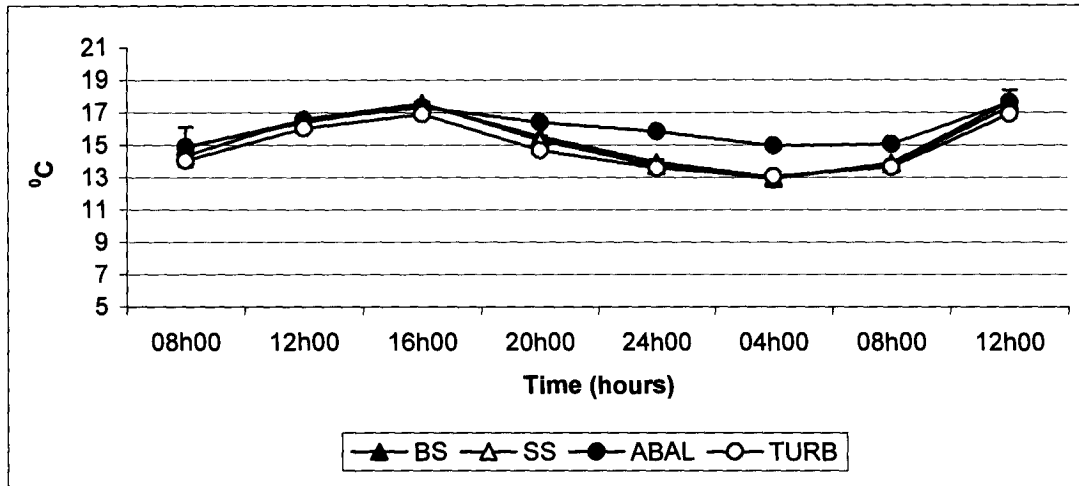
Figure 6.15: Average water temperature in seawater, turbot and abalone (wastewater) culture tanks during a two-day experiment at JSP farm. All values are means ± SD.

BS- medium seawater tanks, SS- small seawater tanks, ABAL- abalone tanks, TURB-turbot tanks.

6.4.3.1.2 pH

There was a large fluctuation in pH level in all the treatments (seawater, turbot and abalone wastewater). The pH level was very high in all treatments between 12h00-16h00 and it was above the critical pH limit (9.0) at 12h00 both on day one and day two. The pH level ranged from 8.72-9.25 in small seawater tanks, from 8.8-9.13 in medium-sized seawater tanks, from 8.54-9.12 in turbot waste and from 8.48-9.08 in abalone waste (see Figure 6.16).
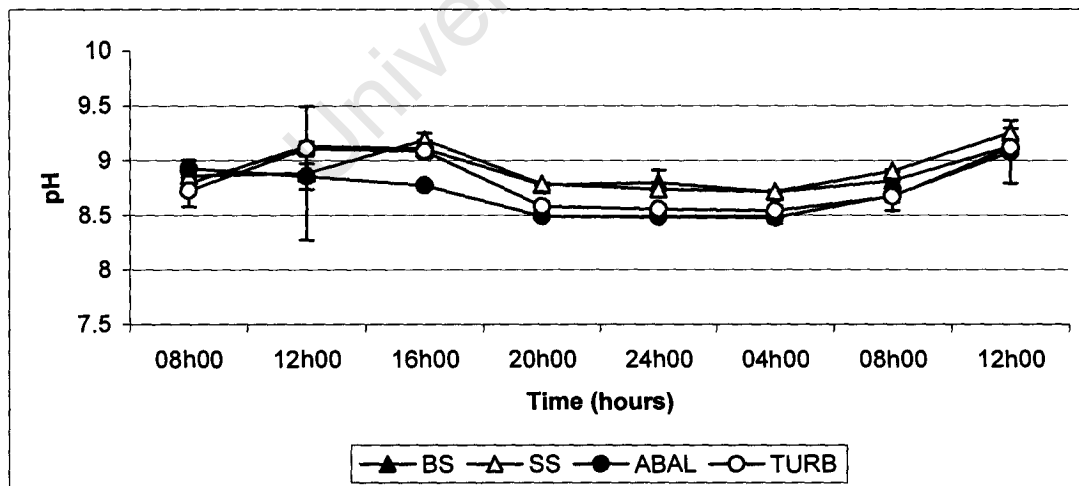
Figure 6.16: Average water pH level in seawater, turbot and abalone (wastewater) culture tanks during a two-day experiment at JSP farm. All values are means ± SD.

6.4.3.1.3 Dissolved oxygen

The dissolved oxygen level in tank water was generally high in the morning (04h00-12h00) and it decreased in the afternoon and was low at night (20h00-24h00) particularly in abalone waste. Seawater (small and medium-sized tanks) had a slightly higher dissolved concentration than turbot and abalone waste at night. The average dissolved oxygen in seawater at night ranged from 8.65-9.1 mg l^{-1} while in turbot and abalone waste it ranged from 8.53-8.72 mg l^{-1} and 7.95-8.23 mg l^{-1}, respectively (see Figure 6.17).
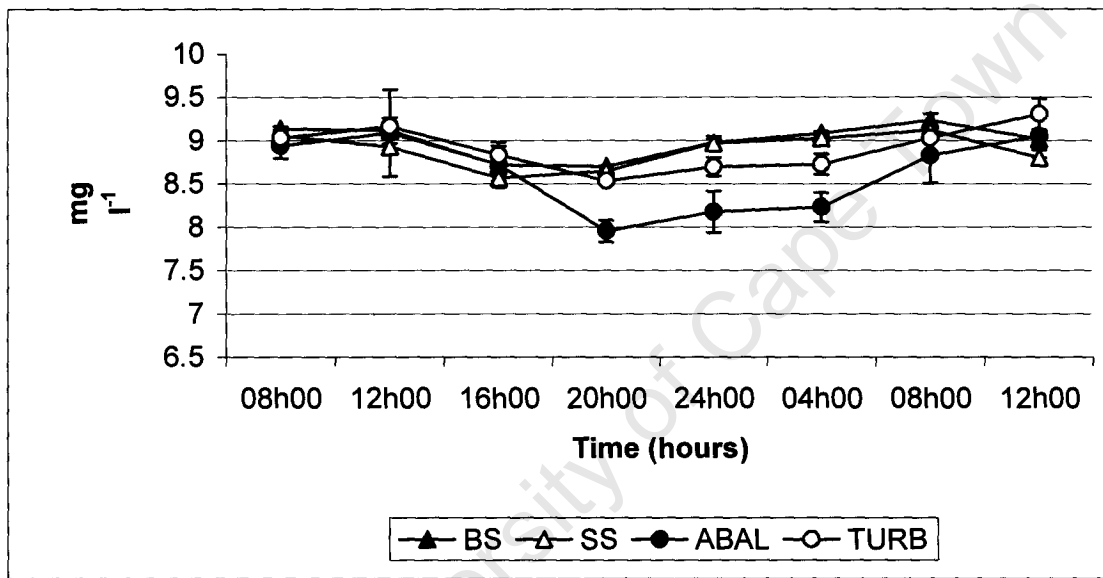
Figure 6.17: Average dissolved oxygen concentration in seawater, turbot and abalone (wastewater) culture tanks during a two-day experiment at JSP farm. All values are means $\pm$ SD.

6.4.3.2 I&J

6.4.3.2.1 Temperature

The water temperature was generally high on the first day (15.8-18.7 ^{0}C) and it declined on the second day (14.2-17.5 ^{0}C) and was low on the third day in all treatments (13.1-15.4 ^{0}C). The highest water temperature (18.7 ^{0}C) was obtained in seawater at 12h00 (midday) on the first day and the lowest water temperature (13.1 ^{0}C) was obtained in fertilized seawater tanks at 08h00 on the third day.

The highest water temperatures were 18.7 ^{0}C, 18.3 ^{0}C and 17.8 ^{0}C and the lowest water temperatures were 14.5 ^{0}C, 13.1 ^{0}C and 13.5 ^{0}C, seawater, fertilized seawater and abalone waste, respectively (see Figure 6.18).
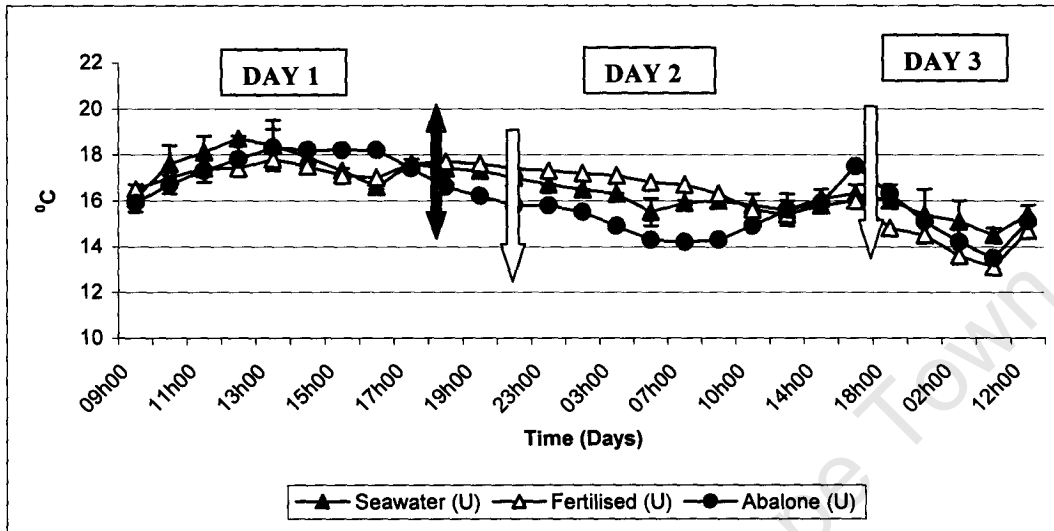
Figure 6.18: Average water temperature in seawater, fertilized seawater and abalone wastewater culture tanks over a three-day experiment at I&J farm. All values are means $\pm$ SD. U- unshaded tanks

↕ Denotes the time for the addition of fertilizer

⇩ Denotes the end and the start of a day

6.4.3.2.2 pH

The water pH level was very high during the day on the first day (11h00-19h00), particularly in seawater (8.97-9.23) and fertilized seawater tanks (8.94-9.14). The pH levels were generally low on the second day but again they reached the critical pH limit (9.0) in seawater tanks at 16h00 and18h00 (9.03 and 9.07), respectively. The pH level in abalone wastewater remained below the critical limit throughout the experiment. The pH level suddenly dropped to about 6.8 in all treatments at 12h00 on the second day (Figure 6.19).
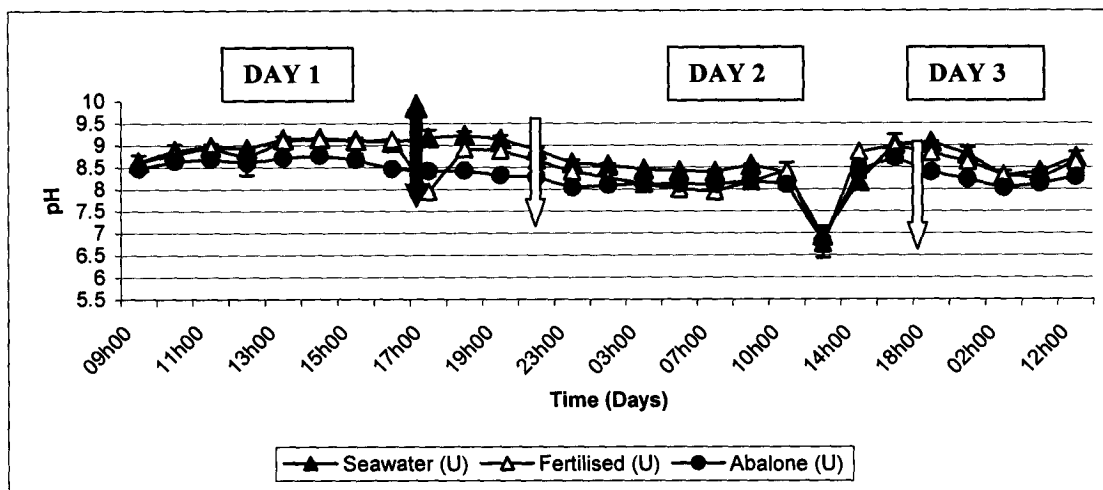
Figure 6.19: Average water pH level in seawater, fertilized seawater and abalone wastewater culture tanks over a three-day experiment at I&J farm. All values are means ± SD. U- unshaded

6.4.3.2.3 Dissolved oxygen

There was high fluctuation in the amount of dissolved oxygen in all treatments. In seawater, DO ranged between 7.51-15.96 mg l^{-1}, between 7.8-5.28 mg l^{-1} (fertilized seawater) and between 7.91-13.46 mg l^{-1} (abalone wastewater) see Figure 6.20.
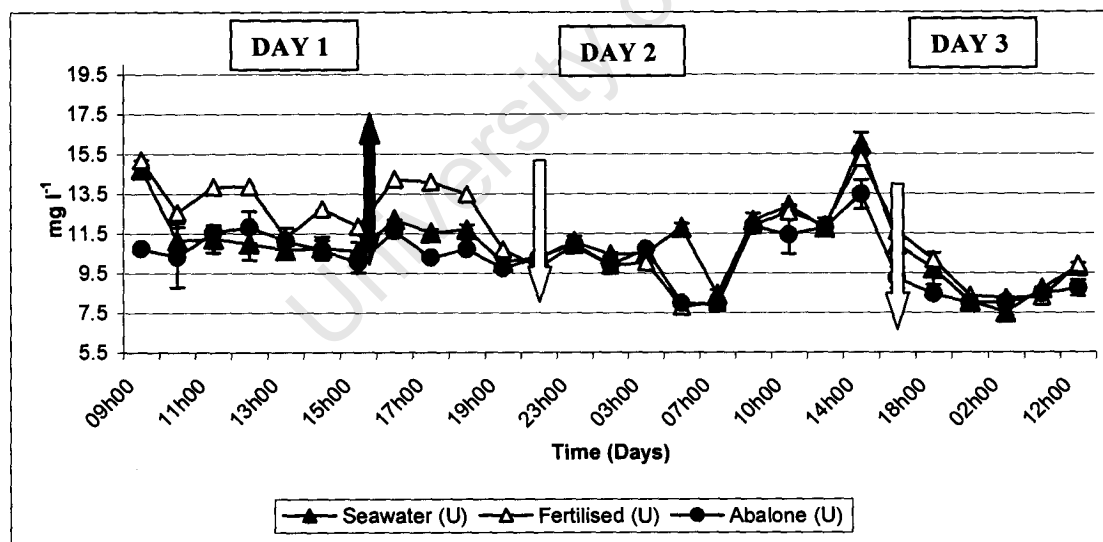
Figure 6.20: Average dissolved oxygen concentration in seawater, fertilized seawater and abalone wastewater culture tanks over a three-day experiment at I&J farm. All values are means ± SD. U-unshaded

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Table 6.8: Nutrient uptake rates of *Gracilaria* cultured in various media over two days experiment at JSP farm.

Culture medium	Inintial conc. ($\mu M l^{-1}$)	Final conc. ($\mu M l^{-1}$)	Removed ($\mu M l^{-1}$)	% Removed	Flow/hr (l/hr)	Removed/hr ($\mu M/hr$)	Dwt of *Grac.* (g)	Uptake rate ($\mu M\ g^{-1}$ dwt h^{-1})
NH$_4$								
Seawater	5.99	1.36	4.63	77.30	68.33	316.38	50.00	6.33
Abalone	10.39	5.76	4.63	44.56	68.33	316.38	50.00	6.33
Turbot	34.37	25.33	9.04	26.30	68.33	617.73	50.00	12.35
NO$_3$								
Seawater	16.91	10.25	6.66	39.38	68.33	455.10	50.00	9.10
Abalone	10.78	9.74	1.04	9.65	68.33	71.07	50.00	1.42
Turbot	21.63	20.30	1.33	6.15	68.33	90.88	50.00	1.82
NO$_2$								
Seawater	0.54	0.13	0.41	75.93	68.33	28.02	50.00	0.56
Abalone	0.90	0.40	0.50	55.56	68.33	34.17	50.00	0.68
Turbot	2.39	1.32	1.07	44.77	68.33	73.12	50.00	1.46

Table 6.9.: The total N uptake g^{-1} dry weight *Gracilaria* during the course of the experiment at JSP farm.

	N	Seawater (μg atoms- N g^{-1} dwt)	Abalone (μg atoms- N g^{-1} dwt)	Turbot (μg atoms- N g^{-1} dwt)
N uptake	NH$_4$	4.03	4.03	7.86
	NO$_3$	2.05	0.32	0.41
	NO$_2$	0.17	0.21	0.44
	Total N	6.25	4.56	8.71

Table 6.10: Table showing % N taken up from each source during experiment at JSP farm.

N (%)	Seawater	Abalone effluent	Turbot effluent
NH$_4$	64.42	88.42	90.19
NO$_3$	32.86	7.04	4.72
NO$_2$	2.73	4.54	5.09

Table 6.11: The percentage uptake of N, from each available N source, by 50 g dwt *Gracilaria* at JSP farm, and the percentage of each of the three forms of N in this uptake.

Medium	Total N Removed (%)	NH$_4$ (%)	NO$_3$ (%)	NO$_2$ (%)
Seawater	57	46.9	46.9	0.07
Abalone	35.9	71	26.1	2.9
Turbot	33	79.6	0.18	0.03

6.5 Discussion

Gracilaria shows high uptake for dissolved nutrients in seawater (Table 6.1). Tissue N concentration of *Gracilaria* appears to be season dependent in seawater (Table 6.3). During winter months *Gracilaria* tissue contains higher nutrient concentration than during summer months. The seasonal trends in nitrogen content of macroalgae can be associated with growth rate i.e. high growth rate leads to low N content. According to Hanisak (1983) these show the seasonality of inorganic nitrogen availability in seawater as well as the ability of macroalgae to store up nitrogen in excess of its growth need. *Gracilaria* tissue nitrogen and phosphate content also follows a seasonal pattern in wastewater (turbot and abalone). However, abalone wastewater shows no difference in tissue PO$_4$ content between summer and winter. The seasonal difference in tissue N concentration can be attributed to the bleaching of thalli that is a natural and biological process associated with high irradiance levels during summer. The bleaching of thalli was observed in both farms and *Gracilaria* thalli began to lose the dark and reddish colour in November 2001. Bleaching of thalli is common in *Gracilaria* cultivation and it occurs when internal N reserves are exhausted due to high growth rates under limiting N concentrations. As a result, algae begin to change from their dark to reddish colour and become pale to yellow in colour. This can reduce the growth of *Gracilaria* (Ryther *et. al*, 1981). However, in this study *Gracilaria* continued to grow and better RGR and yield was obtained during summer in spite of the bleaching of thalli. The

bleaching of thalli was severe in big seawater culture tanks at I&J farm. Bleaching of thalli was not observed in fertilized seawater tanks but it was observed in abalone wastewater tanks. Bleaching was severe in big seawater tanks probably because of low turnover rates (4 volume exchanges d^{-1}) early in summer. However, bleaching was not highly pronounced in big fertilized seawater and abalone culture tanks probably because these tanks were supplied with nutrients and high turnover rates (12 volume exchanges d^{-1}) respectively. According to Bird *et al.* (1982) the loss of the dark-reddish pigmentation in *Gracilaria* is due to the pigments that are being metabolised as a protein source. There is a significantly different tissue nutrient concentration between seawater and turbot treatment i.e. higher concentration in the latter, and this shows a higher nutritional value in turbot treatment than in seawater treatment. There is a marked total-N concentration difference between abalone and seawater treatments and between abalone and turbot treatments, respectively. Total-N tissue concentration in *Gracilaria* is higher in abalone than in seawater treatment; lower in abalone than in turbot treatment but the difference is not significant. PO_4 tissue concentration is also higher in turbot than in abalone and higher in abalone than in seawater treatment. This study shows that *Gracilaria* growth is not only feasible in wastewater (turbot and abalone) culture units but such systems also yield *Gracilaria* with a higher nutrient, and hence nutritional value. However, there is an inverse correlation between tissue N concentrations of *Gracilaria* and its growth, with high tissue N concentrations obtained during winter when biomass is low and vice versa. In small culture tanks, turbot wastewater has proved to be a suitable culture medium for *Gracilaria* growth i.e. gives higher production (Figure 4.2 and Figure 5.2) and higher tissue N concentration (Table 6.3 and Table 6.4) than the other two treatments. However, this is only based on observations; no statistical significance tests were done.

Nitrate is the most abundant form of inorganic nitrogen in seawater. In spite of the abundance of nitrate in seawater, *Gracilaria gracilis* shows uptake preference for ammonium than for nitrate (Table 6.1). This is in agreement with findings by Haglund and Pedersen (1993) in a study on *Gracilaria tenuistipitata*; D'Elia and De Boer (1978) on *G. tikvahiae*; Thomas *et al.* (1987) on *G. pacifica*; Ryther *et al,* (1981), Bird *et al* (1982) on *G. tikvahiae*; Jones *et al,* (1996) *G. edulis*. *Gracilaria* showed relatively higher nutrient uptake rates in nutrient-limited culture medium (seawater) than in nutrient-enriched seawater (abalone effluent). The high affinity for ammonia exhibited by *Gracilaria* at low N concentrations in this study corresponds with the findings by

Fujita (1985) in his study about the role of nitrogen in regulating transient ammonium uptake and nitrogen storage by microalgae. Turbot wastewater appears to be the most suitable culture medium for *Gracilaria* growth in land-based culture systems (only tested in a pilot-scale experiment) with a higher tissue nutrient concentration (good nutritional value as abalone food) (Table 6.4), higher growth rate (biomass production) (Figure 5.2) and a higher total-N uptake capacity (Table 6.9) .

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Chapter 7

General Discussion

The current study demonstrates that land-based cultivation of *Gracilaria gracilis* is technically and biologically feasible in the south-west and west coast of South Africa, both for bioremediation and production purposes. Ammonium (NH_4) and nitrate (NO_3) are regarded as the most easily assimilated forms of N for most macroalgae species (Hanisak, 1983). Similar findings were obtained in the present study (Table 8.4). However, for total N removal, *Gracilaria* displays higher N uptake in NH_4 and NO_3 than in NO_2 (Table 6.11). *Gracilaria* shows higher affinity for NH_4 than for NO_3 except in the seawater treatment where the percent total N removal for NH_4 and NO_3 is the same (Table 6.11). The concentration of nitrite is very low in seawater while the concentration of nitrate is much higher (Table 6.8). This is consistent with findings by D'Elia and DeBoer (1978), who point out that nitrite is present in smaller concentrations in seawater than nitrate hence nitrate is more easily assimilated than nitrite. According to Norton and Kain (1990) nitrate is the most common form of dissolved inorganic nitrogen found in seawater and its concentration level is significantly higher than that of ammonium and nitrite. This corresponds to the findings in the present study (Table 6.8).

Most of the percentage N taken up per gram dry weight *Gracilaria* was in the form of ammonium i.e. 64.42%, 88.42 and 90.19%, from seawater, abalone water and turbot water, respectively (Table 6.10). About 32.86%, 7.04% and 4.72% was taken in the form of nitrate, from seawater, abalone water and turbot water, respectively. There was very minimal percentage N taken up as nitrite per dry weight of *Gracilaria* i.e. 2.73%, 4.54% and 5.09%, from seawater, abalone water and turbot water, respectively (Table 6.8).

Growing *Gracilaria gracilis* in land based tank culture systems at a commercial scale level is feasible and sustainable as shown at I&J abalone farm. However, the use of large tanks for commercial-scale production would require larger volumes of pumped seawater to reach a flow rate of 20-30 volume exchanges d^{-1}. Alternatively, the nutrients would have to be supplemented by adding fertilizer. Small culture tanks, even though not as easy to manage and maintain as large

tanks, are potentially suitable for a commercial scale production of *Gracilaria*. In order to enhance *Gracilaria* growth, it is recommended that a decision about the size and/ or number of tanks to be used be taken depending on the availability of water. A decision should be taken either to have fewer large tanks or several small tanks, where the desired flow rate (e.g. 20-30 volume exchanges d^{-1}) would determine the exact quantity for each.

The present study has shown that there are successful alternative ways of dealing with the constraints that make cultivation of *Gracilaria gracilis* in land-based culture systems very expensive and unsustainable (e.g. pumping of seawater and addition of fertilizer). For an example, the study showed that at half the desired water flow rate of 20-30 volume exchanges d^{-1} (e.g. 12 volumes d^{-1}), *Gracilaria* can remove 33% of total N from turbot water, 36% total N from abalone water and 57% total N from seawater. Availability of sufficient space is also crucial if the need to use surplus seaweed as supplementary feed for abalone arise. Constant monitoring of *Gracilaria* thalli is essential to ensure that any irregularities that might affect the growth of the material can be picked up early enough and corrective measures taken. This may include daily (morning and afternoon) measurements of abiotic factors (e.g. temperature, light, pH and dissolved oxygen); regular harvesting, cleaning and maintainance of the stocking density; removal of fouling species and the measurement of the water exchange rate. Shading of *Gracilaria* thalli reduced growth. Similar RGR results in *Gracilaria* were obtained between large seawater culture tanks and fertilized seawater culture tanks (e.g. 2.8 % d^{-1}). Generally, shading of *Gracilaria* thalli proved detrimental in that a decline in production was observed in shaded culture tanks, irrespective of the growth media used. Furthermore, there were some shortfalls in the experiment that need to be acknowledged, and these include the unavailability of one of the desired culture media (e.g. abalone effluent) at JSP farm at the beginning of the study. This has led to the lack of continuity in the abalone treatment data. This can be avoided by checking the availability of all the desired treatments or culture media and the suitability of the culture set up to run the experiment for the entire duration of the study. The second challenge in the study was that of the lack of sufficient water to be pumped into the culture tanks for seawater and fertilized seawater treatments at I&J farm. This has resulted in very low volume exchange rate per day (4 volume exchanges d^{-1}) that was only one third of the desired water flow rate (12 volume exchanges d^{-1}).

Looking at the average *Gracilaria* yield per tank per day from turbot effluent at JSP (Figure 5.2) which was about 0.2 kg m^{-2} d^{-1}, and considering the average total N concentration in *Gracilaria* tissue from the same culture medium of about 60 mg N g^{-1} (Figure 6.5), there is about 12 g of N m^{-2} d^{-1} that the turbot water system can remove from the 0.2 kg m^{-2} d^{-1} average yield. Therefore, in the short experiment at JSP (Table 6.11) this represented 33% of the total N in turbot water, 79.6% of which was ammonium. These values will allow the farmer to calculate what area of *Gracilaria* tanks is required to remove a given amount of N from a particular volume of effluent. However, it is important to note that small tanks are more expensive (per m^{-2} of growth area) than large tanks, but larger tanks are less efficient for *Gracilaria* growth (maximum yields of more than 0.5 kg m^{-2} d^{-1} were obtained in small tanks at JSP compared to 0.13 kg m^{-2} d^{-1} in larger tanks at I&J). This study thus provides preliminary data, but the success of commercial-scale aquaculture will depend on optimization of the farm design.

In summary, though, the bioremediatory potential of *Gracilaria gracilis* can yield socio-economic and ecological benefits as the success of land based tank cultivation of *Gracilaria* may increase aquaculture production without worsening dissolved nutrient loading of our coastal waters.

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