The potential for *Gracilaria* polyculture at Jacobs Bay Sea Products Pty.

Derek Morgan

HONS 2000
ICD MORG

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

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

University of Cape Town

BOLUS LIBRARY
C24 0007 7514

Contents

Executive Summary

This report investigates the potential for the culture of *G. gracilis*, an indigenous red algae, into the west coast mariculture farm Jacobs Bay Sea Products Pty.. Various methods for cultivating *Gracilaria* as well as environmental parameters, which influence growth of Gracilaria, are discussed below. From these optimal conditions for the polycultivation of *G. gracilis* are determined.

Cultivation can take place either, in open waters, or on land. Open water systems are generally less intensive than land based operations. Open water systems can be either bottom planted or suspended. Suspended cultivation has the advantage of maintaining the plant thalli in optimal light intensities. Land based systems can make use of ponds, raceways or tanks. Tanks and raceways are more productive than ponds, but require higher flow rates and regulated nutrient regimes.

The optimal environmental conditions for *G. gracilis* cultivation are:

i) Temperature: 15 – 20° C, with a significant mortality at 30° C.

ii) Light: 80 µmol photon m^{-2} s^{-1} and above

iii) Salinity: Natural salinity levels of 33-38 $^{0}/_{00}$

(iv) Stocking Density: 2 kg m^{-2}, with significantly decreased growth rates at 4 kg m^{-2} for tank cultivation and 400g m^{-1} for raft cultivation.

Furthermore a mathematical model is used to determine flow rates and nutrient supply, which are dependent on the method of cultivation.

In a polyculture system algae can function as both a biofilter of effluent water and as a food source for animals such as abalone. Nutrient analysis of the farm showed that nutrient loading on the environment was not significant, thus the primary motivation for growing *Gracilaria* would be for abalone feed. A realistic monthly yield of *Gracilaria* is set at 8 tons, which corresponds to 20% of the present 40 ton Kelp diet for the abalone. A number of cultivation techniques are recommended. If tanks are incorporated into the system, a yield of 8t $month^{-1}$ could be achieved with 1512 tanks of the dimension 0.5m X 1m and 0.45m deep, with a corresponding flow rate of 25 volumes $d^{-1}$of sump water. The existing oysters raceway system is modelled at a production of 2.1 t $month^{-1}$, at a water turnover rate of 6.64 volumes d^{-1} (growing in the effluent water from high density stocked turbot tanks). Using a suspension system of netlon ropes at a stocking density of 400g m^{-1}, the holding dams are modelled at producing 8 t $month^{-1}$ while the settling reservoir is modelled at producing 1.2 t $month^{-1}$ *G. gracilis*. However the growth parameter *k* (the amount of growth per nutrient consumed) is estimated, thus the yields for the various systems need to be considered with some caution. It is recommended that pilot scale experiments are initiated until precise growth parameters can be calculated.

1] Introduction:

Gracilaria is cultivated worldwide, primarily as a part of a multi-million dollar phycocolloid industry. The principal extract form *Gracilaria*, agar, is one of the most valuable of all natural colloids obtained from seaweeds and *Gracilaria* supplies approximately half of the international agar demand (Kain 1995). In the late 20th century it became evident that the natural stocks of the majority of exploited seaweeds were all but exhausted in endeavouring to sustain increasing international demands. Consequently research begun to focus on algal mariculture as a viable alternative to providing not only the demand for agarophytes such as *Gracilaria*, but also for algae cultivated specifically for food and fodder, such as *Porphyra*, *Laminaria* and *Eucheuma* (Oliveira and Alveal 1990).

There is a recent trend in mariculture away from monoculture towards integrated polyculture. (Ajisaka and Chiang 1993, Shpigel *et al.* 1993, Neori and Shpigel 1999). In these inventive farm designs, macroalgae are no longer cultivated as isolated crops, but serve as functional components within the farming system. Seaweeds are typically used as biofilters for effluent water, removing excess dissolved organic nitrogen (DIN) and phosphates while simultaneously oxygenating the cultivation medium (Neori *et al.* 1996, Wildman 1999). This process of improving water quality has the sometimes-crucial benefit of extended water residence times with a resulting overall

augmented productivity. Moreover biofiltering lessens the negative impact of nutrient loading on the external environment, a factor that may increasingly become economically significant as companies are forced to carry their external polluting costs.

In South Africa, cultivation of macroalgae, particularly *Gracilaria gracilis* (Stackhouse) Steenhoft, Irvine *et* Farnham, has gradually been shifting not only from the conceptual to the practical but also towards polycultural methodology. Initially, in 1991, suspension raft cultivation experiments were instigated in Saldanha Bay to evaluate both the feasibility of cultivating *G. gracilis* as well as the key environmental and biological variables limiting productivity (Anderson *et al.* 1996). Although this study demonstrated the viability of cultivating *G. gracilis* using a raft culture system in South African waters, to date no commercial structures have been successfully implemented. There has however been some rapid advancement along the south coast, most notably at Marine Growers (Pty.) Ltd. in Port Elizabeth. Here *G. gracilis* is cultivated in tanks alongside abalone (*Haliotis* spp.), providing the abalone with a valuable source of nutrition, while simultaneously functioning as a biofilter for effluent water (see Hampson 1998 for details).

This multifaceted approach is increasingly being implemented in mariculture operations around South Africa. However systems are unique to their

appropriate environments and in order to optimise production, a comprehensive understanding of the immediate interacting physical and biological variables is essential.

The principle aim of this report is to investigate the prospect of incorporating *G. gracilis* cultivation in an existing abalone (*Haliotis midae*) and turbot (*Scopthalmus maximus*) culture system, at the west coast mariculture farm, Jacobs Bay Sea Products Pty. There are two reasons for integrating *Gracilaria* cultivation into Jacobs Bay Sea Products Pty.. Firstly recent analysis by Simpson and Cook (1997) indicated that growth rates of the South African species of abalone (*H. midae*) were significantly higher when cultivated with mixed algal diets compared to single species diets. *G. gracilis* could thus serve to supplement the current, predominantly kelp (*Ecklonia maxima*) based diet of the abalone, at Jacobs Bay Sea Products Pty.. Secondly, and possibly more crucially, toxic algal blooms (red, brown and black tides) are fairly commonplace along the west coast of South Africa (Pitcher 1998). The threat of shellfish poisoning caused by these blooms is considerably reduced with increased water residence times, particularly if temporary autonomy from an external seawater source is possible.

The general taxonomy of *Gracilaria* and various techniques relevant to *Gracilaria* cultivation as well as biological, environmental and physical factors controlling growth are discussed below. A mathematical model predicting the

algal yields at different nutrient and flow rate regimes is investigated for application in a polyculture system. And in conclusion a range of optimal practical conditions are recommended for a viable integrated cultivation system at Jacobs Bay Sea Products.

2] General Overview

2.1] Taxonomy of *Gracilaria gracilis*

Since the early1980's there have been over 80 publications dealing specifically with the problematic taxonomic state of the gracilarioids. At present however the group, in particular the genus *Gracilaria,* still remains largely unresolved (see Bird 1995 for review). This is evident with regards to the South Africa Gracilariaceae species, originally classified as *Gracilaria confervoides* (L.) Grev. (Fox and Stephens 1943, cited by Anderson *et al.* 1989), then subsequently as *Gracilaria verrucosa* (Hudson) Papenf. and more recently as *G. gracilis* (Stackhouse) Steenhoft (Steenhoft *et al.* 1995). The South African *Gracilaria* from Saldanha Bay has been shown to be conspecifc with the European *G. gracilis* by molecular methods (Iyer, *pers. comm.*) Added to this, Fredericq and Hommersand (1989) have reinstated the genus *Gracilaropsis,* which in the past has been misidentified as *G. verrucosa.* The reason for this general uncertainty is partly due to standard generic diagnostic features in the Gracilariaceae, reportedly being mixed in single species (Oliveira and Plastino 1994,

Philippines to Canada (Kain and Destombe 1995). In southern Africa *G. gracilis* extends from Lüderitz (Namibia) to Port Elizabeth and possibly Port Alfred (Smit 1998) along the south coast. Anderson *et al.* (1993) note that the habit of the *G. gracilis* can be free-floating, attached to substrates or anchored to the seafloor by sand. The plants are usually found shallower than 10m. The gracilarioids in general rarely grow in areas of extreme wave action but can be found in partly turbulent waters where they develop attached to shells, loose stones or sedentary animals such as the ascidian *Pyura stolonifera* (Critchley 1993).

2.3] Life History

Typically *Gracilaria* has a three-phase Polysiphonia type life history, with an alteration of isomorphic macroscopic gametophytic and tetrasporophytic generations coupled with a microscopic diploid carposporophytic generation (Fig. 1) (Rueness *et al.* 1987, Critchley 1993, Kain and Destombe 1995).

During fertilization, carpogonia are retained on the thallus of the haploid female gametophytes while the male plants freely release large amounts spermatia into the surrounding water column. The diploid zygote produced from the unison of two gametes remains embedded in the female gametophytic thallus, ultimately developing into a parasitic carposporophyte. The mature carposporophytes or cystocarps are visible to the naked eye as

Fredericq and Hommersand 1989, Bird 1995), as well as evidence of both phenotypic and ecotypic variation at the species level (Oliveira and Plastino 1994). Compounding this, the type of *Gracilaria* (nom. cons.) has been conserved although the original type specimen is *Gracilariopsis* (Chapman *et al.* 1977, Hampson 1998).

G. gracilis, the common South African west coast species, is a member of the genus *Gracilaria*, family Gracilariaceae, order Gracilariales, in the class Florideophyceae (Stegenga *et al.* 1994). Santelices and Doty (1989) describe the general morphology of *Gracilaria* as an often dark coloured, red macroalga, with numerous terete axes resulting in a "knotted, string-like appearance". More specifically though, Stegenga *et al.* (1994) recognize *G. gracilis* in accordance with the reproductive structures. The latter authors confirm *G. gracilis* as having spermatangia occurring in conceptacles and carposporophytes connected with the roof of the cystocarp via tubular nutritive cells.

2.2] Biogeography and Ecology

The gracilarioids in general have a pantropical distribution, with no more than a few species established in the more temperate waters (McLachlan and Bird 1984, Oliveira and Plastino 1994). Of these, *G. gracilis* (as *G. verrucosa*) has been recorded from various locations around the world ranging from the

dark spots rooted on the thallus tissue and can be utilised to differentiate female from male plants (Kain and Destombe 1995). Mitotic division of the zygote within the cystocarp results in the formation of carpospores which are released into the environment. The carpospores attach to a substratum and develop into diploid tetrasporophytes, which are morphologically identical to the dioecious gametophytic plants. Meiotic sporogenesis within the cortical sporangia of the tetrasporangial plants result in the formation of haploid tetraspores. These spores are released into the environment and develop into haploid gametophytes (Kain and Destombe 1995).

Gracilaria is however not confined to sexual reproduction. Critchley (1993) states that attached populations of *Gracilaria* frequently contain fertile individuals, while free-floating populations are generally completely sterile and reproduce vegetatively. Likewise Rueness *et al.* (1987) have shown that there are very few documented instances of sexual reproduction in *G. verrucosa*, and of these none refer to free floating populations. This has valuable implications for cultivation, in that it is often more practical and cost effective to avoid the intricacies of life history simulation and propagate plants through fragmentation.

3] **Cultivation Methods**

As mentioned, *Gracilaria* is typically propagated vegetatively (McLachlan and Bird 1986, Santelices and Doty 1989, Critchley 1993) so that cultivation techniques do not require growing different life history phases. However there are many different practical ways of farming *Gracilaria* (Dawes 1995, Friedlander and Levy 1995, Buschmann *et al.* 1995). Cultivation can take place either in the open ocean or on land, in the latter case it can either be grown in highly regulated environments such as tanks and raceway systems or in less intensive systems such as ponds.

3.1] Open Water Systems

Open water cultivation is usually done in the calm waters of bays and estuaries. There are two basic approaches in growing *Gracilaria*: (i) bottom planting, where the plants are anchored to the sea floor and (ii) suspension, where the thalli are buoyed at varying depths in the water column.

3.1 a] Bottom planting

In the natural environment *Gracilaria* can either settle on a mixture of substrates, ranging from small shells to rocks to blocks of drift wood, or can be anchored in sand (Fig 2). Bottom planting techniques utilise this trait to fix

plants to a farming area (Fig 3). Vegetative thalli already attached to substrate particles can be transplanted into areas where growth is needed (Buschman *et al.* 1995). Plants can also be artificially secured to hard substrates using a variety of fasteners such as netting or tape. In sandy sediments the thalli can be pinned to the bottom substrate using planting forks or weights. Alternatively, in areas of low water movement, plants can be inserted directly into soft sandy sediments (Santelices and Doty 1989). One of the more productive methods is securing plants using tubular polyethylene bags filled with sand. The plants can either be physically tied to the bags or simply held in place by the bag load on the plant. In time the plants become attached to seafloor and the bags disintegrate (Santelices and Ugarte 1987). In China this technique has proved highly profitable with annual yields of 21t dry wt. ha^{-1} y^{-1} (Martinez *et al.* 1990).

An alternative bottom planting technique is to seed small substrate particles with spores (either tetra- or carpospores) (Santelices and Doty 1989, Smith 1989). Following seedling establishment, the seeded material can be replanted and cultivated in the desired area (see below; 3.1c] Spore *versus* Fragmentation Inoculation).

A particular problem associated with bottom planting techniques is that the plants remain at a fixed depth in the water column, which means that light intensity fluctuates with tidal oscillations. Other problems include the risk of

mortality when plants are transported from one environment to another and that thalli may become dislodged from their substrates, which in turbulent waters may cause a significant decrease in yield (Buschman *et al.* 1995).

3.1 b] Suspension

Suspended cultivation can either involve seeding from spores or attachment through vegetative propagation. But generally most commercial systems use vegetative propagation techniques (Dawes 1995). If vegetative material is used, the plants can be attached to ropes either by attaching the plants using any of a variety of ties, or by weaving the plant material through the rope fibres (Fig 4). In Lüderitz (Dawes 1995) and Saldanha Bay (Anderson *et al.* 1996) the use of netlon, a nylon netrope, has proved highly effective as a means of securing thalli. During stocking, the netlon is hung on wire hooks projecting from a "stocking board". The mesh of the net is relaxed while the net is attached to the stocking board, allowing efficient threading of the plant material. When the stocked netlon is suspended in the water column, the tension resulting from the weight of the plant material secures the plants to the netlon material (Oliveira and Alveal 1990, Dawes 1995).

Once the rope (or netlon) is stocked it can be either suspended between two stakes, anchored to the seafloor, or supported by buoys on a raft system (Fig 5). The advantage of a floating raft structure is that the thalli remain at a

consistent depth irrespective of tidal fluctuations, so maximizing light intensity. Anderson *et al.* (1996), in a pilot study on raft cultivation in Saldanha bay, have shown that average annual growth rates of 5% d^{-1} are possible with a projected commercial yield of 26t dry wt. ha^{-1} y^{-1}.

Open water systems as a whole have both the benefit and drawback of being exposed to the natural environment. Temperature, salinity and pH are may be fairly constant, depending on the farm location. Seawater is also in constant motion around the raft, distributing fresh nutrients and disposing of waste products. These factors together with the typically unsophisticated design of the farm structures make open water farming less capital intensive than most land based systems (Dawes 1995). However farming in the sea has the disadvantages associated with an erratic unpredictable natural environment, e.g. Anderson *et al.* (1996) have shown that one of the most important variables associated with raft cultivation in Saldanha Bay is the nutrient stratification in the water column, which occurs during summer months. In addition rafts are restricted to areas where wave action and tidal currents are minimal. In the high wave energy waters of the west coast of South Africa this is particularly relevant as only Saldanha Bay and St Helena Bay are adequately protected to be suitable areas for raft cultivation systems.

3.1c] Spore *versus* Fragmentation Inoculation

Spore and fragmentation inoculation can be implemented in algal cultivation systems (Fig 6), with varying degrees of success. The two primary advantages of spore inoculation are that it is less labour intensive and that genomic uniformity is possible in that plants can be either all haploid or diploid. The disadvantages are more diverse and include: the need for a fertile specimen, the cost of seeding facilities, slow initial production during grow out phase and increased fouling susceptibility during of the longer grow out period (Oliveira and Alveal 1990). These authors however also state that although fragmentation is the preferred method of cultivation, further experimentation in spore inoculation may result in significantly improved *Gracilaria* cultivation techniques.

3.2] Pond Cultivation

Ponds are typically large (0.5 to 2 ha), shallow (less than 1m) uncovered concrete or earthen structures (Critchley 1993). The plants are usually free floating and evenly distributed over the bottom surface of ponds. Once a sufficient biomass has been reached, the plants are removed from the ponds using either rakes or nets. Typically the only inputs in pond cultivation are water pumping and nutrient addition. However, intensive pond farming

involves the addition of aeration and water movement, which enhances yield but can drastically increase capital investment.

One of the problems associated with large pond cultivation is that of temperature extremes. However the effects of temperature as well as light intensity can be buffered by adjusting the water depth of the pond (Critchley 1993). When the air temperature is below 10°C the water depth can be as low as 20-30 cm, but in summer should on average be kept at 60-70 cm. Other problems associated with pond cultivation include the effects of prevailing winds and epiphyte fouling. Prevailing winds can cause the plant thalli to congregate downwind, which in turn causes self-shading. In areas of consistent prevailing winds, either windbreaks need to be erected or the thallus movement needs to be restricted through attachment or restraining nets.

3.3] Tank Cultivation

Gracilaria tank cultivation is a land based intensive operation where free floating plant material is grown in relatively small tanks (from litres to cubic meters) of concrete or industrial material. To ensure intensive production it is of primary importance to keep the plant material in motion, in order to ensure that plants receive sufficient light and nutrient supply. Water motion has the added benefit of decreasing boundary layer effect, which improves metabolite

transfer in and out of the plant. Movement can be supplied either through paddle wheels (Mathieson 1982) or through aeration pipes placed to circulate the water (Fig 7). In these systems environmental and biological variables that influence growth are regulated so as to optimise productivity (see Friedlander and Levy 1995, and Oliveira *et al.* in press, for review). Central to maintaining intensive yields in tanks, is an elevated nutrient supply, particularly nitrogen, phosphorus and carbon. Temperature, light and epiphyte control are additional factors which need to be regulated and will be discussed in more detail in the following section.

Raceways have also proved to be potentially successful systems for *Gracilaria* cultivation (Lapointe *et al.* 1976, Ryther *et al.* 1978). Pilot scale models of this approach have purportedly resulted in high productivity values (2-4 kg (wet). $m^{-2}\,d^{-1}$) (Hampson 1998) but are restricted by high capital investment necessary to construct the raceways. In intensive cultivation systems the costs are on the whole much higher than in other less intensive systems, but these costs can be offset by greater returns. The most substantial cost in tank cultivation is the pumping needed for high water exchange rates. Oliveira *et al.* (in press) state that the expense of pumping water can be so great that intensive monocultivation of *Gracilaria* can generally be considered economically unviable. These authors also recognize however, that when *Gracilaria* is grown in association with other organisms, economic viability

improves, as the cost of pumping water is disseminated over all the organisms.

4] Key Environmental and Biological Parameters

4.1] Temperature

Temperature is not a variable that is easily manipulated in cultivation systems. However an understanding of its effects on growth of *Gracilaria* can be useful in predicting seasonal productivity fluctuations.

Friedlander (1991) and Haglund and Pedersen (1993) recognized a significant positive correlation between temperature and growth rate of both *Gracilaria conferta* and *Gracilaria tenuistipitata* in a range of 15 - 30° C. Likewise Engledow and Bolton (1992) demonstrated that, testing within the range 5 - 25° C, *G. gracilis* (as *G. verrucosa*) had optimal growth at the higher temperatures of 15-25° C and minimal growth between 5-10° C. However they also noted significant mortality at a higher temperature of 30° C.

4.2] Light

Light is seldom a limiting factor in suspended or land based algal cultivation techniques. Friedlander *et al.* (1991) illustrated that for tank cultures of *G. conferta*, light saturation was reached at 5% of total surface irradiance of 1100 µmol photon m^{-2} s^{-1} during summer and 75% of full irradiance of 1600 µmol photon m^{-2} s^{-1} during winter. Likewise Engledow and Bolton (1992) demonstrated that maximum growth rates for *G. gracilis* (as *G. verrucosa*) were reached at and above 80 µmol photon m^{-2} s^{-1}. Friedlander and Ben-Amotz (1991) stated that by filtering out ultra violet radiation (UV), growth rates for *G. conferta* can be increased by as much as 70%. This is due to detrimental effects of UV irradiation on photosynthetic pigments so that photosynthetic capacity of the plants is decreased (Lüning 1981). However Friedlander and Ben-Amotz (1991) also state that a decrease in UV irradiation can significantly increase epiphyte biomass.

4.3] Salinity

Natural seawater salinity levels of around 30 - 40 $^{0}/_{00}$ are optimal for *Gracilaria* cultivation. Optimal salinity for *Gracilaria tikvahiae* has been demonstrated to be in the region of 24 - 36 $^{0}/_{00}$ (Hanisak 1987), while for *G. conferta* a salinity of 30 $^{0}/_{00}$ was recorded as optimal. For *G. gracilis*, Engledow and Bolton (1992) demonstrated maximum growth rates at natural salinity

levels (33-38 $^{0}/_{00}$) and showed a 40 - 50 % decrease in growth at higher and lower levels of 16 and 48 $^{0}/_{00}$. Anderson (unpublished), in considering effective epiphyte control mechanisms, has also demonstrated the capacity of *G. gracilis* to withstand short periods of freshwater treatment.

4.4] Water movement

Efficient water circulation has two important consequences: (i) Nutrients and waste products are evenly distributed in the culture medium and (ii) the boundary layer effect is diminished, thus improving uptake and excretion rates (Friedlander and Levy 1995). Effective rates of circulation can be regulated using flow-through systems, or alternatively, continuous circulation patterns can be set up in cultivation tanks. However because of the costs involved in water exchange systems, high water flow rates are generally only used in tank cultivation systems. Critchley (1993) states that a minimum water exchange rate of 6 - 15 days in pond cultivation is necessary in order to maintain a culture medium suitable for *Gracilaria* growth.

Photosynthetic rates for *Gracilaria* cultivation have been shown to increase by as much as 40 - 50 % with an increase in water velocity form 0 to 8 cm s^{-1} (Gonen *et al.* 1993). However Hanisak and Ryther (1984) and Friedlander and Ben-Amotz (1991) have found that no seawater exchange produces an increase in pH due to the depletion of ambient carbon supplies, and

consequently results in the decrease of *Gracilaria* growth rates. Kremer (1981) noted that at high pH levels (± 9) free CO_2 becomes unavailable to algae, thus limiting photosynthesis. They also noted that by controlling for pH (by adding HCl) but not adding inorganic carbon, the growth rate remains minimal. As a consequence, in cultures with limited water flow exchange rates, it is necessary to control for pH as well as supplement inorganic carbon supplies.

Continuous water circulation can be achieved using aeration systems or paddle wheels. The advantage of these systems is that the plant material is kept in motion, thus evenly exposing all parts to nutrients, CO_2, and light. The rotation also results in abrasion of plant material with the tank walls and with other plant material, thus limiting epiphyte colonisation. However aeration can be expensive, particularly for large cultivation operations. Aeration can also increase oxygen concentrations, resulting in increased oxidative metabolism (Friedlander and Levy 1995). To neutralize this it is possible to use CO_2 for aeration, but this in turn may increase running costs.

4.5] Nutrient Supply

Of all the environmental variables manipulated in algal cultivations nutrient concentrations are often the most decisive in optimising growth rates. There is a range of macro- and micronutrients as well as various trace elements

necessary for algal growth (see De Boer 1981). Most of these are present in concentrations adequate for intensive cultivation, except nitrogen, phosphorous and carbon, which can often be limiting, resulting in decreased growth rates and ultimately productivity (Oliveira *et al.* in press).

Dissolved inorganic nitrogen (DIN) is available to algae in three forms; ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-). Of these, NO_3^- is the most abundant in seawater followed by NH_4^+, while NO_2^- is generally present in very dilute concentrations (De Boer 1981). In Saldanha Bay maximum concentrations of these values have been recorded at 20, 5, 0.6 μM respectively (Smit 1998). Seaweeds differ in their capacities for utilizing different sources of nitrogen. Most reports observe a distinct preference of gracilarioids for NH_4^+ (De Boer 1978, D'elia and de Boer 1978, Ryther et al. 1981), and Smit (1998) has shown that *G. gracilis* is more efficient at absorbing NH_4^+ at low ambient nitrogen concentrations. If NO_3^- and NH_4^+ are present in concentrations of less than 6μM then *G. gracilis* has a preference for NH_4^+ because of the presence of biphasic kinetics, with a strong diffusive component for NH_4^+. This may because NH_4^+ can be assimilated both passively and actively. However because of a rate-saturated uptake mechanism (Michaelis-Menten kinetics) for NO_3^-, *G. gracilis* is able to store NO_3^- when nitrogen levels are in excess, and as a result the efficiency of NO_3^- uptake exceeds that of NH_4^+ at nitrogen concentrations higher than 6μM (Smit 1998).

Lapointe and Ryther (1979) noted that a useful indicator of nitrogen deprivation is thallus colour. Under sufficient nitrogen levels (C:N ratio of less than 10), adequate pigmentation results in a dark brown/red colour in *Gracilaria.* However when nitrogen becomes limiting, a decrease in pigmentation results and the thalli become straw coloured.

Phosphorus is naturally available to marine algae almost exclusively in the form of orthophosphate ions, primarily HPO_4^{2-} (De Boer 1981). Orthophosphates are generally present is seawater at concentrations of 1 - 3 μM, which can easily limit growth rates at high seaweed densities (Guist *et al.* 1982). Friedlander and Levy (1993) also demonstrated that under at a range of seawater N:P ratios (2.5 to 20), high phosphate concentrations may inhibit growth of *G. conferta.* Although no data is available on the effect of phosphate concentrations on the growth of *G. gracilis,* a sensible approach to cultivation may be to follow Friedlander and Levy's (1995) recommendation of maintaining N:P ratios below 10:1 and possibly above 2:1.

G. gracilis is also able to accumulate excess orthophosphates through a rate-saturated uptake mechanism, and in most species of *Gracilaria* (Hanisak 1987). Furthermore *Gracilaria,* unlike most other algae, has the ability to absorb nutrients in darkness at similar rates to daytime uptake rates (Hanisak and Ryther 1984). These attributes have been utilized extensively in cultivation

systems around the world as a mechanism for controlling epiphyte growth. Since *Gracilaria* is able to store nitrogen and phosphorous in excess to growth requirements, to optimise growth, fertilizer can be supplied intermittently in excess and preferably at night. This favours *Gracilaria* as epiphytes will become nutrient starved during non-fertilized periods. Smit *et al.* (1996) have shown that *G. gracilis* out-competes epiphytic algae in a pulsed nutrient regime where nitrogen is supplied once a week at night. *G. gracilis* grows at nitrogen non-limited rates for 7 days in a nitrogen-free medium

Carbon (C) is available to seaweeds in the forms of dissolved CO_2, bicarbonate (H_2CO_3) or as carbonate ions (HCO_3^- and CO_3^{2-}). At natural pH values of seawater (7.8 to 8.2) HCO_3^- constitutes 90% of the available dissolved inorganic C (DIC) in seawater (Kremper 1981). Various species of algae including the diverse group of gracilarioids have different affinities for the various forms of DIC. Haglund *et al.* (1992) have demonstrated that *Gracilaria tenuistipitata* can utilize CO_2 directly but needs to convert HCO_3^- to CO_2 through hydration with the aid of extracellular carbonic anhydrase. Friedlander and Levy (1995) conclude that if plants respond to the addition of CO_2 but not HCO_3^-, the presence of extracellular carbonic anhydrase is unlikely. This can be useful in evaluating the affinity of DIC for species such as *G. gracilis* where it is unknown.

Since HCO_3^- (C) supply is pH dependent, pH can be used as a relative index of culture medium C concentrations. Critchley (1993) states that tanks should be managed at a pH range of 8.3 to 9.0. If pH rises above 9 carbon supply can be improved either by increasing the flow rate of water into the cultivation system or through the addition of CO_2 or HCO_3^-. However increasing flow rate is often the more costly option and consequently it may be more practical to provide carbon through CO_2 enriched aeration.

4.6] Stocking density

Stocking density is important in that if plant material is too concentrated plants compete for resources, predominantly light, whereas if densities are too low resources are wasted and systems often run sub-optimally. Optimal stocking densities are easily determined and most studies agree that optimal for tank cultivated *Gracilaria* is in the region of 2 - 5 kg m^{-2} surface area. (Lapointe and Rhyther 1978, McLachlan 1991, Ugarte and Santelices 1992, Friedlander and Levy 1995). Similarly Smit (1995) obtained a maximum growth rate for *G. gracilis* at 2 kg m^{-2}, however he also noted a 50% decrease in growth rates at stocking densities of 4kg m^{-2}. Dawes (1995)and Anderson *et al.* (1996) have recorded growth rates of 2kg m^{-2} 30 d^{-1} with stocking densities of 400g $m^{-1}$of for *G. gracilis* on raft cultivation systems.

4.7] Epiphytes

Epiphytes, by competing for resources, can seriously decrease productivity of cultivated algae (Friedlander and Levy 1995). A range of epiphytes exist that exploit *Gracilaria* cultivation systems, including *Ulva, Enteromorpha, Cladophora* and *Ceramium* (Oliviera *et al.* in press). There are also an equivalent variety of approaches utilized to prevent their colonisation. Epiphytes often bloom when the cultivation environment is more suited to the epiphyte than that of the cultured species, thus an effective approach is to maintain cultivation environments at optimal conditions for the cultured species. As mentioned above, a possible mechanism is to use night pulse feeding. Anderson *et al.* (1998) have also noted the effectiveness of biological controls such as the isopod *Paridotea reticulata* that has a grazing preference for *Ceramium,* the common epiphyte on *G. gracilis.* Another successful method for controlling *Ceramium* on *G. gracilis* is to immerse the plant material in fresh water for 5 - 10 minutes (Anderson, unpublished). *G. gracilis* but not *Ceramium* is able to withstand the brief hypotonic shock.

Most concern with epiphytes is centred on the decline in the productivity of the cultivated species. However, if *Gracilaria* is cultivated for abalone feed, the impact of epiphyte colonization is not as critical, provided the epiphytic species is palatable to the abalone. Simpson and Cook (1998) have demonstrated that abalone prefer mixed seaweed diets. Although abalone

prefer a single species diet of *Gracilaria* to *Ulva*, when these plants are combined with *Ecklonia*, the preference swaps to the *Ulva, Ecklonia* mixture. If epiphytes are observed on the cultured *Gracilaria* it may be prudent to first present them to the abalone, if they are not eaten then effective controlling mechanisms can be initiated.

5] **Modelled Nutrient Regimes**

A variety of simulation models have been designed to aid in the understanding of mariculture systems (see Ellner *et al.* (1996) for review). The function of these models are threefold: (i) They can aid in scientific understanding of processes occurring in a system, (ii) They can explore the effects of altering parameters in a system design and (iii) They can be utilized in forecasting and optimising yield. Most of these models have ~~being~~ been determined with reference to a specific culture system. However Gold (1977) has designed a relatively simple universal aquaculture model that predicts optimal yields for cultured aquatic microorganisms. In this report Gold's (1977) model has been adapted to *G. gracilis* tank cultivation with the aim of evaluating optimal yields under alternative nutrient and flow rate regimes.

The model, using Michaelis-Menten kinetic parameters, predicts the optimal flow rate and yield of biomass for a range of concentrations of a specific

nutrient. It makes four important assumptions: (i) All other parameters are non-limiting, (ii) the rate of mixing in the cultivation tank is sufficient to evenly distribute the nutrient considered, (iii) growth of an organism is directly related to the amount of the limiting nutrient and (iv) at low nutrient concentrations, the nutrient is absorbed as rapidly as possible, while at high concentrations the rate becomes saturated. The model is specifically designed for the large-scale production of microorganisms. The yield is calculated from the concentration of organisms in the effluent water and is a function of the rate of pumping and growth rate. Furthermore, the growth rate depends on the concentration of organisms in the growth chamber. In order to adapt to *Gracilaria* cultivation, harvesting is assumed to be continuous, so as to optimise growth rates.

The variables included in the model with the respective units of measure have been listed in Table 1.

The model takes the form of two equations. The first predicts maximum yield,

$$Y = kF\frac{k\mu_{max}VgSr - F(K + Sr)}{k\mu_{max}Vg - F}$$

while the second predicts optimal flow rate at Y (maximum yield).

$$F = k\mu_{max} Vg\left(1 - \left(\frac{K}{K + Sr}\right)^{1/2}\right)$$

Since the Michaelis-Menten kinetic parameters of *G. gracilis* for NH_4^+-N and NO_3^--N but not PO_4^{-3} have been determined (Smit 1998), this model cannot be applied to a phosphate concentration regime. However as mentioned above, the concentration of phosphate would be optimal provided the N:P ratios are maintained between 2 and 10. Furthermore Carr *et al.* (1997) state that Michaelis-Menten formulation cannot credibly be utilized for carbon concentrations, as under high concentrations, photosynthesis and thus carbon-saturation occurs more rapidly than predicted by Michaelis-Menten kinetics.

One of the key variables operating in this model is (*k*) the amount of growth per nutrient consumed. For netlon raft cultivation, in order to maintain a net growth of 928g over a 30-day period, *G. gracilis* assimilated 2.9g of nitrogen (or 320g plant tissue. g^{-1} nitrogen, hereafter referred to as 320g g^{-1}), which coincides with a thallus nitrogen concentration of 3.1% (Smit 1998). However Smit (1998) comments that this concentration is unusually high and may be due to the indirect (theoretical) method he used to estimate uptake rates. Wilson (1999) has indicated that these values may indeed be exaggerated and using isotope analysis, calculated %N content in *G. gracilis* to be approximately 1.27% in both fertilized and non-fertilized experiments. For

this report it was therefore assumed, given that Smit's (1998) %N concentration of 3.1% corresponds to a growth of 320g g^{-1}, a more conservative but realistic value of *k* would be 130 g g^{-1}.

Figures 8 and 9 illustrate the hyperbolic relationship between maximum yield and flow rate for 5 µmol NH_4^+-N and 20 µmol NO_3^--N regimes respectively. These concentrations were used as they represent the maximum recorded values of nitrogen in Saldanha Bay (see above) and thus serve as an intermediate between unfertilised and fertilized culture mediums. It was shown that yields increase with an increasing flow rate up until a maximum of 4.2 % (23 volumes d^{-1}) for NH_4^+-N (Fig. 8) and 5.9% (7.3 volume d^{-1}) for NO_3^--N (Fig. 9). The decrease is at higher flow rates are most probably because at these flow rates the nutrients concentrations become limiting, to sustain high yields.

The relationship between nutrient concentrations and optimal yields and associated flow rates are shown in Figures 10 and 11. Theoretically, provided nutrient concentration and flow rate are adequate and that all other variables are kept optimal, growth can continue to increase unabated. The model predicts that for NH_4^+-N, growth rates can be as high as 20 % d^{-1} provided the flow rate is kept at 30 volumes d^{-1}. In reality however flow rates of 30 volumes d^{-1} are rarely maintained due to high costs associated with the pumping of seawater. The model also appears to collapse at very high nutrient

concentrations. Figure 11 shows that for NO_3^--N concentrations of 100µmol an optimal growth rate of 60% d^{-1} is predicted. It is very unlikely that such extreme growth rates are possible even in intensive culture systems, as other micronutrients and trace elements as well variables such as light and aeration may be limiting and will become difficult to manage.

Figures 12 and 13 confirm the linear relationship between nutrient concentration and optimal yield under constant flow rates. Fig 12 shows that with by increasing flow rate a higher optimal yield can be achieved at the same NH_4^+-N concentrations. However the model predicts that, in order to achieve optimal production, high levels of NO_3^--N are ineffectual without a correspondingly high flow rate (Fig. 13). This does not imply that there will be no yield at these NO_3^--N concentrations, it merely suggests that in order to attain optimal yields an increase in flow rates is needed. Because NH_4^+-N nutrition does not show this limitation by flow rate, the optimal flow rate where no additional nutrient supplies are needed can be determined exclusively from NO_3^--N flow rates. If ambient NO_3^--N are at 20 µM, a maximum flow rate of approximately 8 volumes d^{-1} signifies optimal yields in a system where no additional nitrogen is needed (Fig. 14). Furthermore if a flow rate of 8 volumes d^{-1} is applied to the model, the relationship between nutrient concentration and maximum yield can be determined for both NO_3^--N and NH_4^+-N (Fig. 15). From this it is possible to determine maximum yields

from a system where the culture medium nitrogen concentrations are known (see below in application, 6.2] Existing structures).

This model successfully demonstrates the relationship between nutrient regimes, flow rates and yields and provides insight into the diverse aspects of nitrogen assimilation for cultivated *G. gracilis*. However in terms of accuracy it is limited by the available data on growth parameters for *G. gracilis*. Primarily a realistic value for *k* (growth per nutrient) is required, but Smit (1998) also notes that the nitrogen uptake rates of *G. gracilis* are influenced by environmental factors, specifically temperature. Thus different Michaelis-Menten constants apply in different environments. The predicted enhanced yield with NH_4^+-N nutrition (Fig. 15) does however give credibility to the model in that it correlates with Smit's (1998) conclusions on nutrient preferences in *G. gracilis* under low concentrations of NO_3^--N and NH_4^+-N (see above). Nevertheless until more exact data on the growth parameters of *G. gracilis* for specific environments is determined, it would be inappropriate to apply this model in a predictive or managerial capacity.

6] **Recommended cultivation techniques for Jacobs Bay Sea Products Pty**

Jacobs Bay is located along the west coast of South Africa approximately 120km north of Cape Town. The farm, Jacobs Bay Sea Products Pty., is a land based intensive mariculture operation, situated on the point of Jacobs Bay.

The farm has an abalone stock of approximately 2.7 million abalone (±76.8 tons), which range in size from recruits to 6 year old animals. 40 tons of kelp are used each month as abalone feed, which eat approximately 5-7% body weight per day.

= 1.3 t/day of feed.

are other feeds supplied?

But 76.8 t of abalone needs 76.8 × 0.05 /day = 3.8 t?

Figure 17 is a schematic depicting the layout of Jacobs Bay Sea products Pty.. There are four primary components to the farm: the settling and holding dams (a, b and c), the abalone tanks (d), the turbot tanks (e) and the inactive oyster runways (f). Seawater is pumped directly into the top settling reservoir (a) and from there it is gravity fed to either the turbot tanks (e) or the bottom holding dams (b) and (c). Water can also be pumped directly into the holding dams and is consequently heated by solar radiation. Water turnover rate for the top reservoir is 5.6 volumes d^{-1} and 4.5 volumes d^{-1} for the bottom two dams. From these dams water is pumped into the mixing tank (g) where it can be distributed to the abalone tanks, turbot tanks, oyster raceways or returned back to the dams. Effluent water is channelled to the sump (h) where particulates are settled out, and the remaining water is returned to the sea.

Table 2 lists the relative nutrient, temperature, pH and flow rates at various points on the farm recorded during spring (14 September 2000). It is evident that temperature and pH are relatively uniform throughout the farm. Comparisons between total inflow (reservoir) and outflow (sump) give very

little indication of significant levels of nutrient loading. HPO_4^{2-} and particularly NH_4^+ concentrations were slightly higher at outflow, which is noteworthy to cultivation on account of the preference *G. gracilis* has for NH_4^+-N at low nitrogen concentrations (see above). The overall consistency of nutrients levels may be a result of kelp absorbing the nutrients, as live kelp material is fed to the abalone and at any one moment there is more than one ton of kelp present in the system.

O_2 concentrations appear to decline consistently throughout the system (table 2). O_2 is naturally liberated from seawater at a constant rate, dependent on the relative temperatures and/or salinities present in the medium (De Boer *et al.* 1981). Thus the less oxygenated the water becomes the longer the residence time in a cultivation system . O_2 is also lost through oxidative respiration. This may explain the low O_2 values in the sump, where abundant bacterial respiration takes place (Table 2).

The concerns of effluent nutrient loading into the external environment seem unfounded at this stage, because of the minimal distinction between inflow and outflow nutrient concentrations. However in the event that water is required to recirculate through the farm, low O_2 levels may become detrimental to abalone and turbot production. By incorporating *Gracilaria* cultivation into the system, the effluent water could be used as a culture medium for the algae, with the benefit of increased O_2 concentrations.

Two approaches can be considered in incorporating *Gracilaria* production into the farm design at Jacobs Bay Sea Products Pty. Firstly new structures can be introduced into the farm, designed specifically for *Gracilaria* cultivation, or alternatively existing structures can be customized or altered to best suit *Gracilaria* cultivation requirements.

6.1] Additional Structures

Introducing structures specifically for *Gracilaria* cultivation will require a significant capital investment. Thus it is preferable that the system is designed in such a way to optimise productivity. Simpson and Cook (1998) applied a ratio of 80% kelp 20% secondary species in their mixed rotation diet experiments for abalone. If the kelp consumption at Jacobs Bay Sea Products Pty. is at 40 t $month^{-1}$ (wet weight), a realistic optimal productivity goal for *Gracilaria* would be 8 t $month^{-1}$ (wet weight).

Productivity in tanks can range from 25 to 127 t ha^{-1} yr^{-1} (dry weight) (Capo *et al.* 1999). To achieve rates of 127 t ha^{-1} yr^{-1}, relatively small tanks of 55L with high water exchange rates (20 -30 volumes d^{-1}) were used. Nutrient enrichment was minimal, in the region of 10 -100µM nitrogen and 10µM phosphorous and a stocking density of 2-3 kg m^{-2} with a corresponding weekly harvest rate of 35 g $m^{-2}d^{-1}$, was employed (Lapointe and Ryther 1979,

Ryther *et al.* 1979, Critchley 1993) By converting these figures to a production goal of 8 t month^{-1}(wet weight), a total cultivation surface area of 756 m^{-2} is needed. This translates to 1512 tanks of the dimension 0.5 X 1m. For optimal production a flow rate of 234 L h^{-1} per tank or a total of 345 375 L h^{-1} is required if the depth of each tank is set at 0.45 m and an exchange rate of 25 volumes d^{-1} is assumed.

Exchange rates of water in this magnitude can be costly, however there is the resultant advantage that few additional imports (e.g. nutrients) are then required. Theoretically there should be no need for carbon addition, however careful supervision of the pH is recommended. Carbon supply in the form CO_2 can be automated using a pH meter switch, which activates whenever the pH exceeds a set threshold (see Critchley 1993). Likewise relatively little additional nutrients, in the form of nitrogen and phosphorous would be needed to optimise growth. Given the high flow rates it may be difficult to maintain consistent nutrient concentrations. The simplest solution may be to use slow release fertilizers placed in the tanks. However the released nutrient concentration relative to the flow rate will need to be determined.

6.2] Existing Structures

The inactive oyster raceways are a viable option for algal cultivation as the necessary structures for intensive cultivation are already in place. There are 16

raceways with dimensions of 1m X 50m X 50cm. This translates to a total surface area of 800 m^2, which provides enough surface area for intensive cultivation (see above). The flow rates of the raceways can be regulated, thus given the farm nutrient concentrations, optimal yields can be predicted using Gold's (1977) model. Because NO_3^--N and not NH_4^+-N nutrition is limiting at low concentrations, the healthier culture medium will be the one with higher NO_3^--N concentrations. Table 2 shows that the high turbot stocking density effluent water has the highest NO_3^--N concentrations. If these concentrations are integrated into the model, a NO_3^--N concentration of 15.875 will yield an optimal yield of 4.2% d^{-1} at a turn over rate of 6.64 volumes d^{-1}. The model assumes a stocking density of 2kg m^{-2}, thus a 4.2% d^{-1} growth rate translates to a yield of 2.1 tons per month. This in turn translates to 5.2% of the total abalone feed.

The holding dams and reservoir too provide adequate surface area. These structures are however more in line with non-intensive pond cultivation systems. There is a range of reported productivities from *Gracilaria* pond cultivation systems from around the world. In China and Taiwan the most productive non-intensive systems are ponds of around 1 ha in size, with an average depth between 60-70 cm. In these systems the temperature ranges from 15 to 30 °C and salinity from 10 to 20 ppt (Santelices and Doty 1989). A water exchange rate of 2-3 days is recommended but it can be as long as 6-15 days. Using this technique average annual yields of 1.33t dry wt. ha^{-1} have

been recorded (Friedlander and Levy 1995) but have reached a maximum of 40t dry wt. ha^{-1} (Shang 1979). Critchley (1993) proposes a rough sustainable guideline of harvesting a third to a half of the crop every 30–35 days during summer and every 45 days during winter.

Cultivation dams are designed seldom at depths more than 1m. This is in part because plant material is left free floating and may become light limited at greater depths. Anderson *et al.* (1996) indicated that optimal *G. gracilis* production is achieved as close to the sea surface as possible. If the settling dams at Jacobs Bay Sea Products Pty. are to be considered for cultivation, ideally plant material should to be kept in suspension, as holding dams are m deep and the settling reservoir 2m deep. Productivities in the region of 2kg m^{-2} 30 d^{-1} have been reported for raft systems, at a stocking density 400g m^{-1}, with plant material of 0.75m apart (Dawes 1995 and Anderson *et al.* 1996). This can be applied to a pond system if the material is suspended across the breadth of the reservoir. If these figures are applied to the two settling dams at Jacobs Bay yields, with a spacing of 0.5m between each individual netlon, yields of 8t $month^{-1}$ per dam and 1.2t $month^{-1}$ for the reservoir, can be expected.

These yields may be inflated however, due to the growth rates being estimated from open ocean raft systems, where the exchange rate of water is much higher. Likewise these yields cannot be expected for both dams as

water is pumped into holding dam (b) first and then directly into (c). Thus nutrients will become limited in holding dam (c). Smit *et al.*(1996) have demonstrated a 5% d^{-1} growth rate with a weekly pulse of 1200µM NH_4^+-N. This corresponds to a 67.2kg nitrogen addition for each of the holding dams per week. However it is unlikely that *G. Gracilis* will be able to assimilate all the nitrogen immediately, and these high level of nitrogen may prove toxic to the abalone and turbot. A more cautious approach may be to add nutrients at a more consistent but lower concentration. Unlikely that they would want to add N to holding dams!

C concentration can be monitored through pH analysis, and in the event of it becoming limited it can be added using HCO_3^- or possibly CO_2 aeration. However it is unlikely that *G. gracilis* will assimilate all the nutrient supply at once and thus the water may be toxic to the abalone due to the high levels of nitrogen, thus a more uniform nutrient supply may be appropriate.

A final possibility for *G. gracilis* polycultivation is to cultivate algae and abalone in the same tank. The advantage of this system this is that the tank infrastructure is already in place, thus creating a possible intensive algal operation without the additional capital investment. The tanks at Jacobs Bay are at present maintained at a flow rate of 1000L h^{-1}. The dimensions for each tank are 1.35 X 5m and 0.45 m in depth. This corresponds to a flow rate of 7.9 volumes per day, which can be incorporated into the model to predict optimal yields under different nutrient regimes (Fig 16). Table2 shows that NO_3^--N

concentration at the outflow to the high stoking density abalone tanks is 15.46 μM. From Fig. 16 a optimal yield of 3.94 % d^{-1} can thus be expected. Which corresponds to a yield 8.2 tons per month, of at a stocking density of 2kg m^{-2}.

To separate the algae from the abalone, a net containing the plant material can suspended at the surface of the tank, slightly away form the tank walls. However the tank depths may not be adequate to accommodate both algae and abalone particularly for the larger more mature abalone. More importantly though, at present all the abalone tanks are covered by a roofing structure, which will eliminated most light and excludes any possibility of cultivating algae.

In conclusion a sensible approach to initiating *G. gracilis* cultivation in the Jacobs Bay Sea Products environment would be to institute a variety of pilot scale studies. A number of suspension lines could be strung in the holding dams to evaluate growth rates as well as determine optimal stocking densities, in terms of plant material and distances between cultivation ropes. Likewise initial experiments in the oyster runways could be used to established to determine the possibility intensive yields. If these suggest economical viability, they could be scaled up to full-scale production. Possible future plans for expansion could also consider either building abalone tanks without roofing or building with some form of transparent roofing material.

Acknowledgements

I would like to thank Jacobs Bay Sea Products Pty., particularly Kevin Ruck, for his assistance and amenability. I hope algal polycultivation becomes a reality.

I would also like to thank Rob Anderson and John Bolton for their help and supervision as well as Chris Boothroyd, Dylan Robertson and Adam West for their assistance in data collection.

Finally I would like to thank Heidi Hawkins for her intellectual input and revision of this project.

Reference list

Ajisaka T. and Chiang Y.M. 1993 Recent status of *Gracilaria* cultivation in Taiwan. *Hydrobiologia*. 260/261: 335-338

Anderson R.J. Levitt G.J. and A. Share. 1996. Experimental investigations for the mariculture of Gracilaria in Saldanha Bay, South Africa. *J. Appl. Phycol.* 8: 421-430

Anderson R.J., Levitt C.J., Keats D.W., and R. H. Simons. 1993. The role of herbivores in the collapse of the *Gracilaria* resource at Saldanha Bay, South Africa. *Hydrobiologia* 260/261: 285-290

Anderson R.J., Simons R.H., and N.G. Jarman. 1989 Commercial seaweeds in southern Africa: a review of utilization and research. *S Afr J Mar Sci. 8: 277-299.*

Bird C. J. 1995. A review of recent taxonomic concepts and developments in the Gracilariaceae (Rhodophyta). *J. Appl. Phycol.* 7: 225-267.

Buschman A. H.,Westermeyer R., and C. A. Retamales. 1995. Cultivation of *Gracilaria* on the sea-bottom in southern Chile: a review. *J. Appl. Phycol.* 7: 291-301.

Capo T. R., Jaramillo J. C., Boyd A. E., Lapointe B. E., and J.E. Serafy 1999. Sustained high yields of Gracilaria (Rhodophyta) grown in intensive large-scale culture. *J. Appl. Phycol.* 11: 143-147

Carr G. M., Duthie H. C., and W.D. Taylor 1997. Models of aquatic plant productivity: a review of the factors that influence growth. *Aqua. Bot.* 59: 195-215

Chapmann A., Edelstein T. and P.J. Power. 1997. Studies on Gracilaria. I. Morphological and anatomical variation in samples from the lower gulf of St. Lawrence and New England. *Bot. Mar.* 20: 149-153

Critchley, A. 1993. *Gracilaria* (Rhodophyta, Gracilariales): An economically important agarophyte. **In:** *Seaweed cultivation and marine ranching* (Ohno, M. and A. Critchley, Eds). Nagai: Kanagawa Int. Fisheries Training Center and JICA.

D'elia C.F. and J.A. De Boer 1978. Nutritional studies of two red algae. II Kinetics of ammonium and nitrate uptake. *J Phycol.* 14: 266-272

Dawes, C. P. 1995 Suspended cultivation of *Gracilaria* in the sea. *J. Appl. Phycol.* 7: 303-313

De Boer J.A. 1981. Nutrients In. Lobban C.S. and M.J. Wynne *The biology of seaweeds. Biological monographs volume 17.* Blackwell Scientific Publishing London p. 356-392

De Boer J.A. 1978. Effects of nitrogen enrichment on growth rate and phycocolloid content in *Gracilaria foliifera* and Neoargardiella bailleyi (Florideophyceae) *Proceedings of the nineth international seaweed sympodium.* 263-271

Ellner S., Neori A., Krom M. D., Tsai KuenHi, Easterling M. R. and K.H. Tsai 1996. Simulation model of recirculating mariculture with seaweed biofilter: development and experimental tests of the model. *Aquaculture.* 143: 167-184

Engledow H.R., and J.J. Bolton 1992. Environmental tolerances in culture and agar content of *Gracilaria verrucosa* (Hudson) Papenfus (Rhodophyta, Gigartinales) from Saldanha Bay. S *Afr J Bot* 58(4): 263-267

Fredericq, S. and M. Hommersand. 1989. Proposal of the Gracilariales ord. nov. (Rhodophyta) based on an analysis of the reproductive development *of Gracilaria verrucosa. J. Phycol..*25: 213-227.

Friedlander, M. and I Levy. 1995. Cultivation of *Gracilaria* in outdoor tanks and ponds. *J. Appl. Phycol.* 7: 315-324

Friedlander M. (1991). Growth rate, epiphyte biomass and agar yield of *Gracilaria conferta* in an annual outdoor experiment. I. Irradiance and nitrogen. *Bioresource Technol.* 38: 203-208

Friedlander M., and A. Ben-Amotz 1991 The effect of outdoor culture conditions on growth and epiphytes of *Gracilaria conferta. Aqua. Bot.* 39 (3-4): 315-333

Friedlander M., Krom M.D., and A. Ben-Amotz 1991. The effect of light and ammonium on growth, epiphytes and chemical constituents of *Gracilaria conferta* in outdoor cultures. *Bot. Mar.* 34 (3): 161-166

Friedlander M., Galai N. and H. Farbstein1990. A model of seaweed growth in an outdoor culture in Israel. *Hydrobiologia* 204/205: 367-373.

Gold H.J. 1977. *Mathematical modeling of biological systems. An introductory Guidebook.* Wiley-interscience publication. Toronto.

Gonen Y., Kimmel E., and M. Friedlander 1993. Effect of relative water motion on photosynthetic rate of red alga Gracilaria conferta. *Hydrobiologia* 260/261: 493-498

Guist G.G.,Jr., Dawes C.J., and J.R. Castle J.R. 1982 Mariculture of the red seaweed, *Hypnea musciformis*. *Aquaculture*. 28 (3-4): 375-384

Haglund K. and M. Pedersen 1993. Outdoor pond cultivation of the subtropical marine red alga Gracilaria tenuistipitata in brackish water in Sweden. Growth, nutrient uptake, co-cultivation with rainbow trout and epiphyte control. *J Appl Phyc* 5(3): 271-284

Haglund K., Bjoerk M., Ramazanov Z., Garcia-Reina G. and M. Pedersen 1992 Role of carbonic anhydrase in photosynthesis and inorganic-carbon assimilation in the red alga *Gracilaria tenuistipitata*. *Planta*. 187: 275-281

Hampson 1998. Evaluation of effluent water from an abalone mariculture system as culture medium for two strains of Gracilaria gracilis. MSc thesis, University of Port Elizabeth. 148pp

Hanisak M.D. 1987 Cultivation of Gracilaria and other macroalgae in Florida for energy production. **In.** Bird K.T. *Seaweed cultivation for renewable resources*. Amsterdam, Elsevier Science Publishers. p. 191-218.

Hanisak M.D. and J. H. Ryther 1984. Cultivation biology of *Gracilaria tikvahiae* in the USA. *Hydrobiologia*. 116/117: 295-298.

Kain J. M. 1995. Introduction. *J. Appl. Phycol*. 7: 229-230.

Kain J. M. and C. Destombe. 1995. A review of the life history, reproduction and phenology of *Gracilaria*. *J. Appl. Phycol*. 7: 269-281

Kremper B.P. 1981. Carbon Metabolism. **In.** Lobban C.S. and M.J. Wynne *The biology of seaweeds. Biological monographs volume 17.* Blackwell Scientific Publishing London p. 493-533.

Lapointe B.E. and J.H. Ryther 1979. The effects of nitrogen and seawater flow rate on the growth and biochemical composition of *Gracilaria foliifera var. angustissima* in mass outdoor cultures. *Bot. Mar*. 22(8): 529-537

Lapointe B. E., Williams L.D., Goldman M.E. and J. H. Ryther. 1976. The mass outdoor culture of macroscopic marine alga. *Aquaculture* 8: 9-21.

Lüning K. 1981. Light: Algae, ocean environment, photosynthesis. *Bot. Mono*. 17: 326-355

Martinez, C., Barrales H., and M. Molina. 1990 Economic assessment of a successful method for mass field cultivation of *Gracilaria* in Chile. *Aquaculture* 84: 101-116

Mathieson A. 1982. Seaweed cultivation: A review. **In**: *Proceedings of the sixth U.S.-Japan Meeting on aquaculture*, pp. 25-66. (Sinderman, C.J., Ed.), Santa Barbara, U. S. Dep. Commer.

McLachlan J. and C. J. Bird. Geographical and experimental assessment of the distribution of *Gracilaria* species (Rhodophyta: Gigartinales) in relation to temperature. *Helgo. Meer.* 38: 319-334.

Neori A. and M. Shpigel. 1999. Using algae to treat effluents and feed invertebrates in sustainable integrated mariculture. *World Aqua.* 30(2): 46-51

Neori A., Krom M.D., Ellner S.P., Boyd C.E., Popper D., Rabinovitch R., Davison P.J., Dvir O., Zuber D., Ucko M., Angel D., and H. Gordin 1996 Seaweed biofilters as regulators of water quality in integrated fish-seaweed culture units. *Aquaculture.* 141(3-4): 183-199

Oliveira E.C., Alveal K. and R.J. Anderson.(in press). Mariculture of the Agar-producing gracilarioid red algae.

Oliveira, E. C. and E. M. Plastino. 1994. Gracilariaceae, pp. 185-226. **In**: *Biology of economic algae* (Akatsuka, I., Ed.). The Hague: Academic Publishing (1994).

Oliveira, E. C. and K. Alveal. 1990. The mariculture of *Gracilaria* (Rhodophyta) for the production of agar, pp. 553-564. **In**: *Introduction to applied phycology* (Akatsuka, I., Ed.). The Hague: Academic Publishing.

Pitcher G.C., Boyd A.J., Horstman D.A. and B.A. Mitchell-Innes. 1998. Subsurface dinoflagellate populations, frontal blooms and the formation of red tide in the southern Benguela upwelling system. *Mar. Eco. Prog. Ser.* 172: 253-264

Rueness J., Mathisen H. A. and T. Tananger. 1987. Cultue and field observations on *Gracilaria verrucosa* (Huds.) Papenf. (Rhodohyta) from Norway. *Bot. Mar.* 30: 267-276.

Ryther J.H. Corwin N. DeBusk T.A. and L.D. Williams 1981 Nitrogen uptake and storage by the red alga *Gracilaria tikvahiae. Aquaculture.* 26(1): 107-115

Ryther J.H., Williams L.D., Hanisak M.D., Stenberg R.W., and T.A. DeBusk 1979 Biomass production by marine and freshwater plants [Red seaweed *Gracilaria tikvahiae*, *Lemna minor*, *Eichhornia crassipes*, and *Hydrilla verticillata*] *Conference proceedings: The National Biomass Program.* Colorado School of Mines, Golden, Colo.,. Washington, D.C. (USA): U.S. Government Printing Office.

Ryther, J. H., De Boer J.A. and B. E. Lapointe. 1978. Cultivation of seaweeds for hydrocolloids, waste treatment and biomass for energy conversion, pp. 1-16. **In**: *Proceedings of the ninth International Seaweed Symposium* (Jensen, A. and J. R. Stein, Eds.). Santa Barbara, Science Press

Santelices B. and M. S. Doty. 1989. A review of *Gracilaria* farming. *Aquaculture*, 78: 98-133.

Santelices B. and R. Ugarte. 1987. Production of Chilean *Gracilaria*: problems and perspectives. *Hydrobiologia* 151/152: 295-299.

Shang, Y. C. Economic aspect of *Gracilaria* culture in Taiwan. *Aquacultue* 8: 1-7 (1976)

Shpigel M., Neori A., Popper D. M. and H.Gordin. 1993. A proposed model for "environmentally clean" land-based culture of fish, bivalves and seaweeds. *Aquaculture*. 117: 115-128

Simpson B.J.A., and P.A. Cook. 1997 Rotation diets: A method of improving growth of cultured abalone using natural algal diets. *J Shell Res.* 17: 635-640

Smit A.J.1998. Nitrogen environment, ecophysiology and growth of *Gracilaria gracilis* in Saldanha Bay South Africa. Ph.D. thesis. University of Cape Town.

Smit A.J. 1995. Optimising Gracilaria verrucosa production in a laboratory based culture system. M. Sc. Thesis. University of Port Elizabeth.

Smit A.J., B.L. Robertson and D.R. Du Preez, D.R. 1996 Influence of ammonium-N pulse concentrations and frequency, tank condition and nitrogen starvation on growth rate and biochemical composition of *Gracilaria gracilis*. *J. Appl. Phycol.* 8: 473-481

Steentoft M., Irvine L.M. and W.F. Farnham 1995. Two terete species of Gracilaria and Gracilariopsis (Gracilariales, Rhodophyta) in Britain.. *Phycologia.* 34: 113-127

Stegenga H., Bolton J.J. and R.J. Anderson. 1997. Seaweeds of the South African west coast. Contributions from the Bolus Herbarium. University of Cape Town.

Wildman J.C.,Jr. 1999. Reflections on biofilter selection for shellfish culture. J Shell Res. 18: 279.

Willson D.T. 1999. Some Aspects of the nitrogen nutrition and growth of Gracilaria gracilis grown by suspended cultivation in Saldanha Bay, South Africa. Honours Thesis. University of Cape Town.

Table 1: Parameters used in algal growth simulation model (Gold 1977) with respective symbols and units of measure.

Parameter	Symbol	Unit
Nutrient concentration in inflow to cultivation tank	$S_r(t)$	$g\ L^{-1}$
Volume of cultivation tank	V_g	L
Flow rate	F	$L\ h^{-1}$
Yield of cultured species	$Y(t)$	$g\ h^{-1}$
Maximum rate of nutrient consumption per unit biomass cultured species	μ_{max}	$g\ g^{-1}\ h^{-1}$
Nutrient concentration of inflow to cultivation tank, needed for half maximum growth	K	$g\ L^{-1}$
Amount of growth per nutrient consumed	k	$g\ g^{-1}$

Table 2: Flow rates, pH, temperature and nutrient concentrations from various locations of Jacobs Bay Sea Products Pty. recorded on 14 September 2000. High Abalone stocking density recorded at 0.42 kg L^{-1}, medium at 0.21 kg L^{-1} and low at 0.005 kg L^{-1}. High turbot density recorded at 0.01 kg L^{-1} and low 0.005 kg L^{-1}. X denotes no data available.

		pH	Temp (°C)	Flow rate (L h^{-1})	O_2 (mM)	NO_3^- (µM)	NO_2^- (µM)	NH_4^+ (µM)	HPO_4^{2-} (µM)
Reservoir		7.56	12.1	750 000	8.83	17.415	0.33	1.495	1.38
Dams		x	x	280 000	X	x	x	x	x
Abalone high stocking density	In	7.95	11.8	1000	8.78	16.79	0.34	1.945	1.42
	Out	7.62	12.3	1000	6.45	15.465	1.28	6.81	5.4
Abalone medium stocking density	In	7.7	12.2	1000	5.56	17.075	0.33	1.925	1.38
	Out	7.47	11.8	1000	5.95	12.68	0.45	3.11	3.34
Abalone low stocking density	In	6.84	12.6	1000	5.73	17.48	0.38	3.18	1.685
	Out	7.29	11.8	1000	5.82	13.89	0.38	2.27	1.995
Turbot high stocking density	Out	7.87	12	8000	7.79	15.875	0.4	7.225	1.66
Turbot low stocking density	Out	7.7	12.2	8000	9.15	7.75	0.38	5.175	1.445
Sump	In	7.84	12	x	5.23	11.1	0.51	3.455	2.98

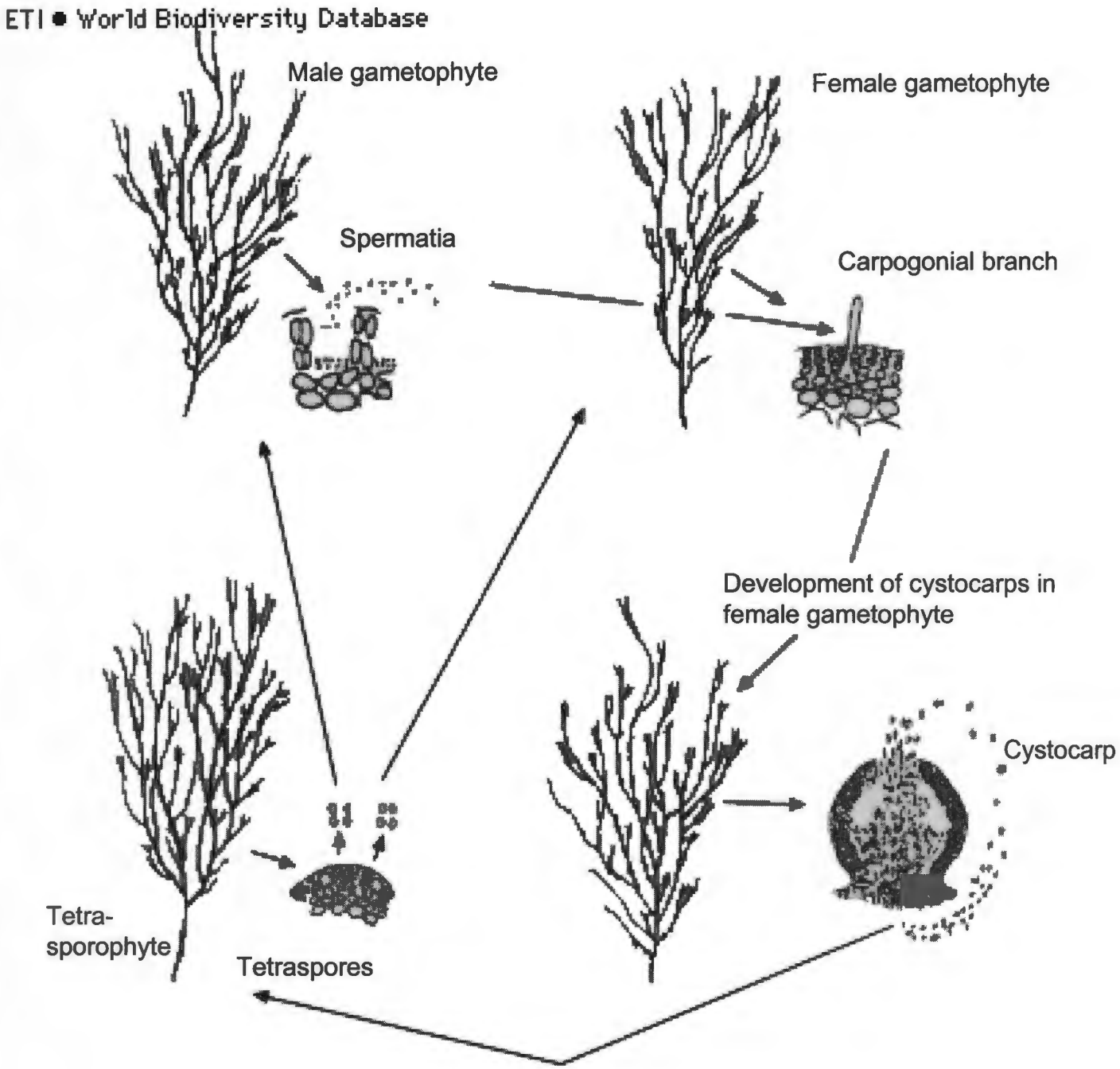

Figure 1: A typical three-phase Polysiphonia type life history of *Gracilaria*, with an alteration of isomorphic macroscopic gametophytic and tetrasporophytic generations coupled with a microscopic diploid carposporophytic generation
Source: ETI Biodiversity Database (after Santelices and Doty, 1989)

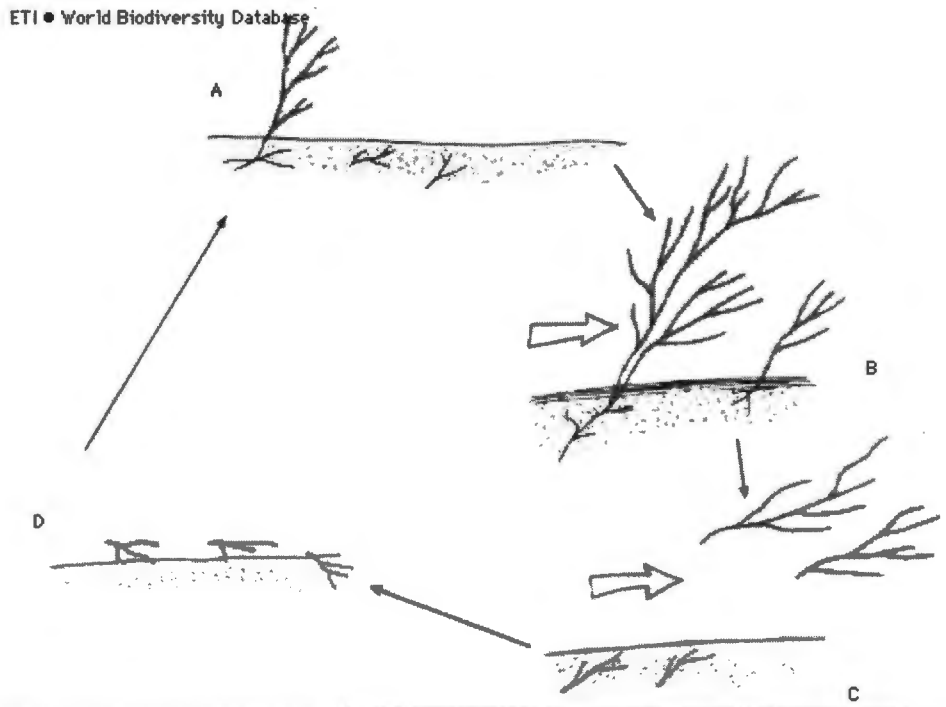

Figure 2: The mechanisms of *Gracilaria* thallus attachment to sandy substrates
A&B: Fragments of the plant are buried in sediment, which anchors the emergent thalli
C: Plant size increases and so does the drag on the thallus. Plants are torn free during storms and heavy swells
D: Fragments of thallus become embedded in the substratum
Source: ETI Biodiversity Database (after Santelices and Doty, 1989)

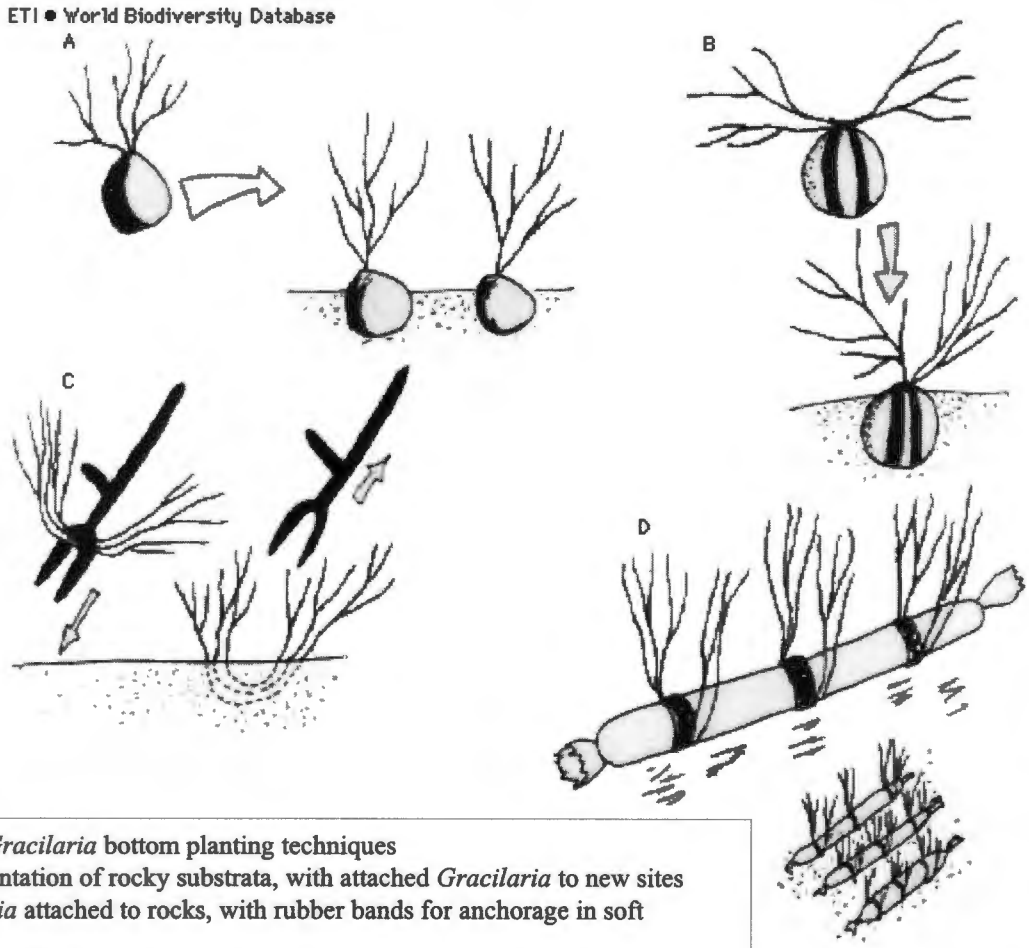

Figure 3: *Gracilaria* bottom planting techniques
A: Transplantation of rocky substrata, with attached *Gracilaria* to new sites
B: *Gracilaria* attached to rocks, with rubber bands for anchorage in soft sediments
C: Insertion of *Gracilaria* into soft sediments using a holding fork
D: *Gracilaria* attached to sand-filled plastic tubes
Source: ETI Biodiversity Database (After: Smith *et al.* 1984, Santelices and Doty 1989, Oliveira and Alveal 1990)

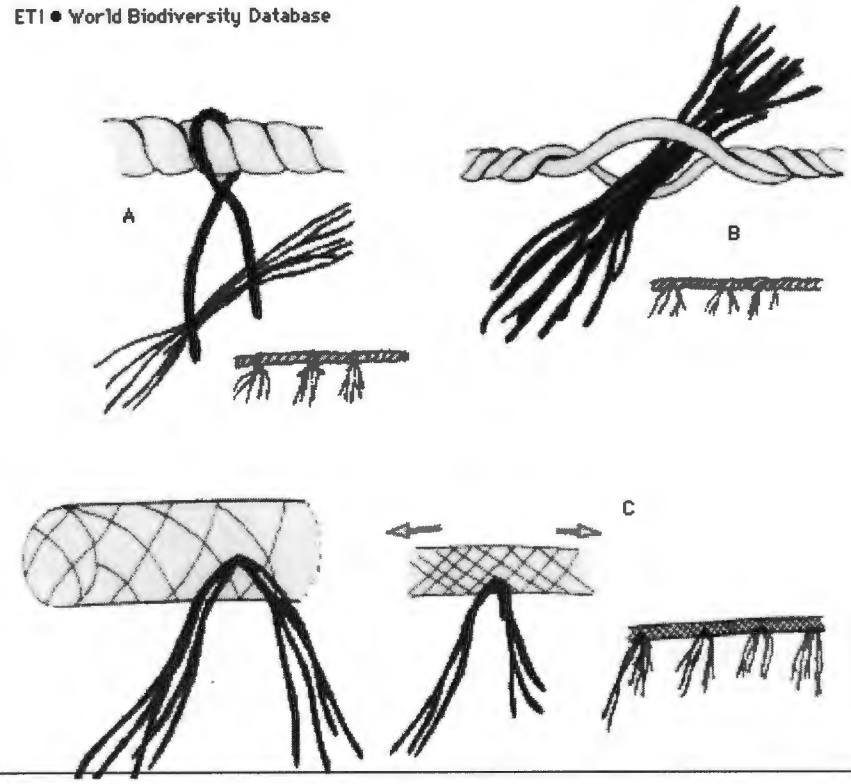

Figure 4: Attachment of *Gracilaria* to ropes for suspended cultivation
A: Plastic tie downs are looped around the main rope and bundles of *Gracilaria* are tied onto the line
B: The lay of the rope is opened and plants inserted into the line
C: Netlon: *Gracilaria* is inserted through the mesh and held in position as the netlon is suspended under tension
Source: ETI Biodiversity Database (After: Critchley, 1993)

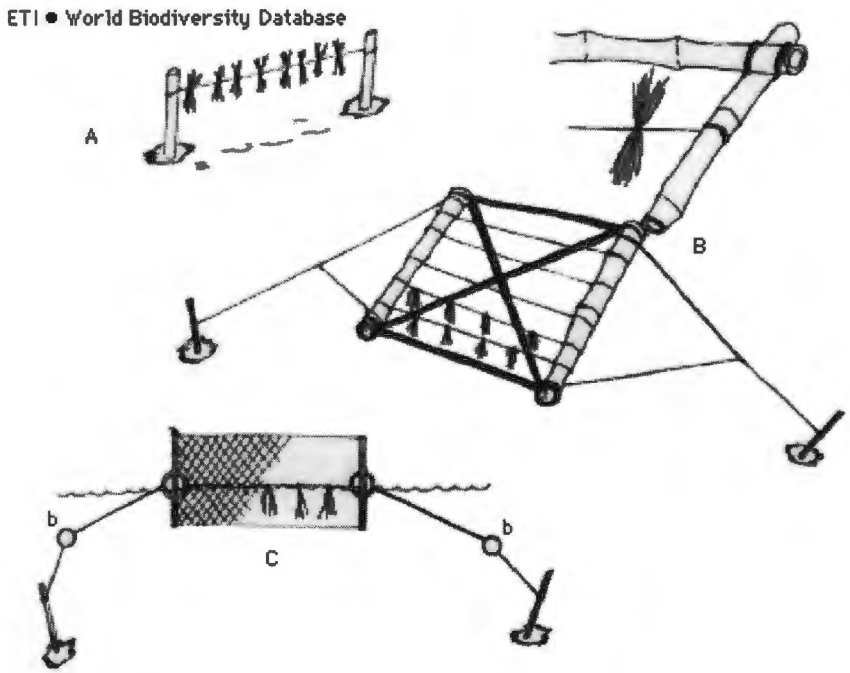

Figure 5: Suspended Gracilaria cultivation techniques
A: Bottom-attached poles
B: Floating-frame construction from bamboo
C: Floating frames may need to be enclosed in plastic-mesh to prevent fish grazing. Buoys (d) may be attached to the anchorage lines.
Source: ETI Biodiversity Database (After Smith, 1989)

ETI • World Biodiversity Database

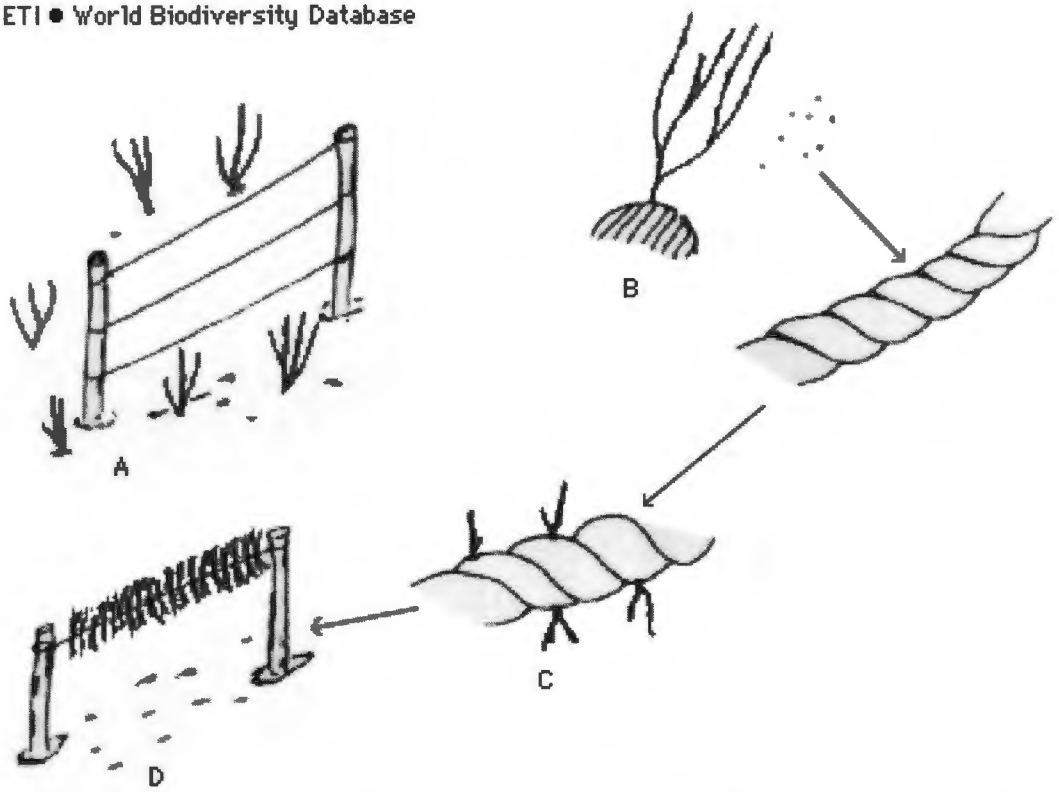

Figure 6: Seed propagation for suspended *Gracilaria* cultivation.
A: Ropes for seeding are placed out in natural populations of *Gracilaria*.
B: Spores (tetra- or carpospores) are released from the fertile plants and settle onto the seed-ropes.
C: Juvenile plants grow attached to the seedrope, which can be transferred to new growing areas, D.
Source: ETI Biodiversity Database (After Smith 1989)

ETI • World Biodiversity Database

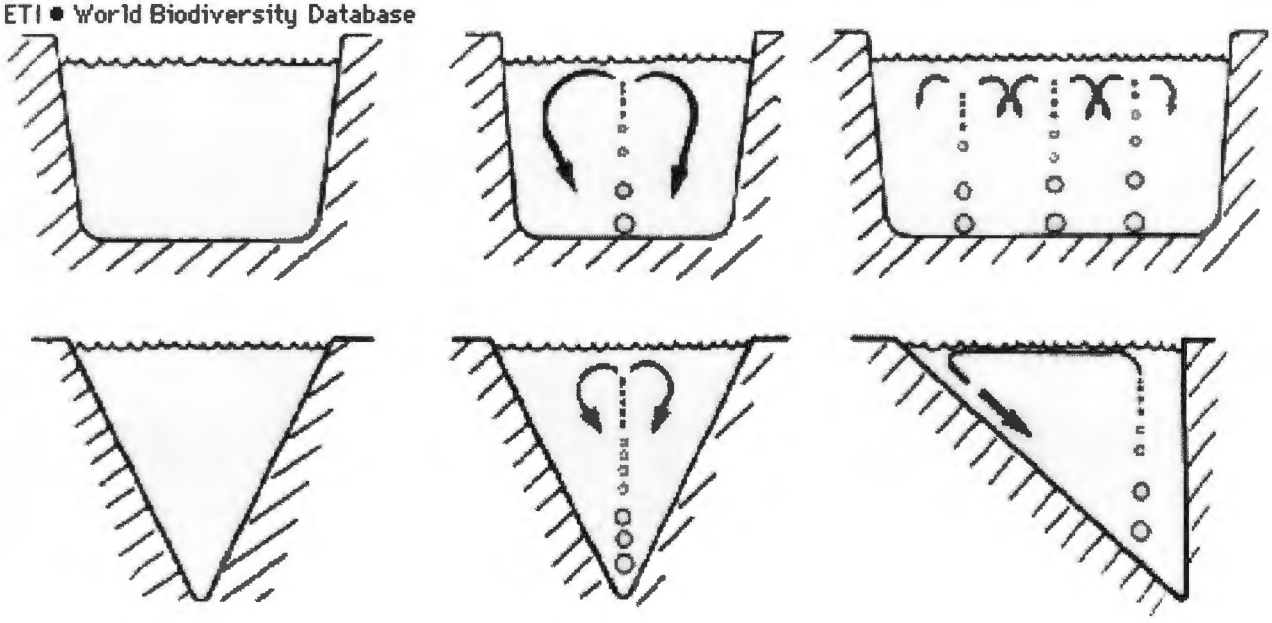

Figure 7: Cross section of various tank designs used in *Gracilaria* cultivation. The arrows depict the circulation pattern created by either paddle wheels or aeration apparatus.
Source: ETI Biodiversity Database (after Critchley, 1993)

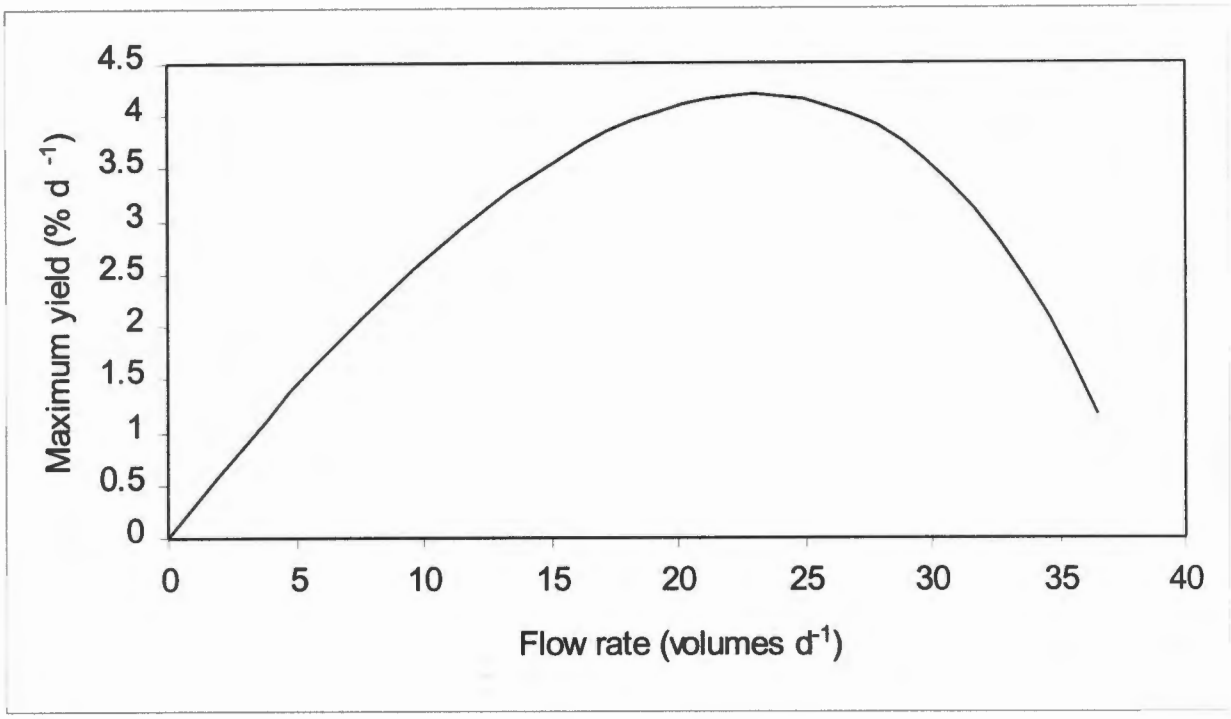

Figure 8: The hyperbolic relationship between maximum yield and flow rate of *G. gracilis*, modelled under a NH_4^+-N nutrient regime of 5µM and k = 130g g^{-1}.

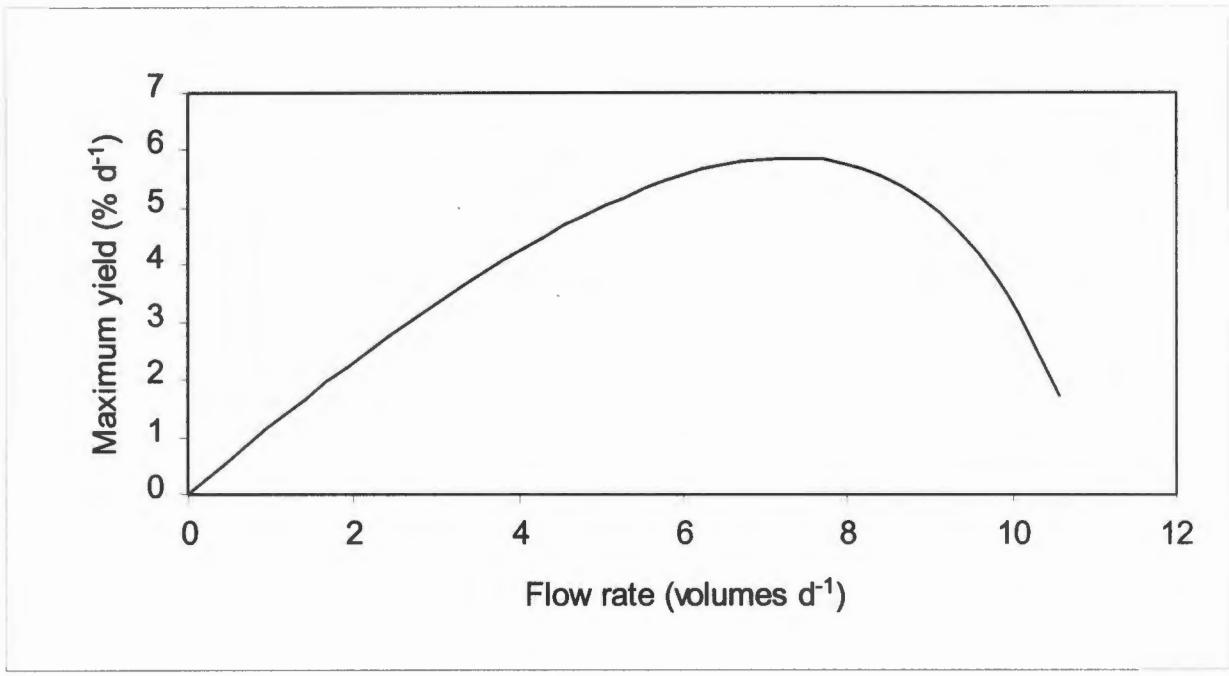

Figure 9: The hyperbolic relationship between maximum yield and flow rate of *G. gracilis*, modelled under a NO_3^--N nutrient regime of 20µM and k = 130g g^{-1}

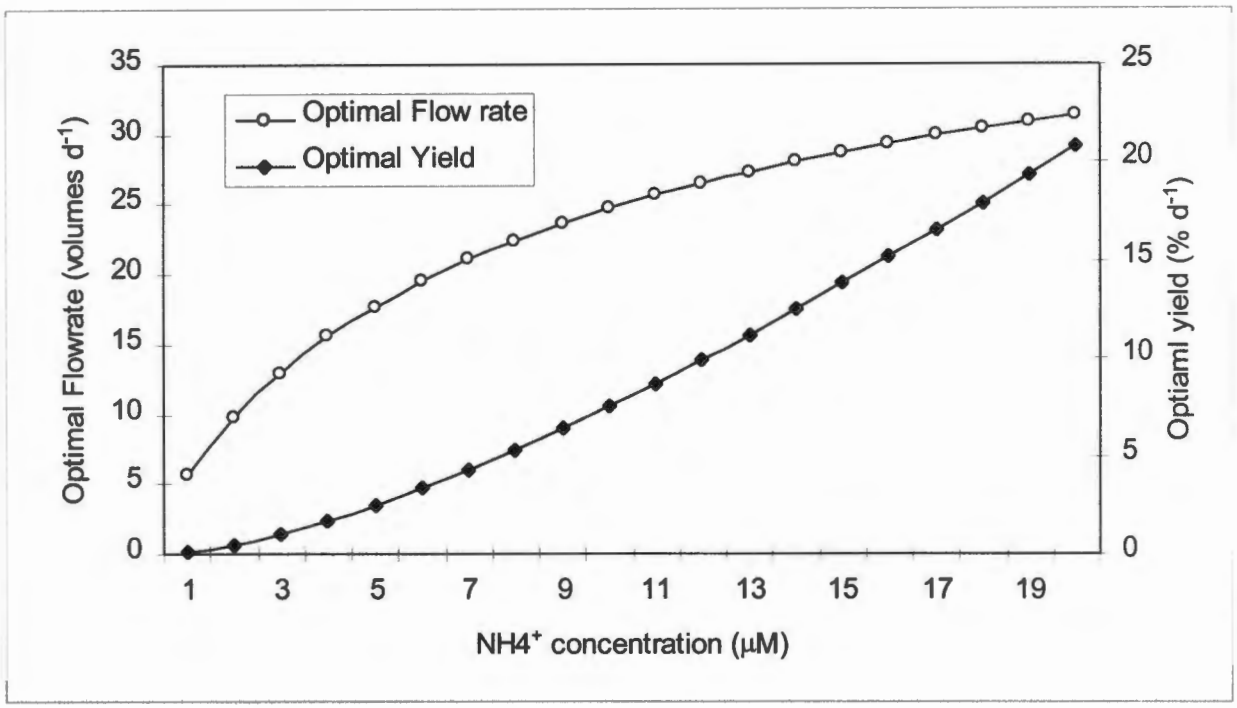

Figure 10: Optimal yields and associated flow rates at various NH_4^+-N concentrations for *G. gracilis*, modelled at $k = 130 g\ g^{-1}$.

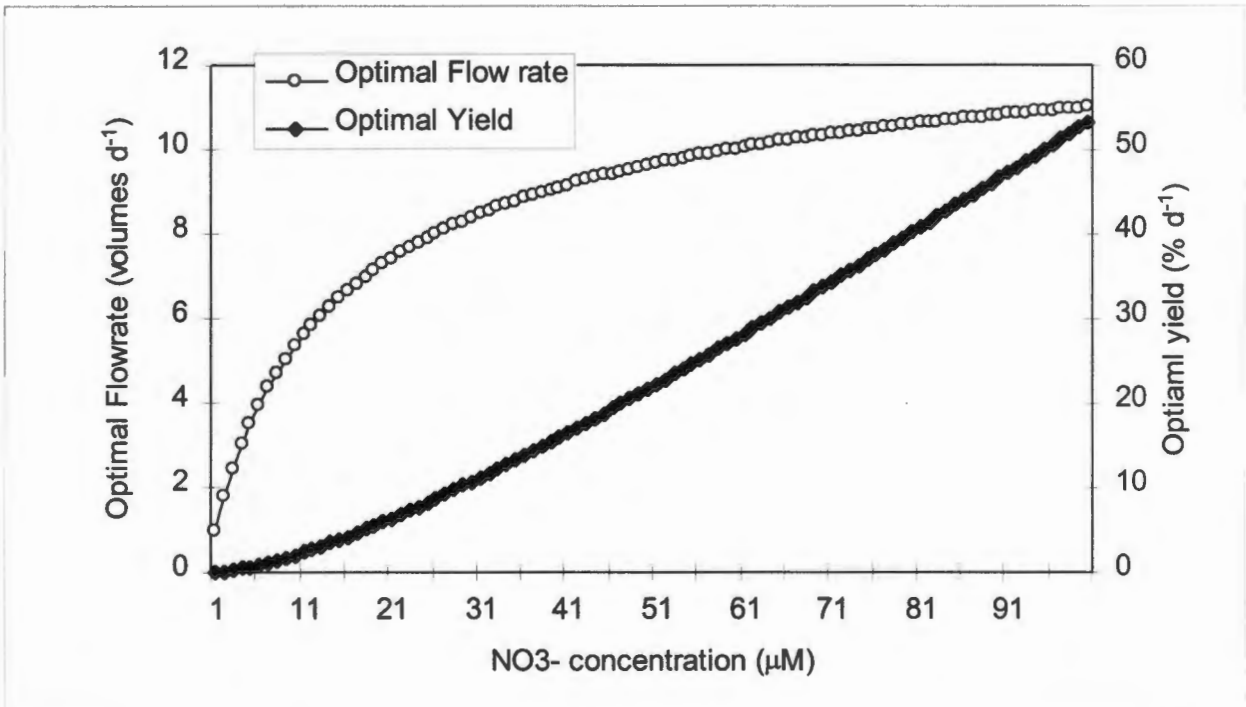

Figure 11: Optimal yields and associated flow rates at various NO_3^--N concentrations for *G. gracilis*, modelled at $k = 130 g\ g^{-1}$.

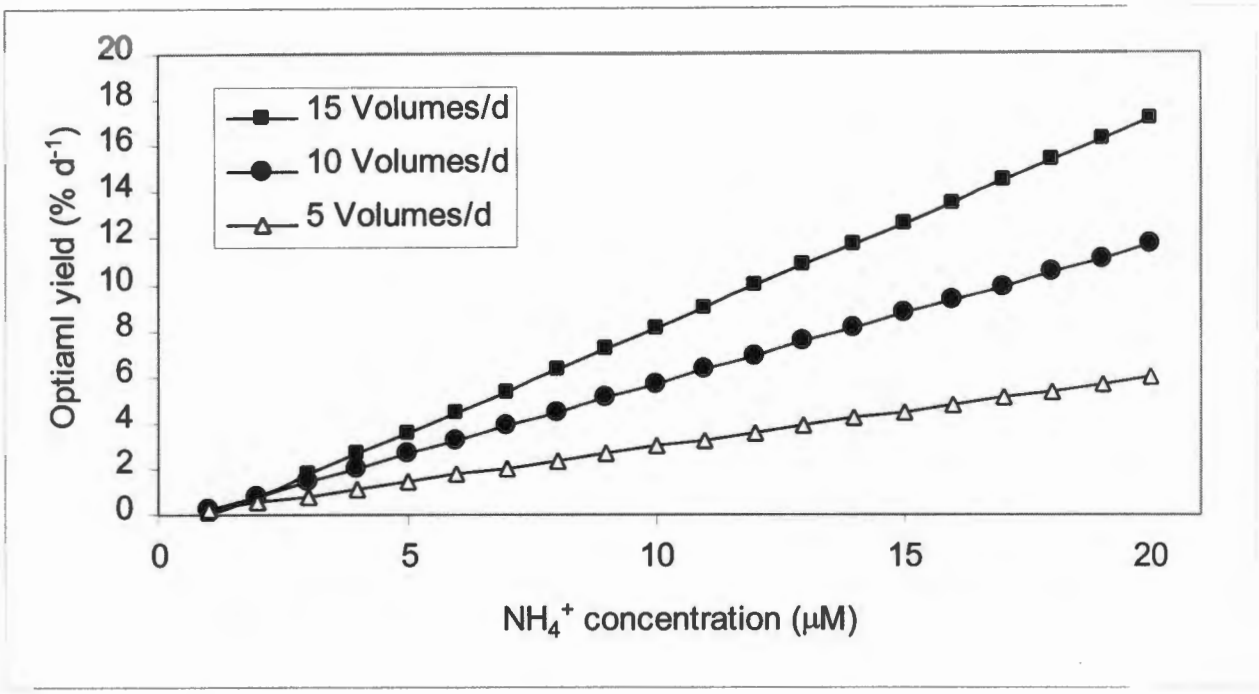

Figure 12: Optimal yields at a range of NH_4^+-N concentrations, under set constant flow rates for *G. gracilis*, modelled at $k = 130g\ g^{-1}$.

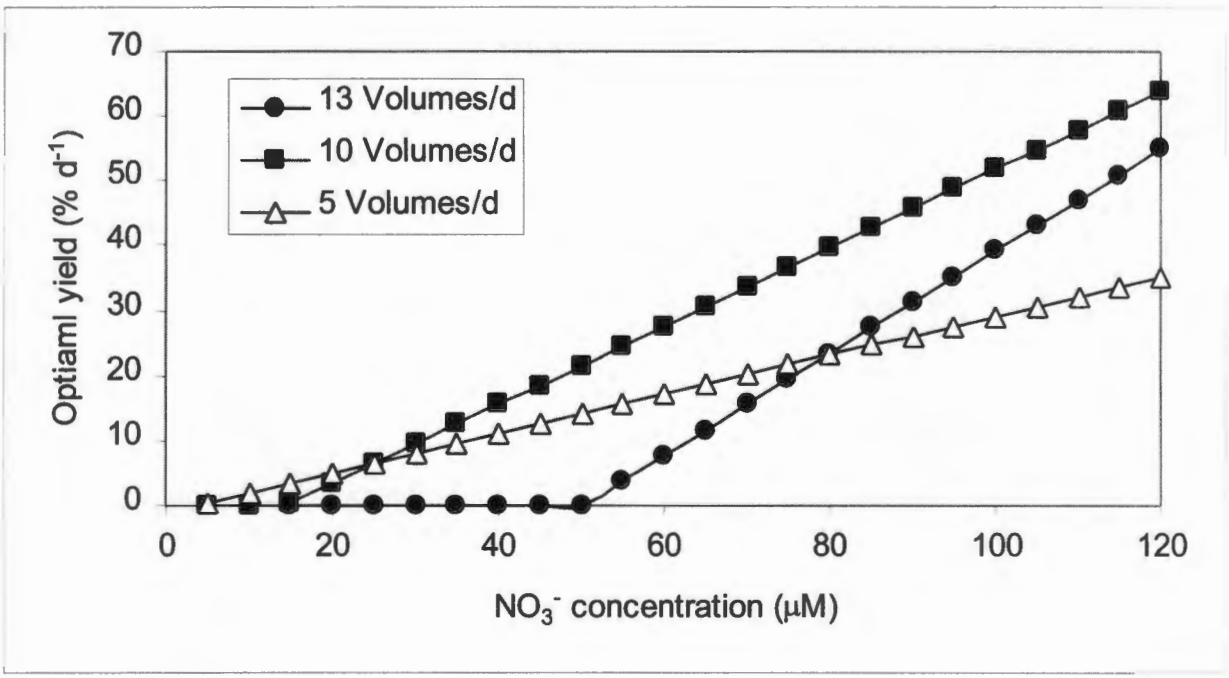

Figure 13: Optimal yields at a range of NO_3^--N concentrations, under set constant flow rates for *G. gracilis*, modelled at $k = 130g\ g^{-1}$.

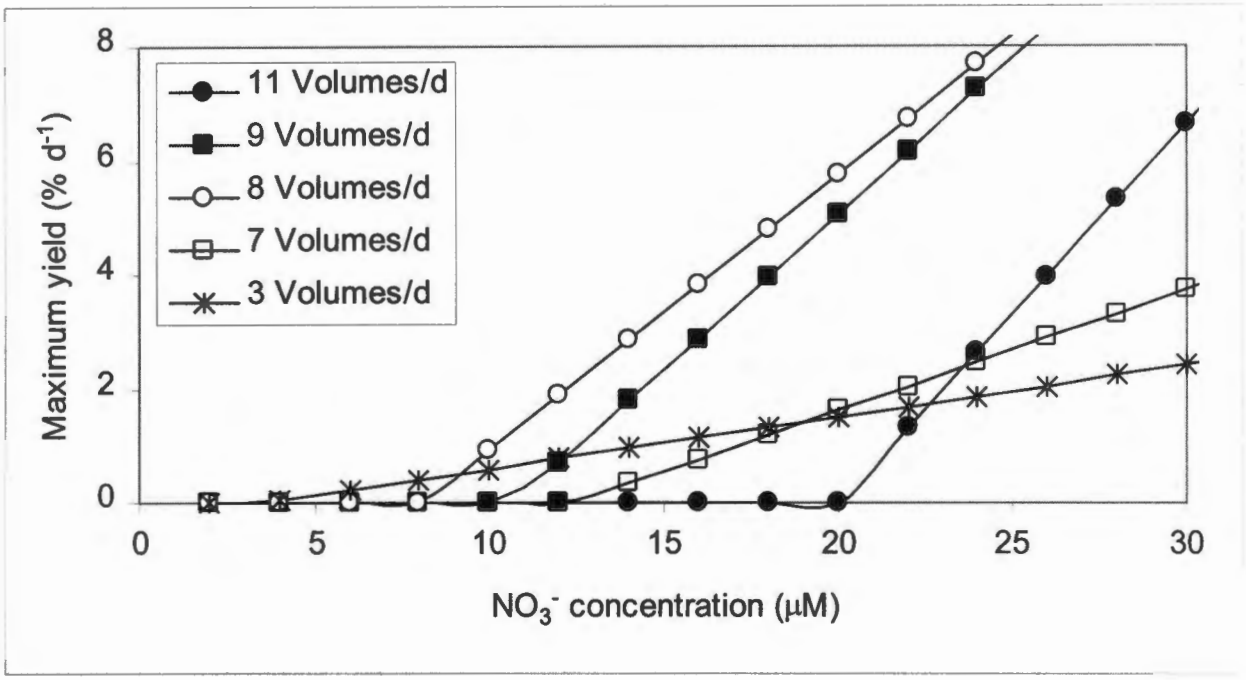

Figure 14: Optimal yields at a range of NO_3^--N concentrations, under set constant flow rates for *G. gracilis*, modelled at $k = 130g\ g^{-1}$.

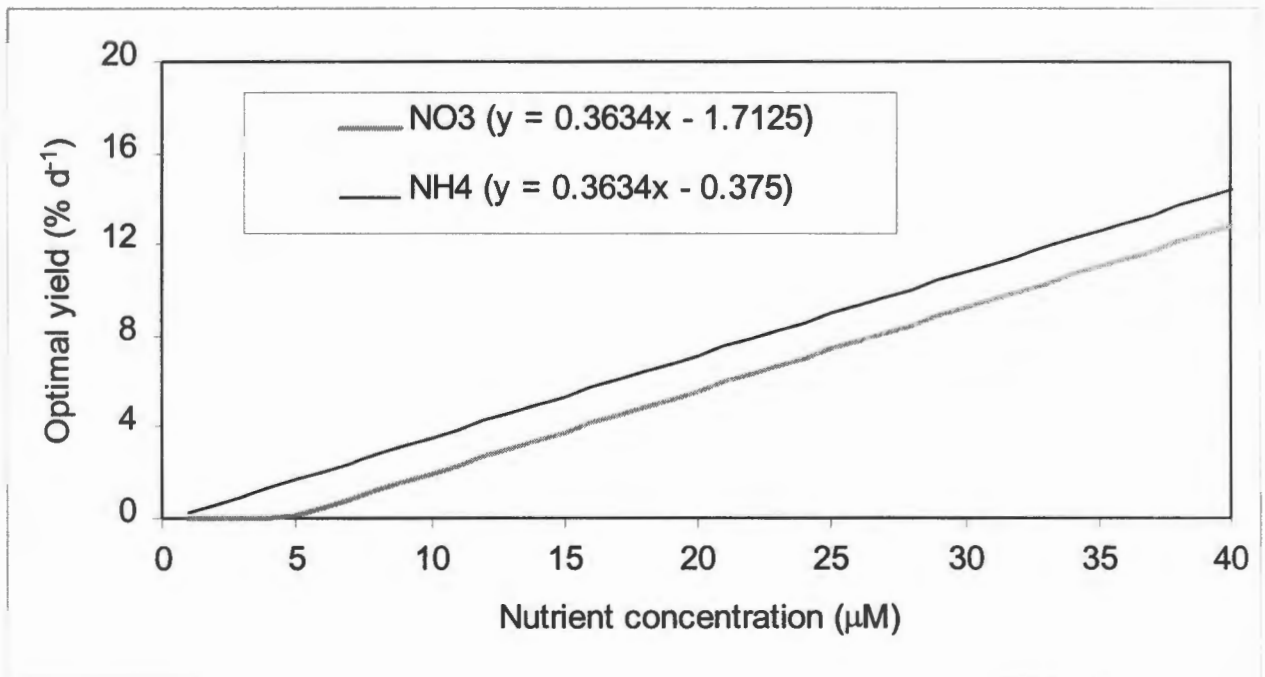

Figure 15: Optimal yields at a range of nitrogen concentrations for *G. gracilis* under a constant flow rate of 8 volumes d^{-1}, modelled at $k = 130g\ g^{-1}$.

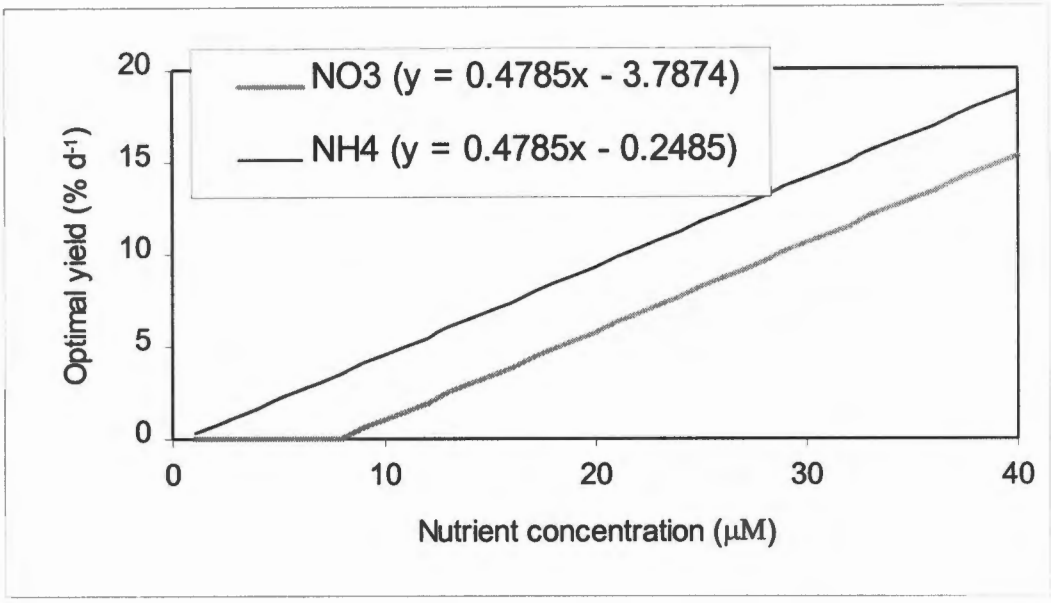

Figure 16: Optimal yields at a range of nitrogen concentrations for *G. gracilis* under a constant flow rate of 7.9 volumes d^{-1}, modelled at $k = 130g\ g^{-1}$.

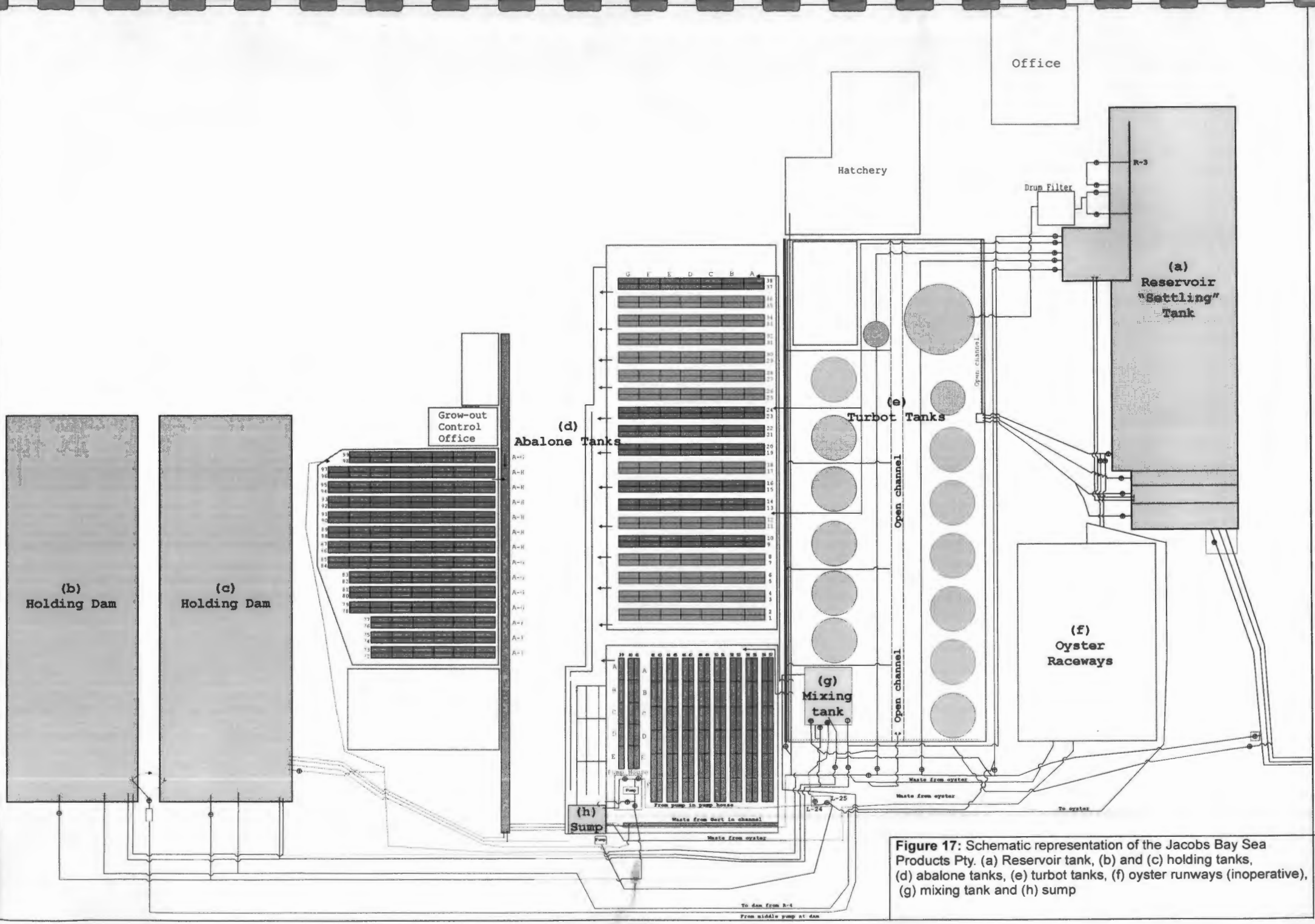

Figure 17: Schematic representation of the Jacobs Bay Sea Products Pty. (a) Reservoir tank, (b) and (c) holding tanks, (d) abalone tanks, (e) turbot tanks, (f) oyster runways (inoperative), (g) mixing tank and (h) sump