Outdoor cultivation of *Gracilaria* in pond and raceway systems at Jacobsbaai Sea Products (Pty) Ltd., South Africa

By
David Miller (MLLDAV027)

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Supervisors:

John J. Bolton
Robert J. Anderson

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Abstract

Two growth experiments were carried out using *Gracilaria gracilis* on a pilot-scale in raceway and pond systems at Jacobsbaai Sea Products, (Pty) Ltd. *Gracilaria* was grown in both turbot effluent water and seawater with aerated treatments in both mediums as well.

No *Gracilaria* remained in the ponds at the end of the first growth period and only negligible amounts remained at the end of the second. A combination of herbivory by isopods (*Paridotea reticulata*) and amphipods (*Jassa falcata*); poor growth conditions and rough weather may have resulted in the lack of growth. These ponds were rejected as possible cultivation systems for *Gracilaria*.

Good growth rates (mean of 4.7%.d^{-1} for aerated effluent treatments) support the possibility of utilising the raceways for *Gracilaria* cultivation. Realistic and conservative growth scenarios based on the results of the growth experiments suggest that a yield of 3.5 tons of *Gracilaria* (8.75% of the total feed requirements of the farm) could be produced per one month in the raceway systems. It is predicted that this could be doubled in the summer months if an effective aeration network is constructed. However, problems such as sedimentation and diatom growth were observed, which could reduce the efficiency of these systems. Epiphytes are not considered to be problematic despite their occurrence in the raceways.

Various factors affecting the growth of *Gracilaria* are considered at the farm to establish the type of growing environment found there. pH values of the seawater and effluent water (~8.1) both lie within the optimal range and are low enough so as not to negatively effect the rate of growth. Raceway water temperatures range between 13 and 17°C over the year. This lies on the lower boundary of the optimum temperature conditions, and during winter months growth may be inhibited slightly due to low temperatures.

Simple modelling is used to project what flow rates would be necessary in the raceways in order to supply the amount of nutrients required to maintain the possible growth rates. Typical hyperbolic relationships between flow rate and nutrient concentration are found. The model predicts that at higher relative growth rates the flow rates become more sensitive to changes in nutrient concentration. It also theorises that a relative growth rate of 20%.d^{-1} could be sustained for *Gracilaria* cultured in turbot effluent water in the raceways, although achieving such massive growth rates would be unlikely.

Economic and ecological benefits and good production rates make the raceways at JSP possible candidates for the cultivation of *Gracilaria* and could go a long way to supplementing the feed requirements on the farm.

Introduction

The Gracilarioid algae comprise a group of the red algae (Rhodophyta) of the order Gracilariales, *sensu* Fredericq and Hommersand (1989). The genus *Gracilaria* is currently the most important alga for the production of agar (Oliveira *et al.*, 2000). Large-scale *Gracilaria* cultivation began when it became clear that natural stocks of the seaweed could not support the growing agar trade and were being placed under considerable stress (see Shang (1976), Santelices and Ugarte (1987), Oliveira (1981) and Lewmanomont (1998) for the description of the overexploitation and depletion of natural beds in various of the Asian and South American countries supporting large seaweed industries). Since agar is the basis of a multi-million dollar industry, the development of farming techniques for *Gracilaria* has progressed rapidly.

Three categories of outdoor *Gracilaria* culture are found. Firstly, *Gracilaria* may be cultivated in open waters by three different methods: bottom planting, farming with nets or lines and raft farming. Open sea cultivation tends to be more of an extensive form of cultivation but requires little capital investment (Oliveira *et al.*, 2000). For further reading on sea culture refer to Oliveira *et al* (2000) and Santelices and Doty (1989).

Secondly, *Gracilaria* may be cultivated in tanks as an intensive cultivation system. This method requires high-energy input and capital investment (Oliveira *et al.*, 2000). Conditions within the tanks are controlled and kept within certain limits with respect to temperature, water flow, nutrients, pH, light and other such factors. In addition epiphytes and predators are controlled. Lapointe and Ryther (1979), Neish and Knutson (1979), Shacklock and Doyle (1983) and DeBusk and Ryther (1984) and many others have undertaken studies to determine optimum growing conditions for this method. For further details on tank cultivation techniques and conditions refer to Huguenin (1976), Neish (1979) and Hanisak (1987) (to name a few) and to Oliveira *et al.* (2000) and Santelices and Doty (1989) for review.

Another method which falls into this category is that of growing seaweed in raceways or similar such systems. Lapointe *et al.* (1976), Ryther *et al.* (1979) and Hampson (1998) have all reported successful results from this method at a pilot scale. However, this high productivity is only achieved with very high input costs and hence development beyond a pilot scale has yet to become a reality (Oliveira *et al.*, 2000). Huge expenses include very high capital investment necessary to construct the raceways and thereafter the substantial cost of pumping needed for high water exchange rates.

The final method of *Gracilaria* cultivation is that of pond cultivation. In pond cultivation the term 'ponds' refers to either large man-made excavations or natural lagoons/brackish water vleis. These vary greatly in size ranging from only a few fractions of a hectare to several hectares (Oliveira *et al.*, 2000). The depth of ponds also varies from just over 20 cm to a few metres. In most cases water motion in ponds is not enough to significantly move the thalli (Santelices and Doty, 1989) and hence nutrient replenishment is low. In fact, in many ponds the only water motion found is wind-driven and often results in the accumulation of materials downwind. Not only is water motion low but also the salinity of the water is often considerably lower than that of seawater. Since *Gracilaria* can grow well under conditions of low water motion and salinity, cultivation of *Gracilaria* in ponds has been successful in many countries. Countries where *Gracilaria* has been grown successfully in ponds at a commercial scale include Taiwan (Shang, 1976 and Chiang, 1981), southern China (Wu, 1998) and Vietnam (Ohno *et al.*, 1997). In the US, Hanisak and Ryther (1984) successfully cultured *Gracilaria* in excavated ponds but only at a very low productivity (lower than that of the local naturally growing populations). Other countries where pond cultivation of *Gracilaria* has been attempted (but not at a commercial scale) include Sweden (Haglund and Pedersen, 1993), Namibia (Rotmann, 1987) and the Philippines (Largo *et al.*, 1989).

In comparison with the more intensive methods of *Gracilaria* cultivation, the process involved in traditional pond cultivation is extremely simple. Planting materials are collected from the wild or nearby ponds and, once cut into smaller pieces, are merely scattered around the ponds and sink to the bottom (Shang, 1976; Chiang, 1981 and Yang *et al.* 1981). In ponds where tidal flow is limited the water has to be exchanged every 2-3 days and occasionally freshwater needs to be added to counter the increase in salinity arising due to evaporation. In some cases effluent from other animal aquaculture systems (such as oysters) is fed into the ponds. In the more simplistic systems fertiliser may be added, but this is often just fermented pig or chicken manure (Santelices and Doty, 1989).

These differences in scale and control between the various methods are reflected in their productivities (Oliveira *et al.*, 2000). Productivities of more than 80 $t.ha^{-1}.y^{-1}$d.wt. are not unusual in tank culture operations. While this amount of productivity has been reported in some open sea ventures, the norm is far closer to 40 $t.ha^{-1}.y^{-1}$d.wt. Productivity is lowest in pond culture operations, with an average of about 20 $t.ha^{-1}.y^{-1}$d.wt. being reported, but in certain cases it has been reported to be as low as 1,5 $t.ha^{-1}.y^{-1}$d.wt.

Various factors have been put forward as having effects on the growth rates and productivity of both cultivated and natural stocks of *Gracilaria*. These include temperature (e.g. Friedlander and Levy, 1995), light (e.g. Friedlander and Ben-Amotz, 1991; Friedlander and Levy, 1995), salinity (e.g. Hanisak, 1987; Engledow and Bolton, 1992), water movement (e.g. Hanisak and Ryther, 1984; Friedlander and Ben-Amotz, 1991; Critchley, 1993), nutrients (e.g. Mclachlan and Bird, 1986; Smit *et al.*, 1996), stocking density and biological factors such as epiphytes (e.g. Buschman and Gomez, 1993) and grazers (see Oliveira *et al.*, 2000).

Gracilaria requires a large number of micro- and macronutrients (in particular nitrogen and phosphorus) for good growth (see Morgan (2000) for a more detailed review of important nutrients). The majority of these nutrients can be found in animal effluent. Animal aquaculture effluent has been used in a number of cases in tank and pond cultivation of *Gracilaria*. In Hawaii, very high production rates have been reported using this practice (Santelices and Doty, 1989). Martinez and Buschman (1996) found that *Gracilaria* grown in effluent water had a lower agar yield, though the agar was of a better quality.

Most of these techniques and studies of factors affecting growth have been aimed at producing algae with high gel strength values for good agar production. However, in many instances, in particular with the more archaic methods where conditions cannot be as finely controlled, the *Gracilaria* produced is more prized as food for abalone or a luxury vegetable (Santelices and Doty, 1989). Likewise, in South Africa in particular, there has been growing interest in the cultivating of *Gracilaria* on abalone farms as a feed supplement. Simpson and Cook (1997) found that mixed algal diets, as opposed to single species diets, resulted in superior growth rates in the South African species of abalone, *Haliotis midae*. This implies that not only does cultivation of *Gracilaria* lead to a reduction in costs as less feed needs to be purchased, but it could well lead to improved production as well.

Morgan (2000) undertook an evaluation of the potential of incorporating *Gracilaria* cultivation in the existing abalone (*H. midae*) and turbot (*Scopthalmus maximus*) culture system at Jacobsbaai Sea Products (Pty) Ltd. (JSP). He proposed that polyculture of this sort would be beneficial to the operation both by lowering feed costs and possibly increasing growth rates as well as possibly providing temporary autonomy from external seaweed resources considering toxic algal blooms that are commonplace along the west coast of South Africa. He concluded that a variety of pilot-scale studies would be necessary before *Gracilaria* cultivation could be fully initiated at

JSP and recommended a number of methods (requiring either new structures or existing ones) that could possibly be used.

This project aimed in the first place to successfully culture the rhodophyte *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine and Farnham for the purpose of abalone feed on a pilot-scale at JSP. This was attempted by two different cultivation techniques. Firstly, *Gracilaria* was grown in the large outdoor 'ponds'. Secondly, a means of cultivating *Gracilaria* in the inactive oyster raceway systems at JSP was devised. In addition to this various physicochemical factors affecting growth of *Gracilaria* are considered at the farm to establish the type of growing environment found there. Mclachlan and Bird (1986) considered temperature and nutrients to be of primary importance for *Gracilaria* cultivation and considered light to be only of secondary importance. Hence the former two factors are focussed on. Possibly effects of pH values were also investigated, as an indicator of available CO_2.

Gracilaria was grown in both effluent water and seawater and it is hypothesised that growth rates of *Gracilaria* would be greater in effluent water than ordinary seawater. Also aerated treatments were used in the raceways and the effect of these treatments is examined. Simple modelling is used to project what flow rates would be necessary in the raceways in order to supply the amount of nutrients required to maintain possible growth rates. Different scenarios of growth rates, water exchange, stocking density and growth period are examined to predict possible yields in the systems. Finally, considering the possible productivity of these methods, the nutrient concentrations and flow rates required, and the impact of aeration on growth rates, the viability of growing *Gracilaria* in these ponds or raceways (effluent water or seawater) as a feed supplement for abalone (*Haliotis midae*) is discussed.

Methods

Study Site

Jacobsbaai Sea Products (Pty) Ltd. (JSP) is a land-based intensive mariculture operation on the South African West Coast approximately 120 km north of Cape Town. Established in the early 1990's the farm currently stocks between 2.5 and 3 million abalone with a feed requirement of roughly 40 tons of kelp per month. Morgan (2000) earmarked two existing components of the farm, the oyster raceways and the holding dams, for possible use in *Gracilaria* cultivation to supplement this large feed requirement.

Ponds

The 'ponds' at JSP are two PVC (polyvinyl chloride)-lined holding dams, both 100m by 40m and between 0.5 and 1.5 m deep, in which water from the adjacent coastline is passed through before reaching the farm. They function mainly in raising the temperature of the water slightly before it reaches the abalone tanks. Each pond has a volume of about 4800m^3 that gets turned over every 4 to 5 hours per pond. This translates roughly to an inflow of 1000 to 1200m^3 of seawater per hour. The clarity of the water entering the dams is variable but is more often than not fairly clear. This high water turnover rate, large size and PVC lining of the dams makes them fairly unique compared to the much smaller, stagnant, earthen ponds traditionally used and more similar, though much larger, to those used by Hanisak and Ryther (1984) in the US. The high water exchange rate means that pH and salinity levels are not likely to be problematic and hence these factors were not looked into in the ponds.

Raceways

Sixteen unused oyster raceways, 50m long and 0,9m wide, are present at the farm. The raceways are 0,4m deep at the start and 0,6m deep at the end giving a slight slope along the entire length of each raceway. Water enters each raceway at the shallow end through two pipes and flows down the length of the raceway to an outlet pipe at the deep end. While no significant current (water movement) is created, the water is exchanged at a high rate. Besides this high water exchange rate, another advantage of the raceways is that fish effluent water from other parts of the farm may be diverted into them.

Growth Experiments

In both the ponds and the raceways, two growth experiments were carried out. The first growth period lasted four weeks (7/8/01 to 5/9/01) while the second lasted only sixteen days (5/9/01 to 21/9/05). Tufts of *Gracilaria* were attached to suspended ropes using cable ties. Each tuft weighed between 18 and 23 grams and tufts were spaced at least 20cm apart. For the first growth experiment *Gracilaria* was obtained from natural populations in Saldahna Bay. For the second, *Gracilaria* from the raceways was used to restock both the ponds and the raceways.

Relative growth rates (RGR) were calculated using the traditional compound interest formula:

$$RGR = ((W_f/W_i)^{1/n}-1)100$$
$$= \% \text{ growth per day}$$

Where: n = no. days; W_i = initial weight; W_f = final weight

<u>Cultivation techniques</u>

Ponds

Due to the uniqueness of the ponds at JSP as locations for seaweed aquaculture, a new technique and a new apparatus design were necessary. While most pond operations grow the *Gracilaria* unattached scattered on the bottom of the ponds the size of the dams is such that the *Gracilaria* will have to be grown attached to ropes as harvesting would be too difficult otherwise and clustering on the upwind side of the dam would have negative impacts on growth and productivity of the seaweed. This meant that in the design of the cultivation system the ponds had to be treated as areas of open sea, the only difference being the depth of the ponds.

Initially, two 1.5 by 2 metre rafts were used, one per dam. The rafts were sunk with diving weights at one end and floated at the other. This allowed for the suspension of ropes at different depths to determine an optimal depth for *Gracilaria* growth. The failure of this method, due to a number of factors that will be discussed later, led to another design being necessary. This took the form of two cube-shaped wooden frames, one, the experiment, had herbivore excluding netting around the sides and bottom (40 % sun-excluding shadecloth) and the other was a control. Each frame was anchored to the bottom with diving weights on the end of ropes and kept at a constant depth by four floats attached to the sides. The floats kept each frame with the uncovered top approximately 20cm above the surface and the weights prevented them moving around the large ponds. Three ropes were suspended below the water in each frame.

Since this water is destined for other mariculture operations it cannot be manipulated to any great extent. For example, the addition of fertilisers, as is traditionally carried out in most successful pond culture operations, is not possible here. This is not a major drawback as this project aims to see whether *Gracilaria* can be cultivated in these systems as they stand and the less manipulation required, the more economically viable the operation becomes.

Raceways

Since at this stage growing *Gracilaria* in the raceways is not likely to be a permanent fixture, it was necessary to construct a means of suspending the *Gracilaria* without altering the raceways in any way. A gird of iron poles was designed which rested on top of the raceways. Ropes were suspended across the raceways from metal bars descending down from the top grid. Four such structures, with four suspended ropes each, were used. Four treatments were applied: seawater, aerated seawater, effluent water and aerated effluent water. The water was aerated by a single air pipe running along the bottom of the raceways. Effluent water came from nearby turbot tanks.

Table 1. Treatments applied in the raceway growth experiments.

	Turbot water	Seawater
Air	**2**	**4**
No air	**1**	**3**

Statistical tests

Statistical tests were carried out using STATISTICA (see StatSoft Inc. (1995) for details of the tests).

- One-way ANOVA

Null Hypothesis: The means of the samples/treatments are equal (i.e. do not differ significantly).

This is a parametric test that makes two assumptions that need to be met:

1. Normal distributions within groups (treatment samples). Normal probability plots were examined to see if the data were normally distributed. While it is good for this assumption to be met, Lindman (1974) found that one-way ANOVA tests are very robust against deviations from normality.

2. Group variances homoscedastic (equal variances). Two different tests were used to check for homoscedasticity of variances: the Levene and Brown-Forsythe Test of Homogeneity of Variances.
Log transformations are often applied to deal the problem of non-homoscedastic variances.

ANOVA tests may be very misleading if the means of the samples being compared are correlated with their standard deviations. Therefore plots were examined to see if any correlations existed.

If these assumptions are not met, non-parametric equivalent tests are necessary.

- Non-parametric tests

Two non-parametric tests were used in the case where the ANOVA assumptions were not met:

1. Kruskal-Wallis ANOVA by ranks.

Null hypothesis: Each sample is drawn from the same distribution or all are from distributions with the same median.

2. Median test.

Null hypothesis: all samples are from populations with identical medians.

- Post-hoc comparisons of means

In the case of significant results for any of the above tests a Tukey honest significant difference (HSD) test (for equal n) was done to establish which treatments differed significantly from the others.

Physicochemical Factors

Staff at JSP took temperature and pH readings each day for eight days during the second growth experiment in both seawater and effluent water raceways. In addition to this monthly average temperatures for the year September 2000 to August 2001 were obtained from K. Ruck (JSP, pers comm.) and nutrient concentrations and flow rates were obtained from Morgan (2000).

Nutrient modelling

Simple nutrient modelling was undertaken in the form of an equation relating nutrient concentrations (NH_4^+) and associated flow rates required to produce a various yields.

Assumptions

As with all models, there are various assumptions that need to be made in order to simplify the plethora of factors impacting on the system. This model assumes that nutrients are the only limiting factor and the primary limiting nutrient is nitrogen in the form of NH_4^+. It is assumed that the seaweed is not space limited and all water in the raceways is available to the seaweed. However common sense is applied in the choosing of stocking densities, the capacity of the raceways and possible yields. As it would require much further research and more complex modelling, uptake kinetics is not considered. It is therefore assumed that at any given nutrient concentration the seaweed takes up nutrients at a rate sufficient enough to utilise half of all available nutrients. In order to produce more realistic results the actual absorption rates at each flow rate for each stocking density would be needed.

Variables

The variables used in this model are described in Table 2 below.

Table 2. Variables used in nutrient modelling.

Variable	Symbol	Units
Day	d	d
Time	t	d
Flow rate	F	$l.d^{-1}$
Relative growth rate	RGR	$\%.d^{-1}$
Quantity of seaweed on day d	Q(d)	g_{sw}
Yield for day d	Y(d)	g_{sw}
Starting stock	S (=Q(0))	g_{sw}
Growth per nutrient absorbed	k	$g_{sw}.g_{nut}^{-1}$
Nutrient concentration required on day d if all nutrients are absorbed	$N_r(d)$	$g_{nut}.l^{-1}$
Nutrient concentration required for given growth period if all nutrients are absorbed	N_r	$g_{nut}.l^{-1}$
Absorption factor	a	-
Nutrient concentration needed in the raceway	N_w	$g_{nut}.l^{-1}$

Obviously as more and more seaweed is produced each day, the nutrient concentration required increases. This model is interested in predicting the maximum nutrient concentration required to support a given growth rate as if this concentration cannot be supplied then the given growth rate is not possible over the whole growth period. As the maximum concentration will always be found on the last day of the growth period of d days, for the purposes of this model we will let $N_r = N_r(d)$.

The growth constant, k, depends largely on the nitrogen content of the seaweed produced. Smit (1998) found a k value of 320 $g.g^{-1}$ for *Gracilaria* with thallus nitrogen content of 3.1%. This gives a thallus nitrogen content to k ratio of 103. The colour of the *Gracilaria* has been considered as an indication of its nitrogen content (Lapointe and Ryther, 1979; Wilson, 1999). Wilson (1999) found a qualitative correlation between seaweed colour and nitrogen content, dark red implying high nitrogen levels, paler yellow implying low levels. The colour of the harvested *Gracilaria* was noted and the thallus nitrogen content of the *Gracilaria* was determined using Wilson's (1999) qualitative correlation. Using this nitrogen content value and the above ratio, the k value for the seaweed in the raceways was calculated.

Equation derivation

$N_r(d) = N_r = Y(d)/kF$ (Gold, 1977)

$Y(d) = Q(d+1)-Q(d)$; where $Q(d) = (S(1+RGR/100)^t)$

Hence, $N_r = [Q(d+1)-Q(d)]/kF$

Finally, $N_w = N_r/a$; where a = 0.5 (half of all nutrients are absorbed). This value was then convert to μM.

Yield scenarios

Five different scenarios were created with respect to growth period, water exchange, starting stock and RGR (see Table 3). Using the equations above for N_r and Q(d), yields per day (=Q(d)/d) and associated N_r's were projected.

Results

Growth experiments

Ponds

At the end of the first four week growth experiment no *Gracilaria* remained on any of the ropes suspended in the ponds. While some seaweed remained at the end of the second sixteen day growth period in both the experiment and the control frames, the amounts were negligible and represented substantial negative growth. The seaweed was also in very poor condition being very yellow, and sometimes black, in patches. More seaweed was found loose on the bottom of the experiment 'cage' than still attached to the ropes. This seaweed, which was also in poor condition, must have fallen off of the suspended ropes.

Raceways

Table 4 below shows the mean RGR's for each treatment during each growth experiment.

Table 4. Mean relative growth rates (%.d^{-1}) obtained in each treatment for each growth experiment.

Treatment	Turbot, no air (1)	Turbot, air (2)	Seawater, no air (3)	Seawater, air (4)
Growth experiment 1	3.5598	4.6767	3.1726	2.5919
Growth experiment 2	3.3538	3.8785	1.1917	2.7799

First growth experiment

Good growth rates (Table 4) were obtained for all of the treatments during the first growth period despite high sedimentation and fouling of the *Gracilaria* by diatoms and other species of seaweed such as *Ulva*, *Enteromorpha* and *Aeodes*. This sedimentation and diatom growth occurred in all treatments and was often so severe that large proportions of the *Gracilaria* were totally covered and needed good rinsing to clean.

As the water was aerated by only a single pipe, the tufts suspended directly above this pipe displayed substantially higher growth rates than the tufts attached on either side of it. This resulted in high variances within the aeration treatments as can be seen in Figure 1. It is expected that should the aeration have been more efficient, the growth rates in the aerated treatments would probably have been even higher (the average RGR of the tufts directly above the air pipe in the effluent treatment came to 6.75%.d^{-1}). It was also noted that these very large tufts often displayed patches of discolouration (yellow), an indication of nutrient limitation.

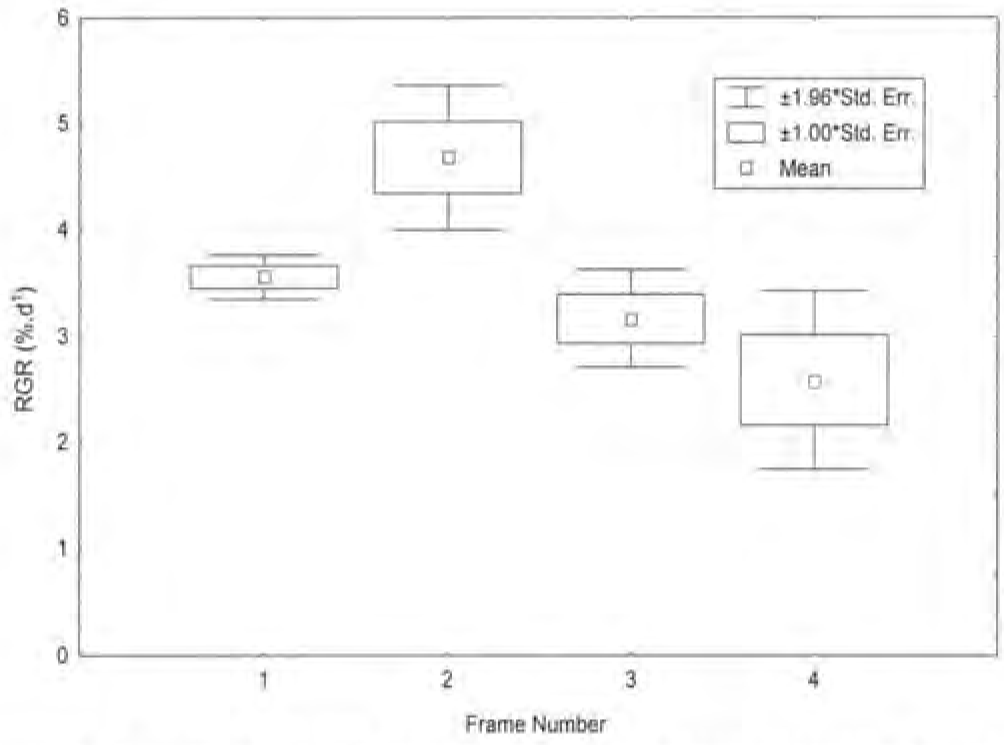

Figure 1. Box plots of the Relative Growth Rates obtained for each of the four treatments during the first growth experiment (growth period = 28 days).

Against expectations, the non-aerated seawater treatment out-performed its aerated counterpart during the first growth experiment (Table 4).

Second Growth Experiment

In order to reduce sedimentation on the *Gracilaria*, the raceways were cleaned every week during the growth period. In addition to this the frames were shaken every day to try knock off excess sediment. This, or perhaps the shorter growth period, did result in reduced sedimentation though a fair amount of sediment was still present. Much less sediment was present on the *Gracilaria* in the seawater treatments than the effluent treatments and large sheets of *Porphyra* were present in the effluent treatments only. Despite this lower growth rates were obtained than the first experiment.

The problem of high variance among tufts in the aerated treatment was less exaggerated in this experiment possibly due to the frames being moved during cleaning and shaking resulting in different tufts being aerated. As a result the variances within in treatment were more similar than during the first growth experiment (Figure 2).

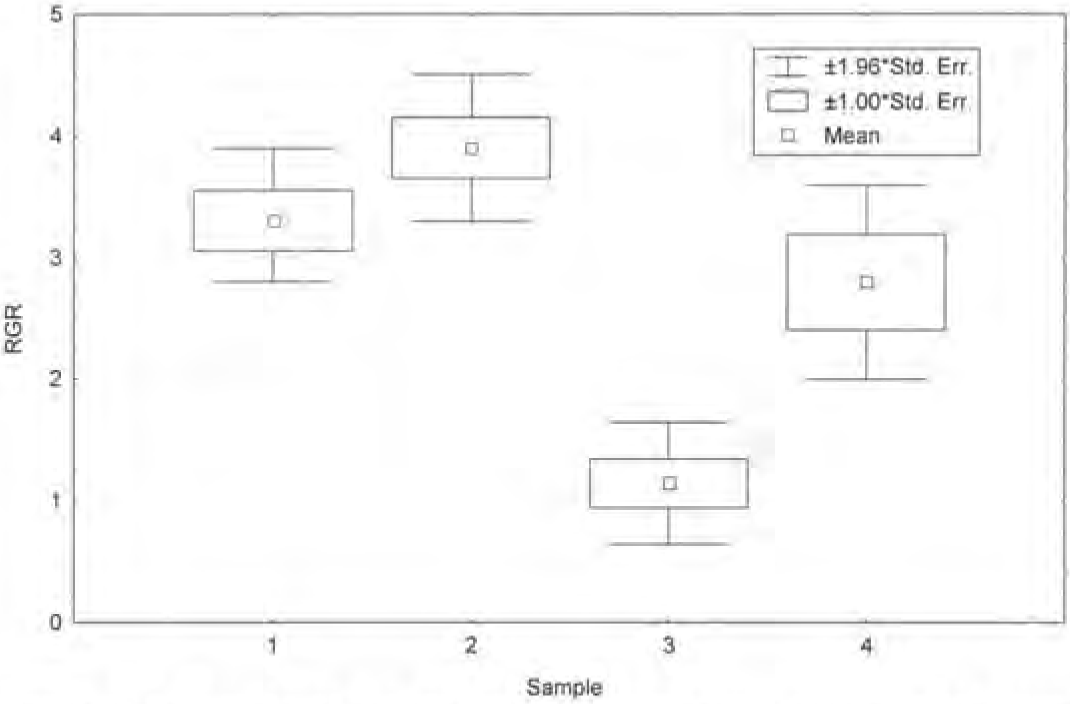

Figure 2. Box plots of the Relative Growth Rates obtained for each of the four treatments during the second growth experiment (growth period = 16 days).

While the growth rates of the first experiment compared favourably with those reported from various sources in

Mclachlan and Bird (1986), the growth rates in the second experiment were slightly lower. This could be due to a number of factors but may well be due to the shorter growth period which allowed the *Gracilaria* less time to acclimatise to the growing conditions and prevented it reaching full growth capacity.

Statistical tests

Table 5. Results of the statistical tests done on the RGR's obtained from the two growth experiments in the raceways.

Growth Experiment	Assumptions				Statistical Tests			
	Normal	Homoscedastic		Means vs. Std. Dev.	ANOVA	Krustal-Wallis	Median test	Significant Difference
		Levene	Brown-Forsythe					
First	Yes	No (p=0.002)	No (p=0.011)	not significantly correlated	-	H=16.98; p=0.0007	χ^2=11; df = 3; p=0.0117	Yes
Second	Yes	Yes (p=0.30)	Yes (p=0.28)	not significantly correlated	p<0.0001	-	-	Yes

In the case of the first growth experiment, log-transformations actually lowered the p-values of both homoscedasticity tests hence making the variances more significantly different. Therefore the transformed data was not used and instead non-parametric tests were applied to the non-transformed data.

Both experiments had significant results and hence post-hoc comparisons of means were necessary.

Table 6. Results of the Tukey HSD test on the first growth experiment. (p-values in **bold** indicate significant differences.)

	1	2	3	4
1		0.056428	0.805507	0.122492
2	0.056428		**0.004995**	**0.000202**
3	0.805507	**0.004995**		0.536411
4	0.122492	**0.000202**	0.536411	

The mean RGR from treatment 2 was significantly higher than that of both treatments 3 and 4. While it was not significantly higher than that of treatment 1, it was very nearly so indicating that the superior treatment was definitely the aerated effluent treatment, as expected.

Table 7. Results of the Tukey HSD test on the second growth experiment. (p-values in **bold** indicate significant differences.)

	1	2	3	4
1		0.654445	**0.000215**	0. 586710
2	0.654445		**0.000156**	0. 082803
3	**0.000215**	**0.000156**		**0. 004744**
4	0. 586710	0. 082803	**0. 004744**	

In the second growth experiment there was no clear superior treatment, but the non-aerated seawater treatment proved to be the most inferior treatment showing a significantly lower RGR than all the other samples. This goes more in line with expectations than the first growth experiment where the aerated seawater treatment had the lowest RGR (though not significantly so).

Physicochemical factors

Table 8. Comparison of physical variables in turbot effluent water and ordinary seawater in the raceways at JSP in the month of September. All data from K. Ruck (JSP, *pers. comm.*) except NH_4^+ and flow rate (Morgan, 2000).

Variable	Turbot effluent (high stocking density)	Seawater	t-tests	Significant difference
pH	8.0075+/-0.1469	8.13625+/-0.1053	p=0.064	No
temperature (°C)	15.4+/-1.5033	15.425+/-1.9234	p=0.977	No
NH_4^+ (μM)	~7.225	1.5	-	-
Flow rate ($l.h^{-1}$)	max. = 8000; avg. =4 500	max. > 10 000; avg. = 3 000	-	-

From Table 8 above it can be seen that the pH values of the seawater are only slightly higher than those of the effluent water. Both are within the optimal range and are low enough so as not to negatively affect the rate of growth.

The temperatures are very similar and lie right of the lower bound of the optimum temperature range. Engeldow and Bolton (1992) found that optimum growth in *Gracilaria gracilis* is experienced between temperatures of 15 and 25°C. Therefore while temperatures are not ideal they should not have any significant negative impact on growth.

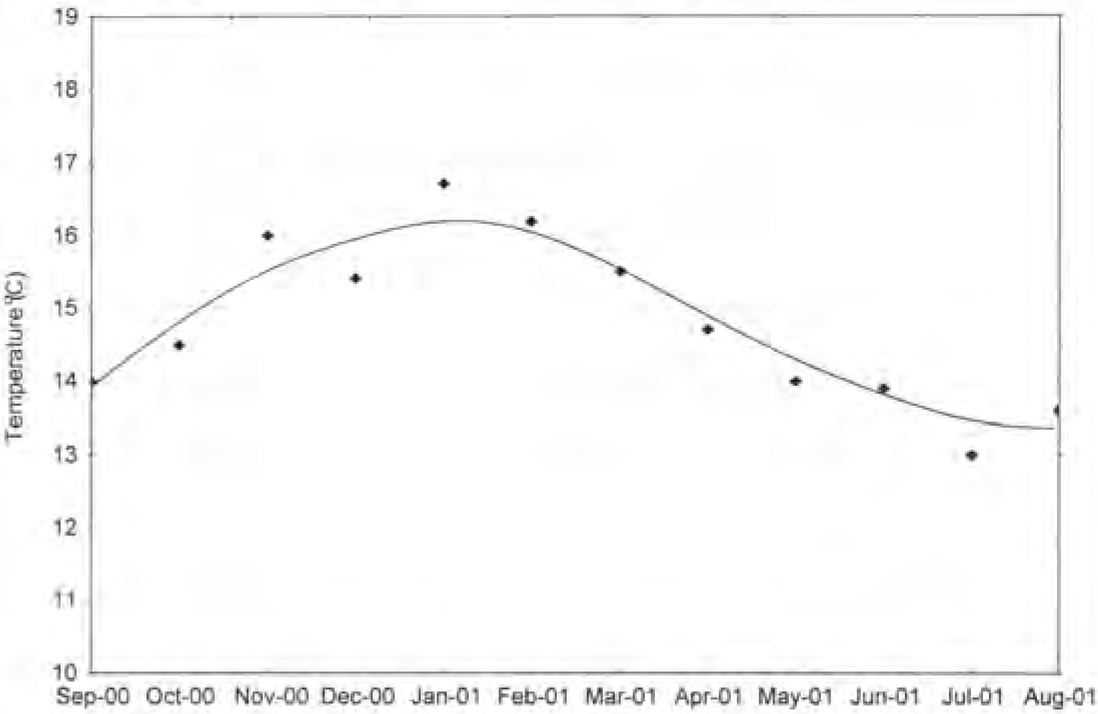

Figure 3. Average monthly temperatures in the raceways at JSP for the year September 2000 to August 20001. Data from K. Ruck, (JSP, pers comm.).

From Figure 3 above it can be seen that temperatures in the raceways are generally confined to the range of 13 to 17°C. Again this lies on the lower boundary of the optimum conditions and during winter months growth may be inhibited slightly due to low temperatures. The two growth experiments were conducted coming out of the coldest time of the year (August to September). Therefore the values obtained are a good indication of the minimum growth rates that can be expected (the 'worst case scenario').

The nutrient concentration from the turbot effluent is almost five times higher than that found in the seawater and while the average flow rate through the seawater raceways was slightly lower the potential for much higher flow rates exists.

Nutrient Modelling

The model developed could not be used to make predictions of yield or RGR, only nutrient requirements (and how this related flow rate and nutrient concentration) at various RGR's. This was because the N_r values produced were dependent on RGR, and therefore yield.

An important factor in this analysis was k, the growth per nutrients consumed. The *Gracilaria* colour was characteristically red, despite the few yellow patches and in general fell in the colour range of 7-9F8 according to the *Methuen Handbook of Colour* (Kornersrup and Wanscher, 1984). According to Wilson's (1999) qualitative correlation this corresponds to a thallus nitrogen content of around 2%. Therefore a k value of 206 $g.g^{-1}$ was used for the purpose of this model.

Figure 4 below relates flow rate and nutrient concentration for given RGR's at commercial stocking density for a 28 day growth period. Typical hyperbolic relationships are found. This is because the amount of nutrient required is the product of the concentration and rate of supply (N_r and F). The area underneath each curve represents therefore represents the amount of nutrients required and hence the curves move further away from the axes (greater areas) as RGR increases.

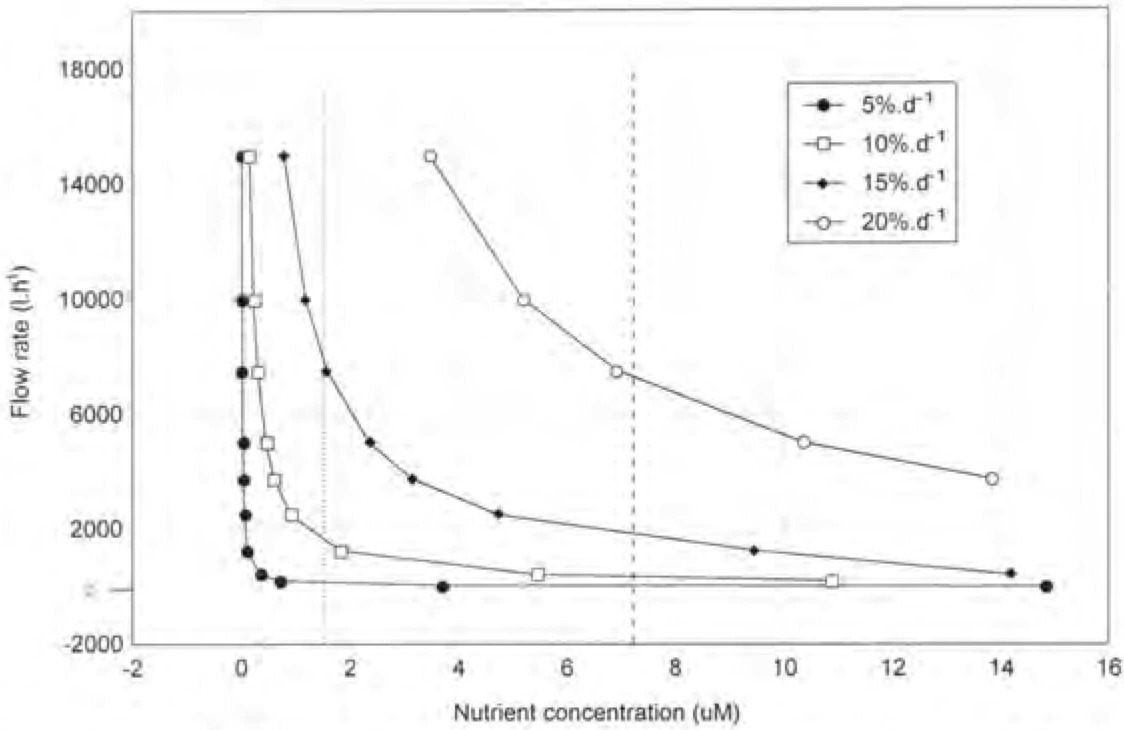

Figure 4. Flow rates required to provide the necessary nutrients to maintain different relative growth rates at varying nutrient concentrations. The nutrient concentrations in high stocking density turbot effluent water (---) and seawater ( ) are included.

As the RGR increases the curves flatten out at an increasing rate. For RGR's of less than 10% flow rates only become important at nutrient concentrations of less than 1 M (less than that of seawater). Above this concentration the curves are very flat and flow rate only decreases very slightly with large increases in nutrient concentration. At higher RGR's the flow rates become more sensitive to changes in nutrient concentration as the curves display more consistent slopes. Therefore at these higher RGR's choosing the correct flow rate becomes somewhat more important.

Various authors, e.g. Parker (1982) and Ryther *et al.* (1977), have reported growth rates as high as $20\%.d^{-1}$ in artificial systems such as aquaria under controlled conditions. However, an RGR of 20% was included in this model not because this is considered as a feasible growth rate for these systems but rather to see in terms of nutrients if this massive growth could be supported. Reading off the graph, at a RGR of

20%.d^{-1}, the nutrient concentration of high stocking density turbot effluent corresponds to a flow rate just over 7 000 l.h^{-1}. As a maximum flow rate of 8 000 l.h^{-1} is possible from the turbot tanks, theoretically the nutrients required to sustain this very high growth rate could be supplied by the turbot effluent. The nutrient concentration of seawater is such that this growth rate could never feasibly be achieved.

Figure 5 below contains much of the same information as Figure 4 but makes a few patterns more visible. Given certain realistic flow rates, it shows the nutrient concentrations that would be required to sustain a range of growth rates. From the effluent concentration line it can be seen that at a flow rate of 8 000 l.h^{-1}, the maximum possible flow rate, a RGR of just over 20%.d^{-1} can be achieved. Therefore while an RGR of 20%.d^{-1} is theoretically supportable, this is very near the maximum possible growth rate that can be achieved using the effluent water.

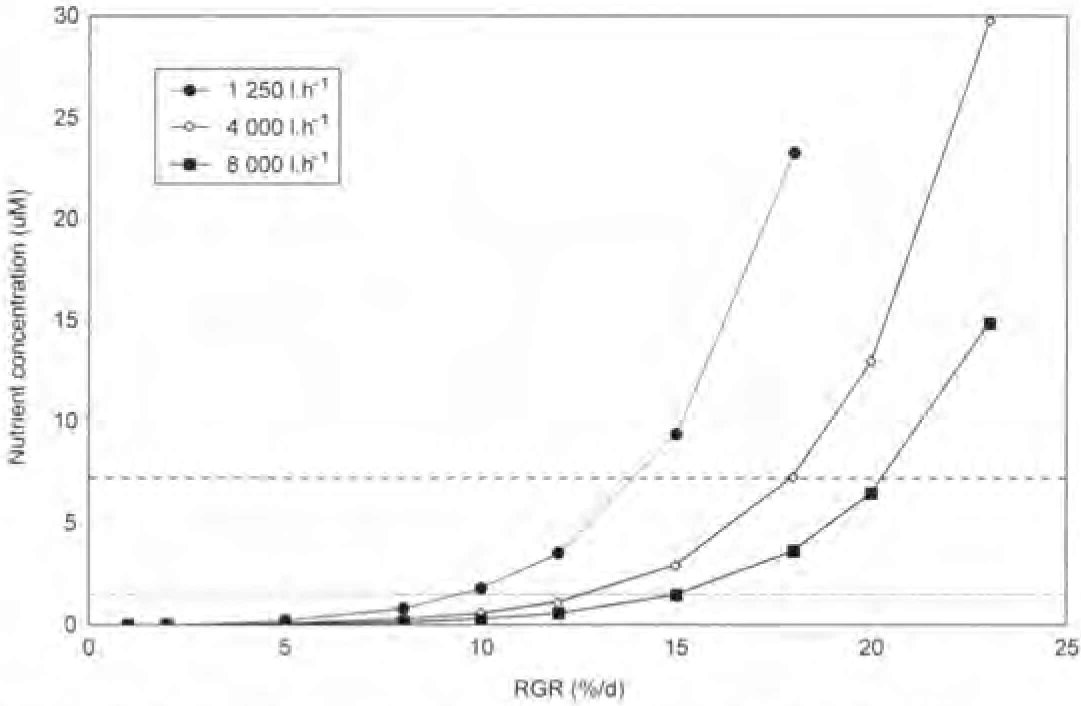

Figure 5. Nutrient concentrations required to sustain different relative growth rates at various given flow rates. The nutrient concentrations in high stocking density turbot effluent water (---) and seawater (·····) are included.

Likewise, only at a RGR of just less than 10% does seawater become an inadequate source of nutrients at low flow rates. After 10%.d^{-1} low flow rates become unfeasible as can be seen in how the 1 250 l.h^{-1} curve begins to increase sharply after this point. High flow rates in the range of 8 000 l.h^{-1} keep required nutrient concentrations within a reasonable range up until very high RGR's.

Yield Scenarios

While RGR's could not be predicted using the above model, at given RGR's simple yield equations could be used to predict the resultant yield over time and the model could predict the nutrients required to support this yield.

Table 3. Predicted yields and required nutrient concentrations for five different scenarios.

Scenario	Growth period (d)	Water exchange		Starting Stock (g)	RGR (%.d^{-1})	Nutrient concentration required (μM)	Yield (g.d^{-1})
		Turnovers (d^{-1})	Flow rate (l.d^{-1})				
A	28	5	4 628	10 666	5	0.01	1 112
B	28	5	4 628	10 666	1	0.0007	122
C	28	5	4 628	70 000	5	0.07	7 300
D	14	5	4 628	70 000	10	0.13	14 000
E	14	2	1875	70 000	10	7.65	14 000

Scenarios A and B represent projected yields for the highest (turbot, air) and lowest (seawater, no air) RGR's found in the growth experiments respectively. The starting stock is equivalent to the same stocking density as was used in the growth experiments. For the remaining scenarios the starting stock was consistent with optimum stocking densities for netlon ropes used on raft culture operations (R. Anderson, Marine and Coastal Management, *pers. comm.*). Both yields are very low, especially that of scenario B.

Scenario C represents a realistic growth scenario. In a fully aerated raceway growth rates of at least 5%.d^{-1} would be achievable when one looks at the results of the growth experiments. This accompanied by commercial stocking densities produced worthwhile yields of *Gracilaria*. The only assumption being made is that increasing stocking density does not impact significantly on the RGR. While RGR may decrease slightly, it was decided that this decrease would be negligible.

Scenarios D and E have very high RGR's and hence shorter growth periods were used. At the high RGR's for these scenarios a growth period of more than two weeks would result in the raceways reaching capacity and various negative feedback mechanisms would then result in much decreased growth. Both produced good daily yields. Scenario E, with its low flow rate, in the only scenario in which nutrient concentration becomes an issue. While in all the other scenarios seawater would have been able to supply the necessary nutrients, in this scenario not even effluent water would be sufficient.

Discussion

The most surprising result of the growth experiments was the failure to achieve any growth whatsoever in the holding dams (ponds). While one would expect very low growth rates under the conditions of low water exchange and movement with no significant nutrient inputs, this complete failure was unexpected. Numerous factors could have caused this loss of seaweed and in particular herbivory and poor general growth environment were considered to be most likely.

Anderson *et al.* (1998) found that when no other preferred food source was available, the isopod *Paridotea reticulata* would consume *Gracilaria gracilis*. A small number of isopods were found in the dams as well as many amphipods, *Jassa falcata*. This amphipod is commonly associated with *Gracilaria* populations (natural or farmed) and may well have contributed to the loss of seaweed biomass through herbivory. It was for this reason that the herbivore exclusion frame was used. However, the frame failed to protect the seaweed and while no *P. reticulata* was found inside, many *J. falcata* were.

This begged the question of why seaweed still remained after the second growth period and not the first. While this may have been due to the shorter growth period it seems more likely that environmental conditions played a role. The loose *Gracilaria* in the exclusion frame suggests that perhaps a combination of herbivory and general bad growth conditions (low temperatures, low nutrients) resulted in slight negative growth. This would have caused the tufts to thin, loosening their attachment to the ropes. Any significant water movement (due to wind for example) could have then easily caused the seaweed to fall off the ropes and be washed away. It is possible (though not known) that the weather was calmer during the second growth period and hence less seaweed was lost.

It must be noted that the growth experiments were carried out during the worst possible growing season in terms of temperatures, which were at their lowest. Combining this and the possible effects of herbivory it is not all too surprising that the growth experiments failed. Whatever the reason for the failure, the results from this experiment do not support the optimistic yields of these systems predicted by Morgan (2000) based on results from other pond systems. The raceways, however, produced some promising results.

Good growth rates, in line with those from other such systems, support the possibility of utilising these systems for *Gracilaria* cultivation. Morgan (2000) estimated that a yield of 2.1 tons could be produced each month in the raceways. Growth scenario C, a very realistic and perhaps even conservative scenario given the results of the growth experiments, suggests that a yield of 3.5 tons could easily be produced in one month by the sixteen raceways. This translates to a substantial 8.75% of the total feed requirements of the farm. Scenario D is even more promising. If an effective aeration network is constructed, this could combine with the high nutrient concentrations in turbot effluent and high turnover rates possible to achieve an RGR of around $10\%.d^{-1}$. This would be even more likely in the summer months when more light is available and temperatures are higher. Then, given the yield from scenario D, almost twice as much *Gracilaria* could be produced than scenario C, representing a contribution of about 17% of the total feed requirements.

These yields are not only fairly realistic, but could prove to be cost effective too. No fertilisation would be necessary and no construction costs apply. Also, while agar yield may be lower in *Gracilaria* grown in effluent water (Martinez and Buschman, 1996), it seems reasonable to assume that the quality of *Gracilaria* as abalone feed would be better if it was grown in effluent water as the greater amount of organic and inorganic nutrients could possibly result in higher protein content.

Besides economic benefits there are also the ecological benefits that accompany such polyculture operations. Oliveira *et al.* (2000) predict that mariculture of *Gracilaria* will certainly have an ecological impact that has to be assessed. However, since no be artificially fertilising is necessary, the mariculture of *Gracilaria* in this instance is unlikely to have any additional impact on the local environment. In fact, by growing seaweeds in such polyculture systems, high nutrient levels of effluent water from animal aquaculture organisms can be significantly reduced by flowing the water through seaweed tanks and hence reducing the environmental impact.

There are of course various practical problems associated with the cultivating of the *Gracilaria* in the raceways and these need to be considered before planning any large scale production. The sedimentation found on the raceway *Gracilaria* could pose a substantial problem. Sedimentation of the degree experienced during the first growth experiment could substantially reduce the amount of light available to the seaweed and may also impact on the seaweed's ability to absorb nutrients. However, effective aeration and the resultant increased water movement could alleviate this problem to an extent, and the cleaning of the raceways and shaking of the frames did also succeed in reducing sedimentation.

Epiphytes may also pose a more significant problem at higher stocking densities. Depending on the intended use of cultured *Gracilaria*, epiphytes may or may not be problematic. If the *Gracilaria* is intended for agar production, the decrease in *Gracilaria* productivity due to epiphytes can be very problematic. However, Fluerence (1999) found that *Porphyra* and *Ulva* have high protein contents (33-47% and 20-26% respectively), which would make them good food sources for the abalone. Simpson and Cook (1997) noted that abalone prefer mixed seaweed diets and hence epiphytes such as *Ulva* and *Porphyra* found on the cultured *Gracilaria* may not decrease the food value of the harvested seaweed and may even provide better feed. Also, while these epiphytes may reduce the productivity of the *Gracilaria*, the productivity of all seaweed in the raceway system would not be significantly reduced and may even be increased.

The raceway experiments also supported the findings that effluent water is a good medium for *Gracilaria* cultivation (e.g. Santelices and Doty, 1989). The highest growth rates obtained were in the effluent water treatments.

Both pH and temperature were within reasonable ranges for good growth. Hanisak and Ryther (1984) and Friedlander and Ben-Amotz (1991) found that in the absence of sufficient water exchange, *Gracilaria* growth rates declined due to an increase in pH. Shang (1976) also found that high pH values were not conducive to good growth rates. However raceway systems are characterised by significant water movement/turnover so pH does not seem to pose a problem in these systems. Therefore, of all the physicochemical factors considered, nutrient concentration, along with aeration/water movement, seemed to be the most important. Hence the nutrient modelling was undertaken which produce some interesting results.

The model showed how effluent water could sustain very high RGR's, much higher than ordinary seawater, at realistic flow rates. Considering that the uptake kinetics of *Gracilaria* would obviously be more favourable to high productivity in effluent water than in seawater, the superiority of this as a culture medium is made clear.

Like all models, assumptions had to be made. The factor being considered, nitrogen concentration, had to be isolated and therefore the effects of other factors had to be ignored. The major drawback of the model is its disregard of uptake kinetics. At different nutrient concentrations the uptake rate of the *Gracilaria* varies but this had to be ignored for the sake of simplicity. Also the nutrient requirements of seaweed increases as growth rate increases, over and above the increased requirements of more seaweed.

Morgan (2000) attempted to take uptake kinetics into account in his modelling of possible yields and necessary flow rates for *Gracilaria* cultivation. However, the Michaelis-Menten equations he employed were not designed with the cultivation of *Gracilaria* in mind. The equations came from a model for the tank culture of micro-organisms. In the case of the micro-organisms, harvesting is a continual process as it is aimed to maintain a constant stocking level. Therefore at increased nutrient concentrations, productivity increases and the flow rate needs to be increased to harvest the extra micro-organisms being produced. So by using these equations Morgan produced an incongruous relationship between nutrient concentration and flow rate where the greater the concentration, the greater the flow rate required. The model produce in this paper results in the more realistic hyperbolic relationship between flow rate and nutrient concentration.

Conclusions

In the past the very high input costs required to grow *Gracilaria* in raceways have precluded the development of this practise (Oliveira *et al.*, 2000). The major costs involved are those necessary to construct the actual raceways themselves and then the huge costs of pumping needed for the high water exchange rates (Morgan, 2000). However, the setup at JSP is such that none of these expenses apply. The raceways are already in place (from a previous attempt at oyster aquaculture) as are the facilities required for pumping water. Since many other organisms are cultivated at JSP, the costs for pumping the water are disseminated over all of the other organisms as well. Effluent water has been shown to be a very good culture medium. By cultivating the *Gracilaria* at a functioning aquaculture farm, effluent water from the other organisms can easily be diverted to flow through the raceways.

The experiments and modelling carried out in this paper show that the raceways at JSP are possible candidates for the cultivation of *Gracilaria*, especially considering that they are currently lying unused. If good growth rates can be maintained, cultivating *Gracilaria* in the raceways could go a long way to supplementing the feed requirements on the farm.

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