Effect of water motion on the cultivation of the economic seaweed *Gracilaria parvispora* (Rhodophyta) on Molokai, Hawaii

Erin Ryder[a], Stephen G. Nelson[a,*], Casey McKeon[a],
Edward P. Glenn[a], Kevin Fitzsimmons[a],
Sherman Napolean[b]

[a]*Environmental Research Laboratory, 2601 West Airport Drive, Tucson, AZ 85706, USA*
[b]*Ke Kua'aina Hanauna Hou, P.O. Box 741, Kaunakakai, Molokai, HI 96748, USA*

Abstract

A cage culture system was previously developed for the red alga *Gracilaria parvispora* Abbott on Molokai, HI; however, yields have shown marked variation, even among cages with identical stocking rates and fertilization treatments. Water motion, which can be affected by location and arrangement of the cages in the grow-out area, was hypothesized to be a factor contributing to the variation in yield. To examine this, the growth rates of thalli and the development of sporelings in relation to water motion were determined in replicated trials both in tanks and in small-scale field experiments.

Generally, water motion had a substantial effect on both thallus growth rate and spore development. In the tank cultures of thalli, water velocities ranged up to 13.7 cm s^{-1}, and relative growth rates (RGRs) ranged from 2.8% to 8.9% day^{-1}. In the lagoon, water velocities ranged between 3.6 and 11.6 cm s^{-1}. Relative growth rates of the thalli in the lagoon trials were 0.02–10.3% day^{-1}. Sporeling density, a measure of spore development, was also significantly affected by water motion. In the tank trials, water motion ranged from near 0 to 6.50 cm s^{-1}, and sporeling densities ranged from 1.4 cm^{-2} at lower water motion levels to 11.6 sporelings cm^{-2} at higher levels. Similar results were obtained in lagoon trials, with sporeling densities ranging from 0.2 to 6.7 cm^{-2}. However, sporeling length was not significantly correlated with water motion. In previous trials, we have been able to achieve high sporeling densities, but elongation of sporelings has been inhibited. In the present trials, we were able to break the apparent sporeling dormancy by incubating the sporelings in tanks enriched in nutrients supplied by fish cultures. Consideration of

* Corresponding author. Tel.: +1-520-626-2386; fax: +1-520-573-0852.
E-mail address: nsteve@ag.arizona.edu (S.G. Nelson).

the effects of water motion is important in designing culture systems for species of *Gracilaria* and other marine algae. The results also suggest that nutrients play a key role in regulating the early development of *G. parvispora* sporelings.

1. Introduction

The red alga *Gracilaria parvispora*, known in Hawaii as "ogo", is highly valued in the seafood markets of Honolulu. On Molokai, HI the non-profit organization Ke Kua'aina Hanauna Hou has established a demonstration project to serve as a model for sustainable, small-scale crop production in rural areas of the tropical Pacific (Glenn et al., 1998). The group is a community-based organization whose goal is to provide native Hawaiians with an income while allowing them to adhere to the traditional ways of life and land use.

The system developed on Molokai incorporates all the life stages of *G. parvispora* from production of spore-coated substrates through the harvesting of thalli (Glenn et al., 1998, 2003; Nelson and Glenn, 2000). The system includes integration of shrimp/fish culture with the production of *G. parvispora* (Nelson et al., 2001; Nagler et al., 2003). In the final stage of culture, thalli that have been fertilized in fish tanks or in shrimp pond effluents are placed in floating cages in a low-nutrient lagoon for production (Nagler et al., 2003). Subsequent research has been aimed at improving both the nursery and grow-out phases of the Molokai system.

Marked variation of yields of *G. parvispora* has been observed among the production cages, even among those that were placed in the same areas of the lagoon and that had identical algal stocking densities (Nagler et al., 2003). Because the thalli in these cases were all adequately fertilized prior to placement in the lagoon and had all been fertilized in the same batch, nutrient limitation was eliminated as a major contributing factor to the variation in yields. Therefore, we tested the hypothesis that differences in water motion among the grow-out cages could be a major influence on production of *G. parvispora* under these conditions.

Similarly, we were also interested in the effect of environmental conditions, including water motion on spore development and sporeling growth. As in the grow-out, the nursery phase of the Molokai ogo culture operation has had highly variable results. For example, it has proven difficult to predict the successful development of harvestable patches of *G. parvispora* from placement of spore-laden substrates at locations within the surrounding reef (Glenn et al., 1999).

Water motion, or relative water motion in cases where thalli are in motion, can effect exchanges of nutrients and carbon dioxide between the water mass and the thallus. This can occur because increased relative water motion reduces the boundary layer through which such exchanges occur (Gonen et al., 1995). Also, increased water motion can enhance rates of photosynthesis via removal of inhibitors, such as OH^- ions, from the algal surface (Gonen et al., 1995). However, previous research with *Gracilaria* species has produced widely varying results among species and conditions (Jones, 1959; Conover,

1968; Santelices, 1977; Wheeler, 1980; LaPointe and Ryther, 1979; Parker, 1981, 1982; Gonen et al., 1993, 1994). Earlier studies with *G. parvispora* in Hawaii failed to find an effect of water motion on growth of thalli either in cages (Glenn et al., 1998) or in the ocean (Glenn et al., 1999). However, these experiments were conducted under conditions of nutrient limitation. Here we present the results of studies designed to eliminate nutrient limitation through the use of well-fertilized thalli. These were subjected to varying degrees of water motion in a series of replicated trials in tanks and in the lagoon.

With regard to the nursery phase of the culture operation, we found little information available on the effects of water motion on the development of spores or sporelings of any species of *Gracilaria*. Friedlander and Dawes (1984) examined sporeling growth from carpospores of *Gracilaria foliifera* in response to light, temperature, and salinity, but not to water motion. Some studies (e.g. Destombe et al., 1990; Pearson and Brawley, 1996; Serrao et al., 1996) examined the effect of water motion on the release of *Gracilaria* spores, but not on sporeling development. To determine the effect of water motion on development of sporelings, additional experiments were conducted, both in tanks and in the lagoon where the seaweeds were being cultivated.

The objectives of this work were to determine the effect of water motion on: (1) the growth rates of *G. parvisopora* thalli, that were fertilized to relieve nutrient limitation; and (2) on the development and survival of spores and sporelings. The data will be of use in the development of culture technologies for this, and related, species.

2. Materials and methods

2.1. Effect of water motion on growth of thalli in tanks

Replicate experiments were carried out in two, 1.9-m diameter, fiberglass tanks, filled to approximately 0.45 m depth with seawater, which had been filtered through a pressurized, sand filter. Thalli of *G. parvispora* were fertilized with a mixture of chemical fertilizers providing both nitrogen and phosphorous for five days before growth trials in order to allow assimilation of nutrients in the thalli. This raises the nitrogen content of the thalli to ca. 4%, sufficient to support rapid growth for $2-3$ weeks under conditions of nutrient restriction (Nagler et al., 2003).

Ten fertilized thalli (15.9 g mean mass) were individually weighed and tagged for each treatment. The thalli for each treatment were contained in a plastic-mesh basket, suspended in one of the tanks. Three baskets were placed in each of the two tanks. Water motion within each basket was controlled by aeration through air stones. Each aerated basket was provided with either one or two air stones. For the lowest water motion treatment, no air stone was provided. Each basket was also supplied with a gypsum block (clod card), which was placed in the basket for 1 day to measure water motion. In order to calculate relative water motion, we used the equation:

$$y = 39.61x - 1.502,$$

where x is the fractional weight loss of the clod card before and after placement in tanks or cages for 24 h, and y is the relative water motion expressed in cm s^{-1}. The equation is

based on controlled studies of weight loss versus water motion and includes corrections for the effects of salinity and temperature on the dissolution rate of the gypsum blocks (Thompson and Glenn, 1994). Clod cards were first weighed dry (ca. 35 g each), placed in treatments and then re-dried at room temperature to constant weight (ca. 3–5 days) before reweighing. After 10 days, thalli were removed from the tanks, and excess water was removed by spinning them in a salad spinner. Each thallus was weighed to determine relative growth rate (RGR) for each treatment. RGR was calculated for thalli with the formula (Leopold and Kriedemann, 1975):

$$RGR = 100[\ln(\text{final weight}) - \ln(\text{initial weight})]/\text{days}$$

where RGR is % increase day^{-1}.

2.2. Effect of water motion on growth of thalli in the lagoon

Field experiments were carried out in Puko'o Pond, a dredged lagoon, on the southeast shore of Molokai, HI. We placed 200 g of fertilized thalli in "pearl baskets" (designed for the culture of pearl oysters) suspended in the water column (Nagler et al., 2003). The basket trays were 34 cm^2 and were made of, and covered by, 9-mm mesh netting. Clod cards were placed in each pearl basket for 24 h during each experiment to determine water motion as described above. The baskets were suspended in the water column by tying the basket to a rope stretched between floats, which were anchored in place with cinder blocks. Baskets were placed at 1.0-m intervals and suspended approximately 60 cm below the surface. Water temperature was approximately 27 °C during the trials. Placement of the baskets was planned so that the thalli would be exposed to a gradient of water motion in one arm of the lagoon. Trade winds induced relatively high water motion at the entrance to the arm of the lagoon, but water motion was attenuated inside, becoming nearly calm at the back of the arm. The growth trials lasted from 10 to 20 days. After the thalli were brought in from the lagoon, excess water was removed with a salad spinner, and they were then weighed to determine relative growth rates.

2.3. Effect of nutrient levels and water motion on sporeling growth in tanks

To determine the effect of water motion, substrate, and nutrients on sporeling growth, we conducted replicated experiments in 1.9-m diameter fiberglass tanks filled to depth with seawater. The two substrates, ropes and tiles, were inoculated with carpospores from cystocarpic thalli collected from the production areas where the seaweeds were being cultivated. Segments of rope (15 cm in length, 12 mm in diameter) and small ceramic tiles (2.54 cm^{-2}) were placed in the tanks under the cystocarpic thalli for 4 days to allow spore fixation to take place (Glenn et al., 1996). Microscope slides were also placed in the tanks to verify the release and attachment of spores to the substrates.

Following successful sporulation, five rope segments and eight tiles were placed in each of eight plastic-mesh fish baskets. Two baskets were placed in each of four tanks. Two of the tanks contained approximately 30 milkfish (*Chanos chanos*), each approximately 25 cm in length, and the other two tanks contained only seawater. The addition of

milkfish ensured an increase of ammonia nitrogen over that found in the low-nutrient lagoon, and which was sufficient to produce optimal growth of thalli when placed in cages (Nagler et al., 2003). Water was exchanged daily.

Water motion was controlled in each basket with air stones, and relative water velocity was measured with clod cards as described previously. Variation in water motion was achieved by varying the number of air stones per basket as in the growth experiments described above. Rope and tile substrates were removed after 47 days, and the number and length (mm) of sporelings per rope segment and density of sporelings per cm^2 on each tile was determined by examining them through a dissecting microscope.

2.4. Effect of water motion on sporeling growth in the lagoon

Spore-covered rope segments and tiles were also placed in the lagoon to allow spore development. Two rope-lines were strung in the lagoon, and pearl baskets were attached at 1.0-m intervals. Five baskets were tied to each line and suspended approximately 60 cm below the surface. Each basket contained four to five rope segments and 16 tiles. The lines were located so as to expose the baskets to a gradient of water motion as in the growth experiments described above. Clod cards were placed in each basket for 24 h to measure the water motion at each location. After 38 days in the lagoon, sporeling development on the tiles and rope segments in each basket was determined by measuring the density on 10 randomly selected 1-cm^2 sections on the rope segments and from eight randomly chosen tiles. Randomization was achieved through the use of a computer-generated random numbers table. In contrast to the tank experiments, the sporelings did not grow sufficiently in the lagoon to allow us to take meaningful length measurements. Instead, we used the density of visible sporelings to indicate the relative degree of spore development.

2.5. Statistical analysis

To compare the growth of thalli and development of sporelings with respect to water motion, we used a one-way analysis of variance (ANOVA). For cases where variances were not equal and transformations to alleviate the heterogeneity were unsuccessful, we used a Kruskal–Wallis, non-parametric, test instead of a one-way ANOVA.

3. Results

3.1. Effect of water motion on growth of thalli in tanks

In both of the tank trials with thalli, growth differed substantially among water velocity treatments ($F_{4,59} = 15.98$, $p < 0.0001$; Kruskal–Wallis $F_{4,58} = 12.44$, $p < 0.0001$, respectively) with RGR generally increasing with increased water motion (Fig. 1). Highest water velocities in the two trials were between 12 and 14 cm s^{-1}. In the first trial, mean RGR ranged from 4.3% to 8.5% day^{-1} with the lowest occurring at 0 cm s^{-1} and the highest RGRs occurring at 8.19 cm s^{-1}. Results of the second trial were
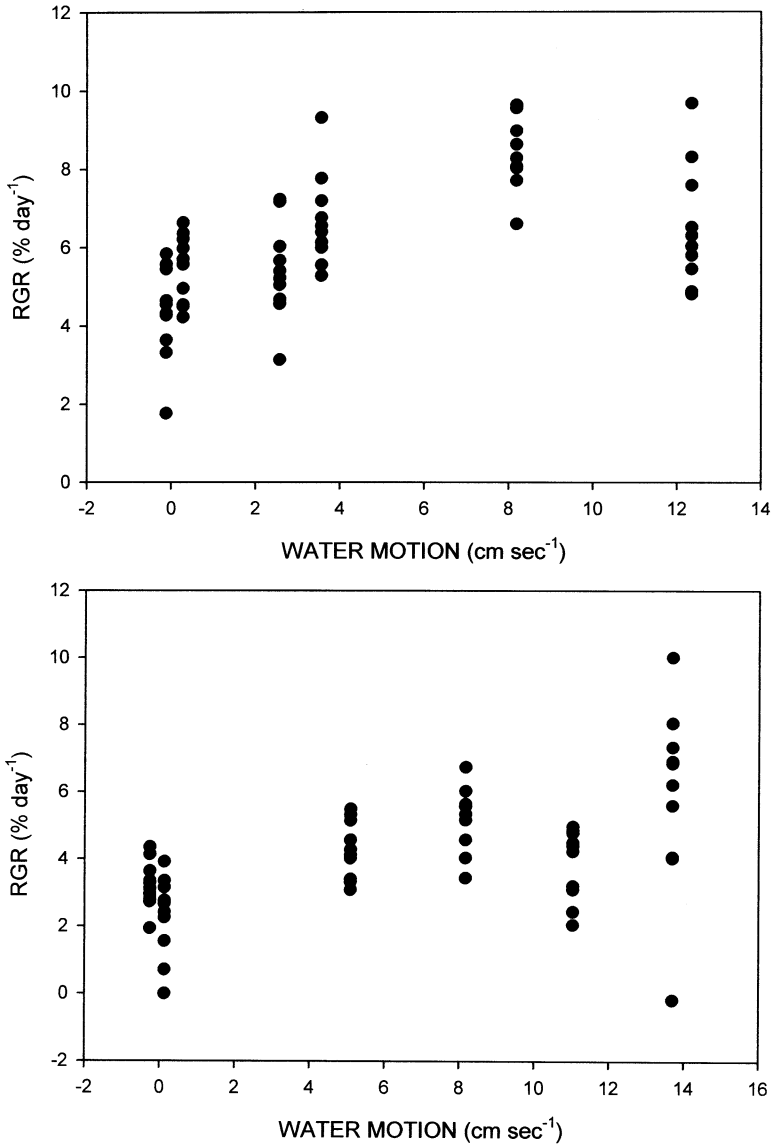
Fig. 1. Relative growth rates of individually tagged thalli exposed to varying water velocities in two tank experiments over periods of 10 (upper figure) and 8 (lower figure) days.

similar where mean RGR ranged from 2.3% to 5.9% day^{-1}, with the lowest at 0.13 cm s^{-1} and the highest at 13.7 cm s^{-1}.

Although growth clearly increased with increasing water motion, the optimal current for *G. parvispora* growth may be beyond the tested range of water velocities, since the growth rates did not reach a clear plateau in either trial.

3.2. Effect of water motion on growth of thalli in the lagoon

Similar results were obtained for thalli grown in the lagoon as shown in Fig. 2. Clearly, water motion had a substantial, and statistically significant ($F_{8,25} = 5.13$, $p = 0.0023$), effect on growth of these thalli. Growth rates were positively correlated with water velocity ($r^2 = 0.5583$). In the lagoon trials, water velocities ranged from 3.6 to 11.6 cm s^{-1}. Low and high RGR of 0.02% and 10.3% day^{-1} occurred at these extremes, respectively. In general, the growth rates obtained in the lagoon were similar to those observed in the tanks. Also, as in the tank experiments, a clear plateau in the relation between thallus growth and water velocity was not reached.

3.3. Effect of nutrients and water motion on sporeling development in tank trials

Sporeling development was followed, when possible, by measuring density as number per square centimeter and length. Spores only developed in the two tanks with milkfish, which had increased nutrient inputs. Mean ammonium levels in the tanks without milkfish were 0.61 mmol m^{-3}, while tanks containing milkfish had mean ammonium levels of 1.54 mmol m^{-3}. Only the data from the tanks with fish were included in further analyses. Clearly, elevated nutrient levels are critical for sporeling development. Water velocities ranged from approximately 0 to 6.5 cm s^{-1} in these trials.

Sporelings grew to lengths sufficient to be measured only on the ropes. Sporeling development on the rope segments did not differ significantly ($p > 0.05$) when measured either as length or as density. The mean lengths of sporelings per rope segment in the tanks ranged from 3 to 37 mm, and 11 of the 20 ropes produced sporelings >10 mm in length.
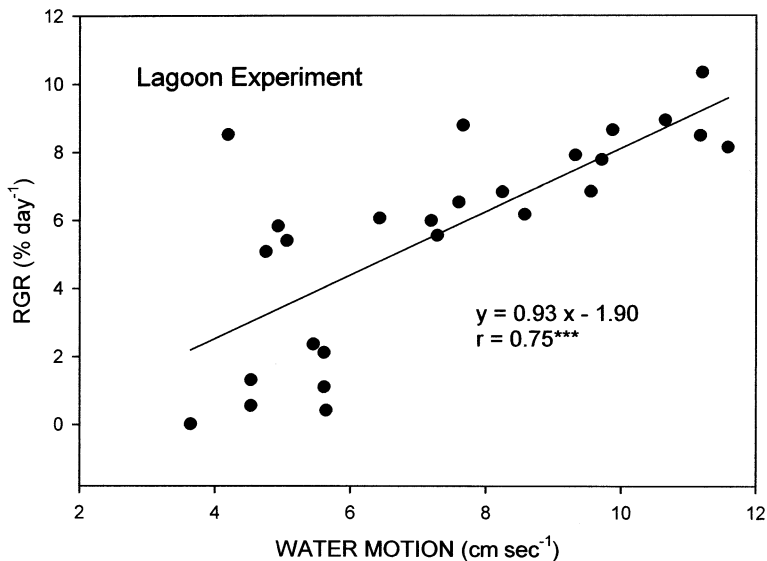

Fig. 2. Relative growth rates of thalli placed in pearl baskets in the lagoon on Molokai, HI.

Although the effect of water motion on density was not significant, at the lowest velocity (0.17 cm s^{-1}), there were 9.8 sporelings per segment, while at the upper velocities there were over 18 sporelings per segment. Results from the rope substrate were highly variable, possibly because of the variable exposure of sporelings within the rope strands. This decreased the power of the statistical tests and, subsequently, the probability of detecting an effect of water motion.

On the tiles, sporelings did not develop to sufficient lengths to be able to gather meaningful measurements, so only densities were compared among treatments. Water motion had a significant effect on sporeling density (Kruskal–Wallis $F_{2,57} = 10.65$, $p = 0.0001$). Mean sporeling density for the lowest water velocity was 1.4 cm^{-2} and was 11.6 cm^{-2} at the highest water velocity (Fig. 3).

3.4. Effect of water motion on sporeling density in lagoon trials

Sporelings cultured on rope and tile substrates in the lagoon did not grow sufficiently to be able to obtain meaningful length measurements, so density was used as a measure of sporeling development. In these trials, water motion ranged from 1.4 to 5.6 cm s^{-1}.

We found that water motion had significant effects on the density of sporelings both on ceramic tiles (Kruskal–Wallis $F_{4,79} = 3.43$, $p = 0.0125$) and on the rope segments (Kruskal–Wallis $F_{4,44} = 3.50$, $p = 0.0152$). The highest mean sporeling density on the tiles, 6.7 sporelings cm^{-2}, occurred at 4.5 cm s^{-1} (Fig. 3), while on the rope segments, the highest density of sporelings, 1.3 sporelings cm^{-2}, occurred at the highest water velocity (Fig. 3).

4. Discussion

Water motion enhances growth of *G. parvispora* in tank and lagoon culture. The maximum water velocities measured here, $10–15$ cm s^{-1}, are low compared to values of $20–40$ cm s^{-1} measured on the open reef in Hawaii (Glenn et al., 1999); our data suggest that we did not reach the optimal water velocity for growth. Increased water flow facilitates the uptake of essential nutrients such as inorganic nitrogen and carbon dioxide (Hurd, 2000). However, in our experiments, thalli were fertilized with nitrogen before being placed in the low-nutrient lagoon, thereby eliminating nitrogen as a limiting factor. We suspect that, in this case, the growth-enhancing effect of water motion may have resulted from increased availability of carbon dioxide. Although the growth of *G. parvispora* is usually limited by nitrogen, carbon dioxide can become limiting when sufficient nitrogen is available. If this is the case, then carbon dioxide limitation may occur in areas of the lagoon where nutrients are high, or the thalli are well fertilized, and water motion is low.

Diffusion of CO_2 in seawater is 10,000 times slower than in air (Denny, 1990); therefore, many tank culture systems, designed for intensive seaweed production, have improved yields by increasing the availability of CO_2 by injection, aeration, or increases in water flow. For example, the mean growth rate of thalli of *G. chilensis* aerated with air and 1250 ppm of CO_2 was over 14% day^{-1} while growth rates of control thalli, aerated just with air, were only about 9% day^{-1} (Gao et al., 1993). A growth study with
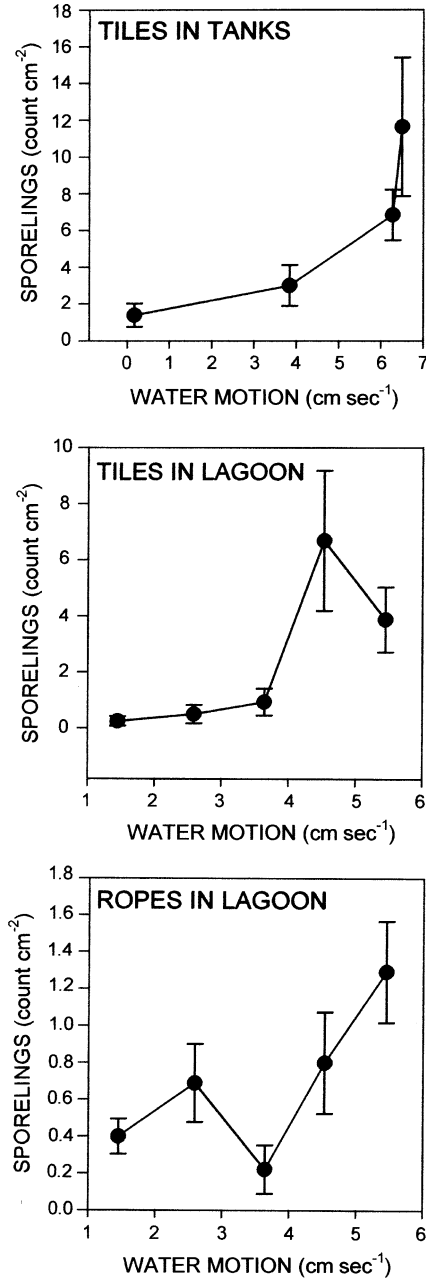
Fig. 3. Top: Sporeling densities on tiles from the tank experiment after a 47-day period. Sporeling densities were higher at higher water velocities. Middle: Sporeling densities on tiles placed for 38 days in the lagoon on Molokai, HI. Lower: Sporeling densities on ropes placed in the lagoon for 38 days on Molokai, HI. Error bars are standard errors.

nitrogen-saturated *Ulva rigida* showed increase growth when enriched with CO_2 (Gordillo et al., 2001). DeBusk and Ryther (1984) found that production of *Gracilaria tikvahiae* in tanks was five times greater in tanks where pH was controlled and supplemental CO_2 was provided.

Other factors may also contribute to the growth response to water motion. In laboratory studies with *Gracilaria conferta*, Gonen et al. (1993) found that rates of photosynthesis are affected by relative water motion, carbon content of the seawater, and plant condition. However, they found that photosynthetic rates were enhanced only at relatively low water velocities. The greatest response to increasing water velocity occurred at water velocities below 2 cm s^{-1} with only minor increases at water velocities between 2 and 8 cm s^{-1}. Subsequent studies (Gonen et al., 1995), based on laboratory experiments and theoretical models of mass transport, concluded that these effects of water motion on photosynthetic rate of *G. conferta* could not be attributed to increased carbon transport. It was suggested that the effect of water velocity was likely due to enhanced removal of photosynthetic inhibitors (OH^- ions) from the thallus surface.

In our study, the effect of water motion on sporeling development was similar to that observed for growth of thalli. In areas of the lagoon with higher water flow, greater densities of sporelings were found on the substrates. Since the substrates for all the treatments were seeded at the same time, the differences in density can be attributed either to the relative number of spores that developed in each treatment or to differential rates of sporeling attrition. High sporeling density is an important early indicator of eventual biomass production because adjacent sporelings can fuse to form more robust thalli in clonal, coalescing seaweeds such as *Gracilaria* (Santelices, 2001). Increased water motion may allow increased survival of newly germinated spores by the increased transport of low levels of nutrients past the surfaces of the sporeling. This would be consistent with the results of the tank trials in which there were no sporelings evident in the tanks without elevated nutrients.

It is likely that small-scale effects of water motion are very important in the development of spores into juveniles. For example, there were marked variations in sporeling densities between tiles and ropes subjected to the same water velocities. These differences may result from variation in the complexity of the substrate and its effect on water flowing past the surfaces at a micro-scale. The surface structure of rope is complex relative to that of tile, and this complexity may have limited the exposure of entwined spores to water motion.

There were also differences in sporeling response to water motion between the tank trials and the lagoon trials. There was a more linear relation between sporeling density and water motion in the lagoon trials. This may be due to the fact that ropes in the lagoon were suspended directly in the water column so the entire rope segment was equally exposed to water flow. In tanks, however, ropes were in baskets with air stones, so water flow may not have been equally distributed within the basket or over the rope-segment lengths, such as where the rope came into contact with the sides of the basket.

Besides water motion, these experiments showed that nutrients have a strong impact on sporeling development. We have previously been unable to get sporelings to consistently increase in length when placed on the reef (Glenn et al., 1998), and others have attributed this to a form of dormancy affecting sporelings when they reach approximately 1 mm in

length e.g., (Santelices, 1977, 1990; Levine, 1986). However, in the present experiments, we were able to obtain rapid growth of sporelings, up to 10–40 cm length, in just a few weeks when they were placed in fish tanks enriched in nutrients. No thalli developed on substrates placed in tanks without fish. Thus, the sporelings appear to behave like the mature thalli with regard to nutrient requirements (Nagler et al., 2003), requiring >1 μM ammonia to grow (Glenn et al., 1999). The waters of most unpolluted reefs in Hawaii typically contain less than 0.5 μM of inorganic nitrogen (Larned, 1998). Unlike mature thalli, they are too small to store enough nitrogen to go from initial size to harvest size with a single fertilization pulse, which is sufficient for a tripling of initial weight for thalli. Therefore, the sporelings require a continuous source of nutrients during early stages of development.

Our results are in contrast to previous field experiments that had shown that growth rates of *G. parvispora* thalli were not correlated with water motion (Glenn et al., 1998, 1999). In the field experiments, *Gracilaria*-bearing rocks were placed at various locations on the reef, most of which were low nutrient areas. In the previous cage experiments (Glenn et al., 1998), thalli were fertilized over a 24-h period once per month and achieved growth rates of only 2–4% even under conditions of high water motion. Subsequent experiments determined that, for optimum *G. parvispora* growth in this culture system, longer periods are needed for nutrient assimilation; and, even then, the thalli will deplete their nutrient stores in about 3 weeks (Nagler et al., 2003). So, in the earlier cage experiments, the thalli were nutrient limited. Similarly, in the field experiments (Glenn et al., 1999), water quality factors such as salinity, water motion, temperature, nitrate, ammonia, and phosphorus were measured to determine the effect on *G. parvispora* growth. Of all the factors analyzed, only ammonia was significantly correlated with growth. However, in these previous field trials, the effects of water motion may not have been significant because most of the algal patches were limited by nutrients, particularly ammonia nitrogen. Our present data clearly show that when the seaweed is adequately fertilized, removing nitrogen as a limiting factor, water motion has an important effect on growth of *G. parvispora*.

There have been a number of studies of the effects of water motion on the growth of *Gracilaria* species, and the results have been highly variable. Perhaps in some these cases there were, as we found, other limiting factors that masked the effects of water motion. Similar to our studies, Parker (1982) found that *G. tikvahiae* growth rates are enhanced at current speeds up to 7.5 cm s^{-1}, but beyond this velocity, growth rates plateau. Similarly, Conover (1968) also reported that water motion enhanced *G. verrucosa* with optimal growth at current speeds of about 50 cm s^{-1}. On the other hand, in another study of *G. verrucosa*, optimal growth rates were obtained at current speeds < 0.03 cm s^{-1} (Jones, 1959). It is likely in the latter case that other experimental variables had a confounding effect on the role of water motion, as in our previous experiments under nutrient-limited conditions. When nutrients are not limiting, we found that water flow is a major factor determining the growth rates of *G. parvispora* thalli. This data could be used to determine optimal locations for production of *G. parvispora* and to determine an optimal design and layout for cages. Existing methods utilize floating cages attached to lines in the lagoon, and the cages may inhibit water flow. Positioning the array with respect to maximizing water flow throughout the culture system could increase production.

Acknowledgements

References

Conover, J.T., 1968. The importance of natural diffusion gradients and transport of substances related to benthic marine plant metabolism. Botanica Marina 11, 1–4.

DeBusk, T.A., Ryther, J.H., 1984. Effects of seawater exchange, pH and carbon supply on the growth of *Gracilaria tikvahiae* (Rhodophyceae) in large-scale cultures. Botanica Marina 27, 357–362.

Denny, M.W., 1990. Terrestrial versus aquatic biology: the medium and its message. American Zoologist 30, 111–121.

Destombe, C., Godin, J., Remy, J.M., 1990. Viability and dissemination of spermatia of *Gracilaria verrucosa* (Gracilariales, Rhodophyta). Hydrobiologia 204/205, 219–223.

Friedlander, M., Dawes, C., 1984. Studies on spore release and sporeling growth from carpospores of *Gracilaria foliifera* (Forsskal) Borgesen var. *angustissma* (Harvey) Taylor: II. Photosynthetic and respiratory responses. Aquatic Botany 19, 233–241.

Gao, K., Aruga, Y., Asada, K., Kiyohara, M., 1993. Influence of enhanced CO_2 on growth and photosynthesis of the red algae *Gracilaria* sp. and *G. chilensis*. Journal of Applied Phycology 5, 563–571.

Glenn, E., Moore, D., Fitzsimmons, K., Azevedo, C., 1996. Spore culture of the edible red seaweed *Gracilaria parvispora* (Rhodophyta). Aquaculture 142, 59–74.

Glenn, E., Moore, D., Brown, J., Tanner, R., Fitzsimmons, K., Akutigawa, M., Napolean, S., 1998. A sustainable culture system for *Gracilaria parvispora* (Rhodophyta) using sporelings, reef growout and floating cages in Hawaii. Aquaculture 165, 221–232.

Glenn, E., Moore, D., Akutagawa, M., Himler, A., Walsh, T., Nelson, S., 1999. Correlation between *Gracilaria parvispora* (Rhodophyta) biomass production and water quality factors on a tropical reef in Hawaii. Aquaculture 178, 323–331.

Glenn, E.P., Gerhart, V., Nelson, S., Moore, D., Nagler, P., Machado, C., 2003. A community-based polyculture system in Hawaii that incorporates all the life stages of *Gracilaria parvispora* (Rhodophyta). In: Chapman, A.R.O., Anderson, R.J., Vreeland, V.J., Davison, I.R. (Eds.), Proceedings of the XVII International Seaweed Symposium, Cape Town, South Africa, Oxford University Press, Oxford, pp. 65–73.

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

Gonen, Y., Kimmel, E., Friedlaner, M., 1994. Attenuation of water velocity and incident light as a function of shape-parameters in *Gracilaria*. Journal of Applied Phycology 6, 381–390.

Gonen, Y., Kimmel, E., Friedlander, M., 1995. Diffusion boundary-layer transport in *Gracilaria conferta* (Rhodophyta). Journal of Phycology 31, 768–773.

Gordillo, F.J.L., Neil, F.X., Figueroa, F.L., 2001. Non-photosynthetic enhancement of growth by high CO_2 level in nitrophilic seaweed *Ulva rigida* C. Agardh (Chlorophyta). Planta 213, 64–70.

Hurd, C.L., 2000. Water motion, marine macroalgal physiology, and production. Journal of Phycology 36, 453–472.

Jones, W.E., 1959. Experiments on some effects of certain environmental factors on *Gracilaria verrucosa* (Hudson) Papenfuss. Journal of the Marine Biological Association of the United Kingdom 38, 153.

LaPointe, B.E., Ryther, J.H., 1979. The effects of nitrogen and seawater flow rate on growth and biochemical composition of *Gracilaria foliifera* var. *angustissima* in mass outdoor cultures. Botanica Marina 22, 529–537.

Larned, S., 1998. Nitrogen-versus phosphorus-limited growth and sources of nutrients for coral reef macroalgae. Marine Biology 132, 409–442.

Leopold, A.C., Kriedemann, P.E., 1975. Plant Growth and Development, 2nd Ed. McGraw-Hill, New York, 531 pp.

Levine, I., 1986. Environmental effects upon the development of the early life history stages of *Gracilaria coronopifolia* and *G. parvispora*. PhD dissertation, Department of Botany, University of Hawaii, 547 pp.

Nagler, P., Glenn, E.P., Nelson, S.G., Napolean, S., 2003. Effects of fertilization treatment and stocking density on the growth and production of the economic seaweed *Gracilaria parvispora* (Rhodophyta) in cage culture at Molokai Hawaii. Aquaculture 219, 379–391.

Nelson, S.G., Glenn, E.P., 2000. Spore and sporeling production in the development of aquaculture systems for species of *Gracilaria* and related seaweeds. In: Fingerman, M., Nagabhushanam, R. (Eds.), Aquaculture: Part A. Seaweeds and InvertebratesRecent Advances in Marine Biotechnology, vol. 4. Science, USA, pp. 1–21.

Nelson, S.G., Glenn, E.P., Conn, J., Moore, D., Walsh, T., Akutagawa, M., 2001. Cultivation of *Gracilaria parvispora* (Rhodophyta) in shrimp-farm effluent and floating cages in Hawaii: a two-phase polyculture system. Aquaculture 193, 239–248.

Parker, H.S., 1981. Influence of relative water motion on the growth, ammonium uptake and carbon and nitrogen composition of *Ulva lactuca* (Chlorophyta). Marine Biology 63, 302–318.

Parker, H.S., 1982. Effects of simulated current on the growth rate and nitrogen metabolism of *Gracilaria tikvahiae* (Rhodophyta). Marine Biology 69, 137–145.

Pearson, G.A., Brawley, S.H., 1996. Reproductive ecology of *Fucus distichus* (Phaeophyceae): an intertidal alga with successful external fertilization. Marine Ecology. Progress Series 143, 211–223.

Santelices, B., 1977. Water movement and seasonal algal growth in Hawaii. Marine Biology 43, 225–235.

Santelices, B., 1990. Patterns of reproduction, dispersal and recruitment in seaweeds. Oceanography and Marine Biology Annual Reviews 28, 177–276.

Santelices, B., 2001. Implications of clonal and chimeric-type thallus organization on seaweed farming and harvesting. Journal of Applied Phycology 13, 153–160.

Serrao, E.A., Pearson, G., Kautsky, L., Brawley, S.H., 1996. Successful external fertilization in turbulent environments. Proceedings of the National Academy of Sciences of the United States of America 93, 5286–5290.

Thompson, T., Glenn, E., 1994. Plaster standards to measure water motion. Limnology and Oceanography 39, 1768–1779.

Wheeler, W.N., 1980. Effect of boundary layer transport on the fixation of carbon by the giant kelp *Macrocystis pyrifera*. Marine Biology 56, 103–110.