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Cultivation of *Gracilaria parvispora* (Rhodophyta) in shrimp-farm effluent ditches and floating cages in Hawaii: a two-phase polyculture system

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Abstract

A culture system for the commercial production of the seaweed *Gracilaria parvispora* using shrimp-farm effluents for fertilization and floating cage-culture for grow-out has been developed on Molokai, HI. This two-phase system produces high-quality products for direct human consumption. The mean relative growth rates (RGRs) of effluent-enriched thalli in the cage system ranged from 8.8% to 10.4% day^{-1}, a significant increase over the growth (4.6% day^{-1}) of thalli fertilized with inorganic fertilizer. Thalli were also grown directly in the effluent ditch, where mean growth rates of 4.7% day^{-1} were obtained, less than in cage-culture. In the cage-culture system, thallus nitrogen content declined without fertilization. Effluent-enriched thalli grown in the cages steadily declined in nitrogen content, to about 1%, and their C:N ratios increased to between 20 and 30. However, when nitrogen-depleted thalli were transferred to the effluent ditch for enrichment, N content rapidly increased over 5 days to approximately 3%, with a C:N ratio near 10. Benefits of this two-phase polyculture system include enhanced growth of *G. parvispora* and the use of effluent from commercial shrimp farms as a resource. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: *Gracilaria parvispora*; Cage-culture; Nitrogen; Effluent; Molokai

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1. Introduction

The benefits of integrating the production of aquatic plants with the production of fish or invertebrates to recapture nutrients are well known (e.g. Neori et al., 1996; Dumas et al., 1998; Mathias et al., 1998; Truell et al., 1997). We found, in previous work, that growth of the edible seaweed *Gracilaria parvispora* is limited by nitrogen on the south reef of Molokai, HI, where the species is cultured commercially (Glenn et al., 1998, 1999). Here we describe a two-phase culture system for this species utilizing ammonium and other nutrients in shrimp effluent ditches to enrich thalli, which are then transferred to cages in a lagoon for grow-out.

The Molokai grow-out system was described in detail by Glenn et al. (1998) and is focused on the production of *G. parvispora*, a seaweed traditionally harvested throughout the Hawaiian islands. In this system, spore-laden substrata are produced in a small-scale, seaweed nursery operated by a local cooperative. These spore-laden substrata are provided to individuals for use in establishing out-plantings of seaweed, which can be harvested periodically, sold to the cooperative, and stocked in cages for grow-out. Typically, the thalli are fertilized weekly with inorganic fertilizer during grow-out. The success of the out-plantings has been highly variable and appears to depend primarily on the availability of ammonium nitrogen (Glenn et al., 1999).

On Molokai and other Hawaiian islands, shrimp farms are required to discharge effluent through drainage ditches in order to reduce the release of nutrients into the coastal waters. In this study, we examine how nutrients discharged from shrimp farms can be used as a resource in the management of nearby seaweed production facilities. In particular, we examine the nutrient concentrations and the production of *G. parvispora* thalli grown in an effluent ditch from a commercial shrimp farm. In addition, for the cage grow-out system, we compare the growth rates of thalli that were enriched in an effluent ditch to those that were fertilized with inorganic nutrients.

2. Materials and methods

2.1. Growth in effluent ditches

To explore the culture of the seaweeds in an effluent source as a viable alternative to the cage-culture system, we measured the growth of thalli in a drainage ditch of a shrimp farm. The ditch was 0.4–0.7-m deep, 1.5–8.2-m wide, extending 350 m from the shrimp farm to the final discharge site at a shallow pond. We divided the ditch, conceptually, into three sections, from nearest to the shrimp ponds (1) to the discharge site (3). For growth trials in the ditch sections, thalli were attached with cable ties to polypropylene ropes suspended across the ditch at a depth of 30 cm. The thalli were damp-dried, by blotting them between paper towels, and weighed. For each thallus, the relative growth rate (RGR, % day^{-1}) was calculated by the formula $\text{RGR} = 100 \times [\ln(\text{final weight}) - \ln(\text{initial weight})]/\text{time in days}$.

2.2. Growth in cage-culture

To determine if the effluent could be used as a source of enrichment as an alternative to the use of inorganic fertilization, we conducted growth trials in the cage-culture system. The cage-culture trials were carried out at the facilities of the Ke Kua'aina Hanauna Hou in Puko'o pond, a dredged, sheltered lagoon of Molokai, HI. An identification card was attached to each thallus with a cable tie. The tagged thalli were placed in $1.52 \times 1.22 \times 0.61$ m floating cages constructed of 1.25-cm mesh netting on PVC frames (Glenn et al., 1998). The treatments were: (1) initial fertilization by immersion in the effluent ditch; (2) regular fertilization with inorganic nutrients; and (3) non-fertilized. The non-fertilized thalli were taken from the cage-culture system and had not been fertilized for 2 weeks. RGRs of the individual thalli were determined each week for 4 weeks. The currently used weekly fertilization at the commercial grow-out facility involves immersing the thalli in shaded tanks supplied with technical grade fertilizer, 33 mg l^{-1} ammonium nitrate and 24 mg l^{-1} diamonium phosphate, for 18 to 24 h (Glenn et al., 1998).

2.3. Reciprocal transfers

Changes in carbon and nitrogen content of *G. parvispora* thalli were determined for thalli after reciprocal transfers between the effluent ditch and cages. Three cages, with approximately 0.4 kg of thalli, were taken from the effluent ditch and placed in the lagoon, and three with unfertilized seaweed from the lagoon were transferred to the ditch. Periodically, samples of approximately 27 g were taken from at least 10 thalli in each basket. Samples were taken immediately prior to transfer and then after 2, 4, 7, 9, 11, and 13 days of culture. Samples of thalli from each basket were pooled, dried to constant weight at 60°C, and analyzed for carbon and nitrogen content with a Carlo Erba NA 1500 CNS analyzer.

2.4. Water quality

All water quality measures were determined by the University of Hawaii's SOEST Analytical Services Laboratory (Honolulu, HI), which specializes in the analysis of seawater. Inorganic nutrients were analyzed with a four-channel, Technicon AA-II continuous-flow system (Technicon, 1977), and turbidity (ntu) was measured against appropriate standards with a turbidometer. Detailed procedures were provided by Glenn et al. (1999). Relative water motion over the exposure period was determined by the weight loss of plaster standards (Thompson and Glenn, 1994).

2.5. Statistical analysis

To compare the growth of thalli among the three sections of the effluent ditch, we used a one-way analysis of variance (ANOVA) with RGR as the dependent variable and ditch section as a fixed factor. To compare the growth of thalli in the cage-culture system, we used a one-way ANOVA with RGR as the dependent variable and fertilization treatment as a fixed factor. In the growth trial, some thalli were broken, so thalli with a final weight less than the original weight were excluded from further analysis.

3. Results

3.1. Thalli growth in the effluent ditch

Growth rates of *G. parvispora* thalli did not differ significantly among the three sections of the effluent ditch (ANOVA $F_{2,182} = 1.823$, $P = 0.164$). Mean RGRs were 4.3% day^{-1} ($n = 44$, s.d. = 2.6), 4.4% day^{-1} ($n = 79$, s.d. = 2.6), and 5.3% day^{-1} ($n = 62$, s.d. = 4.6) for sections 1, 2, and 3, respectively. The overall mean RGR of the experimental thalli in the ditch was 4.7% day^{-1}.

3.2. Thalli growth in cage-culture

When thalli from the ditch were transplanted to cages, their growth rates initially increased. Over the first week, the mean RGRs of thalli transferred from ditch sections 1, 2, and 3 were 9.2% day^{-1} ($n = 57$, s.d. = 3.5), 10.4% day^{-1} ($n = 84$, s.d. = 6.7), and 8.8% day^{-1} ($n = 84$, s.d. = 4.3), respectively. There was a significant effect of the source of thalli on the RGRs of thalli in cage-culture (ANOVA $F_{4,223} = 4.875$, $P = 0.001$). Table 1 shows that, in addition, the thalli transplanted from the ditch had a significantly higher mean relative rate of growth than thalli stocked from out-plantings. The mean RGR of tank-fertilized Thalli was 4.6% day^{-1} ($n = 9$, s.d. = 1.7) during the first week. This is about the same as the growth rates of the thalli in the effluent ditch and about half the mean growth rate of the thalli transplanted from the ditch to the cages. Also shown in Table 1 is that, without further fertilization, the growth rates of thalli from all groups declined over the next 3 weeks; and the thalli in the unfertilized group began to disintegrate.

3.3. Environmental conditions in the effluent ditch and cage-culture

There were no significant differences for any of the measures of water quality among the three sections of the ditch; so the data were pooled, and the overall means presented

Table 1
RGR (% day^{-1}) of *G. parvispora* thalli in cage-culture at Molokai over 4 weeks. The data are mean (standard error) and sample size (n). Cells with no data indicate that the cultures had deteriorated

Week/source	Ditch section 1	Ditch section 2	Ditch section 3	Cage fertilized	Cage un-fertilized
Week 1	9.2 (0.5), $n = 57$	10.4 (0.7), $n = 84$	8.8 (0.5), $n = 84$	4.6 (0.6), $n = 9$	1.7 (0.3), $n = 3$
Week 2	5.3 (0.6), $n = 52$	4.4 (0.3), $n = 71$	5.0 (0.8), $n = 71$	1.5 (0.5), $n = 4$	deteriorated
Week 3	2.7(0.4), $n = 33$	2.4(0.6), $n = 48$	2.3(0.4), $n = 45$	deteriorated	deteriorated
Week 4	1.9 (0.3), $n = 24$	2.8 (0.4), $n = 37$	2.0 (0.3), $n = 36$	deteriorated	deteriorated

Table 2
Water quality in the effluent ditch from a commercial marine shrimp farm and in the pond where production cages for the seaweed *G. parvispora* are located on Molokai, HI

Measure	Location	*n*	Units	Range	Mean	s.d.
PO_4	ditch	26	mmol m^{-3}	1.5–7.8	3.7	1.5
NO_3	ditch	26	mmol m^{-3}	0.1–12.7	2.9	3.4
NH_4	ditch	26	mmol m^{-3}	2.1–200.8	61.9	58.5
Turbidity	ditch	26	NTU	0.8–9.0	4.0	2.8
Motion	ditch	9	cm s^{-1}	2.3–3.3	2.6	0.5
PO_4	pond	3	mmol m^{-3}	0.9–1.4	1.1	0.3
NO_3	pond	3	mmol m^{-3}	0.3–2.6	1.1	1.3
NH_4	pond	3	mmol m^{-3}	0.8–1.3	1.1	0.3
Turbidity	pond	3	NTU	0.6–2.5	1.3	1.1

in Table 2. The means were associated with relatively large standard deviations, presumably a result of the activities related to the management of the shrimp ponds, such as the periodic exchange of water and draining the shrimp ponds during harvest. However, all of the nutrient levels were higher in the ditch than in the lagoon where the cages were located. Of particular interest was ammonia, with a mean concentration approximately 60 times higher in the effluent ditch (61.9 mmol m^{-3}) than in the lagoon (1.1 mmol m^{-3}). Mean turbidity was almost four times higher in the effluent ditch (4.0 ntu) than in the lagoon (1.3 ntu). Water motion in the ditch ranged from 2.3 to 3.3 cm s^{-1}, with higher rates usually being found in the last, narrowest, section.

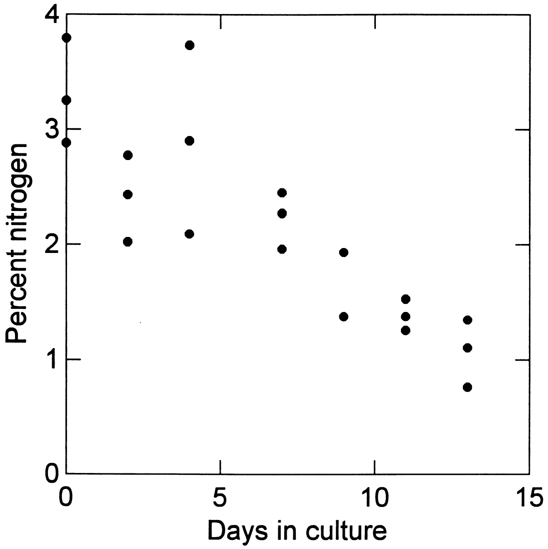

Fig. 1. The decrease in nitrogen content of *G. parvispora* thalli after being taken from the effluent ditch of a commercial shrimp farm and placed in a cage-culture system.

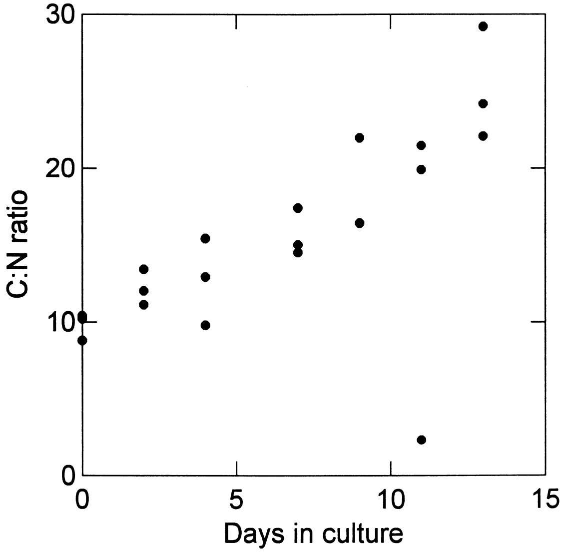

Fig. 2. The increase in C:N ratio of *G. parvispora* thalli after being taken from the effluent ditch of a commercial shrimp farm and placed in a cage-culture system.

3.4. Nutrient levels in transferred seaweed

Transferring seaweeds from the ditch to the cages at the pond site, or vice versa, resulted in changes in thallus nitrogen contents and C:N ratios. The mean nitrogen

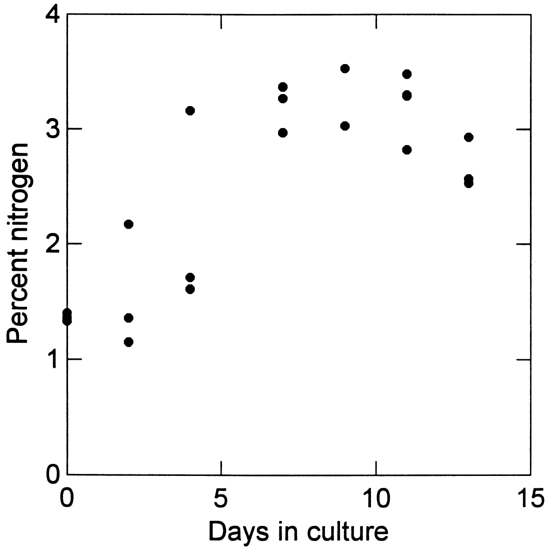

Fig. 3. The increase in nitrogen content of *G. parvispora* thalli after being taken from a cage-culture system and placed in the effluent ditch of a commercial shrimp farm.

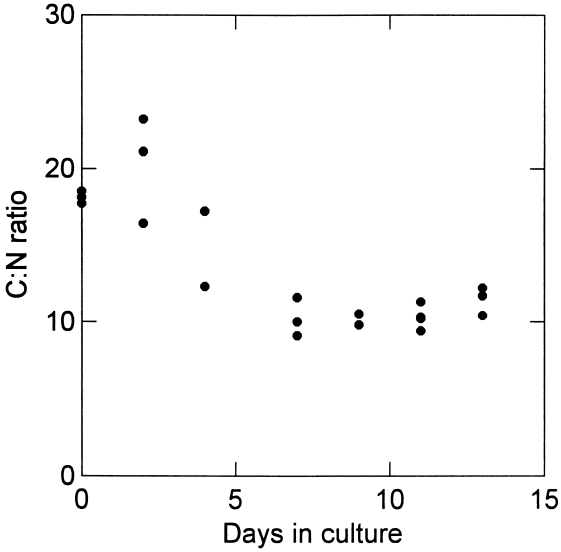

Fig. 4. The decrease in C:N ratio of *G. parvispora* thalli after being taken from a cage-culture system and placed in the effluent ditch of a commercial shrimp farm.

content and the C:N ratio of *G. parvispora* thalli within the effluent ditch were 3.1% ($n = 7$, s.d. = 0.9) and 10.7% ($n = 7$, s.d. = 3.1), respectively, on the day of transfer. When thalli from the ditch were grown in the cages at the lagoon site, their nitrogen content steadily declined to about 1% in 2 weeks (Fig. 1), and their C:N ratio increased steadily to levels between 20 and 30 (Fig. 2). However, when thalli grown in the cages were transferred to the ditch, their N content rapidly increased over 5 days and then remained near 3% (Fig. 3), while their C:N ratio declined rapidly over 5 days and stabilized around 10 (Fig. 4).

4. Discussion

4.1. Integrated aquaculture

In previous research, we found that the single most important factor limiting *G. parvispora* production on the reefs of Molokai is the availability of ammonia (Glenn et al., 1999). Of the reef out-plantings used to provide stock for the cage-culture operation, only about 15% were productive, and these were at sites that received some form of ammonia enrichment from the land. Our work illustrates how extensive seaweed aquaculture can be linked to the nutrient-rich effluent from land-based, shrimp production.

Species of seaweeds best suited for integrated aquaculture may differ depending on the type of culture operation. In Hawaii, we found *G. parvispora* to be well suited to culture in pond effluent in extensive production systems: it becomes established in the effluent ditches and persists as a dominant species without direct management. In contrast, Neori et al. (1996) found that for treating fish culture effluent with tank-cultured seaweeds, the green algae *Ulva* was highly effective, but *Gracilaria* performed poorly (Neori et al., 1996). In Chile, the siting of *G. chilensis* cultures in locations where they would receive flows of water from adjacent salmon cages allowed increased seaweed production (Truell et al., 1997).

4.2. Environmental benefits of growing seaweeds in effluent ditches

The discharge of ammonia-rich effluent from fish and shrimp can result in the eutrophication of nearshore areas (Costa-Pierce, 1996; Hopkins et al., 1995a,b; Wu, 1995). In Hawaii, the required effluent ditches from fish and shrimp farms not only provide environmental safeguards but can also be a valuable resource for the commercial cultivation of seaweeds. The culture of *Gracilaria* is promising in this regard as thalli of these species are capable of rapid uptake and storage of nitrogen (Rhyther et al., 1981). This allows thalli to exploit pulses of elevated dissolved nitrogen and to store the excess to support growth during periods of lower nutrient availability (D'Elia and DeBoer, 1978; Hanisak, 1987).

4.3. Effluent from shrimp farms as a resource

We found that an effective use of the effluent nutrients is for enrichment of the thalli, which are then transferred to other sites for grow-out. Thalli within the ditch had high nitrogen contents but relatively slow rates of growth, probably because of high turbidity within the ditch. In addition, thalli maintained in the ditch become covered with thin layers of fine silt, which is difficult to remove for marketing. However, the thalli increase in nitrogen content within the ditch, and this enrichment prior to their transfer to the cage-culture system results in enhanced growth compared to the current practice of fertilization with inorganic fertilizers. From the growth trials, it is apparent that the thalli in the cage-culture system require nitrogen fertilization. When thalli were transferred from the ditch to the cages, they exhibited increased rates of growth, which declined over time as the nitrogen stores of the thalli were depleted. If the increased growth of these thalli is sustained through periodic enrichment, substantially reduced times to harvest could be achieved. The average growing time in the system for thalli that are tank-fertilized with inorganic nutrients is 44 days (Glenn et al., 1998). The use of shrimp effluent for enrichment in place of the usual tank-fertilization would reduce the mean time in culture to approximately 21 days. Therefore, for Hawaii, we propose a two-stage polyculture system in which *Gracilaria* harvested from ditches is cleaned and multiplied in the ocean through cage-culture prior to sale.

Within the effluent ditch, the thalli were subjected to fertilization in pulses related to management of the shrimp ponds. Pulse fertilization has been effectively employed in the management of other seaweed culture systems. For example, Friedlander et al. (1991) found that pulse fertilization of *G. conferta* with ammonium resulted in increased growth and reduced epiphyte loads. This type of fertilization regime may be among the reasons that epiphyte problems have not developed in the cage-cultures of *Gracilaria* on Molokai.

However, results from one aquaculture system may not be directly applicable to others, particularly to those in other environments. In Israel, Friedlander and Ben-Amotz (1991) found that the result of fertilization on the growth of *G. conferta* varied considerably among seasons. Although seasonal variations in environmental conditions are much less pronounced in Hawaii, our results may be not be applicable under all environmental conditions.

Various strategies for integrating seaweed cultivation with fish culture have been successful. Buschmann et al. (1994) found that effluents from intensive tank cultures of salmon in Chile were effective in the production of *G. chilensis* in tank cultures; although they noted that epiphytes were a problem. In Sweden, Haglund and Pedersen (1993) found that *G. tenuistipitata* worked well in co-cultivation with rainbow trout, particularly during the warmer months of the year. In Israel, the green alga *Ulva lactuca* was found to be an attractive candidate for production with effluent from the culture of the gilthead bream *Sparus aurata* (Vandermuelen and Gordin, 1990). Also, Truell et al. (1997) reported that, in the coastal waters of Chile, the RGRs of *G. chilensis* thalli within 10 m of an array of salmon cages were 40% higher than those 150 m from the cages.

In this work, we illustrate how the concept of integrated production can be applied in the management of commercial aquaculture systems in Hawaii. As has been shown in the models developed by Petrell et al. (1993) for the culture of *Laminaria saccharina* near salmon cages in Canada, seaweeds used for human consumption have relatively high economic value and can contribute substantially to the economic viability of integrated systems. The integration of *G. parvispora* farming with land-based aquaculture offers similar opportunities for Hawaii and other areas with similar environmental conditions.

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