A sustainable culture system for *Gracilaria parvispora* (Rhodophyta) using sporelings, reef growout and floating cages in Hawaii

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Abstract

A culture system for the edible, red seaweed, *Gracilaria parvispora* Abbott (long ogo), was developed in Hawaii that utilized a hatchery to produce tetrasporophyte and gametophyte life stages of the seaweed, reef growout of sporelings to harvest size adults, and multiplication of the harvested thalli in floating cages prior to sale. A central cooperative operated the hatchery and floating cages, and marketed the product. Sporelings from the hatchery were distributed to coastal residents who established patches of seaweed on the reef and sold their harvest to the cooperative. Mean relative growth rate of seaweed in the cages over 52 weeks was 2.64% d^{-1} and productivity was 14.8 g m^{-2} d^{-1} (dry weight), within the range of intensive culture systems. Cage cultures were not sensitive to water motion over the range of 4–14 cm s^{-1} but growth and productivity tended to be higher in summer and spring than in winter. The culture system potentially overcomes problems that have hindered development of a sustainable supply of this species: low availability of wild stocks due to overharvesting; low productivity of spore cultures; and deterioration of vegetative cultures over time. Some of the elements may be applicable to other areas where wild stocks of *Gracilaria* have been overharvested. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Gracilaria parvispora Abbott (Abbott, 1985), or long ogo, was formerly the most important edible seaweed on Hawaii's reefs (Fortner, 1978). Due to overharvesting (Hoyle, 1978; Doty et al., 1986), it has not been available in large quantities in recent years. We previously developed a culture method for *G. parvispora* on Molokai, HI, in which sporelings were planted on a pond bottom for growout to harvest size (Glenn et al., 1996a,b). However, the productivity of the system was low due to the slow development of *G. parvispora* sporelings in the pond. We have modified the system so that hatchery products are now used to establish extensive patches of seaweed in favorable locations on Molokai's open, south reef; these are harvested periodically by coastal residents and sold back to the hatchery. Part of the harvest is used as a source of spores in the hatchery, but the majority is stocked into floating cages to increase the yield, then marketed (Fig. 1). This is intended to be a sustainable, community-based system that enhances rather than diminishes the reef stocks (Glenn et al., 1996b).

We report on the operation of the culture system over one year at a pilot stage on Molokai. Data is presented on the overall performance of the system, and on individual components of the system, including sporeling initiation in the hatchery, the establishment of reef populations, and effects of water motion and seasonality on cage cultures.

2. Materials and methods

2.1. Location of experiments and operation of hatchery

Experiments were carried out from October 1995–July 1997 on the extensive, south reef of Molokai, HI (Glenn and Pfund, 1992). *G. parvispora* was first introduced from sporelings brought from Oahu in 1984 (Doty et al., 1986) and has been in continuous culture on Molokai since 1992 (Glenn et al., 1996a,b). The hatchery and cage cultures were carried out at Puko'o pond, a dredged, sheltered lagoon with connection to the open reef, operated by Ke Kua'aina Hanauna Hou, a nonprofit organization developing aquaculture enterprises for coastal residents. Spores were settled onto substrates using modifications of our previous procedures (Glenn et al., 1996a), which were based on methods developed by Doty et al. (1986), Levine (1986) and Doty and Fisher (1987).

The hatchery consisted of four rectangular tanks (0.6 m deep, 3 m² area) filled with sand-filtered, aerated seawater and covered with 75% shade cloth. Approximately 750 flat, river rocks (5–10 cm diameter) were placed on the bottom of each tank in plastic trays (40 cm × 40 cm) and overlain with adult, spore-releasing plants. *G. parvispora* has a three phase life history typical of floridian red algae, with free-living gametophytic and tetrasporophytic phases while carposporophytes (cystocarps) develop as visible bumps on female gametophytes (Sze, 1993). We used a mixture of cystocarpic plants and tetrasporophytes as the source of spores to ensure that all life stages were present in the culture products, to develop self-regenerating populations of plants on the reef. Substrates were left in hatchery tanks for 48–72 h. Microscope slides were placed in each
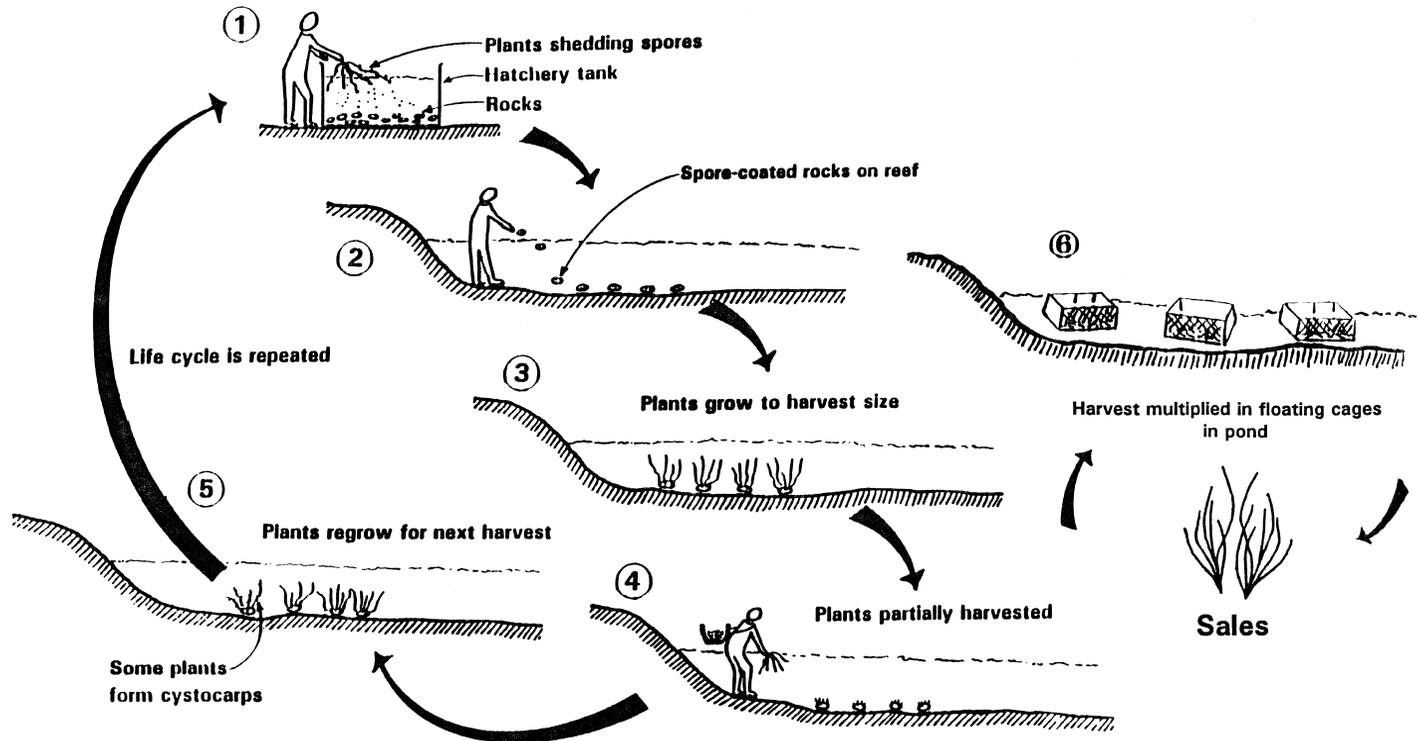
Fig. 1. Steps in growing *G. parvispora* on Molokai, HI: (1) rocks are inoculated with spores in a hatchery tank operated by a central cooperative; (2) spore-coated rocks are distributed to coastal residents, who establish patches of seaweed on the reef; (3) some of the patches produce plants which (4) are harvested and sold to the central cooperative, which (5) uses them to start new hatches or (6) multiplies them in cage cultures before sale in Honolulu.

tank and examined at the end of the hatch to confirm that spore release occurred (Glenn et al., 1996a,b).

2.2. *Quantifying spore release from tetrasporophytes and cystocarpic female gametophytes*

We conducted experiments on spore release to determine the minimum amount of adult material required to initiate a hatch. Variable amounts of smooth thalli (assumed to be a mixture of tetrasporophytes and male gametophytes) or cystocarpic thalli were placed in 20-l buckets (30 cm diameter) of filtered, aerated seawater over two microscope slides per bucket for 48 h. Three experiments each with smooth and cystocarpic thalli were conducted in June–July 1996, using five levels of seaweed in one bucket each. The levels tested were 0.1–0.5 kg in 0.1 kg increments of smooth, or 0.05–0.25 kg in 0.05 kg increments of cystocarpic thalli; the same levels were repeated each experiment, hence $n = 3$ buckets per level over three experiments. For data analysis, density of inoculum was expressed as kilogram per square meter of bucket bottom area so the results could be extrapolated to tanks (1.4–7.0 and 0.7–3.5 kg m^{-2} of smooth or cystocarpic thalli, respectively). Buckets were placed in a rectangular tank partially filled with water, which served as a water bath to control temperatures during the experiment. Control buckets which contained only slides and filtered seawater were included in two experiments.

Microscope slides were rinsed in filtered seawater to remove unattached spores and debris, then examined under a compound microscope to determine the extent of spore recruitment. Attached spores were not distributed homogeneously on slides but tended to occur in clumps, hence standard, algal cell enumeration methods that assume a random distribution pattern could not be used to estimate mean spore density over the slide (Guillard, 1973). Therefore we adapted the procedure of Doty et al. (1986) for counting *Gracilaria* sporelings on rocks, in which the most densely populated areas of substrate are selected for counting to estimate maximum spore density per substrate. Rinsed slides were scanned for settled spores at 100 × magnification at the end of the experiment, and a field of view judged to contain the maximum density of spores was chosen for counting under 400 − magnification. Although this procedure estimates maximum rather than mean spore density, it is a useful indicator of *Gracilaria* spore settling success because viable plants tend to emerge from areas of initial high spore density, through coalescence of filaments (Santelices, 1990).

2.3. *Nursery stage and outplanting sporelings onto the reef*

Rocks were removed from hatch tanks and planted in a shallow area of the pond (less than 0.3 m at low tide) that served as a nursery area. The purpose of the nursery was to allow sporelings to develop to visible size in a sheltered location before the substrates were placed on the reef, and to cull out substrates that did not develop sporelings. Outplanting of sporelings onto the reef was conducted in cooperation with local residents who were recruited to become seaweed growers. Starting in October, 1995, Ke Kua'aina conducted workshops on *Gracilaria* culture. Residents who expressed an

interest in growing seaweed were provided with spore-coated rocks which they placed in patches on the reef, either in traditional shoreline fishponds which have fallen into disrepair and are partially open to the sea, or on the reef itself.

Approximately 250 rocks with vigorous sporeling growth were chosen from the nursery area for outplanting at each of 24 locations. They were transported in ice chests containing seawater and placed out in water no deeper than 1 m at low tide to facilitate harvesting, using approximately 1 rock per square meter. Growers harvested seaweed from the patches for sale to Ke Kua'aina when thalli reached 20 cm or longer. The holdfasts were left on the rocks to grow another crop.

2.4. Floating cage cultures

Cage cultures were conducted for 52 weeks (June 1996 through June 1997) based on methods described by Hanisak (1987), but incorporating a weekly fertilizer treatment originally developed for tank cultures (Hanisak, 1987). Loose thalli were stocked into floating cages framed with 3.1 cm diameter schedule 200 PVC tubing and covered with light, 1.25 cm mesh, flexible plastic netting (bird netting, Memphis Net and Twine, Memphis, TN). Small holes were drilled in the PVC tubes to allow water to enter and air to exit, thereby reducing buoyancy so that all but the top surface of the cage was below the water surface when it was loaded with seaweed. The netting was attached to the frame with cable ties. Netting extended over the top of the cage as well as the sides and bottom to prevent turtles from entering. Cages were 1.52 m × 1.22 m on top and bottom and 0.61 m deep. Materials for each cage cost approximately US$20 and 1 h was required for construction.

Cages were stocked initially with 4.5 kg of seaweed (Hanisak, 1987) and floated in 1.5–2.0 m depth of water in sheltered bays in Puko'o pond, with 10–30 cages tied together in a pod and anchored to the bottom by a rope tied to a concrete block. As many as 140 cages were kept in the pond at one time. Each cage was returned to shore once a week. Seaweed from a pod of cages was pooled, rinsed with fresh water from a hose to remove silt and epiphytes then weighed. The amount of seaweed that exceeded the restocking requirement for the cages was cleaned and sold. The remaining seaweed was placed in 1.67 m^3 outdoor, aerated tanks of seawater (22 kg seaweed per tank) containing 33 mg l^{-1} ammonium nitrate (34% N) and 24 mg l^{-1} diammonium phosphate (53% P_2O_5, 21% N) as technical-grade fertilizers. Tanks were covered with 75% shade cloth and the seaweed was left in the tanks for 18–24 h, then restocked into cages and replaced in the pond. Cages were used for 4 weeks, then allowed to dry for a week on shore to kill epiphytes before putting them back in service. Data for 46 of the 52 weeks were available for analysis; data records for 6 weeks were lost.

2.5. Determining the effect of water motion on growth rates

In determining the best spots to anchor cages in the hatchery pond, the degree of water motion appeared to be a primary variable that could affect growth. Water motion varied from generally still water in sheltered locations, to choppy in areas exposed to the prevailing trade winds and to waves entering the pond through the inlet. Five cages were

anchored in the pond at five different locations which received different degrees of water motion due to wave action; growth rates and water motion were measured weekly for three weeks in June and July 1996. Cages were stocked with 4.5 kg seaweed, which was removed and weighed weekly for three weeks. Water motion was measured once a week by placing two plaster standards in each basket for 24 h (Glenn and Doty, 1992; Thompson and Glenn, 1994). Average water motion over the exposure period was estimated by the fraction of weight lost. The standards were calibrated by placing them on a rotating arm in a seawater tank (Thompson and Glenn, 1994).

2.6. Calculations and statistical analyses

Relative growth rates (RGR, % d^{-1}) of seaweeds in cages were calculated by the formula, $RGR = 100 \times [\ln(\text{final weight}) - \ln(\text{initial weight})]/\text{time in days}$. The residence time of seaweed in cages when RGR and weight increase were known was calculated by rearranging the formula to solve for t: $t = [\ln(\text{final weight}) - \ln(\text{initial weight})]/(RGR/100)$. Productivity of cages in grams dry weight per square meter per day was calculated by the formula gdw m^{-2} $d^{-1} = 0.18 \times [(\text{final weight} - \text{initial weight})/1.85 \text{ m}^2]/\text{time in days}$, where 0.18 is the proportion of dry weight to wet weight (mean of 10 samples of whole thalli) and 1.85 m^2 is the area of the top of the basket. Except for cages that were part of experiments, growth data were not taken on each individual cage but for all cages at a given location in the pond, since the cage contents were pooled before weighing each week.

Statistical tests were based on methods in Li (1964) and a computer statistics program (CoStat). Spore counts on slides were analyzed by linear regression to estimate the number of spores that could be expected to be settled per kilogram of each type of inoculum. Due to uneven variances among cages, the water motion experiment was analyzed using the nonparametric, Kruskal–Wallis test with RGR and water motion as dependent variables and location in the pond as the independent variable (treatment).

3. Results

3.1. Spore release by smooth and cystocarpic thalli

Sporeling counts on slides were pooled by type of inoculum across the three experiments to determine sporelings settled for each kilogram per square meter of inoculum (Fig. 2). For smooth thalli, 1 kg m^{-2} inoculum settled 421 sporelings cm^{-2} onto high-density areas of slides whereas 1 kg m^{-2} of cystocarpic thalli settled 3204 sporelings cm^{-2}, eight times higher. Initial spore counts greater than 200 cm^{-2} are normally sufficient to produce one or more viable plants per rock (Glenn et al., 1996a). Based on these results, 1 kg m^{-2} of inoculum containing an equal mixture of smooth and cystocarpic thalli was used in hatch tanks to inoculate rocks. This inoculum should theoretically produce a high density of sporelings consisting mainly of tetrasporophytes, the market product, but containing 10% gametophytes to complete the life cycle.
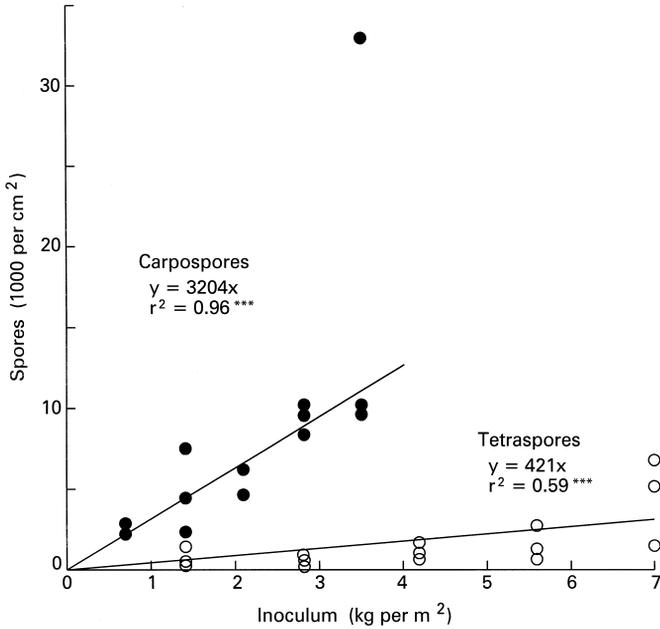
Fig. 2. Regression equations of sporelings density on slides versus inoculum density using thalli releasing carpospores (closed circles) or tetraspores (open circles) as source of spores. The outlying point at 33,000 carpospores cm^{-2} was not used in the regression equation.
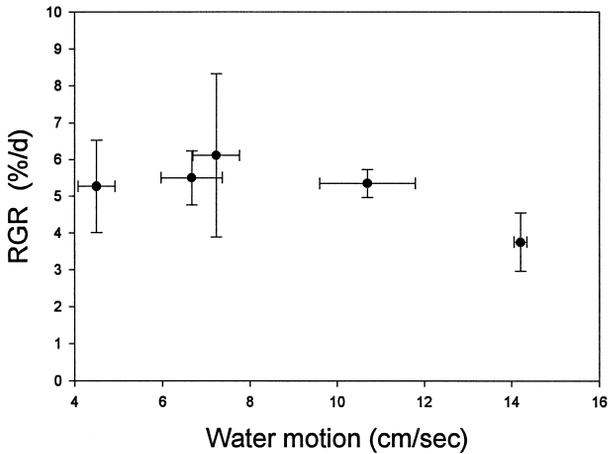
Fig. 3. Relative growth rates (RGR) and water motion over 3 weeks in floating cages of *G. parvispora* anchored at five locations in a pond on Molokai, HI. Data points are the means of the weekly measurements; error bars show S.E.M.

3.2. Establishing Gracilaria patches on the reef

By June, 1996, 4 of the original 24 reef plantings were producing significant amounts of seaweed, which was harvested periodically by the growers and sold to Ke Kua'aina. The remaining plantings failed to produce harvestable thalli although they were inspected for growth through June 1997. The successful patches were all in shallow, silty water within 100 m of shore, but the factors controlling productivity were not determined in this study. Although tetrasporophytes were expected to dominate the hatchery products, from 23–27% of thalli in each batch of seaweed harvested from outside patches were cystocarpic.

3.3. Effect of water motion on floating cage cultures

Water motion varied significantly ($P < 0.01$) among the five study sites in Puko'o pond, ranging from 4 cm s^{-1} to 14 cm s^{-1}, but RGRs did not differ significantly ($P > 0.05$), with all sites supporting RGRs $> 3.5\%$ d^{-1} regardless of water motion
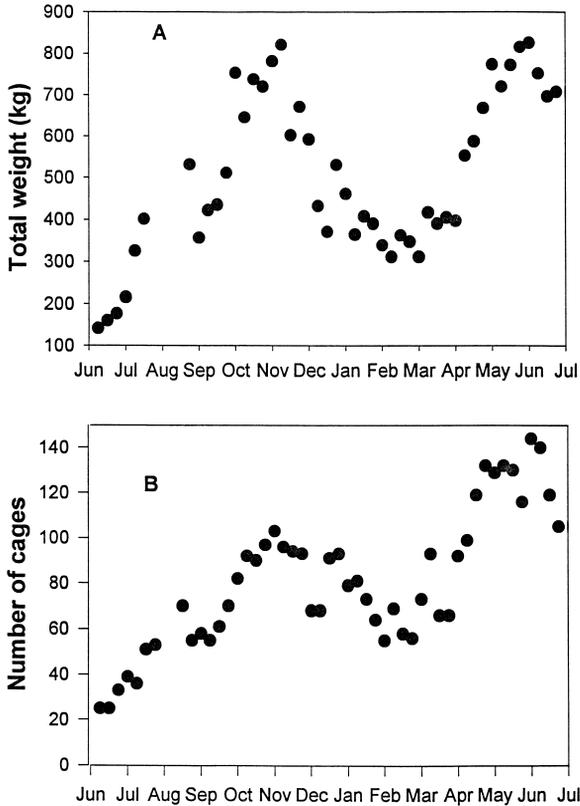
Fig. 4. Standing crop (a) and number of cages (b) of *G. parvispora* grown in floating cage culture over 52 weeks in a pond on Molokai, HI.

(Fig. 3). The five locations became the anchoring points for cages for the remainder of the pilot production period.

3.4. Performance of the pilot system

The standing crop in cages (Fig. 4a) increased from 150 kg in June 1996 to 850 kg in November 1996 as more seaweed from outside sources became available and more cages were added (Fig. 4b). The total standing crop decreased in winter due to slowing growth rates, but increased again to 800 kg by June 1997. Cumulative sales of seaweed and purchases of new material from the four outside growers over the study are in Fig. 5. The pilot system was able to maintain relatively stable levels of sales and purchases over the experiment, indicating that the system is potentially capable of sustained production. Weekly production data from the pilot system was analyzed from September 1996, when stocks were first built up to 500 kg, to June 1997 (Table 1). Over this
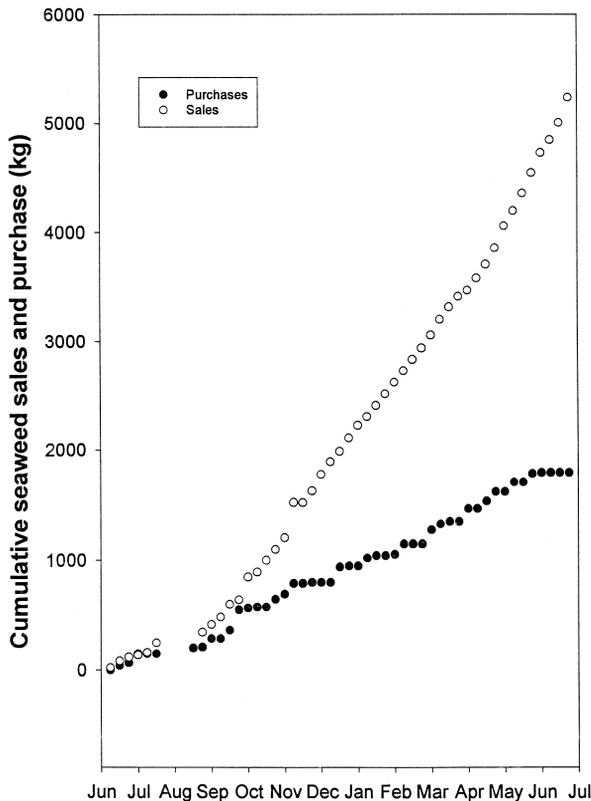
Fig. 5. Cumulative sales (open circles) and purchases (closed circles) of *G. parvispora* grown over 52 weeks in a pilot system on Molokai, HI. Sales represent the amount of seaweed harvested from cages and sold to Honolulu wholesalers; purchases represent the amount of replacement stock supplied by local growers for growout in cages.

Table 1
Performance of a pilot culture system for *G. parvispora* on Molokai, Hawaii, showing mean values of production parameters, standard errors of means (S.E.M.) and the number of weeks for which data were available (*n*)

	Mean	S.E.M.	*n*
Purchases from growers (kg week^{-1})	41.3	7.4	43
Sales (kg week^{-1})	131.1	7.9	43
Stocking density in cages (kg m^{-2})	5.2	0.23	43
Relative growth rate in cages (% d^{-1})	2.64	0.17	46
Productivity in cages (gdw m^{-2} d^{-1})	14.8	1.0	46

period, weekly imports averaged 41.3 kg while sales were 131.1 kg week^{-1}, indicating that the stocks were multiplied 3.2 times in the cages; stocking density in cages over this period was 5.2 kg m^{-2}. From June, 1996 to June, 1997, mean RGR and productivity were 2.64% d^{-1} and 14.8 gdw m^{-2} d^{-1} (Table 1). The mean residence time of seaweed in cages was calculated to be 44 days based on the mean RGR and a mean weight
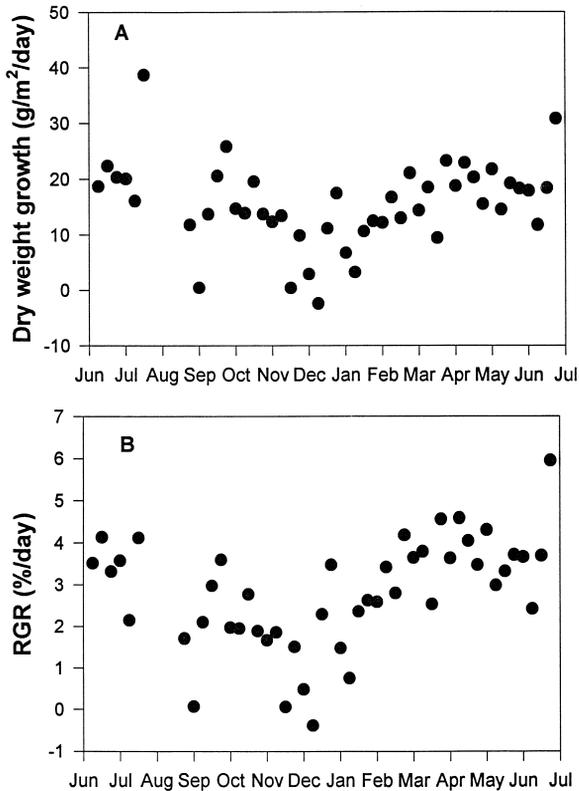
Fig. 6. Dry weight productivity (a) and relative growth rate (b) of *G. parvispora* grown in floating cage cultures over 52 weeks in a pond on Molokai, HI. Values are the means across locations in the pond.

increase factor of 3.2 over the study. Both productivity (Fig. 6a) and RGR (Fig. 6b) dipped in winter.

4. Discussion

Although the output from the pilot system was relatively modest, it provided a consistent source of long ogo to Honolulu wholesale markets for the first time since the wild stocks were overharvested (Glen Tanoue, Tropic Seafood and Vegetable; Guy Tamishiro, Tamishiro's Market, private communications). All the culture elements in the pilot system were necessary for sustainable and productive culture of *G. parvispora*. When we tried to grow the seaweed from sporelings alone, production levels were low. Floating cages could be used to multiply the harvest, but loose thalli in cages tend to deteriorate over several months (Hanisak, 1987), so a continual supply from outside was needed. Puko'o pond did not support sufficient production to keep the cages stocked, so outside patches on the reef were necessary. Without a hatchery to establish and replenish those outside patches, the reef stocks would be overexploited, similar to what happened on Oahu (Hoyle, 1978; Doty et al., 1986). In contrast, the present culture system enhanced the reef stocks of *G. parvispora* on Molokai.

Our low success rate in establishing new reef patches (approximately 17%) was similar to the results of Doty et al. (1986). *Gracilaria* sporelings are subjected to predation, overgrowth by other organisms, and siltation. Hence, it is not surprising that the sporelings disappeared at the majority of sites. Once established, however, the patches appear to be resilient and capable of supporting sustained harvesting. The finding that 23–27% of harvested plants were cystocarpic, despite the predominance of tetrasporophytes in the hatchery products, indicates that gametophytes were produced from tetrasporophytes within the patches. Twenty-five percent cystocarpic thalli, the mean of the observed results, are expected in an ideal population in which all the plants are in a reproductive state, with 50% tetrasporophytes, 25% male gametophytes and 25% fertilized, female gametophytes (Hoyle, 1978; Glenn et al., 1996a).

The cage culture results were similar to those obtained for *Gracilaria tikvahiae* in Florida, which could be cultured in cages for several weeks in nutrient-rich water or for up to 8 months in oligotrophic water before thalli deteriorated (Hanisak, 1987). Growth rates of 2.64% d^{-1} and production rates of 14.8 gdw m^{-2} d^{-1} are within the range of intensive tank cultures of *Gracilaria* while production per unit area is 2–5 times higher than results from ponds or other types of bottom culture (LaPointe et al., 1976; Hanisak, 1987; Santelices and Doty, 1989; Ugarte and Santelices, 1992). For example, Bravo et al. (1992) obtained yields of *Gracilaria chilensis* equivalent to 4.9 gdw m^{-2} d^{-1} from intertidal enclosures in Chile, stocked at approximately the same density as our cage cultures. Cage culture of seaweeds in general is a hybrid method (e.g., Hirata and Kohirata, 1993), more intensive than pond culture yet much less expensive to establish and maintain than land-based tank cultures, which require aeration and up to 30 pumped water exchanges per day for maximum productivity. Floating cage cultures utilize the water exchange provided by the sea. *G. parvispora* was productive even under conditions of low water motion, by contrast to *Eucheuma* and *Kappaphycus*, two other red

seaweeds grown for gels on tropical reefs, that showed a positive response to water motion up to 20 cm s^{-1} (Glenn and Doty, 1992).

The present method of establishing reef populations for harvest by outside growers appears to be workable on Molokai, and may be a good model for *Gracilaria* culture in other locations around the world where wild stocks are under pressure from overharvesting. This method should be sustainable over time, since it includes enhancement of wild stocks as an essential part of the overall aquaculture method.

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