Sustained high yields of *Gracilaria* (Rhodophyta) grown in intensive large-scale culture

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Abstract

Gracilaria ferox J. Agardh was grown continuously in large, outdoor tanks under a pulse-fed nutrient regime for four years. Productivity ranged from 21.4 to 59.2 g d. wt m^{-2} d^{-1} with a mean of 39.7 g d. wt m^{-2} d^{-1} over the entire study period. Because the cultures were maintained under non-nutrient limiting conditions, productivity was regulated primarily by seasonal changes in light and temperature, which accounted for 75% of the variability of growth in algal yields. Salinity ranged from 31.0 to 36.5‰ and had insignificant effects on growth within this range. The original vegetative strain was maintained over the entire study without the need for additional supplementation from field-collected stock. Because of the pulse-fed nutrient supply, epiphytic growth on the target species was negligible (< 3% total biomass) throughout the study. The yields attained in this study rank among the highest reported for any intensively managed photosynthetic crop and demonstrate the feasibility of growing red macroalgae like *Gracilaria* at a sustained high yield in a large-scale, land-based culture system.

Introduction

Macroalgae have been an important, commercially valuable resource for food and chemicals for centuries (Bird & Benson, 1987). Historically, seaweeds were harvested from natural populations as a source food, fodder, soil conditioners and pharmaceuticals. The increasing demand for hydrocolloid extracts during the past 40 years has led to dramatic growth of the seaweed industry (McLachlan et al., 1986). Consequently, seaweeds have become the second largest aquaculture product worldwide in terms of biomass (FAO, 1996). This impressive production is made possible by the high intrinsic growth rates of macroalgae which allow them to rank among the most productive plants on earth whether in nature (Mann, 1973) or in cultivation (Lapointe et al., 1976).

Because of the increasing demand for seaweeds, studies have explored the limits of macroalgal productivity in a parallel manner to that conducted with commercially valuable terrestrial crops (Bugbee & Salisbury, 1988). During the 1970s, J.H. Ryther and co-workers evaluated numerous species of red, green and brown macroalgae for their potential growth rates and dry weight (d. wt) yields. They demonstrated that the genus *Gracilaria* was the most attractive candidate because of its ability to achieve high yields and also produce commercially valuable extracts (Lapointe & Ryther, 1978). Lapointe and Ryther (1978) obtained year-round yields of *Gracilaria foliifera* (= *G. tikvahiae*) of 35 g d. wt m^{-2} d^{-1} with maximal yields of 45 to 50 g d. wt m^{-2} d^{-1} in small-scale, nutrient-enriched cultures. *Gracilaria tikvahiae* growth has been shown to be maximal at high irradiance and temperatures between 25°C and 30°C (Lapointe et al., 1984b), with vigorous agitation (Haglund & Pedersén, 1993) and high seawater exchange rates (Lapointe & Ryther, 1978; DeBusk & Ryther, 1984). A se-

ries of studies have demonstrated the intensive culture of *G. tikvahaie* for hydrocolloids, wastewater treatment, and biomass for energy conversion (Ryther, 1977; Lapointe & Ryther, 1978; Hanisak & Ryther, 1986; Hanisak, 1987). Several species of *Gracilaria* have also been grown on rafts to produce 'seamoss', a seaweed-based drink popular in the Caribbean area (Smith et al., 1984).

Although small-scale, intensive cultivation of seaweeds has demonstrated some very high yields, there are bioengineering concerns with the extrapolation of such yields to large-scale systems. Huguenin (1976) evaluated the problems and potential of intensive seaweed systems and stressed the uncertainty of achieving sustained high growth rates at large scales. In addition, continuous nutrient enrichment of *Gracilaria* cultures causes problems of overgrowth by undesirable epiphytic species which reduce yields of the targets species and can result in culture loss (Lapointe & Ryther, 1978). Pulsed nutrient supply strategies have been used to minimize epiphyte contamination while producing high yields of *G. tikvahiae* both in land-based tank cultures (Ryther et al. 1981) and in raft cultures in the Florida Keys (Lapointe, 1985; Lapointe & Ryther, 1978). Long-term studies demonstrating the sustainability of stocks and the feasibility of large-scale, pulse-fed culture systems are needed to demonstrate the commercial potential for intensive large-scale production.

This study describes a land-based, pilot-scale raceway culture system that produced highly productive cultures of *Gracilaria ferox* J. Agardh at the University of Miami's Experimental Hatchery for four years without replenishing or supplementing the original inoculum. The objective of this study was to determine whether *Gracilaria* could be grown in a sustained manner at a rate sufficient to supply a reliable year-round source of food for a large-scale culture of the mollusk *Aplysia californica* in South Florida (USA).

Materials and methods

The study was conducted from October 1992 to June 1995 at the Experimental Hatchery of the University of Miami, Florida, USA (25° 44.0′N; 80°9.4′W). Fifteen kg of a vegetative strain of *Gracilaria* were obtained from the Marine Resources Laboratory in Key Largo, Florida. The strain, tentatively identified as *Gracilaria ferox* J. Agardh (C.J. Dawes, pers. comm.) was first isolated at Harbor Branch Oceanographic Institution Inc., Fort Pierce, Florida, where it has been referred to as 'stubby brown' (M. Hommersand, pers. comm.). It has been maintained in continuous culture at the Experimental Hatchery ever since October, 1989.

The *Gracilaria* was grown in seven, white, gel-coated fiberglass raceway tanks with inside dimensions of 6.63 m long, 1.85 m wide, and 1.22 m deep. Each raceway was installed at ground level with a 1.2 m tall (12 cm diameter) polyvinyl chloride (PVC) standpipe to control water levels. Raw sea water was pumped from an adjacent coastal inlet to a settlement tank and then passed through sand filters which removed particles >50 μm in diameter. Filtered water was then pumped to an elevated head tank (10 m high) and gravity-fed to the raceways. A gate valve regulated water flow into each tank at a rate 38 L min^{-1} (i.e., equivalent to six tank turnovers d^{-1}). A 10 horse-power blower provided aeration and agitation at a rate of 0.9 m^3 min^{-1} through perforated PVC pipes positioned at the bottom center of each raceway. The drainage systems of the seven raceway tanks converged into an open cement box (1.5 m long, 1.5 m wide, and 1.5 m deep) set below ground level which served as a harvest basin. Macroalgae were managed in two groups of three tanks; the production cycles of these groups were staggered 7 d apart. The remaining tank served as a food storage container for the *Aplysia californica*.

Prior to inoculation, tanks were cleaned with 10% sodium hypochlorite or 10% HCl to sterilize and remove organic matter and then rinsed thoroughly with fresh water. The tanks were then filled with seawater and inoculated with 54.4 kg of wet weight (w. wt) of *Gracilaria*, at an initial stocking biomass of 4.46 kg w. wt m^{-2} (5.88 g w. wt L^{-1}). Nutrients were added in the form of NH_4NO_3 (400 g), $(NH_4)_2HPO_4$ (375 g) and 600 ml of Fritz Chemical (Dallas, Texas, USA) f/2 enriching solution (Guillard, 1975). The inoculum was then allowed to 'feed' in a static system for 16 to 17 h (Lapointe, 1985). After the pulse-feeding, seawater flow was restored and maintained for 7 d before a final nutrient pulse feeding on day 8. Harvesting was conducted on day 14 and involved draining the individual tanks into the cement harvest basin and gathering the algae in plastic baskets. Each basket's capacity was approximately 15 kg of w. wt algae. The baskets were allowed 15 to 20 min to drain excess water before weighing on a digital balance. After weighing, 163.2 kg w. wt of algae was selected from the tank considered to be the cleanest (i.e., terms of least epi-

phytic organisms) to re-inoculate and to continue the production cycle.

Light incident on the culture system was measured at intervals of 60 s, averaged, and logged every hour using a LI-COR datalogger model LI-1000 equipped with a quantum sensor (LI-190SA, LI-COR Inc., Lincoln, NE, USA). Temperature in each tank was recorded every 5 s, averaged, and logged hourly using the same datalogger connected to a water temperature thermistor (071, YSI, Yellow Springs, OH, USA). All data were transferred weekly to a computer spreadsheet. Incident light (mol photon m^{-2}) in the photosynthetically active range was summed and temperatures were averaged every two weeks. Salinities were measured daily with a temperature-compensated refractometer (Reichert-Jung, Buffalo, New York, USA), and averaged every two weeks to be consistent with the temporal resolution of the temperature, light, and biomass measurements.

Primary productivity was determined from the increase in dry weight over a two-week interval and expressed as g d. wt m^{-2} d^{-1}. To determine dry weight, duplicate 500 g samples of wet algae were randomly sampled from each tank on day 14 after inoculation, rinsed with fresh water to remove excess salt, and dried to constant weight at 60°C. Quantities of epiphytes in the culture system were measured once during May 1993, by selecting 50 random plants and manually stripping them of all attached plant material. The separated macroalgal and epiphyte samples were then dried to constant weight and their relative percentages calculated.

Multiple linear regression was performed to evaluate the combined effects of the factors light, temperature and salinity and all possible interactions on productivity. In the event of a factor being found to be non-significant ($p > 0.05$), it was removed and regression re-run until all (highest order) model terms were significant (Hocking, 1976; Draper & Smith, 1981).

Results

During the period of this study, water temperatures in the system ranged from 18.2°C to 29.0°C with an average of 23.7°C. Light levels ranged from a high for a 2-week period of 701.9 m photon m^{-2} in May 1993, to a low of 223.4 m photon m^{-2} in November 1993. The average biweekly level for the entire duration of the study was 474.6 mol photon m^{-2}. Salinity ranged from 31.0 to 36.5‰ throughout the study. Mean salinity over the entire study period was 34.4‰.

Productivity from October 1992 to June 1995 averaged 39.7 g d. wt m^{-2} d^{-1}. The maximum value recorded was 59.2 g d. wt m^{-2} d^{-1} in May 1993, and the minimum was 21.4 g d. wt m^{-2} day^{-1} in January, 1994. Dry weight to wet weight ratios were relatively stable throughout the study ranging from 6.7 to 7.8% with an average of 7.2%. Net wet weight yield of algae, or the amount of algae produced in two weeks of intensive culture, averaged 95.1 kg or 175% of the original inoculum (54.4 kg). The highest yield was 143.9 kg (264%) and the lowest was 53.0 kg (97.4%).

The most common epiphyte taxa in the culture system were *Griffordia johnsoni* and *Entomorpha* sp. These were only found attached to the oldest portion of the plants. Mean epiphyte biomass was 2.8% of the whole plant's dry weight. Although we quantified epiphyte biomass only once during May, 1993, qualitative observations suggested that this level of epiphyte biomass was typical of the entire study period.

Multiple regression analysis indicated that salinity, within the ranged observed, was insignificant ($p > 0.05$) in its effect on *G. ferox* productivity. Light, temperature and the light x temperature interaction term were all significant factors (i.e., $p < 0.05$) and together accounted for 75% of the variability in *G. ferox* productivity (Figure 1). The resulting regression equation was

$$P = -60.02 + (2.76)L + (3.10)T + (-0.082)(LT) \qquad r^2 = 0.76$$

where *G. ferox* productivity, P, is measured in g d. wt m^{-2} d^{-1}, L is light level in mol photon m^{-2} d^{-1}, and T is temperature in degrees Celsius.

Discussion

This study has demonstrated the feasibility of producing high yields of macroalgae in large-scale, land-based intensive raceway systems for an extended period of time in South Florida, USA. At 39.8 g d. wt m^{-2} d^{-1}, the mean productivity rate obtained exceeded the values reported for *G. tikvahiae* by Lapointe and Ryther (1978: 34.8 g d. wt m^{-2} d^{-1}) Ryther et al. (1979: 31.0 g d. wt m^{-2} d^{-1}) and Hanisak (1986: 25 g d. wt m^{-2} d^{-1}). As in previous studies on *G. tikvahiae* under non-nutrient limited conditions (Lapointe et al., 1976; Lapointe & Ryther, 1978; Lapointe

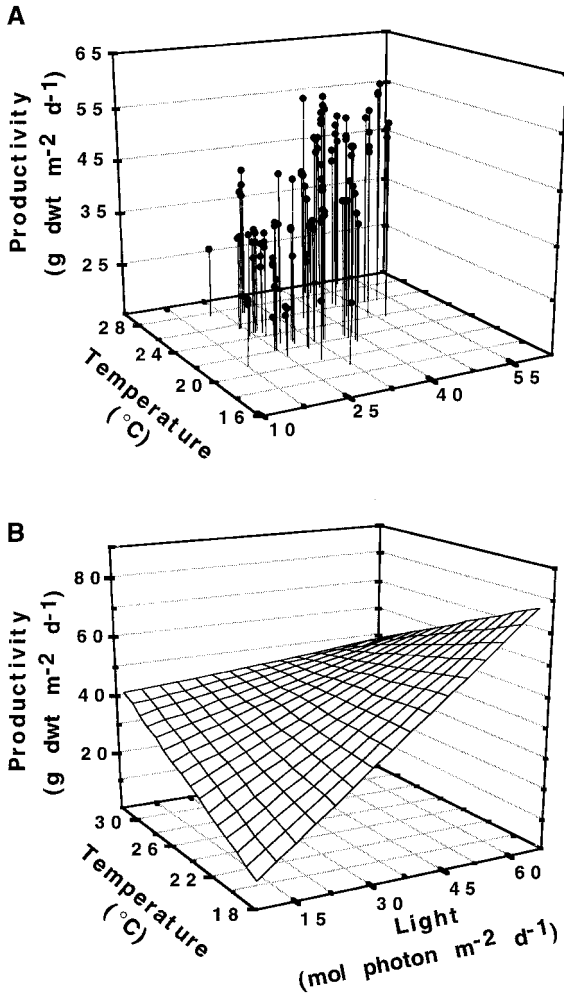

Figure 1. Plots showing the relationship between temperature (°C), light (mol photon m^{-2} d^{-1}) and productivity (g d. wt m^{-2} d^{-1}) of *Gracilaria ferox* in culture: (A) scatter plot of observed productivities; (B) fitted light-temperature response surface.

et al., 1984b), light was the overriding controller of *G. ferox* yields. Temperature may have become limiting in our study during winter months when temperatures of approximately 18°C were observed. Lapointe et al. (1984a) showed that the maximum photosynthetic rate at light saturation (P_{max}) of *G. tikvahiae* was reduced at temperatures below 25°C, and became suppressed below 20°C. Similarly, Hanisak (1987) reported maximum growth rates of *G. tikvahiae* between 24°C and 30°C. This effect is due to the temperature-dependence of the enzyme-controlled dark reactions of photosynthesis and similar temperature-limitation of maximum growth rates have been described for phytoplankton populations under natural conditions in the sea (Eppley, 1972) and in outdoor mass cultures (Goldman, 1979).

In contrast to light and temperature, salinity had no significant effects on *G. ferox* growth in this study. *Gracilaria tikvahiae* grows over a wide range of salinities, 6 to 42‰, but maximally between 24 and 36‰ (Hanisak, 1987). Lapointe et al. (1984a) showed that hyposmotic shock from 26 to 16‰, which could occur in an outdoor system during a major storm event, had only transient, short-term effects (i.e., hours to days) on *G. tikvahiae* photosynthesis and respiration, while longer term growth rates (weekly) were unchanged. Daugherty & Bird (1988) have reported that low salinities (17‰) result in low productivities and low agar strength in *G. verucosa* and that the optimal salinity range was 25 to 33‰. The small changes in salinity that we observed had no detectable effects on *G. ferox* productivity.

This study has demonstrated the feasibility of sustaining *G. ferox* in culture for four years without the need for restocking. Such an extended culture period was achieved by growing a vegetative strain with a low epiphyte biomass (< 3%). Although always present in small amounts, epiphytes did not noticeably reduce the growth of our strain and never overgrew a batch culture as it did in the experiments by Hanisak (1987). Furthermore, diatoms never became a major component of the epiphytic community as was observed by Hansen (1984). We found that our nutrient pulse-feeding technique was the key to preventing chronic epiphytic blooms and thereby enabled production of consistently high yields of *G. ferox*. The maintenance of low nutrient levels during the majority of algal culture period appears to be critical for high sustainable yields in large-scale production systems.

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