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Integrating Intensive Aquaculture of the Red Seaweed *Chondracanthus exasperatus*

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Abstract Washington State has a significant history of experimental seaweed aquaculture. Early experiments involved the carrageenophytes *Mazzaella splendens* and *Chondracanthus exasperatus* in both open water net culture and semi-closed tank systems on land. Later, net culture of *Porphyra* for nori and long line culture of the edible kelps *Nereocystis* and *Macrocystis* were tested successfully. Further development of these culture systems was halted due to combinations of market, regulatory, political and social considerations. When a Seattle company developed a high value product from the Turkish Towel Seaweed, *Chondracanthus exasperatus*, there was renewed interest in intensive tank and pond based aquaculture because Washington has a long term moratorium on commercial seaweed harvesting from wild seaweed populations. The initial phase of this research was conducted at Mukilteo, Washington, where strategies for long term cultivation in tanks were tested, and a new custom cultivation tank design was developed for pilot scale cultivation research at a larger site on the shore of Clam Bay near Manchester, Washington. Long term cultivation is now being tested in tanks of up to 5,000L volume supplied with natural seawater, nutrient supplemented seawater, and seawater effluent from nearby fish culture tanks. Seawater from Clam Bay is naturally rich in nutrients from tidal driven upwelling and nearby commercial salmon aquaculture operations. Supplemental nutrients (commercially available "f/2" enrichment and agricultural fertilizers) and halibut culture tank effluent have both been tested for their ability to support *C. exasperatus* growth with relatively low seawater turnover rates. Compared to seawater at the Clam Bay site, halibut tank effluent differs in both nutrient composition and quantities. Initial results indicate that halibut tank effluent is a satisfactory source of nutrients for *C. exasperatus* in intensive culture and that this seaweed scrubs significant quantities of nutrients from halibut tank effluent, especially ammonium. Recent experiments with several bioreactor designs have investigated the culturing of *C. exasperatus* at very high loading densities in recirculated natural and artificial seawater in both submerged and spray culture.

Key words: seaweed, red algae, *Chondracanthus,* aquaculture

Introduction

Washington State has a history of experimental seaweed aquaculture. In the 1970s net and tank cultures of the carrageenan producing red seaweeds *Mazzaella* (then known as *Iridaea*) and *Chondracanthus* (then known as *Gigartina*) were tested. In the 1980s, the emphasis shifted to *Porphyra* species, both Japanese and Washington species which were cultivated on raft nets to produce edible nori (Mumford *et al.*, 1985; Merrill & Olson, 1988).

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Late in the 1980s, long line cultivation of the kelps *Nereocystis* (as a wakame substitute) and *Macrocystis* (for the herring roe on kelp fishery) were tested on long lines (Merrill, 1989). Recently interest in cultivation of *Chondracanthus exasperatus* was renewed when a Seattle company discovered and developed a high value cosmetic product from this seaweed. Since Washington State has a moratorium on commercial seaweed harvesting from natural populations and harvest limits elsewhere in this species' range limit harvests from the wild, aquaculture was viewed as the best option for obtaining raw material to support this developing industry. As the earlier open water aquaculture activities had elicited considerable opposition from shoreline property owners, open water cultivation was not considered a viable option. Therefore, intensive tank or pond cultivation was viewed as the best option for obtaining a sustained raw material supply to support this new industry. Furthermore, intensive aquaculture offers opportunities for optimizing product yield through strain selection, genetic manipulation, control of certain growth parameters and protection from pests and competition. A joint university, industry and agency project was initiated to develop this option.

In earlier research, both open water net cultivation and tank culture of *Chondracanthus exasperatus* had been tested on a limited basis as *Mazzaella splendens* (as *Iridaea cordata*) was the species which received the most attention in the 1970s research (Waaland, 1973, 1976). What was known was that *C. exasperatus* exhibited dependable growth in tank culture and could be cloned and propagated perennially from vegetative fragments. Additional data were available about loading densities, seawater turnover and mixing required for sustainable growth in intensive tank culture (Waaland 1977).

This report includes both field and laboratory aspects of recent research which has progressed through three phases. The first phase investigated strategies for long term maintenance of seaweed stocks and explored experimental and pilot scale tank designs for cultivation of *C. exasperatus*. The second phase focused on increasing stock quantity and quality by selection and management of growth parameters and integrating water from fish (Pacific Halibut) culture tanks. The third and most recent phase has emphasized reducing water use and pumping through recycling and spray culture.

Methods

The *Chondracanthus exasperatus* (Turner & Bailey) J. Hughey plants used to initiate these experiments were obtained from natural populations at Clam Bay near Manchester, Washington in Sept. 1999. After the initial collection, most material used in large-scale experiments came from the tank cultures with occasional use of freshly collected wild plants.

In certain smaller scale laboratory and field experiments, plants from uni-algal laboratory experiments were sometimes used. These were typically maintained in natural seawater enriched with commercially available Guillard's f/2 enrichment (Kent Co. marketed by Aquatic-Ecosystems), grown in controlled environment chambers at 15℃ with irradiance of ～50 μE・m^{-2}・s^{-1} from cool white fluorescent lamps (Merrill & Waaland 1979). In many experiments, artificial seawater (Instant Ocean®, Aquarium Systems Inc., Mentor, OH) made with reverse osmosis water to salinity 30o/oo (typical of Puget Sound) and supplemented with Guillard's f/2 enrichment (Kent Co. chemicals as above) was used successfully.

Outdoor Experiments

Several types of tanks were used depending on the experiment. The tanks at the first outdoor site, Mukilteo, Washington were cylindrical fiberglass fish culture tanks 1.2m diameter, 1.0m deep, volume 1500L, with a center drain (Fig. 1). Water motion was provided by a central aerator.

At the Manchester, Washington site, most tanks were horizontal half cylinders with

hemispherical ends (4.7m long, 1 m deep, 1.5m wide, 5500L volume) with a well screen equipped drain near each end (only one drain was active, the other was used for instruments, cross connecting tanks and other purposes) (Fig. 2). A longitudinal aeration pipe circulated the seawater and seaweeds. Smaller versions of these tanks were used in a few experiments. An array of ten 75L semi-square polyethylene tanks was used for small scale field experiments; they were equipped with well screen center drains and central aeration (Fig. 3). Seawater was pumped from an intake on a nearby pier (visible in background in Fig. 3). At the Manchester site, effluent seawater from Pacific Halibut culture tanks was also available. Typical salinity is 28-30o/^{oo} at these sites.

Typical tank culture conditions involved ambient daylight and ambient temperatures of the pumped seawater. At this latitude (~47 ℕ), winter days are short (~8.5 hr light) and often cloudy and summer days long (16 hr light). Pumped seawater temperatures ranged from 4-6 ℃ in winter to 14-15℃ in summer. Solar heating may raise water temperatures one or two degrees in large tanks depending on flow rate. Light and temperature in or near the large tanks were monitored with data loggers (Onset Computer Corp.). Other experimental details will be given with each type of experiment. Nutrient analyses (nitrate, nitrite, ammonium, phosphate) were performed by the Marine Chemistry Laboratory, Department of Oceanography.

Greenhouse Experiments

In the greenhouse, plants were housed in an hexagonal plexiglass (0.50m long×0.45m wide, 100L volume aquarium) turned on its side and fitted with a plastic mesh platform to support the plants and equipped with drain openings and a removable panel for sealing the opening and providing for sea water supply and drain pipes (Fig. 4).

Plastic spray nozzles were connected to an external polyethylene reservoir recirculating 80L of UV sterilized (QL-25, Rainbow

Fig. 1. Vertical cylindrical tanks used at Mukilteo field station

Fig. 2. Horizontal half cylinder tanks used at Manchester field station

Fig. 3. Semi-square tanks used for outdoor culture at Manchester and indoor spray culture in controlled environment room

Aquarium Systems), 35 μm filtered (Aquatic Ecosystems VF-125), and chilled sea water. The chiller (Aqualogic 0.23Kw) was equipped with a titanium heat exchanger. The seawater medium was pumped at approximately 12-15L•min^{-1} through the spray nozzles. The chiller maintained the seawater at temperatures between 10-15°C depending on the experiment. From late spring through mid autumn, the greenhouse was covered with 30% shade cloth to reduce the solar heat load. In addition to the greenhouse shade cloth, plastic window screen often was used as a neutral density filter on the tank. The ambient light is supplemented on cloudy days and daylength extended in winter with 600W high pressure sodium greenhouse lamps. Data loggers (Onset Computer Corp.) were used to track temperature and light in this system. This culture unit has a relatively low density of seaweed per seawater volume because of the volume needed to cover the chiller probe in the reservoir. The large volume of chilled medium also adds a brief period of buffer time to react to failure of the cooling system in the high ambient heat of the greenhouse environment. The unit has operated satisfactorily for months at a time with weekly shut offs to remove the plants for weighing.

Typical Operating Conditions:

Light: ambient greenhouse, shading in summer, ~150 μE•m^{-2}•s^{-1}

Temp: 10-12°C

Nutrients: 0.1×f/2 (low biomass/medium volume)

Seawater: replaced ~7-8weeks

Pumping rate: ~12-15L•min^{-1} (for spray)

Growth rate: ~4-5% d^{-1}

Controlled Environment Rooms

In controlled environment rooms, two types of tanks were used for most experiments: 320L rectangular polyethylene tanks (1.0m × 0.67m × 0.65m) (Fig. 5) and 0.6m × 0.6m, 75L semisquare tanks (described above) equipped with a plastic screen platform to support the plants and using a recirculating pump (20L in circulation), 25 watt UV sterilizer (25 watt) and 35 μm particle filter and plastic lawn sprinkler spray nozzles attached to a distribution manifold (Fig. 6). In the controlled environment rooms, the temperature of the circulating water was maintained in a range (10-15°C) encountered by *C. exasperatus* in natural communities in the Puget Sound region during spring, summer and early autumn.

The 320L rectangular polyethylene tanks were used primarily as holding tanks for plants to be used in other experiments. Nevertheless, they demonstrated that high densities of plants may be maintained for up to 16 months in batch culture with little seawater

Fig. 4. Hexagonal chamber used for spray culture in greenhouse environment

Fig. 5. Rectangular plastic tanks used in controlled environment room

turnover (once every 3-6 months) provided that mineral nutrients are replenished and salinity controlled (by addition of reverse osmosis water) as required. For faster growth, addition of carbon dioxide (via a pH controller and sparger) resulted in higher growth rates. Particle filtration (35 μm) and UV sterilization (25 watt) were essential to control phytoplankton species in such a system and to reduce the survival of spores of competing seaweeds (e.g., *Ulva* and *Enteromorpha*, etc.) which might be circulating in the system, introduced with the *C. exasperatus* from natural populations. Growth rates up to 5 % d^{-1} day were observed in this system. It would be relatively simple to integrate this type of culture system with a finfish or shell culture system.

Laboratory Benchtop Bioreactor

Cytolift® Bioreactor (Kontes Glass Co.) vessels were used for the most intensely monitored experiments (Fig. 7). These are cylindrical, water-jacketed bioreactors with an internal volume of ~500mL. Water circulation was provided by a multi-channel peristaltic pump (Cole-Parmer/MasterFlex Model 7553-70). Seawater was chilled in a countercurrent heat exchanger chilled by a 0.12Kw Lauda K-2/R chiller circulating chilled tap water. Circulating seawater was UV treated by circulating the water through quartz tubing in a cylindrical aluminum chimney housing a 25 Watt UV lamp. A computer interfaced data logger (pH, dissolved oxygen, temperature and light) and controller (Remote Measurement Systems, Inc., "EnviroMac" unit) was used to monitor experimental conditions and to add CO_2 as the plants consumed it via photosynthesis.

Fig. 6. Semi-square tank with recirculating pump, UV sterilizer and particulate filter as used for spray culture in controlled environment room

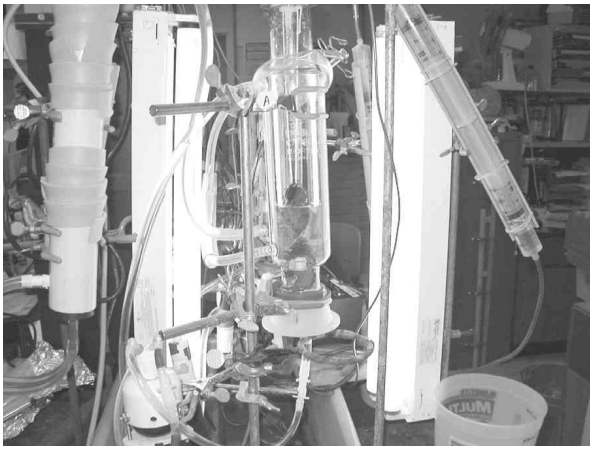

Fig. 7. Cytolift® bioreactor used in laboratory

An Olympus 620L digital camera was used to record the appearance of individual tagged plants at weekly intervals. Fresh weights were measured after plants were drained and blotted.

Results

The results presented here are representative of the performance, as measured by relative growth rate, of plants raised under the conditions specified for each series. These represent the potential performance of such systems but do not detail experiments that were not productive.

Outdoor tanks

At present, the large outdoor tanks

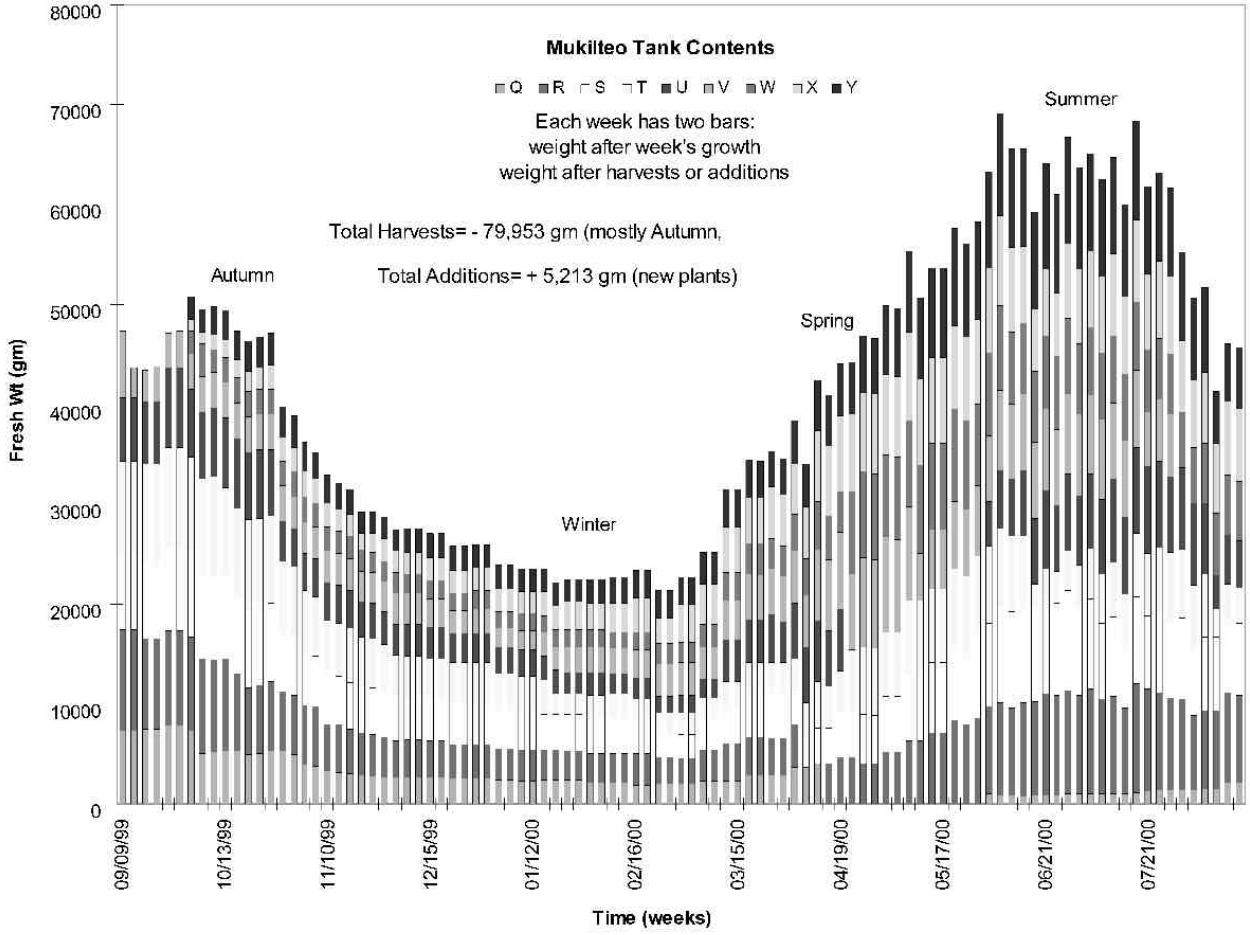

Fig. 8. Summary graph for Mukilteo tank array

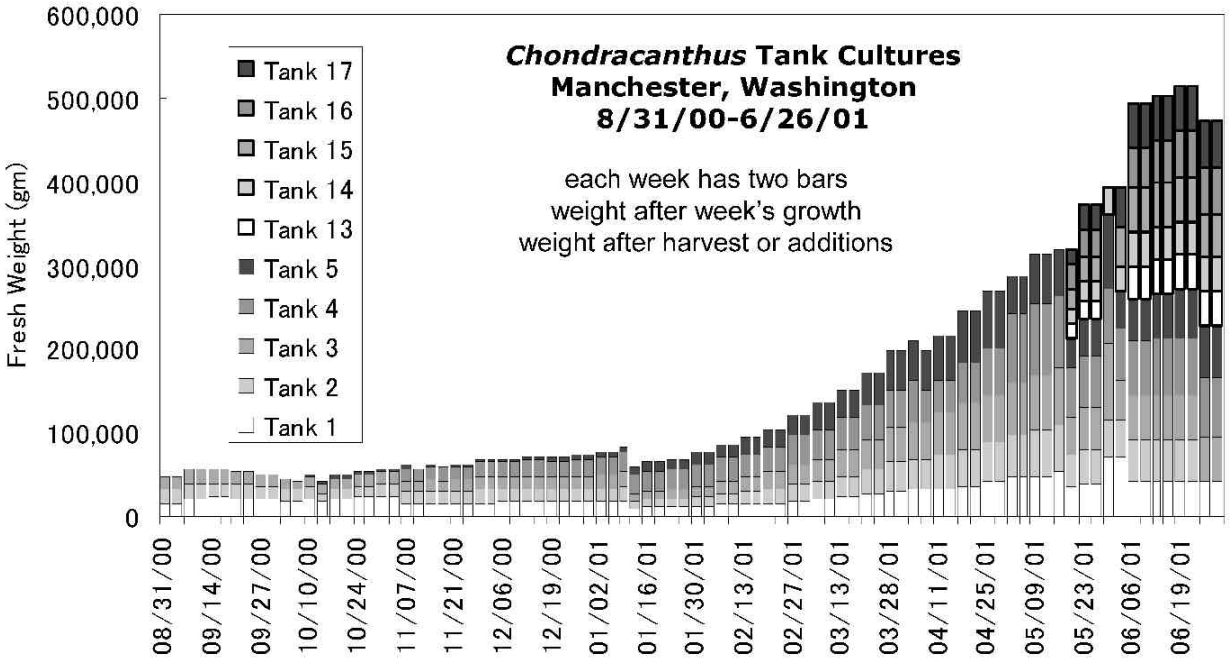

Fig. 9. Summary graph for Manchester tank array

represent the most realistic option for pilot or small commercial scale production. Several years of experience have been obtained encompassing all seasons and a variety of weather conditions (from September 1999 through late August 2000 at Mukilteo and late August 2000 through mid-June 2001 at Manchester for our experiments and subsequently to the present by Söliv personnel at Manchester).

A summary of production in the Mukilteo tanks is seen in Fig. 8. A similar summary for the Manchester large tank array is seen in Fig. 9.

For some experiments some or all of the Manchester tanks were supplied with effluent water from adjacent halibut rearing tanks. Fig. 10 shows the ammonium supplementation that occurred as a result of the halibut in the water and the reduction in ammonium that occurred as a result of *C. exasperatus* growing in the water.

Greenhouse experiments

Several tank and container designs were tested in greenhouse conditions, but only one is shown here, the hexagonal enclosure used for spray culture (Fig. 4). Early experiments did not use particle filtration or UV sterilization of the medium. Such filtration (to prevent nozzle clogging) and sterilization (to inhibit epiphyte and phytoplankton growth) proved essential for long term operation (months) of culture units using recirculated medium. Without such treatment, diatoms, green flagellates and ulvoids were recurring problems. Typical operating conditions for the recirculating hexagonal enclosure system were:

Light: 150 μ E • m^{-2} • s^{-1} (ambient greenhouse light, shaded and/or supplemented with sodium vapor lamps)
Temp: 12℃
Nutrients: 0.15×f/2/week
Seawater: replaced ~every 8-20 weeks
Pumping rate: 12L • min^{-1} for circulation and spray operation
Growth rate: ~1-2% d^{-1}
A representative plant growing in this system is shown in Fig. 11.

Controlled Environment Room Experiments

Controlled environment rooms provide a convenient way of maintaining seaweed cultures at temperatures similar to those of their natural environment. UV sterilization of the seawater medium pumped using an airlift system, proved essential to prevent growth of competing algae (e.g., diatoms, green flagellates and ulvoids). With a pH controller to add carbon di-

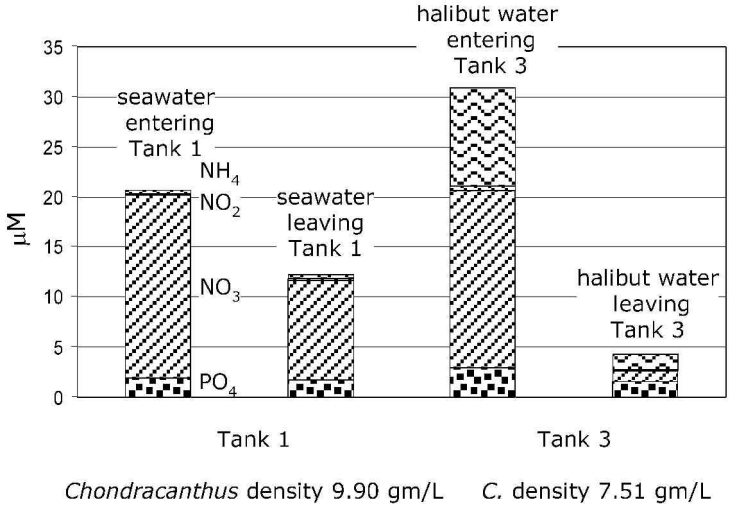

Fig. 10. Seawater and halibut tank inflowing and effluent water. Samples were taken near mid-day. Loading density was as shown. Note the preferential use of ammonium over nitrate. Plants were actively growing although the plants in Tank 1 were at a very high density and may have been light limited compared to those in Tank 3

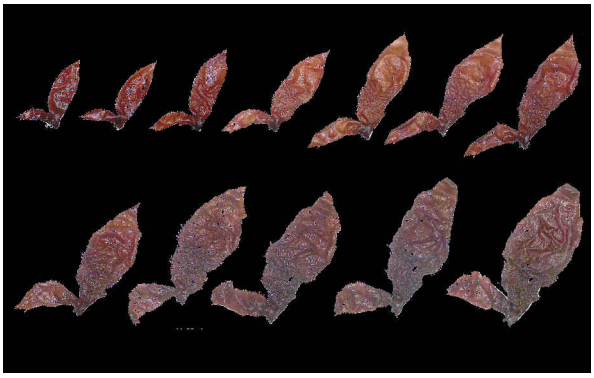

Fig. 11. Representative plant grown in spray culture in hexagonal enclosure in greenhouse

oxide as the pH rose above 8.2 and mineral nutrient supplementation by periodic additions of f/2, it was possible to maintain plants in an actively growing state at very high densities for several months in the same batch of seawater. Typical operating conditions for the 320L tanks follows:
Light: 150 μE・$m^{-2}s^{-1}$ (metal halide and cool white fluorescent lamps; 16 hr L: 8 hr D)
Temp: 12℃
Nutrients: 0.15×f/2/week
Seawater: replaced ～every 8-20 weeks
Pumping rate: aeration for circulation, air lift pump for UV sterilizer
Growth rate: ～1-2% d^{-1}

Semisquare Spray Culture System

This system has a very small volume of water (～20L) circulating in a chamber equipped with a plastic mesh rack to support plants and plastic spray nozzles to keep the plants damp and supply mineral nutrients. This system circulates the seawater medium through a UV sterilizer to inhibit competing algae and a particle filter which prevents fouling of the spray nozzles and also removes competing algae. This system has proved quite successful.
Typical Operating Conditions:
Light: 150 μE・$m^{-2}s^{-1}$ (metal halide and cool white fluorescent lamps; 16 hr L: 8 hr D)
Temp: 12℃
Nutrients: 1.5×f/2/week
Seawater: replaced ～every 4-10 weeks

Fig. 12. (Top): Representative plant ER-3 grown in spray culture in controlled environment room (Bottom): Representative plant 02-05-31 growing in benchtop Cytolift Bioreactor

Pumping rate: ～12L/min for spray
Growth rate: ～4-5%/d
A representative plant is shown in Fig. 12 (top).

Cytolift Bioreactor

The Cytolift® Bioreactor units have a very high plant density per volume of circulating medium. The temperature and light monitors mainly reported on the functioning of the lamp timer and circulating chiller. The pH monitor could add CO_2 to the closed system via a low volume pumping system. The oxygen monitor was useful only when plants were entirely submerged with no air space to provide an oxygen reservoir and sink. Most useful for these experiments was the pH monitor and regulator as it provided rapid feedback and also compensated for carbon dioxide uptake by rapidly growing plants. Mineral nutrient additions were done manually with the frequency and amount depending on the experiment being conducted. Similar growth rates are obtained for immersed or emersed plants. A representative plant is shown in Fig. 12 (Bottom).
Typical Operating Conditions:
Light: 150 μE・$m^{-2}s^{-1}$ 16L: 8D & 20L: 4D (fluorescent)
Temp: 15-20℃
Nutrients: pH maintained at 8.0-8.2; f/2 enrichment 1.2×per week (proportional to biomass)
Seawater: replaced ～every 4 weeks
Pumping rate: ～0.2L/min
Growth rate: ～4-5%/d

Discussion and Conclusions

This report describes several scales of culture method for use with the red seaweed *Chondracanthus exasperatus*. Many other seaweeds are likely to flourish in such systems as well. The larger scale flow through outdoor systems are presently used as a pilot scale research system for commercial production associated with a high value product extracted from *Chondracanthus*. The smaller scale experimental systems can be modified for immersed or emersed culture of this seaweed and probably other seaweeds. All the tested systems offer opportunities for reducing the volume of seawater used. All the laboratory scale systems have been successfully tested with both natural and artificial seawater medium. Recirculation offers significant economies in reduced pumping and materials handling costs. All tested systems benefit from nutrient supplementation, particularly at low seawater turnover rates. Mineral nutrient supplementation is essential in low volume and/or turnover systems to maintain plant growth. Carbon dioxide supplementation improves growth rate where CO_2 limits growth as it does in all these systems as shown by pH monitoring and CO_2 supplementation. These systems offer many opportunities for integrating seaweed culture with finfish or shellfish culture to use the seawater more efficiently and to benefit from the seaweed's extractive metabolism in removing nitrogenous compounds from the animal culture effluent. With appropriate integration with fossil fueled power plants, seaweeds may also contribute to carbon dioxide scrubbing from the flue gases. The effectiveness of such integration will likely depend on seasonal effects on growth in outdoor systems. One of the larger challenges in such integration will likely be obtaining rapid feedback on the mineral nutrient demands of the seaweeds. While pH provides rapid and convenient feedback on carbon dioxide uptake, methods for similarly rapid analysis (i.e., sufficiently rapid to supplement the limiting nutrient[s] within a few hours) of the numerous mineral nutrients that might limit seaweed growth in a closed or semi-closed system are either non-existent or very expensive.

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