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Preliminary examination of the bioremediation and mariculture potential of a Northeast U.S.A. and an Asian species of *Porphyra*

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Abstract Finfish and shrimp mariculture operations produce nutrient-rich effluent that can threaten the health of coastal ecosystems if not properly managed. As part of an effort to develop an economically viable system of integrated polyculture, we have begun to evaluate the bioremediation and mariculture potential of Northeast U.S.A. and Asian species of *Porphyra*. We present here preliminary results based on short- and long-term experiments. Short-term nitrogen (N) uptake measurements were conducted over ca. 20 min in 50 mL tubes at 5-15 ℃ and at high (10 g FW L^{-1}) stocking density. During long-term (28-d) experiments at 15 ℃ and at 0.4 g FW L^{-1}, we examined the growth, N assimilation into *Porphyra* tissue, and phycobiliprotein contents at three- to seven-day intervals as a function of N concentration (25, 75, 150, 300 μM). Performance (growth rate and bioremediation) was maximal at 150-300 μM inorganic N. Induction of archaeospore production reduced growth rates. *Porphyra purpurea* removed 96-100 % of N within 3.5 days at 150 μM NH_4^+. Overall, *Porphyra* appears to be an excellent choice for bioremediation of moderately eutrophic effluents, with the added benefit that tissue may be harvested for sale.

Key words: *Porphyra*, eutrophication, aquaculture, mariculture, nitrogen, phosphorus, bioremediation, seaweed

Introduction

Finfish and shrimp mariculture produces effluent that is rich in inorganic nitrogen (N) and phosphorus (P). These nutrients derive from the bacterial release of inorganic N and P from non-consumed animal food, and from excretory waste products of the cultured animals (Beveridge, 1987). These additions of N and P can threaten ecosystem health by degrading critical functions of coastal ecosystems (McVey *et al.*, 2002). The detrimental effects of eutrophication include blooms of harmful phytoplankton and unwanted macroalgae (Cuomo *et al.*, 1993), as well as development of hypoxia and anoxia (Sfriso *et al.*, 1987). The ecological incentive for remediating eutrophic effluents is clear. Moreover, there are also economic incentives to reduce the poss of nutrients. The N and P that are flushed from the system represent a loss of opportunity for the aquaculturist, since these nutrients could be channeled into the production of valuable products. In addition, governmental agencies charged with reducing coastal eutrophication are developing regulations limiting the release

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of N and P (c.f. U.S.A. Environmental Protection Agency Proposed Effluent Guidelines Program Plan, Federal Register (June 18, 2002, Volume 67, Number 117); Jordan, 2002). In the future, aquaculturists can expect to incur financial penalties if they cannot regulate the release of their waste effluent.

One solution to the problem of eutrophic effluent that addresses both ecological and economic issues is the development of integrated aquaculture, in which seaweeds are grown downstream from animals. Systems of integrated aquaculture are ideal because the N and P in the animal effluent are necessary requirements for the growth of the seaweeds. This is not a new idea (for review see Chopin *et al.*, 2001). What distinguishes this project from many of the prior studies is the selection of the algal component. Obviously, the best seaweed to integrate into an animal aquaculture operation is one characterized by rapid growth, the accumulation of N and P to high levels in tissue, and some added value. We are investigating the genus *Porphyra* as the seaweed partner for a number of reasons (Yarish *et al.*, 1998; Chopin *et al.*, 1999). All species of *Porphyra* produce gametophytes that are flat sheets one or two cell layers thick. This combination ensures an extremely high surface area-to-volume ratio, with all cells involved in the uptake of nutrients and production of new tissue. In part for these reasons, *Porphyra* is capable of rapid growth; preliminary measurements show this genus capable of at least 20 % d^{-1} (Kraemer, pers. obs.). Species of *Porphyra* also are efficient nutrient concentrators. In situations where nutrients are readily available, N can constitute up to 7.4 % of the dry tissue biomass (Chopin and Yarish, 1998, 1999). Finally, several species of *Porphyra* form the basis for a multi-billion dollar (U.S.) global business in the production of nori for human consumption. A recent added value under development is the use of R-phycoerythrin as the fluorescent conjugate for immunological dectection of target molecules in molecular biological research (Mumford and Miura, 1988).

Previous studies have focused on the integration of other macroalgal species into finfish culture (e.g., *Ulva lactuca*; Cohen and Neori, 1991). While other species may be efficient nutrient filters, the biomass that is produced has limited application after harvest (e.g., organic composting). Consequently, *Porphyra* not only has potential for bioremediation of aquaculture effluents, but also production of a crop that may have economic value.

Growth, accumulation of N and P, and high value by-products in tissue are dependent on the environmental factors that regulate production: temperature, nutrient availability, irradiance, water motion. As part of an effort to develop an economically viable system of integrated aquaculture, we have been evaluating the bioremediation and mariculture potential of Northeast U.S.A. and Asian species of *Porphyra*. We have included Asian species in the comparison since they represent in many ways the industrial benchmarks. However, we do not advocate the use of non-native species of *Porphyra* in open culture in New England coastal waters. We present here preliminary results that describe the influence of temperature and nutrient availability on N uptake, tissue production, phycoerythrin and N tissue content, and nutrient removal efficiency.

Materials and Methods

We have been working with aquaculturists from GreatBay Aquaculture, LLC. (GBA; Portsmouth, New Hampshire, U.S.A.) in the development of the integrated system. GBA raises summer flounder (*Paralichthys dentatus*) and cod (*Gadus morhua*) in a land-based system. Effluent from the grow-out tanks has an average NH_4 concentration of 150 μM, and temperatures that range from 10 to 19°C over the course of the year. These values were used to determine the conditions for the laboratory experiments.

All blade (gametophyte) tissue used for these experiments was generated from the

conchocelis stage. The experimental work was conducted under controlled environmental conditions of irradiance, temperature, and photoperiod in walk-in growth chambers at the University of Connecticut, Stamford. We pre-sent here data from the Northeast U.S.A. species *P. purpurea* (New York strain for long-term experiment and Maine strain for short-term N uptake measurements) and *P. yezoensis* (an Asian cultivar). Short-term measurements of NH_4^+ uptake rate were performed on tissue grown at 5 , 10 , or 15 ℃. Samples (ca. 0.3 g FW) were placed in 50 mL transparent plastic screw-top tubes containing 30 mL of artificial seawater ("MBL" formula, Cavanaugh 1975). Ammonium levels were adjusted to 10, 20, 40, 75, or 150 μM while inorganic phosphorus was added to a constant N:P molar ratio of 10:1. Tissue was incubated at the growth temperature. At 7 and 17min after the introduction of tissue into the tubes, samples of incubation medium were collected and analyzed for NH_4^+ concentration (Liddicoat *et al.*, 1976). Five to seven replicates were used per N concentration.

Long-term (28-d) experiments were also carried out at the University of Connecticut facility in 1-L flasks. *Porphyra* gametophytic tissue (ca. 0.4 g FW L^{-1}), grown at 10 ℃ and acclimated at 15 ℃ for seven days, was placed in 800 mL of von Stosch-enriched culture medium. The N level in the incubation medium was adjusted to either 25, 75, 150, or 300 μM, with P added to constant 10:1 N:P molar ratio. Three replicates at each N concentration were used. At 3-4 d intervals the culture medium was changed, with a sample from each flask retained for N and P analysis (via Technicon autoanalyzer). At 7-d intervals, the tissue in each flask was weighed (FW). Newly produced tissue was removed so that each 7-d growth period began with the same stocking density (0.4 g FW L^{-1}). From the excised tissue, samples were conserved for CHN (by means of a Perkin-Elmer CNS analyzer) and phycobiliprotein analyses (Beer and Eshel, 1985).

Results

Porphyra purpurea demonstrated differences in the rate of NH_4^+ uptake that were a function of temperature (Fig. 1). The highest rates of NH_4^+ uptake by P. purpurea occurred at 10 ℃. *Porphyra yezoensis* took up NH_4^+ roughly twice as fast at 15 ℃ than did *P. purpurea.*

Over the 28-d long-term experiments, growth was not constant for either species (Fig. 2). *Porphyra yezoensis* grew well during the first week (18 % d^{-1}), but archaeospore production ensued and the tissue began to disintegrate. By the experiment's end, the *P. yezoensis* growth rate was negative as losses due to reproduction exceeded new growth. *Porphyra* purpurea appeared to enter a brief period of archaeospore production but growth rebounded by the experiment's end.

By the experiment's end, phycobiliprotein concentration in *P. purpurea* blade tissue showed approaching saturation (Fig. 3). Half saturation values at occurred at ca. 120 μM NH_4^+. Unlike phycobiliprotein content, tissue N content did not appear to be close to an asymptote, even when grown at 300 μM (Fig. 4).

Preliminary results for *P. purpurea* demonstrate its efficacy as a bioremediator of eutrophic effluent (Fig. 5). During 3.5 d batch cultures, *P. purpurea* removed on average 96-98 % of the NH_4 in the incubation medium. This

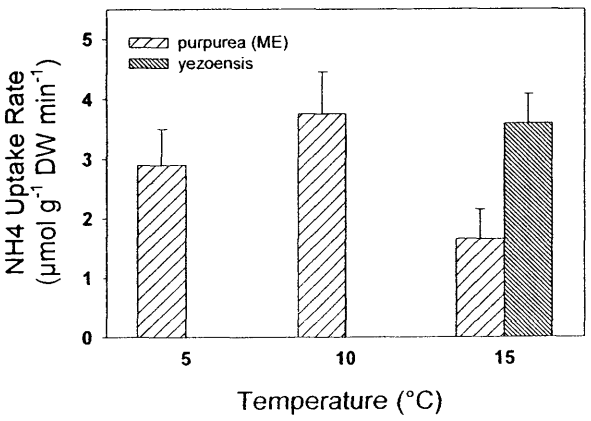

Fig. 1. Short-term NH_4^+ uptake rates measured as a function of temperature. All measurements conducted at 150 μM ammonium. Errors bars are standard deviations.

removal declined to 77 % at 300 μ M. Interestingly, the efficiency with which *P. purpurea* removed inorganic P declined as N concentrations increased (molar N:P ratios constant at 10: 1). At 150 μ M NH_4 and 15 μ M PO_4^{3-} *P. purpurea* removed 46 % of the inorganic P.

Discussion

The short-term measurements of NH_4 uptake rate demonstrated interspecific differences in performance. Clearly, not all *Porphyra* species are equally efficient in the uptake of NH_4, an argument for the evaluation of a range of possible species before selecting the best bioremediator. *Porphyra yezoensis* outperformed *P. purpurea*, at least at 15 ℃, but we do not yet know whether other local species will compare with the Asian species. Some have argued that short-term uptake measurements are to some extent artifactual due to "surge" uptake (e.g., Dy and Yap, 2001). However, previous work with *P. yezoensis* suggests that this phenomenon is not common to all macroalgal species (Yamamoto, 1992). The fact that a definite temperature optimum was identified for one *Porphyra* species suggests that further ex-

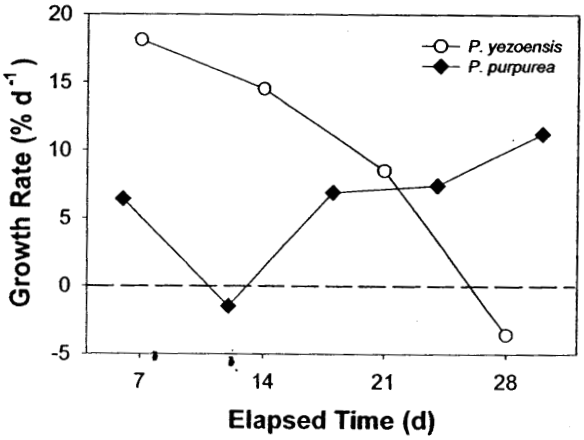

Fig. 2. Average growth rates (n=3) during long-term experiments (150 μ M NH_4). Negative growth rates occur when tissue becomes reproductive and fragments

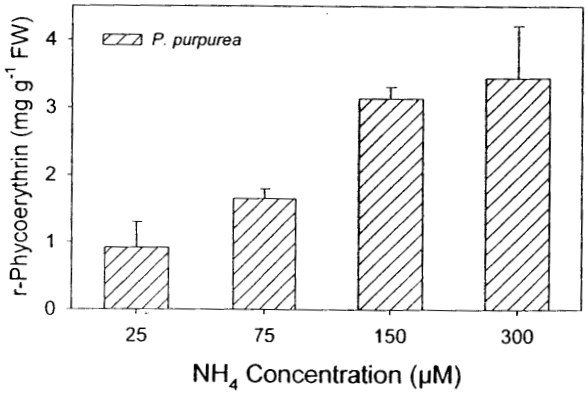

Fig. 3. Ammonium concentration vs. *Porphyra purpurea* r-phycoerythrin concentration at end of 28-d experiment (n=3; error bars are standard deviations)

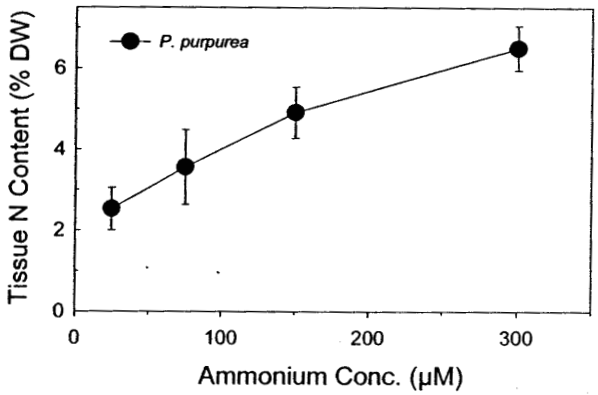

Fig. 4. Relationship between N availability (as NH_4 concentration at start of each 3-4 d growth period) and tissue N content at the end of the 28-d experiment (n=3; error bars are standard deviations)

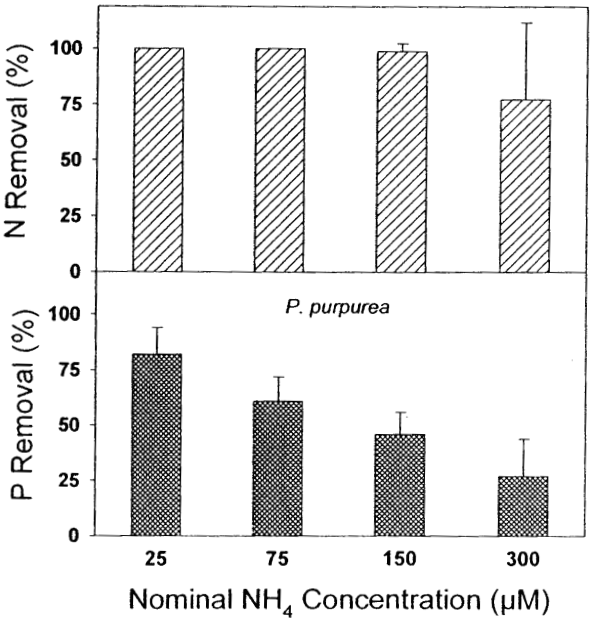

Fig. 5. Efficiency of nutrient removal from incubation media. Values are averages over entire 28-d experiment (n=12; error bars are standard deviations)

panding the pool might provide a high- or low temperature tolerant species. This would enable seasonal crop rotation to take advantage of temperature optima for each particular species. Alternatively, project engineering could be employed to moderate the differences between the operating and optimal temperatures for bioremediation of nutrient-rich aquaculture effluent.

When grown during the long-term experiments (effectively 3.5 d batch culture at 15 °C, 150 μ mol photons m^{-2} s^{-1}, and a stocking density of 0.4 g FW L^{-1},) the performance indicators indicated that different physiological functions respond differently to the availability of inorganic nitrogen. Data for *P. purpurea* regarding phycobiliprotein concentration indicates that production of pigments is not restricted under our experimental conditions in the same way that growth is limited. That pigments continue to accumulate under nutrient-replete conditions suggests that these compounds can act both as light absorbers and as nitrogen stores, something reported for other red macroalgae under N-sufficient conditions (Bird *et al.* 1982; Vergara *et al.*, 1995). Presumably, as growth causes an increase in biomass density the extra pigments are advantageous in light of the diminution of the irradiance regime. Second, tissue nitrogen accumulates in an apparently linear function of nitrogen availability, with no indication of saturation even at 300 μ M NH_4. This argues that nitrogenous compounds other than pigments are important in the storage of nitrogen. Amino acids are a candidate. Our unpublished data demonstrate such a response to nitrogen availability, though the increases in amino acids concentrations may not account for all of the increase in tissue nitrogen.

Our short-term data indicates that *P. purpurea* (Maine isolate) is capable of removing ca. 120 μ mols NH_4 g^{-1} FW d^{-1} (assuming 10: 1 FW: DW ratio and uptake during 12 lighted hours of a day). The long-term experiment using *P. purpurea* (New York isolate) indicates an average uptake rate during a 3.5 d period of 66 μ mols NH_4 g^{-1} FW d^{-1}. This difference may be due to true differences in performance between strains. However, the average uptake rates during the long-term experiments reflect an integration of the effects of changing NH_4 concentration during the 3.5 d period. The uptake rates in a functioning, flow-through system will be much higher, since the concentration will be correspondingly higher. The actual bioremediation (i.e., % N and P removed) will depend on *Porphyra* stocking density and effluent residence time, in addition to concentration-specific uptake rates. These are variables to be investigated at larger scales.

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