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## Evaluation of the bioremediatory potential of several species of the red alga *Porphyra* using short-term measurements of nitrogen uptake as a rapid bioassay

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**Key words:** ammonium, integrated aquaculture, nitrogen, *Porphyra*, uptake

### Abstract

Rates of inorganic nitrogen uptake by three Northeast US and three Asian species of *Porphyra* were compared in short-term incubations to evaluate potential for longer term and larger scale examination of bioremediation of nutrient-loaded effluents from finfish aquaculture facilities. The effects of nitrogen (N) species and concentration, temperature, acclimation history, and irradiance were investigated. Uptake rates increased ca. nine-fold from 20 to 150  $\mu\text{M}$  N. Nitrate and ammonium uptake occurred at similar rates. Irradiance had a strong effect, with uptake at 40  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  only 55% of uptake at 150  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . N-replete tissue took up inorganic nitrogen at rates that averaged only 60% of nutrient-deprived tissue. Although there were species (*P. amplissima* > (*P. purpurea* = *P. umbilicalis*)) and temperature effects (10 °C > 5 °C > 15 °C), interactions among factors indicated that individual species be considered separately. Overall, *P. amplissima* was the best Northeast US candidate. It took up ammonium at faster rates than other local species at 10 and 15 °C, two temperatures that fall within the expected range of industrial conditions for finfish operations.

### Introduction

Marine finfish aquaculture has become a multi-billion dollar industry (New, 1999) with a diverse group of fishes, including summer flounder, salmon, and cod, being successfully cultivated (Stickney & McVey, 2002). However, an emerging problem associated with aquaculture activities is the introduction of inorganic nutrients into coastal waters (Chopin et al., 2001; Naylor et al., 2000; Paez-Osuna et al., 2003). This occurs because fish do not consume all their

feed, with the remainder degraded by bacteria that release inorganic nitrogen (N) and other nutrients. Fish also excrete other nitrogenous wastes. Together, these processes contribute to an effluent rich in  $\text{NH}_4^+$ . Many coastal areas already suffer from nutrient-driven blooms of phytoplankton and weedy macroalgae, creating aesthetic problems, the development of severe hypoxia in bottom waters, and the death or departure of ecologically and economically important biota (Briand, 1987; Sfriso et al., 1987; Cuomo et al., 1993). Aquaculture has the potential to exacerbate coastal

nutrient loading if not properly balanced (McVey et al., 2002).

A promising approach to reduce the impact of eutrophic effluent is the development of polyculture systems that integrate the culture of finfish with macroalgae (e.g. Cohen & Neori, 1991; Chopin et al., 2001; Schuenhoff et al., 2003; Neori et al., 2004). Macroalgae can concentrate nutrients by a factor of up to  $10^5$  over seawater levels (Lobban & Harrison, 1997). Further, macroalgae can respond to increased nutrient availability by augmenting internal stores. For example, *Porphyra purpurea* tissue from the area of anthropogenic nutrient loading contained 6.3% dry weight (DW) N while tissues from a pristine site contained only 4.6% DW at the same time of year (Chopin & Yarish, 1998, 1999). This natural concentration phenomenon has practical application in an aquaculture context. The integration of the agarophyte *Gracilaria* into salmon aquaculture in Chile reduced the release of nitrogen (N) and phosphorus (P) by 56 and 94%, respectively (Kautsky et al., 1996; Troell et al., 1997).

The rhodophyte *Porphyra* is the most valuable cultured seaweed, with an annual value of over US\$ 1.2 billion (FAO, 2002). It is primarily used as the wrapping around sushi rolls (nori) but is also a source of taurine, proteins, vitamins, trace minerals, and dietary fiber (Tsuji et al., 1983; Noda, 1993). *Porphyra* is the preferred source of the pigment, *r*-phycoerythrin, utilized as a fluorescent tag in biotechnological applications (Mumford & Miura, 1988).

The morphology of *Porphyra* makes it an efficient agent of bioremediation. The thin blade of the gametophyte is composed of 1 or 2 cell layers, with all cells involved in nutrient absorption. In morphotypes with a high surface area-to-volume ratio, such as *Porphyra*, the coupling between ambient nutrient levels and internal pools is tight, enabling a rapid response to environmental nutrient availability (Neori et al., 2004). While this coupling often determines field growth rates, nutrient concentrations in fish farm effluent should be high enough that growth and, hence, rates of nutrient removal remain optimal (Schuenhoff et al., 2003).

The efficacy with which algae such as *Porphyra* remediate nutrient-rich effluent depends on the rapid sequestration of nutrients into algal tissue. The rate of mass removal of N equals growth rate multiplied by tissue nutrient concentration. Therefore, evaluation of the bioremediatory potential requires consideration of both growth rate and tissue nutrient concentration. In fact, the genus *Porphyra* attains some of the highest values of these parameters of any macroalga. Tissue grown

in batch cultures pulsed with  $300 \mu\text{M}$  N produced tissue containing 5–7% N DW (Carmona, unpublished data). *Porphyra* is fast growing, with increases of up to 15–35% per day, translating into doubling times as low as 2–5 days (Hafting, 1999; Kraemer et al., unpublished). The high productivity and nutrient accumulation, and market potential make polyculture systems that include *Porphyra* valuable for the abatement of coastal nutrient loading by finfish aquaculture (either in the sea or on the land), while also providing a potentially valuable product upon harvest (Chung et al., 2002).

In any evaluation of candidates for fish farm effluent bioremediation, measurement of N concentration in the growth medium is relatively easy and rapid (Chopin & Yarish, 1999; Chopin et al., 1999). The measurement of growth rate, however, requires significant time and resources (space, growth media, etc.). Since the genus *Porphyra* has a simple sheet-like morphology, limited storage potential, and exhibits rapid growth, taxa in this genus must be capable of supporting that growth with the uptake and assimilation of nutrients. This work used short-term measurements of N uptake rate as a rapid bioassay to evaluate the bioremediatory potential of various species of *Porphyra*. Results presented here and work in review elsewhere support the idea that the rate of N uptake in the short term by *Porphyra* can predict maximum growth rates ( $r = 0.70$ ,  $p = 0.051$ ,  $n = 8$ ; results not shown). We have identified a local candidate now being tested in scaled-up systems of land-based integrated finfish aquaculture operations, and compared this with Asian species.

## Materials and methods

*Porphyra amplissima* (strain ME7-4), *P. purpurea* (strain NY4-1), *P. purpurea* (strain ME40-4), *P. umbilicalis* (strain ME6-9), *P. dentata* (origin of strain unknown from Pusan, South Korea, courtesy of J. Lee), *P. katadai* (strain PKTF99), and *P. yezoensis* (strain PYWT2001039A) were grown from conchospores at  $15^\circ\text{C}$  and under 12:12 h; L:D photoperiod at the Marine Biotechnology Laboratory of the University of Connecticut at Stamford laboratories. The first three species are native to the northeast coast of North America, while the latter three species are Asian in origin. Blades were cultured for at least two weeks at the measurement temperature in von Stosch-enriched (Ott, 1965) Long Island Sound seawater (collected at Avery Point, CT) at  $50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  under a 12:12 h; L:D photoperiod. The stocking density during culture was

approximately  $0.5 \text{ g FW L}^{-1}$ . During the two week acclimation period the culture medium was changed every 3–4 days, with the last change done one day before the uptake measurements. These blades were considered nutrient replete because the acclimation incubation medium contained  $500 \mu\text{M}$  inorganic nitrogen (N) and tissue N contents were approximately 5% DW. For some incubations (N deprivation treatment), blade tissue was left in the growth medium for seven days after a media change, after which tissue N levels were down to 2.9% DW and there had been a visible decrease in pigmentation.

Blades were separated into lots of ca. 0.3 g fresh weight (FW). These blades were kept moist with growth medium and covered to protect from high light. The incubation medium for the measurements was artificial seawater (Harrison et al., 1980) that was pH-adjusted to 8.2 with 1 M NaOH and spiked with  $\text{NH}_4^+$  or  $\text{NO}_3^-$  to concentrations ranging from 10–150  $\mu\text{M}$  N. The inorganic N stock (5000  $\mu\text{M}$ ) was prepared fresh before each uptake measurement.

A volume of 30 mL of spiked incubation medium was placed in a translucent plastic 50 mL tube. Five–seven replicate tubes were prepared for each N concentration. The blade portions of ca. 0.3 g FW were introduced, the tubes shaken, and the timer started. The irradiance incident on the blades was  $150 \mu\text{mol m}^{-2} \text{ s}^{-1}$  for most measurements after absorption of a portion of the incoming light by the plastic. This exceeded intensities required to saturate photosynthesis in these cultures (Kraemer & Yarish, 1999). An irradiance of  $40 \mu\text{mol m}^{-2} \text{ s}^{-1}$  was used to test for a low versus high light effect on N uptake. The contents of the tubes were mixed by rolling every two minutes to prevent the build-up of boundary layers around the blade surfaces.

Samples of the medium were taken at 7 and 17 min after introduction of the *Porphyra* tissue. Samples were placed into acid-washed 5 mL glass tubes. For  $\text{NH}_4^+$  concentrations of 10–40  $\mu\text{M}$ , 1 mL samples were taken, while 0.2 mL samples were taken from the concentrations  $\geq 75 \mu\text{M}$  and these were diluted to 1.00 mL using the artificial seawater solution. Six replicate standards at 0 and 20  $\mu\text{M}$  inorganic N (made with artificial seawater) were also analyzed.

The  $\text{NH}_4^+$  concentrations were analyzed using the procedures outlined in Liddicoat et al. (1975), with volumes reduced to analyze 1 mL seawater samples. To each of the glass tubes containing samples of the incubation medium, 40  $\mu\text{L}$  of phenol-alcohol reagent (1 g phenol in 5 mL 95% ethanol) were added and the tubes were vortexed. Next, 100  $\mu\text{L}$  of the oxidizing reagent

(0.040 g sodium dichloroisocyanurate in 4 mL of 1 M NaOH, 4 mL 50%, (w/v) trisodium citrate, and 2 mL  $\text{dH}_2\text{O}$ ) were added and the tubes mixed. Finally, 40  $\mu\text{L}$  of catalyst (0.050 g potassium ferrocyanide in 5 mL  $\text{dH}_2\text{O}$ ) were added and mixed. The oxidizing reagent and catalyst were both made fresh for each experiment. The tubes were placed in open-mesh metal racks and left for 1 h under UV illumination in a sterile hood to drive color development. Concentrations were obtained from the absorbances measured at 640 nm.

Nitrate concentrations were estimated using procedures modified from Jones (1984). Spongy cadmium (Cd) was generated by placing zinc bars in 20% (wt/vol)  $\text{CdSO}_4$ . The precipitated Cd was removed, washed with water and broken into small pieces. The pieces were washed with 6N HCl and rinsed with copious quantities of distilled water (until  $\text{pH} > 5$ ) and maintained under water with no air contact. Samples of the incubation medium (1 mL) were placed in 1.5 mL Eppendorf microcentrifuge tubes. An aliquot (160  $\mu\text{L}$ ) of 0.7 M  $\text{NH}_4\text{Cl}$  (pH adjusted to 8.5) was added to each sample and mixed. A piece of Cd metal (ca. 35  $\text{mm}^2$ ) was removed from the water, blotted briefly onto a paper towel to remove the excess water, and placed into each Eppendorf tube. The sealed tubes were gently shaken at 5-min intervals for 40 min, during which time the  $\text{NO}_3^-$  was reduced to  $\text{NO}_2^-$ . A volume of 1.0 mL was removed from each Eppendorf tube and transferred to a glass test tube and mixed with 60  $\mu\text{L}$  of 2% sulfanilamide (w/v 10% HCl). This mixture was allowed to stand for about 5 min and then 60  $\mu\text{L}$  0.2% N-1-naphthyl-ethylenediamine (w/v  $\text{dH}_2\text{O}$ ) were added and mixed. Absorbances were read at 543 nm after allowing at least 10 min for color development.

*Porphyra* blade tissue from each tube was rinsed in distilled, deionized  $\text{H}_2\text{O}$ , dried for 48 h at  $60^\circ\text{C}$  overnight, and weighed. Uptake rates (during the 7–17 min interval) were standardized to dry weight. Initial comparisons were made to ascertain the effects of irradiance during incubation,  $\text{NO}_3^-$  versus  $\text{NH}_4^+$ , and N-deficient versus N-replete tissue on rate of uptake. Rates were analyzed using ANOVA procedures (Statistica<sup>®</sup>) and, when the main effects were significant, pairwise mean comparisons were made using Tukey's Honest Significant Difference test. The data from all species were first analyzed together, and then separated according to species. Data from 10 and  $15^\circ\text{C}$  were analyzed to predict performance under conditions most closely approximating effluent conditions reported by our partner, Great Bay Aquaculture, LLC (G. Nardi, personal communication).

Table 1. Results of ANOVA examining the effects of various treatments on the uptake of inorganic nitrogen (N) by *Porphyra purpurea*.

Treatment	Factor	F-value	p-level	Rank order of main factors
A. Low light vs. high light	Light (L)	13.2	0.0010	150 > 40 ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )
	$\text{NH}_4^+$ conc (AC)	63.2	<0.00005*	150 > 40 > 20 ( $\mu\text{M}$ )
	L $\times$ AC	0.70	0.51	
B. $\text{NO}_3^-$ vs. $\text{NH}_4^+$ uptake	Source (S)	3.0	0.11	
	N conc (NC)	6.1	0.0078*	(20 = 40) < 75 < 150 ( $\mu\text{M}$ )
	S $\times$ NC	0.2	0.87	
C. Deprived vs. replete	Pre-acclimation (PA)	4.9	0.036*	Deprived > replete
	$\text{NH}_4^+$ conc (AC)	10.1	0.005*	75 > 40 > 20 ( $\mu\text{M}$ )
	PA $\times$ AC	1.9	0.089	

A Michaelis-Menten curve was fit to the relationship between  $\text{NH}_4^+$  concentration and uptake rate at 15 °C, assuming a non-zero intercept (Naldi & Viaroli, 2002). The ratio  $V_{\text{max}}/K_S$ , the slope of the Michaelis-Menten equation at low N concentrations, was also calculated.

## Results

The rate of  $\text{NH}_4^+$  uptake by *P. purpurea* blades, pre-acclimated to produce nutrient-replete tissue, varied significantly under the three incubation irradiances (20 versus 150  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ ; Table 1; Figure 1). However, uptake rate was significantly ( $p < 0.00005$ ) affected by the concentration of  $\text{NH}_4^+$  in the media. Average uptake rates were 71% higher under 150  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  irradiance than under the lower irradiance. The uptake of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  did not differ

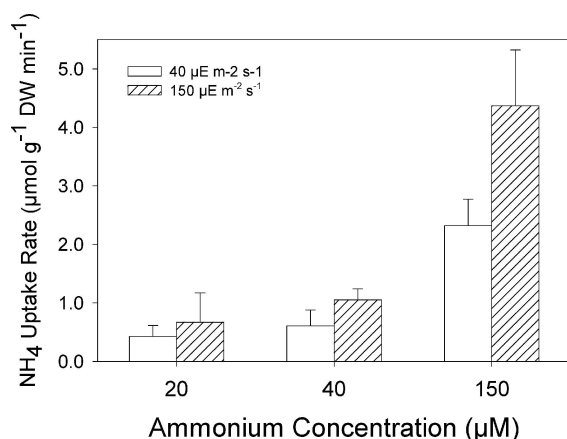


Figure 1. Effects of ammonium concentration and irradiance on the rate of uptake by *Porphyra purpurea* at 10 °C ( $n = 5 - 6$ ). Error bars represent  $\pm$  one SD.

over the four concentrations examined when tissue had been N-deprived for a week (Table 1, Figure 2). Pre-treatment had a significant effect on the rate of  $\text{NH}_4^+$  uptake; N-deprived blades had average uptake rates that were 30–178% higher than blades from cultures whose medium had been replaced the day prior to measurement (Table 1, Figure 3).

The most complete data set included data for three species, three temperatures, and five N concentrations. Analysis of these data revealed significant main treatment effects, as well as significant interactions (Table 1). The rate of N uptake increased with increasing N concentration, ranking 150  $\mu\text{M} > 75 \mu\text{M} > 40 \mu\text{M} > 20 \mu\text{M} > 10 \mu\text{M}$ . Although there were species (*P. amplissima* > *P. purpurea* = *P. umbilicalis*) and temperature effects (10 °C > 5 °C > 15 °C), the interactions argued that the data be examined by individual species to better understand the effects of temperature and N concentration on N uptake rate. The

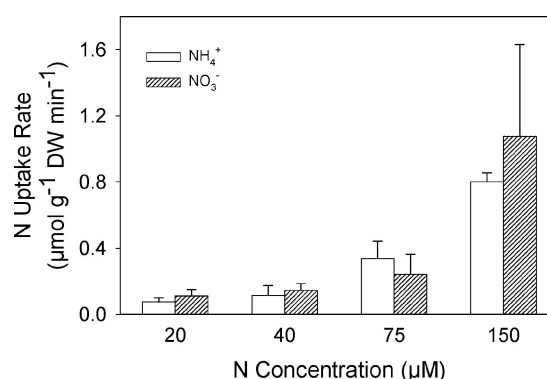


Figure 2. Effects of nitrogen source (ammonium vs. nitrate) and concentration on the rate of nitrogen uptake by *Porphyra purpurea* at 15 °C ( $n = 3$ ). Error bars represent  $\pm$  one SD.

Table 2. Results of ANOVA examining effects of various treatments on the uptake of  $\text{NH}_4^+$ .

Data set	Factor	F value	p-level	Rank order of main factors
A. Complete set	Species (S)	38.0	<0.0001*	See text
	N conc (NC)	24.1	<0.0001*	
	Temperature (T)	302.8	<0.0001*	
	S × NC	13.8	<0.0001*	
	S × T	5.7	<0.0001*	
	NC × T	3.8	0.00035*	
	S × NC × T	1.7	0.043*	
B. <i>P. amplissima</i>	NC	146.1	<0.0001*	150 > 75 > 40 > 20 > 10 ( $\mu\text{M}$ )
	T	10.2	0.00017*	10 > (15 = 5) ( $^{\circ}\text{C}$ )
	NC × T	1.90	0.078	
C. <i>P. purpurea</i>	NC	82.4	<0.0001*	150 > 75 > 40 > 20 > 10 ( $\mu\text{M}$ )
	T	25.9	<0.0001*	(5 = 10) > 15 ( $^{\circ}\text{C}$ )
	NC × T	0.6	0.769	
D. <i>P. umbilicalis</i>	NC	88.1	<0.0001*	(150 = 75) > 40 > 20 > 10 ( $\mu\text{M}$ )
	T	15.1	<0.0001*	15 > (10 = 5) ( $^{\circ}\text{C}$ )
	NC × T	0.2	0.94	
E. 10 $^{\circ}\text{C}/150 \mu\text{M NH}_4^+$	Species	15.7	<0.0001*	(amp = purp) > (kat = yezo = dent) > (umb)
F. 15 $^{\circ}\text{C}/150 \mu\text{M NH}_4^+$	Species	13.5	<0.0001*	(amp = yezo = kat) > (kat = umb) > (umb > purp)

Note: amp: *P. amplissima*; dent: *P. dentata*; kat: *P. katadai*; purp: *P. purpurea*; umb: *P. umbilicalis*; yezo: *P. yezoensis*.

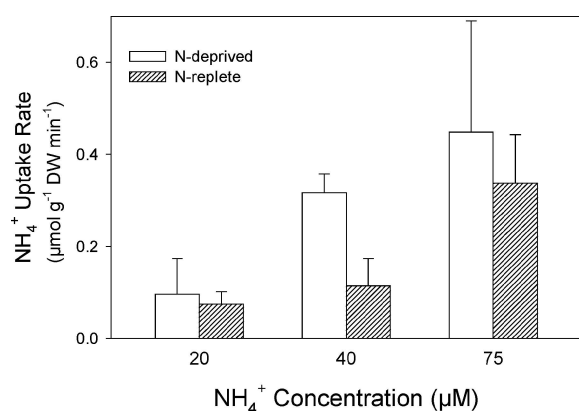


Figure 3. Effects of nutrient pre-treatment and incubation concentration on the rate of ammonium uptake by *Porphyra purpurea* at 15  $^{\circ}\text{C}$  ( $n = 3$ ). Error bars represent  $\pm$  one SD.

effect of N concentration and temperature remained significant for all species (Table 2). The pattern of the temperature effect differed among the species; averaged over all N concentrations, *P. amplissima* showed a 10  $^{\circ}\text{C}$  uptake optimum (10  $^{\circ}\text{C}$  > 5  $^{\circ}\text{C}$  = 15  $^{\circ}\text{C}$ ; Figure 4). *P. umbilicalis* took up  $\text{NH}_4^+$  most rapidly at 15  $^{\circ}\text{C}$  (15  $^{\circ}\text{C}$  > 10  $^{\circ}\text{C}$  = 5  $^{\circ}\text{C}$ ), while *P. purpurea* took up  $\text{NH}_4^+$  least rapidly at 15  $^{\circ}\text{C}$  (5  $^{\circ}\text{C}$  = 10  $^{\circ}\text{C}$  = 15  $^{\circ}\text{C}$ ). The best performers at 10  $^{\circ}\text{C}$  were *P. am-*

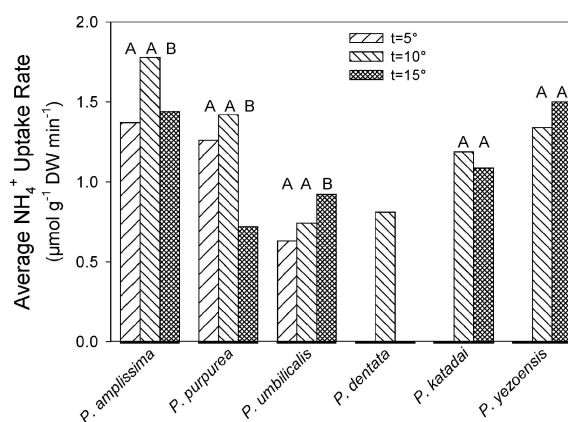


Figure 4. Effects of temperature and species on the uptake of ammonium. Bars represent averages over all ammonium concentrations (10, 20, 40, 75, 150  $\mu\text{M}$ ). Within a species, different letters signify statistically different values, indicating an effect of temperature on uptake.

*plissima* and *P. purpurea*, with similar, high uptake rates. At the warmer temperature (15  $^{\circ}\text{C}$ ), *P. amplissima*, *P. yezoensis*, and *P. katadai* all took up  $\text{NH}_4^+$  at similar rates. Excluding non-native (i.e. Asian) species, *P. amplissima* was clearly the best performer, with highest  $\text{NH}_4^+$  uptake rates under both temperatures.

Michaelis–Menten kinetics, fitted to uptake rate data obtained from measurements at the application-relevant temperature of 15 °C, gave estimates ( $V_{\max}$ ), the half-saturation  $\text{NH}_4^+$  concentration ( $K_S$ ), and the predicted intercept ( $S_T$ ). Uptake of  $\text{NH}_4^+$  by *P. amplissima* did not saturate at concentrations up to 150  $\mu\text{M}$ ; hence, the data could not be fitted to the Michaelis–Menton equation. Rather, uptake rates (10 and 20  $\mu\text{M}$  only) were linearly regressed onto N concentration to estimate the concentration intercept ( $S_T$ ) only. Of the remaining species, *P. yezoensis* showed the highest average  $V_{\max}$  (Figure 5). With the exception of *P. yezoensis*,  $S_T$  values were similar at ca. 6  $\mu\text{M}$   $\text{NH}_4^+$ . Half-saturation constants averaged 55  $\mu\text{M}$ , with the exception of that for *P. katadai* which was roughly three-times the average value. No species showed a particularly high alpha ( $V_{\max}/K_S$ ).

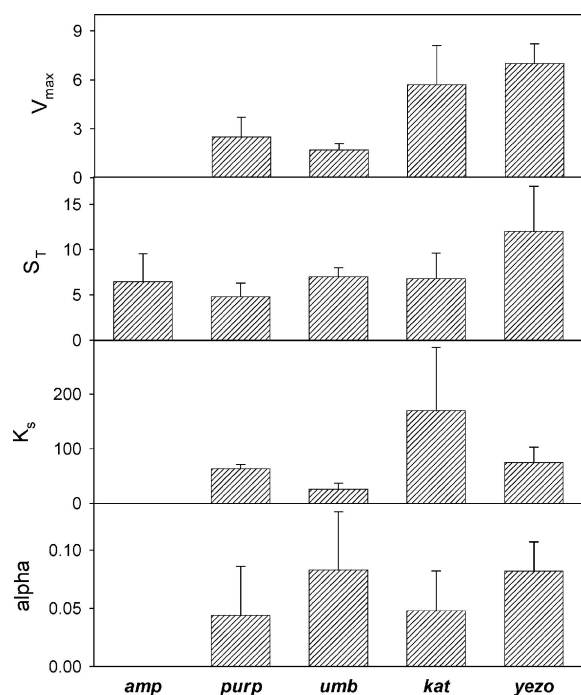


Figure 5. Average values of the maximum rate of ammonium uptake ( $V_{\max}$ ;  $\mu\text{mol NH}_4^+ \text{ g}^{-1} \text{ DW min}^{-1}$ ), the ammonium concentration at which uptake ceases ( $S_T$ ;  $\mu\text{M}$ ), the half-saturation constant ( $K_S$ ;  $\mu\text{M}$ ), and the uptake efficiency (alpha;  $\mu\text{mol NH}_4^+ \text{ g}^{-1} \text{ DW min}^{-1} \mu\text{M}^{-1}$ ). Data are derived from uptake curves under aquaculture conditions (15 °C, 150  $\mu\text{M}$   $\text{NH}_4^+$ ). Error bars represent standard deviations. Since uptake by *Porphyra amplissima* did not saturate over the concentration range, only  $S_T$  was estimated (linear regression of 10, 20  $\mu\text{M}$  data). amp = *P. amplissima*, purp = *P. purpurea*, umb = *P. umbilicalis*, kat = *P. katadai*, yezo = *P. yezoensis*.

## Discussion

The objective of this study was to collect physiological measurements to guide the selection of an appropriate species of *Porphyra* for a system of integrated aquaculture that couples the growth of marine macroalgae and finfish. We have focused here on the bioremediatory performance of the algal partner, although a full evaluation of the suitability of candidate species must include consideration of market potential (i.e. chemical properties determining taste and texture), as well as biotechnological applications (Tamura et al., 1998; Levine, 1998).

The results indicated that *Porphyra amplissima* is the best candidate of the northeast American species tested. *Porphyra amplissima* outperformed the other taxa at 10 and 15 °C, two temperatures that fall within the expected range for existing finfish operations at Great Bay Aquaculture, LLC. (e.g., cod, halibut, black sea bass; G. Nardi, personal communication). Significantly, the rate of nutrient uptake by *P. amplissima* compared quite well with that of *P. yezoensis*, one of the most important species cultivated in Asia. During periods of maximal growth, rates of new tissue production by these two species were similar (within 20%; Carmona, Kraemer & Yarish, unpublished data).

The northeast American species showed definite temperature optima. Results from larger scale (50 L) experiments support the concept of temperature optima (Day, 2003). Since aquaculture operations seek to minimize costs, temperatures are not likely to be modified beyond that which is necessary to optimize production by the most valuable component of the system (here, the finfish). Given this constraint, crop rotation could optimize macroalgal production. Even though our data point to *P. amplissima* as the best performer under any of the temperatures employed, the existence of temperature optima suggests that other *Porphyra* species (or geographical isolates) could prove more valuable under specific conditions.

In short-term measurements, the uptake of N was not influenced by the form of inorganic N; ammonium uptake rates were equivalent to rates of nitrate uptake when presented separately. The ability of the macroalgae to remediate  $\text{NH}_4^+$ -enriched effluent leaving flow-through aquaculture systems is critical in meeting upgraded water discharge standards. Additionally, some of today's aquaculture systems are recirculating designs that include a bacterial biofilter to convert effluent  $\text{NH}_4^+$  to  $\text{NO}_3^-$  before recirculating the water

back into the fish grow-out tanks. Finfish introduce  $\text{NH}_4^+$  into the tank effluent, but can generally tolerate no more than 25–50  $\mu\text{M}$  unionized ammonium before metabolic effects and reduced growth occur (e.g., Espey, 2001; Fuller et al., 2003; Lemaire et al., 2004). Ammonium is unlikely to accumulate; *P. amplissima* assimilated both forms of inorganic N when presented simultaneously, removing  $\text{NH}_4^+$  six times faster than  $\text{NO}_3^-$  even when  $\text{NO}_3^-$  supply exceeds  $\text{NH}_4^+$  supply (data not shown). There is general concurrence that growth on  $\text{NH}_4^+$  should in theory enable faster growth rates than  $\text{NO}_3^-$  due to the energy savings associated with the direct assimilation of  $\text{NH}_4^+$  into amino acids ( $\text{NO}_3^-$  must first be reduced to  $\text{NH}_4^+$ ). However, there is little agreement regarding the efficacy of the two forms of nitrogen in promoting growth of rhodophytes. Hafting (1999) measured higher growth rates under  $\text{NO}_3^-$  than  $\text{NH}_4^+$  when cultured under high irradiance (160  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ), but equivalent growth at lower intensities (50  $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$ ). *Agardhiella subulata* and *Gracilaria tikvaiae* grew faster with  $\text{NH}_4^+$  as a N source (DeBoer et al., 1978), while Hanisak (1990) reported identical growth rates for *G. tikvaiae* grown under the two conditions.

Blades deprived of abundant inorganic N exhibited higher rates of N uptake than did N-replete blades. This finding is in line with other earlier work (D'Elia & DeBoer, 1978; Smit, 2002). In part,  $\text{NH}_4^+$  may be diffusing into the cell walls and intercellular spaces before it is taken up as the apparent free space comes into equilibrium with the external medium. However, the rapid influx of ions in *P. perforata* was limited to "a few minutes" (Epply & Blinks, 1957). Higher rates of uptake by N-deprived individuals (and during early phases of incubation; Harrison & Hurd, 2001) also represent the filling of internal pools (Lobban & Harrison, 1997).

High values of the maximum uptake rate and/or low half-saturation constants are desirable in an integrated aquaculture application. The initial slope of the concentration-uptake rate curve ( $\alpha$ ) provides one indication of bioremediatory ability. The  $K_S$  value might not seem relevant for the ultimate application because under aquaculture conditions effluent entering *Porphyra* tanks is rich in inorganic N. However, N concentrations will fall during bioremediation, a function of residence time, macroalgal stocking density, and the physical conditions of temperature and light, all factors influenced by the engineering and operating requirements of the system (Hernandez et al., 2002;

Neori et al., 1998; Schuenhoff et al., 2003). Parallel semi-batch experiments at lower stocking densities (0.3 g FW  $\text{L}^{-1}$ ) showed a 95% reduction in inorganic N after only 3 days, making lower  $K_S$  (and higher  $\alpha$ ) more desirable since this supports higher growth rates. The irradiance effect, visible in the comparison of uptake rates at 40 and 150  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , has obvious implications to the aquaculture of macroalgae. Hafting (1999) reported that growth rates were elevated at higher light levels, an effect requiring increased supply of N. Macroalgal yield and, hence, bioremediation under nutrient-enriched conditions depend on the interaction between stocking density and growth rate. Increased culture density raises the amount of the biomass capable of producing new tissue, but reduces the tissue-specific growth rate through a reduction in irradiance. Work to identify the stocking density that optimizes yield is underway as part of an effort to model the production and bioremediation by *Porphyra* grown under conditions of integrated aquaculture.

*P. yezoensis* (and probably other *Porphyra* species) demonstrate limited storage of N (Hafting, 1999; Hernandez et al., 2002). Some N, not devoted immediately to growth, can be sequestered as photosynthetic pigments, free amino acids, and proteins (cf. Martinez & Rico, 2002; Naldi & Wheeler, 1999; Smit et al., 1997). The relationships among external N availability, the form in which N is sequestered internally, and growth rate can be complex. However, since the primary goal of this project is the bioremediation of nutrient-loaded effluent from finfish aquaculture, the ultimate destination of assimilated N is now only of secondary concern. To accurately predict the economic benefits of integrating seaweed and finfish culture, future studies are needed to evaluate the fates of N and their impacts on the biomass as a food or biochemical product.

*P. amplissima* proved to be the best performer of the northeast American species tested in these short-term measurements of inorganic N uptake. We have begun to test this and other species at larger spatial and temporal scales, finding correspondence in the scale-up. For example, over 28 days in 1-L volumes renewed every 3–4 days, *P. amplissima* demonstrated the fastest rates of growth and N removal from the culture medium (Carmona et al., in press). A pilot scale project is currently underway using a flow-through system of 3000-L *Porphyra* growth tanks to further validate the results of prior work (Yarish et al., 2001).



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