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Exploring Northeast American and Asian species of *Porphyra* for use in an integrated finfish–algal aquaculture system

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Abstract

Many aquaculture industries generate a nutrient-rich waste stream that can lead to eutrophication of coastal waters. To address this environmental issue, the bioremediation potential of several native Northeast American species of *Porphyra* was assessed and compared to the well-known Asian species. *Porphyra* thalli were cultured over 4 weeks at 15 °C at a stocking density of 0.4 g FW L^{-1}. At 3- to 4-day intervals nutrient uptake, tissue N accumulation and phycobiliprotein concentration (PBP) were determined as functions of nitrogen (N) concentration (25–300 μM) and N source (nitrate vs. ammonium). Growth rates were measured weekly. Growth and tissue N reached maximal levels at inorganic N concentrations of 150–300 μM. Maximum growth rates ranged from 10% to 25% day^{-1}, although induction of archeospores reduced average growth rates in many cases. No evidence of ammonium toxicity (reductions in growth rate) was observed; in fact, similar values were found with both N sources. Ammonium generally yielded higher PBP and tissue N contents than nitrate. *Porphyra amplissima* presented the highest growth rate, followed by the Asian *Porphyra yezoensis*. Under the experimental conditions, *Porphyra* spp. removed 70–100% of N within 3–4 days at N concentrations up to 150 μM, but was less efficient in removing inorganic phosphorus (35–91% removal). The highest tissue N and PBP concentrations were found at 150–300 μM of N, with N values close to 7% DW. 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.

Keywords: Integrated aquaculture; *Porphyra*; Nutrient uptake; Nitrogen content; Bioremediation

1. Introduction

Finfish mariculture along the Northeast American coast continues to grow, however, there are some constraints (Naylor et al., 2000; Troell et al., 2003). On local to regional scales, finfish aquaculture may significantly contribute to the nutrient loading of coastal waters because of effluents rich in inorganic nitrogen (N) and phosphorus (P) (Kautsky et al., 1997). 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; Chopin et al., 2001). The detrimental effects of eutrophication include blooms of harmful phytoplankton and unwanted macroalgae

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(Cuomo et al., 1993; Naylor et al., 2000), as well as development of hypoxia and anoxia (Bonsdorff et al., 2002; Sfriso et al., 1992). The ecological incentive for remediating eutrophic effluents through balance ecosystem management is clear (McVey et al., 2002). Besides this ecological aspect, there are also economic incentives. The N and P that are flushed from the system represent the loss of opportunity for the aquaculturist, since these nutrients could be channelled into the production of valuable products (Chopin et al., 2001). In addition, governmental agencies charged with reducing coastal eutrophication are developing regulations to limit the release of N and P. In the future, U.S.A. aquaculturists can expect to incur financial penalties if they cannot regulate the release of the waste effluent (Anonymous, 2002).

One solution to the problem of eutrophic effluent, addressing both ecological and economic issues, is the development of integrated aquaculture, in which seaweeds are grown downstream from animals (McVey et al., 2002; Chung et al., 2002). 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 (e.g., Ryther et al., 1978). Previous studies have focused on the integration of other macroalgal species into finfish culture, as *Ulva lactuca* (Coen and Neori, 1991) and *Ulva rotundata*, *Enteromorpha intestinalis* and *Gracilaria gracilis* (Hernández et al., 2002; Martınez-Aragón et al., 2002). While these species may be efficient nutrient filters, the use of the harvested biomass is limited to organic composting and low-profit agar extraction. One thing that distinguishes this study from prior ones 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 (Neori et al., 2004). We investigated the genus *Porphyra* as the seaweed component for a number of reasons. 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 (Neori et al., 2004). In part for these reasons, *Porphyra* is capable of rapid growth. Species of *Porphyra* are also efficient nutrient concentrators. In situations where nutrients are readily available, N can constitute 6% of 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 (FAO, 2003). Another added value development is the use of *R*-phycoerythrin as the fluorescent conjugate for immunological detection of target molecules in molecular biological research (Mumford and Miura, 1988). Several studies of native Northeast American *Porphyra* species have clarified the taxonomic status and ecological requirements (Klein et al., 2003; Neefus et al., 2002; Yarish et al., 1998), as well as their potential productivity (Kraemer and Yarish, 1999). The positive role of *Porphyra* in removing N and P in sites of experimental nori/salmon integrated aquaculture has also been reported (Chopin et al., 1999; McVey et al., 2002).

Growth, accumulation of N and P, and high value by-products in tissue are dependent upon the environmental factors that regulate production: temperature, nutrient availability, irradiance and water motion. As part of an effort to develop an economically viable system of integrated polyculture, we have been evaluating the bioremediation and mariculture potential of Northeast American 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 water culture. We present here results that describe the influence of N source and concentration on growth, nutrient uptake, *R*-phycoerythrin and tissue N contents.

2. Materials and methods

2.1. Plant material

Gametophytic blades of *Porphyra* species used in the experiments were generated from the conchocelis stage, after inducing the formation and release of conchospores. The experimental work was conducted under controlled environmental conditions of irradiance, temperature, and photoperiod in walk-in growth chambers at the Marine Biotechnology Laboratory of the University of Connecticut at Stamford. Under those conditions, both foliose and conchocelis phases of all the strains are maintained in continuous culture in von Stosch's seawater enrichment (Ott, 1965). The Northeast American species included *Porphyra amplissima* (strain ME32), *Porphyra leucosticta* (strain CT23-1), *Porphyra purpurea* (strain NY4-1) and *Porphyra umbilicalis* (strain ME6-9), while Asian species included *Porphyra haitanensis* (origin of strain unknown), *Porphyra katadai* (strain PKTF99) and *Porphyra yezoensis* (strain PYWT2001039A).

2.2. Experimental design

Porphyra tissue was acclimated at 15 °C for 7 days at 150 μmol m^{-2} s^{-1}, 12:12 L/D photoperiod without a media change. Approximately 0.3 g FW were then placed in 1-L flasks containing 800 mL of Von Stosch-enriched seawater culture medium, with air bubbling for providing agitation and breaking down any boundary layers. The N level in the incubation medium was adjusted to 25, 75, 150 and 300 μM of either ammonium (NH_4^+) or nitrate (NO_3^-). These concentrations were chosen taking into account the average NH_4^+ concentration of ca. 150 μM in a finfish effluent (Great Bay Aquaculture, LLC., Portsmouth, New Hampshire, USA, Nardi, pers. comm.). Phosphorus (PO_4^{3-}) was also added to maintain a constant 10:1 N:P molar ratio. In an additional experiment with *P. umbilicalis*, N and P levels were raised by a factor of roughly five (120, 360, 720, 1440 μM) to investigate growth in highly eutrophic effluents. Three replicate flasks were used for each treatment (nutrient source × concentration).

2.3. Growth rate and chemical analyses

At 7-day intervals, the tissue in each flask was blotted on paper towel, weighed (fresh weight, FW), and restored to the initial stocking density (ca. 0.4 g FW L^{-1}) by removing biomass. This prevented growth inhibition due to an excess of biomass or an increase in pH. Specific growth rate (SGR) was then calculated from:

$$\mu(\%\ \text{day}^{-1}) = [\ln(B_2/B_1)]/(t_2 - t_1) \times 100,$$

where B_2 and B_1 were algal biomass at time t_2 and t_1 respectively.

From excised tissue, samples were oven-dried at 60 °C for 48 h and ground to a fine powder for total tissue N analysis using a Perkin-Elmer Series II 2400 CHNS/O Analyzer. For phycobiliprotein analysis, approximately 0.1 g FW was frozen and later ground using a ball mill. When there was sufficient biomass, three replicate samples were used. Phycobiliproteins were extracted overnight in phosphate buffer (0.1 M pH 6.5) at 4 °C, and phycoerythrin was spectrophotometrically quantified in the supernatant after centrifugation of the extracts, according to the equations given by Beer and Eshel (1985).

2.4. Nutrient uptake analyses

At 3–4-day intervals the culture medium was changed, and a sample from each flask and from the initial medium was filtered through Whatman GF/F glass-fiber filters to remove organic debris. Filtered medium was frozen for analysis of inorganic N and P by the Environmental Research Institute, University of Connecticut, using a Four Channel Auto Analyzer equipped with High-Sensitivity Seawater Cartridges (Lachat-QuikChem AE Ion Analyzer). Uptake rates were then calculated as:

$$\mu\text{mol g}^{-1}\text{FW day}^{-1} = [(C_a + C_b) \times V]/B \times t,$$

where C_a and C_b were the nutrient concentrations removed from the medium in two consecutive medium change intervals; V was the culture volume (0.8 L), B was the average biomass for each time period; and t was the time period (7 days). The values shown in the results are the average for the 28-day experimental period.

The removal efficiency (in the medium change period) was expressed as percentage of the incoming amount of the nutrient and values are shown as the average for the 28-day period.

2.5. Statistical analysis

For all the treatments, three independent replicates were analyzed (unless stated otherwise), and means and standard deviations were calculated. For each species, differences among treatments (N source and concentration) were tested for significance using two-way ANOVA. Multiple post-hoc comparisons among means were tested by the SNK test. Data that did not comply with normality or equal variance were transformed (log x or $x^{1/2}$). In all cases, the null hypothesis was rejected at the 5% significance level, according to Sokal and Rholf (1981).

3. Results

3.1. Growth

Over the 28-day long-term experiments, growth was not constant for all species, with periods where blades became reproductive, released spores and consequently lost some biomass. Fig. 1 shows the SGR during the experimental period for the 150 μM NH_4^+ treatments. *P. amplissima* grew rapidly (25% day^{-1}) at the outset of the experiment and declined only slightly during the subsequent three weeks. The same pattern was observed in *P. haitanensis*, though this species reached a maximum weekly SGR of only 16% day^{-1}. *P. yezoensis* also grew well during the first week (18% day^{-1}), but archeospore production ensued and the tissue began to

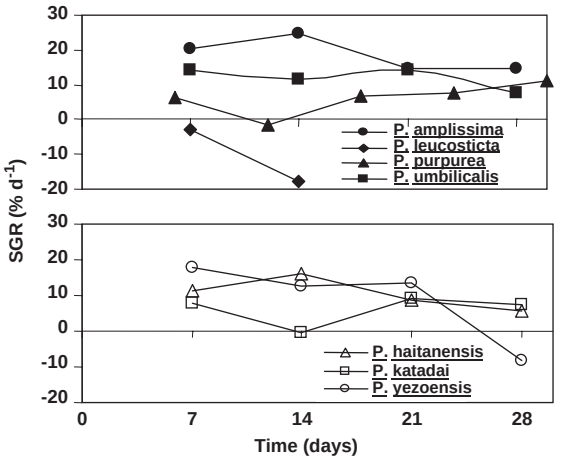

Fig. 1. Average growth rates (n=3) of Northeast American *Porphyra* species (black symbols) and Asian species (white symbols) during long-term experiments (at 150 μM NH_4^+). Negative growth rates occur when tissue becomes reproductive and fragments. For variance of data see Table 1.

Table 1
Specific growth rates of *Porphyra* species during 28-day experiments at 15 °C and 150 μM NH_4^+ (n=8–12)

Species	Growth rate (% day^{-1})		
	Average	Maximum[a]	Variance
P. amplissima	17.9	25.2	7.2
P. leucosticta	[b]	−3.7[b]	[b]
P. purpurea	6.1	14.3	38.9
P. umbilicalis	13.1	17.8	12.2
P. haitanensis	10.5	16.3	24.4
P. katadai	6.1	9.6	21.7
P. yezoensis	10.1	18.4	111.2

[a] Maximum observed for a single culture during a 1-week period.
[b] Production of archeospores began immediately and tissue fragmented completely.

disintegrate. By the experiment's end, the SGR of *P. yezoensis* was negative as losses due to reproduction-linked tissue fragmentation exceeded new growth. *P. katadai* and *P. purpurea* appeared to enter a brief period of reproduction but growth had rebounded by the experiment's end. On the other hand, *P. leucosticta*

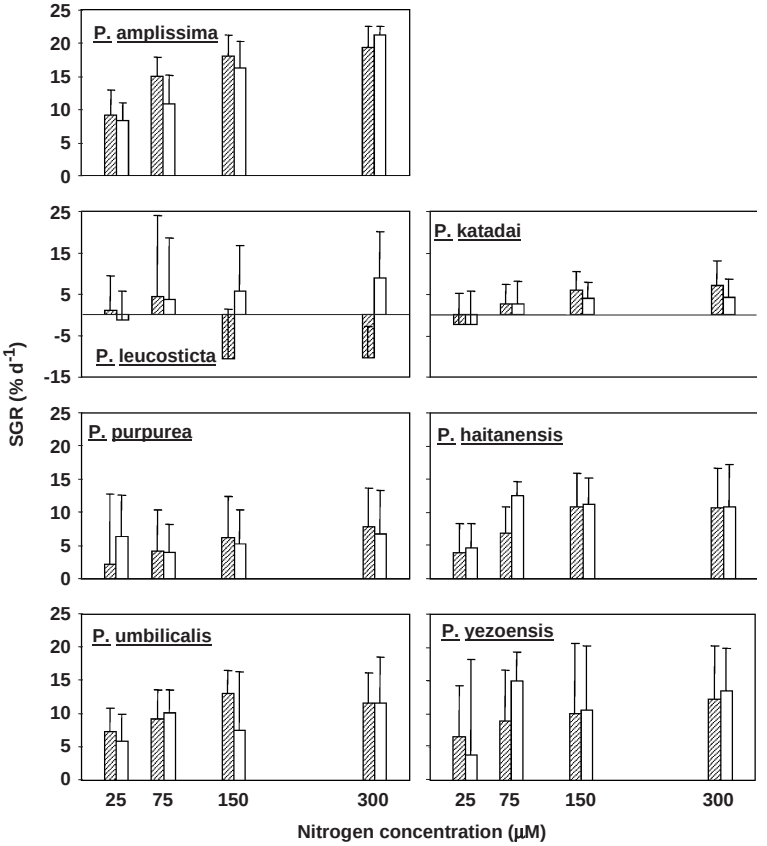

Fig. 2. Growth rates of *Porphyra* species cultured at different N concentrations of NH_4^+ (lined bars) and NO_3^- (white bars). Left and right side are Northeast American and Asian species, respectively. Data are averaged values for the 28-day culture period and error bars are standard deviation (n=8–12).

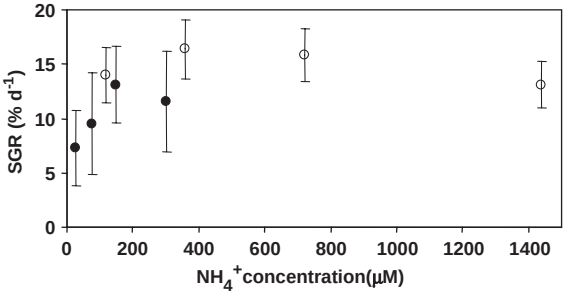

Fig. 3. Relationship between NH_4^+ concentration and SGR of *P. umbilicalis* (averaged over the entire 28-day experiment). Black and open circles correspond to the experiments with the standard (25, 75, 150 and 300 μM) and higher (120, 360, 720 and 1440 μM) N concentrations, respectively. Error bars are standard deviation ($n=12$).

blades started archeospore release during the first days of culture with high NH_4^+ concentrations (150 and 300 μM) and disintegrated completely after 2 weeks.

Fig. 2 shows the SGR pooled across the entire experimental period with the two N sources. Nitrogen source did not affect SGR of *Porphyra* species, except in *P. leucostica*, that presented a higher SGR with NO_3^- at the highest concentrations, while NH_4^+ triggered archeospore release. In *P. amplissima*, growth saturated at 150 μM NH_4^+ and did not when the N source was NO_3^-. Two Asian species, *P. katadai* and *P. haitanensis*, and also *P. umbilicalis* were growth saturated at lower N concentrations (75 μM N), while *P. purpurea* and *P. yezoensis* showed similar SGR at all N concentrations. Overall, *P. amplissima* grew fastest, both on average and in terms of maximal rate achieved (Table 1). The influence of reproduction was reflected in the size of the variance term. *P. amplissima* growth rates were relatively constant over the 28-day experiment, while at the other end of the scale those of *P. yezoensis* varied greatly. When data from the experiments with *P. umbilicalis* employing the standard (25–300 μM) and elevated (120–1440 μM) NH_4^+ concentrations were combined, SGR was roughly 20% less at 1.44 mM, though statistically similar, to the value at 300 μM (Fig. 3).

3.2. Nutrient uptake rate and removal efficiency

Nitrogen and phosphorus depletion from the culture medium provided an estimate of the bioremediation potential of *Porphyra*. Values of uptake were averaged for the entire experimental period of 28 days. Nitrogen uptake is shown in Fig. 4. In general, nitrogen source did not affect N uptake, except in *P. yezoensis*, which presented higher N uptake with NH_4^+. The highest uptake rates were observed in *P. purpurea* and *P. haitanensis*, at 150 and 300 μM N, respectively. On the other hand, the N uptake was not saturated in *P. amplissima* and *P. haitanensis*, regardless the N source,

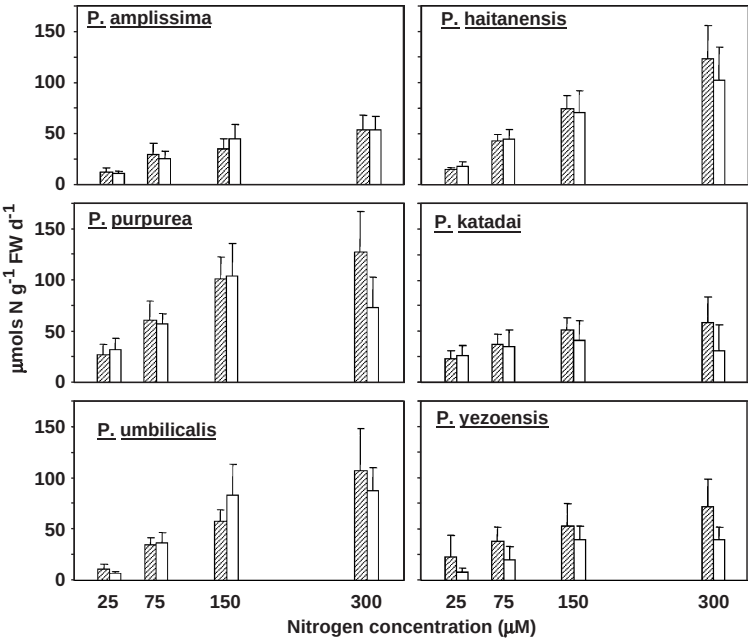

Fig. 4. Nitrogen uptake rates (averaged values for the 28 days) by *Porphyra* species at different N concentrations of NH_4^+ (lined bars) and NO_3^- (white bars). Left and right side are Northeast American and Asian species, respectively. Error bars are standard deviation ($n=10$–12).

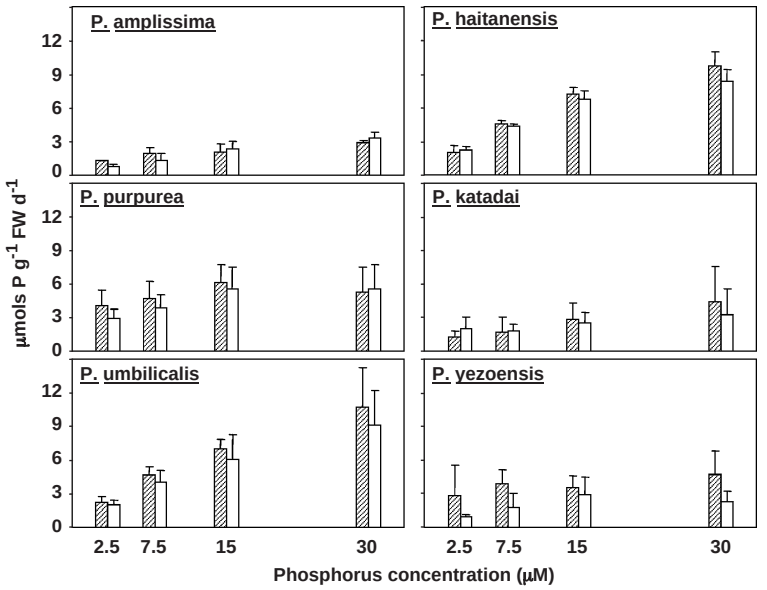

Fig. 5. Phosphorus uptake rates (averaged values for the 28 days) by *Porphyra* species at different N concentrations of NH_4^+ (lined bars) and NO_3^- (white bars). Left and right side are Northeast American and Asian species, respectively. N/P ratio was 10. Error bars are standard deviation (n=10–12).

nor was NH_4^+ uptake by *P. umbilicalis*. On the contrary, NH_4^+ and NO_3^- uptake by *P. purpurea*, *P. yezoensis* and *P. katadai* were saturated at 150 µM. Phosphorus uptake was higher when NH_4^+ was the N source in *P. purpurea*, *P. umbilicalis* and *P. yezoensis* (Fig. 5). Uptake was not saturated up to 30 µM P in *P. umbilicalis* and *P.*

Table 2
Nitrogen and phosphorus removal (%) in 3–4 days of *Porphyra* species at different concentrations of NH_4^+ and NO_3^-, averaged for the 28-day culture period

Species	N concentration (µM)	NH_4^+ treatment		NO_3^- treatment	
		N removal (%)	P removal (%)	N removal (%)	P removal (%)
P. amplissima	25	91 (6)	87 (8)	96 (4)	56 (23)
	75	97 (2)	86 (11)	94 (13)	57 (26)
	150	99 (1)	84 (23)	99 (2)	69 (19)
	300	88 (18)	80 (23)	93 (13)	57 (20)
P. purpurea	25	97 (6)	82 (12)	99 (1)	62 (13)
	75	98 (3)	61 (11)	100 (1)	52 (11)
	150	96 (4)	46 (10)	91 (12)	41 (10)
	300	71 (16)	27 (17)	44 (24)	25 (19)
P. umbilicalis	25	100 (0)	83 (13)	87 (19)	76 (25)
	75	100 (0)	88 (15)	94 (10)	79 (28)
	150	99 (4)	91 (13)	87 (26)	71 (31)
	300	77 (35)	81 (19)	72 (34)	76 (24)
P. haitanensis	25	95 (4)	77 (28)	93 (10)	83 (21)
	75	98 (1)	87 (14)	97 (10)	89 (15)
	150	99 (1)	88 (11)	99 (2)	83 (13)
	300	87 (20)	65 (17)	72 (28)	56 (17)
P. katadai	25	83 (15)	35 (31)	92 (14)	65 (31)
	75	94 (8)	43 (22)	89 (15)	48 (31)
	150	91 (13)	55 (18)	69 (25)	49 (22)
	300	65 (28)	41 (19)	27 (25)	34 (20)
P. yezoensis	25	88 (7)	83 (23)	77 (23)	83 (23)
	75	93 (7)	85 (10)	92 (8)	81 (19)
	150	92 (20)	74 (25)	92 (18)	72 (33)
	300	90 (15)	56 (20)	78 (29)	59 (25)

The N/P ratio was always 10. Standard deviations in parenthesis (n=12).

haitanensis, while uptake rate by *P. purpurea* was saturated at 15 μM P, regardless the N source, and at even lower P concentrations in the other species.

In terms of biofiltering efficiency, at the three lower NH_4^+ concentrations (25, 75 and 150 μM), 83% to 100% of this nutrient was removed from the medium over the 3–4-day periods by *Porphyra* species, with slightly lower but not significantly different NO_3^- removal values (Table 2). At 300 μM either NH_4^+ or NO_3^-, N removal efficiency decreased in most of species tested, especially in *P. katadai* where a decrease was suggested at 150 μM NO_3^- and only 27% and 65% of the NO_3^- and NH_4^+, respectively, was taken up at 300 μM. *P. amplissima*, *P. yezoensis* and, to a lesser extent, *P. haitanensis*, showed only slight decreases in removal efficiency at the highest (300 μM) N concentrations. Phosphorus biofiltering efficiency was higher when NH_4^+ was supplied, except in *P. katadai* and *P. haitanensis* (Table 2). In all species, P removal was lower than for N (64% compared to 87%, averaging all the values in Table 2). The efficiency with which *P. purpurea* removed inorganic P decreased gradually with increasing P concentrations, declining from 82% at 2.5 μM P to only 27% at the highest P concentration (30 μM). This drop in P biofiltering efficiency was also observed in *P. yezoensis*, *P. katadai* and *P. haitanensis* (Table 2).

3.3. R-phycoerythrin and tissue N content

By the end of the experiments, *R*-phycoerythrin concentration in native Northeast American species was higher when NH_4^+ was supplied than when NO_3^- was the N source (Table 3). Overall, *P. purpurea* showed the highest values of this pigment. On the other hand, Asian species presented similar RPE content with both N sources. Pigment content appeared saturated at 150 μM N in *P. purpurea* and *P. umbilicalis*. In *P. amplissima*, RPE concentration increased linearly with NH_4^+ concentration, and appeared to saturate at 75 μM NO_3^- (Table 3). *P. katadai* was the only Asian species that did not show saturation of RPE content. In *P. yezoensis* and *P. haitanensis*, insufficient material was available for RPE extraction in all the treatments, thus the only possible comparison was done at 150 and 300 μM N in *P. haitanensis*, with similar contents.

Tissue nitrogen content was not dependent on the N source in *P. umbilicalis*, *P. amplissima* and *P. yezoensis* (Fig. 6). In the native American species, tissue N content increased linearly with NH_4^+ concentration, while in *P. yezoensis* it saturated at 150 μM NH_4^+ (data not available for NO_3^-). When differences between N sources existed, NH_4^+ treatments gave higher N contents, compared to NO_3^- cultures, presenting saturation at 150 μM NO_3^- in *P. purpurea* and at 75 μM NO_3^- in *P. haitanensis*. The highest average value was found in NH_4^+–grown cultures of *P. purpurea* (Fig. 6).

Table 3
R-phycoerythrin (RPE) content at the end of the culture period (28 days) of *Porphyra* species at different concentrations of NH_4^+ and NO_3^-

Species	N concentration (μM)	NH_4^+ treatment RPE content (mg g^{-1} FW)	NO_3^- treatment RPE content (mg g^{-1} FW)
P. amplissima	25	0.08 (0.04)	0.06 (0.02)
	75	0.26 (0.08)	0.44*
	150	0.57 (0.1)	0.76 (0.29)
	300	1.35 (0.54)	0.67 (0.10)
P. purpurea	25	0.92 (0.38)	0.27 (0.09)
	75	1.65 (0.15)	1.04 (0.34)
	150	3.14 (0.17)	2.82 (0.52)
	300	3.44 (0.77)	3.25 (0.35)
P. umbilicalis	25	0.55 (0.14)	0.11 (0.0)
	75	1.03 (0.2)	0.3*
	150	2.33 (0.32)	nd
	300	2.82*	2.45*
P. haitanensis	25	nd	nd
	75	0.44*	0.29*
	150	0.59 (0.31)	0.81*
	300	1.15 (0.56)	0.73 (0.52)
P. katadai	25	nd	nd
	75	0.19 (0.01)	0.16 (0.11)
	150	0.52 (0.06)	0.54 (0.11)
	300	0.75 (0.04)	0.70 (0.08)
P. yezoensis	25	0.12*	nd
	75	nd	0.26*
	150	nd	0.68*
	300	1.02*	1.08*

Standard deviations in parenthesis (n=2–3; *n=1; nd=not available data).

4. Discussion

All the *Porphyra* species studied here, except perhaps for the *P. leucosticta* strain that we used, appear to be good candidates for bioremediation, given the high nutrient removal efficiency and growth rate values obtained. The promise of these *Porphyra* species as tools for bioremediation was pointed out by Kraemer and Yarish (1999), reporting higher photosynthetic rates by *P. umbilicalis* and *P. purpurea* than by *P. yezoensis*, the commercially cultured Asian species (Zhang et al., 1997). *P. amplissima* exhibited the highest average and maximum growth rates, consistent with its performance in measurements of short-term NH_4^+ uptake (Kraemer et al., 2004). The growth of *P. amplissima* was also the least variable of all species investigated. All these factors identify *P. amplissima* as a prime candidate for

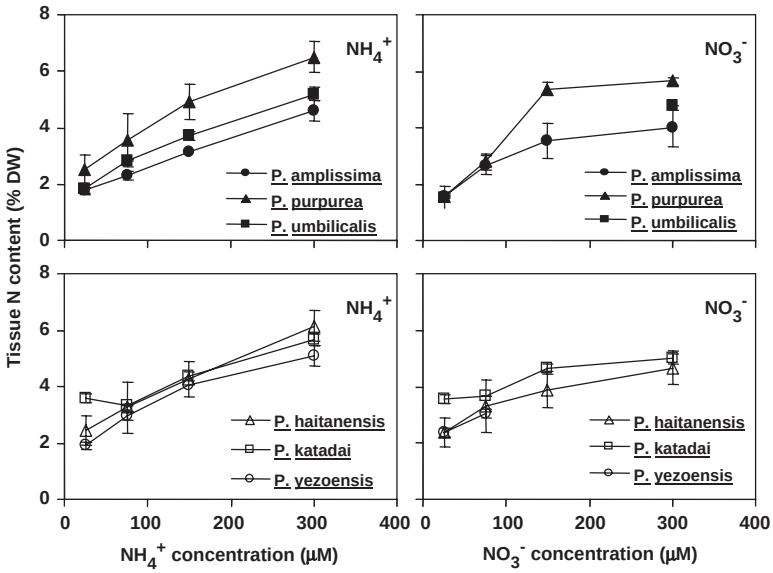

Fig. 6. Relationship between N availability as NH_4^+ or NO_3^- concentration and tissue N content at the end of the 28-day experiment in Northeast American (black symbols) and Asian (white symbols) *Porphyra* species. Data are means and error bars are standard deviation ($n=3$).

the next phase (larger scale) of development of integrated aquaculture systems.

Descriptions of the effect of N source effect on the growth of rhodophytes are sometimes contradictory and depend on the species and other variables as irradiance and physiological history. Hafting (1999) reported that NO_3^- is a better N-source for growth of *P. yezoensis* than NH_4^+ in high light (160 μmol m^{-2} s^{-1}), but no differences in growth under NO_3^- and NH_4^+ were seen in low light conditions (50 μmol m^{-2} s^{-1}). *Gracilaria foliifera* and *Neogardhiella baileyi* grew faster with NH_4^+ as N source (DeBoer et al., 1978), while *Gracilaria tenuistipitata* and *Gracilaria cornea* presented similar growth rates under both N sources (Haglund and Pedersén, 1993; Navarro-Angulo and Robledo, 1999). *Porphyra* species tested here showed this last response, with similar growth rates with the two N sources. Although NH_4^+ is the main N source excreted by fish, the similar growth rates obtained with NO_3^- supports the use of these species in eutrophic coastal waters.

P. leucosticta was the only species where growth rate depended significantly on N source. Growth in NH_4^+ appeared to trigger archeospore production in this species. If so, this would be a significant drawback species considered for an integrated aquaculture system. The rates of thallus maturation, spore production and release, are all processes regulated by species-specific combinations of temperature, light irradiance and photoperiod (Lüning, 1990; Notoya and Miyashita, 1999; Yarish et al., 1998). However, less information is available on nutrient effect on reproduction in macroalgae. A pioneering study showed that high concentrations of N favored vegetative growth and asexual reproduction, while low N levels favored gamete formation in *Ulva fasciata* (Mohsen et al., 1974). On the other hand, nutrient enriched conditions induced reproduction in *Laminaria saccharina* (Lüning, 1988) and tetrasporangia formation in *Falkenbergia*-phase of *Asparagopsis armata* (Guiry and Dawes, 1992), whereas Azanza and Aliaza (1999) did not find a significant effect of nutrient enrichment on spore release in *Kappaphycus alvarezii*. Inhibition of this phenomenon could override the biomass loss problem and studies at the genetic level are underway.

Although average N concentration in fish farm effluent is approximately 150 μM NH_4^+ (Great Bay Aquaculture, LLC., Nardi, pers. comm.), higher concentrations assayed in this study represent potential transient elevations. Northeast American species may yield more biomass than Asian ones at high N levels, since no growth saturation was observed at 300 μM. Work with *P. umbilicalis* raises the possibility that high (i.e., millimolar) concentrations of NH_4^+ may decrease growth and the potential for bioremediation. Hence, dilution of effluent may be necessary for optimal function of the seaweed biofilter system.

In general, *Porphyra* showed a good nutrient removal capabilities, with 96% of NH_4^+ and 73% of PO_4^{3-} removed during the 3–4-day periods at 150:15 μM N/P (averaged values for all the species). Higher

uptake rates have been observed with NH_4^+ as N source compared with NO_3^- in *G. tenuistipitata* (Haglund and Pedersén, 1993) and in *U. lactuca* (Neori, 1996). In our study, greater removal of NH_4^+ than NO_3^- was only observed at all concentrations for *P. yezoensis*, while in most cases, as happened with growth, N source had no effect on N uptake. This lack of an N source effect on uptake rates by *Porphyra* was also found in the short-term uptake measurements (Kraemer et al., 2004). The fact that N uptake rate was not saturated at 300 μM or saturated at higher N concentration than growth suggests the N removed from the media is not entirely incorporated into new algal biomass, but accumulates intracellularly, as reported in other red macroalgae (Hafting, 1999; Naldi and Wheeler, 1999; Ryther et al., 1981; Vergara et al., 1995). On the contrary, P uptake was saturated in most *Porphyra* species of this study at low P concentrations, suggesting that P storage apparently did not occur (over the P range assayed), as also observed by Hafting (1999) in *P. yezoensis*.

Like *Porphyra* species of this study, the lower P removal by *U. lactuca*, relative to N, was explained by the lower molar N/P ratio in the medium than in the seaweed tissue (Neori et al., 1996). Chopin et al. (1999) gave N/P values of 21–32 for three *Porphyra* species, while the N/P ratio in our growth medium was 10. Therefore, the need for N relative to P (defined by the tissue N/P ratio) was greater than the relative molar supply (defined by the medium N/P ratio). The N was exhausted first, leaving quantities of P in the medium unused. Nevertheless, P removal efficiencies reported here were comparable or even higher to those found in other macroalgae used in fish aquaculture systems (Buschmann et al., 1996; Neori et al., 1998; Martınez-Aragón et al., 2002).

The higher P removal efficiency in some species when NH_4^+ was supplied would support their use in integrated aquaculture systems, withdrawing the two main waste nutrients. The inverse relationship found between total N uptake rate and uptake efficiency (Fig. 4 and Table 2) in the Asian species and *P. purpurea* was already presented by other authors (Coen and Neori, 1991; Neori et al., 1991; Ryther et al., 1975). The optimal seaweed biofilter would include a high uptake rate (and with it, a high biomass and protein yield) together with a high nutrient removal efficiency. This dilemma has been recently solved by Neori et al. (2003) by means of a three-stage seaweed biofilter design. The design used the finding that the performance of seaweed ponds depended on the flux of total NH_4^+–N through them, and that therefore effluents with reduced total NH_4^+–N could provide the seaweed with a high total NH_4^+–N flux if the water flow increased proportionally. The effluent was passed through a series of three successively smaller ponds, increasing the water exchange rates inversely to their sizes, increasing the biofiltering efficiency by up to 80%.

In *Gracilaria edulis* and in *Gracilaria pacifica*, pigments, total tissue nitrogen and aminoacids accumulated in response to the supply of NH_4^+ more than to NO_3^- supply (Jones et al., 1996; Naldi and Wheeler, 1999). Opposite to this response, Asian species of this study showed a similar RPE content with both N sources. In *P. amplissima*, our best candidate for integrated aquaculture systems from the growth point of view, the RPE content increased with NH_4^+ concentrations, and in *P. purpurea* and *P. umbilicalis* the accumulation saturated at relatively high concentration (150 μM). The accumulation of pigments under conditions that saturate growth demonstrates the well-known N storage role of these compounds (Lapointe, 1981; Smit et al., 1997; Vergara et al., 1995). As growth causes an increase in biomass density, the extra pigments maximize light absorption in the impoverished light regime. On the other hand, tissue nitrogen accumulates as an apparently linear function of NH_4^+ availability, in Northeast American species of *Porphyra* (Fig. 6). This non-saturation of tissue N accumulation up to 300 μM in media demonstrates that these species are good candidates as nutrient scrubbers, especially for NH_4^+-enriched waters. This argues that N-containing compounds other than pigments are also important in the storage of N. Tissue amino acid levels have been shown to increase with increasing N availability (Hariskov and Yarish, in prep.), though they may not account for all the increase in tissue N.

We can conclude that native Northeast American species of *Porphyra* can be good choices for reducing the N load in fish farm effluents. At this point, we do not advise the use of *P. leucostica* for bioremediation purposes, due to the sporulation problems we encountered in the NH_4^+ enriched media. The native Northeast American species appeared to give growth rates and nutrient removal values comparable and even higher than those of the commercially exploited Asian ones. *P. amplissima* is the best candidate in terms of biomass production and nutrient removal efficiency, while *P. purpurea* would also be useful for its high N and RPE content (both important for added value products).

Nevertheless, the controlled laboratory environment of this study limits the extrapolation of these growth and nutrient removal values to the real conditions in a flow-through finfish–seaweed integrated aquaculture system.

We recognize that the effluent residence time in seaweed tanks will be less than the 3–4-day periods used in this semi-batch design, and that the exact values of nutrient removal under these conditions can only be determined in flow-through systems. Scaling up from 1-L flasks to the 1000-L tanks to be used in the ultimate application may also alter the performance of the system (e.g., Ehleringer and Field, 1993), however, we are encouraged by similar approaches of using small scale systems in the development of the commercial land-based culture of *Chondrus crispus* described by Craigie (1998) and Craigie et al. (1999).

Therefore, assuming uptake rates similar to those measured in this study, we can estimate required seaweed tank volumes. Great Bay Aquaculture, LLC. discharges ca. 190 L min^{-1} at concentrations of 10 μM P and 143 μM N (Nardi, pers. comm.) for a daily total of 2.7 mol phosphorus and 39.1 mol nitrogen. Assuming a relative growth rate of 25% day^{-1} by *P. amplissima* (up to 40% day^{-1} has been measured; Kraemer, unpub. data), 6% tissue nitrogen (DW basis), and 0.1 g DW g^{-1} FW, a *Porphyra* tank volume of about 328 m^3 would be required to remove 90% of the N in the effluent. If tanks are 1.5 m deep, this volume translates into 218 m^2 of tank area, a significant commitment of space. Clearly, the final analysis of system feasibility will require consideration of the economics of location. If aquaculture facilities can be sited in areas currently not highly valued (e.g., blighted urban zones or rural environments), this system of bioremediation may prove very viable.

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