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The effects of temperature and nutrient concentrations on nitrate and phosphate uptake in different species of *Porphyra* from Long Island Sound (USA)

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Abstract

Uptake rates of nitrate and phosphate were measured for four species and one variety of *Porphyra* from Long Island Sound (USA) at two temperatures and two nutrient medium concentrations at increasing intervals over a 24- or 48-h period. Maximum uptake rates found were: $V^{0-1\ h}_{30\ \mu M}$=73.8 μmol NO_3 g^{-1} DW h^{-1} and $V^{0-1\ h}_{3\ \mu M}$ =16.7 μmol PO_4 g^{-1} DW h^{-1}, in the two thinnest *Porphyra*. We found that the nitrate uptake rates were significantly greater at 30 μM than 3 μM NO_3 concentration, and that the uptake rates decreased with time of exposure. Temperature (5, 15, and 25 °C) did not have as strong an effect on nitrate uptake rates as did nutrient concentration. Q_{10} values and uptake rates at four different nitrate concentrations indicated that nutrient uptake at 5 °C was initially an active process. After 24 h, the processes involved appeared passive as Q_{10} values were between 1.0 and 1.3 and nitrate uptake curves were linear. Nitrate uptake rates correlated positively with the surface area/volume (SA/V) ratio. No coherent trends were found for uptake of phosphate, except that the uptake rates were significantly higher in 30 μM NO_3 medium as opposed to 3 μM NO_3. We did not find any significant difference in uptake rate and pattern between the summer species *Porphyra purpurea* (Roth.) C. Agardh, the eurythermic *Porphyra suborbiculata* Kjellm., the winter species *Porphyra*

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rosengurttii J. Coll and J. Cox, and the two varieties of *Porphyra leucosticta* Thur. Le Jol. (both winter species).

Keywords: *Porphyra* spp.; Nitrate; Phosphate; Uptake; Ecophysiology; ANW—Atlantic North West, New England; LIS—Long Island Sound

1. Introduction

The nutrients nitrate (N) and phosphate (P) are two of the most important elements required for algal growth, and one or the other often limits growth (DeBoer, 1981; Lapointe, 1987). Uptake involves both passive and active transport (D'Elia and DeBoer, 1978). However, in the natural environment, active transport through the plasmalemma is the most common mechanism as the concentrations inside the cells are usually in the millimole range, while concentrations outside are typically 1000-fold lower (micromole range; Lobban and Harrison, 1994). Physical, chemical, and biological factors all affect uptake rates of nutrients. Of the physical factors, the most important ones are light, temperature, water motion, and desiccation (Lobban and Harrison, 1994). Irrradiance has a direct effect on the uptake of nitrate (Wheeler, 1982), while spectral quality also affects the uptake and reduction of nitrate or phosphate (Lopez-Figueroa and Ruediger, 1991; Rai and Rai, 1997). Temperature influences the uptake of nutrients through Q_{10} effects on algal metabolism (Raven and Geider, 1988). However, algal metabolic response rates vary with species (Harlin and Craigie, 1978; Topinka, 1978). Many studies have demonstrated the strong influence of temperature on uptake of different nutrients (Asare and Harlin, 1983; Duke et al., 1989; Kautsky, 1990; Peckol et al., 1994; Chopin et al., 1995; Rivers and Peckol, 1995; Davison and Pearson, 1996; Gerard, 1997; Kinney and Roman, 1998; Ozaki et al., 2001). In general, the optimum temperature for uptake coincides with the optimum temperature for growth.

Desiccation is a normal but stressful event in the midlittoral zone and even more so in the supralittoral zone (Hurd and Dring, 1991; Hernández et al., 1995). Some desiccations enhance short-term (10–30 min) nutrient uptake rates upon rewetting (Thomas and Turpin, 1980; Thomas et al., 1987). Nutrient concentration in the medium and its chemical species is also of great importance (DeBoer et al., 1978; DeBoer, 1981; Chopin et al., 1990; Peckol et al., 1994; Sfriso, 1995; Braga and Yoneshigue Valentin, 1996; Harrison and Hurd, 2001) as well as the intracellular nutrient concentration (Gerard, 1982; Wheeler et al., 1984; Wheeler and Srivastava, 1984; Pedersen and Borum, 1996; Viaroli et al., 1996). High internal concentrations of a specific nutrient will repress uptake of the same nutrient (Chopin et al., 1990; Lobban and Harrison, 1994). One nutrient (e.g., ammonium) can have an antagonistic effect on the uptake of another (e.g., nitrate) when added simultaneously (DeBoer, 1981). Another biological factor that influences nutrient uptake is algal morphology [i.e., shape quantified as surface area/volume (SA/V) ratio] (Rosenberg and Ramus, 1984; Wallentinus, 1984; Hein et al., 1995; Taylor et al., 1998; Neori et al., 2004). Several specific enzymes are involved in the assimilation of each nutrient. However, the enzymes involved in both nitrate and phosphate uptake and assimilation are primarily

regulated by concentration of substrate, internal concentrations, and temperature (Syrett, 1981; Hanisak, 1983).

From an ecological perspective, the occurrence of species at different times of the year and in different zones on the shorelines implies that their rates of uptake and assimilation might reflect their spatial and temporal occurrence on the shorelines. Long Island Sound (LIS), USA, is inhabited by several species of *Porphyra* including *Porphyra suborbiculata* Kjellm., *Porphyra purpurea* (Roth) C Agardh., *Porphyra rosengurttii* J. Coll and J. Cox, and *Porphyra leucosticta* Thur. Le Jol. The latter taxon has at least one distinct morphological type; A (sensu Neefus et al., 2000). *P. rosengurttii* was described as *P. leucosticta* type C in Neefus et al. (2000); however, it was shown by Blodgett et al. (2002) to resemble *P. rosengurttii* J. Coll and J. Cox by DNA sequencing, hence referred in this paper as so. The occurrence of these species differs both temporarily and spatially (Pedersen et al., personal communication). Their ability to take up and assimilate nitrate and phosphate may vary as their access to these nutrients differs. In this study, we examined whether temperature or nutrient concentration is related to the uptake characteristics of five types (four species plus one variety) of *Porphyra* and if any differences could reflect the spatial and temporal occurrences of the different types.

2. Materials and methods

Nutrient uptake measurements were performed on five types of *Porphyra* collected from three different locations in LIS. *P. suborbiculata*, a eurythermic species occurring from fall to spring, was collected in January and February 2001 and December 2002, at Cove Island Park, Stamford, CT (N: 41°2, 644′ ; W: 73°30, 133′). *P. rosengurttii*, a winter species occurring from November to May, was collected in January 2002 at Cove Island Park, Stamford, CT. *P. purpurea*, a late summer species, was collected in September 2001 east of Port Jefferson, NY (N: 40°57, 862′ ; W: 73°2, 689′). *P. leucosticta*, found in the midlittoral to shallow sublittoral from March to late May, was collected in early May 2001 from White Point, Waterford, CT (N: 41°18, 180′ ; E: −72°08, 050′). Another strain of *P. leucosticta* was collected at Cove Island Park, Stamford, CT. The strain, type A (sensu Neefus et al., 2000), is mainly distinguished from the *P. leucosticta* holotype by its thallus morphology, thallus thickness, reproductive areas, and DNA. The thickness of the vegetative thallus of *P. leucosticta* type A and the holotype is 18–24 and 35–45 μm, respectively. *P. leucosticta* type A, which occurs in low littoral to shallow sublittoral from January to May, was collected for the experiments in March 2001. Based on these differences and those found by Neefus et al. (2000), type A was treated as a different strain of *P. leucosticta*.

The algae were kept in aerated autoclaved seawater (ASW) at 5 °C except for *P. purpurea*, which was kept in 15 °C, followed by acclimatization for 2 days at the appropriate test temperatures in aerated ASW. A short daylength light regime (8:16; L:D) was applied to all species except for the summer species *P. purpurea*, which as run during uptake experiments in 25 °C in neutral day photoperiods (12:12; L:D). Circular disks of either 20 or 14 mm in diameter, depending on the size of the blades, were then punched out of the thallus and kept in aerated ASW for another day before being transferred into aerated artificial seawater (Tropic Marine®; Dr. Biener Aquarientechnik, Germany), with

no inorganic nitrate or phosphate enrichment (detection levels were 0.7 and 0.3 μmol, respectively). The disks were kept in aerated artificial seawater for 5 days before the uptake experiments at a light irradiance of 100 μmol m^{-2} s^{-1}. To prevent or delay spore formation in the blades, the light attenuation was reduced to 50 μmol m^{-2} s^{-1} during the uptake experiments.

The uptake experiments were performed in two aerated artificial seawater media: one a low-concentration medium (LCM) augmented with 0.5 μmol of $NaHPO_4$ and 3 μmol of $NaNO_3$, and the other a high-concentration medium (HCM) with 3 μmol of $NaHPO_4$ and 30 μmol of $NaNO_3$. These concentrations are in the range of nutrient concentration found in local marine coastal areas (National Oceanic and Atmospheric Administration/National Ocean Service: http://www.co-ops.nos.noaa.gov/pub.html). Experimental temperatures were 5 and 15 °C for all species except for the experiment with the summer species *P. purpurea*, which were run at 5 and 25 °C. Experiments with disks of *P. leucosticta* type A were run at two additional concentrations of 7 and 15 μmol of $NaNO_3$ with 0.7 and 1.5 μmol of $NaHPO_4$, respectively.

During the experiments, the algae were placed in aerated 250-ml Erlenmeyer bottles with 200 ml of incubation medium. Water samples (10 ml) for nutrient measurements were taken at 1, 4, 8, 24, and 48 h for *P. leucosticta* and *P. leucosticta* type A and 1, 3, 6, 10, and 24 h for all others types. The total incubation volume of the bottles was replaced at every sampling. The calculated uptake rates are the average rates between these sampling times (i.e., 1 h represents uptake at 0–1 h interval, and the 24-h uptake occurred at 10–24 h interval). Four replicate tests flasks were used for each nutrient medium. However, they represented pseudo-replicates for temperature as all were incubated in the same chamber. Samples from the incubated media were analyzed for 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; Hach, Loveland, CO).

Samples for dry weight (DW) were taken prior to the experiments and at the end. DW was measured after drying the samples for 48 h at 55 °C. Statistical differences between uptake rates and time were tested, when appropriate, by parametric paired *t* tests (MINITAB; Minitab). When testing statistical differences between temperatures, all *Porphyra* spp. were joined and tested as a group by a parametric paired *t* test as temperature in these experiments was to be treated as pseudo-replicates. p values >0.05 were considered nonsignificant (n.s.) Regression analyses were based on linear ($y=ax+b$) and hyperbolic equations resembling Michaelis–Menten uptake kinetics [$f=ax/(b+x)$] in Sigmaplot 8 and MINITAB.

3. Results

3.1. P. suborbiculata

Uptake of nitrate in *P. suborbiculata* was 1.3 times higher at 15 °C than at 5 °C ($p<0.001$) and, on average, sevenfold higher in HCM than in LCM ($p<0.001$) (Fig. 1A). The rates varied from $V_{3\ \mu M}^{10-24\ h}=0.8$ to $V_{30\ \mu M}^{6-10\ h}=36.7$ μmol NO_3 g^{-1} DW h^{-1}. The initial

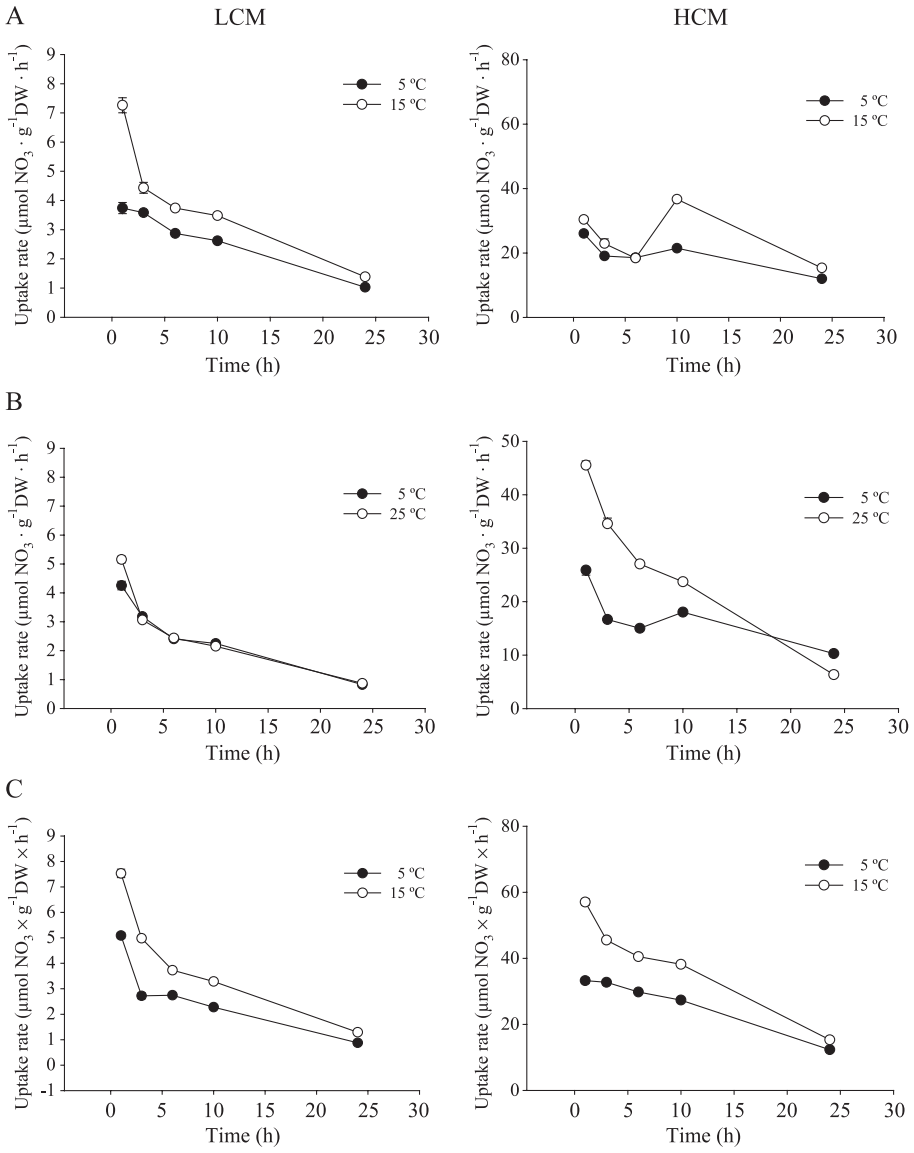

Fig. 1. Nitrate uptake rates in *P. suborbiculata* (A), *P. purpurea* (B), and *P. rosengurttii* (C) in two concentrations of medium; LCM (3 µmol NO_3+0.5 µmol PO_4) and HCM (30 µmol NO_3+3 µmol PO_4) at 5 and 15 °C for *P. suborbiculata* and *P. rosengurttii*, and at 5 and 25 °C for *P. purpurea*. Error bars are standard error.

uptake rates in HCM were two times higher during the first hour than during the last period (10–24 h) at both 5 and 15 °C, and three to six times higher in LCM. In HCM, the uptake rates decreased except for a temporary increase in uptake rates from 6 to 10 h, especially at 15 °C.

The uptake of phosphate in *P. suborbiculata* did not show the same patterns as for nitrate (Fig. 2A) except that the uptake rates were significantly higher in HCM than in LCM ($p<0.005$ and $p<0.003$ in 5 and 15 °C, respectively). The rates varied from 0.2 to 3.2 and from 0.18 to 2 μmol of PO_4 g^{-1} DW h^{-1} in 5 and 15 °C, respectively. At 15 °C in HCM, the initial uptake rates were lower than at 5 °C, and instead of a decline in rates as in 5 °C, the rates temporarily increase before decreasing toward low rates after 10 h.

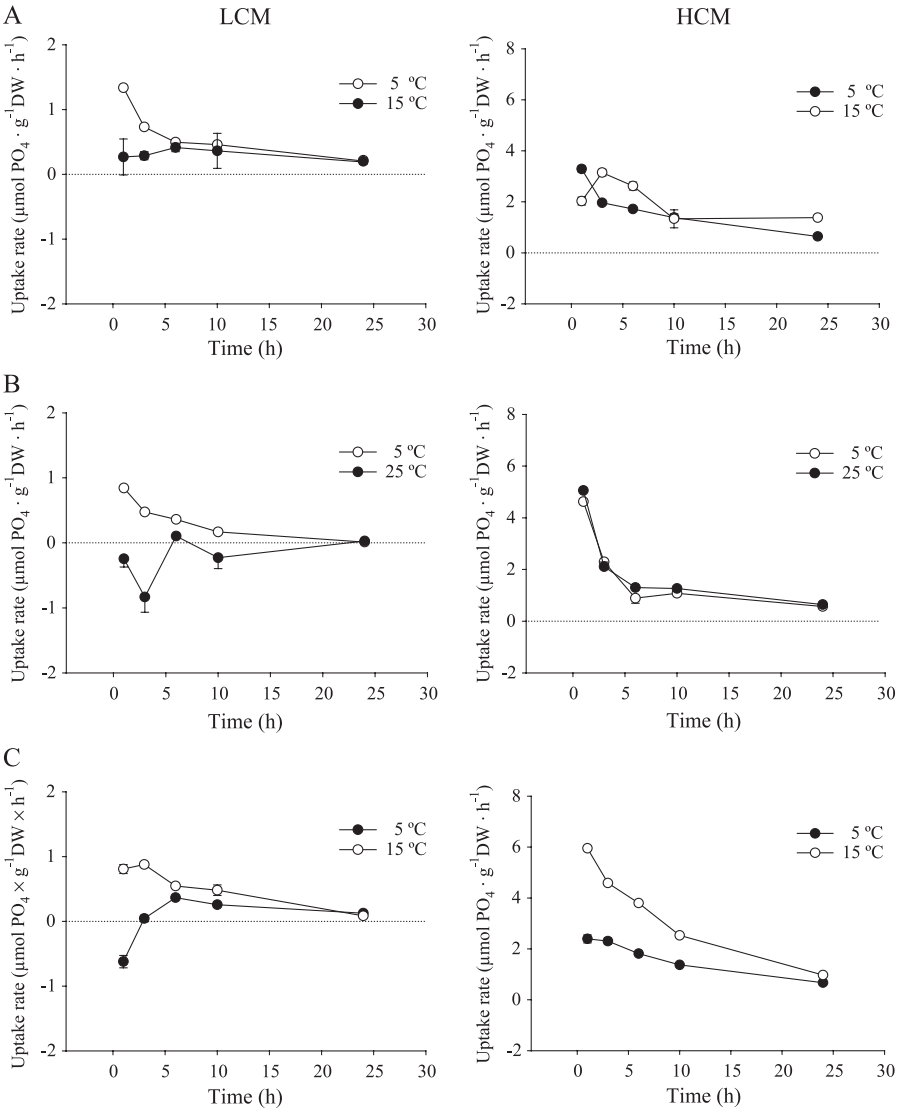

Fig. 2. Phosphate uptake rates in *P. suborbiculata* (A), *P. purpurea* (B), and *P. rosengurttii* (C) in two concentrations of medium; LCM (3 μmol NO_3+0.5 μmol PO_4) and HCM (30 μmol NO_3+3 μmol PO_4) at 5 and 15 °C for *P. suborbiculata* and *P. rosengurttii*, and at 5 and 25 °C for *P. purpurea*. Error bars are standard error.

3.2. P. purpurea

Uptake rates of nitrate by *P. purpurea* were also higher in HCM than in LCM at both 5 °C ($p<0.001$) and 25 °C ($p<0.006$) (Figs. 1B and 2B). The initial uptake rates of nitrate in HCM at 25 °C for *P. purpurea* were higher than the initial rates of *P. suborbiculata* in 15 °C and did not show the distinct peak in rates at 10 h. However, at 5 °C, the uptake rate of nitrate in HCM showed the same temporarily peak after 10 h in HCM. The highest uptake rate was measured as $V_{30\ \mu M}^{0-1\ h}$=46 μmol NO_3 g^{-1} DW h^{-1} at 25 °C and the lowest as $V_{3\ \mu M}^{10-24\ h}$=1.2 μmol NO_3 g^{-1} DW h^{-1} at both 5 and 25 °C (Fig. 1B). The phosphate uptake rates of *P. purpurea* (Fig. 2B) were also significantly lower in LCM than in HCM at 5 °C ($p<0.034$) and 25 °C ($p<0.026$). In HCM, the initial phosphate uptake rates were similar at both temperatures and the rates dropped quickly to about 25% of the initial rates just after 6 h, unlike the phosphate uptake in *P. suborbiculata* (Fig. 2B).

3.3. P. rosengurttii

Initial nitrate uptake rates in *P. rosengurttii* showed a high uptake followed by a subsequent decline in rates over time and, after 24 h, nitrate uptake rates were only 16–33% of initial rates. The rates at 15 °C were higher than at 5 °C (Fig. 1C). The nitrate uptake rates were also significantly higher in HCM than in LCM at 5 °C ($p<0.001$) and 15° ($p<0.012$). The highest nitrate uptake rate was measured as $V_{30\ \mu M}^{0-1\ h}$=57 μmol NO_3 g^{-1} DW h^{-1}. The highest phosphate uptake rate in *P. rosengurttii* was found at 15 °C at $V_{30\ \mu M}^{0-1\ h}$=6 μmol PO_4 g^{-1} DW h^{-1}. Initial loss of phosphate was found in LCM at 5 °C and at a low rate of 0.3 μmol PO_4 g^{-1} DW h^{-1}.

3.4. P. leucosticta: holotype and type A

The nitrate uptake in *P. leucosticta* (Fig. 3A) shows a different pattern than in type A (Fig. 3B). In LCM, the nitrate uptake rates at 5 °C were constant during the first 24 h and dropped from 4 to 0.6 μmol NO_3 g^{-1} DW h^{-1} over the next 24 h. In LCM at 15 °C, the tissue of *P. leucosticta* appears to initially release some nitrates into the water, resulting in a negative uptake rate. After 3 h in LCM, the uptake of nitrate started and increased up to the same level as in 5 °C at 24 h with a subsequent drop to the same level as in 5 °C at 48 h (Fig. 3A). In HCM, nitrate uptake rates were higher than in LCM at both 5 and 15 °C. The uptake of nitrate at 15 °C in HCM showed a high initial nitrate uptake rate followed by a subsequent decline, opposite to the trends in LCM. At 24 h, the nitrate uptake rates at 5 and 15 °C concurred at a similar level of 26–27 μmol NO_3 g^{-1} DW h^{-1} to decline during the next 24 h to 9 μmol NO_3 g^{-1} DW h^{-1} at 48 h (Fig. 3A). The nitrate uptake rates in *P. leucosticta* varied from a maximum of $V_{30\ \mu M}^{0-1\ h}$=60 μmol NO_3 g^{-1} DW h^{-1} at 15 °C to $V_{3\ \mu M}^{24-48\ h}$=0.8 μmol NO_3 g^{-1} DW h^{-1} at both 5 and 15 °C.

The rates of nitrate uptake by type A of *P. leucosticta* showed a different pattern than did the holotype. All nitrate uptake experiments for this type of *P. leucosticta* showed a high initial uptake rate with subsequent decline in rates over time (Fig. 3B). The nitrate uptake rates were higher in HCM than in LCM at 5 °C ($p<0.003$) and at 15° (n.s.). In type A, the initial (0–1 h) uptake rates were 15–22 times higher than rates measure between 10

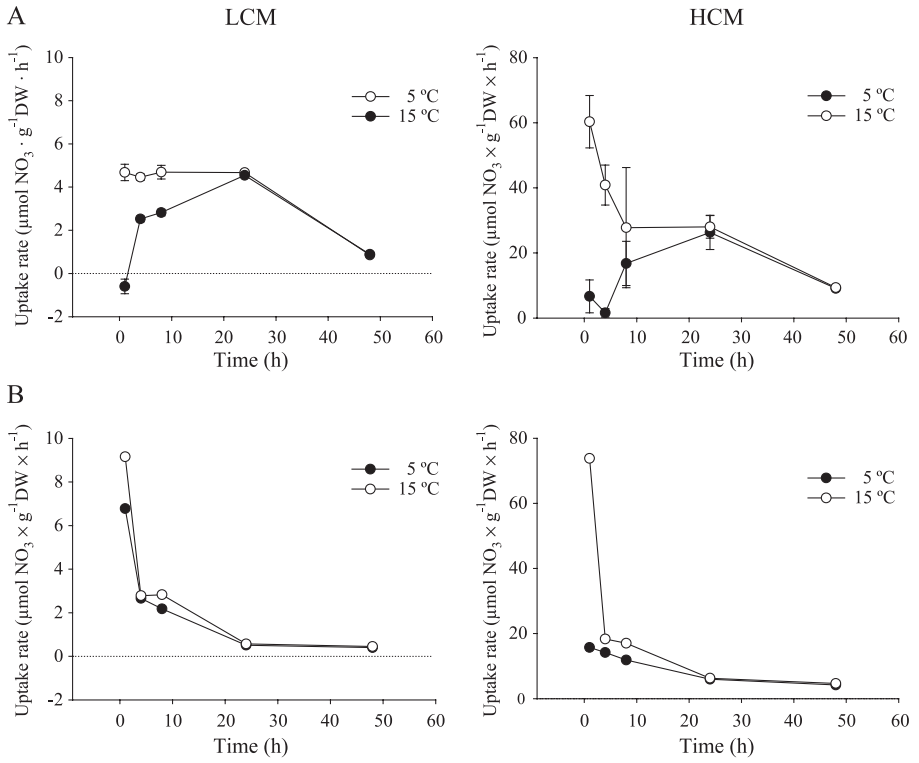

Fig. 3. Nitrate uptake rates in *P. leucosticta* (A) and *P. leucosticta* type A (B) in two concentrations of medium; LCM (3 µmol NO_3+0.5 µmol PO_4) and HCM (30 µmol NO_3+3 µmol PO_4) at 5 and 15 °C. Error bars are standard error.

and 24 h, except for the rates in 5 °C and HCM where initial rates were four times higher than at 24 h. The maximum nitrate uptake rate was found in type A at 15 °C: $V_{30\ \mu M}^{0-1\ h}$=73.8 µmol NO_3 g^{-1} DW h^{-1}.

Phosphate uptake rates by *P. leucosticta* were significantly higher in HCM than in LCM at both 5 °C (p<0.012) and 15 °C (p<0.018), and the phosphate uptake rate at 15 °C exceeded the rate in 5 °C in HCM (Fig. 4A). In LCM and at 5 °C in HCM, the phosphate uptake rates were similar to those of *P. Suborbiculata*, *P. purpurea*, and *P. rosengurttii*. However, the phosphate uptake rates in HCM at 15 °C surpassed the rates found in the same species. As found for nitrate uptake rates, the phosphate uptake rates in *P. leucosticta* had a peak in rates after 10 h, except at 15 °C in HCM. Notable was the loss of phosphate from the algae into the medium during the first hours in LCM especially in *P. leucosticta* type A after 1 h in the medium of $V_{3\ \mu M}^{0-1\ h}$=11 µmol PO_4 g^{-1} DW h^{-1} (Fig. 4B). In HCM, only *P. leucosticta* type A showed a loss of phosphate into the medium at 5 °C (Fig. 4B). In HCM at 15 °C, the phosphate uptake rates in the holotype and type A showed the same initial high values with a subsequent drop in rates (Fig. 4B); however, the drop was much more pronounced in *P. leucosticta* type A than in *P. leucosticta*. The highest uptake rates of phosphate in were

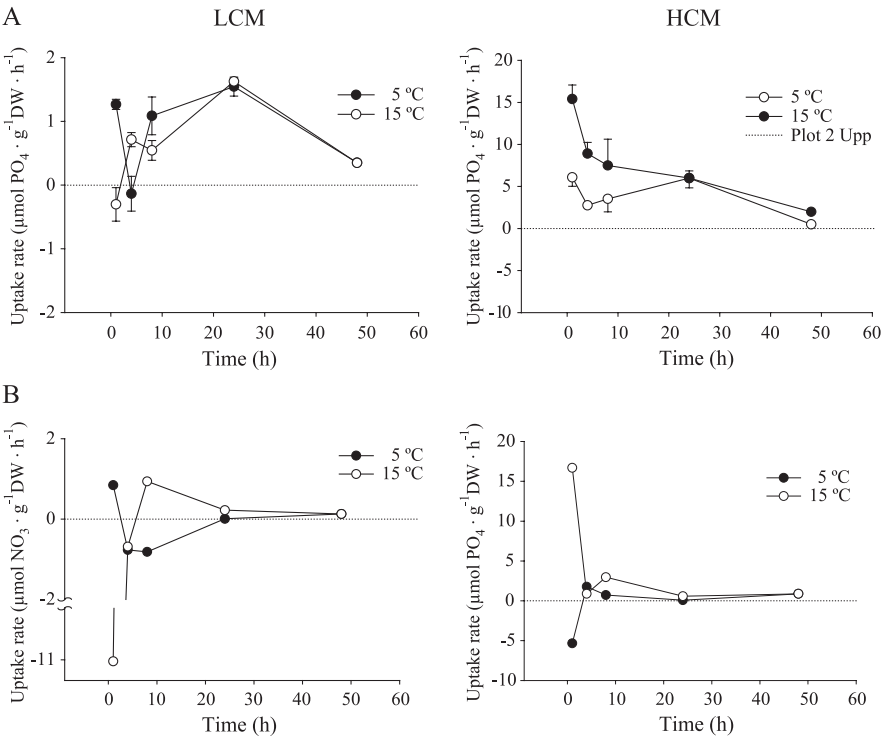

Fig. 4. Phosphate uptake rates in *P. leucosticta* (A) and *P. leucosticta* type A (B) in two concentrations of medium; LCM (3 µmol NO_3+0.5 µmol PO_4) and HCM (30 µmol NO_3+3 µmol PO_4) at 5 and 15 °C. Error bars are standard error.

found at 15 °C at $V_{30\ \mu M}^{0-1\ h}$=17 and 16 µmol PO_4 g^{-1} DW h^{-1} in *P. leucosticta* type A and holotype, respectively. After the first 8 h, the uptake of phosphate in type A was less than 1 µmol PO_4 g^{-1} DW h^{-1} (Fig. 4B).

3.5. Uptake vs. area, biomass, and SA/V ratio

There were no significant differences in uptake rates of nitrate (0–24 h) among the species when uptake rates at both temperatures were normalized to area (nmol NO_3 cm^2 h^{-1}; Table 1). However, the uptake in HCM was eight to nine times higher than in all the LCM experiments. When calculated on a volume basis, the uptake rates were different among species. The uptake rate is negatively correlated in these *Porphyra* species with the thickness of the thallus. Uptake rates (in µmol NO_3 g^{-1} DW h^{-1}) show that the rates increase with increasing SA/V ratio (Fig. 5) for all uptake rates based on 1–24 h (treated separately). Even though the regression coefficients were both positive (a=0.016 and 0.099 in $F=y_0+ax$), they were not significant by difference ($p>0.05$) due to the few and morphologically very similar species examined. *P. suborbiculata* represented the outliers in the regression with the lowest uptake rates and the second highest SA/V ratio of 160 (Fig. 5).

Table 1
Average thickness, surface area, and volume of five different *Porphyra* species from LIS and their uptake of nitrate (NO_3) at two temperatures adjusted to area or volume

	Average thallus thickness (μm)	Surface area/ volume ratio	Uptake$^{0–24\ h}$ (nmol) cm^{-2} h^{-1}				Uptake$^{0–24\ h}$ (μmol) cm^{-3} h^{-1}			
			LCM		HCM		LCM		HCM	
			5 °C	15 °C	5 °C	15 °C	5 °C	15 °C	5 °C	15 °C
P. suborbiculata	25	160	15.6	17.3	129.2	138.2	1.3	2.4	17.7	19.0
P. purpurea	45	89	15.9	12.9	130.0	127.6	1.2	1.0	9.9	9.7
P. leucosticta	40	100	15.4	12.9	89.2	138.4	1.3	1.1	7.6	11.9
P. leucosticta type A	21	190	17.5	18.3	139.9	160.5	2.9	3.0	22.8	26.2
P. rosengurttii	32	125	14.2	17.1	164.6	181.0	1.5	1.8	17.6	19.4

LCM and HCM is low and high nutrient concentration, respectively.

3.6. Uptake vs. media concentrations

Nitrate uptake by *P. leucosticta* type A increased with concentration of nitrate in media (Fig. 6). The only exception was at 5 °C for the initial uptake rates (1 h) where rates in 7 and 15 μmol of nitrate solution were higher than in 30 μmol of nitrate solution. Nitrate uptake rates decreased with time at both temperatures. Correlation analyses between uptake rates and concentrations showed a linear response after 8 h in 5 °C and after the first hour in 15 °C (Fig. 5, Table 2). At 5 °C, the uptake during the first hours showed better conformity with Michaelis–Menten uptake kinetics (r^2=0.97, 0.73, and 0.99 for

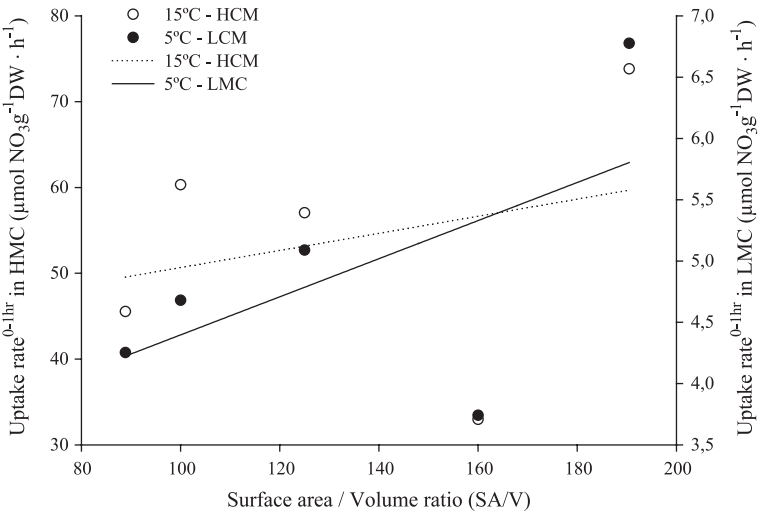

Fig. 5. Biomass-specific rate of nitrate at 0–1 h vs. surface area/volume ratio (SA/V, cm^2:cm^3) for *Porphyra* spp. from LIS. Rates in LCM at 5 °C and in HCM at 15 °C (25 °C for *P. purpurea*) are included. For specific species, refer to SA/V ratios for the respective species in Table 3.

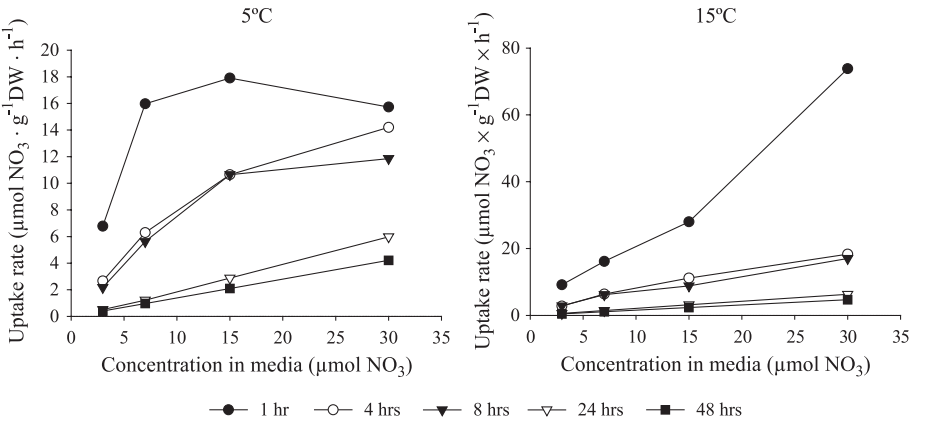

Fig. 6. Uptake rates of nitrate in *P. leucosticta* type A at 5 and 15 °C vs. concentration in medium. Uptake rates are measured over five different time intervals.

uptake at 5 °C during 0–1, 1–3, and 3–6 h, respectively) than for passive linear uptake (Table 2).

3.7. Q_{10}

The Q_{10} values for nitrate uptake varied among species (Table 3). Average Q_{10} values over all sampling times were less than 2, except for the initial $Q_{10}^{1\,h}$ values for *P. suborbiculata*, which were 2.4 in HCM. Initial Q_{10} values were higher than the subsequent ones and declined over time to about 1 at the end of the experiments (after 24 or 48 h). *P. purpurea* even had a Q_{10} value that was slightly below 1 after 24 h at 15 °C. The average Q_{10} values for phosphate varied considerably among species and time. The initial rates for nitrate uptake showed, in general, an elevated uptake during the first hour followed by a

Table 2
Three different tests of curve fitness between uptake rates in *P. leucosticta* type A and nutrient concentration in media

Temperature (°C)	Time (h)	Linear correlation coefficient (r^2)	Michael–Menten correlation coefficient (r^2)	Linear regression ANOVA (p)	Michael–Menten ANOVA (p)	Linear power test of α
5	1	0.29	0.72	n.s.	n.s.	0.09
	4	0.92	0.99	0.039	0.002	0.49
	8	0.80	0.95	n.s.	0.025	0.31
	24	1.00		0.0002		0.99
	48	1.00		<0.0001		1.00
15	1	0.97	0.97	0.013		0.71
	4	0.99		0.006		0.81
	8	0.99		0.006		0.83
	24	0.99		<0.0001		1.00
	48	1.00		<0.0001		1.00

The power test for α should to be above 0.8.

Table 3
Q_{10} for the nitrate and phosphate uptake rates as an average over all sampling intervals

Species	Q_{10}[a]	
	Nitrate rates	Phosphate rates
P. suborbiculata	1.35	1.00
P. purpurea	1.27	0.50
P. leucosticta	1.90	1.00
P. leucosticta type A	1.55	2.87
P. rosengurttii	1.47	n.a.

[a] Q_{10} for *P. purpurea* is calculated over a 20 °C range. n.a.=not applicable.

subsequent decrease over time. The initial uptake rates of phosphate were, in general, much lower and, in many cases, the uptake rates were negative; however, after 24 h, they stabilized at 5 °C and averaged 0.16 and 0.6 μmol PO_4 g^{-1} DW h^{-1} in LCM and HCM, respectively. At 15 °C, the rates were, on average, 0.13 and 1.14 μmol PO_4 g^{-1} DW h^{-1} in LCM and HCM, respectively.

4. Discussion

4.1. Nitrate uptake rates by Porphyra spp.

The uptake rates of nitrate (in μmol NO_3 g^{-1} DW h^{-1}) obtained in these experiments show large variations with both nutrient concentration and time intervals over which rates were calculated. Uptake rates of 15 μmol NO_3 g^{-1} DW h^{-1} have previously been described for *Porphyra perforata* by Thomas and Harrison (1985) from British Colombia, Canada. Similar or higher rates, up to 169 μmol NO_3 g^{-1} DW h^{-1}, have only been reported for green algae (Harlin, 1978; O'Brien and Wheeler, 1987; Lavery and McComb, 1991). Rates of nitrate uptake by brown algae have been reported in the range of 3–27 μmol NO_3 g^{-1} DW h^{-1} (Harlin and Craigie, 1978; Thomas et al., 1985; Harrison et al., 1986; Ahn et al., 1998), while uptake by red algae falls within the range 10–35 μmol NO_3 g^{-1} DW h^{-1} (D'Elia and DeBoer, 1978; Haines and Wheeler, 1978; Thomas and Harrison, 1985; Smit, 2002). Directly comparing these values can be misleading as uptake rates depend on the concentration in the medium, time over which the uptake rates are measured, previous nutritional history of algae (Chopin et al., 1990), and SA/V ratios of the species. We have shown that the uptake rates decreased over time possibly after internal pools filled up. Uptake rates then decrease, possibly as a result of a feedback response. Such patterns have also been reported by others (Harrison and Druehl, 1982; Harrison et al., 1989; Gordillo et al., 2002). Nitrate uptake rates also increase with increasing concentration in the medium (Thomas and Harrison, 1985; Harrison and Hurd, 2001; Ozaki et al., 2001; Naldi and Viaroli, 2002; Smit, 2002) as shown in this paper, but are also dependent on the length of the starvation period before uptake rates are measured (Thomas and Harrison, 1985). The morphology of the *Porphyra* species also affected the uptake of nutrients. Thin leaf-like species will be more efficient at taking up nutrients as the surface area over which the uptake process take place is, relative to the volume, much

larger for thick globular or cylindrical-shaped species (Rosenberg and Ramus, 1984; Taylor et al., 1998). All the *Porphyra* spp. in LIS have very high SA/V ratios as they are only one or two cell layers thick. Consideration of the surface area-to-volume ratio as a morphological index must be taken into account when comparing uptake rates between different species (Rosenberg and Ramus, 1984; Taylor et al., 1998). The uptakes per area in this study were almost identical among the species; the differences in uptake on a volume basis reflected more so the differences in thallus thickness among the species than differences in nitrate uptake at 0–24 h among them.

V_{max} denotes the maximal uptake rate at which no increase in rate occurs even with an increase in the media concentration. Such rates have not been measured in these experiments (with the possible exception of uptake at 5° in *P. leucosticta* type A) as the rates calculated here are uptake rates representing natural seawater concentration (i.e., a nitrate concentration in the range of 0–30/35 μM NO_3). Preliminary studies have shown that *Porphyra* species have much higher uptake rates than those reported here at an elevated media concentration of 3 mM NO_3 (Pedersen, unpublished data). As seen from our experiments, the nitrate uptake rates varied significantly with the time at which measurements were made, and rates also increased with concentration of the incubation medium. Hence, the most informative way to express these rates when comparing with others should be with the notation $V^{0-1\ h}_{(max)-3\ \mu M}$, where both time of nutrient exposure and nutrient concentration are cited, as described by Lobban and Harrison (1994) and Thomas and Harrison (1987).

4.2. Nitrate uptake and temperature effect

Temperature did not have as strong an effect on the uptake rates as did nutrient concentration. In our experimental set-up, temperature had to be treated as pseudo-replicates for each type. However, for all species of *Porphyra* tested as one group, the uptake rate at 15 °C (25 °C for *P. purpurea*) was significantly higher than at 5 °C ($p<0.000$). The notation Q_{10} describes the increase in metabolism that accompanies a temperature increase of 10 °C. Q_{10} values of 2.0–2.5 for nutrient uptake characterize active uptake processes across cell membranes. Passive processes, on the other hand, are not greatly affected by temperature (Q_{10} range between 1.0 and 1.2 (Lobban and Harrison, 1994). The average Q_{10} values calculated from our experiments are much less than 2.0. However, the Q_{10} values were higher in HCM than in LCM, and the values declined over time. The average $Q^{1\ h}_{10}$ value in HCM was 2.4, indicating that active processes might be involved during the initial uptake by the algae. However, such high Q_{10} values were not found during the initial phase in LCM. After 24 h, the Q_{10} values were between 1.0 and 1.2 in both medium concentrations for all the tested *Porphyra*.

4.3. Uptake of phosphate

Phosphate uptake varied dramatically among species, temperatures, and medium concentration. In many cases, phosphate seemed to leak out of the algae after 1 h in the medium, especially in LCM, as the concentration in the medium increased. Hurd and Dring (1991) found that phosphate was rapidly taken up in fucoids during the first 30 min,

followed by near-zero uptake rates during the next 30 min and then intermediate uptake rates over several hours. This might explain the considerable variation found in uptake rates of phosphate, especially during the first hours. No apparent correlation existed between uptake of nitrate and uptake of phosphate. The only coherent pattern was that uptake rates in LCM were significant lower than in HCM ($p<0.000$). The uptake rates after 24 h in HCM had a Q_{10} value of almost 2 as an average for all species, which support Eppley's (1958) findings that the uptake process of phosphate might be an active process. However, this was not the case for phosphate uptake in LCM.

4.4. Difference in uptake rates among species of Porphyra

Although the initial nitrate uptake rates varied between species, they did not after 24 h (i.e., $V_{3\ \mu M}^{0-24\ h}$ and $V_{30\ \mu M}^{0-24\ h}$ were very similar across species). There were no obvious differences between the nitrate uptake patterns between the eurythermic species and the stenothermic *P. purpurea.* Neither were there any significant differences among the stenothermic species, *P. suborbiculata*, *P. rosengurttii*, and *P. leucoticta* type A. The only species that seemed to have a different uptake pattern was the *P. leucosticta* holotype. As mentioned earlier, the previous nutritional history of the algae used in the experiment is of importance. *P. leucosticta* (holotype) was the only stenothermic species that was collected from a different location in LIS than the other stenothermic types. As we have no data from the initial internal nutritional status of the species, the 5-day starving of *P. leucosticta* may have been insufficient for this species to empty its storage compartments compared to the other *P. leucosticta* types. The *P. leucosticta* thallus is 90% thicker than thalli of type A, suggesting that a longer starvation period may have been needed. However, Hafting (1999) found that *Porphyra yezoensis* tissue concentrations fell during 5 days of starvation as nitrogen was used to support growth. After 5 days, the growth rate started to decline and the algae began to die after 12 days. A nitrogen content of 0.40% of FW was reported as a subsistence level of N concentration (Hafting, 1999). *P. yezoensis* has a blade stage that is even thicker than *P. leucosticta*. If loss or uptake is negatively correlated with thickness of thallus, 5 days of starvation should have been sufficient for starving *P. leucosticta* to a possible subsistence level. The initial nitrate uptake rates in *P. leucosticta* at 5 °C in both HCM and LCM were lower than for type A. In LCM at 15 °C, the uptake was negative (i.e., nitrate was lost to the medium during the initial phase). Similar patterns, however, for nitrite were found by Brinkhuis et al. (1989), who reported that in the kelp *Laminaria saccharina* (L.) J.V. Lamour., nitrite was taken up during a few minutes before being released back into the medium. They suggested that this complicated form of uptake was due to surge filling of the nitrite pool within the cells of the kelp. This was due to an increase in nitrite reductase (NiR) activity that rapidly lowered the internal nitrite pool. They also suggested that the next drop in uptake of nitrite could be due to the filling up of an ammonium pool that activates the glutamine synthetase (GS) and the glutamine–oxoglutarate amino transferase (GOGAT) activity and inhibited the nitrite uptake.

Uptake of nitrate by *P. leucosticta* type A indicated that uptake rates at 5 °C during the first three time intervals (1, 4, and 8 h) was an active process as it also showed good fitness ($0.72<r^2<0.99$) to the Michealis–Menten uptake equation for active enzyme kinetics rather than a linear correlation. At 15 °C, the uptake kinetics resembled a passive uptake with no

maximal uptake rates achieved within the nutrient concentration used in our experiments. All uptake regressions showed almost perfect fit (r^2>0.99 with power test α>0.80) except for the first hour, where r^2 was 0.97. Linear fit indicates that within our nutrient range and at 15 °C, we were not able to detect any saturation with the medium nitrate concentrations used. Active uptake kinetics was shown at 5 °C but not at 15 °C, as we were able to show indications of a saturated uptake kinetics at 5 °C and not at 15 °C. However, the passive diffusion is not a temperature-dependent transport, while active transport is. Hence, at 15 °C, the uptake is much more efficient than in 5 °C and with our nutrient concentrations of only 30 μM NO_3, no saturation in nitrate uptake rates could be detected at 15 °C. On the other hand, the active uptake rate at 5 °C is slower and we were able to show a saturated uptake at this temperature.

In our experiments, we were not able to show differences in uptake kinetics in summer and winter species, which thrive under most different ambient nutrient levels. Neither did we find significant differences in nitrate uptake rates between stenothermic and eurythermic species of *Porphyra.* However, average initial nitrate uptake rates by *Porphyra* spp. were rather high (max $V_{30\ \mu M}^{0-1\ h}$=73.8 μmol NO_3 g^{-1} DW h^{-1}), demonstrating that *Porphyra* spp. have the ability to quickly take up nitrate from the medium. This rapid uptake of nitrate from the medium suggests that *Porphyra* species are highly competitive in clearing nutrients from the water column. Hence, *Porphyra* should be considered an important species in bioremediation of eutrophic environments and in integrated aquaculture (Chopin et al., 2000; Chung et al., 2002; Zertuche-Gonzalez et al., 2001).

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