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Metabolic Plasticity of Nitrogen Assimilation by *Porphyra umbilicalis* (Linnaeus) Kützing

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Abstract The physical stresses associated with emersion have long been considered major factors determining the vertical zonation of intertidal seaweeds. We examined *Porphyra umbilicalis* (Linnaeus) Kützing thalli from the vertical extremes in elevation of an intertidal population (*i.e.* upper and lower intertidal zones) to determine whether *Porphyra* thalli acclimate to different vertical elevations on the shore with different patterns of nitrate uptake and nitrate reductase (NR) and glutamine synthetase (GS) activities in response to different degrees of emersion stress. We found that the nitrate uptake and NR recovery in the emersed tissues took longer in lower intertidal sub-population than in upper intertidal sub-population; and GS activity was also significantly affected by emersion and, interestingly, such an activity was enhanced by emersion of thalli from both upper and lower intertidal zones. These results suggested that intra-population variability in post-emersion recovery of physiological functions such as nutrient uptake and NR activity enables local adaptation and contributes to the wide vertical distribution of *P. umbilicalis*. The high GS activity during periodic emersion stress may be a protective mechanism enabling *P. umbilicalis* to assimilate nitrogen quickly when it again becomes available, and may also be an evidence of photorespiration during emersion.

Key words emersion; glutamine synthetase; nitrate reductase; nitrogen assimilation; *Porphyra*

1 Introduction

Intertidal organisms experience environmental stresses during emersion, including extremes of photo-synthetically active radiation (PAR), ultraviolet light, temperature, salinity, nutrient availability and desiccation (Doty, 1946; Davison and Pearson, 1996). Emersion is a major factor constraining the upper limit of intertidal seaweeds, and responses to emersion stress have been intensively studied (Johnson *et al.*, 1974; Quadir *et al.*, 1979; Dring and Brown, 1982; Johnston and Raven, 1986; Thomas *et al.*, 1987; Hurd and Dring, 1990; Lipkin *et al.*, 1993; Gao *et al.*, 1999). A number of mechanisms have been proposed to explain the tolerance of intertidal seaweeds to emersion, including stimulation of nitrate and phosphate uptake following emersion (Thomas *et al.*, 1987; Hurd and Dring, 1990), maintenance of photosynthetic activity at high rates during emersion (Johnson *et al.*, 1974; Dring and Brown, 1982; Lipkin *et al.*, 1993) and the ability of rapidly recovering physiological activities during rehydration (Quadir *et al.*, 1979; Dring and Brown, 1982; Johnston and Raven, 1986; Gao *et al.*, 1999; Kim *et al.*, 2008; Kim *et al.*, 2009). In a short term, emersion enhances the uptake rate of nitrate, ammonium and phosphate in some intertidal species following resubmergence (Thomas *et al.*, 1987; Hurd and Dring, 1990). Thomas *et al.* (1987) reported that algal species inhabiting high intertidal zone, for example *Pelvetiopsis limitata* (Setchell) N.L. Gardner and *Fucus distichus* Linnaeus, can double their nitrogen (N) uptake upon resubmergence after more than 30% emersion. However, their response to emersion appears to be species-specific; N uptake of the low intertidal red algal species, *Gracilaria pacifica* I.A. Abbott, is not enhanced after emersion (Thomas *et al.*, 1987) while the uptake rates of nitrate and phosphate of *Porphyra* (or *Pyropia*) species (*P. leucosticta* (=*Pyropia leucosticta*), *P. umbilicalis* and *P. yezoensis* (=*Pyropia yezoensis*)) are negatively influenced regardless of tidal position (Kim *et al.*, 2008).

The rate of water loss during emersion varies in different species, depending mainly on the morphology. Ji and Tanaka (2002) measured the photosynthetic and respiratory rates of 12 macroalgal species from different vertical zones. Although most species showed initial increases in photosynthetic rates following emersion, no correlations existed between the vertical habitat and the photosynthetic or respiration rates during emersion. The rate of recovery of physiological functions, such as photosynthesis and

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nutrient uptake after resubmergence, appears to be more important than tolerance of emersion in determining the vertical distribution of seaweeds (Dring and Brown, 1982; Johnston and Raven, 1986; Lipkin *et al*., 1993; Gao *et al.*, 1999; Kim *et al*., 2008).

Nitrogen metabolism occurs within cytosol, chloroplast, mitochondrium, and involves various biochemical pathways. Nitrate reductase (NR) and nitrite reductase (NiR) mediate the reduction of nitrate (NO_3^-) to ammonium (NH_4^+) in sequential reactions. The conversion of ammonium into glutamate proceeds *via* two pathways. In the primary biochemical cycle, ammonium is incorporated first into glutamine by glutamine synthetase (GS), and then glutamine donates one of its amine groups to oxoglutarate yielding two glutamate molecules *via* amine transfer catalyzed by glutamate synthase (GOGAT). Although glutamate dehydrogenase (GDH) can also catalyze the incorporation of ammonium into glutamate (Inokuchi and Okada, 2001), the dominant pathway in seaweed protein synthesis is GS/GOGAT (Gayler and Morgan, 1976; Sato *et al*., 1984). Therefore, NR activity provides a measure of nitrate assimilation, and GS activity is a proxy for the total N assimilation. The activities of these enzymes in N metabolism can vary with environmental factors, including light intensity and quality, nutrients and emersion (Kenis and Trippi, 1986; Gao *et al*., 1992; Figueroa, 1996; Kraemer *et al*., 1997; Thompson and Valiela, 1999, Lopes *et al*., 2002; Teichberg *et al*., 2007). NR activity in green alga *Ulva fenestrata* Postels & Ruprecht showed a very clear diurnal rhythm, with maximum rate during the period of greatest light availability (Gao *et al*., 1992), but this process also is affected by emersion (Jun and Chung, 1996). NR and GS activities in seaweeds, such as *Cladophora, Ulva* and *Gracilaria*, can increase in response to increased N availability in local environment (Thompson and Valiela, 1999; Teichberg *et al.*, 2007).

As a genus, *Porphyra* (and its sister genus, *Pyropia*) occupies a broad range of intertidal and subtidal environments in coastal New England (Yarish *et al*., 1998; Chopin *et al*., 1999; Neefus *et al*., 2002; West *et al.*, 2005; Bray *et al.*, 2006; Neefus *et al*., 2008; Sutherland *et al*., 2011). *Porphyra umbilicalis* grows in a wide range of vertical habitats year round, from upper to lower intertidal zones (Villalard-Bohnsack, 1995; Chopin *et al.*, 1999; West *et al*., 2005; Neefus *et al*., 2008). Along wave-exposed shorelines in the southern Gulf of Maine, *P. umbilicalis* can occupy an intertidal range of about 4 m (West *et al*., 2005). This wide vertical distribution indicates that subsets of a *P. umbilicalis* population will experience periods of emersion of a few hours while others experience as much as 6 h emersion each tidal cycle.

In this study, we examined *P. umbilicalis* thalli from the extremes of intertidal distribution (*i.e.*, upper and lower intertidal zones) of a Maine population to determine whether conspecifics from different vertical elevations on the shore respond differently to the emersion stress. We gauged responses to emersion by measuring nitrate uptake, and nitrate reductase and glutamine synthetase activities. In particular, we tested the hypothesis that metabolic plasticity of *P. umbilicalis* enables this species to occupy a broad range of intertidal habitats. We also investigated the efficiency with which thalli take up and assimilate inorganic N during resubmergence following emersion.

2 Materials and Methods

2.1 Algal Materials and Culture

Porphyra umbilicalis was collected in the upper (>3m MLW) and lower (<1 m MLW) intertidal zones at Schoodic Point, Schoodic Peninsula, ME, USA (44°21′01″N, 68°03′29″W; Gulf of Maine), in September, 2007, for NR experiments and in March, 2008, for GS experiments. Specimens were immediately transported to laboratory on ice in a cooler. Tissues were cleaned of epiphytes by rinsing them with running seawater and rubbing with cotton balls. Ten thalli from each end of the distributional continuum were randomly selected for cross-sectional thickness measurements. Twenty additional thalli from each end of the distributional continuum were also selected for the area measurements. The thallus area was calculated from the digital images using the UTHSCSA ImageTool for Windows, Version 3 software (University of Texas Health Science Center, San Antonio, Texas, USA; http://ddsdx.uthscsa.edu/dig/itdesc.html).

Experiments were carried out in a greenhouse at the University of Connecticut at Avery Point. A culture medium (0.45 μm-filtered seawater) was prepared with von Stosch's enrichment (VSE; Ott, 1965) made without nitrogen (N) or phosphorus (P). N and P levels were regulated by addition of nitrate and phosphate at 30 and 3 $\mu mol\,L^{-1}$, respectively, to provide sufficient nutrients at all conditions during experiments. The maximum photon flux rate measured in the greenhouse by a Li-Cor LI-1000 light meter (Li-Cor, Lincoln, Nebraska, USA) was 1320 μmol photons $m^{-2}s^{-1}$. During all experiments, the photoperiod was similar (*i.e.* sunrise approx. at 7:00 and sunset approx. at 19:00). The water temperature was maintained at 10℃, the optimum for the growth of *P. umbilicalis* (Kim *et al*., 2007). The air temperature and humidity during exposure were 18℃±3℃ and 40%±20%, respectively. Stocking density for each treatment was $0.5\,g\,L^{-1}$.

2.2 Acclimation

Porphyra umbilicalis tissues were acclimated to the experimental conditions (simulated tidal cycle including emersion, or constant submergence; see below) for 5–7 d. Filtered seawater containing VSE with 30 $\mu mol\,L^{-1}$ of nitrate and 3 $\mu mol\,L^{-1}$ of phosphate was replaced daily to ensure a stable and sufficient nutrient status in algal tissues during acclimation. Those thalli assigned to the emersion treatment were exposed to air for 4 h twice a day using a tide simulating apparatus (Kim and Yarish, 2010). The tide simulating apparatus, and the identical apparatus for constant submergence controls, sat in a large outer tank connected to a chiller to maintain con-

stant water temperature.

2.3 Experimental Design

The effect of periodic emersion on NR activity was determined using thalli collected in September 2007 while the effect on GS activity used thalli collected in March 2008. Nitrate uptake was measured during both the NR and GS experiments. Within each experiment, two independent trials were conducted, one using thalli from the upper intertidal zone and the other using thalli from the lower intertidal zone. These trials were separated by a 7 d-interval. The experiments were performed from 07:00 to 21:30 using a tide simulating apparatus for an experimental condition and an identical apparatus without a motorized elevator as control (Kim and Yarish, 2010). Samples under the emersion treatment were exposed to air from 10:00 to 14:00, resulting in a final water loss of 90%, while controls remained submerged.

Sampling was conducted to ensure true replication across each experiment. Each apparatus consisted of 18 independent compartments (three rows of six compartments), each containing *ca.* 2.5 liters of seawater. Just before exposure, at the end of exposure (excluding N uptake at emersion condition), and 0.5, 1.5, 3.5 and 7.5 h after re-submergence, tissue and water samples were collected from each of three compartments (each of the three selected at random from a different row). Therefore, three independent samples of water and tissue were collected at each time point. At each time point, all three compartments were completely removed (true replication). During the experiments, the culture medium was changed at 07:00, 10:00, 14:00 and 17:30 each day to ensure sufficient nutrients in the culture media.

2.4 Nitrate Uptake, Nitrate Rductase and Gutamine Synthetase Activity

Water samples from incubation medium were analyzed for inorganic nitrate by using a SmartChem Discrete Analyzer (Westco Scientific Instruments, Inc. Brookfield, CT, USA). An *in vivo* protocol for determining NR activity was utilized in this study following the methodologies of Maier and Pregnall (1990), Thompson and Valiela (1999), and Teichberg *et al*. (2007). Fresh tissue samples (0.5 g) removed from the compartments were immediately incubated at room temperature in 22 mL of incubation medium in a dark flask (0.06 mol L^{-1} KNO_3, 0.1 mol L^{-1} KH_2PO_4 and 0.5% 1-propanol, pH 7.0). To ensure all tissues were completely bathed in incubation medium, the tissue samples were cut into smaller pieces but similar size (< 1 cm^2). The medium was briefly and gently flushed with N gas to purge oxygen, and the top was sealed with parafilm. One mL samples were removed at a half-hour interval for 1.5 h, and one mL of stop buffer was added (0.5 mL of 0.1% napthylethylenediamine in 1 molL^{-1} HCl, 0.5 mL of 5% sulfanilamide in 1 mol L^{-1} HCl). The media were reflushed with N gas and resealed with parafilm after sampling. The nitrate converted to nitrite was quantified by measuring the sample absorbance at 540 nm with a Spectronic Genesys 5 spectrophotometer (Spectronic Instruments, Rochester, NY, USA). Absorbance readings were calibrated against a nitrite standard curve in a concentration range (0, 2.5, 5, 10, 20 and 30 μmol L^{-1}). The reaction rate of all samples was linear over the 1.5 h assay incubation period. Final data are presented as μmol N converted g^{-1}FW h^{-1}.

GS activity experiments were performed separately from the NR experiments. The GS activity was measured by an *in vitro* GS assay (Pregnall *et al*., 1987; Kraemer *et al*., 1997) because the *in vivo* GS assay (Kraemer and Mazzella, 1996) was unsuccessful when applied to *Porphyra umbilicalis*. Tissue (0.2 g) was ground in 2 mL of ice-cold extraction buffer (50 mmol L^{-1} imidazole, pH 7.3, 0.14% 2-mercaptoethanol, 10 mmol L^{-1} $MnCl_2$, 10% glycerol, 0.03% Tween-20, 1% PVP). Homogenates were centrifuged at 2500 × g for 30 min at 4℃ to clear cell debris. An aliquot (0.5 mL) of the resulting tissue extract was added to 3.5 mL reaction cocktail (final concentrations: 470 mmol L^{-1} imidazole, pH 7.3, 26 mmol L^{-1} glutamine, 3 mmol L^{-1} $MnCl_2$, 0.4 mmol L^{-1} ADP, 20 mmol L^{-1} arsenate, 26 mmol L^{-1} hydroxylamine) and incubated at 35℃. Aliquots of 0.5 mL were removed from the reaction mixture at 20–30 min intervals, added to an equal volume of stop reagent (2 mol L^{-1} HCI, 5% trichloroacetic acid, 13.3% $FeCl_2$) and quantified by spectrophotometry at 540 nm and compared to fresh solutions of gamma- glutamyl hydroxamate. Final data are presented as μmol N converted g^{-1}FW h^{-1}.

2.5 Statistical Analysis

A two-way ANOVA was used to evaluate the influence of tidal elevation on thallus area, thickness, nitrate uptake and NR and GS activities. The ANOVA tests compared thallus area and thickness as functions of two fixed factors (season and vertical habitat), while nitrate uptake rate and NR and GS activity were examined as functions of the fixed factors (time and emersion). For nitrate uptake and NR and GS activity, two vertical levels (upper and lower) were not compared statistically since the experiments for each vertical level were performed a week apart. In addition, repeated measures ANOVA were not used because three randomly selected compartments from each sample were true replicates (*i.e.*, thalli were not resampled). Tukey's HSD analysis was used as a *post hoc* test to make pairwise comparisons of treatment means. Regression was used to determine whether NR activity or GS activity was dependent on the uptake of nitrate. All statistical analyses were done using SPSS 15.0 (SPSS Inc. Chicago, IL, USA.).

3 Results

3.1 Area and Cross-Sectional Thickness of Thalli

The vertical position of *Porphyra umbilicalis* from Schoodic Point, Maine, had a significant influence on thallus area (Table 1). Though of similar shape, thalli from the upper intertidal limit of *P. umbilicalis* distribu-

tion were smaller (22.2 $cm^2 \pm 11.3\ cm^2$) than those from lower intertidal limit (47.3 $cm^2 \pm 24.6\ cm^2$) ($P<0.001$). The cross-sectional thicknesses differed as a function of season. The September thalli of *P. umbilicalis* (91.6 μm ± 10.3 μm) were thicker than those collected in March (84.7 μm ± 8.3 μm) ($P = 0.027$). Cross-sectional thickness did not differ significantly between vertical habitats (89.6 μm ± 10.7 μm *vs*. 86.6 μm ± 9.0 μm, upper *vs*. lower) and the effect of vertical position and season did not interact ($P>0.05$).

Table 1 Analysis of variance determining the effects of vertical height and season on thalli area and cross-sectional thickness and emersion and time on nitrate uptake, NR and GS activities of *Porphyra umbilicalis* from upper and lower intertidal zones

Variable		Factor	*F*	*P*-value
Area		Vertical height (upper *vs*. lower intertidal)	15.44	**<0.001**
		Season (September *vs*. March)	0.55	0.469
		VH X S	0.00	0.991
Cross-sectional Thickness		Vertical height	1.00	0.323
		Season	5.31	**0.027**
		VH X S	0.10	0.921
Nitrate Uptake from NR Experiment		Emersion (control *vs*. 90% water loss)	0.70	0.411
	Upper intertidal	Time of day	13.03	**<0.001**
		E X T	3.98	**0.014**
		Emersion	12.52	**0.002**
	Lower intertidal	Time of day	9.06	**<0.001**
		E X T	16.54	**<0.001**
NR activity		Emersion	1.32	0.261
	Upper intertidal	Time of Day	37.92	**<0.001**
		E X T	1.95	0.107
		Emersion	5.03	**0.032**
	Lower intertidal	Time of day	5.35	**0.001**
		E X T	5.99	**<0.001**
Nitrate Uptake from GS Experiment		Emersion	51.10	**0.023**
	Upper intertidal	Time of day	5.87	0.146
		E X T	3.41	**0.047**
		Emersion	5.99	**<0.001**
	Lower intertidal	Time of Day	1.84	**0.001**
		E X T	2.87	**0.026**
GS Activity		Emersion	15.78	**<0.001**
	Upper intertidal	Time of day	1.06	0.410
		E X T	1.58	0.191
		Emersion	14.01	**0.001**
	Lower intertidal	Time of day	2.13	0.081
		E X T	0.79	0.586

Note: Significant differences are shown in bold with *P* values.

3.2 Nitrate Uptake *vs*. Nitrate Reductase (NR) Activity

Both nitrate uptake and NR activity varied across the experiment (Table 1). Time significantly influenced both nitrate uptake and NR activity of both upper and lower intertidal *Porphyra umbilicalis* thalli collected in September ($P<0.001$; Figs.1 and 2). However, emersion significantly affected only thalli from the lower intertidal zone; both nitrate uptake and NR activity ($P=0.002$ and 0.032, respectively). The interaction of time and emersion affected significantly both nitrate uptake and NR activity in lower intertidal thalli ($P<0.001$) but influenced only nitrate uptake in the upper intertidal thalli ($P=0.014$). Nitrate uptake 0.5 h post-emersion by thalli from both upper and lower intertidal zones were significantly lower than that of control ($P=0.029$ and 0.001, respectively). Nitrate uptake rate in emersed samples, however, was recovered to similar rates as controls within 1.5 h post-emersion. Nitrate uptake was highest in the upper intertidal controls (2.25 μmol N g^{-1} FW h^{-1}), followed by upper intertidal emersed (2.08 μmol N g^{-1} FW h^{-1}), lower intertidal control (2.08 μmol N g^{-1} FW h^{-1}) and lower intertidal emersed (0.54 μmol N g^{-1} FW h^{-1}) (Fig.1).

NR activity at the end of emersion (upper intertidal *Porphyra*) was significantly lower than that of control ($P=0.009$; Fig.2) but recovered to the control level in 0.5 h post-emersion. Interestingly, NR activity of lower intertidal *Porphyra* showed a longer recovery (1.5 h; Fig.2). NR activity in each treatment was the highest among up-

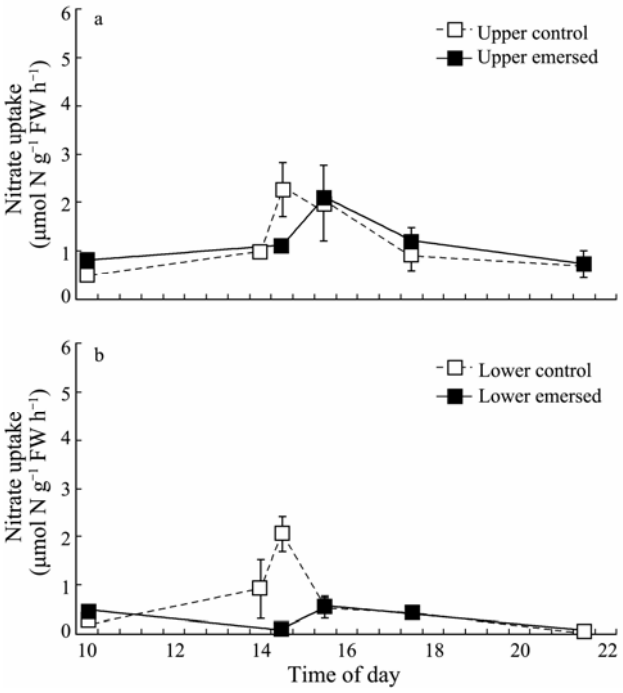

Fig.1 Nitrate uptake of *Porphyra umbilicalis* from upper (a) and lower (b) intertidal zones collected in September, 2007. Sunrise was approximately at 07:00 and sunset was approximately at 19:00. Filled squares represent a desiccated treatment which was exposed to air for 4 h (10:0–14:00). All controls, open squares, from upper and lower zones remained submerged. Error bars represent ± one standard deviation.

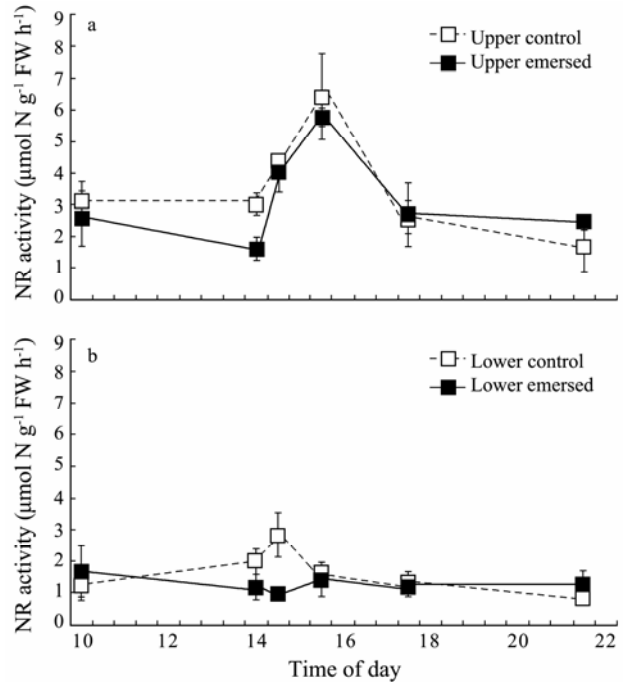

Fig.2 Nitrate reductase activity of *Porphyra umbilicalis* from upper (a) and lower (b) intertidal zones collected in September, 2007. Sunrise was approximately at 07:00 and sunset was approximately at 19:00. Filled squares represent desiccated treatment which was exposed to air for 4 h (10:00–14:00). All controls, open squares, from upper and lower zones remained submerged. Error bars represent ± one standard deviation.

per intertidal controls (6.41 μmol N g^{-1} FW h^{-1}), followed by those of upper intertidal emersed (5.77 μmol N g^{-1} FW h^{-1}), lower intertidal control (2.84 μmol N g^{-1} FW h^{-1}) and lower intertidal emersed (1.66 μmol N g^{-1} FW h^{-1}). Nitrate uptake and NR activity showed a positive correlation in upper intertidal thalli (control, $P = 0.014$; emersed, $P < 0.001$). However, the lower intertidal thalli showed a significant correlation only in control ($P < 0.001$; Fig.3).

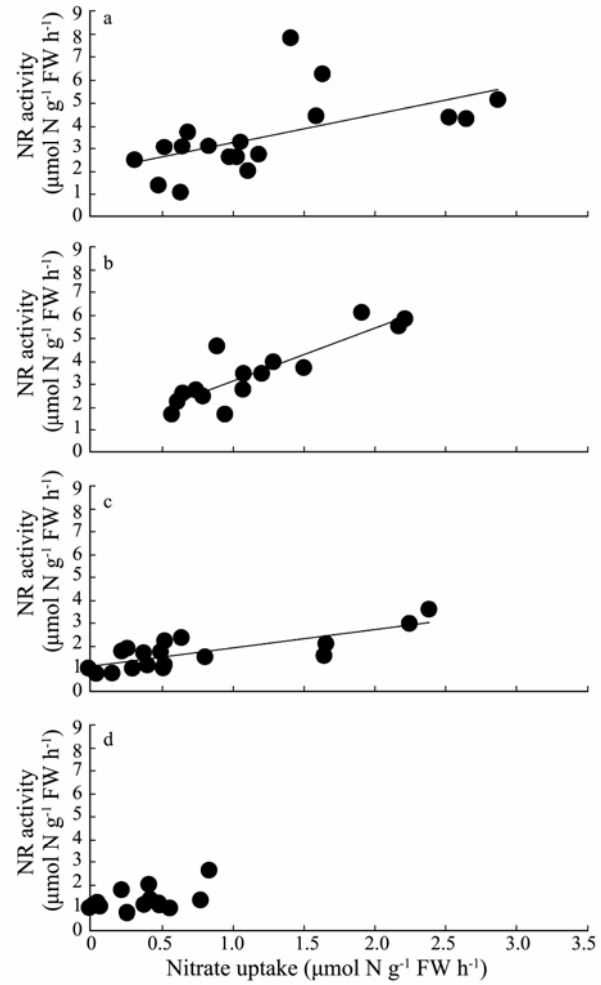

Fig.3 Regression of nitrate uptake *vs*. NR activity of *Porphyra umbilicalis* from upper and lower intertidal zones collected in September, 2007. (a), upper intertidal control ($P = 0.014$); (b), upper intertidal desiccated ($P < 0.001$); (c), lower intertidal control ($P < 0.001$); and (d), lower intertidal desiccated ($P = 0.057$).

3.3 Nitrate Uptake *vs*. Glutamine Synthetase (GS) Activity

Emersion significantly influenced nitrate uptake and GS activity of *Porphyra umbilicalis* thalli from both upper and lower areas of the intertidal zone collected in March ($P = 0.023$, nitrate uptake and $P < 0.001$, GS upper; $P < 0.001$, nitrate uptake and $P = 0.001$, GS lower, respectively; Table 1), whereas time influenced only nitrate uptake in the lower intertidal thalli ($P = 0.001$). The interaction of time and emersion influenced the nitrate uptake of both upper and lower thalli ($P = 0.047$, upper and $P = 0.026$, lower, respectively). Nitrate uptake of lower inter-

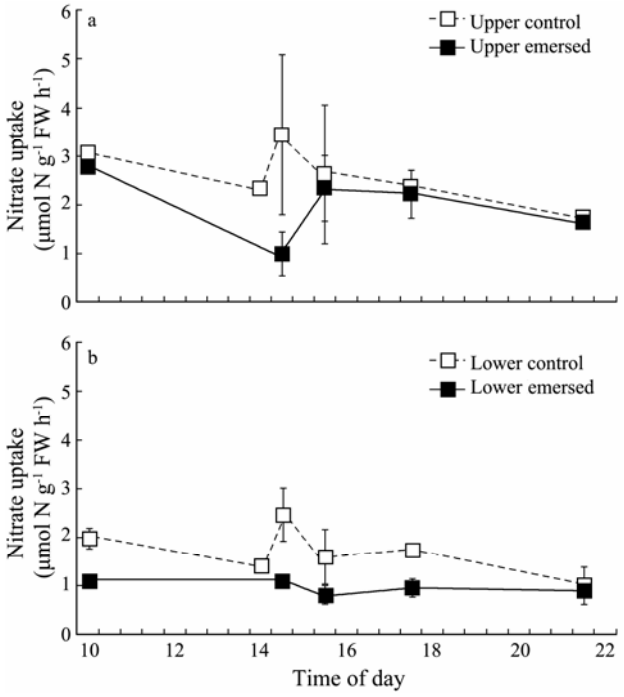

Fig.4 Nitrate uptake of *Porphyra umbilicalis* from upper (a) and lower (b) intertidal zones collected in March, 2008. Sunrise was approximately at 07:00 and sunset was approximately at 19:00. Filled squares represent a desiccated treatment which was exposed to air for 4 h (10:00–14:00). All controls, open squares, from upper and lower zones remained submerged. Error bars represent ± one standard deviation.

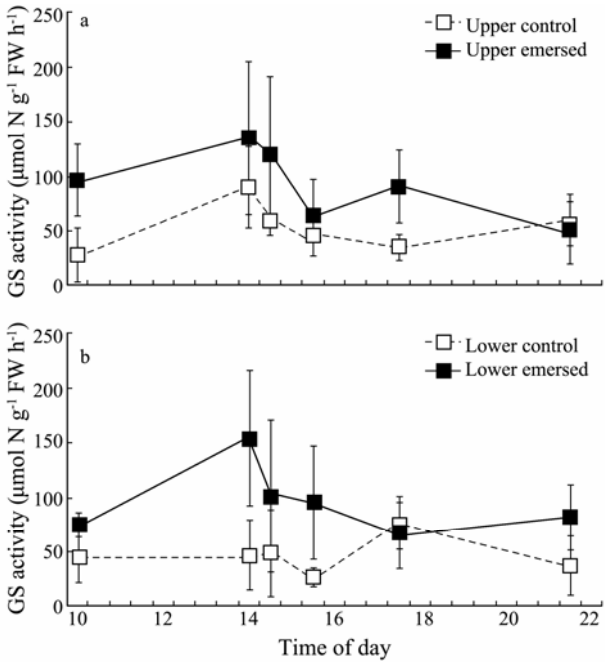

Fig.5 Glutamine synthetase activity of *Porphyra umbilicalis* from upper (a) and lower (b) intertidal zones collected in March, 2008. Sunrise was approximately at 07:00 and sunset was approximately at 19:00. Filled squares represent a desiccated treatment which was exposed to air for 4 h (10:00–14:00). All controls, open squares, from upper and lower zones remained submerged. Error bars represent ± one standard deviation.

tidal thalli exposed to emersion was significantly lower than that of control at the time points before emersion and 0.5 h and 3.5 h post-emersion ($P = 0.03$, 0.15 and 0.02, respectively; Fig.4). Emersion of both upper and lower intertidal thalli resulted in higher GS activities compared with controls ($P < 0.001$ and $P = 0.001$, respectively; Fig.5). GS activity was the highest in the upper intertidal emersed (91 μmol N g^{-1} FW h^{-1}), followed by lower intertidal emersed (90 μmol N g^{-1} FW h^{-1}), upper intertidal control (49 μmol N g^{-1} FW h^{-1}) and lower intertidal control (46 μmol N g^{-1} FW h^{-1}) (Fig.5). There was no correlation between nitrate uptake and the GS activity in both upper and lower intertidal thalli, indicating no correlation between NR and GS activity.

4 Discussion

The metabolism involved in the uptake and assimilation of N by *Porphyra umbilicalis* was affected by emersion as an adaptation linked with its elevation in the intertidal zone. This elevation dependency of N metabolism is undoubtedly part of a suite of adaptations that enables *P. umbilicalis* to occupy such a broad range of intertidal habitats. For example, the rate of recovery of other physiological functions such as photosynthesis and nutrient uptake following emersion is a major factor determining the upper limits to the vertical distribution of *Porphyra* (Lipkin *et al*., 1993; Kim *et al*., 2008; Kim *et al.*, 2009). Kim *et al*. (2008) reported interspecific differences in emersion effect; nitrate uptake by intertidal *Porphyra umbilicalis* and *Porphyra leucosticta* (= *Pyropia leucosticta*) recovered within a few hours following resubmergence, while nitrate uptake by subtidal *Porphyra yezoensis* (=*Pyropia yezoensis*) did not recover even after 7.5 h. They also reported that emersion did not affect the phosphate uptake by intertidal *P. umbilicalis*, but negatively affected the phosphate uptake by lower intertidal and subtidal species, *Py. leucosticta* and *Py. yezoensis,* respectively.

Here, we report an analogous metabolic phenomenon with a population of the broadly distributed species *P. umbilicalis* that emersion of thalli from the upper intertidal zone had little effect on N uptake and assimilatory metabolism, while metabolism of thalli from the lower limits of the distribution of this species was strongly impaired. For example, the N uptake of upper intertidal *P. umbilicalis* in September and March populations averaged 1.28 and 2.64 (control), and 1.16 and 1.98 μmol N g^{-1} FW h^{-1} (emersed), respectively. On the otherhand, the activity of the lower intertidal thalli was 0.69 and 1.73 (control), and 0.33 and 0.94 μmol N g^{-1} FW h^{-1} (emersed), respectively. The reduction of N uptake due to emersion was 9%–15% in upper and 45%–52% in lower intertidal thalli. There was also a lag time in nitrate uptake response in the emerged thalli compared to controls in all experiments. Emersed upper and lower populations collected in September showed the same pattern, recovering nitrate uptake to control level within 1.5 h post-emersion. However, the population collected in March showed a different pattern.

The lag time in tissues from the lower intertidal was more than 3.5 h while the emersed upper sub-population was recovered its nitrate uptake within 1.5 h. This result suggested that fall (summer experienced) and spring (winter experienced) population have different physiological responses (tolerance) to the emersion stress especially at the lower intertidal sub-population. The reduction of NR activity of thalli from each *P. umbilicalis* sub-population, also exhibited similar patterns. The NR activity of upper intertidal *P. umbilicalis* in September population averaged 3.16 (control) and 2.93 μmol N g^{-1} FW h^{-1} (emersed), respectively, while the activity of the lower intertidal thalli was only 1.53 (control) and 1.24 μmol N g^{-1} FW h^{-1} (emersed), respectively. The reduction of NR activity due to emersion was 7% in upper and 19% in lower intertidal thalli. In addition, the lag time of emersed thalli compared to controls in the lower intertidal sub-population was 1.5 h longer than the upper intertidal sub-population (0.5 h). Together, these results support the hypothesis that rapid recovery of physiological function is a major factor involved in the adaptation of this and other intertidal seaweeds to life in the upper intertidal zone.

Phillips and Hurd (2004) also reported that species growing in different vertical habitats have different strategies of meeting their N requirements. They cultivated *Stictosiphonia arbuscula* (Harvey) King & Puttock, *Apophlaea lyallii* Hook. f. & Harvey, *Scytothamnus australis* Hook. f. & Harvey, and *Xiphophora gladiataalso*, all occurring in the intertidal zone, and exposed these seaweeds to nitrate, ammonium or urea for 30 min to test their competitive ability of N uptake, and found that species growing at the highest shore position showed higher nitrate and urea uptake and had unsaturable ammonium uptake in both summer and winter.

Bray (2006) collected numerous *Porphyra* (some now included in the sister genus *Pyropia*) specimens from approximately 100 different sites, as well as at different tidal elevations, in the Northwest Atlantic, sequenced *rbc*L, *rbc*L-*rbc*S spacer, SSU rRNA gene and ITS1, and found no obvious differences vertically across populations within the intertidal zone. This suggested that the broad intertidal population of *Porphyra* (and *Pyropia*) species is not genetically differentiated by elevation. In coastal New England, *P. umbilicalis* occurs over a wide range of vertical habitats (West *et al*., 2005), implying a range of emersion stress from the lower to the upper intertidal zone (15%–96% WL; Kim, 2008). In addition to influencing N metabolism, the level of emersion stress associated with emersion appears to influence the morphology of *P. umbilicalis*. The upper intertidal *P. umbilicalis* is smaller in area than that collected from the lower intertidal. The blade from upper intertidal zone is also typically densely ruffled to reduce the rate of water loss by reducing the effective SA:V ratio when thalli collapse at low tide and/or to increase self-shading during emersion. On the other hand, the lower intertidal blades are less folded (Sears, 2002). In other words, not only do upper thalli not have the potential to dry out as much, but they also suffer less from emersion. The red alga *Gelidiella acerosa* (Forsskål) Feldmann & G. Hamel also showed similar morphological variations in thalli from different vertical distributions (Ganzon-Fortes, 1997). The subtidal and tide pool plants were taller, more frequently branched (higher SA:V ratio) with longer branches than the intertidal plants.

These morphological and metabolic differences between lower and upper intertidal thalli effectively extend the distribution of *P. umbilicalis* across a broader range of intertidal zone. What was not investigated by our research was the source of variability. That is, the differences in N metabolism observed here could represent, like Bray's work (2006) suggested, phenotypic plasticity (though developmentally channelized) within a broadly variable species rather than genetic differences within the intertidal thalli (*i.e.*, upper and lower thalli may be ecotypes; *e.g.* Innes, 1988; Bhattacharya and Druehl, 1989; Chapman, 1995; Zardi *et al*., 2011; Olsen *et al*., 2004; West *et al*., 2005). Here, the application of molecular techniques could deepen our understanding of the ecology of this common intertidal seaweed. The metabolic plasticity found in the present study could also be confirmed by reciprocal transplants of thalli from the upper and lower distributional limits. This would allow us to determine whether long term differences in growth and/or metabolic activities exist.

Nitrate uptake and NR activity normally exhibit diurnal patterns in photosynthetic organisms, even though the timing of peak activity varies. The highest nitrate uptake and NR activity were found during the light period, and most often peaked at midday, with minimal activity in dark period (Davison and Stewart, 1984; Gao *et al*., 1992; Lopes *et al*., 1997; Thevanathan *et al*., 2002; Granbom *et al*., 2004; Kim *et al*., 2008). Although light was not an experimental variable in the present study, *Porphyra umbilicalis* also appeared to follow the general pattern where the nitrate uptake and NR activity were maximal 7.5–8.5 h after sunrise. Light is one of the major regulatory factors in nitrate assimilation, but from this study, it is clearly not the only signal for NR activation. When *P. umbilicalis* from the lower intertidal zone experienced emersion stress, it lost the diurnal pattern of NR activity and nitrate uptake. However, upper intertidal *P. umbilicalis* thalli did not lose the midday uptake and NR maxima. This protection against the loss of the cycle maximum is significant because it enables upper intertidal *P. umbilicalis* to use carbohydrates, abundant during midday, for N assimilation.

When nitrate concentration decreases in the ambient environment and then intracellular NR activity rapidly declines. Thompson and Valiela (1999) found no significant direct relationship between NR activity and ambient nitrate concentration, but a positive relationship between the enzyme activity and internal N of *Cladophora vagabunda* (Linnaeus) Hoek, *Fucus vesiculosus* Linnaeus and *Gracilaria tikvahiae* McLachlan. Our observation of correlation between nitrate uptake and NR activity is consistent with that reported. The low NR activity under high nitrate availability may associate with ammonium sup-

pression in the field as has been reported in other organisms (Conway, 1977; DeBoer *et al*., 1978; Thomas and Harrison, 1985; Teichberg *et al*., 2007). In fact, when cultivated with nitrate as the sole source of inorganic N, a positive correlation between NR activity and external nitrate concentration was reported for phytoplankton (Joseph and Villareal, 1998), seaweed (Lopes *et al*., 1997; Gordillo *et al*., 2001), and seagrasses (Touchette and Burkholder, 2000). NR activity is also correlated with growth (Berges and Harrison, 1995) and nitrate uptake (Gordillo *et al*., 1997; Gordillo *et al*., 2001). The NR activity during emersion decreased in the present study, and in a previous study, the tissue N in *Porphyra* species, including *P. umbilicalis*, also decreased during emersion (Kim *et al*., 2008).

NR and NiR reduce nitrate to ammonium. Glutamine synthetase assimilates ammonium into organic form (glutamine). When nitrate is the only N source for seaweeds, GS activity should depend on NR activity to provide ammonium. Teichberg *et al*. (2007) reported that GS activity of *Ulva lactuca* directly linked to N reduced by NR when nitrate was the major N source. However, nitrate uptake and its assimilation (*i.e.*, GS activity) were not closely coupled in the present study (in which ammonium was not provided in medium). While nitrate uptake and NR activity were tightly linked to each other and were significantly influenced by emersion, GS activity was not limited and even appeared to be stimulated by emersion. The high level of GS activity in *Porphyra umbilicalis* may also be a protective mechanism, enabling *P. umbilicalis* to assimilate N quickly when it again becomes available. This high level of GS activity may also indicate photorespiration in emersed thalli. During emersion, proteins may be converted to ammonium in mitochondria, and the photorespiratory NH_4^+ is transferred to chloroplasts and re-assimilated by glutamine synthetase (GS) (Keys *et al*., 1978).

Kim *et al*. (2008) demonstrated that the rate of recovery of nutrient uptake following emersion may be an important factor involved in setting the intertidal distribution of different species of *Porphyra*. In the present study, we have found ecophysiological differences among thalli from the vertical extremes in elevation of an intertidal population of *P. umbilicalis*. Thalli growing in the upper intertidal zone were less impacted by emersion stress than lower intertidal thalli when nitrate uptake and NR activity were the metrics used.

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