Journal of Experimental Marine Biology and Ecology 365 (2008) 75–85

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Physiological activity of *Porphyra* in relation to eulittoral zonation

Jang K. Kim [a,b,*], George P. Kraemer [c], Charles Yarish [a]

[a] Departments of Ecology and Evolutionary Biology and Marine Sciences, University of Connecticut, 1 University Place, Stamford, CT, 06901, USA
[b] Departments of Ecology and Evolutionary Biology and Marine Sciences, University of Connecticut, 1080 Shennecossett Road, Groton, CT, 06340, USA
[c] Department of Environmental Studies, Purchase College, 735 Anderson Hill Road, Purchase, NY, 10577, USA

ARTICLE INFO

Article history:
Received 24 June 2008
Received in revised form 22 July 2008
Accepted 22 July 2008

Keywords:
Emersion
Eulittoral Zonation
Nitrogen
Phosphate
Porphyra

ABSTRACT

Eulittoral seaweeds at different tidal elevations are exposed to various frequencies and durations of emergence. Their physiological activities (e.g. nutrient uptake) may be affected by water loss during emersion. We used three *Porphyra* species from different tidal elevations to test whether species at different vertical elevations on the shore respond differently to the increasingly non-marine environment, in terms of their physiological activities including nutrient uptake, tissue carbon, nitrogen and phycoerythrin contents. Simulated tidal cycles produced water losses of 0%, 40±10% and 90±5% tissue water. Emersion was stressful for all species regardless of their habitat. It was more stressful to nitrate and phosphate uptake for the sublittoral species *P. yezoensis* than eulittoral species, *P. umbilicalis* and *P. leucosticta*. Interestingly, tissue N for thalli that had been emerged and then re-submerged was significantly higher than those of continuously submerged individuals. During exposure, tissue N contents of all species declined but recovered quickly (e.g. within 30 min) after re-submergence. This result suggests that emersion-induced N release may constitute an undescribed biogeochemical pathway linking marine, terrestrial, and atmospheric N reservoirs.

1. Introduction

Eulittoral zonation is reflected in the distinctive vertical patterns of organism distribution. Marine ecologists and physiologists have studied eulittoral zonation for nearly a century (Baker, 1909, 1910; Lewis, 1964; Edwards, 1977; Hurd and Dring, 1990; Davison and Pearson, 1996; Beach and Smith, 1997; Skene, 2004). Organisms in the eulittoral zone are regularly exposed to the air by the ebb and flow of the tide. Species living in a particular environment (e.g., at one elevation on the shore) may be better adapted to live, grow, and reproduce in that environment than organisms that grow elsewhere. While emerged, eulittoral organisms experience various environmental challenges including high light, exposure to ultraviolet light, desiccation, extremes of temperature and salinity, and nutrient unavailability. We expect that eulittoral species are better adapted to tolerate such environmental challenges than sublittoral species (Lüning et al., 1990).

Eulittoral seaweeds at different tidal elevation are exposed to different frequencies and durations of emergence. Their growth and physiological activities, such as photosynthesis and nutrient uptake, may be affected by emersion. The ability to withstand emersion and to recover physiological function following emersion is likely a major determinant of suitability for life within the eulittoral zone. Recently, Kim et al. (in review) found that desiccation was more stressful to a sublittoral species of *Porphyra* than a eulittoral one in terms of growth. When the sublittoral *P. amplissima* experiences longer emersion, it loses biomass, while the eulittoral species *P. umbilicalis* grew at rates approximately 70% of those of continuously submerged tissues. Kim and Yarish (in review) also found that lower littoral species, *P. leucosticta* and another sublittoral species *P. yezoensis* lost weight under longer period of emersion.

No relation is reported to exist between the ability to retain water and the vertical distribution of eulittoral seaweeds (Ji and Tanaka, 2002). The resistance of photosynthesis to desiccation also does not explain the distribution of seaweeds in the eulittoral zone (Dring and Brown, 1982). However, losses of inorganic and organic N and P after desiccation have been reported for eulittoral seaweeds, and the degree of N and P release was dependant on vertical habitats of seaweeds (Thomas et al., 1987a; Hurd and Dring, 1991). For example, *Gracilaria pacifica* from an upper littoral habitat had lost nitrogen after 50% water loss, while sublittoral plants lost nitrogen after only 10% water loss (Thomas et al., 1987a). The release of N and P (organic and inorganic) may indicate the disruption of the cell membrane (Thomas et al., 1985; Hurd and Dring, 1991). Photosystems are also damaged by desiccation, which causes a decrease in the production of reductant and reduces carbohydrates available for nitrogen assimilation (Proctor and Smirnoff, 2000). Therefore, the rate of recovery of physiological functions such as carbon metabolism and nutrient uptake following

* Corresponding author. Departments of Ecology and Evolutionary Biology and Marine Sciences, University of Connecticut, 1080 Shennecossett Road, Groton, CT, 06340, USA. Tel.: +1 860 405 9089; fax: +1 860 405 9153.
E-mail address: jang.kim@uconn.edu (J.K. Kim).

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doi:10.1016/j.jembe.2008.07.040

emersion of eulittoral seaweeds is more important in determining patterns of vertical distribution than tolerance to desiccation (Quadir et al., 1979; Bidwell and McLachlan, 1985; Johnston and Raven, 1986; Gao et al., 1999; Kim et al., in review).

While post-emersion phosphate uptake was not enhanced, the rate of recovery of phosphate uptake was correlated with the vertical zonation of seaweeds (Hurd and Dring, 1990). Emersion also appears to enhance short-term uptake of nitrogen upon resubmersion in some eulittoral species (Thomas et al., 1987b). An increase in N uptake following emersion could be a mechanism to replenish N stores depleted when thalli are isolated from the source of nutrients. The degree of stimulation of N uptake following emersion may be correlated with observed vertical distribution patterns. Upper shore species may exhibit greater stimulation of N uptake following emersion, may achieve maximum uptake at higher levels of desiccation, and may be able to take up N following longer period of emersion that inhibits uptake by low-shore species. The difference in a seaweed's ability to acquire a resource following a period of limitation (i.e., emersion) may be another critical physiological factor controlling the vertical distribution patterns of seaweeds (Thomas et al., 1987b; Hurd and Dring, 1990; Davison and Pearson, 1996).

Seaweeds take up N from seawater and store it in organic and inorganic forms for later use, especially during periods of N depletion (Bird et al., 1982). For example, *Gracilaria tikvahiae* can store enough N to allow non-limited growth when N was added every 2 weeks. The tissue N contents of *Gracilaria* just prior to N addition were significantly higher (3-5% DW) than the contents indicating N deficiency (1.5–2% DW; Ryther et al., 1981; Fujita, 1985). *Laminaria longicruris* also took up nitrogen when available during the winter. The sequestered nitrogen was stored in organic pools and used to support growth later in the spring (Chapman and Craigie, 1977). Unlike species with a low surface area:volume (SA:V) ratio (e.g., *Gracilaria tikvahiae)* that can maintain high growth rate over 14 days without external N supply, the growth of *Ulva lactuca* with extremely high SA:V ratio decreased or stopped only 6 to 10 days after nitrogen pulse (Ryther et al., 1981; Fujita, 1985). Pigments are also sensitive indicator of the N status in seaweeds (Chopin et al., 1999; Carmona et al., 2006). In red algae, phycobiliprotein is major N storage compartment (Bird et al., 1982; Chopin et al., 2001; Harrison and Hurd, 2001; Sampath-Wiley and Neefus, 2007). Kim et al. (2007) recently reported that phycoerythrin and tissue N contents are positively correlated, which suggests a storage function for this pigment.

Algal morphology significantly influences the capacities of N uptake and storage (Bird et al., 1982; Fujita, 1985). Seaweeds with high SA:V ratios have higher biomass-specific rates of nutrient uptake than species with low ratio. However, in contrast, species with low

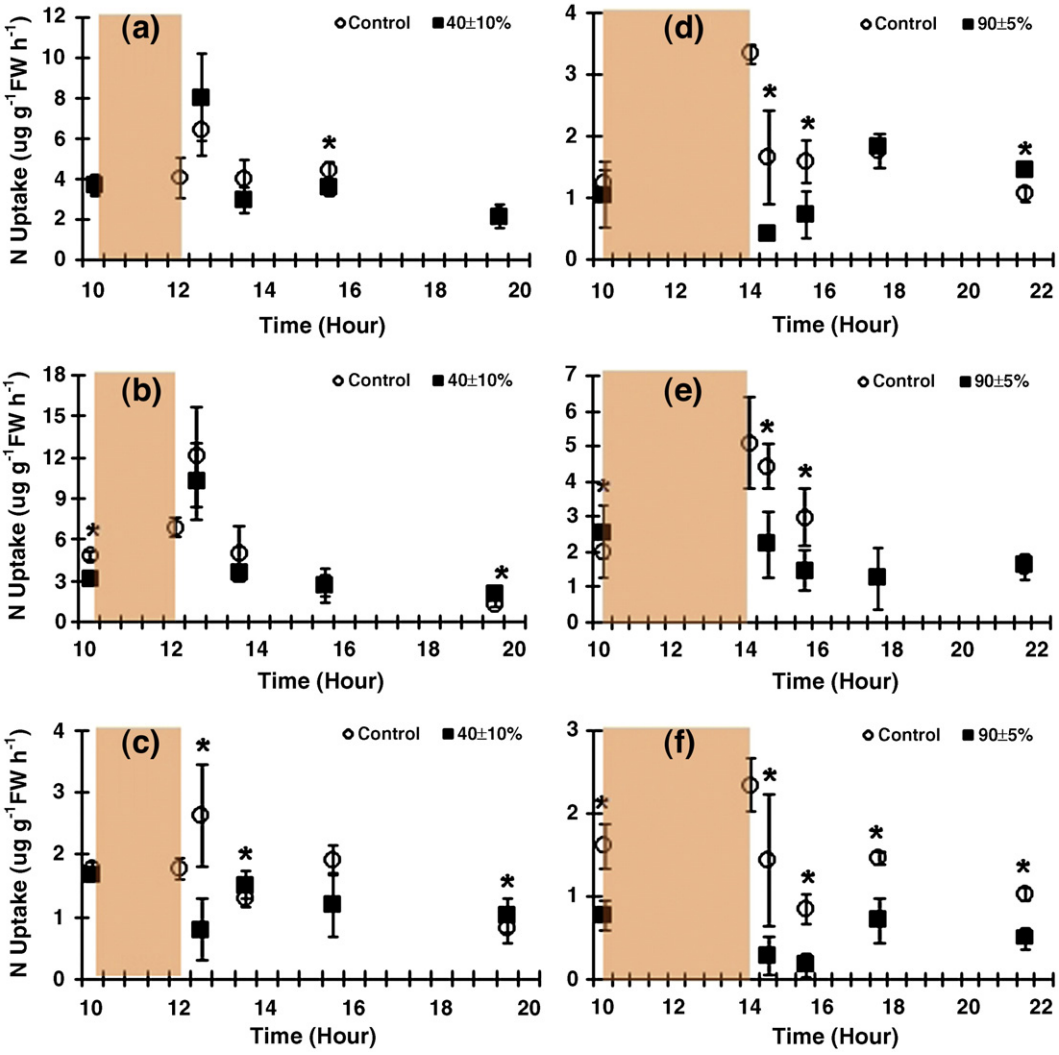

Fig. 1. Nitrate uptake rates of *Porphyra* species from different tidal elevations and different emersion treatments (0, 40±10% and 90±5% water loss). (a) and (c): mid-upper littoral *P. umbilicalis* (shorter and longer emersion, respectively), (b) and (d): lower littoral *P. leucosticta* (shorter and longer emersion, respectively), (c) and (f): sublittoral *P. yezoensis* (shorter and longer emersion, respectively). Shaded areas represent exposure time. Error bars represent standard deviation. Bars with an asterisk are significantly different ($p<0.05$).

SA:V ratios can provide greater N storage capacity. Unfortunately, most previous studies of nutrient uptake by eulittoral seaweeds have mixed taxa and morphologies, making interpretation of strategies for dealing with elevation-related issues difficult (Thomas et al., 1987b; Hurd and Dring, 1990; Hurd and Dring, 1991; Phillips and Hurd, 2003, 2004).

All *Porphyra* species consist of 1 or 2 cell layers, and have an extremely high surface area to volume ratio with all cells capable of rapid uptake of nutrients (Kraemer et al., 2004; Neori et al., 2004). Twelve *Porphyra* species have been reported in New England and the southern Maritime Provinces of Canada (Yarish et al., 1998; Chopin et al., 1999; Neefus et al., 2002; West et al., 2005; Bray et al., 2006; Neefus et al., in review). New England *Porphyra* species occur seasonally, except for *P. umbilicalis*. As a group, *Porphyra* species show a wide vertical distribution in the eulittoral and sublittoral zones. For example, *P. umbilicalis* is the most abundant species, both spatially and temporally, and occurs throughout the year within the eulittoral zone. *Porphyra leucosticta* grows within the lower littoral zone from late fall to spring (West et al., 2005; Blouin et al., 2007). By early summer, the thalli of *P. leucosticta* disappear. An introduced species, *Porphyra yezoensis* occurs during winter through spring within coastal areas of northern New England and Long Island. This species appears to be most abundant within the low littoral and sublittoral zones through that time of the year (Villalard-Bohnsack, 1995; Chopin et al., 1999; West et al., 2005; Neefus et al., in review).

The eulittoral species are exposed to air as much as 8 of every 12 h while sublittoral species do not naturally experience exposure. Therefore, species in the upper littoral zone may have specific adaptations to deal with the increasingly non-marine environment than species in lower littoral or sublittoral zones. In the present study, three congeneric *Porphyra* species with similar SA:V ratios but from different intertidal elevation were used to test whether populations at different vertical elevations on the shore respond differently in terms of their physiological activities including nutrient uptake, tissue carbon, nitrogen and phycoerythrin contents.

2. Materials and Methods

2.1. Algal materials and culture condition

Porphyra leucosticta was collected in the lower littoral zone (<0.5 m MLW) at Groton, Connecticut, USA in March 2007. *Porphyra umbilicalis* was collected in the mid to upper littoral zone (>2 m MLW) at Rye, New Hampshire, USA in April 2007. *Porphyra yezoensis* was collected in the sublittoral zone at Winnapaug Pond outlet, Weekapaug, Rhode Island, USA in May 2007.

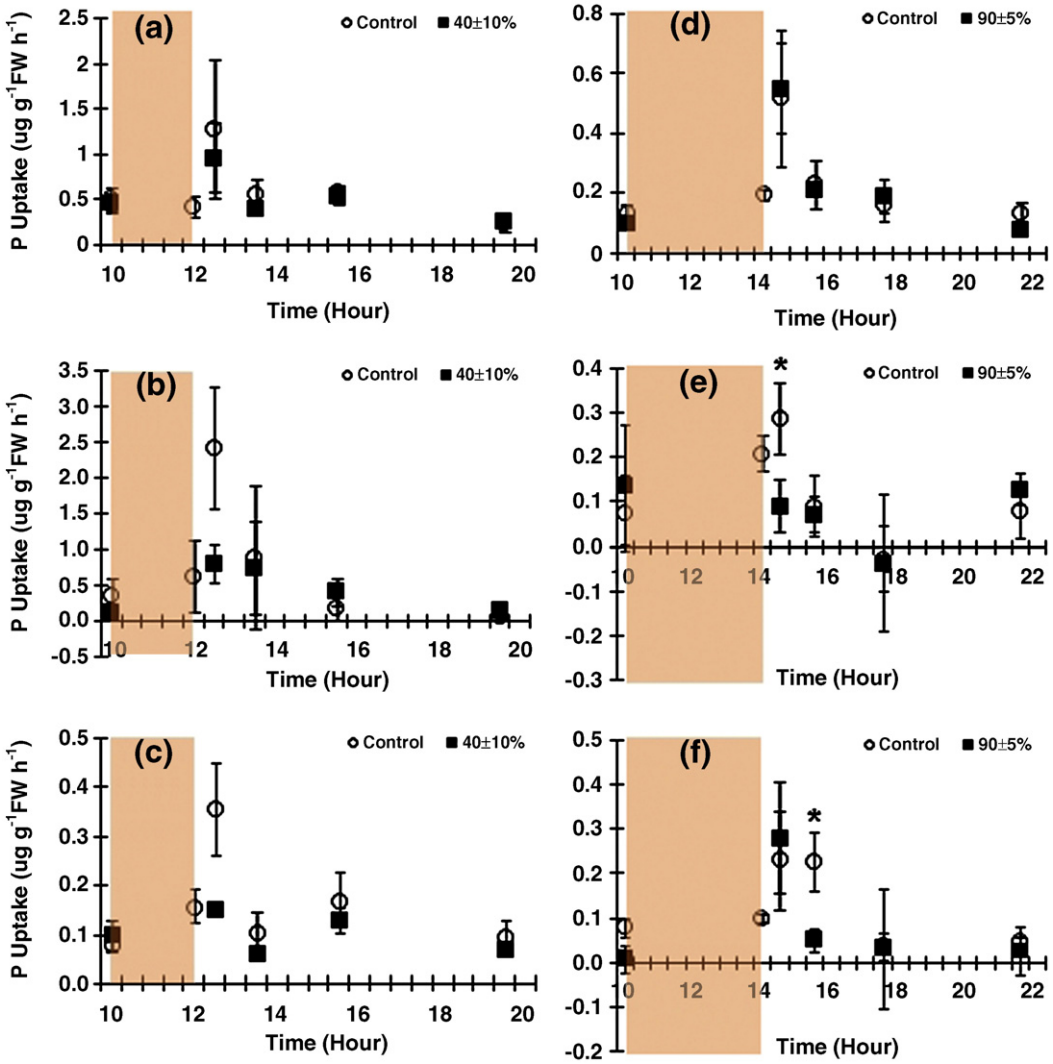

Fig. 2. Phosphate uptake rates of *Porphyra* species from different tidal elevations and different emersion treatments (0, 40±10% and 90±5% water loss). (a) and (c): mid-upper littoral *P. umbilicalis* (shorter and longer emersion, respectively), (b) and (d): lower littoral *P. leucosticta* (shorter and longer emersion, respectively), (c) and (f): sublittoral *P. yezoensis* (shorter and longer emersion, respectively). Shaded areas represent exposure time. Error bars represent standard deviation. Bars with an asterisk are significantly different ($p<0.05$).

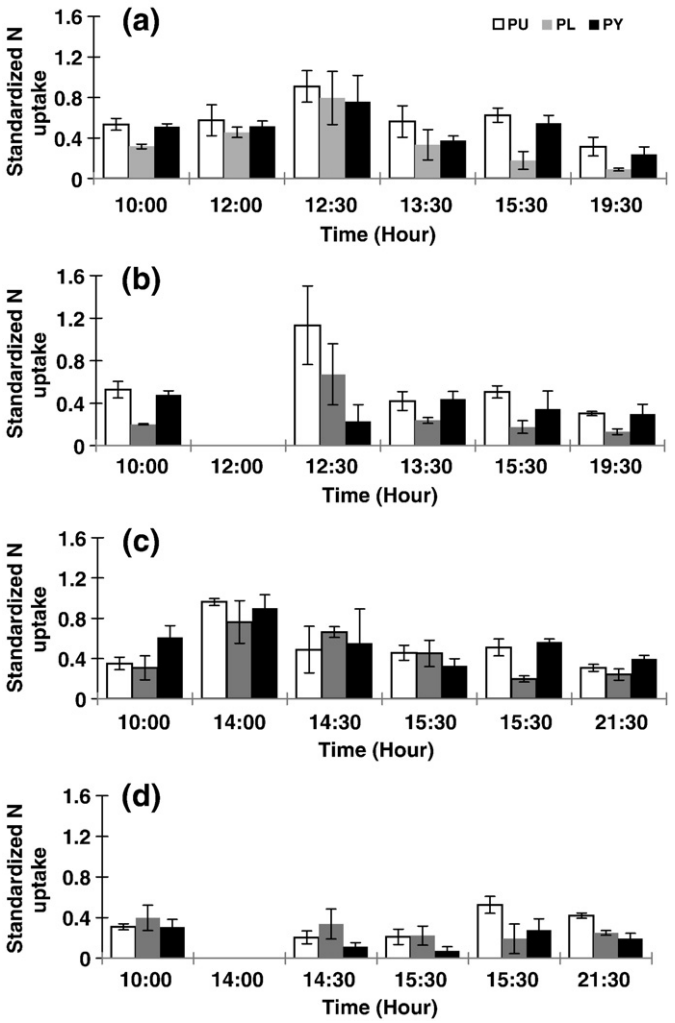

Fig. 3. Relative nitrate uptake of *Porphyra* species from different tidal elevations and different emersion treatments. Values were calculated by standardizing the highest control values for each species and each treatment. The highest values for each species and each treatment were considered to be "1." (a): relative nitrate values of control of three *Porphyra* species for shorter emersion experiment, (b) relative nitrate values of 40±10% water loss treatment, (c) relative nitrate values of control of three *Porphyra* species for longer emersion experiment, (d) relative nitrate values of 90±5% water loss treatment. Error bars represent standard deviation.

Experiments were carried out in a greenhouse at the University of Connecticut at Avery Point (Groton, CT) on cloudless or partly sunny days to keep the water loss similar in all species. Samples were exposed to a semi-diurnal cycle using a tide simulating apparatus (Kim and Yarish, in review). The apparatus consists of 18 compartments (3 rows of 6 compartments). The culture medium was 0.45 μm-filtered seawater with von Stosch's enrichment (Ott, 1965) without nitrogen (N) and phosphorus (P). Nitrogen and P levels were regulated by addition of nitrate and phosphate at a molar N:P ratio of 10:1. To prevent nutrient depletion, the culture medium was changed at every sampling except for 0.5 h post-emersion. The nitrate and phosphate concentrations (30 and 3 μM, respectively) reflect the highest N and P concentrations in the Long Island Sound, USA during winter season (Capriulo et al., 2002; Pedersen et al., 2004; Balch et al., 2008). Water temperature of 10 °C is reflective of what all species would experience in the field. The maximum light intensity measured in the greenhouse by a Li-Cor LI-1000light meter (Li-Cor, Lincoln, Nebraska, USA) was 1300 μmol photons $m^{-2}s^{-1}$. The air temperature and humidity during exposure were 15-27 °C and 32-80%, respectively. Stocking density for each treatment was approximately 0.5 g/L.

2.2. Acclimation

All three species were acclimated to the experimental conditions with treatments for 5-7 days. Filtered seawater containing nutrients was replaced daily to maintain a stable nutrient status in the algal tissues during acclimation. During acclimation, samples assigned to the emersion treatment were exposed at semi-diurnal cycle by using the tide simulating apparatus whereas, all controls remained submerged.

2.3. Water loss calculation

Water loss (WL) of samples was controlled to 0, 40±10% (2 h exposure), and 90±5% (4 h exposure), WL was estimated as follows:

$$\text{WL}\ (\%)=\frac{\text{FW}-\text{TW}}{\text{FW}-\text{DW}}\times 100$$

where FW is the fresh weight obtained after blotting the thalli dry with paper towels. TW is the desiccated weight after known time interval. DW is the dry weight measured by drying a sample of the biomass at 60 °C to constant weight.

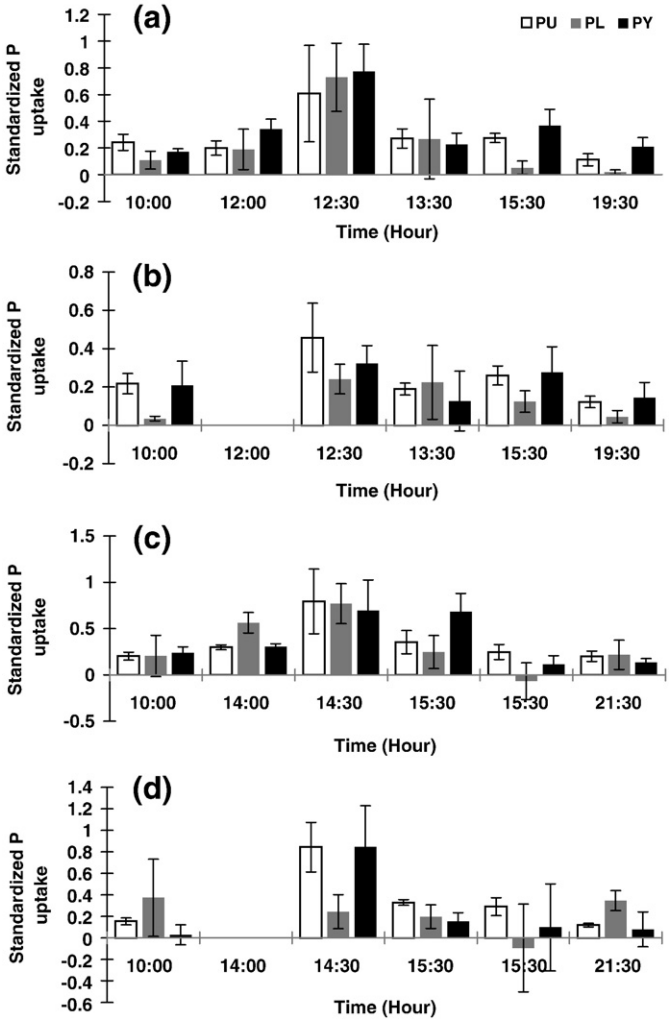

Fig. 4. Relative phosphate uptake of *Porphyra* species from different tidal elevations and different emersion treatments. Values were calculated by standardizing the highest control values for each species and each treatment. The highest values for each species and each treatment were considered to be "1." (a): relative phosphate values of control of three *Porphyra* species for shorter emersion experiment, (b) relative phosphate values of 40±10% water loss treatment, (c) relative phosphate values of control of three *Porphyra* species for longer emersion experiment, (d) relative phosphate values of 90±5% water loss treatment. Error bars represent standard deviation.

Table 1
Results of analysis of variance examining the effects of emersion and time on the standardized nitrate uptake and phosphate uptake, the tissue carbon and nitrogen contents, and phycoerythrin contents of *Porphyra* species

Species	Water loss	Factor	Standar. nitrate uptake		Standar. phosphate uptake		Tissue carbon		Tissue nitrogen		Phycoerythrin	
			F	p-value	F	p-value	F	p-value	F	p-value	F	p-value
P. umbilicalis	40%	time	27.65	**<0.001**	11.27	**<0.001**	3.96	**0.007**	1.41	0.244	1.15	0.362
		emersion (control vs. 40%)	0.07	0.801	1.23	0.281	0.06	0.803	18.79	**<0.001**	0.02	0.886
		time X emersion	2.07	0.123	0.33	0.857	0.96	0.456	1.13	0.363	0.34	0.850
	90%	time	4.36	**0.011**	24.97	**<0.001**	4.25	**0.005**	7.80	**<0.001**	5.85	**0.003**
		emersion (control vs. 90%)	6.73	**0.017**	0.26	0.619	13.28	**0.001**	74.75	**<0.001**	60.92	**<0.001**
		time X emersion	5.08	**0.006**	0.61	0.660	1.00	0.432	0.65	0.662	4.07	**0.015**
P. leucosticta	40%	time	24.66	**<0.001**	11.84	**<0.001**	4.24	**0.004**	1.99	0.105	1.69	0.193
		emersion (control vs. 40%)	1.68	0.210	1.49	0.236	18.52	**<0.001**	5.87	**0.021**	0.02	0.888
		time X emersion	0.56	0.694	2.82	0.053	0.39	0.853	1.62	0.180	0.65	0.634
	90%	time	7.79	**0.001**	5.45	**0.004**	2.61	**0.045**	1.77	0.150	1.93	0.145
		emersion (control vs. 90%)	5.94	**0.024**	0.56	0.462	166.45	**<0.001**	216.67	**<0.001**	2.00	0.173
		time X emersion	4.41	**0.010**	1.30	0.303	0.59	0.706	1.21	0.329	0.91	0.475
P. yezoensis	40%	time	3.53	**0.025**	10.90	**<0.001**	2.31	0.068	1.38	0.260	1.69	0.193
		emersion (control vs. 40%)	8.12	**0.010**	9.38	**<0.001**	0.17	0.682	5.07	**0.032**	1.80	0.195
		time X emersion	6.17	**0.002**	3.59	**0.023**	0.99	0.438	1.06	0.402	1.32	0.297
	90%	time	4.00	**0.015**	9.88	**<0.001**	1.30	0.292	1.54	0.207	1.84	0.160
		emersion (control vs. 90%)	42.36	**<0.001**	2.56	0.125	40.51	**<0.001**	154.24	**<0.001**	0.04	0.837
		time X emersion	0.73	0.582	1.92	0.147	0.86	0.518	1.75	0.154	1.45	0.255

Significant differences are shown in bold with p values.

2.4. Measurements

Tissue and water samples were taken before exposure, and 0.5 h, 1.5 h, 3.5 h and 7.5 h after re-submergence. Samples were taken from three compartments, one selected at random from each row (n=3). Water samples from the incubation medium were analyzed for inorganic nitrate by using a SmartChem Discrete Analyzer (Westco Scientific Instruments, Inc. Brookfield, CT, USA). Inorganic phosphate concentration in media was determined using a color developing method by Parsons et al. (1984).

Phycoerythrin (PE) was extracted using a modification of the method of Beer and Eshel (1985). Approximately 100 mg FW of tissue was ground in a mortar with pestle in 0.1 M phosphate buffer (pH 6.5) and kept at 4 °C before being centrifuged at 19,000 g for 15 min. The supernatant was analyzed with a Spectronic Genesys 5 spectrophotometer (Spectronic Instruments, Rochester, NY, USA). PE content was calculated according to the equations used in Beer and Eshel (1985). For the analysis of tissue carbon and nitrogen contents, samples were dried at 60 °C before being ground. The powder was analyzed using a Perkin Elmer 2400 series II CHNS/O elemental analyzer (Waltham, MA, USA).

Time use efficiency (TUE) integrates the net assimilation of N and the net production of biomass during the available time for nitrogen uptake (i.e., the submerged time). The TUE (expressed as % tissue nitrogen and biomass change per hour) was calculated as followed:

$$\mathrm{TUE}\left(\mathrm{gN\,h^{-1}}\right)=\frac{(N_f \times B_f)-(N_i \times B_i)}{T_f-T_i}$$

where N_f and N_i are the tissue nitrogen contents and B_f and B_i are the biomass at time T_f (final) and T_i (initial), respectively. Initial samples were collected at 7:00AM and the final samples were collected at 7:30PM for shorter and 9:30PM for longer emersion experiments. Fresh weight based biomass was converted by multiplying 0.2 for all species which is the average ratio of DW to FW in all three species.

2.5. Statistical analysis

Two-way ANOVA ($\alpha=0.05$) was used to analyze tissue carbon and nitrogen, and PE contents. For these analyses, the immediately post-emersion samples (0.5 h following emersion) were excluded from analysis to determine the cumulative impact of emersion. When ANOVA indicated treatment effect of temperature or an interaction between time and emersion level, Tukey's HSD analysis ($\alpha=0.05$) was used as a post hoc test to determine pairwise comparison probabilities between treatment level means. Prior to analyses, homogeneity of variances was checked via F-test. In those data sets for which heteroscedasticity was found, data were ln-transformed. In one case the F-test of the transformed data indicated still significant differences in sample variances. We conducted ANOVA on the transformed data, relying on the relative robustness of ANOVA to this violation under balanced designs. The tissue C, N and phycoerythrin values at the end of emersion period were also compared with the pooled data from all other time periods by using a t-test. To determine how quickly the uptake rate returned to the control values following emersion, t-tests of control and emerged samples at the same time period were used for nitrate and phosphate uptakes. Two-way ANOVA ($\alpha=0.05$) was also used to analyze the standardized nitrate and phosphate uptake data. All values within experiment were normalized to the single highest value in that experiment. All statistical analyses were done using Statistica v5.1 (Statsoft, Tulsa, OK, USA) or SPSS 15.0 (SPSS Inc. Chicago, Illinois USA).

3. Results

3.1. Nitrate and phosphate Uptake

Short period of emersion did not affect the nitrate uptake of the two eulittoral species (Fig. 1a, b). However, following shorter emersion, nitrate uptake rate of sublittoral *P. yezoensis* was lower than the control rate immediately after re-submergence. The nitrate uptake rate recovered within 0.5 h (Fig. 1c). Long period of emersion affected the nitrate uptake of all three species (Fig. 1d-f). Following longer emersion, the nitrate uptake rates of the two eulittoral species were significantly lower than those of controls (*P. umbilicalis*: p=0.032, *P. leucosticta*: p=0.003) and recovered within a few h (Fig. 1d, e). The nitrate uptake rates of desiccated *P. umbilicalis* and *P. leucosticta* were approximately 26% and 50% of the controls, respectively, at 0.5 h after re-submergence. The sublittoral *P. yezoensis*, however, did not recover nitrate uptake as compared to the control even after 7.5 h of submergence. The nitrate uptake rates of desiccated *P. yezoensis* remained less than 50% of the controls during whole culture period.

Under shorter emersion condition, the phosphate uptake rates of desiccated tissues were not significantly different from the controls in all three species during whole culture periods (Fig. 2a-c; $p>0.05$). Even under longer period of emersion, phosphate uptake by the upper littoral species, *P. umbilicalis*, was not significantly different from the control (Fig. 2d; $p>0.05$). However, the phosphate uptake rates of *P. leucosticta* and *P. yezoensis* with longer emersion were significantly lower than those of controls at 0.5 h and 1.5 hour, respectively, after re-submergence (Fig. 2e, f; $p=0.004$ and $p=0.043$, respectively).

The relative uptake values were calculated by standardizing all uptake rates to the highest control value for each species and each treatment (Figs. 3 and 4). The controls of all species showed diurnal patterns in both nitrate and phosphate uptake. Peaks in uptake were observed from 5.5 to 7.5 h after sunrise (Figs. 3a, c and 4a, c). Under shorter emersion, all three species had similar diurnal patterns of phosphate uptake. However, only *P. umbilicalis* and *P. leucosticta* exhibited diurnal patterns with nitrate uptake while the sublittoral *P. yezoensis* lost its diurnal pattern. Under longer emersion, all three species lost their diurnal pattern in nitrate uptake while only *P. leucosticta* lost its diurnal pattern with phosphate uptake (Figs. 3 and 4).

At shorter emersion, the nitrate uptake was significantly affected by time in *P. umbilicalis* and *P. leucosticta* ($p<0.001$) and by time, emersion and the combination of these two factors in *P. yezoensis* (Table 1; $p=0.025$, $p=0.010$ and $p=0.002$, respectively). Under longer period of emersion, the nitrate uptake was affected by time and emersion in all three species ($p\leq0.024$) and the combination of these two factors influenced the nitrate uptake of *P. umbilicalis* and *P. leucosticta* (Table 1; $p=0.006$ and 0.010, respectively). Under shorter period of emersion, the phosphate uptake is affected by only time for P. *umbilicalis* and *P. leucosticta* ($p\leq0.001$), while *P. yezoensis* was affected by time, emersion and a combination of two (Table 1; $p=0.001$, 0.001 and 0.023, respectively).

3.2. Tissue carbon (C)

The immediately post-emersion data (0.5 h following emersion) were excluded for the statistical analysis to see the cumulative impact of emersion. At short period of emersion, the tissue carbon (C) contents were significantly affected by time in *P. umbilicalis* ($p=0.007$) and by time and emersion in *P. leucosticta* (Table 1; $p=0.004$ and 0.001, respectively). The tissue C contents at the end of exposure period were significantly lower than the pooled data from all other time periods after short period of emersion (Fig. 5a-c; *P. umbilicalis* and *P. leucosticta*: $p<0.001$, *P. yezoensis*: $p=0.003$). The tissue C content at the end of emersion was on average 91-93% of the pooled desiccated data. When the value at the end of emersion period was removed, the tissue C contents of tissues that had experienced

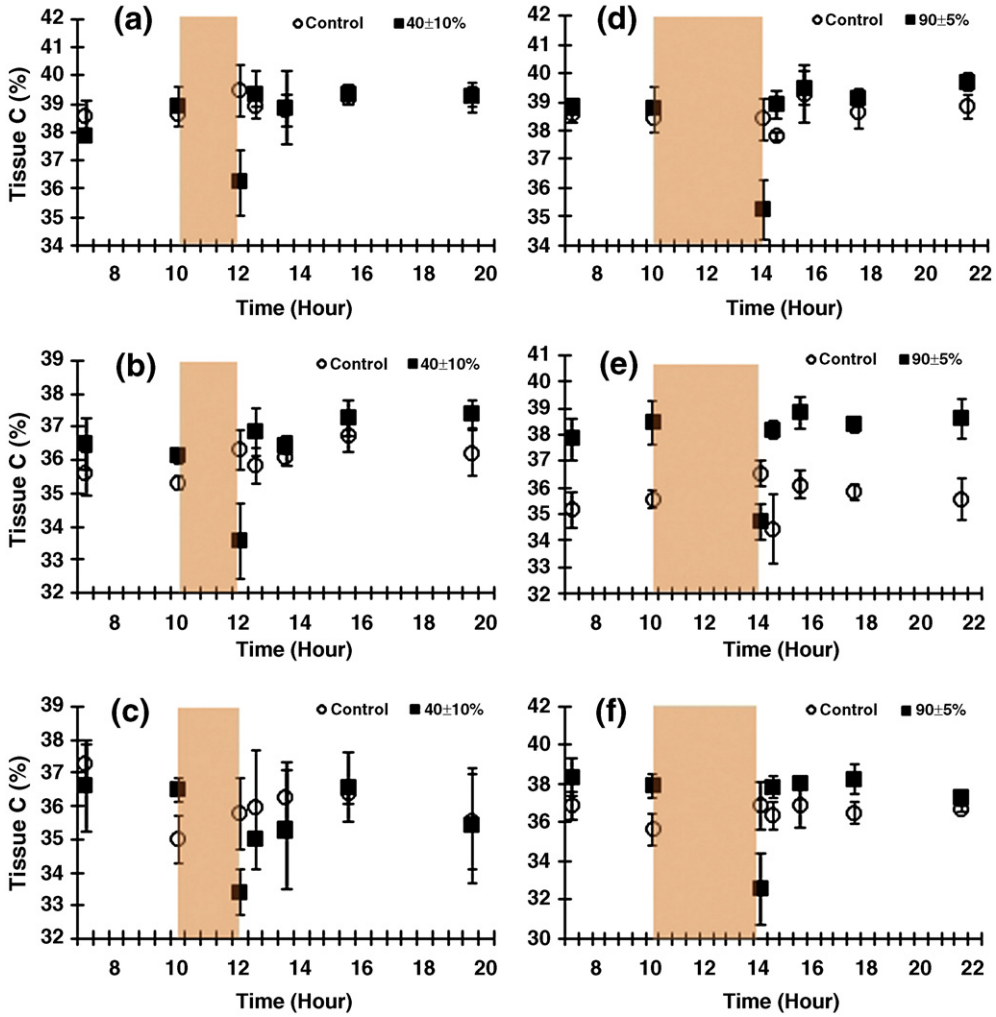

Fig. 5. Tissue carbon contents of *Porphyra* species from different tidal elevations and different emersion treatments (0, 40 ± 10% and 90 ± 5% water loss). (a) and (c): mid-upper littoral *P. umbilicalis* (shorter and longer emersion, respectively), (b) and (d): lower littoral *P. leucosticta* (shorter and longer emersion, respectively), (c) and (f): sublittoral *P. yezoensis* (shorter and longer emersion, respectively). Shaded areas represent exposure time. Error bars represent standard deviation.

emersion were significantly higher than tissue C of control samples of *P. leucosticta* ($p<0.001$), but not the other species.

At longer period of emersion, the tissue C contents of *P. umbilicalis* and *P. leucosticta* were significantly affected by time ($p=0.005$ and 0.045, respectively) and emersion ($p\leq0.001$). In *P. yezoensis*, emersion was the only significant factor for tissue C (Table 1; $p<0.001$). During longer period of emersion, the tissue C contents also dropped, but returned to control values within 0.5 h after re-submergence in all three species (Fig. 5d-f). The C contents at the end of emersion period were on average only 86-91% of the pooled desiccated value. When the value at the end of emersion period was removed, the tissue C contents of emersion experienced tissues were significantly higher than those of control in all three species (*P. umbilicalis*: $p=0.001$, *P. leucosticta* and *P. yezoensis*: $p<0.001$). On average, the absolute tissue C values were 0.5-3.0% DW^{-1} higher in desiccated tissues than the controls (Fig. 5d-f).

3.3. Tissue nitrogen(N)

Like the analyses for tissue C, the immediately post-emersion data (0.5 h following emersion) were excluded for the statistical analysis to see the cumulative impact of emersion stress. At short period of emersion, the tissue N contents were significantly affected by emersion (*P. umbilicalis* ($p=0.001$), *P. leucosticta* ($p=0.021$), *P. yezoensis* ($p=0.032$); Table 1). During short period of emersion, the tissue N contents of the eulittoral *P. umbilicalis* and *P. leucosticta* dropped and recovered within 0.5 h after re-submergence while the sublittoral *P. yezoensis* did not show any significant change (Fig. 6a-c). The values at the end of emersion period were on average 86-96% of the pooled desiccated value of other sampling periods in two eulittoral species. When these values were removed, the tissue N contents of short period of emersion experienced tissues were significantly higher than those of control in *P leucosticta* and *P. yezoensis* ($p<0.001$ and $p=0.027$, respectively).

At longer period of emersion, the tissue N contents were significantly affected by time and emersion in *P. umbilicalis* ($p<0.001$), emersion only in *P. leucosticta* and *P. yezoensis* (Table 1; $p<0.001$). Longer emersion of all three species caused a significant drop in tissue N contents, and returned to control levels within 0.5 h after re-submergence (Fig. 6d-f). The values at the end of emersion period were on average 82-90% of the pooled value of other sampling periods. When the value at the end of emersion period was removed, tissue N contents at longer period of emersion were significantly higher than those of control in all three species (all three species: $p<0.001$). On average, the tissue N values were 0.45-1.27% DW^{-1} higher in desiccated tissues than the controls.

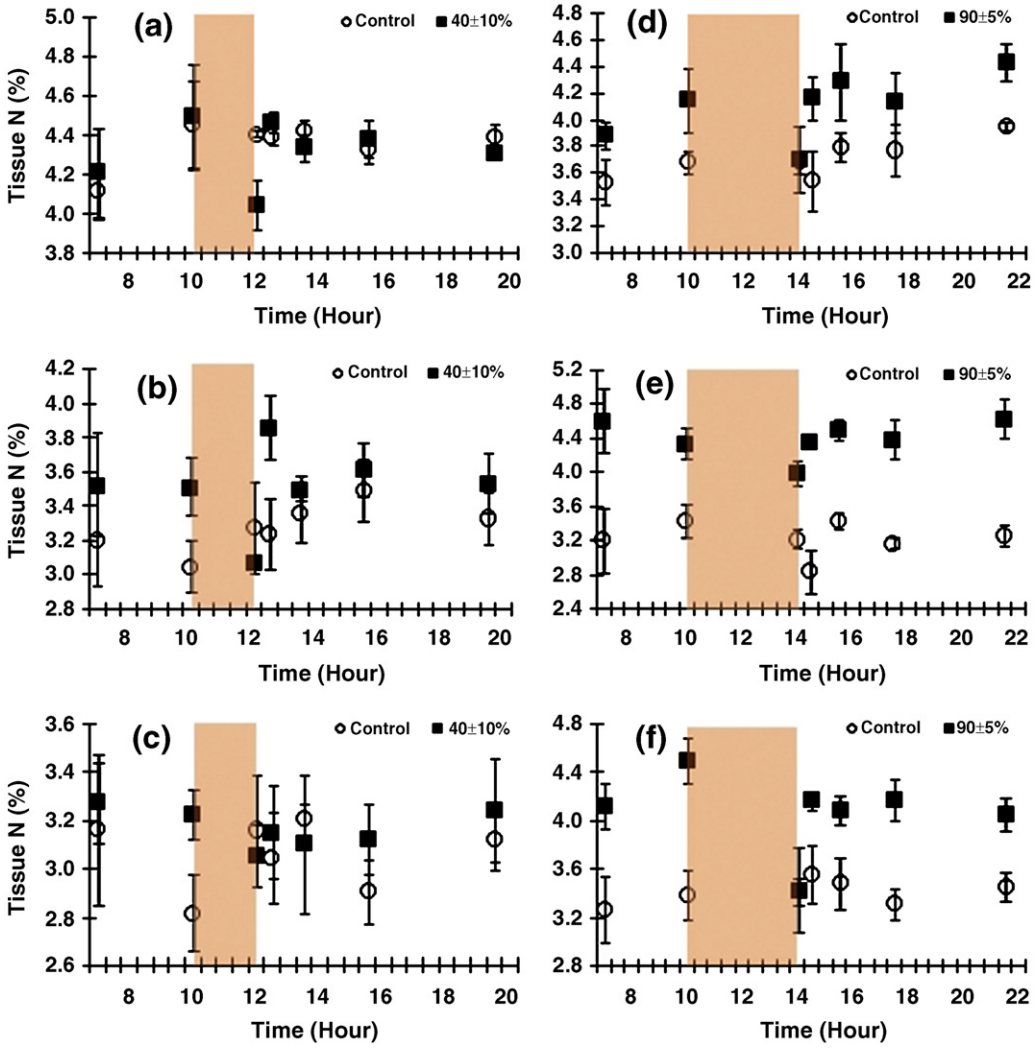

Fig. 6. Tissue nitrogen contents of *Porphyra* species from different tidal elevations and different emersion treatments (0, 40 ± 10% and 90 ± 5% water loss). (a) and (c): mid-upper littoral *P. umbilicalis* (shorter and longer emersion, respectively), (b) and (d): lower littoral *P. leucosticta* (shorter and longer emersion, respectively), (c) and (f): sublittoral *P. yezoensis* (shorter and longer emersion, respectively). Shaded areas represent exposure time. Error bars represent standard deviation.

3.4. Phycoerythrin

Short period of emersion did not affect phycoerythrin (PE) levels in all three species when the pooled control values were compared with each individual desiccated value, nor was time a significant factor (Table 1; Fig. 7a-c; $p>0.05$). Under longer period of emersion, however, the PE contents of *P. umbilicalis* were significantly affected by time ($p=0.003$), emersion ($p<0.001$) and combination of these two factors (Table 1; $p=0.015$). The phycoerythrin content of *P. umbilicalis* decreased during emersion and recovered within 1.5 h after re-submergence (Fig. 7d). When the value at the end of emersion period was removed, the pooled phycoerythrin value of longer emersion experienced *P. umbilicalis* was significantly higher than the pooled control (Table 1; $p<0.001$). On average, the control value was approximately 62% of the values of emersion experienced tissues (Fig. 7d).

3.5. Time Use Efficiency (TUE)

Neither short period of emersion nor species affected TUE (Fig. 8a; Table 2; $p>0.05$). However, at longer period of emersion, the TUE was affected by species ($p=0.002$) and emersion ($p=0.004$) and combination of these two factors (Fig. 8b; Table 2; $p=0.001$). The post-hoc test indicates that the TUE at 90% WL in sublittoral *P. yezoensis* was significantly lower than that of control ($P=0.001$) while emersion did not affect the TUE of other two eulittoral species (Fig. 8b; $p>0.05$). When *P. umbilicalis* was removed from the 90% analysis (since this species results differed qualitatively from the other two species), emersion was the significant factor for the TUE ($p=0.001$).

4. Discussion

In some eulittoral species, interestingly, emersion enhances rates of nitrate uptake upon re-hydration. Thomas et al. (1987b) studied five species with different morphologies and found that eulittoral *Pelvetiopsis limitata* and *Fucus distichus* could more than double nitrogen uptake upon re-submergence following up to 30% water loss. However, in the present study, uptake in three *Porphyra* species with the same basic morphologies from different elevations was not stimulated by emersion. Instead, we found a difference among the species in terms of the time required before uptake rates were similar to control (i.e., continuously submerged) rates. The eulittoral *Porphyra* species recovered nitrate uptake within a few hours following longer emersion, while the sublittoral species did not recover their nitrate uptake during the following 7.5 h, which was 0.5 h before the following emersion. Longer period of emersion did not affect phosphate uptake by eulittoral *P. umbilicalis*, but negatively affected

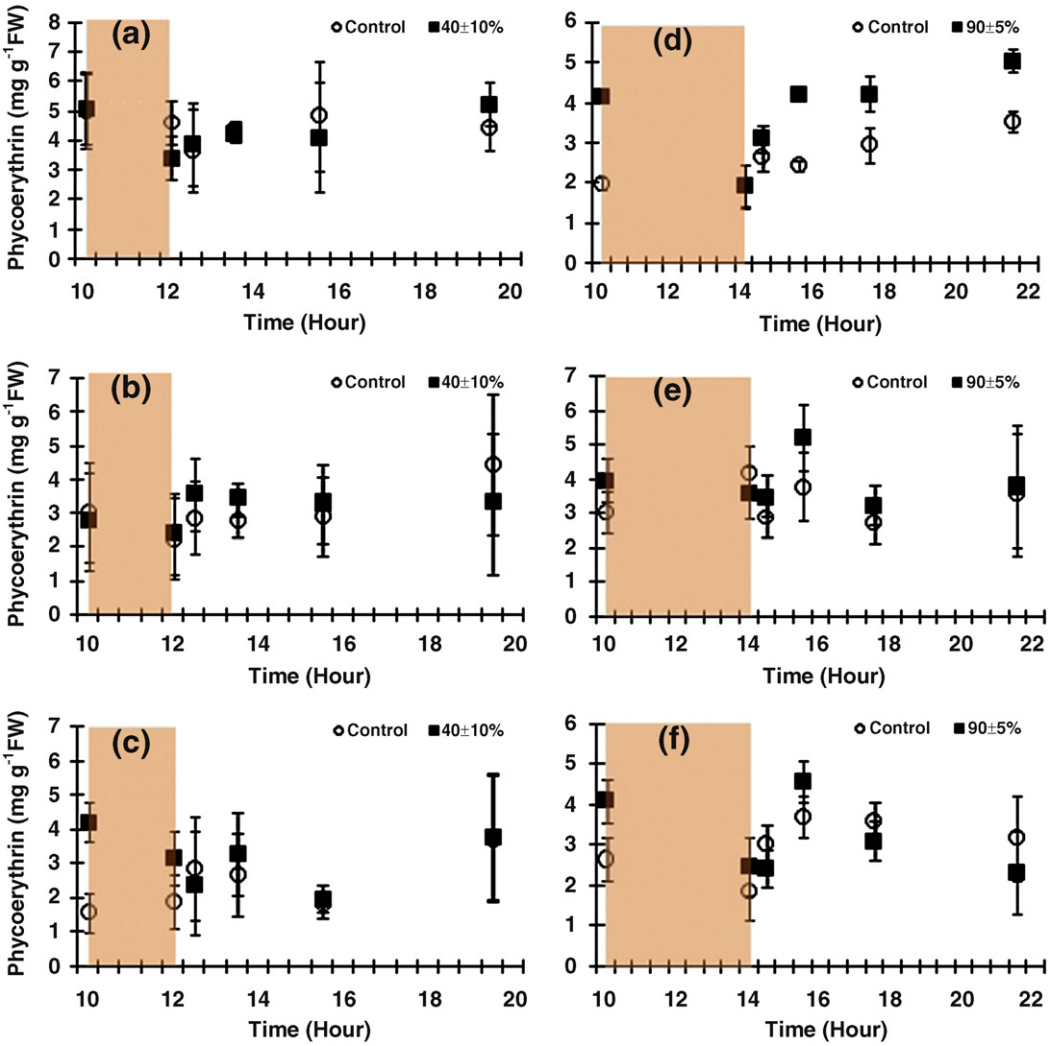

Fig. 7. Phycoerythrin contents of *Porphyra* species from different tidal elevations and different emersion treatments (0, 40±10% and 90±5% water loss). (a) and (c): mid-upper littoral *P. umbilicalis* (shorter and longer emersion, respectively), (b) and (d): lower littoral *P. leucosticta* (shorter and longer emersion, respectively), (c) and (f): sublittoral *P. yezoensis* (shorter and longer emersion, respectively). Shaded areas represent exposure time. Error bars represent standard deviation.

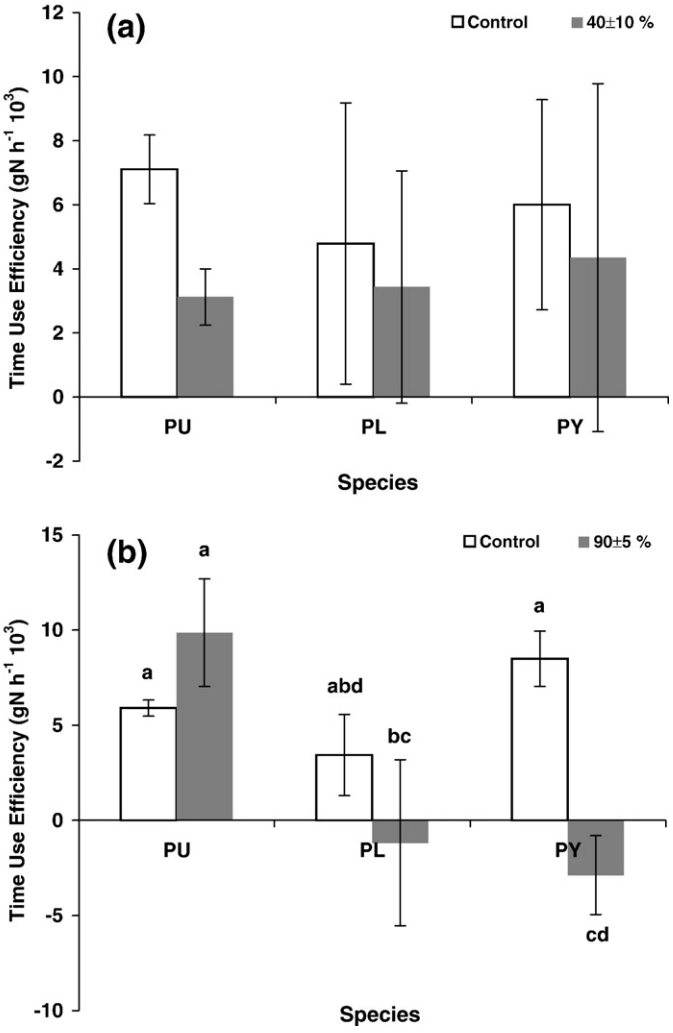

Fig. 8. Time use efficiency of *Porphyra* species from different tidal elevations and different emersion treatments (0, 40 ± 10% and 90 ± 5% water loss). (a): TUE from shorter emersion experiments. (b) TUE from longer emersion experiments. Error bars represent standard deviation.

the phosphate uptake by lower littoral and sublittoral species, *P. leucosticta* and *P. yezoensis,* respectively. Hurd and Dring (1991) also found that the rate and extent of recovery of phosphate uptake following longer emersion are closely related to the height on the shore. The upper littoral *Pelvetia canaliculata* was not affected by emersion. However, other species, *Fucus spiralis, Ascophyllum nodosum* and *F. serratus,* even lost phosphate from the tissues following longer emersion stress, and the rate of phosphate loss after resubmersion was closely related to their position on the shore (*F. spiralis*<*A. nodosum*<*F. serratus*). These results strongly suggest that eulittoral and sublittoral species have different nutrient uptake strategies for different nutrients, adaptations that have been driven by the different environmental challenges across the littoral gradient.

Plants living in environments characterized by low resource availability (e.g. limited nutrients, light, etc.) may have resource conservation traits such as long lifespans, high concentrations of defense compounds, thinner leaves, or desiccation tolerance (e.g. via dehydrin proteins) to maximize their resource use efficiency (Chapin, 1980; Vitousek, 1982; Ndima et al., 2001). The resource use efficiency (RUE) is generally defined as the products (e.g. biomass increase, product of N, photosynthesis, etc.) per resource inputs (e.g. light, nutrient, water, etc.). A slow growing species may have lower resource requirements while fast growing species may require more resources. Therefore, at least in theory, RUE may not be phenotypically different between slow growing species (fewer products) and fast growing species (more products). However, this concept may determine the distribution of organism. For example, Funk and Vitousek (2007) tested RUE with 19 native and 19 invasive terrestrial plants, and found that invasive species had significantly higher RUE. Since time is an important resource (Loreau, 1989, 1992), the vertical distribution of eulittoral seaweeds may also be partially determined by time use efficiency. In this study, the TUE was defined as the integrated net production of biomass and tissue N per unit of available time for N uptake. When *Porphyra* experienced emersion simulating the upper littoral zone, the growth and tissue N-based TUE of upper littoral *P. umbilicalis* was significantly higher than those of the other two species from lower littoral and sublittoral zones (Fig. 8b). Under control conditions, and those of shorter emersion that simulate sublittoral and lower littoral zones, respectively, the TUEs of all three species were not significantly different. Fast-growing *Porphyra* species in the eulittoral zone have evolved the ability to rapidly take up nutrients during when they are submerged and, especially, to rapidly recover uptake rates upon resubmersion after exposure to air. These results support the concept that the upper limit of a species is determined by tolerance of environmental stresses, such as the unavailability of nutrients or desiccation, while the lower limit is controlled by biological factors such as predation and competition (Lobban and Harrison, 1994).

When continuously submerged, all three *Porphyra* species showed diurnal patterns in uptake rates of nitrate and phosphate, with uptake peaks approximately 5.5-7.5 h after sunrise and then the uptake rates decreased (Figs. 3a and 4a). Three brown algal species, *Fucus vesiculosus, F. serratus* and *Laminaria digitata*, also showed similar N and P uptake patterns, and had peaks in the middle of day light period. Activity of an enzyme involved in nitrate uptake (nitrate reductase) also showed a clear diurnal pattern with a maximum in the middle of day in algae and terrestrial plants (Goodwin and Mercer, 1983; Turpin, 1991; Gao et al., 1992a; Gevaert et al., 2007). Our recent findings with upper littoral species *P. umbilicalis* and *P. linearis* (unpublished) also showed similar diurnal patterns in nitrate reductase activity. However, when tissues were stressed by longer emersion, all three species lost their diurnal patterns in terms of nitrate uptake. Interestingly, the nitrate uptake rate remained high in dark period in all three species (Figs. 3b and 4b). These results suggest that emersion definitely influenced the nitrate uptake pattern of *Porphyra*.

Inorganic C in seawater has not been considered as a limiting factor for macroalgal photosynthesis (Beer and Koch, 1996). When tissues are submerged, both HCO_3^- and CO_2 are available, and many algal species, including *Porphyra* can use both forms, but mostly HCO_3^-, of carbon sources for photosynthesis since approximately 97% of dissolved inorganic carbon is present as HCO_3^- (Mercado et al., 1997; Larsson and Axelsson, 1999). During emersion, however, CO_2 is the only available C source for the photosynthesis of eulittoral seaweeds. The initial increase of photosynthesis during emersion of some eulittoral species probably occurs because of a reduction in the aqueous diffusion barrier for CO_2 (Quadir et al., 1979; Johnston and Raven, 1986; Lipkin

Table 2
Results of analysis of variance examining the effects of emersion and species on Time Use Efficiency (TUE) of *Porphyra* species

Water loss	Factor	F	p-value
40%	species	0.34	0.716
	emersion (control vs. 40%)	2.64	0.130
	species X emersion	0.21	0.815
90%	species	10.79	**0.002**
	emersion (control vs. 90%)	12.54	**0.004**
	species X emersion	13.06	**0.001**

Significant differences are shown in bold with p values.

et al., 1993; Davison and Pearson, 1996; Ji and Tanaka, 2002). The atmospheric CO_2 will also dissolve into the aqueous layer on emerged thalli and be available as HCO_3^-. Therefore, the initial increase of photosynthesis during emersion may also be due to the reduction in the aqueous layer across which HCO_3^- must diffuse, and for those species that can only use HCO_3^-, the drop in photosynthesis rate may come as this water evaporates. Overall, however, atmospheric CO_2 may limit photosynthesis by eulittoral species during emersion. Gao et al. (1999) reported that photosynthetic uptake of CO_2 by the eulittoral seaweeds *Ulva linza*, *Ishige okamurae* and *Gloiopeltis furcata* increased 25-40% when the ambient CO_2 level was doubled.

CO_2 loss may also increase during emersion. Zou and Gao (2002) recently reported that the CO_2 compensation point of *Porphyra haitanensis,* cultured at 20-30 °C temperature range, increased dramatically when the water loss was more than 50%. There was no significant effect of temperature. These authors interpret the higher compensation point as a result of increased carbon loss. *Porphyra yezoensis* increased dramatically in the CO_2 compensation point as the period of emersion increased (Gao et al., 1992b). We found that C contents in tissue in the three *Porphyra* species dropped during emersion and recharged quickly after re-submergence. This suggests that the net balance of carbon loss and photosynthetic C gain in *Porphyra* is negative during emersion.

When they experience desiccation stress associated with emersion, plants form protective enzymes such as catalase, superoxide dismutase (SOD), ascorbate peroxidase (AP), glutathione reductase (GR), Dehydrins and LEA proteins that minimize desiccation damage (Li et al., 1998; Vicre et al., 2004; Wolfe-Simon et al., 2005; Ross and Van Alstyne, 2007). In the higher plant *Xerophyta viscose*, activities of AP, GR and SOD increased during drying (Sherwin and Farrant, 1998), and these enzymes reacted similarly in a brown alga *Fucus* (Collén and Davison, 1999). Dehydrin proteins are also expressed in both higher plants and algae during dehydration (Li et al., 1998; Ndima et al., 2001). The role of these proteins in desiccation tolerance is not clear yet. In this study, we found that desiccated *Porphyra* contained more N than tissues that were remained submerged. This increase in N may reflect elevated levels of similar desiccation tolerance proteins used as a protective mechanism by *Porphyra* when tissues experience periodic emersion.

The phycoerythrin contents of *P. umbilicalis* under longer emersion condition dropped during emersion, and recharged very quickly after re-submergence. The tissue N contents of *Porphyra* (% of biomass) also dropped during emersion, and recovered quickly after re-submergence. This result is consistent with the role of these pigments as an N storage reservoir (Bird et al., 1982; Harrison and Hurd, 2001; Kim et al., 2007). Carmona et al. (2006) and Kim et al. (2007) also found PE increase with tissue N content increase in different *Porphyra* species including *P. umbilicalis*. Phycoerythrin as a ratio of total protein decreased when tissue N content fell below 1.8% (Bird et al., 1982). These pigments may be preferentially utilized to support continued growth at incipient N limitation (Kim et al., 2007).

Nitrogen loss during emersion indicates that emersion causes a loss of N, or that stored N becomes diluted by a biomass increase. For *Porphyra* to channel stored N into growth, and increase in biomass to dilute the tissue N contents to the observed degree, this seaweed would have to grow at a rate of 50% - 160% d^{-1}. However, there was no evidence that the dry weight of *Porphyra* increased during emersion. Therefore, we conclude that *Porphyra* lost N during emersion, though the route of N loss is not clear. Nitrogen from organic compounds in the tissue could have been converted to ammonia, which then outgassed from the thallus (under investigation). If so, some of the N taken up by eulittoral seaweeds during submersion is subsequently released into the atmosphere during emersion. The nitrogen loss during emersion represents an induced N release which may constitute a novel biogeochemical pathway linking marine, terrestrial, and atmospheric N reservoirs.

In summary, we found that eulittoral and sublittoral species of *Porphyra* have different strategies for nutrient uptake and TUE. The nitrate and phosphate uptake function was recovered quickly after emersion of upper littoral *P. umbilicalis*, while lower- or sub-littoral species could not recover their functions. This suggests that the rate of recovery of physiological functions such as nutrient uptake following emersion is important in determining the vertical distribution of seaweeds. The TUE also showed similar patterns with the nutrient uptake; high TUE in upper littoral *P. umbilicalis* and low TUE in lower- or sub-littoral species. The carbon loss during emersion indicates that atmospheric CO_2 may limit algal photosynthesis. The net balance of carbon loss and photosynthetic C gain in *Porphyra* is negative during emersion. In addition, nitrogen is lost during emersion. However, tissue N content is higher in *Porphyra* acclimated to emersion than thalli that are continuously submerged. This may reflect elevated levels of desiccation tolerance proteins (dehydrins) used as a protective mechanism by *Porphyra* when tissues experience periodic emersion. There is a need to study more eulittoral species to determine if N loss during exposure is a general characteristic of eulittoral seaweeds. In addition, N storage in tissue in forms such as free amino acids and proteins should be examined during emersion to determine what categories of organic compounds are also altered during emersion. Photosynthetic ability should also be measured during emersion to determine whether carbon supply limits photosynthesis during emersion.

Acknowledgements

We wish to thank P. Boardman, G. Grenier and D. Arbige for assistance with tide simulating apparatus management in Rankin Lab, University of Connecticut at Avery Point. We also thank R. Galdych and Y. Abdu who assisted in the Seaweed Marine Biotechnology Laboratory, University of Connecticut at Stamford. Finally, we thank to the anonymous reviewers for invaluable comments. This study was supported by grants to C. Yarish from the Perkin Elmer Analytical Division of E,G & G, Wellesley, MA, USA, Connecticut Sea Grant College Program, a grant to C. Yarish and G.P. Kraemer from National Oceanic and Atmospheric Administration's National Marine Aquaculture Initiative (DOC/U.S.A.), and awards to J.K. Kim from the Department of Ecology and Evolutionary Biology, University of Connecticut (Ronald Bamford Award) and from the Connecticut Museum of Natural History (Henry N. Andrew and Francis Rice Trainor Awards). **[SS]**

References

Baker, S.M., 1909. On the causes of zoning of brown seaweeds on the shore. New Phytol. 8, 196–202.

Baker, S.M., 1910. On the causes of zoning of brown seaweeds on the shore II. The effect of periodic exposure on the expulsion of gametes and on the germination of the oospore. New Phytol. 9, 54–67.

Balch, W.M., Drapeau, D.T., Bowler, B.C., Booth, E.S., Windecker, L.A., Ashe, A., 2008. Space–time variability of carbon standing stocks and fixation rates in the Gulf of Maine, along the GNATS transect between Portland, ME, USA, and Yarmouth, Nova Scotia, Canada. J. Plankton Res. 30, 119–139.

Beach, K.S., Smith, C.M., 1997. Ecophysiology of a tropical rhodophyte. 3. Recovery from emersion stresses in *Ahnfeltiopsis concinna* (J Ag) Silva et DeCew. J. Exp. Mar. Biol. Ecol. 211, 151–167.

Beer, S., Eshel, A., 1985. Determining phycoerythrin and phycocyanin concentrations in aqueous crude extracts of red algae. J. Mar. Freshwat. Res. 36, 785–792.

Beer, S., Koch, E., 1996. Photosynthesis of marine macroalgae and seagrasses in globally changing CO_2 environments. Mar. Ecol. Prog. Ser. 141, 199–204.

Bidwell, R.G.S., McLachlan, J., 1985. Carbon nutrition of seaweeds: photosynthesis, photorespiration, and respiration. J. Exp. Mar. Biol. Ecol. 86, 15–46.

Bird, K.T., Habig, C., DeBusk, T., 1982. Nitrogen allocation and storage patterns in *Gracilaria tikvahiae* (Rhodophyta). J. Phycol. 18, 344–348.

Blouin, N., Xiugeng, F., Pengb, J., Yarish, C., Brawley, S.H., 2007. Seeding nets with neutral spores of the red alga *Porphyra umbilicalis* (L.) Kützing for use in integrated multi-trophic aquaculture (IMTA). Aquaculture 270, 77–91.

Bray, T.L., Neefus, C.D., Mathieson, A.C., 2006. Morphological and molecular variability of *Porphyra purpurea* (Roth) C. Agardh (Rhodophyta, Bangiales) from the Northwest Atlantic. Nova Hedwig. 82, 1–22.

Capriulo, G.M., Smith, G., Troy, R., Wikfors, G., Pellet, J., Yarish, C., 2002. The planktonic food web structure of a temperate zone estuary, and its alteration due to eutrophication. Hydrobiologia 475/476, 263–333.
Carmona, R., Kraemer, G.P., Yarish, C., 2006. Exploring Northeast American and Asian species of *Porphyra* for use in an integrated finfish-algal aquaculture system. Aquaculture 252, 54–65.
Chapin, F.S., 1980. The mineral nutrition of wild plants. Annu. Rev. Ecol. Syst. 11, 233–260.
Chapman, A.R.O., Craigie, J.S., 1977. Seasonal growth in *Laminaria longicruris:* Relations with dissolved inorganic nutrients and internal reserves of nitrogen. Mar. Biol. 40, 197–205.
Chopin, T., Buschmann, A.H., Halling, C., Troell, M., Kautsky, N., Neori, A., Kraemer, G.P., Zertuche-Gonzalez, J.A., Yarish, C., Neefus, C., 2001. Integrating seaweeds into marine aquaculture systems: A key toward sustainability. J. Phycol. 37, 975–986.
Chopin, T., Yarish, C., Wilkes, R., Belyea, E., Lu, S., Mathieson, A., 1999. Developing *Porphyra*/salmon integrated aquaculture for bioremediation and diversification of the aquaculture industry. J. Appl. Phycol. 11, 463–472.
Collén, J., Davison, I.R., 1999. Reactive oxygen metabolism in intertidal *Fucus* spp. (Phaeophyceae). J. Phycol. 35, 62–69.
Davison, I.R., Pearson, G.A., 1996. Stress tolerance in intertidal seaweeds. J. Phycol. 32, 197–211.
Dring, M.J., Brown, F.A., 1982. Photosynthesis of intertidal brown algae during and after periods of emersion: a renewed search for physiological causes of zonation. Mar. Ecol. Prog. Ser. 8, 301–308.
Edwards, P., 1977. An investigation of the vertical distribution of selected benthic marine algae with a tide-simulating apparatus. J. Phycol. 13, 62–68.
Fujita, R.M., 1985. The role of nitrogen status in regulating transient ammonium uptake and nitrogen storage by macroalgae. J. Exp. Mar. Biol. Ecol. 92, 283–301.
Funk, J., Vitousek, P.M., 2007. Resource-use efficiency and plant invasion in low-resource systems. Nature 446, 1079–1081.
Gao, Y., Smith, G.J., Alberte, R.S., 1992a. Light regulation of nitrate reductase in *Ulya fenestrata* (Chlorophyceae). Nature 112, 691–696.
Gao, K., Aruga, Y., Asada, K., Ishihara, T., Akano, T., Kiyohara, M., 1992b. Photorespiration and CO2 fixation in the red alga *Porphyra yezoensis* Ueda. Jpn. J. Phycol. 40, 373–377.
Gao, K., Ji, Y., Aruga, Y., 1999. Relationship of CO_2 concentrations to photosynthesis of intertidal macroalgae during emersion. Hydrobiologia 398/399, 355–359.
Gevaert, F., Barr, N.G., Rees, T.A.V., 2007. Diurnal cycle and kinetics of ammonium assimilation in the green alga *Ulva pertusa*. Mar. Biol. 151, 1517–1524.
Goodwin, T.W., Mercer, E.I., 1983. Introduction to Plant Biochemistry. Pergamon, New York.
Harrison, P.J., Hurd, C.L., 2001. Nutrient physiology of seaweeds: Application of concepts to aquaculture. Cah. Biol. Mar. 42, 71–82.
Hurd, C.L., Dring, M.J., 1990. Phosphate uptake by intertidal algae in relation to zonation and season. Mar. Biol. 107, 281–289.
Hurd, C.L., Dring, M.J., 1991. Desiccation and phosphate uptake by intertidal fucoid algae in relation to zonation and season. Br. Phycol. J. 26, 327–333.
Ji, Y., Tanaka, J., 2002. Effect of desiccation on the photosynthesis of seaweeds from the intertidal zone in Honshu, Japan. Phycol. Res. 50, 145–153.
Johnston, A.M., Raven, J.A., 1986. The analysis of photosynthesis in air and water of *Ascophyllum nodosum* (L.) Le Jol. Oecologia 69, 288–295.
Kim, J.K., Kraemer, G.P., Neefus, C.D., Chung, I.K., Yarish, C., 2007. The effects of temperature and ammonium on growth, pigment production and nitrogen uptake in four species of *Porphyra* native to the coast of New England. J. Appl. Phycol. 19, 431–440.
Kim, J.K., Kraemer, G.P., Yarish, C., In Review. A comparison of growth and nitrate uptake by New England *Porphyra* species from different tidal elevations in relation to desiccation. Phycological Research.
Kim, J.K., Yarish, C., In Review. Development of a tide-simulating apparatus for macroalgal blades. Bot. Mar.
Kraemer, G.P., Carmona, R., Chopin, T., Neefus, C., Tang, X.R., Yarish, C., 2004. Evaluation of the bioremediatory potential of several species of the red alga *Porphyra* using short-term measurements of nitrogen uptake as a rapid bioassay. J. Appl. Phycol. 16, 489–497.
Larsson, C., Axelsson, L., 1999. Bicarbonate uptake and utilization in marine macroalgae. Eur. J. Phycol. 34, 79–86.
Lewis, J.R., 1964. The Ecology of Rocky Shores. English Universities Press, London.
Li, R., Brawley, S.H., Close, T.J., 1998. Proteins immunologically related to dehydrins in Fucoid algae. J. Phycol. 34, 642–650.
Lipkin, Y., Beer, S., Eshel, A., 1993. The ability of *Porphyra linearis* (Rhodophyta) tolerate prolonged periods of desiccation. Bot. Mar. 36, 517–523.
Lobban, C.S., Harrison, P.J., 1994. Seaweed Ecology and Physiology. Cambridge University Press, New York. 366 pp.
Loreau, M., 1989. Coexistence of temporally segrated competitors in a cycle environment. Theoretical Population Biology 36, 181–201.
Loreau, M., 1992. Time scale of resource dynamics and coexistence through time partitioning. Theoretical Population Biology 41, 401–412.
Lüning, K., Yarish, C., Kirkman, H., 1990. Seaweeds. Their Environment, Biogeography and Ecophysiology. Wiley, New York.
Mercado, J.M., Niell, F.X., Figueroa, F.L., 1997. Regulation of the mechanism for HCO_3^- use by the inorganic carbon concentration in *Porphyra leucosticta* Thur. in Le Jolis (Rhodophyta). Planta 210, 319–325.
Ndima, T.B., Farrant, J.M., Thomson, J.A., Mundree, S.G., 2001. Molecular characterization of XVT8, a stress-responsive gene from the resurrection plant *Xerophyta viscosa* Baker. Plant Growth Regul. 35, 137–145.
Neefus, C.D., Mathieson, A.C., Bray, T.L., Yarish, C., The occurrence distribution, morphology and ecology of three introduced Asiatic species of *Porphyra* (Bangiales, Rhodophyta) in the northwestern Atlantic. J. Phycol., In Review.
Neefus, C.D., Mathieson, A.C., Klein, A.S., Teasdale, B.W., Bray, T., Yarish, C., 2002. *Porphyra birdiae* sp. nov. (Bangiales, Rhodophyta): A new specie from the North West Atlantic. ALGAE (Kor. J. Phycol.) 17, 203–216.
Neori, A., Chopin, T., Troell, M., Buschmann, A.H., Kraemer, G.P., Halling, C., Shpigel, M., Yarish, C., 2004. Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modem mariculture. Aquaculture 231, 361–391.
Ott, F.D., 1965. Synthetic media and techniques for the xenic cultivation of marine algae and flagellate. Va. J. Sci. 16, 205–218.
Parsons, T.R., Maita, Y., Lalli, C.M., 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Oxford.
Pedersen, A., Kraemer, G., Yarish, C., 2004. The effects of temperature and nutrient concentrations on nitrate and phosphate uptake in different species of *Porphyra* from Long Island Sound (USA). J. Exp. Mar. Biol. Ecol. 312, 235–252.
Phillips, J.C., Hurd, C.L., 2003. Nitrogen ecophysiology of intertidal seaweeds from New Zealand: N uptake, storage and utilization in relation to shore postion and season. Mar. Ecol. Prog. Ser. 264, 113–122.
Phillips, J.C., Hurd, C.L., 2004. Kinetics of nitrate, ammonium, and urea uptake by four intertidal seaweeds from Nea Zealand. J. Phycol. 40, 534–545.
Proctor, M.C.F., Smirnoff, N., 2000. Rapid recovery of photosystems on rewetting desiccation-tolerant mosses: chlorophyll fluorescence and inhibitor experiments. J. Exp. Bot. 51, 1695–1704.
Quadir, A., Harrison, P.J., DeWreede, R.E., 1979. The effects of emergence and submergence on the photosynthesis and respiration of marine macrophytes. Phycologia 18, 83–88.
Ross, C., Van Alstyne, K.L., 2007. Intraspecific variation in stress-induced hydrogen peroxide scavenging by the ulvoid macroalga *Ulva lactuca*. J. Phycol. 43, 466–474.
Ryther, J.H., Corwin, N., DeBusk, T.A., Williams, L.D., 1981. Nitrogen uptake and storage by the red alga *Gracilaria tikvahiae* (McLachlan, 1979). Aquaculture 26, 107–115.
Sampath-Wiley, P., Neefus, C.D., 2007. An improved method for estimating R-phycoerythrin and R-phycocyanin contents from crude aqueous extracts of *Porphyra* (Bangiales, Rhodophyta). J. Appl. Phycol. 19, 123–129.
Sherwin, H.W., Farrant, J.M., 1998. Protection mechanisms against excess light in the resurrection plants *Craterostigma wilmsii* and *Xerophyta viscosa*. Plant Growth Regul. 24, 203–210.
Skene, K.R., 2004. Key differences in photosynthetic characteristics of nine species of intertidal macroalgae are related to their position on the shore. Can. J. Bot.-Revue Canadienne De Botanique 82, 177–184.
Thomas, T.E., Harrison, P.J., Taylor, E.B., 1985. Nitrogen uptake and growth of the germlings and mature thallus of *Fucus distichus*. Mar. Biol. 84, 267–274.
Thomas, T.E., Harrison, P.J., Turpin, D.H., 1987a. Adaptations of *Gracilaria pacifica* (Rhodophyta) to nitrogen procurement at different intertidal locations. Mar. Biol. 93, 569–580.
Thomas, T.E., Turpin, D.H., Harrison, P.J., 1987b. Desiccation enhanced nitrogen uptake rates in intertidal seaweeds. Mar. Biol. 94, 293–298.
Turpin, D.H., 1991. Effects of inorganic N availability on algal photosynthesis and carbon metabolism. J. Phycol. 27, 14–20.
Vicre, M., Farrant, J.M., Driouch, A., 2004. Insights into the cellular mechanisms of desiccation tolerance among angiosperm resurrection plant species. Plant Cell Environ. 27, 1329–1340.
Villalard-Bohnsack, M., 1995. Illustrated Key to the Seaweeds of New England. The Rhode Island Natural Survey, Kingston.
Vitousek, P., 1982. Nutrient cycling and nutrient use efficiency. Am. Nat. 119, 553–572.
West, A.L., Mathieson, A.C., Klein, A.S., Neefus, C.D., Bray, T.L., 2005. Molecular ecological studies of New England species of *Porphyra* (Rhodophyta, Bangiales). Nova Hedwig. 80, 1–24.
Wolfe-Simon, F., Grzebyk, D., Schofield, O., Falkowski, P.G., 2005. The role and evolution of superoxide dismutases in algae. J. Phycol. 41, 453–465.
Yarish, C., Wilkes, R., Chopin, T., Fei, X.G., Mathieson, A.C., Klein, A.S., Neefus, C.D., Mitman, G.G., Levine, I., 1998. Domestication of indigenous *Porphyra* (nori) species for commercial cultivation in Northeast America. World Aquaculture 29, 26-29, 55.
Zou, D., Gao, K., 2002. Effects of desiccation and CO_2 concentrations on emersed photosynthesis in *Porphyra hatanensis* (Bangiales, Rhodophyta), a species farmed in China. Eur. J. Phycol. 37, 587–592.