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Effects of temperature and ammonium on growth, pigment production and nitrogen uptake by four species of *Porphyra* (Bangiales, Rhodophyta) native to the New England coast

Jang K. Kim · George P. Kraemer · Christopher D. Neefus · Ik Kyo Chung · Charles Yarish

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Abstract *Porphyra* is one of the world's most valued maricultured seaweeds and has been cultivated for several hundred years in Asia. The objective of this study was to produce critical information as a guide for the selection of an appropriate *Porphyra* species from coastal New England for the development of a land-based aquaculture system. Four Northwest Atlantic *Porphyra* species: *P. leucosticta, P. amplissima, P. linearis* and *P. umbilicalis,* were cultivated for 1 and 2 weeks at saturated light intensities (100–150 μmol photons $m^{-2}s^{-1}$) and six combinations of ammonium (25 and 250 μmoles L^{-1}) and temperature (10, 15 and 20°C). Specific growth rate (SGR) increased with decreasing temperature in *P. leucosticta, P. linearis* and *P. umbilicalis* and increased with increasing temperature in *P. amplissima.* The SGR of all species was greater at the higher ammonium concentration. *Porphyra linearis* had the highest SGR, increasing in biomass by approximately 16% day^{-1}. Phycoerythrin (PE) content was higher at 10°C and 250 μmoles L^{-1} in all species except *P. amplissima*. The PE content, measured as fresh weight (FW), of *P. linearis* (29 mg g^{-1} FW^{-1}) and *P. umbilicalis* (26 mg g^{-1} FW^{-1}) was significantly higher than the other two species. Tissue nitrogen content of all species measured in dry weight was on average 1.45% higher at 250 μmoles L^{-1} than at 25 μmoles L^{-1} ammonium concentration. *Porphyra umbilicalis* had the highest tissue nitrogen contents (6.76%) at 10°C and 250 μmoles L^{-1} ammonium. Based on these results, *P. linearis* and *P. umbilicalis* should be considered as potential candidates for bioremediation with finfish and shellfish mariculture.

Key words *Porphyra* · nutrient uptake · temperature · ammonium · bioremediation · mariculture

J. K. Kim (✉)
Department of Ecology and Evolutionary Biology,
University of Connecticut,
1080 Shennecossett Road,
Groton, CT 06340, USA
e-mail: jang.kim@uconn.edu

G. P. Kraemer
Department of Environmental Science, SUNY Purchase College,
735 Anderson Hill Road,
Purchase, NY 10577, USA

C. D. Neefus
Department of Plant Biology, University of New Hampshire,
8 College Road, G32 Spaulding,
Durham, NH 03824, USA

I. K. Chung
Division of Earth Environmental System,
Pusan National University,
Busan 609-735, South Korea

J. K. Kim · C. Yarish
Department of Ecology and Evolutionary Biology,
University of Connecticut,
1 University Place,
Stamford, CT 06901, USA

Introduction

An emerging problem of coastal fish mariculture is the loading of inorganic nutrients into local waters (Beveridge 1987), which contributes to blooms of phytoplankton and weedy macroalgae (Cuomo et al. 1993). Reducing the net release of nutrients into the environment is, therefore, an important issue; bioremediation of fish mariculture effluent is an option that provides both ecological and economic incentives. Integrated aquaculture, in which seaweeds are grown downstream from the animals, is a process of

bioremediation. It can reduce nutrient loading in coastal waters because the nitrogen (N) and phosphorus (P) in the animal effluent enable rapid growth of the seaweeds. Obviously, the best seaweed to integrate into an animal aquaculture operation is the one characterized by rapid growth, high accumulation of N and P in tissue (ecological value), and high commercial price (economic value; Troell et al. 2003; Kraemer et al. 2004; Neori et al. 2004; Carmona et al. 2006; Pereira et al. 2006). Therefore, knowledge of ecological and physiological aspects of seaweed growth are a very important part of the development of an integrated aquaculture system.

Nitrogen has traditionally been considered the limiting nutrient in temperate oceans both for phytoplankton (Twomey and Thompson 2001) and seaweed communities (Lobban and Harrison 1994; Harrison and Hurd 2001). Generally, nitrogen addition increases photosynthetic activity and growth of seaweeds. Nitrogen is an indispensable element incorporated in to many organic macromolecules (proteins, nucleic acids and pigments). Synthesis of chlorophyll *a* (Chl *a*) and phycoerythrin (PE) also requires N (Lobban and Harrison 1994). On average, proteins contain about 15% N, nucleic acids about 13% N and PE is comprised of up to 20% N (e.g., Harrison and Hurd 2001). Depending upon the polysaccharide content, algae may contain between 3–10% N by dry weight (Ryther et al. 1981; Harrison and Hurd 2001; Carmona et al. 2006; Pereira et al. 2006).

In general, algae can use a wide variety of nitrogenous compounds to fulfill their N requirements: ammonia, nitrate, urea, amino acids and nucleosides may be taken up from the growth medium (Lobban and Harrison 1994), with NO_3^- and NH_4^+ being the primary sources in most circumstances. Some seaweeds, such as *Gelidium nudifrons* (Bird 1976) and *Laminaria groenlandica* (Harrison et al. 1986), take up NO_3^- and NH_4^+ simultaneously and at the same rate. Thus, these species have the potential to assimilate more N per unit time if provided both forms of N than if limited to only one N form for uptake. However, in many species, NH_4^+ inhibits the uptake of NO_3^- by up to 50% (Conway 1977; DeBoer 1981). For instance, NH_4^+ concentrations in the range 0.5–10.0 μmoles L^{-1} suppress NO_3^- uptake in phytoplankton cells by 50% (Conway 1977). Thalli of *Gracilaria foliifera* and *Neoagardhiella baileyi* grown with NH_4^+-N showed a greater biomass yield than did NO_3^--cultured thalli and appeared to be capable of storing more N (DeBoer et al. 1978; Bird et al. 1982). Exposure of *Gracilaria tikvahiae* to NH_4^+-enriched seawater also led to greater levels of phycoerythrin than did exposure to the same level of NO_3^- (Bird et al. 1982). *Porphyra yezoensis* also prefers NH_4^+ in preference over other nitrogen sources, such as NO_3^-, NO_2^-, amino acid and urea (Amano and Noda 1987).

The genus *Porphyra* (Bangiales, Rhodophyta) is capable of rapid growth and is an efficient nutrient concentrator. Recently Carmona et al. (2006) measured specific growth rates of *Porphyra* species exceeding 25% day^{-1}, and more recent studies suggest this genus is capable of 45% day^{-1} in the short-term (Kraemer, personal communication). Since it consists of one or two cell layers, *Porphyra* has an extremely high surface area to volume ratio. With all cells taking up nutrients, it is capable of rapid assimilation and growth (Kraemer et al. 2004; Neori et al. 2004; Carmona et al. 2006; Pereira et al. 2006). In addition, *Porphyra* is a valuable source of food and, in Asia, this seaweed is commercially maricultured to supply a billion dollar (U.S.) annual market (Yarish et al. 1999; FAO 2003; He and Yarish 2006). *Porphyra* contains high levels of protein (25–50%), vitamins (higher vitamin C than in oranges), trace minerals and dietary fibers (Noda 1993). Eighteen types of free amino acids have been reported, including taurine, which has been found to reduce blood cholesterol levels (Noda 1993). This alga is also a preferred source of the red pigment *r*-phycoerythrin, which is utilized as a fluorescent "tag" by the medical diagnostic industry (Mumford and Miura 1988).

Porphyra has been cultivated for the past several hundred years in Japan and has become one of the most successful aquaculture industries in Japan, Korea and China (Mumford and Miura 1988; FAO 2003). In coastal New England, a commercially valuable Asian taxon, *P. yezoensis*, was selected for aquaculture trials in part because little was known of the biology of the native New England *Porphyra* species. *Porphyra yezoensis* was not grown successfully because the gametophyte was not well adapted for the temperature and nutrient regimes of northeastern Maine's coastal environment (Yarish et al. 1998). Therefore, selection of an appropriate local *Porphyra* cultivar is necessary for a successful integrated aquaculture system.

A wide variety of biological factors, such as inter-individual variability, nutritional history, type of tissue, life history stage/age, surface area:volume ratio of the thallus, and blade morphology, may influence the nutrient uptake and growth. Young tissue exhibit higher rates of uptake and growth than older tissue and therefore one must be careful when uptake rates are determined only on portions of the thallus (Kraemer and Yarish 1999). For example, the kelp *Laminaria groenlandica* is a perennial plant wherein the first year plants have higher uptake rates than the third year plants (Harrison et al. 1986). Younger blades and small size tissue of *Porphyra yezoensis* also grow at a significantly higher rate than mature blades and large size tissue (Hafting 1999). To avoid these biological effects, materials size and age should be considered.

The primary objective of this study was to collect physiological measurements to guide the selection of an

appropriate species of *Porphyra* (*P. leucosticta, P. linearis*, *P. umbilicalis* and *P. amplissima*) from coastal New England to be used in the development of an integrated land-based aquaculture system. This will enable us to compare the efficiency of different species of *Porphyra* as nutrient scrubbers to decide which candidate would be best suited for bioremediation. We present here results that describe the influence of temperature and nutrient availability (ammonium concentration) on growth rate, phycoerythrin content, tissue N content and N removal from the media.

Materials and methods

Algal material and culture

Porphyra amplissima (Kjellman) Setchell et Hus (ME-32p) used in this study was in culture at the Marine Biotechnology Laboratory of the University of Connecticut at Stamford. The strain of *Porphyra amplissima* was originally collected from Gore Point, Cobscook Bay, Maine, USA. *Porphyra leucosticta* Thuret in Le Jolis was collected in the mid intertidal zone at Groton and Waterford, Connecticut, USA, in March and May 2002. *Porphyra umbilicalis* Kützing and *Porphyra linearis* Greville were collected in the upper intertidal zone at Rye, New Hampshire, USA, in March 2003.

Experiments were carried out for 1 or 2 week(s) in 50L tanks at saturating light intensities (100–150 μmol photons $m^{-2}s^{-1}$; Kraemer and Yarish 1999). Two 50L tanks were contained in each of 9 water baths. Temperature in each water bath was controlled independently via 1,000-W immersion heaters. Light was supplied by 400W Ceramalux lamps (Philips, Somerset, N.J.) placed above each pair of tanks. Irradiance was measured by a light meter (LI-185A, Li-Cor) and adjusted with neutral density filters. Photoperiod was 12:12 h L:D. The culture medium was filtered (0.45 μm) and UV-irradiated seawater with von Stosch's enrichment (Ott 1965) without nitrogen and phosphorus. Nitrogen and phosphorus levels were regulated by addition of ammonium (NH_4Cl) and phosphate (Na_2HPO_4) to cultures twice a week at a molar N:P ratio of 10:1. The initial stocking densities of each tank for *P. leucosicta, P. linearis P. umbilicalis* and *P. amplissima* were 0.08, 0.1, 0.14 and 0.08 g L^{-1}, respectively.

Acclimation

Porphyra leucosticta, P. umbilicalis and *P. linearis,* were acclimated for 4–6 days in gently aerated, Avery Point (Conn.) filtered seawater at 10°C and 50–100 μmol photons $m^{-2}s^{-1}$ under a 12:12 L:D photoperiod. Filtered seawater was renewed daily to maintain a stable nutrient status in the algal tissues during acclimation. After acclimation, discs (20 mm diameter) of *P. leucosticta* and *P. umbilicalis* were punched from different blades and cultivated for 2 days under the same conditions to allow recovery from wounding effects (Drew 1983). Whole blades (5×1 cm) of *P. linearis* and *P. amplissima* were used. As the *Porphyra amplissima* had been grown in von Stosch enriched seawater, an acclimation period was considered unnecessary.

Experimental design

The experiments were conducted using a split-plot, randomized complete block design, with temperature (three levels) as main plots and ammonium (two levels) as subplots, resulting in a total of six treatment combinations, each with three replicates. The design accommodated practical limitations in temperature and ammonium control: three levels of temperature (10, 15 and 20°C) and two ammonium concentration (25 and 250 μmoles L^{-1}). The ammonium and temperature levels reflect the range in marine aquaculture (Day 2003; Carmona et al. 2006).

Measurements

At 1-week intervals, all of the biomass in each tank was weighed (fresh weight; FW) and samples were taken for dry weight and pigment analysis. The FW were obtained after blotting the thalli dry with paper towels. Dry weight (DW) and moisture content were calculated by drying a sample of the biomass at 60°C to constant weight. Specific growth rate (SGR, expressed as % increase day^{-1}) was calculated as follows:

$$\mathrm{SGR} = \frac{\ln S_2 - \ln S_1}{T_2 - T_1} \times 100$$

where S_1 and S_2 are the fresh weight at days T_1 and T_2, respectively. 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. PE content was calculated according to the equations used in Beer and Eshel (1985). For the analysis of tissue total N and C content, samples were dried at 60°C before being ground. The powder was analyzed using a Perkin Elmer 2400 series II CHNS/O elemental analyzer. Specific growth rate, tissue PE, nitrogen and carbon content were determined weekly.

N removal was calculated using the equation,

$$\text{N removal}\left(\text{mg N g}^{-1}\text{day}^{-1}\right) = \frac{(B_t \times \text{Tissue } N_t) - (B_0 \times \text{Tissue } N_0)}{\left(\frac{B_t + B_0}{2}\right) \times t} \times \frac{DW}{FW}$$

where B_t and B_o are the biomass at days t and 0 respectively. The difference in biomasss-specific nitrogen removal ability between species and between conditions can be compared with this method.

Statistical analysis

Two-way split-plot ANOVA (α=0.05) was used to analyze data. When ANOVA indicated treatment effect of temperature or an interaction between ammonium level and temperature, Tukey's HSD analysis (α=0.05) was used as a post hoc test to determine pairwise comparison probabilities between treatment level means. All statistic analyses were done using Minitab (release 13, Minitab. State College, Pa., USA).

Results

Growth

Temperature significantly influenced the growth rate of all species. The growth rate of *Porphyra leucosticta* was higher at 10 and 15°C than at 20°C (Fig. 1; p=0.01). *Porphyra linearis* and *P. umbilicalis* grew fastest at 10°C (Fig. 1). However, *P. amplissima* showed the higher growth rate at 20°C than that at the lower temperatures (Fig. 1).

Nutrient availability also significantly influenced growth rate. The growth rates of *Porphyra linearis* and *P. umbilicalis* were significantly higher at 250 μmoles L^{-1} ammonium than that at 25 μmoles L^{-1} ammonium concentration (Fig. 1; $p \leq 0.022$). Of the four species studied, *Porphyra linearis* had the highest growth rate, increasing in biomass by about 16% day^{-1} over 14 days at 10°C, while other three species grew at about 10% day^{-1} at optimum conditions.

Phycoerythrin content

The phycoerythrin (PE) content was affected by temperature ($p \leq 0.027$) at high ammonium concentration in all species

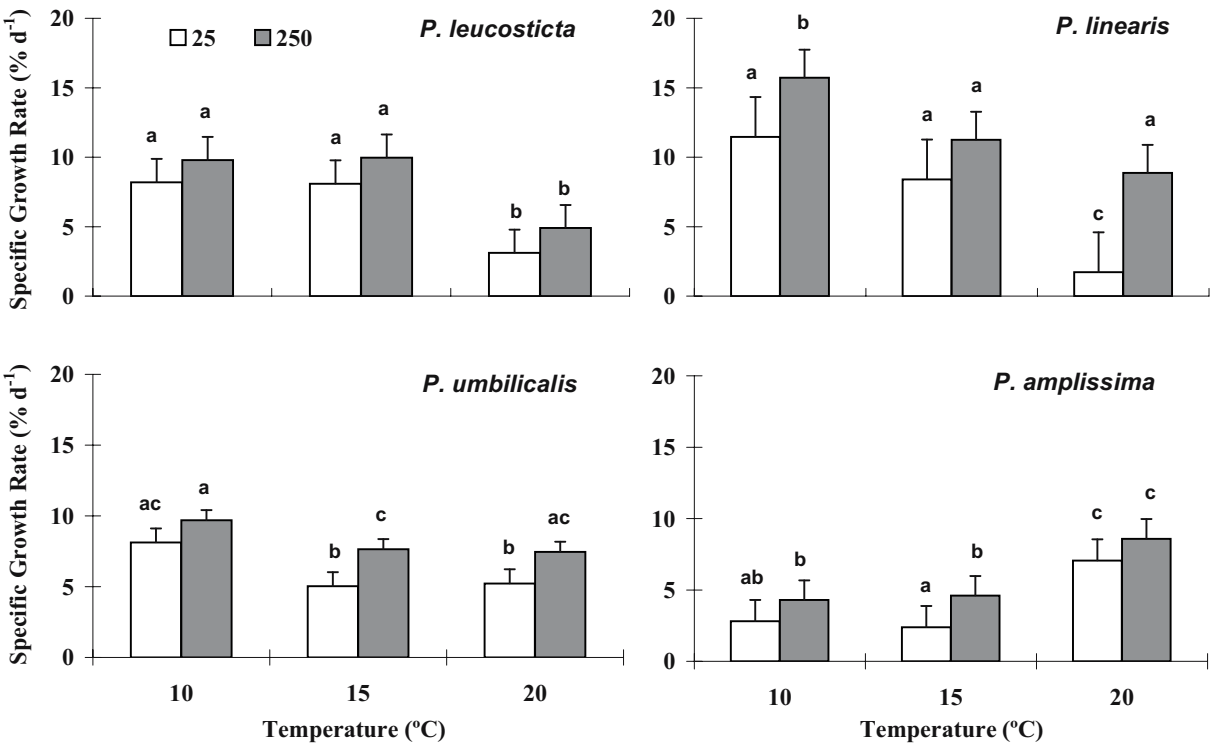

Fig. 1 Growth rate of *Porphyra leucosticta**, *Porphyra linearis***, *Porphyra umbilicalis*** and *Porphyra amplissima** grown at 10, 15 and 20°C and 25 and 250 μmoles L^{-1} ammonium. *Error bars* represent standard error. Bars with the same letter are not significantly different (p>0.05; *first week's results, **average results over first 2 weeks)

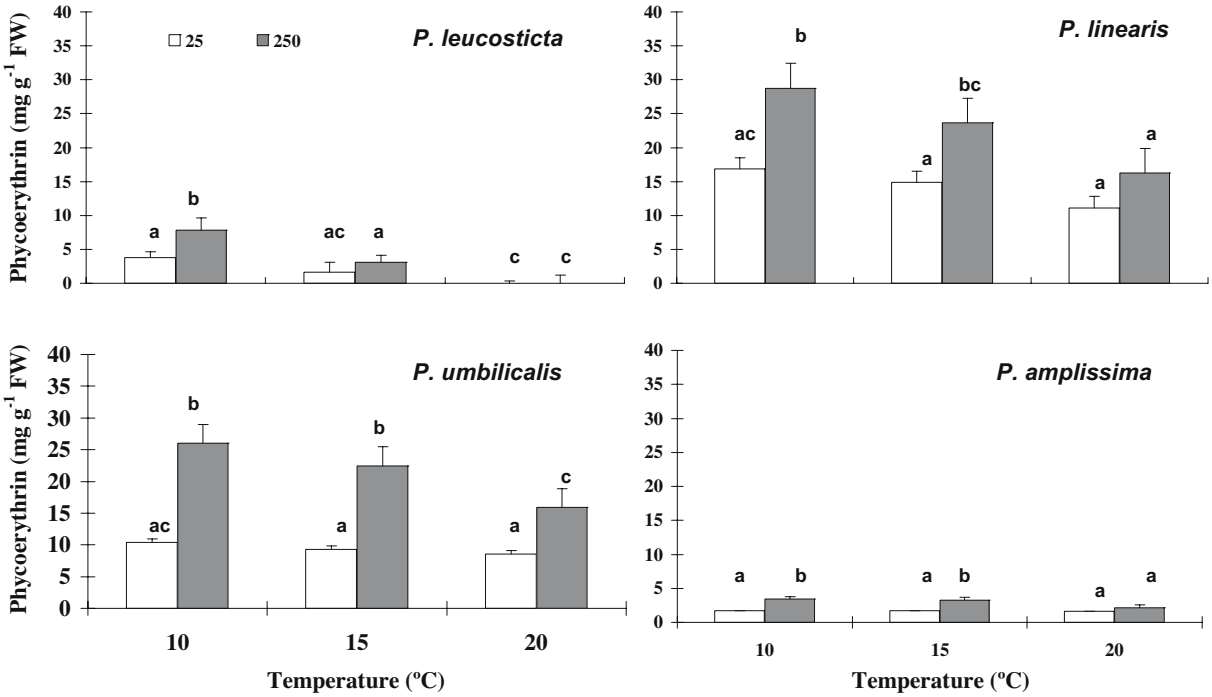

Fig. 2 Phycoerythrin contents of *Porphyra leucosticta**, *Porphyra linearis***, *Porphyra umbilicalis*** and *Porphyra amplissima** grown at 10, 15 and 20°C and 25 and 250 μmoles L^{-1} ammonium. *Error bars* represent standard error. Bars with the same letter are not significantly different ($p>0.05$; *first week's results, **average results over first 2 weeks)

except *Porphyra amplissima* (Fig. 2). The PE contents of *P. leucosticta* were higher at 10°C than at other temperatures and *P. linearis* and *P. umbilicalis* showed significantly higher PE contents at 10 and 15°C. The PE content of *P. linearis* and *P. umbilicalis* was on average 90% higher at 250 μmoles L^{-1} than at 25 μmoles L^{-1} ammonium concentration at all temperature conditions (Fig. 2; $p\leq0.012$). The PE content of *P. linearis* (29 mg g^{-1} FW^{-1}) and *P. umbilicalis* (26 mg g^{-1} FW^{-1}) was much higher than *P. leucosticta* and *P. amplissima*. For *P. linearis*, the effect of temperature on PE content was greater at high than at low ammonium levels; similar interactions were not found in the other species.

Table 1 Tissue C and N contents and C:N ratio of *Porphyra leucosticta**, *Porphyra linearis***, *Porphyra umbilicalis*** and *Porphyra amplissima** (Means±S.D; * : 1st week's results, ** : average results over 2 weeks)

	C (%)		N (%)		C:N	
	25 μM	250 μM	25 μM	250 μM	25 μM	250 μM
P. leucosticta						
10°C	38.31 (±0.10)	39.64 (±0.17)	2.63 (±0.06)	4.95 (±0.11)	14.58 (±0.33)	8.02 (±0.15)
15°C	51.13 (±8.64)	39.59 (±0.23)	3.58 (±0.64)	4.69 (±0.12)	14.40 (±0.23)	8.47 (±0.18)
20°C	38.06 (±0.30)	39.84 (±0.03)	2.25 (±0.08)	4.59 (±0.04)	16.98 (±0.61)	8.68 (±0.08)
P. linearis						
10°C	37.82 (±0.43)	35.94 (±2.11)	4.60 (±0.52)	5.66 (±0.18)	8.27 (±0.84)	6.34 (±0.19)
15°C	37.08 (±0.78)	37.61 (±0.27)	4.73 (±0.15)	5.81 (±0.06)	7.85 (±0.09)	6.47 (±0.09)
20°C	38.62 (±1.66)	37.41 (±1.44)	4.79 (±0.45)	5.57 (±0.24)	8.02 (±0.54)	6.71 (±0.05)
P. umbilicalis						
10°C	37.19 (±0.64)	38.82 (±0.30)	3.89 (±0.07)	6.76 (±0.27)	9.57 (±0.01)	5.75 (±0.28)
15°C	38.00 (±0.85)	39.64 (±1.85)	4.24 (±0.23)	6.62 (±0.31)	8.97 (±0.31)	5.99 (±0.01)
20°C	37.22 (±2.14)	39.46 (±0.55)	4.36 (±0.31)	6.31 (±0.43)	8.55 (±0.41)	6.27 (±0.47)
P. amplissima						
10°C	37.09 (±1.48)	36.93 (±0.78)	3.71 (±0.12)	3.92 (±0.04)	10.00 (±0.08)	9.42 (±0.11)
15°C	32.42 (±6.06)	36.75 (±1.32)	3.28 (±0.41)	4.08 (±0.21)	9.86 (±0.61)	9.02 (±0.65)
20°C	34.91 (±2.34)	37.04 (±1.20)	3.56 (±0.34)	3.87 (±0.03)	9.85 (±0.86)	9.57 (±0.38)

Carbon, nitrogen contents, and C:N ratio in tissue

Carbon contents of all species did not differ at different temperature and ammonium concentrations ($p>0.05$), except that carbon content of *P. leucosticta* at 25 μmoles L^{-1} and 15°C (51.13%) was higher than at other conditions. Nitrogen values in tissue varied from 2.25% (*P. leucosticta* at 25 μmoles L^{-1} and 20°C) to 6.76% (*P. umbilicalis* at 250 μmoles L^{-1} and 10°C). Temperature alone had no effect on tissue nitrogen content of any *Porphyra* species ($p>0.05$). Ammonium concentration significantly affected nitrogen content ($p\leq0.001$) in all species. The tissue nitrogen content of all species was on average 1.45% higher in the DW at 250 μmoles L^{-1} than at 25 μmoles L^{-1} ammonium concentration. This percentage represents the absolute difference between two values. *Porphyra umbilicalis* had the highest tissue nitrogen contents (6.76%) at 10°C and at 250 μmoles L^{-1} ammonium (Table 1). The starting C:N ratio of the algae was between 7.2 and 9.5. Lower C:N ratios were observed in all *Porphyra* species grown at 250 μmoles L^{-1} than at 25 μmoles L^{-1} ammonium concentration over 14 days. *Porphyra leucosticta* showed the highest ratio (up to 16.98) under 25 μmoles L^{-1} ammonium (Table 1).

Nitrogen removal

The nitrogen removal ability of *Porphyra* was affected by temperature and ammonium concentration. In *P. leucosticta, P. linearis* and *P. umbilicalis*, N removal decreased with increasing temperature and was higher at high ammonium concentration than at low ammonium concentration (Fig. 3). The highest N removal capability of *P. leucosticta* occurred at 10 and 15°C; for *P. linearis* and *P. umbilicalis* it was at 10°C, and for *P. amplissima* it was at 20°C. The N removal of *P. umbilicalis* (1.30 mg N g^{-1} day^{-1} DW) and *P. linearis* (1.28 mg N g^{-1} day^{-1} DW) was markedly higher than *P. leucosticta* at 10°C and *P. amplissima* at 20°C (0.92 and 0.56 mg N g^{-1} day^{-1} DW, respectively) and 250 μmoles L^{-1} ammonium concentration.

Discussion

Porphyra linearis grew at over 16% SGR, which was significantly higher than that of other seaweeds being used for integrated aquaculture, e.g., *Laminaria saccharina* (9% day^{-1}; Subandar et al. 1993), *Gracilaria parvispora* (10% day^{-1}; Nelson et al. 2001) and *Ulva pertusa* (12% day^{-1}; Kim and Han 1999). *Porphyra umbilicalis* has a high capacity for nitrogen accumulation (6.76% tissue nitrogen at optimal conditions). This value is also much higher than that of other maricultured seaweeds, including *Chondrus crispus* (4.8%; Asare and Harlin 1983), *Gracilaria pacifica* (4.18–4.59%; Naldi and Wheeler 1999) and *Laminaria saccharina* (3.42%; Gevaert et al. 2001). It is even markedly higher than other efficient nutrient scrubbers

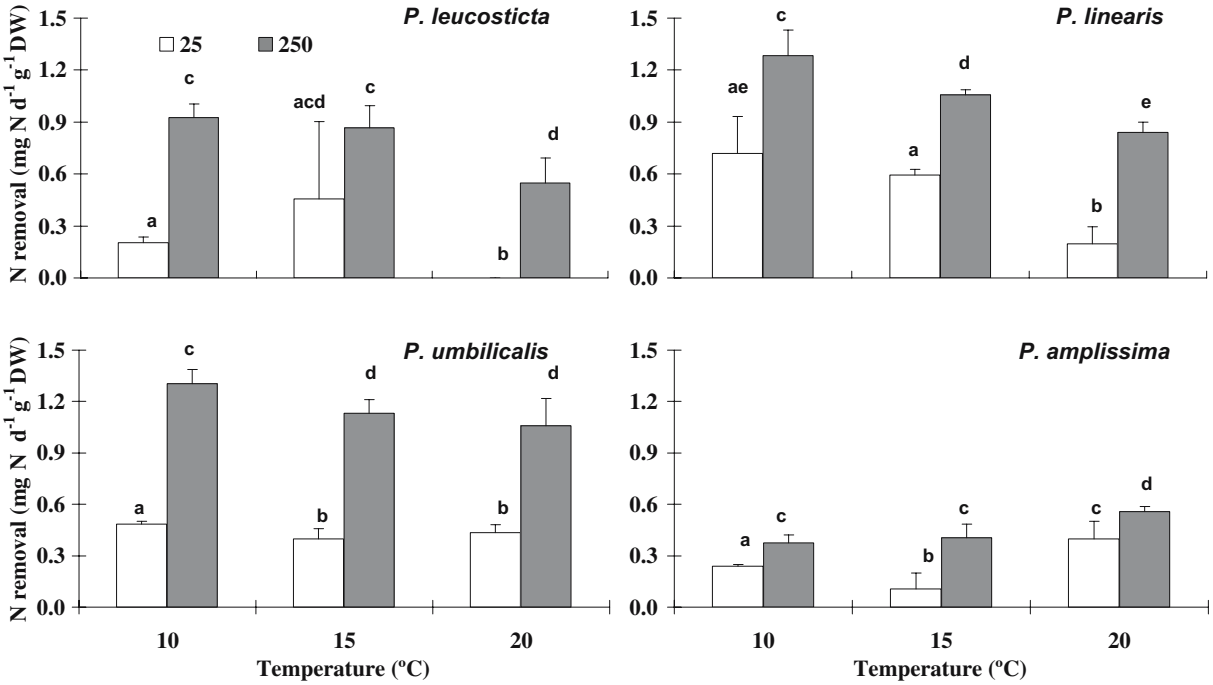

Fig. 3 Nitrogen removal by *Porphyra leucosticta**, *Porphyra linearis***, *Porphyra umbilicalis*** and *P. amplissima** grown at 10, 15 and 20°C and 25 and 250 μmoles L^{-1} ammonium. Error bars represent standard error. Bars with the same letter are not significantly different ($p>0.05$; *first week's results, **average results over first 2 weeks)

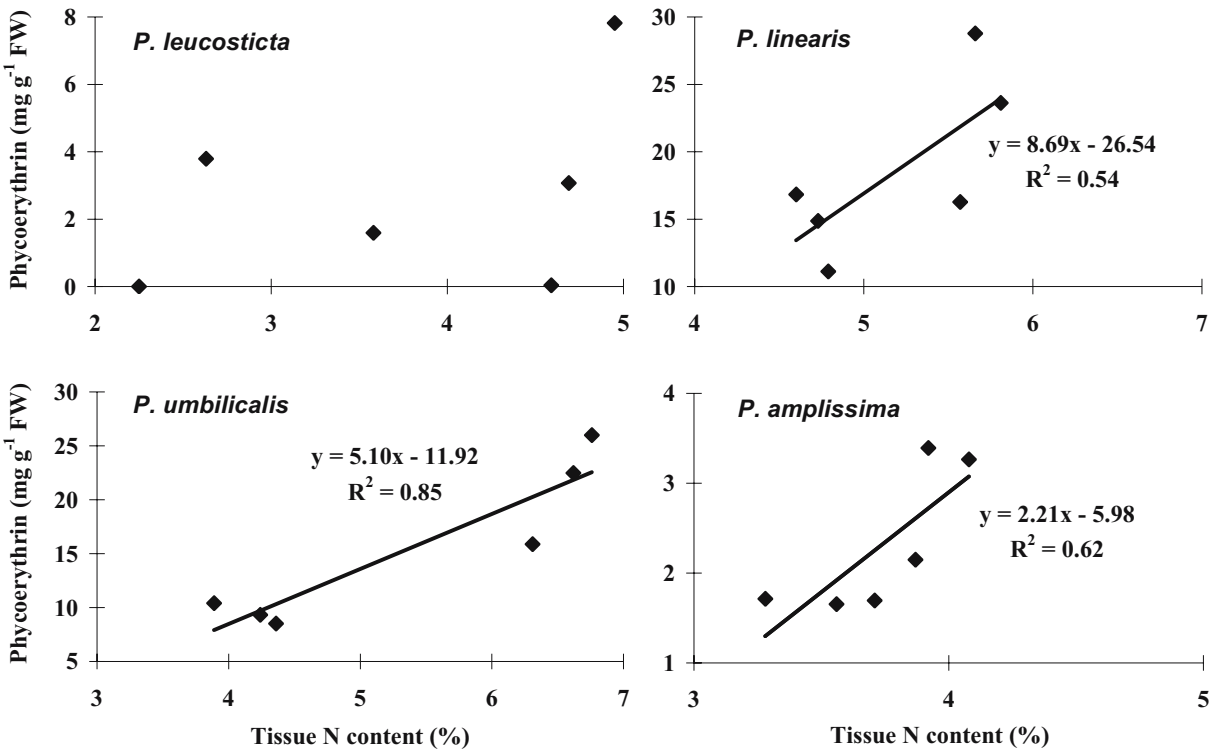

Fig. 4 Phycoerythrin versus tissue N contents of *Porphyra leucosticta* ($p>0.05$), *Porphyra linearis* ($p<0.05$), *Porphyra umbilicalis* ($p<0.05$) and *Porphyra amplissima* ($p<0.05$)

such as *Ulva rotundata*, *Ulva intestinalis, Ulva fenestrate* and *Ulva pertusa* (3.06%, 3.35%, 4.71% and 5.07%, respectively; Liu and Dong 2001; Hernández et al. 2002).

Some species grow well under conditions in laboratory that never occur in their native environment. For example, the distribution of the red alga, *Polyneura hilliae*, indicates that it grows well between 11 and 15°C. However, Yarish et al. (1986) reported that the alga grew very well from 10 to 20°C under laboratory conditions. Below 5°C and above 25°C, temperatures never experienced in nature, *Polyneura hilliae* died. Another red alga, *Calliblepharis ciliata*, showed similar tendencies (Yarish et al. 1986). The fundamental niche describes the environmental "space" within which a species can survive and reproduce in the absence of biotic interactions. Competition, predation, and parasitism restrict organisms to the realized niche, only a part of the fundamental niche (Hutchinson 1958). Temperature is an important part of the ecological niche, a concept that is often used to describe the range of tolerance (Lampert and Sommer 1997). Hutchinson (1958) emphasized that organisms have ranges of tolerance for many environmental factors, rather than only a single factor. Stress in one abiotic factor may reduce the tolerance range of another; the optimal environment in aquaculture most probably broadens the environmental tolerance ranges of some seaweeds. In the present study, *P. leucosticta, P. linearis* and *P. umbilicalis* grew well at low temperature similar to those of the natural habitat of its gametophytes. However, *P. amplissima* attained the highest growth rate at 20°C, a temperature above the highest reached in the field (Chopin et al. 1999).

Porphyra amplissima gametophyte is present from spring through autumn along the coast of Maine, where sea surface temperatures generally range between 0–15°C (Chopin et al. 1999; Yarish et al. 1999). *Porphyra leucosticta* is common in winter/spring in Connecticut, (USA) when water temperatures range between 1–16°C. Therefore, *P. leucosticta* was expected to be at least as tolerant of higher temperatures as *P. amplissima* because *P. amplissima* is rarely found south of New Hampshire (Chopin et al. 1999). However, *Porphyra amplissima* grew well at high temperature, which demonstrated higher temperature tolerance than the other three species. *P. amplissima* gametophytes came from conchospores grown in the lab without the effects of biotic interactions while other three species came from the field which was previously exposed to biotic interactions.

Recently, Day (2003) and Carmona et al. (2006) reported for *P. amplissima* a specific growth rate of over 32% day^{-1} at 12°C and 25% day^{-1} at 15°C, respectively. However, the growth rate of *P. amplissima* measured during the present study was much lower, possibly due the effect

of tissue age. *Porphyra amplissima* used in this study was mature (over 10 cm), while Day (2003) and Carmona et al. (2006) used younger blades. Young tissue has much higher growth and nutrient uptake rate than older tissue (Kraemer and Yarish 1999; Harrison and Hurd 2001).

The ability to store N has ramifications in management techniques in algal aquaculture. Previous studies have shown that *Gracilaria* is able to take up ambient N very rapidly and store it in organic form for later use during periods of N-limitation (Bird et al. 1982). *Gracilaria* can obtain and store enough nitrogen for non-limited growth if given a single pulse of nitrogen every 2 weeks (Ryther et al. 1981). This storage is reflected in thallus N contents (3–5%), which can be substantially higher than those indicating N deficiency (1.5–2%; Ryther et al. 1981). Pigments are sensitive to the N status of the algae and probably decline due to growth and a lack of sufficient ambient N for continued synthesis of the new pigments. Increases in the chlorophyll *a* content with increases in cellular N are well known for algae (Fogg 1965). Studies of the red alga *Gracilaria tikvahiae* indicated that chlorophyll and carotenoid pigments did not contribute greatly to the overall N content (Bird et al. 1982).

Our results indicate a storage function for phycoerythrin (PE) pigments in *Porphyra* tissue (except for *P. leucosticta*; Fig. 4). At 250 μmoles L^{-1} ammonium level, pigment and tissue N contents were significantly greater than those at lower level of ammonium. This supports other studies that arrived at the same conclusion (Carmona et al. 2006). PE comprised 20% of total N but decreased markedly under N-limitation (e.g., Harrison and Hurd 2001). PE as a ratio of total protein decreased when tissue N content fell below 1.8% (Bird et al. 1982). Perhaps at incipient N limitation these pigments are preferentially utilized to support continued growth. *Porphyra linearis* and *P. umbilicalis* exhibited much higher PE contents (276 and 205 mg g^{-1} DW^{-1} respectively; calculated from PE contents FW^{-1}) than that reported for *P. yezoensis* (40–60 mg g^{-1} DW^{-1}; Yan et al. 2000) grown under nutrient enriched conditions. The high PE and tissue N contents may explain how *P. umbilicalis* and *P. linearis* can remove N at higher rates (1.30 mg N g^{-1} day^{-1} DW and 1.28 mg N g^{-1} day^{-1} DW respectively) than the other two species (Fig. 3).

In many coastal areas, blooms of fast-growing opportunistic seaweeds have replaced the previously dominant species. Such blooms may be due to increased loading of inorganic nutrient into the seawater, decreased herbivore activity, global warming, or a combination of these effects (Raffaelli et al. 1998). The former is potentially controllable. Fish farms are a major source of released nutrients (Ackefors and Enell 1994). All species of *Porphyra* used in this study thrived in ammonium concentrations as high as those measured in fish farm effluent (Day 2003), and had much higher growth rates (10–16% day^{-1}) than under natural lower ammonium concentration.

The efficiency of the different species of *Porphyra* as nutrient scrubbers, as well as the economic potential, temperature range and growth ability may be examined to decide which candidate(s) would be best suited for bioremediation of aquaculture effluent. *Porphyra leucosticta* and *P. linearis* exhibit excellent gustatory properties (Chopin et al. 1999), while *P. umbilicalis* and *P. amplissima* may be marketable for a variety of industrial and biotechnological uses (taurine and r-phycoerythrin; Chopin et al. 1999; Carmona et al. 2006). Our results indicate the possibility of inter-specific variation in growth, N accumulation and PE content as functions of temperature and ammonium availability. For year-round ammonium removal and production of *Porphyra* and phycobiliprotein, several species should be used in rotation according to their seasonality (Day 2003; Kraemer et al. 2004). *Porphyra* has two phages, the gametophytic blade and a microscopic conchocelis (sporophytes). The transition between life history stages may limit the use of *Porphyra* for bioremediation throughout the year. The rotation of different species may be a solution to set off the disadvantage of having the conchocelis phage for aquaculture use.

Our study demonstrated that *P. linearis* and *P. umbilicalis* had high PE contents, as well as high nitrogen uptake and fast growth, making these species candidates for bioremediation of effluents from land-based finfish and shellfish mariculture. The growth of *Porphyra* may be affected by contaminations such as heavy metals or persistent organic compounds. Therefore, *Porphyra* may not be suited for the open water system or sewage treatment, but can be used for an integrated land-based aquaculture system which can control most of contaminations.

Porphyra amplissima grew well at the high temperature (20°C) condition, but the apparent low nutrient uptake capability and low PE content seem to make this species less suitable for bioremediation. Early life history stages usually have higher nutrient uptake and growth rates than mature thalli of the same species (Kraemer and Yarish 1999). Therefore, further study of the effect of temperature on the bioremediatory performance of young *P. amplissima* tissue should be performed if this species is to be utilized at high temperature.

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References

Ackefors H, Enell M (1994) The release of nutrients and organic matter from aquaculture systems in Nordic countries. J Appl Ichthyol 10:225–241

Amano H, Noda H (1987) Effect of nitrogenous fertilizers on the recovery of discolored fronds of *Porphyra yezoensis*. Bot Mar 30:467–473

Asare SO, Harlin MM (1983) Seasonal fluctuations in tissue nitrogen for five species of perennial macroalgae in Rhode Island Sound. J Phycol 19:254–257

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

Beveridge MCL (1987) Cage aquaculture. Fishing News Books, Farnham

Bird KT (1976) Simultaneous assimilation of ammonium and nitrate by *Gelidium nudifrons* (Gelidiales: Rhodophyta). J Phycol 12:238–241

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

Carmona R, Kraemer GP, Yarish C (2006) Exploring Northeast American and Asian species of *Porphyra* for use in an integrated finfish-algal aquaculture system. Aquaculture 252:54–65

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

Conway HL (1977) Interactions of inorganic nitrogen in the uptake and assimilation by marine phytoplankton. Mar Biol 39:221–232

Cuomo V, Merrill J, Palomba I, Perretti A (1993) Systematic collection of *Ulva* and mariculture of *Porphyra*: biotechnology against eutrophication in the Venice Laggon. Int J Environ Stud 43:141–149

Day JP (2003) Effects of light and ammonium on growth, N uptake and pigmentation of *P. umbilicalis* Kützing, *Porphyra linearis* Greville, *P. leucosticta* Thuret in Le Jolis, *Porphyra amplissima* Kjellman. MS thesis, University of New Hampshire

DeBoer JA (1981) Nutrients. In: Lobban CS, Wynne MJ (eds) The biology of seaweeds. Blackwell Scientific, Oxford, pp 365–391

DeBoer JA, Guigli HJ, Israel TL, D'Elia CF (1978) Nutritional studies of two red algae. I. Growth rate as a function of nitrogen source and concentration. J Phycol 14:261–266

Drew EA (1983) Physiology of *Laminaria* I. Use of excised lamina discs in short and long term experiments. Mar Ecol 4:227–250

FAO (2003) Food and Agriculture Organization of the United Nations. http://www.fao.org/unfao/bodies/conf/c2003/c2003-e.htm

Fogg GE (1965) Algal cultures and phytoplankton ecology. The University of Wisconsin Press, Madison

Gevaert F, Davoult D, Creach A, Kling R, Janquin MA, Seuront L, Lemoine Y (2001) Carbon and nitrogen content of *Laminaria saccharina* in the eastern English Channel: biometrics and seasonal variations. J Mar Biol Assoc UK 81:727–734

Hafting JT (1999) A novel technique for propagation of *Porphyra yezoensis* Ueda blades in suspension cultures via monospres. J Appl Phycol 11:361–367

Harrison PJ, Hurd CL (2001) Nutrient physiology of seaweeds: application of concepts to aquaculture. Cah Biol Mar 42:71–82

Harrison PJ, Druehl LD, Lloyd KE, Thompson PA (1986) Nitrogen uptake kinetics in three year-classes of *Laminaria groenlandica* (Laminariales: Phaeophyta). Mar Biol 93:29–35

He P, Yarish C (2006) The developmental regulation of mass cultures of free-living conchocelis for commercial net seeding of *Porphyra leucosticta* from Northeast America. Aquaculture 257:373–381

Hernández I, Martínez-Aragón JF, Tovar A, Pérez-Lloréns JL, Vergara JJ (2002) Biofiltering efficiency in removal of dissolved nutrients by three species of estuarine macroalgae cultivated with sea bass (*Dicentrarchus labrax*) waste waters 2. Ammonium. J Appl Phycol 14:375–384

Hutchinson GE (1958) Homage to Santa Rsalia, or why are there so many kinds of animals. Am Nat 91:145–159

Kim JK, Han T (1999) Effects of inorganic nutrients and heavy metals on growth and pigmentation of the green alga, *Ulva pertusa* Kjellman. Korean J Environ Biol 17:427–438

Kraemer GP, Yarish C (1999) A preliminary comparison of the mariculture potential of *Porphyra purpurea* and *Porphyra umbilicalis*. J Appl Phycol 11:473–477

Kraemer GP, Carmona R, Chopin T, Neefus C, Tang X, 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

Lampert W, Sommer U (1997) Limnoecology: the ecology of lakes and streams. Oxford University Press, pp 48–49

Liu JW, Dong SL (2001) Comparative studies on utilizing nitrogen capacity between two macroalgae *Gracilaria tenuistipitata* var. liui (Rhodophyta) and *Ulva pertusa* (Chlorophyta). I. Nitrogen storage under nitrogen enrichment and starvation. J Environ Sci (China) 13:318–322

Lobban CS, Harrison PJ (1994) Seaweed ecology and physiology. Cambridge University Press, New York

Mumford TF, Miura A (1988) *Porphyra* as food: cultivation and economics. In: Lembi CA, Waaland JR (eds) Algae and human affairs. Cambridge University Press, London, pp 87–117

Naldi M, Wheeler PA (1999) Changes in nitrogen pools in *Ulva fenestrata* (Chlorophyta) and *Gracilaria pacifica* (Rhodophyta) under nitrate and ammonium enrichment. J Phycol 35:70–77

Nelson SG, Glen EP, Conn J, Moore D, Walsh T, Akutagawa M (2001) Cultivation of *Gracilaria parvispora* (Rhodophyta) shrimp-farm effluent ditches and floating cages in Hawaii: a two-phage polyculture system. Aquaculture 193:239–248

Neori A, Chopin T, Troell M, Buschmann AH, Kraemer GP, Halling C, Shpigel M, Yarish C (2004) Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. Aquaculture 231:361–391

Noda H (1993) Health benefits and nutritional properties of nori. J Appl Phycol 5:255–258

Ott FD (1965) Synthetic media and techniques for the xenic cultivation of marine algae and flagellate. Va J Sci 16:205–218

Pereira R, Yarish C, Sousa-Pinto I (2006) The influence of stocking density, light and temperature on the growth, production and nutrient removal capacity of *Porphyra dioica* (Bangiales, Rhodophyta). Aquaculture 252:66–78

Raffaelli DG, Raven J, Poole L (1998) Ecological impact of macroalgal blooms. Oceanogr Mar Biol Annu Rev 36:97–125

Ryther JH, Corwin N, DeBusk TA, Williams LD (1981) Nitrogen uptake and storage by the red alga *Gracilaria tikvahiae* (McLachlan, 1979). Aquaculture 26:107–115

Subandar A, Petrell RJ, Harrison PJ (1993) *Laminaria* culture for reduction of dissolved inorganic nitrogen in salmon farm effluent. J Appl Phycol 5:455–463

Troell M, Halling C, Neori A, Chopin T, Buschmann AH, Kautsky N, Yarish C (2003) Integrated mariculture: asking the right questions. Aquaculture 226:69–90

Twomey L, Thompson P (2001) Nutrient limitation of phytoplankton in a seasonally open bar-built estuary: Wilson Inlet, Western Australia. J Phycol 37:16–29

Yan X, Fujita Y, Aruga Y (2000) Induction and characterization of pigmentation mutants in *Porphyra yezoensis* (Bangiales, Rhodophyta). J Appl Phycol 12:69–81

Yarish C, Breeman AM, van den Hoek C (1986) Survival strategies and temperature responses of seaweeds belonging to different biogeographic distribution groups. Bot Mar 29:215–230

Yarish C, Wilkes R, Chopin T, Fei XG, Mathieson AC, Klein AS, Neefus CD, Mitman GG, Levine I (1998) Domestication of indigenous *Porphyra* (nori) species for commercial cultivation in Northeast America. World Aquac 29:26–29, 55

Yarish C, Chopin T, Wilkes R, Mathieson AC, Fei XG, Lu S (1999) Domestication of nori for Northease America: the Asian experience. Bull Aquacult Assoc Can 1:11–17