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Developing *Porphyra*/salmon integrated aquaculture for bioremediation and diversification of the aquaculture industry

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Abstract

For rapid growth and appropriate pigmentation, *Porphyra* requires the constant availability of nutrients, especially in summer when temperate waters are generally nutrient depleted. Cultivation near salmon cages allows the alleviation of this seasonal depletion by using the significant loading of fish farms, which is then valued (wastes become fertilisers) and managed (competition for nutrients between desirable algal crops and problem species associated with severe disturbances). *Porphyra*, being an extremely efficient nutrient pump, is an excellent candidate for integrated aquaculture for bioremediation and economic diversification. Frequent harvesting provides for constant removal of significant quantities of nutrients from coastal waters, and for production of seaweeds of commercial value. The production of *P. yezoensis* being limited in the Gulf of Maine, an assessment of the potential of seven native north-west Atlantic *Porphyra* species is presently in progress. To enable the production of conchospores for net seeding, the phenology of these species and the conditions for their vegetative conchocelis exponential growth, conchosporangium induction, and conchospore maturation were determined. The development of integrated aquaculture systems is a positive initiative for optimising the efficiency of aquaculture operations, while maintaining the health of coastal waters.

Introduction

Porphyra is a major source of food for humans throughout the world and is one of the most valued maricultured seaweeds with an annual value of over US$1.61 billion (Hanisak, 1998). *Porphyra*, commonly known as nori, is primarily used in the food industry as the reddish-black wrapping around the Japanese delicacy 'sushi'. It is also a major source of taurine that controls blood cholesterol levels (Tsujii et al., 1983), and is a staple in health food diets (Mumford & Miura, 1988). *Porphyra* contains high levels of proteins (25–50%), vitamins, trace minerals and dietary fibre (Noda, 1993). It also serves as a preferred source of the red pigment r-phycoerythrin, which is utilised as a fluorescent 'tag' for fluorescence in situ hybridisation (Mumford & Miura, 1988).

As the United States and Canada are primarily dependent upon China, and to a lesser extent Japan, Taiwan, and South Korea, for imports of nori, the development of a competitively produced North American product would be a major asset (Bergdahl, 1990; Bird, 1990). Previous attempts at cultivating nori on the West Coast of the United States were unsuccessful. The failure was solely due to the inability of nori farmers to obtain aquaculture lease permits in the coastal waters of Washington State because of political pressures from riparian land owners and commercial fishermen (Merrill, 1989; Mumford, 1990). These political forces have not been present in coastal New England and the Canadian Maritimes. The initiative has received strong support of local, state and federal agencies, as well as a popular interest. The establishment of a labour intensive sea vegetable industry may reduce unemployment and the reliance on a single dominant source of employment, salmonid farming.

The development of integrated aquaculture practices appears necessary and timely as the present approach to aquaculture is at a crossroad. The economic and environmental limitations of mono-specific operations are being realised and their sustainability questioned. For rapid growth and appropriate marketable pigmentation, *Porphyra* requires constant availability of nutrients, especially in the summer when temperate waters are generally nutrient depleted. At a time when there is mounting evidence associating finfish monoculture activities with significant loading of inorganic nutrients in coastal waters (Beveridge, 1987; Kautsky et al., 1997), common sense would suggest that integrating the culture of *Porphyra* to salmonid culture is a promising, balanced ecosystem approach providing mutual benefits to the co-cultured organisms. Cultivation of nori in the proximity of salmon cages allows the alleviation of the summer nutrient depletion by using the nutrient loading of fish farms, which is then valued (wastes become fertilisers) and managed (competition for nutrients between desirable algal crops and problem species associated with severe disturbances). As a consequence this would represent one of the schemes for the development of integrated coastal zone management (ICZM; Black et al., 1997).

With the lack of understanding of the biology of native New England and Canadian Maritimes *Porphyra* species, PhycoGen, Inc. initially used a commercially valuable Asiatic taxon, *P. yezoensis*. This taxon was developed during the 1960s by strain improvement programmes on *P. tenera* and *P. yezoensis* (Patwary & van der Meer, 1992). Although *P. yezoensis* has many desirable features, it had been selected for growth conditions in warm-temperate waters, and is having difficulty adapting to the coastal environment of north-east Maine. Therefore, it was logical to establish a cultivar improvement programme for local *Porphyra* species, just as has been done in Japan as these cultivars should be better adapted to local conditions.

The research programme is developing the co-culture of *Porphyra* with salmonid aquaculture for food, eutrophication abatement, and biochemical components, and is co-ordinating a field and culture assessment of native north-west Atlantic *Porphyra* species (from Long Island Sound to the Canadian Maritime Provinces). This paper compares the nutrient and pigment data of three species of *Porphyra*, at sites remote from salmon aquaculture activities and at the sites of the integrated aquaculture trials, and presents information on the phenology of seven New England and Canadian Maritime species of *Porphyra*.

Materials and methods

Development of nori/salmon integrated aquaculture

Samples of different species of *Porphyra* and of seawater were collected, between December 1996 and March 1999, at 8 locations:

- Dipper Harbour and Beaver Harbour, New Brunswick, Canada, two sites remote from salmon aquaculture activities;
- Huckins, the *Porphyra* nursery site of PhycoGen, Inc., in Cobscook Bay, Maine, USA. This part of Cobscook Bay was the subject of intense scallop dragging during the autumn of 1996 and 1998, but not 1997. After a few days of dragging at the beginning of November 1997 it became apparent that scallops were not abundant and, consequently, dragging stopped (S. Crawford, pers. comm.). The relationship of this fishery to seaweeds is that dragging puts nutrients trapped in interstitial waters of sediments back into suspension;
- Mathews Island, an intermediate station in Cobscook Bay;
- Deep Cove, Cobscook Bay, where PhycoGen, Inc., was allowed by Connors Aquaculture, Inc., to experiment during autumn 1996, on a pilot scale, integrated farming of *P. yezoensis* Ueda on nets adjacent to cages of *Salmo salar* Linnaeus (Atlantic salmon);

- Treats Island, also in Cobscook Bay, where PhycoGen, Inc., carried out another integrated farming trial with Treats Island Fisheries in the fall of 1998;
- West Ross Island, where two individuals from Grand Manan Island, New Brunswick, Canada, developed on their own initiative a nori site using weir poles;
- East Ross Island, a salmon aquaculture site.

Nutrient analysis

Triplicate tissue samples were taken to determine tissue total phosphorus (P) and nitrogen (N) contents. Tissue total P content was measured by the method of Murphy & Riley (1962) after acidic mineralization (H_2SO_4 and HNO_3) in Büchi 430 and 435 digester units. For the analysis of tissue total N content, portions of the samples were dried for 48 hours at 60 °C before being ground to a fine, homogeneous powder using a Retsch mixer mill. The powder was again dried for a minimum of 48 hours before being analysed using a Perkin Elmer 2400 Series II CHNS/O elemental analyser.

Dissolved inorganic P (DIP; as PO_4^{3-}) and dissolved inorganic N (DIN; as the sum of NH_4^+ + NO_3^- + NO_2^-) concentrations in seawater were measured by the methods of Murphy & Riley (1962) and Grasshoff et al. (1983), respectively, using a Technicon Autoanalyzer II segmented flow analyser.

Pigment analysis

Pigments were extracted using a modification of the method of Beer & Eshel (1985). Blotted dry tissue (0.3–0.5 g) was ground in a mortar and pestle in a 0.1 M phosphate buffer (pH 6.8). It was then frozen and thawed before being centrifuged at 3000 g for 20 minutes. The supernatant was filtered through a 0.45 μm membrane filter and the filtrate used for phycobilin analyses. Triplicate samples were analysed with a Spectronic 1201 spectrophotometer using Milton Roy's SpecScan and μ-Quant software. Pigment contents were calculated according to the equations used in Beer & Eshel (1985).

Phenology of native north-west Atlantic Porphyra *species*

At least seven species of native *Porphyra* occur in New England and the southern Maritime Provinces of Canada, including: *Porphyra amplissima* (Kjellman) Setchell *et* Hus *in* Hus, *P. miniata* (C. Agardh) C. Agardh, *P. umbilicalis* (Linnaeus) J. Agardh, *P. linearis* Greville, *P. purpurea* (Roth) C. Agardh, *P. leucosticta* Thuret *in* Le Jolis, and *P. carolinensis* Coll *et* Cox (Schneider et al., 1979; Mathieson & Hehre, 1986; Mathieson, 1989; Bird & McLachlan, 1992; Hehre & Mathieson, 1993). Species composition and seasonality of each of these taxa were characterised over a four year period at sites from Long Island Sound (LIS) to the coastal and estuarine habitats of the Bay of Fundy. Mostly random samples were taken and returned to the laboratories for identification. Representatives of all collections are held in the Herbarium at the University of New Hampshire, Durham, USA.

Cultures

Unialgal cultures of the different species of *Porphyra* were initiated from carpospores released by field collected specimens at the culture facilities of the University of Connecticut. Small portions of blades were scrubbed with sterile cotton swabs, and then immersed in von Stosch's enriched (VSE) seawater medium (von Stosch, 1964). After spore discharge (~24 h), the foliose sections were extracted and the carpospores were cultured in sterile VSE seawater. The resulting conchocelis stages were maintained and mass cultured in the laboratory, using enriched seawater at 10 °C, 10–40 μmol photon m^{-2} s^{-1}, and under variable photoperiods: 12:12; 8:16; and 16:8 hour Light:Dark (L:D) cycles. Cultures of 8 strains, belonging to 7 species, were used in these experiments: *P. amplissima*, ME9-1 collected on 7 June 1995, at Gore Point, Seward Neck, Lubec, Maine, USA; *P. umbilicalis*, ME40-6 collected on 11 September 1996, at Mathews Island, Cobscook Bay, Maine, USA; *P. linearis*, ME14-1 collected on 1 February 1996, at Rachel Carson Salt Pond Preserve, Maine, USA; *P. leucosticta*, CT11-3 collected on 17 December 1996, at Cove Island Park, Stamford, Connecticut, USA; *P. miniata*, NS14-1a collected on 29 June 1997, at West Sandy Cove, Digby Neck, Nova Scotia (NS), Canada; *P. purpurea*, NS9-1br collected on 30 September 1996, at Avonport, Minas Basin, Nova Scotia, Canada; *P. purpurea*, NY4-1a collected on 24 July 1997, at South Manursing Island, Rye, New York, USA; *P. carolinensis* CT1-6 collected on 30 October 1998, at Waterford, Connecticut, USA.

Upon isolating the conchocelis of several *Porphyra* taxa in culture, a variety of physiological investigations were conducted, including comparisons of light, temperature and photoperiod using temperature

Table 1. Nutrient (phosphorus and nitrogen) concentrations in seawater, and nutrient and pigment (phycoerythrin and phycocyanin) contents in *Porphyra purpurea*, *P. yezoensis* and *P. umbilicalis* in natural habitats, on nets, and in nori/salmon integrated aquaculture

Date	Location	Species	P in tissue (mg P g DW^{1})	N in tissue (mg N g DW^{-1})	PO_4^{3-} in seawater (μM P)	$NH_4^+ + NO_3^- + NO_2^-$ in seawater (μM N)	Phycoerythrin in tissue (mg PE g FW^{-1})	Phycocyanin in tissue (mg PC g FW^{-1})
06-12-96	Dipper Harbour	*P. purpurea*	3.2 ± 0.1	46.3 ± 0.0	0.68	6.87		
13-12-96	(1) Huckins	*P. yezoensis*	8.5 ± 0.4	72.7 ± 1.1	0.82	10.81	4.29 ± 0.08	0.98 ± 0.12
	Deep Cove	*P. yezoensis*	7.9 ± 0.4	66.4 ± 1.4	0.93	11.23	4.40 ± 0.10	1.24 ± 0.20
17-07-97	W Ross Island	*P. yezoensis*	1.6 ± 0.1	23.1 ± 0.1	0.43	1.31		
28-07-97		*P. purpurea*	2.8 ± 0.3	32.3 ± 0.7	0.31	1.79		
20-09-97	(2) Huckins	*P. yezoensis* (nets)	2.7 ± 0.2	39.2 ± 0.1	0.80	4.32		
		P. purpurea (shore)	2.9 ± 0.3	43.2 ± 0.5	0.81	4.46		
	Mathews Island	*P. purpurea*	3.0 ± 0.3	50.7 ± 1.4	0.80	6.45		
	Deep Cove	*P. purpurea*	3.4 ± 0.1	50.3 ± 0.2	0.85	7.25		
21-11-97	(3) Huckins	(4) *P. yezoensis* (nets)	8.1 ± 0.3	73.9 ± 0.8	0.91	10.54		
		P. purpurea (shore)	3.3 ± 0.5	44.8 ± 0.6			1.00 ± 0.02	0.11 ± 0.01
	Deep Cove	*P. purpurea*	7.1 ± 0.8	62.5 ± 0.4	0.91	10.59	2.81 ± 0.06	0.45 ± 0.06
20-08-98	(2) Huckins	*P. purpurea* (shore)	2.0 ± 0.3	24.7 ± 0.1	0.69	2.98		
	Mathews Island	*P. purpurea*	2.5 ± 0.2	29.9 ± 0.3	0.80	3.52		
	Deep Cove	*P. purpurea*	3.3 ± 0.1	40.6 ± 0.1	0.87	6.70		
17-11-98	(5) Huckins	*P. yezoensis*	7.8 ± 0.1	63.6 ± 0.1	0.99	9.40	2.96 ± 0.06	0.68 ± 0.10
	Treats Island	*P. yezoensis*	7.6 ± 0.3	72.2 ± 0.2	1.07	11.46	3.08 ± 0.06	0.55 ± 0.08
02-12-98	Huckins	*P. yezoensis*	7.3 ± 0.3	65.1 ± 0.1	1.01	9.59		
	Treats Island	*P. yezoensis*	6.7 ± 0.2	66.0 ± 0.2	0.87	11.81		
17-12-98	(6) Huckins	*P. yezoensis*	6.3 ± 0.4	56.9 ± 0.7				
	Treats Island	*P. yezoensis*	6.2 ± 0.4	57.8 ± 0.1				
02-03-99	Beaver Harbour	*P. umbilicalis*	4.1 ± 0.2	38.6 ± 0.4	0.97	10.33	0.26 ± 0.01	0.12 ± 0.01
	E Ross Island	*P. umbilicalis*	5.6 ± 0.4	48.2 ± 0.6	1.02	13.76	0.98 ± 0.03	0.50 ± 0.01

Notes: (1) Intense scallop dragging near Huckins in December 1996.
(2) No scallop dragging at that time of the year.
(3) Reduced scallop dragging in November 1997.
(4) Daily fertilization of nori nets with 2.5 μM N (NH_4NO_3) and 0.25 μM P (triple phosphates 42%).
(5) Intense scallop dragging near Huckins in November 1998.
(6) Reduce scallop dragging in December 1998.

and photoperiod-controlled, lighted incubators (with cool-white fluorescent lamps) and 3 aluminium light-temperature gradient tables. The gradient plates were used to define light and temperature optima for each of the taxa, as well as selected strains (Yarish et al., 1979; Yarish & Edwards, 1982; Egan et al., 1989). Conchocelis fragments (around 50–70 μm) of each strain were placed in Corning Costar cell-wells (6-welled with a lid). About 20 mL of culture medium (VSE) were placed in each cell-well containing approximately 30 conchocelis fragments. Fully factorial experiments were employed using combinations of light (10, 20, 40 μmol photon m^{-2} s^{-1}) and temperature (5, 10, 15, 20 °C) on the temperature gradient tables at 12:12, L:D. Exponential growth was determined from the increase in projected area of the filamentous tufts measured weekly. Conchocelis specific growth rates (SGR) were calculated as the percentage of colony area increase per day, using the formula:

$$SGR = (\ln A_2 - \ln A_1)/(t_2 - t_1),$$

where A = area and t = time which assumes exponential growth (DeBoer et al., 1978; Stekoll et al., 1999).

Results

Integrated aquaculture

In December 1996, *Porphyra yezoensis* collected from Huckins (intense scallop dragging) and Deep Cove (salmon farming) had total P and N contents higher than those of *P. purpurea* from Dipper Harbour (remote from aquaculture activities). The differences corresponded to variations in P and N concentrations of seawater between the first two locations and the latter one (Table 1). The phycoerythrin and phycocyanin contents of *P. yezoensis* were similar at both sites.

Table 2. Morphological characteristics and seasonality of the species of *Porphyra* in New England and the Canadian Maritimes

Species	Cell layer(s)	Cross sectional thickness	Thallus shape	Gametangial position	Fertile blade seasonality
P. amplissima	2	50–80 μm	lanceolate/oblong	Male/female intermingled at margins	spring/summer/autumn^{1}
P. miniata	2	30–70 μm	ovate/round	Male/female in distinct longitudinal halves	spring/summer^{1,2}
P. linearis	1	25–50 μm	linear/flat	Apical male preceding female streaks	winter^{1,2,3,4,5}
P. leucosticta	1	25–50 μm	round/oblong	Male/female in distinct patches at margin	winter/spring^{2,3,4} spring/summer^{1}
P. umbilicalis	1	>80 μm	short round	Male/female intermingled at margins	all year^{1,2,3,4,5}
P. purpurea	1	35–50 μm	linear/ovate	Male/female in distinct longitudinal halves	summer/autumn^{1,2,3,4,5}
P. carolinensis	1	20–30 μm	ovate/lanceolate (serrulate edges)	Male/female in distinct marginal patches	autumn, winter^{4}

Notes: ^{1} Canadian Maritimes and Maine; ^{2} Maine; ^{3} Massachusetts; ^{4} Connecticut; ^{5} New York.

In July 1997, *P. yezoensis* collected from nets at West Ross Island had low levels of tissue P and N corresponding to low levels of P and N in seawater. In the same environment, native *P. purpurea*, growing on the weir poles, had higher levels of tissue P and N, and survived the summer better.

In September 1997, at a time when the scallop season was closed, both *P. yezoensis* on nets, and *P. purpurea* on shore, at Huckins, had relatively low levels of tissue P and N corresponding to relatively low levels of P and N in seawater. *Porphyra purpurea* on shore at Mathews Island, and on buoys of the anchorage system of salmon cages at Deep Cove, had higher tissue N content corresponding to increasing N concentrations in seawater.

In November 1997, because of the much reduced scallop dragging activity the nori nets at Huckins were fertilised daily at low levels [2.5 μM N (as NH_4NO_3) and 0.25 μM P (as triple phosphates 42%)]. This treatment allowed *P. yezoensis* on nets to reach similar P and N contents as in the previous year. This low level of fertilisation did not diffuse much beyond the nets because *P. purpurea* from the shore, approximately 200–300 m away, displayed much lower tissue P and N levels. However, *P. purpurea*, on the buoys of salmon cages at Deep Cove, showed high levels of tissue P and N. There was also a marked difference in phycobiliprotein contents in *P. purpurea* between the two locations.

In August 1998, there was an increase of tissue P and N contents in *P. purpurea* from Huckins to Mathews Island and Deep Cove concomitant with an increase of seawater P and N concentrations.

In November 1998, intense scallop dragging resumed at Huckins and high levels of tissue P and N were again recorded in *P. yezoensis*. Nets with the same species were installed at the Treats Island salmon aquaculture site on November 2, 1998. Two weeks later, similar P concentrations in seawater led to similar tissue P contents; the tissue N content at Treats Island became higher, associated with higher seawater N concentration. Phycobiliprotein contents were similar at both sites. During the late autumn, tissue P and N contents in *P. yezoensis* decreased at both sites, at a time when seawater temperature was decreasing by 1 °C per week and growth of *P. yezoensis* ceased at the end of November (A. Stevenson, pers. comm.).

In March 1999, *P. umbilicalis*, growing in conditions of high nutrient levels in seawater, displayed lower tissue P and N contents than *P. yezoensis* and *P. purpurea* exposed to similar conditions. Nutrient and phycobiliprotein contents at the aquaculture site (East Ross Island) were higher than at the site remote from such activity (Beaver Harbour).

Phenology of native north-west Atlantic Porphyra *species*

Porphyra umbilicalis is by far the most abundant species, both spatially and temporally, within the Gulf

Table 3. Conditions for vegetative conchocelis exponential growth of New England and Canadian Maritime species of *Porphyra*

Species	Growth rate range (% area $d^{-1} \pm$ SE)	Irradiance range (μmol photon m^{-2} s^{-1})	Temperature range (°C)	Upper lethal temperature (°C)
P. amplissima	8.82 ± 0.77	10–40	5–15	22
P. miniata	unknown	10–40	5–15	20
P. linearis	2.67 ± 0.45	10–40	5–20	22
P. leucosticta	6.42 ± 0.57	10–40	5–20	22
P. umbilicalis	6.98 ± 0.51	10–40	5–20	25
P. purpurea (NS)	6.41 ± 0.90	10–40	5–15	20
(LIS)	8.42 ± 1.00	10–40	10–20	25
P. carolinensis	unknown	10–40	10–20	25

of Maine and LIS (Table 2). It occurs throughout the year within the eulittoral zone. *Porphyra amplissima* is most abundant within the northern Gulf of Maine, occurring particularly during spring-summer within coastal and disjunct estuarine environments. It is most abundant within the low eulittoral and sublittoral zones. *Porphyra linearis* occurs in winter along the coast of north-east America and extends as far south as eastern LIS within the upper eulittoral zone on open coastal habitats.

Young fronds of *P. leucosticta* are initiated in late autumn. Typically they are found growing as epiphytes on fronds of *Chondrus crispus, Fucus vesiculosus* and *Polysiphonia lanosa* (which is itself growing on *Ascophyllum nodosum*) but as winter progresses they may also be found on other algae and occasionally on rocks within the lower eulittoral and upper sublittoral zones. By early summer, the foliose thalli disappear in eulittoral habitats, but may persist subtidally. The species is found from Newfoundland to LIS.

Limited knowledge is available about the phenology of *P. miniata* and *P. purpurea*. The former has been found in early autumn and winter from Nova Scotia to the coast of New Hampshire. The latter is enigmatic in its distribution, with initial reports only occurring in the Canadian Maritimes; however, we have found the taxon as far south as LIS throughout the summer. Plants in the northern part of the range are common throughout the year in coastal habitats, with populations in the Bay of Fundy and the Minas Basin being most common in late autumn and early winter. There appears to be distinct genetic differences between northern and southern populations, as the LIS populations are only found during the summer months.

Porphyra carolinensis grows epilithically (primarily on barnacles) in LIS during late autumn and winter. This appears to be the northern most limit of this taxon in Northeast America.

Physiological experiments

The growth rates, irradiance and temperature ranges, and the upper lethal temperature for vegetative conchocelis exponential growth of eight strains of seven species of *Porphyra* from New England and the Canadian Maritimes are given in Table 3. The conditions (irradiance and temperature ranges and time) for conchosporangium induction and conchospore maturation are indicated in Table 4.

Discussion

An emerging consequence of increasing finfish aquaculture activities is significant loading of inorganic nutrients in coastal waters (Beveridge, 1987). Ackefors and Enell (1994) estimated that 9.5 kg P and 78 kg N per ton of fish are released into the water column per year when the feed conversion coefficient is 1.5 and the contents in the feed are 0.9% P and 7.2% N. With improvements in feed composition, digestibility, and feed conversion efficiency in recent years, the discharge is probably now reduced to 7.0 kg P and 49.3 kg N per ton of fish per year (Ackefors, pers. comm.). If considering only the world farmed salmon production that expanded to 644,092 tons in 1996 (New 1999), this represents a worldwide nutrification of coastal waters by 4,509 t P and 31,754 t N. It is obvious that locally each habitat can carry only a certain level of monoculture before dis-equilibrium develops. When aquaculture exceeds

Table 4. Conditions for conchosporangium induction and conchospore maturation of New England and Canadian Maritime species of *Porphyra*

Species	Irradiance range (μmol photon m^{-2} s^{-1})	Temperature range (°C)	Time (weeks)
P. amplissima	10–40	5–15	3–4
P. miniata	unknown	unknown	unknown
P. linearis	10–40	5–15	4–6
P. leucosticta	10–40	5–15	4–6
P. umbilicalis	10–40	5–20	4–6
P. purpurea (NS and LIS)	10–40	10–15	4–6
P. carolinensis	10–40	5–15	4

the carrying capacity of coastal waters, severe disturbances including diseases, eutrophication, harmful algal blooms, and green tides – can occur in the receiving waters (Folke & Kautsky, 1989). Moreover, intense biofouling of fish cages represents additional problems for the finfish aquaculture industry itself (restriction of water/oxygen/nutrient circulation patterns and displacement of cages and their anchoring systems).

Approximately 72% N and 70% P in feed are not retained by fish (Ackefors & Enell 1990). Nutrient release may be reduced by controlling leaching from food and trapping or stabilising faecal matter (Phillips et al., 1993). The development of polyculture systems represents a promising solution by integrating the culture of macroalgae into finfish/shellfish culture (e.g. Ryther et al.,1978; Subandar et al., 1993; Troell et al., 1997; Neori & Shpigel, 1999). Kautsky et al. (1996) developed the concept of 'ecological footprint', defined as the area of open coastal waters required to cancel the eutrophication effects of each square metre of aquaculture activity. The N released by 1 m^2 of salmon aquaculture requires 340 m^2 of pelagic production for its assimilation, while the P production requires 400 m^2 of pelagic production. By integrating *Gracilaria* culture (for the agar market) with salmon aquaculture in Chile, these authors were able to reduce the ecological footprints to 150 m^2 for N and 25 m^2 for P. Not only does integrated seaweed production benefit coastal ecosystems by reducing nutrient loading, macroalgal production also represents a saleable product. Our research programme is aimed at developing a similar integrated system using *Porphyra* (for direct human consumption and biotechnology markets).

Seaweeds are able to take up actively and accumulate nutrients in large quantities (P concentration factor up to 10^5; Chopin et al., 1990a). Variations in tissue nutrient content in field-collected macroalgae reflect seasonal variations in ambient seawater nutrient concentration (Chopin et al., 1990b), as well as inherent species-specific differences in the ability to sequester nutrients. Our results demonstrate that *P. yezoensis* and *P. purpurea* respond to high nutrient loading in coastal waters resulting from anthropogenic activities (salmon aquaculture and intense scallop dragging). It is also worth noting that the nutrient levels reached by these two species (8.5 mg P g DW^{-1} and 73.9 mg N g DW^{-1}) are very high. For comparison, the highest P content recorded by Chopin et al. (1996) in *Ascophyllum nodosum* was 3.2 mg P g DW^{-1}, 3.7 mg P g DW^{-1} in *Polysiphonia lanosa*, and 5.4 mg P. g DW^{-1} in *Pilayella littoralis*. The highest P content recorded by Gallant (1993) in *Chondrus crispus* was 5.2 mg P g DW^{-1}. The highest N content recorded by Chopin et al. (1996) and Gallant (1993) were 26.8, 43.0, 41.4, and 45.1 mg N. g DW^{-1} in *A. nodosum, Polysiphonia lanosa, Pilayella littoralis*, and *C. crispus*, respectively.

Photosynthetic production and growth by marine macroalgae depend to a large extent on thallus morphology. Littler and co-workers (1982, 1983) identified the flat sheet as the most productive morphotype. Thallus thickness has been strongly and negatively correlated with the maximum rate of photosynthesis (Enriquez et al., 1995). The high levels of production and nutrient accumulation displayed by *Porphyra* (thin blade with 1 or 2 layers of cell and all involved in nutrient absorption) are sufficiently great to make *Porphyra* an excellent choice for eutrophication abatement via polyculture, while also providing a valuable product upon harvest (e.g. Merrill et al., 1992; Cuomo et al., 1993).

It could be argued that a thallus of high surface area to volume ratio, while creating an efficient nutrient "scrubber", does not allow storage in reserve tissues like those of large brown algae (i.e. Laminariales and Fucales). However, the advantage of *Porphyra* is its rapid growth (less than 40 days from seeding to first harvest in net culture; Merrill, 1989), which may permit repeated harvesting of a net-grown crop every 9 to 15 days (I. Levine, pers. comm.). Consequently, frequent harvesting amounts to a continuing removal of significant quantities of nutrients from coastal waters, hence, validating the concept of using *Porphyra* as a biological nutrient removal system integrated with finfish aquaculture (Chopin & Yarish, 1998).

The previous estimates of 37 and 35 nets required to remove 9.5 kg P and 78 kg N, respectively, per ton of fish per year (Chopin & Yarish, 1999) need revising to reflect improvements in controlling P and N discharges (Ackefors, pers. comm.). The new values for the bioremediation of the P and N nutrification process per ton of fish per year are now 27 and 22 nori nets, respectively. It must be emphasised that these numbers would be for the complete scrubbing of P and N, which is not the ultimate goal. The latter should be to reduce nutrient concentrations in seawater below the threshold triggering devastating and costly hypertrophic events; consequently, it is anticipated that fewer nets would be necessary.

The efficiency of the different species of *Porphyra* as nutrient scrubbers remains to be compared in order to decide which candidate(s) would be best suited for bioremediation. Our results indicate the possibility of variations in P and N absorption and phycobiliprotein production at different seasons, within and between species. *Porphyra yezoensis* and *P. purpurea* appear to be at the high end of the range for total P and N tissue and phycobiliprotein contents in high nutrient environments. Beer & Eshel (1985) reported that *Gracilaria* sp. had only 1.25 mg g FW^{-1} of phycoerythrin and 0.11 mg g FW^{-1} of phycocyanin. There was, however, no indication that the tissues of these field-collected plants were P- and/or N-saturated to evaluate if they had reached their maximal nutrient removal capabilities, and maximal production of these commercially important, nitrogen-rich pigments.

Aquaculture of *P. yezoensis* in Maine and the Bay of Fundy is problematic. Production is not only severely limited during summer months by low levels of inorganic nutrients in seawater, it also ceases during cooler months. This represents a significant loss of potential income. If the emerging North America nori industry is to grow and mature, enhanced cultivation techniques and new cultivars are required, including the cultivation of native species at different time of the year and in diverse habitats. Such an enhanced usage of native taxa requires detailed and basic knowledge regarding controls of growth and nutrient accumulation (Thomas & Harrison, 1985), propagation, and husbandry of the conchocelis and foliose stages, plus the selection of strains containing valuable pharmaceutical and biotechnology compounds.

Four native *Porphyra* taxa, each of which is now in unialgal culture at the University of Connecticut (Yarish et al., 1998, 1999), are likely choices for bioremediation and nori crop production in New England and the Canadian Maritimes. *Porphyra linearis* grows from late November to March, *P. leucosticta* from late autumn to early summer, *P. amplissima* grows at a sustained rate in late spring/early summer, while *P. purpurea* is prolific throughout the warmer months depending upon location (southern or more northern population). *Porphyra leucosticta* and *P. linearis* exhibit excellent gustatory properties, while *P. purpurea* and *P. amplissima* may be of marketable quality for a variety of industrial and biotechnological uses (taurine and r-phycoerythrin).

Current nori farming technology relies on conchocelis growing in bivalve shells to produce conchospores to seed nets (Mumford, 1990). Being able to optimise the growth of free conchocelis under varying regimes of light, temperature and photoperiod have enabled the mass culture of conchocelis for shell inoculation, as has been reported by Waaland et al. (1990) in Washington State. Experimental commercial cultivation with hard shelled clams (*Mercenaria mercenaria*) that have been inoculated with conchocelis of *P. amplissima*, *P. leucosticta*, *P. linearis* and *P. purpurea* is now possible. However, controlling the development of the conchocelis, conchospore formation and release from these shells, as has been achieved for the free-living LIS population of *P. purpurea*, is still needed. This is significant for these taxa since it is the first time that successful cultures have been cycled to produce thalli via conchocelis 'seeded' shells. However, Stekoll et al. (1999) emphasised that caution must be exercised, as environmental responses may be species and strain specific. Therefore an ongoing selection process for strains that would be most appropriate for aquaculture is required.

An extensive nori culture collection and cultivar improvement programme has been established for local *Porphyra* species (Yarish et al., 1999). It is hoped that by gaining better knowledge of the ecological requirements of these native species, viable commercial entities can be identified. Through such a programme, genetically improved nori cultivars will be developed, just as has been done in Japan (Bergdahl, 1990). Ultimately, the most promising plants (i.e. the ones that have the most advantageous shapes, taste, appropriate maturation periods for particular sites, sufficient monospore production, and superior nutrient absorption and pigment composition) will be made available for 'grow-out' cultivation.

One of the primary benefits derived from this research programme will be its contribution to the reduction of inorganic nutrient discharge into coastal waters, decreasing the potential for outbreaks of devastating and costly hypertrophic events (Bruno et al., 1989; Reguera et al., 1998). An additional benefit, to operators of finfish aquaculture, is that the currently discharged (unassimilated and/or excreted) P and N, which represent a loss of money in real terms, will be captured and converted into the production of saleable nori and biochemicals. This in turn can generate revenue that more than compensates for the added expenses associated with growing *Porphyra* (Troell et al., 1997). Furthermore, as legislative controls on the discharge of inorganic nutrients into coastal waters become more stringent, bioremediation via the production of nori will aid the finfish aquaculture industry avoid non-compliance. Finally, results from this research programme should assist in the economic development and job diversification of this sector by developing a new, sustainable, and environmentally responsible approach to optimising the efficiency of aquaculture operations, while maintaining the health of coastal waters.

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