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Introduction

The most valuable algae of commerce belong to the genus *Porphyra*, a genus of marine red algae utilized mainly as the human food supplement, nori. Worldwide annual sales of *Porphyra* are estimated at $1-2 billion (U.S.). The current North American retail value of imported nori is $20-30 million (U.S.) per year, with a projected growth of 10% per annum (Merrill 1993). This high growth rate is partially fuelled by an increased awareness of the dietary benefits of consuming marine plants. Health conscious consumers are turning from animal products, and are searching for diverse vegetable alternatives. Cultivated *Porphyra* from Japan, China, Korea and the Philippines accounts for approximately 90% of the world's commercial supply and exceeds one billion U.S. dollars in annual retail value (Jensen 1993).

The cultivation and utilization of *Porphyra* in China as a food and medicine was first recorded during 533-544 A.D. (Tseng 1981) and its use in Japan was reported over a thousand years ago (Mumford and Miura 1988). During the 1600s the coastal people of Japan managed to collect *Porphyra* spores in nature by using bamboo twigs called "hibi", while the Chinese in southeastern coastal waters used the "rock cleaning" method, applying unslaked lime to remove unwanted species thus permitting conchospores to recolonize (Tseng 1981). Both methods were used to foster increased crop production. Such primitive methods depended entirely on natural spore release and settlement for success; consequently, nori production fluctuated greatly from year to year. Early in the present century the horizontal floating net method, promoted through the efforts of the Japanese government, was introduced to replace the old "hibi" method. Also, the Chinese government supported their scientists in developing *Porphyra* aquaculture in coastal waters to help fishermen propagate crops in the sea.

Significant improvements became possible in 1949 after Kathleen Drew's discovery that the conchocelis was the alternate generation of the foliose thallus phase of *Porphyra* (Drew 1949). The subsequent development of nori as a major aquacultured crop in Japan has proceeded rapidly due to the fact that the growers learned to produce and store seedlings thus making juvenile plantlets available over

an extended season. Commercial cultivation of nori became truly modernized in the 1960s, when floating-net cultivation techniques were further improved. Three species presently used for the commercial production of nori are *Porphyra haitanensis, P. tenera* and *P. yezoensis*. These are being cultivated in China, Korea and Japan. A closely integrated system of aquaculture, technology and modern processing machinery for hoshi-nori (the dried rectangular paper-like sheets of cultivated *Porphyra* thalli) forms the basis for the present industry in Japan and elsewhere (Tseng 1981, Kain 1991, Oohusa 1993).

The rapid development of heavy industries in Japan during the 1960s led to pollution of the air and sea and the loss of *Porphyra* habitat. For example, Tokyo Bay, formerly the site of luxuriant *Porphyra* cultivation, became greatly polluted necessitating the abandonment of the "hibi" cultivation grounds which had been successful for centuries (Aruga 1990). Dredging and filling of nearshore areas, increased ship traffic and oil pollution have further reduced habitat for nori propagation. It was reported that in the 1991-1992 season the Japanese production of seaweeds was reduced by almost 50% due to a combination of poor weather conditions and pollution.

The longstanding importance of hoshi-nori in the Japanese diet, the recognized natural and man-made threats to the *Porphyra* crop, and the potential for further gradual or catastrophic loss of Asian nori habitat made it reasonable to consider regional alternatives for nori production. Small-scale commercial trials of transplanted *P. yezoensis* utilizing Japanese aquaculture technology were successfully conducted in Puget Sound, Washington State (Mumford and Hanson 1987, Waaland et al. 1986). Further expansion of the culture of nori in Puget Sound was not possible for socio-political reasons. Similar attempts to propagate *P. yezoensis* in the coastal waters of Maine by Coastal Plantations have been made since 1993. Success of this aquaculture venture remains to be seen.

The transfer of aquaculture technology for the cultured species of *Porphyra* from Japan and China to Atlantic Canada is problematic for a number of reasons. First, these exotic species are usually grown in comparatively warmer waters than we

find along Maritime coasts during the winter season. Second, transplanted species have the potential for contaminating the local environment by introducing unwanted organisms such as fungi, bacteria and other disease-causing agents as well as foreign plant and animal species. Such chance introductions may be deleterious to the local marine community. By comparison, native species that are already adapted to our environment will be more suitable for propagation under aquaculture conditions.

While none of the commercial species is endemic to the Atlantic coasts, six species of *Porphyra* are reported for the Maritime Provinces (Bird and McLachlan 1992). One of these, *P. linearis*, was identified as a potential source of high quality nori with superior flavour and texture when compared to *P. tenera* and *P. yezoensis* (Craigie 1971, McLachlan et al. 1972, Guptill 1994). The factors responsible for the desirable flavour of *P. linearis* include a relatively high concentration of isofloridoside and several free amino acids such as alanine, glutamic acid and glycine, as well as the absence of amines that impart unpleasant, fishy odours (McLachlan et al. 1972). The characteristics of high quality nori (taste, colour and texture) possessed by *P. linearis* will be advantageous in providing a competitive edge in both the local and export markets since it must compete directly with an established product (Guptill 1994). Prior to our work, attempts have not been made to domesticate this potentially superior species for commercial nori production .

Ecological studies show that *Porphyra linearis* is a winter annual, growing from late October to early April along the north Atlantic coasts at exposed sites in the upper intertidal spray zones (Edelstein and McLachlan 1966). As the fronds mature they produce carpospores and, through an alternation of generations (Fig. 1), spend the rest of the year in a microscopic "conchocelis" phase (Bird et al. 1972, Bird 1973). Conchospore release appears to occur in late summer as the seawater temperature drops to approximately 13-14°C and can continue to some degree until the following June (Bird 1973). The life cycle of this species has been demonstrated for five generations under controlled environmental conditions (Bird et al. 1972). It was found that in most cases, 1-3 months elapsed between transfer to 13°C and release of conchospores. Considerable variations in the growth rate, colour, degree of

branching, morphology of conchosporangia and ability to release conchospores suggest that genetically different strains may exist within this species.

Available information indicates that the fronds of *P. linearis* do not produce neutral spores as do the commercially cultured Oriental *Porphyra* species (Mumford and Miura 1988). Because of this lack of neutral spores in *P. linearis*, repeated harvests of vegetatively produced fronds during the growing season can not be expected. In an effort to create "artificial neutral spores", studies were carried out on the formation of protoplasts from fronds of *P. linearis* (Chen et al. 1988, Chen et al. 1994). The mechanisms by which the protoplasts regenerate into either new fronds or, in some cases, conchocelis filaments are not clear. Further studies are required to elucidate the development of fronds from isolated protoplasts. Laboratory-scale axenic cell-suspension cultures of regenerated *P. linearis* derived from isolated protoplasts have been achieved (Chen 1989, Chen and McCracken 1993), and it has been suggested that mass cell suspension culture could produce hoshi-nori directly (Chen et al. 1990).

Porphyra linearis is a native species, is a renewable resource and poses no threat to the environment. The development of nori aquaculture in this region is considered to be environmentally "neutral" or even positive since *Porphyra,* like other phototrophs, fixes carbon dioxide, releases oxygen and assimilates fertilizer nutrients from the seawater. If grown in conjunction with animal aquaculture systems, these plants would ameliorate the effects of increased nitrogen and phosphorus loads resulting from animal husbandry. Atlantic Canadian coastal waters are relatively free of industrial and human pollution making them ideal for the culture of cold water species such as *P. linearis*.

The collapse of the Atlantic fishing industry underscores the need for greater diversification of marine products from the region. These events have resulted in our receiving some 35 requests for technology to cultivate marine plants, in particular, to establish a Canadian *Porphyra* aquaculture industry. A market study supporting the development of *Porphyra linearis* cultivation was prepared (Guptill 1994). In addition, a detailed research plan with an estimation of costs for the development of *P. linearis*

aquaculture technology was requested by, and supplied to, the Halifax based company 8-veg-8. A copy of the research design and work plan appears in Appendices 1 and 2.

A successful *P. linearis* aquaculture industry would create a new marine food product designated for the export market. Since aquaculture is typically a labour intensive operation, a mature nori industry would create jobs in coastal communities for aquaculturalists, seasonal fisher-growers, and workers in food processing, sales, marketing and other related services.

The Institute for Marine Biosciences (IMB), National Research Council of Canada, has been internationally recognized for its studies of marine algae since it was established in 1952. IMB scientists have developed extensive knowledge on various aspects of the ecology, taxonomy, life history, natural resource production, cultivation, as well as the chemical composition of marine plants. The land-based algal aquaculture farm operating at Charlesville, N.S. was developed by Acadian Seaplants Limited with advice and collaboration from IMB scientists. IMB researchers have accumulated much basic scientific knowledge of *P. linearis*, including life history, ecology, distribution and other aspects of chemistry and physiology. This valuable information can be used to develop *P. linearis* for commercial nori production.

In order to advance the aquaculture of *P. linearis*, it is imperative that studies be conducted on all phases of the life-history. Special attention must be directed to conchocelis growth within seashell matrices, conditions that cause large-scale release of conchospores must be elucidated and methods must be developed to propagate fronds biomass. The following report covers work conducted from January to December 1994.

Materials and Methods

Field observations and sample collections of *P. linearis* were made at Peggy's Cove, Halifax County, Nova Scotia from November to June during low tide and favourable sea conditions. Selected fertile fronds were washed thoroughly in sterile seawater and placed in 60 X 20 mm disposable petri dishes containing D-11 medium (Chen 1988) supplemented with 10 $mg \cdot L^{-1}$ GeO_2 to suppress diatom contamination. The fronds were incubated at 15°C and 12:12 and 16:8 h (light:dark) photoperiod with a photon flux density of 16-40 $\mu mol \cdot m^{-2} \cdot s^{-1}$. After release, the carpospores were washed and collected using a refrigerated centrifuge and seeded onto sterilized scallop and oyster shells for germination and conchocelis development. The shells and developing conchocelis were maintained at 10 and 15°C as well as room temperature and under various light intensities and photoperiods. After two to three months shells with well developed conchocelis were transferred to tank cultures at the Aquaculture Research Station at Sandy Cove. Most of the shells were hung vertically in two 900-L fibre glass culture tanks with maximum temperatures of 10°C while a number of experiments were conducted in 5-L polyethylene culture buckets. Natural light was reduced to 8-20% of ambient irradiance with black fly-screen covers. Experiments were also conducted to determine the effect of culture conditions on frond growth and to minimize epiphyte fouling.

Research Progress to Date

1) Field observations

Periodic observations were carried out at Peggy's Cove from September 1993 to December 1994. Microscopic examination of rock samples collected from the lower margin of the intertidal zone revealed that very young plantlets of *Porphyra linearis* were present as early as the middle of October. In November a few fronds were collected from the lower portion of the intertidal zone. At least two frond morphotypes have been attributed to *P. linearis* (Bird 1973). The broad morphotype was most common at this time, and a few of these were already fertile. By early December, sizable patches of rock in the intertidal zone were becoming covered with fronds.

Fifty individual fronds from a sample of *P. linearis* collected on December 24 had an average length of 185 mm, width of 4.7 mm and blotted fresh weight of 0.04 g. The air-dried weight of these fronds was 24% of the blotted fresh weight.

From December to March, patches of *P. linearis* were observed growing at progressively higher levels in the intertidal zone. By late March fronds were growing in the highest splash zones. Meanwhile, it was noticed that fronds growing in the mid-intertidal zone were deteriorating due to mature carpospore discharge and the spent fronds were being washed from the rocks by wave action. The *P. linearis* cover in these areas was replaced by filamentous red algae, *Bangia spp.* In early April, *P. linearis* was only found in the upper splash zone growing together with abundant *Bangia spp.* The morphology of *P. linearis* changed greatly during April as the fronds became more yellowish-brown in colour and much reduced in length compared to fronds found in January and February. By mid-May, only a few greatly deteriorated fronds remained, and by early June no more *P. linearis* fronds were found.

2) Carpospore isolation and inoculation

a. Oyster and scallop shells were prepared by boiling for a minimum of one hour in 0.5 M NaOH to remove organic matter, rinsed and washed thoroughly with several changes of fresh water. They were then sterilized in an autoclave and packaged for subsequent use.

b. Apparently fertile *P. linearis* fronds were selected from sample collections taken during January to April, the basal holdfast areas excised and discarded, and their surfaces brushed to dislodge epiphytes. The frond segments were treated with GeO_2 (10 $mg \cdot L^{-1}$) solution overnight and placed in D-11 medium to release carpospores under a constant temperature of 10 or 15°C and a photoperiod of 16:8 h (light:dark) with a photon flux density of 16-30 $\mu mol \cdot m^{-2} \cdot s^{-1}$. While carpospore release can be achieved with several temperatures and photoperiods, the present conditions provided for rapid germination of the discharged carpospores.

c. Observations to detect carpospore release were made three times daily (morning, noon and evening). Once carpospores were discharged, the fronds were removed from the dishes and the carpospores were resuspended, collected by centrifugation, rinsed and washed several times with sterilized seawater to reduce potential contaminants.

d. The washed carpospores were inoculated onto previously sterilized shells for germination and conchocelis development at 10 and 15°C with a photoperiod of 16:8 h (light:dark) and a photon flux density of 16-30 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

e. Two days after inoculation some carpospores germinated and elongated to form conchocelis filaments on the surfaces of the shells. Within two weeks the conchocelis developed many branches with some penetrating the shell matrix. Culture media was changed weekly and unwanted contaminants in the cultures were removed. After a month, regions within the shells had become noticeably red indicating that the developing conchocelis had penetrated the shell matrix and were propagating filaments throughout the entire shell.

3) Conchocelis propagation at Sandy Cove

After two or three months, shells with fully developed conchocelis were selected and transferred to tank culture at the Aquaculture Research Station at Sandy Cove. Two fibre glass culture tanks each containing 900 L of filtered (1.0 μm) and UV-sterilized seawater were maintained with a maximum temperature of 10°C by recirculating the water to a temperature controlled sump tank (100L) containing

titanium cooling coils. The recirculation also provided water movement within the main culture tank. Surfaces of the culture tanks were covered with five layers of black fibre glass fly-screen which permitted approximately 8% of the natural incident light to enter the tanks. Approximately 500 shells containing conchocelis were transported to the Station in early May and held in one of the tanks. The alternate tank was readied to receive the shells when media changes were made at two to three week intervals. Six to eight shells were attached to a 70 cm length of 3 mm nylon string threaded through small holes (0.5 mm in diameter) drilled near the hinge area of each shell. The shells were spaced 10 cm apart on the strings which were suspended vertically from small stainless steel wire hooks attached to 1.9 cm PVC pipes bolted in position across the top of the tank. A total of 100 hooks were provided by attaching ten hooks evenly spaced on each of ten pipes. The seawater nutrients were supplemented with a solution of commercial fertilizers (0.5 mM N and 0.03 mM P) (Staples et al.1995). During the first three weeks small amounts of filtered (0.2 μm) seawater were added to maintain the culture volume. In mid-June diatom contamination was observed. The contaminated shells were treated with GeO_2 (10 $mg \cdot L^{-1}$) and reduced light levels by covering the tank with ten layers of fly-screen. After two weeks in GeO_2, the shells were transferred to clean, filtered (1.0 μ), UV-sterilized seawater (supplemented with commercial fertilizer) for one week. This two week/one week alternation was effective in reducing diatoms and was continued until mid-September. At this point both tanks were used to culture the conchocelis with each tank containing approximately 250 shells. On October 3, seawater flushing into one tank was initiated to remove oil resulting from a refrigeration leak. In an attempt to capture conchospores, nylon nori-nets purchased from China were suspended in the tanks, just below the surface. Seawater flushing for both tanks was in effect by mid-October.

The first evidence of conchospore release and possible germination on the nets was noted on October 14. Examination in late-October revealed that plantlets failed to develop on the nets, although the appearance of reddish areas comprised of very small plantlets were observed on the walls of the sump in the splash zone just beneath the return pipe from the main culture tank (900 L). Some of these fronds had

grown to a length of almost 2 cm by late November, but by January most had become fouled with diatoms. Some fronds were found growing attached to a portion of the fly-screen cover that had sagged and was immersed in seawater. Approximately 30 fronds were found growing attached to the concrete below the outflow where they were continually flushed with nutrient supplemented seawater. These fronds grew to an average length of approximately 70 cm and width of 3 cm in two months.

Experiments to study maturation and discharge of conchospores were conducted in 5-L air-agitated culture buckets. Sets of four shells containing conchocelis which germinated from carpospores inoculated in January, February, early March or late March were placed in separate buckets and maintained in the greenhouse in a water-bath system at 10°C (Table 1). The buckets were continuously flushed with freshly pumped seawater at the rate of six volume changes per day. Nutrients were supplied with commercial grade fertilizer twice a week to give initial nutrient levels of 0.5 mM N and 0.03 mM P. Duplicate buckets were set up in a system that was maintained at a maximum of 12°C. All buckets were covered with three layers of fly-screen which cut the incident light to approximately 20%. Additional experiments were set up to determine the effect of various nutrient levels on conchospore release (Table 2). Sets of four shells inoculated in early March were placed in air-agitated buckets as described above. Four nutrient levels were tested: normal (i.e. 0.5 mM N and 0.03 mM P), 50% of normal, 25% of normal and no addition. As above, one set of buckets was maintained at 10°C and the other controlled at a maximum of 12°C. Approximately every two weeks the shells were temporarily removed from the buckets and cleaned using a quick freshwater spray. Any green algae were spot-treated with alcohol using a cotton applicator. The buckets were also cleaned before the shells were returned.

By the end of August all conchocelis in the inoculation time series were well developed and brownish-purple in colour. Fouling by diatoms was most severe on the shells inoculated in January and least on those inoculated in late March. Green algal fouling was light to moderate on all shells. In the nutrient level experiments the conchocelis receiving only seawater (no nutrient addition) were greenish-yellow in

colour and the shells had little diatom or green algal fouling. All remaining conchocelis in the 25%, 50% and normal nutrient supplementation were brownish-purple in colour and the shells were fouled lightly with diatoms and green algae.

In early October, small *P. linearis* fronds were found growing in some of the buckets just below the surface and in the splash zone just above the water line (Table 1). Buckets containing conchocelis seeded in January had the most developed fronds. Nylon nori-nets were placed in the buckets in an attempt to capture released spores. These nets were not effective in obtaining plantlets from germinated conchospores.

4) Cultivation of *P. linearis* in tanks

Experiments were conducted to determine whether or not *P. linearis* fronds could be propagated totally submerged in air-agitated culture tanks. Fifteen young fronds collected from Peggy's Cove in early January with an average length of 7.9 cm propagated for a four week period in a 5-L culture tank increased to an average of 36.8 cm in length. Eight of the fronds survived an additional four weeks and increased to an average length of 52.9 cm. These results indicate that *P. linearis* can be propagated to sizes much larger than those in natural populations, and suggests that future nori aquaculture could utilize totally submerged nets in the sea.

Tests were conducted to determine whether the harvested crops could be increased in fresh weight biomass through additional propagation in tanks. The results shown in Table 3 indicate that the biomass of wild populations can be doubled within two weeks when cultured in tanks under natural conditions with nutrient supplementation. Prolonged culture and high densities of fronds in a tank resulted in reduced growth.

5) Epiphyte fouling on various developing stages

Epiphyte fouling will be one of the major barriers in producing high quality nori and strategies must be developed to overcome the problem. During the winter, *P. linearis* grows in the intertidal zone of open coastlines, a high energy environment that

markedly reduces competition from other species. In addition, the fronds are exposed to the air at low tide which results in drying and freezing, while during high tide the fronds are re-immersed and thaw. This frequent freeze-thaw cycle that *P. linearis* fronds experience in nature undoubtedly minimizes fouling. For example, fronds collected particularly in January and February are virtually free of fouling organisms. When these plants were maintained in culture tanks they eventually became fouled. Preliminary observations indicate that the extent of fouling appears to depend on culture density, nutrient supply, irradiance and degree of aeration or water movement.

Several strategies were investigated to control contaminating species and epiphytes on *P. linearis* including: a) reduced pH; b) reduced salinities, and c) freezing and thawing.

A. Fronds

a) Reduced pH - Concentrations of 0.05 to 0.50 M citric acid in fresh water at pH ranging from 2 to 4 at temperatures between 4 and 7°C for 30 min were effective at killing diatoms, but adversely affected *P. linearis*. Citric acid in seawater did not result in acceptable diatom kills, even at 0.1 M (pH 2).

b) Fresh water soak - Immersion in fresh water for 15 to 60 min damaged *P. linearis* and was not effective against diatoms. Fronds supplemented with N and P were damaged to a greater extent than nutrient deficient fronds.

c) Freezing and thawing - A 15 min fresh water pre-soak followed by a 15 min freezing in seawater to -15°C was somewhat more effective in killing diatoms than a simple seawater freeze treatment. Effects of the pre-soak on *P. linearis* were variable. Fronds tested in mid-February showed little or no damage, while fronds tested in mid-March sustained low to high mortality depending on their prior growth conditions. Fronds collected from the field not more than three days before treatment were affected the least by freezing and thawing. Tests in which plants were pre-soaked in 25% seawater provided good diatom kill with only minimal effects on *P. linearis*. This treatment appears to be the most promising and must be examined in detail.

B. Carpospores

a) Fresh water soak - A 15 min fresh water soak was not lethal to *P. linearis* carpospores. Subsequent germination of treated spores was marginally suppressed relative to the control.

b) Freezing and thawing - Carpospores died rapidly when submitted to a 15 min fresh water pre-soak followed by 15 min of freezing. Carpospores would survive a pre-soak in 25% seawater for 15 min followed by a 15 min freeze.

C. Germlings

Germinating carpospores and well developed filaments (presumably free-living conchocelis) were more sensitive than developing or newly released carpospores. Filaments were destroyed by freezing and thawing in both fresh and 25% seawater pre-soak treatments.

Discussion

It appears that the *Porphyra* populations at Peggy's Cove are not genetically uniform since morphologically distinct forms (broad and narrow) and fronds with different reproductive characteristics are found, in some cases, growing side-by-side throughout the growing season. Our field observations confirm earlier ecological studies on *P. linearis* (Bird 1973). However, it was noted that the majority of fronds collected during January and February from the high energy spray zone were narrow-form fronds. This indicates that strong wave action and severe environmental conditions may also affect the morphology of *Porphyra* populations. Further studies are needed to clarify whether the morphologies of the *Porphyra* fronds are related to genetic differences or merely reflect the effects of environmental conditions on growth.

Populations of *P. linearis* exist for almost eight months from early October to May. It is noteworthy that, as the season advanced, samples of fronds could be collected from sites progressively higher in the intertidal zone. It was noticed that individual frond growth lasts only a few weeks (4-8) before carpospores are discharged and the entire frond is eroded. The fronds did not reappear at the same site until following season. Bird et al. (1972) reported that *P. linearis* lacks neutral spores in its life history, unlike commercially cultivated species which are able to produce repeated crops within a season from neutral spores. If this is the case for *P. linearis* populations in nature, the extended period of population development must be through delayed or extended conchospore release. It was noted (Table 1), that plantlets derived via conchocelis seeded in January and February were much larger than plantlets derived via conchocelis seeded in March. Because fronds grow rapidly (2-3 weeks), the results suggest that conchocelis seeded at various time intervals release conchospores in the order in which they were seeded. This may account for the existence of *P. linearis* populations over extended periods during the growth season. If this is so, one would assume that *P. linearis* does not respond to specific conditions that trigger conchospore release as with the commercially cultivated oriental *Porphyra* species. Without the ability to control conchospore release, it may be

difficult to obtain seedlings for aquaculture at predicted times. It is important in any form of aquaculture to be able to control seed stock supplies, thus further studies are needed on the conditions for conchospore maturation and mass discharge. However, it would still be possible to develop commercial aquaculture of *P. linearis* by staggering the time of carpospore seeding to provide conchospore production over an extended period.

During the 1993-1994 season we used scallop and oyster shells as host substrates for conchocelis propagation. The scallop shells were so thin that the conchocelis either penetrated through the shells or grew on the shell surface which made them difficult to clean and maintain. Oyster shells were too friable to permit cleaning. For future conchocelis propagation we suggest that thicker, sturdier shells be used for carpospore inoculation.

Our preliminary attempts to seed *P. linearis* conchospores indicate that commercial nets used for *P. yezoensis* seedlings were unsuitable. Further tests are needed to define what net materials and seeding conditions are acceptable for use with this species. In addition, the mesh size of the nets should be redesigned to determine optimal size for the relatively narrow fronds of *P. linearis.*

Control of epiphyte fouling organisms is critical to the successful production of aquacultured nori. Samples collected in January and February, during periods of extremely low temperatures when the fronds were subjected to freezing during low tides, contained little contamination. Initial taste tests indicate that samples collected during this period are noticeably better than those collected in March and April. This may be due to the degree of fouling or simply a cold temperature effect. Fortunately, *P. linearis* is tolerant of extremely cold conditions and this may be used to advantage to produce high quality nori products through aquaculture. While severe weather conditions make it difficult for aquaculturists to work, these extreme environments may be the key to the production of high quality nori products with a competitive advantage over available nori products. As a result, the aquaculture technology developed for *P. linearis* would not be transferrable out of this region, thereby protecting regional economic development.

The biomass of naturally collected fronds propagated at optimum densities in tanks with nutrient supplementation can be doubled within two weeks (Table 3) with a notable increase in frond size. These results indicate that the crop biomass can be increased over a short period through the use of post-harvest land-based cultivation. It also indicates that constantly submerged net cultures may be used to propagate *P. linearis* in the sea.

Propagation of conchosporelings to significant sizes was achieved in a flowing nutrient enriched seawater system. This technique of biomass production could be an alternate method to conventional nori net-cultures in the sea. Whether such a system will be economical on a large scale remains to be established.

An estimate of biomass density for commercial culture of *P. linearis* on nets is approximately 20 conchosporelings per centimetre of string net (mesh size 10 cm^2). This calculation is based on fronds collected from nature having an average size of 18.5 cm X 0.47 cm (length X width). Using an average frond fresh weight of 0.04 g, a 1 m^2 net could produce 1.76 kg fresh weight.

In order to develop commercial aquaculture of *P. linearis*, research will be necessary as outlined in the work plan presented in Appendices 1 and 2.

Conclusions

1. The varying morphotypes of *P. linearis* populations observed side-by-side at Peggy's Cove during the course of this study may reflect the effects of environmental factors or genetic differences.
2. Populations of *P. linearis* can exist in nature for almost eight months, although individual fronds last only a few weeks before becoming fertile, releasing carpospores and eroding. This suggests that several crops of *P. linearis* could be harvested from an aquaculture system during a growing season.
3 Fronds collected during extremely cold weather in January and February were found to have very little contamination. Tidal cycles act on *P. linearis* populations by alternately submerging and exposing the fronds resulting in episodic freezing and thawing. Such cycles may contribute to a reduction in fouling and contamination.
4. The use of freezing and thawing techniques appear promising for controlling epiphyte fouling of cultured *P. linearis*. Our experiments suggest that, for maximum diatom kill, consistent with minimal damage to fronds of *P. linearis*, a 15 min pre-soak in 25% seawater followed by freezing for 15 min at -15°C is beneficial.
5. To establish clean unialgal conchocelis stock cultures, it is suggested that the isolation of carpospores be conducted early in the season when fouling is minimal.
6. Scallop and oyster shells are inferior substrates for developing conchocelis cultures. In order to maintain high density and clean seedling cultures it is recommended that the shells be relatively thick, sturdy and smooth to facilitate manual cleaning.
7. Conchocelis developing within shells held in tank cultures released conchospores which germinated to form plantlets as early as October. Conchospore release continued for several weeks, but factors that stimulate conchospore release remain to be determined.
8. The commercially available nets for nori seedling culture cannot be used for *P.*

linearis without a reduction of the mesh size.

9. Biomass density must be considered in seeding conchospores on nets. According to our calculations, seeding density should approximate 20 spores·cm^{-1} of net string with a mesh size of 10 cm^2.
10. The growth of young fronds collected from nature can be enhanced to increase the frond size more than three-fold by propagation in aerated nutrient supplemented tanks.
11. Conchosporelings of *P. linearis* propagated indoors, in a continuous flow of nutrient enriched seawater, reached significantly large frond size (70 cm X 3 cm, length X width) within two months. It is possible that such an indoor culture technique can be developed for *P. linearis* aquaculture.

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Table 1. **Observation of *P. linearis* plantlets[a] germinated from conchospores released from conchocelis seeded at various dates.**

Observation Date	12-Oct-94		02-Nov-94	
Culture Conditions	A[b]	B[c]	A[b]	B[c]
Date of Isolation[d]	Plantlet rating[e]			
14-Jan-94	+++	+++	++[1]	-[1]
18-Feb-94	-[2]	+++	+[2]	+++
06-Mar-94	+++[2]	+++	++[2]	+++
25-Mar-94	+++[2]	+	+++[2]	++

[a] It was noted that plantlets derived via conchocelis seeded in January and February were much larger than plantlets derived via conchocelis seeded in March. Because fronds grow rapidly (2-3 weeks), the results suggest that conchocelis seeded at various time intervals release conchospores in the order in which they were seeded.

[b] A, cultures maintained at 8-10°C.

[c] B, cultures maintained below a maximum of 12°C.

[d] Carpospores were isolated for conchocelis germination.

[e] Plantlet rating:

+++ Abundant *P. linearis*

++ Moderate number of *P. linearis*

\+ Few *P. linearis*

\- No *P. linearis*.

[1] Shells were placed in a clean tank 12-Oct-94 to detect the occurrence of conchospore colonization.

[2] Tank cleaned 30-Sep-94.

Table 2. **Effect of various levels of nutrient supplementation on conchospore release.**

Observation Date	12-Oct-94		02-Nov-94	
Culture Conditions	A[a]	B[b]	A[a]	B[b]
Nutrient Treatment	Plantlet rating[c]			
Normal[d]	+	++	+++	++
50%	++	++	++	+++
25%	+	+++	+++	+++
none	-	+	-	++

[a] System A, cultures maintained at 8-10°C.
[b] System B, cultures maintained below a maximum of 12°C.
[c] Plantlet ratings:
+++ Abundant *P. linearis*
++ Moderate number of *P. linearis*
+ Few *P. linearis*
- No *P. linearis.*
[d] Normal fertilizer: 0.5 mM N and 0.03 mM P.

Table 3. **Record of biomass increases of natural *P. linearis* fronds propagated in a 5-L air-agitated bucket.**

Day	Fresh weight (g)
0	23.4
14	46.2
28	63.1
34	63.6

Table 4. **The effect of freezing (-15°C) and thawing on diatoms and *P. linearis* fronds.** [1,2]

Mortality of Diatoms (%)

Treatment	*Licmorpha*	Filament	Tube	Naviculoid
1) FW only	90	90	75	<5
2) FW; Frz	100	100	100	100
3) SW; Frz	100	95	75	90
4) SW50; Frz	100	95	90	90
5) SW25; Frz	100	100	100	100

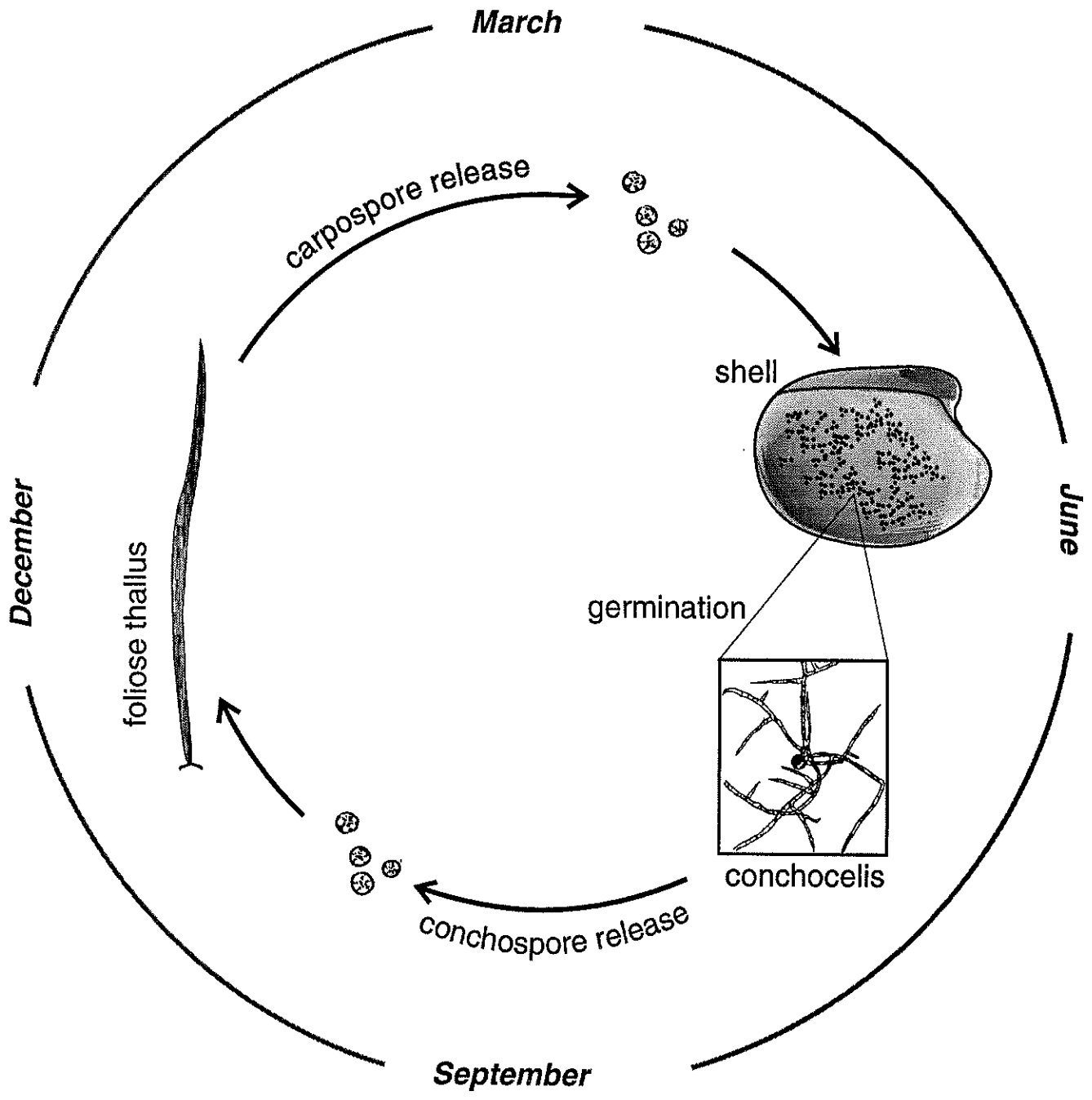
Mortality of *Porphyra* (%)

Treatment	Type I	Type II	Type III
1) FW only	0	0	0
2) FW; Frz	50	<75	10
3) SW; Frz	5	5	5
4) SW50; Frz	0	0	0
5) SW25; Frz	0	0	0

[1] Samples were treated at various salinities for 15 min.
[2] Pre-treatments: FW= fresh water, SW= seawater, SW50= 50% seawater, SW25= 25% seawater, Frz= After treatment, samples were frozen for 15 min at -15°C.
Type I = fronds slightly epiphytized, nutrient replete, water batch changed twice per week.
Type II = fronds moderately epiphytized, high flush rate with no nutrient supplements.
Type III = freshly collected fronds, apparently epiphyte free.

Figure 1

Diagram of *Porphyra linearis* life cycle



Appendix 1

Experimental Design

A number of research activities need to be initiated to facilitate *Porphyra linearis* aquaculture. The following detailed steps outline a research approach that would achieve this goal.

(1) Develop techniques for producing large quantities of conchocelis germinated in shell matrices.

1-a. *Observe and collect natural fronds.* Fronds are present on the seashore only during the winter months, from November to April. For safety reasons a minimum of two people are required to make the observations and collections at each site. Sampling fronds from the intertidal zone must be accomplished during low tide and under favourable sea conditions. For these reasons samples can only be collected during one or two hours on a few days per month. Some of the best collections might be obtained on days when the seas are calm but the air temperature is extremely cold (as low as -20°C). It will be important to begin collecting samples early in the growing season since the quantity and quality of the fronds can change rapidly in nature. Fronds are most abundant during January and February, but as time passes they become increasingly fouled with other algae (i.e. diatoms, *Bangia spp.* and blue-green algae) which contaminate isolated carpospores making unialgal conchocelis culture extremely difficult. By April most of the fronds have become reproductive and deteriorated. Also, it is possible that a storm might destroy entire *P. linearis* populations due to ice-scouring, thus leaving little material for

carpospore isolation.

1-b. *Sort mature reproductive fronds.* Once the fronds are collected they must be transported to the laboratory and placed in vessels of aerated seawater. Individual healthy fronds must then be selected and, using a microscope, screened for signs of reproductive maturity. This procedure is tedious and labour intensive.

1-c. *Treat to minimize contaminating species.* As a precaution and to minimize contamination by other organisms a number of steps must be taken to establish unialgal conchocelis cultures. Once a fertile frond is selected, its basal portion is excised as this region is a major source of fouling algal contamination. The fronds themselves are brushed to remove epiphytic organisms, sonicated and rinsed numerous times with filter-sterilized seawater. This is followed by treatment with germanium dioxide to eliminate diatoms, and with antibiotic solutions to reduce bacterial and cyanobacterial contamination. Alternative methods to clean the fronds without affecting the viability of the spores should be tested.

1-d. *Obtain and clean suitable shells.* During 1994, oyster and scallop shells were obtained and used as substrates for conchocelis growth. During initial inoculation tests it was found that these shells were not suitable for the conchocelis filaments to develop properly. Thicker, sturdier shells such as the quahog or offshore clam shells need to be obtained and tested. Shells must first be cleaned of all organic material and sterilized before they can be used. The best method for cleaning large numbers of shells has to be determined.

1-e. *Collect released carpospores and inoculate cleaned shells.* Clean fertile fronds (1-c,

above) are then placed in sterile seawater medium and exposed to a range of light and temperature conditions in an effort to induce carpospore release. (The optimum conditions for spore release have not yet been determined.) Every two hours the fronds should be checked for released spores and, if present, the carpospores collected by the use of a refrigerated centrifuge. The spores will be washed several times, resuspended in medium, and the spore density determined before they are used to inoculate the shells. The density of spores per shell needs to be ascertained to determine the optimal numbers for seeding.

1-f. *Germinate and propagate conchocelis in shells.* It is estimated that at least 3000 seeded shells will be required for the first year. The seeded shells will be placed under conditions suitable for germination of the carpospores and conchocelis growth. Small-scale experiments will be conducted to determine optimal conditions (temperature and photoperiod) for this process. The culture medium in each dish should be changed weekly and contaminated shells treated and/or cleaned. Heavily contaminated shells should be discarded.

1-g. *Maintain shells with developing conchocelis in culture tanks and attempt to induce early production and maturation of conchosporangia.* Shells containing established conchocelis filaments will be transported to the Aquaculture Research Station at Sandy (Fink) Cove and placed in seawater tanks. The majority of shells will be held in 1000 litre tanks (up to 5 tanks in year 1) and maintained at a maximum temperature of 10°C. The conchocelis will be exposed to natural photoperiods, but at low irradiances (5-10% of natural). The culture media will consist of filtered seawater enriched with nitrogen and

phosphorus nutrients and will be changed on a weekly basis. Germanium dioxide will be added to the medium as required to control diatom contaminants.

On a smaller scale, experiments will be conducted to evaluate conchospore maturation. Separate groups of individual shells (5) containing conchocelis filaments will be maintained under a range of conditions using such variables as light, temperature and nutrient supplements to determine if early maturation of conchosporangia can be induced.

1-h. *Investigate conchocelis growth at two temperatures (10 and 15°C) to determine if maturation of conchosporangia can be advanced.* Small numbers of conchocelis will be grown at 10 and 15°C in the laboratory to determine the effect of temperature on the maturation of the conchosporangia. Nutrient enriched seawater media will be changed on a weekly basis.

(2) Determine conditions for mass conchospore release and attachment to substrates.

2-a. *Observe conchospore release in tank cultures.* A procedure to detect conchospore release in the culture tanks involves taking water samples for microscopic examination after filtration or centrifugation. It will be necessary to sample each culture system on a daily basis starting early in August.

2-b. *Determine the time of day for conchospore release and the duration of release period (hours or days).* Once spore release is detected, five shells containing conchocelis will be cultured separately to observe and determine whether conchospore release occurs in the morning, afternoon or at night. In addition, the duration of the spore release period

will be determined. These shells will be maintained for one year to observe regeneration of the conchocelis, information that will be valuable to operators of a commercial seeding facility.

2-c. *Quantify spore release under "large" (900L) tank culture conditions.* Water samples will be concentrated and the number of released spores will be determined either by microscopic observation or with a Coulter Counter.

2-d. *Prepare and test various substrates and techniques for promoting conchospore settlement.* At the present time there is no information about the most suitable methods for spore settlement and attachment of *P. linearis* spores. Preliminary experiments indicate that commercially available nets designed for *P. yezoensis* are not suitable for *P. linearis* without modification. A seeding protocol must be developed and adapted for use with *P. linearis.*

2-e. *Determine the density of conchospores settled on the substrates.* This procedure entails microscopic examination and determination of the numbers of attached spores.

2-f. *Determine the density of germlings developed (i.e. success of "germination").* This step requires microscopic examination of the substrate to determine the numbers of developing attached sporelings.

2-g. *Storage and culture of sporelings attached to substrates.* To determine the feasibility of storing young sporelings by freezing, small groups of attached young sporeling will be frozen and thawed at various time intervals and then propagated to determine their viability.

(3) Develop cultivation methods for large scale propagation of fronds.

3-a. *Prepare apparatus for testing culture of fronds.* Apparatus for spray and drip culture systems will be designed, constructed and tested.

3-b. *Evaluate growth using tank, spray and drip culture methods as well as conduct limited tests in the sea, depending on site availability.* The biomass produced will be measured and the results from the various culture methods will be compared to determine their effectiveness. Two trials in the sea will be attempted, one at a commercial aquaculture site on the Eastern Shore, and the other in the Bay of Fundy.

3-c. *Test methods for minimizing contamination with weed species.* Contaminated cultures will be subjected to physical and chemical procedures to determine how effectively contaminating species can be eliminated or controlled.

3-d. *Develop methods to harvest, dry and store the biomass.* A low-cost device for rapidly drying the fresh *P. linearis* at ambient or somewhat higher (to 50°C) temperatures must be designed and constructed.

Appendix 2.

Work plan for *Porphyra linearis* aquaculture.