Summary:

The "Seaweed (Porphyra) Aquaculture Enhancement" project represented a synthetic effort by diverse specialists, an industrial partner (Phycogen: formerly Coastal Plantations International, Inc.), and consisted of five subprojects: (1) field and culture evaluations (C. Yarish & A. C. Mathieson); (2) physiological responses to nutrient limitations (I. R. Davison); (3) population genetics (C. D. Neefus, A. S. Klein, A. C. Mathieson and C. Yarish); (4) strain improvement via genetic engineering (D. P. Cheney) and gene cloning (S. Minocha & A. C. Mathieson); and (5) technology transfer and aquaculture extension (I. A. Levine). An assessment of both the native and anintroduced Asiatic species (i.e. Porphyra yezoensis strains U51 and H25) was conducted.

Yarish and Mathieson evaluated the species composition, seasonality, and habitat preferences of native taxa, utilizing detailed seasonal and spatial collections from diverse coastal and estuarine habitats between the Canadian Maritimes and Long Island Sound. Seven previously described species were recorded (i.e. P. amplissima, P. carolinensis, P. leucosticta, P. linearis, P. miniata & P. umbilicalis) plus at least six "cryptic" taxa that are either unknown or undescribed. The various taxa were delineated by a variety of traditional morphometric, cytological, and molecular techniques (see below). Porphyra umbilicalis was by far the most abundant species, both spatially and temporally; four other previously recorded taxa also exhibited cosmopolitan distributional patterns, while one taxon was more northern (P. amplissima) and another more southern (i.e. P. carolinensis). The latter species is newly recorded from New England, with its northern distribution extending to Long Island Sound. Varying patterns of phenology (winter versus spring or summer) and vertical distribution (littoral versus sublittoral) were also apparent for the different taxa, while at least one taxon (P. purpurea) exhibited variable phenological and karyological patterns spatially (i.e. N versus S), suggesting genetic differences. The field samples were also used for various physiological and molecular evaluations (see below), including the preparation of over 130 unialgal cultures of different taxa. The culture studies also demonstrated variable life history patterns, physiological responses, and potentials for domestication by traditional Asiatic cultivation techniques. That is, optimal temperature and light conditions
were assessed, as well as their reproductive potentials via monosporous
and/or conchospores.

In Davison’s subproject dealing with nutrient responses in Porphyra
growth, light absorption by pigments, photosynthesis, and rates of protein
synthesis were enumerated. Nitrogen limitations can reduce all four of
these properties, and it has important implications for commercial nori
aquaculture. For example, Coastal Plantations Inc. (CPI) had problems with
reduced growth and poor crop quality (coloration) when plants were grown
during the summer in Cobscook Bay. The latter period is a time when
nitrogen levels are depleted by “blooms” of microscopic phytoplankton-
i.e. during the transition to warmer water temperatures and higher light
levels. As seaweeds lack specialized absorptive structures (i.e. roots as in
higher plants), they can only absorb nutrients from the surrounding water.
Another important finding was the occurrence of a rapid and dramatic
increase in protein synthesis after nutrient supplements, which suggests
that recovery from low-nitrogen conditions can be rapid. Hence, nitrogen
concentrations, or the ability to increase nutrients via carefully targeted
applications of fertilizers, must be considered during site selection;
nutrients must also be monitored frequently during the growing season in
order to evaluate productivity and crop quality.

During the genetic studies by Neefus and colleagues (see above)
isozyme evaluations of the different Porphyra taxa were initially done,
followed by amplification and sequencing of the ribulose biphosphate
carboxylase large subunit gene (rbcL). Most “cryptic” taxa were initially
designated as “leucosticta-like” plants, due to the occurrence of whitish
spermatangial streaks or bleaches that “characterizes” this taxon. In
retrospect much greater variation was evident within or between
populations of this “species” would be expected; hence, they do not
represent a single taxon. Such results confirm the findings of Bird and
McLachlan (NRC Atlantic Regional Laboratory, Halifax) who noted that
Porphyra species were too broadly interpreted and that they were probably
represented by “form species” composed of multiple entities. The
phylogenetic and phytogeographic relationships of these cryptic taxa were
also closely related to the Asiatic taxon P. yezoensis (see below).
Ultimately a taxonomic monograph of the genus is needed, as well as
enhanced information regarding their genetics, which will aid in future
selection and domestication of native Porphyras.
The gene cloning studies by Minocha & colleagues have primarily emphasized the development of reliable gene transfer to cells, the selection of transformed cells, and regeneration of protoplasts into whole plants. Thus, protocols were developed for regeneration of protoplasts in six native species (i.e. except P. carolinensis), while electroporation procedures for transfer of foreign DNA into protoplasts were perfected for the green and red algae Ulva lactuca and P. miniata, respectively. Data from these transformation studies indicate that the 35S CaMV promoter is also quite effective. However, it only involves a transient expression (i.e. as diagnosed by assays of the GUS gene) and it does not produce a stable transformation. Toxicity studies on the growth of protoplasts of the same six taxa were compared with several antibiotics and herbicides in order to test for the selection of transgenic cells. Spectinomycin is quite suitable, while the potential use of zeocin and blasticidin is still being evaluated. Using PCR techniques collaborators at the University of Connecticut (Professor Thomas Chen) have demonstrated the presence of foreign DNA in Porphyra. Hence, the successful transfer of DNA into the cells/protoplasts, the regeneration of protoplasts into whole plants, and the use of potential selection agents is quite possible. Recent attention has focused upon the cloning of genes and promoters of highly expressed genes. The sequence of two actin genes in all native and some non-native species of Porphyra has almost been completed in order to trace the evolution of this two-gene family and to generate data regarding the phylogeny of different species. Partial DNA sequence information has been obtained for 23 of these cDNAs and several have been sequenced fully. A genomic library is under construction that will be useful for obtaining genomic DNA regulatory domains associated with additional cDNAs.

Strain improvement studies by Cheney and colleagues were primarily directed at developing genetically improved plants of Porphyra that would be specifically adapted for farming in northern Maine. Thus, an attempt was made to produce new strains of P. yezoensis that had the key commercial traits of this species (i.e. excellent taste, color, etc.) and the improved growth potential of one of the local Porphyras, without altering the basic reproductive characteristics of the plant. Protoplast fusion techniques were primarily used to produce somatic hybrids between P. yezoensis and two local species having varying ecologies, namely P. amplissima & P. umbilicalis (see above). Prior to the initiation of these studies only chimeras and "possible" (i.e. unproven) hybrids had been produced. A new protoplast fusion method, which has recently been
 patented (see below), was developed that provides an opportunity to produce either polyploids of an individual taxon (e.g. P. vezoensis) or hybrids between multiple taxa (e.g. P. amplissima and P. umbilicalis). The plants produced by protoplast fusion were initially screened using isoenzyme electrophoresis (using PGI) in order to identify any plants that might have an unusual banding pattern. Subsequently they were analyzed molecularly using both chloroplast (rbcL) and nuclear gene markers (rRNA-ITS1). One hybrid strain between P. vezoensis and P. umbilicalis (i.e. #19-3) had the morphology of the latter species, a lighter pigmentation, and a greater DNA content. Using the same procedures outlined above a polyploid strain of P. vezoensis (#9-13) was produced, based upon protoplast fusion between P. vezoensis and P. umbilicalis. It has a blade shape (long & narrow) and isoenzyme pattern like P. vezoensis, but its chromosomes and DNA compliment are double (i.e. n= 6 versus 3 & 0.35 pg versus 0.175 pg); further it has rbcL and ITS1 PCR patterns that are identical to those of P. vezoensis. The similarity of its reproduction and freezing tolerance to strain U-51, plus its enhanced growth and pigmentation offers several advantages for nori farming in northern Maine and Canada. At the same time it is environmentally safe. Lastly it should be noted that the methods are applicable to any species of Porphyra, producing new and reproducible plants.

The primary goal of the technology transfer/aquaculture extension subproject (Levine) was to establish a commercially viable seaweed aquaculture industry in New England. At this point the final evaluation is incomplete, with the former corporate partner (Phycogen or formerly Coastal Plantations International, Inc.) withdrawing from active marine agronomic activities. Currently the concept is continuing via bioremediation and polyculture activities in Connecticut, involving the University of Connecticut, several corporate partners, and I. Levine (see below). The technology transfer part of the original project concentrated on Japanese nori technologies; it involved multiple trips to Japan by CPI personnel in order to review the activities of farmers and processors. Further, during the stay of Dr. Xiugen Fei’s (Professor and Director of the Experimental Marine Biology Laboratory, Institute of Oceanology, Chinese Academy of Sciences)at the University of Connecticut he visited CPI’s Eastport facility, which was followed up by a visit by I. Levine to China, establishing a technology transfer relationship for Chinese nori farming technologies. Aquaculture extension efforts were divided into four parts: recruitment of farmers; a course in nori farming; a training manual; and an
extension course. CPI completed a nori farming course that ran for six months; it covered the biology, economics, equipment, assembly, seeding, cultivation, and harvesting of nori. Recruitment efforts included a nori training seminar, plus advertisements in local newspapers (Quoddy Times) and other professional publications (Commercial Fisheries News). Additional exposure came from various news articles, radio interviews and television coverage. A total of 82 individuals contacted the company from New England, New York, Canada, Israel, Korea, South Africa and Vietnam. Development of a Sea Grant Extension training manual and the translation of the Japanese Nori Farmer handbook were the two major written products of this subproject.

The effects of Porphyra yezoensis cultivation on native vegetation within Cobscook Bay were also evaluated (Klein, Mathieson, Neefus & Cheney), utilizing detailed molecular methods perfected during the earlier "Enhancement" studies, plus ecological assessments of in situ populations at Huckins Ledge and Mathews Island- i.e. where CPI previously cultivated P. yezoensis. Two "cryptic" taxa were found in Cobscook Bay, with one being similar to P. leucosticta and the other being more closer to P. purpurea or P. umbilicalis. The genetic similarity between P. yezoensis (strain U-51) and P. leucosticta was previously noted. Thus, it only differed by three nucleotide substitutions in the rbcL gene, which is an evolutionarily conserved trait. Such observations raise two important questions: (1) Have there been recent introductions of Pacific Porphyras into the North Atlantic as a result of trans-global commerce over the last few centuries?; (2) Did these North Atlantic Porphyras, which are genetically related to the Japanese species, arrive during much earlier biogeographic dispersal events, extending across the Arctic Ocean during glacial cycles? To address these evolutionary and biogeographic questions, a collaborative effort is proposed between A. Klein, A. Mathieson and C. Neefus, plus Drs. Sandra Lindstrom (University of British Columbia), Masahiko Kunimoto (National Fisheries University, Japan), and Yuzuru Mizukami (National Fisheries University, Japan). The submission of a recent NSF proposal entitled "Systematic revision of North American Porphyras" should also be noted.

Detailed monthly surveys of in situ Porphyra populations were also conducted during a two year period at Huckins Ledge and Mathews Islands in Maine, plus at two New Hampshire sites (A. West, M.Sc. candidate), including a nearshore open coastal area (Fort Stark) and a mid-estuarine
site (Dover Point). The latter studies were conducted by Andrew West, an M. Sc. candidate at UNH (see below). No documentation of “escapee” Porphyra yezoensis were documented within Cobscook Bay by UNH personnel (Mathieson & Klein). Similarly Kathy Watson’s field studies in Cobscook Bay (M.Sc. thesis, Northeastern University) also showed that the plant could not overwinter or establish a permanent population. Andrew West’s studies documented the occurrence of one the same two “cryptic” taxa recorded for northern Maine and the Canadian Maritime Provinces. Overall, the detailed ecological and molecular studies have fostered our understanding of P. yezoensis plus our “native”species; they also provide an important baseline for future strain selection, domestication and/or potential introductions. See the attached synopsis for an extensive documentation of published papers, talks, abstracts, theses, etc.

As noted previously, Phycogen (formerly Coastal Plantations International, Inc.) withdrew from active marine agronomic activities in 1999. Basically they had obtained inconsistent productivity and quality of Porphyra materials on their nets within Cobscook Bay, which may have been associated with nutrient stress or inadequate site/material selection. It should be noted that P. yezoensis is a warm temperate (i.e. Asiatic) plant that grows most abundantly in the spring and summer within its native habitat. Licensing restrictions required that it be grown during the summer and fall within Cobscook Bay, when the temperature and photoperiodic regimes would not allow it to overwinter. Using this growth strategy the plant did not overwinter (see above), but it was forced to grow under periods of diminishing light and temperature, which were not optimal. Nutrient stress may have also confounded the issue, diminishing the plant’s growth, reducing its phycobilin (i.e. pigment) content, and resulting in a rather irregular and corrugated blade morphology during certain years. At best the productivity and optimal stature/coloration were quite variable between multiple years, presumably because of differing patterns of environmental stress, including nutrient deficiency. On more than one occasion CPI personnel attempted to “fertilize” the farm sites by spreading solubilized fertilizer, but the high current velocities and ensuing dilution probably made this a prohibitive effort.

Within the P.I.’s associated with the Enhancement project there were areas of both consensus and differing views concerning how to solve some of the problems outlined above. Basically most individuals agreed that
environmental optimization of the species was a key consideration (i.e. enhanced nutrient, proximity to salmonid culture sites, variable outplanting dates, differential times and depths of planting). On the other hand there was no consistent wisdom regarding the use of different source materials (i.e. native versus introduced), with some individuals emphasizing the growth of *Porphyra yezoensis* under more favorable conditions, while others felt it would be preferrable to use native materials that were already adapted to ambient conditions. The possibility of providing hybrid material combining physiological and growth traits of *Porphyra yezoensis* with a native species was a compelling suggestion (see above). The concerns of some individuals regarding the use of an introduced species in northern Maine can probably be dismissed due to its incapacity to overwinter. However, if it were to be grown in southern New England, which is more environmentally similar to its native habitat, it might become more invasive. Hence, this should be resolved in any future cultivation of an introduced taxa in the latter area.

As noted previously the concept of growing native *Porphyra* in more southerly habitats (i.e. Connecticut) is continuing via bioremediation and polyculture activities. Basically a consortium of individuals is attempting to develop an integrated system in which some of the costs of nutrients are defrayed and enhanced growth may occur via a coordinated *Porphyra* / salmonid cultivation facility (i.e. farm). At this point, most of the species being grown were selected based upon previous assessments of optimal growth and recruitment- e.g. monospore production for the seeding of nets.

**Publications:**


Abstracts and Presentations:


Watson, K. and D. Cheney. 1999. Can the non-indigenous red alga, Porphyra


, , , , and . 1996b. Field and culture studies of several New England Porphyra species in order to characterize the distributional ecology and develop strains. J. Phycol. suppl. 32 (3): 52.


Papers in Preparation:


Graduate Students Associated with the Project and Completed Theses:


Teasdale, Brian. 2002. Ph.D. candidate, Department of Plant Biology, University of New Hampshire, Durham, NH.

Wallace, Aaron L. 2002. Ph.D. candidate, Department of Molecular Biology and Biochemistry, University of New Hampshire, Durham, NH.


Patents:

UNH Final Report
National Sea Grant Aquaculture Enhancement Grant Project

"Strain Improvement of Porphyra for Cultivation in Maine by Protoplast Fusion - Somatic Hybridization"

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Marine Science Center
Northeastern University
Nahant, MA 01908

Summary
Funding from our part of the overall Aquaculture Enhancement Grant supported one primary study (entitled "Strain improvement of Porphyra by protoplast fusion") and two smaller studies that led to graduate theses on related topics. Although results of our primary investigation will be emphasized in this report, the major findings of the two graduate theses will also be briefly described.

The primary objective of our part of this grant was to develop new, genetically improved strains of Porphyra that would be especially adapted for farming in the waters of northern Maine. The rationale behind our study was that the plant being farmed by Coastal Plantations International (CPI), Porphyra yezoensis strain U-51, was developed in Japan and was not an ideal cultivar for CPI's farm sites in Cobscook Bay. Our goal was to produce one or more new strains that had the key commercial traits of P. yezoensis (i.e. excellent taste, color, etc.) and the improved growth potential of one of the local Porphyra species, without altering the basic reproductive characteristics of the plant.

The method of strain improvement we utilized was protoplast fusion. Specifically, we used the technique of protoplast fusion to attempt to produce somatic hybrids between P. yezoensis and two local Porphyra species, P. umbilicalis and P. amplissima. At the time our study began, protoplast fusion in Porphyra was still in the development phase. It had not been applied to any of the local Porphyra species, and with only limited success to some Japanese species. That is, using previously described methods, only chimeras and "possible" (i.e. unproven) hybrids had been produced.

This project was extremely successful; basically, we accomplished everything we had hoped to accomplish. Not only did we develop new protoplast fusion methods that we believe can be applied to any Porphyra species, but we also produced at least one new, improved strain of P. yezoensis (strain # 9-13) that we believe offers advantages for nori farming in waters of northern Maine and Canada, and at the same time is environmentally safe. Highlights of these and other accomplishments of this project are described in more detail below.

Major accomplishments or highlights of this project
1. **The first report of successful protoplast fusion in *Porphyra*.**

We developed techniques for the protoplast fusion of *Porphyra yezoensis* with itself (to produce a polyploid) and with either *P. umbilicalis* or *P. amplissima* (to produce a hybrid). Our methods for protoplast fusion in *Porphyra* include several innovations over past techniques. A patent has been filed to protect our methods. With our new methods, we have been able to produce for the first time both true hybrids and polyploids. The ability to produce such new strains in a practical and reproducible manner could revolutionize strain improvement efforts in the genus.

2. **The first report of a true somatic hybrid being produced in *Porphyra* or any seaweed.**

Plants produced by protoplast fusion were initially screened using isoenzyme electrophoresis (using PGI) to identify any plants that might have an unusual banding pattern. After which they were analyzed molecularly using both chloroplast (rbcL) and nuclear (rRNA - ITS1) gene markers. In some cases, plants also had their number of chromosomes counted and their DNA content quantified by a collaborator, Donald Kapraun of the University of North Carolina at Wilmington. A total of 12 interesting plants were identified using the above protocol, several of which we have proof of being true somatic hybrids. One example of such a hybrid is strain # 19-3. Strain #19-3 was produced in a protoplast fusion experiment between *P. yezoensis* and *P. umbilicalis*. The plant has the morphology of *P. umbilicalis*, but is lighter in pigmentation than *P. umbilicalis*. It has a PGI isoenzyme pattern that is different from that of either parent and a DNA content that is greater than that of either parent. Most significantly, it produces two ITS 1 bands upon PCR of 600 bp and 700 bp, which correspond to those found in *P. umbilicalis* and *P. yezoensis*, respectively.

3. **The first report of a valuable new strain of *Porphyra* being produced by protoplast fusion (or any other modern biotechnological technique).**

Using the protocol described above, we have also identified a polyploid strain of *Porphyra yezoensis* that shows enhanced growth characteristics. The strain, called #9-13, was produced in a protoplast fusion experiment between *P. yezoensis* and *P. umbilicalis*. It has a *P. yezoensis* blade shape (long and narrow) and PGI isoenzyme pattern. However, its blades have a chromosome number of n= 6 instead of n=3, the normal chromosome number for *P. yezoensis* blades. It also has a DNA content of approximately 0.35 pg (D. Kapraun, personal communication), which is approximately double that of a normal *P. yezoensis* blade. Furthermore, #9-13 has rbcL and ITS1 PCR patterns identical to those of *P. yezoensis*. Therefore, #9-13 must be a polyploid, which would make it the first polyploid reported for *Porphyra*. Polyploids have been shown to be valuable cultivars in many land crop species, oftentimes exhibiting enhanced growth rates and/or yield. In laboratory tests, #9-13 has exhibited a significantly faster growth rate than its parental (U-51) strain (20% per day vs 13% per day, respectively). Its carotenoid-antioxidant content is currently being investigated in collaboration with experts at Tufts Medical School. Its tolerance to freezing was tested in the laboratory and appears to be identical to that of strain U-51. CPI tested its ability to produce conchospores and seed nets commercially,
and found them to be similar to that of U-51. Therefore, we believe that #9-13 is similar to U-51 in its reproductive and freezing-tolerance characteristics. We feel it can offer the nori farmer an advantage over growing U-51 and should be permitted to be farmed in the same habitats as U-51, at least on a trial basis.

4). Biomonitoring of *Porphyra yezoensis* in Cobscook Bay, ME, shows that its farming there does not pose a environmental threat.

In the summer of 1996, two graduate students from my laboratory initiated a biomonitoring study to determine if the *Porphyra yezoensis* being farmed by CPI could recruit and establish permanent populations in Cobscook Bay. This study was continued by Katherine Watson as part of her Masters Degree thesis research and was further supported by a grant to Support Non-Indigenous Species Research to the UNH/UME Sea Grant Program. Using a variety of field collecting techniques and molecular analyses, the conclusion of her extensive investigation was that *P. yezoensis* can not overwinter and establish a permanent population in Cobscook Bay, ME. This suggests that *P. yezoensis* can in fact be safely farmed in areas like Cobscook, ME, and that this species should still be considered a good candidate for nori farming in the waters of northern Maine and eastern Canada.

5). *Porphyra* rapidly accumulates copper from seawater and could be used for remediating copper-contaminating marine systems.

A second graduate student supported on this project, Kathryn Roberts, is about to complete her Masters Degree on an investigation into the "phyto-remediation" capability of *Porphyra*. Specifically, she has been examining the ability of *Porphyra* to accumulate two heavy metals, copper and cadmium. She has found that *Porphyra yezoensis* exhibits a very rapid rate of copper accumulation, but that the majority of copper removed from the seawater appears to be bound to ligands in the plant's cell walls and can be easily removed. These two abilities to rapidly take up and release copper make *Porphyra* an idea candidate for use in remediating copper-contaminated marine habitats.

6). List of Publications, Abstracts, Theses and Patents for UNH Sea Grant Projects

Publications


Paper Presentations and Published Abstracts


Proceedings, p 100


**Patents**


**Theses**


-5-

Papers in Preparation

Watson, K. and D. Cheney. Biomonitoring of an introduced, aquacultured seaweed shows it does not pose an environmental threat. (In preparation for Marine Biology)

RESULTS

Transformation: - Successful genetic engineering of any species by gene transfer depends upon:
(1) the availability of commercially useful genes, (2) the availability of regulatory sequences
(promoters, enhancers, etc.) for controlled expression of foreign genes, (3) a reliable means of
gene delivery to cells; and (4) a reliable means of selection of transformed cells and regeneration
of transformed plants. The objectives of this project were related to steps 3 & 4.

Of the several techniques used to transfer foreign genes into plant cells (direct uptake, micro-
jection, electroporation, biolistic bombardment, and *Agrobacterium* vectors), we had shown
earlier that *Agrobacterium* is not a suitable vector for gene transfer in algae. For electroporation,
which depends upon the availability of single cells (preferably protoplasts) that are capable of
regeneration into whole plants, we have developed protocols for regeneration of prootoplasts into
whole plants for all six native *Porphyra* species - *P. umbilicalis*, *P. linearis*, *P. amplissima*, *P.
miniata*, *P. leucosticta* and *P. purpurea* (Larsen, 1999). We have also developed electroporation
protocols for transfer of foreign DNA into protoplasts and partially digested cells of *Ulva lactuca*
and *P. miniata*. Data from transformation studies indicate that the 35S CaMV promoter that is
commonly used for plant transformations is quite effective in both *U. lactuca* and *P. miniata* cells
in transient expression assays of the GUS gene. However, it loses its activity quickly and is not
expressed on long term basis, i.e. stable transformations. All attempts at detecting the expression
of reporter genes in long term cultures have failed.

Selection of transformed cells is often done by transferring a gene that imparts resistance to a
toxic compound, e.g. an antibiotic or an herbicide and plating the treated cells on that compound.
We have conducted detailed toxicity studies on growth of protoplasts of all 6 native New England
species of *Porphyra* on various antibiotics that are commonly used for selection of transgenic cells
and shown that spectinomycin is quite suitable for selection of transformed cells. We are currently
testing the use of a gene for resistance to zeocin and blasticidin; the former has been successfully
used for selection of transgenic cells of microalgae. We have also tested the biolistic
bombardment technique for transformation of *Porphyra* thallus with the GUS gene. The results
show that the intact thallus is not directly suitable for transformation using the GUS gene, since its
expression is not detectable under the microscope under normal lighting conditions. However,
bleaching the thallus should alleviate this problem and make it possible to monitor gene expression
of the GUS as well as the GFP protein, since there will be reduced interference from refraction
(for GUS) and fluorescence from the chloroplasts (for GFP).

Our collaborators at U. Conn. have demonstrated by PCR the actual presence of the foreign
DNA in the *Porphyra* cells. Thus we believe that we have in our hands the required techniques for
successful transfer of DNA into the cells/protoplasts and also the regeneration of protoplasts of all
native species into whole plants. In addition we have identified potential selection agents. Based upon these conclusions, we have focused our current attention on cloning of genes and promoters of highly expressed genes in *Porphyra*.

**Cloning of genes and promoters:** Since the red algae branched off from the other plant groups very early in evolution, and our attempts to transform *Porphyra* using vectors designed for higher plants did not result in stable transformation, we concluded that in order to achieve red algal transformation, higher plant transformation technology will have to be modified, as has been the case for several microalgae. In particular, the transformation vectors will require the use of red algal DNA regulatory elements (i.e. promoters, transcription terminators, etc.) in constructs used to express heterologous genes. Toward that end, we have constructed cDNA libraries of transcripts obtained from the gametophytic blades of *P. purpurea*.

Using a combination of techniques, we have cloned, sequenced and characterized several cDNAs from the *P. purpurea* library. The sequence of two actin genes in all native and some non-native *Porphyra* species has almost been completed in an effort to trace the evolution of this two-gene family and, generate data regarding the phylogeny of *Porphyra* species. A total of 40 randomly selected cDNAs have been screened for their abundance relative to actin. Partial DNA sequence information has been obtained for 23 of these cDNAs and several have been sequenced fully. Using the BLAST search, it was possible to identify the proteins encoded by several of the cDNA while in other cases the similarity to any known gene was insufficient. A number of interesting cDNAs have been identified by this method including a very abundant integrin-like cDNA as well as a calnexin-like and tenacin-like cDNA, β-tubulin, EF1α, and an abundant cell wall-associated “polysaccharide binding protein”. Several of these cDNAs have been used to amplify DNA from the desired genomic regulatory domains (promoters and 3' signals). Efforts to use a similar approach to clone the 5' genomic DNA are continuing. In addition to the genomic PCR approach, a genomic library is under construction that will be useful for obtaining genomic DNA regulatory domains associated with more of the cDNAs. The 5' and 3' flanking genomic sequences isolated from this library will be used to assemble *Porphyra* transformation constructs along with the coding sequences of selectable marker genes and reporter genes. The 3' genomic DNAs for both actin genes have already been combined with selection/reporter genes to study their effectiveness for expression in *Porphyra*. We are also testing the use of “promoterless vectors” that we have constructed to “tag” actively transcribed genes in *Porphyra*.

While the progress of research on genetic engineering with marine macroalgae has been slow, these organisms have certain unique aspects of their growth and development that create major impediments to experimentation. These include a specialized cell wall, extremely slow growth from single cells, the need for seawater for growth, lack of callus formation, etc. Based upon published information and personal knowledge of the current work in different laboratories around the world, it is apparent that our laboratory is in a leading position in this research.

We are currently negotiating a formal cooperative agreement with U. Conn, involving the laboratory of Prof. Thomas Chen, Director of U. Conn. Biotechnology Center for continuing our efforts with transformation of *Porphyra*. Under this agreement most of the gene transfer experiments will be done at U. Conn., Storrs, thus allowing us to focus on the cloning of genes and their regulatory domains. Dr. Chen's laboratory has tremendous experience with the transformation of marine animals and also funding to support the collaborative effort.
Publications and Presentations


* Peer-reviewed

Professional Development - Postdocs and Students Supported/Trained

1. Dr. Xiaohang Huang - Postdoctoral Research Associate
2. Dr. Dennis Mathews - Postdoctoral Res. Associate - currently Research Asst. Prof.
4. Marc Valyo - Undergraduate Student, Biology
5. Christina Ryan - Undergraduate student, Biology
6. Daria Hahn - Undergraduate student, Biology
7. Neil Ganem - Undergraduate student, Biology
8. Kelly Burney - Graduate student. Plant Biol


Outreach and Industrial Impacts: Our laboratory is in a leading position in the area of genetic engineering and gene cloning in seaweeds. This is evident from the fact that the P.I. (SCM) has been invited to present symposium lectures at three international marine biotechnology conferences and two internationally recognized research laboratories for lectures and discussions. Negotiations are underway for research collaboration with a major company involved in seaweed products worldwide.
Project Impact Form

**Project Title:** Nori technology transfer and aquaculture extension programs

**Investigators and Institutions:** Ira Levine, Coastal Plantations International

**Start Date:** 02/01/95  
**End Date:** 01/31/98

A. Publications

Abstracts and Presentations:


Publications:


B. Students
Graduate students were not included on this grant

C. Professional Support

<table>
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<tr>
<th>Name</th>
<th>Role</th>
<th>Time</th>
<th>Project</th>
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<tr>
<td>Ira A. Levine</td>
<td>P. I.</td>
<td>1/3</td>
<td>Project Mgmt</td>
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<tr>
<td>Andrew Stevenson</td>
<td>Associate P. I.</td>
<td>2/3*</td>
<td>Extension Course</td>
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<tr>
<td>Stephen Crawford</td>
<td>Associate P. I.</td>
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<tr>
<td>T. Hiramatsu</td>
<td>Research Assistant</td>
<td>1/3</td>
<td>Translation</td>
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Mr. Stevenson replaced Mr. Crawford during the latter stages of the grant

Andrew Stevenson is presently Wet Lab manager of Mook Sea Farms
Stephen Crawford is presently President of his own aquaculture consulting firm
T. Hiramatsu is molecular biologist in Tokyo, Japan

Grants: 


Other Impacts

Over a dozen newspaper and magazine articles prepared along with two segments on Maine Public Television.
Nori Technology Transfer and Aquaculture Extension Program

Developing a Commercially Viable Seaweed Aquaculture Industry in New England

Ira A. Levine, Coastal Plantations International, Inc.

This research contract through the University of Maine was a small part of a multiphasic award. The goal, to establish a commercially viable seaweed aquaculture industry in New England. The final evaluation is still incomplete. Although the original corporate partner, Phycogen (formerly Coastal Plantations International, Inc.) has withdrawn from active marine agronomic activities, the University of Connecticut along with several corporate partners including this P.I., has continued the effort to develop nori farming in Long Island Sound. The concept is alive and growing to include bioremediation and polyculture.

This project as written had two separate components; technology transfer and Aquaculture extension. Technology transfer concentrated on the Japanese nori technologies. To achieve the stated goals the following was accomplished. Four trips to Japan by CPI personnel were undertaken throughout the grant period to review technologies with Japanese farmers and processors. Additionally, two Japanese farmers, Urano from Yuge Island and Kagawa from Shikoku and a processor, Shiraha from Nagoya visited our facilities in Eastport Maine.

Dr. Xiugeng FEI, Professor and Director of the Experimental Marine Biology Laboratory, Institute of Oceanology, Chinese Academy of Sciences during his stay at the University of Connecticut, visited our Eastport facilities which was followed up by a visit to his lab in China to establish a technology transfer relationship for Chinese nori farming technologies.

Aquaculture extension efforts were divided into four levels of effort: farmer recruitment, nori farming training course; training manual; and extension course. CPI completed a nori farming training course. This course ran six months and covered the biology, economics, equipment, assembly, seeding, cultivation, and nori harvesting (non Sea Grant funding sources). Our recruitment efforts have included a nori training seminar, advertisements in local newspapers (Quoddy Tides) as well as professional publications (Commercial Fisheries News). Additional exposure came from local, state, national, and international news articles, radio interviews (CBS) and television coverage (Maine Public Television - Quest) and Japanese TV. Our nori farming training list included 82 persons who contacted the Company. Geographic diversity included; California, Connecticut, Hawaii, Maine, Massachusetts, New Hampshire, New York, Canada, Israel, Korea, South Africa, and Vietnam.
Development of the Sea Grant Extension training manual and the translation of the Japanese Nori Farmer handbook were the two major written products of this grant and were submitted to the Maine and New Hampshire Sea Grant office's nearly 20 months ago.