Completion Report: "Effects of Nori Aquaculture on the Marine Flora of Cobscook Bay and Selected Sites" (R/FMDF - 152)


Project accomplishments:

1. Assess the success of meeting each objective identified in the project proposal.

Objectives: Assess the impact of the fledgling nori aquaculture industry on the marine flora of Cobscook Bay and selected sites in the Gulf of Maine.

In the early 1990's Coastal Plantations International (CPI) began to commercially farm nori in Cobscook Bay, Maine. The company received permits from local, state, federal and international agencies to grow two cultivars of the Japanese species *Porphyra yezoensis* strains U51 and H25. Since both *Porphyra yezoensis* cultivars are nonindigenous to Cobscook Bay, there was widespread concern among marine ecologists as to whether *Porphyra yezoensis*, an introduced species, could 1) over winter in Cobscook Bay and 2) become invasive.

We have taken several different approaches to addressing these questions. First, and most importantly, we needed much more information about the indigenous of *Porphyra* species in the Northwest Atlantic and adequate molecular tools to distinguish *Porphyra yezoensis* from the local species. Development of *Porphyra* molecular markers was one of the goals of a multi-investigator project funded by Sea Grant from 1995-1998, to help in the development of a Nori Aquaculture Industry in Northern Maine. As part of this project, we established molecular methods to identify native Northwest Atlantic *Porphyra* species (Teasdale et al. 2002), and uncovered two cryptic species of *Porphyra* (Klein et al. 2003; Neefus et al. 2002). We also described the distribution and floristic patterns of native *Porphyra* species (Hehre et al. submitted; West et al. in revision).

To determine whether *Porphyra yezoensis* could over-winter and pose a threat to the environment where it was being cultivated, we conducted two different types of detailed monthly field surveys of *Porphyra* populations over a two year period at Huckins Ledge and Mathews Islands in Cobscook Bay, Maine (Watson et al. 2000), plus at two New Hampshire sites including a near-shore 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) (West et al. in revision; West, M.Sc. thesis).

*Porphyra* samples were collected from intertidal transects and artificial substrata constructed of Japanese nori netting for over two years (Watson, 2000). *Porphyra* plants were identified to species using morphological characteristics, as well as DNA markers. The Rubisco large subunit (rbcL) and rRNA genes were used to distinguish putative *P. yezoensis* plants from native species (Watson 2000). The results of both the intertidal transect studies conducted by the UNH team (Mathieson and Klein) and the nori-netting studies conducted by the Northeastern University team (Watson and Cheney) led to the same conclusion - that *Porphyra yezoensis* could only recruit in the Coastal Plantations farm sites during the summer and autumn seasons and could not over-winter in significant numbers. There was no evidence that *P. yezoensis* could establish a permanent population in Cobscook Bay. No plants of *Porphyra yezoensis* were found in transects or on nori netting in the vicinity of the farm sites six months after Coastal Plantations ceased farming nori in Cobscook Bay in 1998. Thus, our investigations show that the permitting process for the introduction of this particular exotic species worked and that *P. yezoensis* does not appear to pose a threat where it was being farmed. These results are described in one thesis (Watson, 2000) and one publication (Watson et al., 2000 submitted).

Andrew West's studies in New Hampshire documented the occurrence of two "cryptic" taxa recorded for northern Maine and the Canadian Maritime Provinces (West 2001; Teasdale et. al., 2002; Neefus et al. 2002; Klein et al. 2003). Overall, the detailed ecological and molecular studies supported by this project have shown the value of conducting a detailed bio-monitoring investigation on an introduced marine species, and have fostered a better understanding of the "native" *Porphyra* species found in the western Atlantic.

Coastal Plantations International (which changed their name to PhycoGen Inc.) ceased Nori farming operations in Cobscook Bay in 1998. Their nori production experience was very uneven, and the
emphasis of the company changed. Similar efforts by a local fisherman (Dennis Moran) to farm *Porphyra*
at a second site near Castine (i.e. Penobscot Bay) met with limited success: one crop was sold for
phycotoxin and attempts were made to grow native North Atlantic *Porphyra amphissa* and *P.
purpurea*. This too has been discontinued. To the best of our knowledge, there are no current efforts
underway to farm the introduced species, *Porphyra yezoensis*, anywhere in Maine or the western Atlantic.

2. Identify new research directions pursued during the course of the project and reasons for modifying
original research plans:

During the current NOAA project, we have resolved a number additional cryptic species, all of
which are morphologically similar to *P. leucosticta* (abstract, Neefus et al. 2000). Importantly, we have
evidence that the *Porphyra yezoensis*, which was cultivated by CPI, is closely related genetically to the
Northwest Atlantic *Porphyra* (Klein et al. 2003).

One cryptic North Atlantic *Porphyra* species, *Porphyra* sp. D, identified at Dover Pt., New
Hampshire, differs by only three nucleotide substitutions from *P. yezoensis* H25 in an evolutionarily
conservative gene, ribulose bisphosphate carboxylase oxygenase Large subunit (rbcL). This raises an
important question: Have there been recent introductions of Pacific *Porphyra* species to the North Atlantic,
as a result of trans-global commerce over the last few centuries? Or did these North Atlantic *Porphyra* taxa,
which are genetically related to the Japanese species, arrive during much earlier biogeographic dispersal
events, from across the Arctic Ocean during inter-glacial cycles? To address these evolutionary and
biogeographic questions, we (Klein, Mathieson and Neefus), in collaboration with Dr. Sandra Lindstrom of
the University of British Columbia, have written several research proposals (as yet unsuccessful) to the
National Science Foundation for systematic revision of North American *Porphyra*. As part of that proposed
studies, we have established collaborations with Drs. Masahiko Kunimoto and Dr. Yuzuru Mizukami of the
National Fisheries University, Japan, in an attempt to compare Japanese (Western North Pacific) and
Northern Atlantic *Porphyra*.

3. Describe major problems encountered during the study. Of particular interest are problems associated
with experimental protocols and if and how they were resolved.

Correct identification of vegetative *Porphyra* thalli based solely on morphology is still
problematic. In original methodology we had proposed to use allelic specific Polymerase Chain Reaction
(AS-PCR) as a molecular screen to distinguish between native Northwest Atlantic *Porphyras*. AS-PCR
turned out to be an unnecessarily complicated way to sort out the >eight different taxa now known to be
native in the Gulf of Maine. Instead, because of the relatively high level of sequence divergence in the
*rbcL* gene between the native *Porphyras*, it was possible to design a molecular screen using PCR
amplification with Bangia specific *rbcL* primers, and diagnostic restriction digests (Teasdale et al. 2002).
Using molecular screens to aid morphological studies, we have been able to improve the morphology,
ecology and phenology of native species of *Porphyras* (Neefus, et al. 2002; West et al. in revision; Neefus
et al. in preparation).

In order to discriminate between different cultivars of *Porphyra yezoensis* that were grown by CPI
in Cobscook Bay, we had planned to use Amplified Fragment Length Polymorphisms (AFLP) to develop
genetic profiles for different strains. To our knowledge CPI only introduced two *P. yezoensis* cultivars in
Cobscook Bay: U51 and H25. However with culminating of CPI’s operation, it became unnecessary to
conduct these AFLP studies. However we have recognized a general problem with PCR-based studies of
genetic diversity using DNAs prepared field accessions of this alga: the thalli are coated with various
epiphytic and endophytic organisms. It is difficult or impossible to use mechanical and chemical methods
to remove all of the endophytes and epiphytes, and thus isolated *Porphyra* DNAs are contaminated with
foreign DNAs. PCR based analysis of genetic diversity that use ‘random primers’ (RAPDs, AFLPs,
ISSRs) or ‘universal’ primers will likely amplify bands corresponding to contaminants as well as the
Porphyra host organisms. For example, Brian Teasdale has identified DNAs corresponding to sea lice, and
to *Acrosiphonia* sp. a common algal endophyte, when he amplified *Porphyra* templates with PCR primers
designed to amplify the nuclear ribosomal internal transcribed spacer (ITS). We recommend that future
molecular studies of population diversity for *Porphyra* be conducted only with PCR methods that target
known *Porphyra* genes.
Publications:


Abstracts:


Teasdale B.W., Neefus, C. D., West A., Klein, A.S., Tirado-Mercado, S., Yarish, C. Sequence divergence of the *rbcL* and *rbcL-rbcS* spacer in isotypes of rhodophyte alga *Porphyra* (Rhodophyta: Bangiales) suggest increased diversity in the Northwest Atlantic NEAS 2000


Papers in preparation, submitted, or in press:


Watson, K. and D. Cheney. Biomonitoring of the intentional introduction of a non-indigenous, aquacultured seaweed (*Porphyra yezoensis*, Rhodophyta) into Cobscook Bay, Maine, USA. Marine Ecology, Progress Series, submitted

Names and majors of students supported.

Aaron Wallace, Ph.D. in Biochemistry and Molecular Biology Program, UNH

Andrew West, MS in Genetics Program UNH

Brian W. Teasdale, Ph.D. in Plant Biology, UNIH

Katherine L. Watson, MS in Biology, Northeastern University

Theses:

Brian W. Teasdale, Phylogeography of the cosmopolitan red seaweed *Porphyra umbilicalis* (Bangiales, Rhodophyta) in the North Atlantic Ocean, *in progress*.

Aaron Wallace, Using microsatellite loci to resolve taxonomic issues in fucoid algae and their ecds, and to examine the genetic structure of fucoid algae in the Northwest Atlantic, *in progress* (Wallace assisted with some of the *Porphyra* studies).
