Today's date: April 4, 2011

Project number: AISR-05-094

Project title: Aquatic Invasive Species Research: tracking origins, examining population structure and economic impact of the invasive green alga *Codium fragile* in the Northwest Atlantic.

Project initiation date: Sept. 30, 2007*

* This project was initially funded in June 2005, just as the PI began a two year leave of absence to work with the National Science Foundation. The National Sea Grant Office gave permission to delay the project's initiation and permit a no-cost extension. Research was not started until the fall of 2007, when the PI returned to University of New Hampshire, recruited and then trained new graduate students.

Principal investigator: Anita Klein

Affiliation: Department of Biological Sciences, University of New Hampshire

Associate investigator(s) and affiliation(s):
Arthur Mathieson, Department of Biological Sciences;
Kelly Cullen, Department of Natural Resources and the Environment

Brief project overview/Abstract:

*Codium fragile* is an invasive green seaweed that arrived in Long Island Sound in the late 1950’s. It has a large (>1-2 ft.) dichotomous thallus. Growing from a holdfast on rocks or molluscs, the buoyant thallus can lift and carry molluscs away in heavy seas; hence, *C. fragile*’s common name, “Oyster thief”. *Codium fragile* may also smother molluscs or urchins. At the Isle of Shoals *Codium fragile* has overgrown urchin barrens, displacing native kelps that are an important habitat for juvenile fish (cf. Mathieson et al. 2003; Expansion of the Asiatic green alga *Codium fragile* subsp. *tomentosoides* in the Gulf of Maine. Rhodora 105:1-53). The purpose of this project was to document the current distribution of the alga in New England and the Canadian Maritimes, use molecular tools to identify the origins of *C. fragile* populations in the NW Atlantic, and model the economic impacts of the invasive alga on mollusc and sea urchin fisheries.

Phycologists recognize multiple subspecies of *Codium fragile* from temperate coastal areas around the world. It is hard to distinguish some of these different subspecies based solely on grow morphology; however, they can be delineated by chloroplast gene sequences (Provan et al. 2008, Diversity and Distributions 14:343-354). The taxonomic nomenclature for these subspecies has recently been revised so that what has been known as *C. fragile* subsp. *tomentosoides* is now identified as *C. fragile* subsp. *fragile* (Maggs and Kelly in Brodie et al. 2007; Green Seaweeds of Britain and Ireland, British Phycological Society, pgs.189-201).

Only *C. fragile* subsp. *fragile* is considered invasive, but several reports (Garbary and Hubbard 2002, Botanica Marina 45:476-485, and Kusakina et al. 2006, Botanica Marina 49: 1-9) have suggested that *C. fragile* subsp. *atlanticum*, has also spread along Prince Edwards Island Canada and parts of Nova Scotia. One goal of this project has been to verify whether or not there has been more than one introduction of *C. fragile* to the NW Atlantic. Chloroplast DNA
polymorphisms distinguish the subspecies C. fragile subsp. atlanticum from C. fragile subsp. fragile (Provan et al. 2008, op. cit.).

Objectives: The specific objectives of this project were six fold:
1. Develop a coordinated field/ecological and genetic evaluation of C. fragile ssp. tomentosoides for Northwest Atlantic populations, documenting their distribution, abundance and population structure.
2. Develop molecular markers that will confirm whether another strain or a second introduced subspecies (i.e. subsp. atlanticum) occurs within in the Canadian Maritimes and has contributed to the localized explosion of Codium in the Gulf of Maine.
3. Use nuclear and plastid microsatellite markers to compare genetic structures of early and more recently established populations- i.e. establish whether they are differentiated (e.g. closely or distantly related) and whether hybridization has occurred.
4. Evaluate the economic impact of Codium fragile in the Gulf of Maine.
5. Use the results from 1-4 to make recommendations to prevent further spreading of Codium, which will be disseminated as a report to managers of coastal/estuarine reserves.
6. Develop educational materials (website, pamphlets) for coastal resource groups, State Fish and Game staff, etc. that monitor coastal resources, to train them to recognize new populations of Codium.

Research findings/accomplishments/progress to date:
1. (Art Mathieson, Anita Klein and graduate students Chris Benton and Lucy Pleticha) During the summer and early fall of 2008, collections of C. fragile were made at 24 sites from the Canadian Maritimes south to Long Island Sound. Significant reductions in C. fragile populations were noted for the Northumberland Strait area near Prince Edwards Island (C.S. Benton, L. Pleticha pers. comm.), as compared to previous surveys (cf. Mathieson et al. 2003; Rhodora 105:1-53; Garbary and Hubbard 2002, op.cit.). This population decline was confirmed during a follow-up survey by Garbary and Benton during August 2009. However in the summer of 2010, Garbary noted rebounding of Codium populations along the shores of PEI (pers. Comm)

2. (Lucy Pleticha, Art Mathieson, and Anita Klein) Over 330 plants were collected. Representative plants from each population were pressed and deposited in the Albion Hodgdon Herbarium (NHA). The swollen terminal portion or utricle of the plant's thallus was evaluated microscopically. Different subspecies of C. fragile are commonly distinguished by morphometric measurements of the utricle: length, width at narrowest point; width at widest point, the length of its pointed tip, the mucron, and presence and size of gametangia (Maggs and Kelly 2007, op.cit.). Utricle attributes were evaluated for each population. While there was significant variation within and between different plant populations, various statistical analyses of utricle features indicated that the plants from the Canadian Maritimes and the Gulf of Maine were more similar to each other, and distinct from plant populations in Long Island Sound. At this time it is unclear whether this subtle phenotypic variation is due to environmental or genetic differences between populations.

3. (Chris Benton, graduate student Renee Eriksen, undergraduate Matt MacKenzie, and Anita Klein) Provan and coworkers (Provan et al. 2005, Molecular Ecol. 14: 189-194; Maggs and Kelly 2007, op. cit.; Provan et al. 2008, op.cit) identified polymorphisms in two chloroplast genes that distinguished Codium fragile subspecies (rps3-rpl16) and/or populations of C. fragile subspecies fragile (trnG). DNAs were extracted from 107 accessions, covering the full geographic range of surveyed sites. The DNAs were split into two tubes and stored at -80°C for additional studies.
A subset of the plants that were evaluated morphologically were screened for their chloroplast haplotype at these two loci. The chloroplast haplotype for the \textit{rps3-rpl16} gene region was determined for 50 individuals representing populations from Nova Scotia Canada to Long Island Sound. All plants had identical haplotypes (GenBank accessions FJ754465- FJ754478), which closely matched the chloroplast haplotype for \textit{C. fragile} subsp. \textit{fragile} (GenBank accession EU045560). A single polymorphism that distinguished NW and NE Atlantic haplotypes, was determined to be a sequencing artefact in Provan and coworkers original studies (Benton and Provan pers. comm.) Sequence analysis revealed a single \textit{trnG} haplotype in the Northwest Atlantic for 26 \textit{Codium} individuals (Figure 3,GenBank accessions GQ274899-GQ274924) spanning the entirety of our study region. This matches the \textit{C. fragile} subsp. \textit{fragile} haplotype from Northeastern Atlantic and Japanese populations from Kochi, Sagami Bay, and Nakagi (Provan et al., 2005, op. cit.). This is the same haplotype of \textit{C. fragile} subsp. \textit{fragile} that is common in Northern Europe.

Recently a second chloroplast haplotype for \textit{C. fragile} collected from PEI in the summer of 2009, and 2010. This haplotype varies by 3 nucleotides (insertion/deletion) compared to the most common chloroplast haplotype found for \textit{C. fragile} subsp. \textit{fragile}. Samples collected in Malpeque Bay have the insertion while samples collected along the southern coast of PEI have the common \textit{C. fragile} subsp. \textit{fragile}. Either the \textit{C. fragile} populations from Malpeque Bay represent a new mutation localized to the Canadian Maritimes or they represent a separate introduction of \textit{C. fragile} subsp. \textit{fragile}  

Although we’ve not observed much genetic variation for the two chloroplast genes in the NW Atlantic, this does not necessarily mean that \textit{C. fragile} populations in are genetically homogenous. A much earlier study (K. C. Malinowski. \textit{Codium fragile}- the ecology and population biology of a colonizing species. Yale University. -127+, 1974) screened isozymes from \textit{C. fragile} populations in Long Island Sound and Boothbay Harbor ME. The populations in Long Island Sound had a single heterozygous genotype while t those from Boothbay Harbor had several distinct alleles. Additional genetic evaluations are in progress.

4. (KGC) The potential economic impact of expansion of \textit{Codium fragile} in the Gulf of Maine was modeled by Assoc. Prof. Cullen. The focus was on shell-fisheries and urchin operations; data on landings and values were obtained from Maine’s Department of Marine Resources, Massachusetts’ Division of Marine Fisheries and the New Hampshire Department of Fish and Game. Impacts included not only direct effects on shellfisheries and urchin but also indirect effects for example to suppliers and induced effects on employees, and regional tax base. The direct effects of further expansion of \textit{Codium fragile} populations to the Gulf of Maine economy could be as high as $154 million, based on 2007 landing values; combined with indirect effects, the impact could exceed $333 million. See the attached detailed report by Prof. Cullen: Region Economic Impacts of \textit{Codium fragile} in the Gulf of Maine.

5. Additional ecological and genetic investigations are ongoing; when these studies are complete we will make recommendations to prevent further spreading of \textit{Codium}; these recommendations will be disseminated as a report to managers of coastal and estuarine reserves.

6. In an effort to engage the public’s help to identify new populations of \textit{C. fragile} in the NW Atlantic, a web site (\texttt{www.Codium.unh.edu}) describing this project was launched in May 2008. To date the web site has attracted 62 visits and 204 page views. To enhance contacts with the public, an educational pamphlet modeled on the web site was distributed to NH and ME Fish and Game Departments, Seabrook Science and Nature Center, plus the Great Bay Discovery Center, Pemaquid Watershed Association, and the Casco Bay Estuary Partnership.
Impacts to date:

The major impacts of this research project are three fold:
1) Scientific:
   a. Continued monitoring of the status of *Codium fragile* populations in the Canadian Maritimes and Gulf of Maine documented significant declines of *C. fragile* in the Northumberland Straits area near Prince Edwards Island, while *C. fragile* populations are either stable or expanding in the Gulf of Maine and Long Island Sound.
   b. Morphological variation was observed for ‘diagnostic’ characters of *C. fragile* subspecies. Statistical analysis suggested that *C. fragile* populations from Long Island Sound differed from more northern *C. fragile* populations. Most plants evaluated to date have chloroplast haplotypes consistent with the *C. fragile* subsp. *fragile* haplotype, which is common to Kochi, Sagami Bay, and Nakagi in Japan, and to invasive populations from the Northeast Atlantic. A second distinctive haplotype of *C. fragile* subsp. *fragile* was identified in Malpeque Bay PEI.

2) Economic: The potential impacts of *C. fragile* to shell-fisheries and urchin harvesting were modeled for the Gulf of Maine. Continued expansion of *C. fragile* would have a multi-million dollar effect on shell-fisheries and urchins in the Gulf of Maine economy.

3) Educational: A website (www.Codium.unh.edu) and educational materials were developed to inform the public about the ecological and economic impacts of invasive seaweeds, especially *Codium fragile*.

Related grants and contracts (other grants and contracts that funded this research or that were obtained as a result of this research): NH Agricultural Experiment Station H499 2007-2010.

Problems encountered: One of the Co-PIs (ACM) was incapacitated during the summer and fall of 2008, limiting the detail of ecological surveys which were carried out by less experienced graduate students. The time table for molecular studies was reduced from the 24 months outlined in the original proposal to ~18 months. Given the novice molecular skills of the two new graduate students, genetic assessments were limited to determining the chloroplast haplotypes of *C. fragile* based on the markers established by Provan et al.(op.cit.).

Publications

Peer reviewed publications: manuscript under revision for Botanica Marina

Other communications products (manuals, tech reports, videos, etc.): An educational web site www.codium.unh.edu was launched in May 2008 and similar pamphlet has been distributed to the public.

A technical report on the regional economic impacts of *Codium* has been prepared by Prof. Cullen. The report is attached.

Presentations to date, with published abstract citation if applicable:
Students Supported:

**Student name:** Lucy Pleticha  
Institution/Department: University of New Hampshire, Plant Biology,  
Duration of support: January 2008-December 2008  
Type of support (stipend, travel, supplies, etc.): stipend, travel, supplies  
Type of degree (undergrad, masters, PhD): MS  
Year degree awarded: in progress  
Title of thesis (if applicable): Morphological variation in *Codium fragile* in the Northwest Atlantic.  
Where is he/she now? Will complete thesis during the Fall of 2009

**Student name:** Chris S. Benton  
Institution/Department: University of New Hampshire, Biochemistry and Molecular Biology  
Duration of support: January 2008-December 2008  
Type of support (stipend, travel, supplies, etc.): stipend, travel, supplies  
Type of degree (undergrad, masters, PhD): Ph.D.  
Year degree awarded: in progress  
Where is he/she now? Continuing student

**Student name:** Renee Eriksen  
Institution/Department: University of New Hampshire  
Duration of support: September –December 2008  
Type of support (stipend, travel, supplies, etc.): partial stipend  
Type of degree (undergrad, masters, PhD): Ph.D.  
Where is he/she now? Continuing student

**Student name:** Matt MacKenzie  
Institution/Department: University of New Hampshire, Biochemistry and Molecular Biology  
Duration of support: July 2008-December 2008  
Type of support (stipend, travel, supplies, etc.): part-time salary, supplies  
Type of degree (undergrad, masters, PhD): undergrad  
Where is he/she now? Continuing student