PROJECT COMPLETION REPORT

97-3 "Use of Molecular Probes for Detection of *Bonamia ostreae* in Oysters, *Ostrea edulis*"

Termination Report Period: March 1997-November 1999

NRAC Total Funding: $57,366 (March 1, 1997 – May 30, 1999)
(No-Cost Ext'n through November 30, 1999)

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REASON FOR TERMINATION: Objectives completed; funding terminated.

PROJECT OBJECTIVES:

1. To develop a molecular probe for sensitive and accurate diagnosis of *Bonamia ostreae*, a pathogen of European oysters, *Ostrea edulis*.

2. To compare the diagnostic sensitivity of fluorescent *in situ* hybridization with both PCR assay and present diagnostic techniques (standard histology and stained blood smears) with oysters submitted from growers throughout the Northeast.

3. To communicate the availability of the probe throughout the Northeast so that interested industry members can utilize it to their advantage. This will be accomplished by the production of a Fact Sheet describing molecular approaches to shellfish diseases, using the *B. ostreae* probe (both its development and utilization) as an example.

ANTICIPATED BENEFITS:

The development of a PCR assay for *Bonamia ostreae* will have three applications for the Northeast aquaculture industry. First, *Ostrea edulis* broodstock, hatchery-produced larvae, and seed may be certified as *B. ostreae*-free, preventing the introduction of *B. ostreae* into disease-free areas. Second, disease-free areas may be identified and approved as "safe" areas for *O. edulis* culture. And third, areas in which *B. ostreae* is enzootic may be closely monitored for disease development, providing growers with management options.

The improved management of bonamiasis made possible by the PCR assay will allow more widespread culture of *O. edulis* in the Northeast, and thus further diversification of the aquaculture industry in this region.

PRINCIPAL ACCOMPLISHMENTS:

1) We have designed a specific and sensitive PCR assay for *Bonamia ostreae*. The presence of *B. ostreae* is indicated in this assay by the amplification of a unique 760 base pair small subunit ribosomal DNA (SSU rDNA) sequence.

2) Experimental trials have demonstrated that this PCR assay has a strong advantage in sensitivity over standard cytological techniques. Many very light *B. ostreae* infections undetected by cytology were unambiguously detected using the PCR. Forty-four of 116 (37.9%) oysters in which *B. ostreae* was undetected cytologically were found by PCR to contain *B. ostreae* DNA.

3) We have demonstrated the specificity of the PCR assay to *B. ostreae* from both Europe (Ireland, Spain, and Holland) and Maine. The PCR has

also verified that *B. ostreae* is not endemic to Spinney Creek, Maine.

4) We have used parsimony analysis of DNA sequence data to establish the phylogenetic affinity of *B. ostreae* to the Phylum Haplosporidia.

5) We have developed a novel technique by which parasite rDNA may be amplified from a bulk host-parasite DNA mixture. We designed conserved PCR primers to amplify a fragment of 18S rDNA and the ITS 1 spacer region that follows the 18S in this gene complex. The ITS 1 region is variable in length across taxa, so amplification products from different species in the bulk mixture may be separated by size on an agarose gel. In our experiments, a *B. ostreae* 18S-ITS 1 fragment (528 bp) was easily distinguished from an oyster amplicon (~1000 bp).

IMPACTS:

The PCR assay for *Bonamia ostreae* represents a powerful new weapon in the arsenal for management of bonamiasis in the Northeast. The superior sensitivity of this assay makes it more likely than histopathology or histocytology to detect *B. ostreae* at very low intensities in adult or seed oysters. It should thus be the method of choice for screening oysters specifically for this pathogen.

RECOMMENDED FOLLOW-UP ACTIVITIES:

Development of an *in situ* diagnostic technique for *Bonamia ostreae*, ongoing in our laboratory, will be continued. Such a tool is essential for resolution of the life history of *B. ostreae*, which is incomplete to date. Our poor understanding of the life cycle makes effective management of bonamiasis difficult.

Quantitative PCR assays represent the "next generation" of this powerful tool, and the development of such an assay for *B. ostreae* should be a priority. A quantitative assay will tell us not only whether or not *B. ostreae* is present, but will quickly tell us *how much* is present as well. This additional knowledge will allow increased flexibility in management of bonamiasis. For example, if we know *B. ostreae* is present at a certain prevalence in an area, but always at low intensity, we may be willing to accept a certain degree of risk in using such an area for *Ostrea edulis* aquaculture.

PUBLICATIONS, MANUSCRIPTS, AND PAPERS PRESENTED:

Published Abstracts

Carnegie, R.B., B.J. Barber, D.L. Distel, and S.C. Culloty. 1999. Development of PCR and in situ hybridization assays for detection of *Bonamia ostreae* in flat oysters, *Ostrea edulis*. J. Shellfish Res. 18(2): 711-712.

Carnegie, R.B., B.J. Barber, and D.L. Distel. 1998. *Bonamia* research in Maine: an update. J. Shellfish Res. 17 (1): 350.

Carnegie, R.B., D.L. Distel, and B.J. Barber. 1997. Amplification and sequencing of the *Bonamia ostreae* 18S rDNA gene: phylogenetic considerations and applications. J. Shellfish Res. 16 (1): 328.

Manuscripts

Carnegie, R.B., B.J. Barber, S.C. Culloty, A.J. Figueras, and D.L. Distel. Development of a PCR assay for detection of the oyster pathogen Bonamia ostreae *(Pichot et al. 1980), and support for its inclusion in the Haplosporidia. Submitted to Dis. Aquat. Org.*

Papers Presented

Carnegie, R,B., B.J. Barber, D.L. Distel, and S.C. Culloty. Development of a PCR assay for detection of *Bonamia ostreae* in flat oysters, *Ostrea edulis*" 92nd Annual Meeting of the National Shellfisheries Association. Seattle, WA. March 2000.

Carnegie, R.B., B.J. Barber, D.L. Distel, and S.C. Culloty. Development of PCR and *in situ* hybridization assays for detection of *Bonamia ostreae* in flat oysters, *Ostrea edulis*. 3rd International Conference on Shellfish Restoration. Cork, Ireland. September 1999.

Carnegie, R.B., B.J. Barber, and D.L. Distel. *Bonamia* research in Maine: an update. 18th Milford Aquaculture Seminar. New Haven, CT. February 1998.

Carnegie, R.B., D.L. Distel, and B.J. Barber. Amplification and sequencing of the *Bonamia ostreae* 18S rDNA gene: phylogenetic considerations and applications for management of bonamiasis in European oysters, *Ostrea*

edulis. Maine Biological and Medical Sciences Symposium. Waterville, ME. May 1997.

Carnegie, R.B., D.L. Distel, and B.J. Barber. Amplification and sequencing of the *Bonamia ostreae* 18S rDNA gene: phylogenetic considerations and applications. 89th Annual Meeting of the National Shellfisheries Association. Fort Walton Beach, FL. April 1997.

Carnegie, R.B., D.L. Distel, and B.J. Barber. *Bonamia ostreae* investigations at the University of Maine: assessment of the distribution of *Bonamia*, and progress towards the development of a molecular diagnostic tool. Maine Aquaculture Conference. Rockport, ME. March 1997.

The Full Report with all the data and tables is available at the NRAC office upon request.