Abbreviated Progress Report

Project Code: 03-17

Subcontract/Account No. 556808
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Project Title: "Development of diagnostic and management techniques to select cod broodstocks and hatchery stocks free from nodavirus"

Reporting Period: November 2003- April 2004

Funding Level: $124,612

Participants:

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Project Objectives:

1. Continue the development and refinement of a Real-time PCR assay for the detection of cod nodavirus in cod broodstocks, gametes, fertilized eggs and larvae.
2. Validate the use of the Real-time PCR assay for detecting nodavirus in infected tissues (brain, eyes, eggs, larvae and juveniles) and for the non-lethal detection of nodavirus in cod broodstock (via blood and/or gametes).
3. Screen captive broodstocks and progeny in New England to test the Real-time PCR assay in an industry setting and explore the development of a reagent kit.
4. Develop egg disinfection methods that effectively eliminate nodavirus on the surface of eggs and possible vertical transmission.
5. Develop extension and outreach materials and presentations for nodavirus detection and disinfection.

Anticipated Benefits

Upon completion of this research, we will be able to provide the aquaculture industry important information on disease diagnostic and egg disinfection techniques for cod nodavirus. If egg surface disinfection can be proven to be an effective control against nodavirus infections, it will be a valuable safety measure, in tandem with broodstock screening, to virtually eliminate the disease from having an economic impact in cod culture. Our work to date, also suggests that we will be able to provide similar information on other commercially important finfish (i.e. haddock, grouper).

Progress and Principal Accomplishments

The quantitative molecular detection methods for cod nodavirus have been developed using dual-labeled probe and SYBR-Green technologies. In addition, a NASBA (Nucleic Acid Based Amplification) protocol is underdevelopment in order to compare the advantages and disadvantages of the different molecular detection methods. All of the assay development work has been facilitated with nodavirus positive cod samples provided by Stewart Johnson.

We have screened a number of cod tissue samples from the region, and to date, no fish tested have been infected with nodavirus. This includes verifying the absence of nodavirus in cod fingerlings stocked into sea cages in Canada. Samples from other fish species have been provided from participants and tested for nodavirus using a more 'universal' set of oligonucleotide primers. For instance, we have screened 7 of 30 haddock broodstock from URI, Narragansett. These 7 fish were sacrificed due to their poor condition, and suspected of carrying nodavirus. Only 1 of the 7 appears to actually have had detectable nodavirus. We are continuing to collect samples of eggs, sperm and larvae from current and future spawns of cod and haddock larvae from our New England cooperators. These will be tested for nodavirus at a later date.

We have conducted two disinfection trials to date. Both trials have been conducted with iodine as the disinfecting agent. Preliminary analysis indicates that there was no effect on survival of the treatment concentration at any of the treatment times tested. However, hatching time was delayed in the longer treatment times despite normal embryo development possibly indicating a hardening of the egg chorion with the extended treatments. Samples of eggs pre-treatment and larvae post treatment have been preserved for later nodavirus testing.

A webpage has been set-up (http://www.mbl.edu/aquaculture/nrac/) for technology transfer and dissemination of techniques for disease diagnostics and egg disinfection methods.

Work Planned

Future work related to nodavirus detection will focus on testing gametes, fertilized eggs, larvae and blood. The sensitivity of the assay will continue to be fully optimized with the goal of being able to develop a non-lethal detection method. Samples from multiple species will continue to be sent to the MBL for testing. We expect more eggs from Great Bay Aquaculture and URI, Narragansett this spring to continue trials using ozone and RNase A. In addition, Stewart Johnson will be able to provide infected cod eggs this fall for disease diagnostics and egg disinfection trials.

Impacts

One of the most significant impacts to date is our testing of cod fingerlings stocked into sea cages in Canada. Without the assay results we provided to managers, a large number of fish might have been destroyed based on the belief that these fish had become infected with nodavirus.

Support

	NRAC-USDA Funding	Matching Support (MBL)	Total Support
Year 1	$63,017	$7,063	$70,080
Year 2	$61,549	$7,063	$68,658
TOTAL	$124,612	$14,126	$138,738

Publications, manuscripts, or paper presented

Steven Roberts, Rick Goetz "*Quantitative real-time molecular methods for disease detection in marine fish"* AQUACULTURE 2004, Honolulu, Hawaii March 1-5 2004