Termination Report[1]

Project: Viral Hemorrhagic Septicemia (VHS)

Key Word(s): Viral Hemorrhagic Septicemia (VHS)

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Total Funds Committed: $197,960

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Reason for Termination: Project objectives completed and funds have been terminated.

[1]This 2-year funded project is chaired by Jeffrey A. Malison and it began September 1, 2008.

Project Objectives:

1. Determine the safety and efficacy of iodine disinfection on walleye and northern pike eggs infected with VHS.
2. Prepare and electronically disseminate a VHS "response" packet that specifically targets fish farm producers. The packet would address aspects of the disease (clinical signs, routes of transmission) and prevention practices to minimize introduction and spread. The packet will also contain Web sites and information sources where fish farmers can obtain the most current, up-to-date status of the disease.
3. Conduct a series of six biosecurity workshops held at different fish farms across the region, targeting different production systems (flow-through, pond, and recirculation systems).
4. Utilize the existing Aquatic Invasive Species (AIS) Hazard Analysis Critical Control Point (HACCP) Training Curriculum to develop specific fish disease HACCP plans for each of the six facilities involved in the workshops.
5. Develop and distribute three model fish disease HACCP plans (one each for flow-through, pond, and recirculation systems), relying heavily on the specific plans developed under Objective 4.
6. Produce a fish farm biosecurity video that incorporates different system types and footage shot at the workshops and distribute this video to end users via DVD and internet streaming videos.

Project Summary:

Cool and warmwater fish eggs are cultured in large numbers at private, state, and federal hatcheries. The introduction of VHS in natural waters has caused extensive fish mortalities and the virus may be spread to other fish throughout the U.S. by the exchange of fish or their eggs among commercial fish suppliers or state and federal hatcheries. Fish culturists want to avoid introducing diseases from wild brood stock to their captive fish populations. If VHS would infect fish in an aquaculture facility, all fish would probably have to be destroyed and the facility would have to be disinfected.

This project evaluated the use and safety of iodophor disinfection of fish eggs for 3 species of fish. In the first study, VHS-challenged walleye and northern pike eggs were disinfected with iodophor for different lengths of time, and then checked for the presence or absence of the VHS virus. In the second trial, wild caught lake herring were spawned and their eggs disinfected with iodophor. They were cultured to determine if the disinfection process affected survival.

These trials were important since the threat of VHS to the aquaculture industry is potentially devastating. Following detection of VHS, direct losses can occur from facility closures, the restriction of movement orders and quarantines (Bebak 1998). Although some fish may survive an outbreak, they may have reduced growth rates, lower yields, or reduced product quality, which also impacts market value. A facility's reputation may also be damaged following identification of a disease on the premises.

The threat of VHS, as well as other aquatic animal diseases, to the aquaculture industry is potentially devastating. Direct losses can occur following the death of fish from the disease, facility closures, and possible

restriction of movement orders or quarantines following detection of a disease.

One approach to this problem is to apply the Hazard Analysis and Critical Control Point (HACCP) concept similar to that used by the seafood industry to minimize seafood consumption health risks. The advantages of this system are that it can effectively deal with a diverse industry, it has proven to be a good partnership between industry and government regulators, and it is effective when properly applied. The HACCP approach concentrates on the points in the process that are critical to the safety of the product, minimizes risks, and stresses communication between regulators and the industry.

Technical Summary and Analysis:
Objective 1.— Tests with walleye and northern pike were conducted at the Upper Midwest Environmental Sciences Center (UMESC). Adult walleye and northern pike collected by the Genoa National Fish Hatchery from the Upper Mississippi River, transported to UMESC where eggs and semen were collected. Fertilization occurred in a secure lab where eggs were divided into two equal groups and exposed to the VHSv (105 or 108 PFU/mL) immediately concurrent with sperm activation. VHSv (strain IVb) used was isolated by the USFWS La Crosse Fish Health Center (LFHC) from emerald shiners, Notropis atherinoides, collected from Lake Erie in 2006. Eggs were challenged with VHSv for 30 min. Walleye egg adhesion was reduced by immersing the eggs in a bentonite solution for approximately 2 minutes during VHSv challenge then rinsed with well water and then placed back into the virus for the remainder of the challenge.

Fertilized VHSv-challenged eggs (~25 mL/jar) were divided among 48 egg jars from each of the four treatment groups, of which six jars were from each of the two virus titer. One of four treatments was assigned to one side of each of the four systems (two VHSv virus titers per system). Eggs were equally divided into four disinfection treatment groups: (1) a VHSv-challenged nontreated control group; 2) a VHSv-challenged group which received a 30 min-100 mg/L iodophor disinfection 30 min after fertilization; 3) a VHSv-challenged group which received a 60 min-100 mg/L iodophor disinfection 30 min after fertilization; or 4) a VHSv-challenged group which received a 10 min-100 mg/L iodophor disinfection 90 min after fertilization. Eggs were maintained in egg jars until hatch with no other chemical treatments applied. Egg and fry samples were collected and the presence or absence of VHSv determined using the USFWS Standard Procedures for Aquatic Animal Health Inspections/American Fisheries Society Fish Health Section Blue Book (2007) procedures.

VHSv was not isolated from any iodophor-disinfected treatment groups. However, VHSv was isolated from control eggs immediately after challenge and for up to four days after challenge in northern pike eggs challenged at 108 PFU/mL. The virus was not detected in positive control eggs one day post-challenge for either northern pike or walleye eggs challenged at 105 PFU/mL and was not detected in fry of either control or iodophor-disinfected treatment groups. Though some iodophor treatments reduced hatch, eggs and fry appeared to develop normally. Iodophor disinfection did not substantially reduce northern pike egg hatch but walleye egg hatch was reduced when eggs were held for 30 or 60 min in the iodophor disinfection solution. Egg iodophor disinfection appears to effectively eliminate VHSv (strain IVb) from the surface of walleye and northern pike eggs.

Although iodophor egg disinfection reduced walleye egg hatch in this study, previous UMESC toxicity studies indicated that when applied shortly after fertilization (~5 min), iodophor disinfection did not alter egg hatch. Incorporation of iodophor disinfection at 100 ppm during gamete collection from non-salmonid fishes immediately post-fertilization (<5 min) for 30 min or at 90 min after fertlization for 10 min may reduce VHSv (strain IVb) transmission without affecting egg hatch.

Personnel from Northern Aquaculture Demonstration Facility (NADF) spawned wild lake herring from Lake Superior and then disinfected the eggs on the boat. Fertilized eggs from three separate matings were split into four disinfection schemes, resulting in three groups of eggs for each disinfection assignment. Control eggs were water hardened and rinsed with well water. Treated eggs were disinfected with iodine as follows: 50 mg/L for 30 min or 100 mg/L for 30 min or 100 mg/L for 15 min. Eggs were transported in separate labeled containers in coolers to UMESC. Samples of ovarian fluid, fertilized eggs, and the spawned fish themselves were collected by NADF personnel, separately bagged and transported to LFHC for VHS testing; all samples tested were negative for VHS.

Treated eggs received a second iodophor disinfection (100 mg/L for 10 min) upon arrival at UMESC before placement in egg jars (~25 mL of eggs measured by volume displacement per jar. A sample of eggs for VHS testing was taken from each group. The control eggs, processed last, were sham treated (placed in rearing water for 10 m) then placed in assigned jars.

Saprolegnia hyphae were observed on dead eggs 7 d after receipt at UMESC, subsequently seven once-daily hydrogen peroxide treatments (500 mg/L for 15 min) were administered to all egg jars (including controls) over a 15-d period. Eye spots were visible in viable embryos at 19 d post fertilization; the percentage of embryos with a visible eye spot was determined at 24 d post fertilization. All viable embryos (i.e., those with a visible eye spot) were transferred back to NADF at 25 d post fertilization.

The mean percent of embryos with a visible eye spot at 24 d post fertilization were: control - 76.2% (65.4 – 82.2%); iodophor disinfection at 50 mg for 30 min - 69.3% (63.4 – 80.2%); iodophor disinfection at 100 mg for 15 min - 69.2% (62.2 – 80.9%); iodophor disinfection at 100 mg for 30 min - 80.43% (74.5 – 81.5%). Iodine disinfection of lake herring eggs did not adversely affect eye-up of the eggs. Shipment of eyed lake herring eggs did not affect survival.

Objective 2.— The VHS "response" packet was developed by Iowa State University in April 2009. The packet is an 18-page PDF document containing information for aquaculture producers on the signs, susceptible species, and prevention of VHS. A "Biosecurity for Aquaculture Facilities" PowerPoint® presentation (36 slides with speaker notes) was also developed in April 2009. All of the materials were forwarded to other Project Leaders (Malison and Kinnunen) to be incorporated into the biosecurity workshop objective of this project (Objective 3). Additionally, these materials have been posted for download on the Center for Food Security and Public Health (CFSPH) Web site (http://www.cfsph.iastate.edu/DiseaseInfo/MoreInfo/VHS.htm) and the Focus on Fish Health Web site (www.focusonfishhealth.org).

Objective 3.— In 2009/2010, eight planned VHS-biosecurity workshops were conducted at aquaculture facilities in the NCR.

Michigan State University and University of Wisconsin Extension Aquaculture Specialists partnered with local and regional animal health professionals to present information on fish disease transmission, VHS and HACCP planning specific to developing a biosecurity plan for aquaculture facilities.

Objective 4.— Bodin State Fish Hatchery already had a HACCP biosecurity plan in place. Comments were made to improve a few critical control points (visitor access and logs).

- Crystal Lakes Fisheries had their own biosecurity plan which was used as a basis for drawing up a HACCP biosecurity plan.
- Michigan Bait and Fish Farms already had a HACCP biosecurity plan in place from previous work with Michigan State University Sea Grant Extension.
- Gollon Bait and Fish Farm had their own biosecurity plan which was used as a basis for drawing up a HACCP biosecurity plan.
- U.S. Geological Survey UMESC had a biosecurity plan developed which was reviewed and recommendations for improvement were made.
- Keweenaw Bay Indian Fish Hatchery is working on developing biosecurity measures and recommendations were made on critical control points.
- Calala's Water Haven produces and sells softshell crayfish and an AIS-HACCP plan was developed for this part of their bait operation.
- Porter's Bait Farm produces and sells fathead minnows and an AIS-HACCP plan was developed for this part of their bait operation.

Objective 5.— Three model fish disease HACCP plans (one each for flow-through, pond, and recirculation systems), relying heavily on the specific plans developed under Objective 4 were developed and used in DVD produced in Objective 6

Objective 6.— Production of the HACCP biosecurity video completed. Completed biosecurity workshop video and model HACCP plans will be posted by ISU for free access on the CFSPH and Focus on Fish Health Web sites as well as the NCRAC web site.

Principal Accomplishments:

Objective 1.— Incorporation of iodophor disinfection at 100 ppm during gamete collection from northern pike and walleye immediately post-fertilization (<5 min) for 30 min or at 90 min after fertlization for 10 min may reduce VHSv (strain IVb) transmission without affecting egg hatch.

Iodine disinfection of lake herring eggs for at 50 mg/L or 100 mg/L for 30 min did not affect eye-up of the eggs. This trial also showed that shipment of eyed lake herring eggs did not affect survival.

Objectives 2-6.— The combination of workshops combined with HACCP Plans have resulted in increased awareness of and associated strategies in dealing with current and future diseases as well as improvement in biosecurity.

Impacts:

- The project demonstrated that iodophor disinfection may safely and effectively reduce the risk associated with VHSv exposure during spawning/egg take operations from wild brood fish.
- The project demonstrated that coolwater fish eggs retain VHSv for up to 4 days following immersion challenge but that eggs may not retain VHSv through egg hatch (all fry, including controls, were VHSv negative).
- Caution should be used when disinfecting eggs of species whose eggs have not been evaluated for potential sensitivity to iodophor disinfection.
- Evaluations of the biosecurity workshops indicated that the participants found the information helpful (average score of 4.56 on a scale of 5), intended to use the information (average score 4.58), and the information was presented in an easy to understand format (average score 4.57).
- HACCP plans were developed for each of the hosting facilities with special emphasis on system type (pond, recirculating, or flow-through) and business activities (wild stocking, egg and fingerling production, or grow out for food).
- The majority of the attendees at the workshops indicated that they would implement biosecurity/AIS-HACCP plans at their facilities based on the information learned at the workshops.
- Through workshops and educational materials on biosecurity, farmers have become aware of the risks and potential hazards diseases from outside sources bring.
- State agencies have responded with their own set of rules requiring additional testing and fish certifications. Farmers have been able to utilize biosecurity strategies to minimize the impacts these rules have or they have been able to continue business by complying with requirements in new rules when biosecurity plans are mandatory.
- The VHS-HACCP instructional DVD will further increase the ability of aquaculture producers to develop effective and economical HACCP-based biosecurity plans to control the spread of VHSV as well as address potential AIS and disease concerns in the future.

Recommended Follow-Up Activities:

Information garnered from this project should be used to guide future disease related projects. The combination of a basic research approach to address specific diseases and extension projects that address biosecurity issues can result in improved industry responses for managing future diseases.

Publications, Manuscripts, or Papers Presented:

See the Appendix for a cumulative output for all NCRAC-funded Viral Hemorrhagic Septicemia (VHS) activities.