VIRAL HEMORRHAGIC SEPTICEMIA (VHS)

Chairperson: Jeffrey A. Malison, UW-Stevens Point Northern Aquaculture Demonstration Facility

Industry Advisory Council Liaison: Christopher Weeks, Rives Junction, Michigan

Extension Liaison: Ronald E. Kinnunen, Michigan State University

Funding Request: $197,916

Duration: 2 Years (September 1, 2008 - August 31, 2010)

Objectives: The overall goal is to minimize the risk of spreading VHS and other fish diseases into fish farms and wild waterways throughout the North Central Region (NCR). Specific objectives are:

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 websites 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.

Proposed Budgets:

Institution	Principal Investigator	Objec-tive(s)	Year 1	Year 2	Total
USGS Upper Midwest Environmental Sciences Center	Jeffrey J. Rach	1	$60,063	$51,897	$111,960
Iowa State University Center for Food Security and Public Health	Glenda D. Dvorak	2 - 3	$19,520	$5,480	$25,000
Michigan State University	Ronald E. Kinnunen	3 - 6	$2,100	$2,100	$4,200
University of Wisconsin-Stevens Point Northern Aquaculture Demonstration Facility	Jeffrey A. Malison	3 - 6	$35,187	$21,613	$56,800
		Totals	**$116,870**	**$81,090**	**$197,960**

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JUSTIFICATION

Viral hemorrhagic septicemia (VHS) is an extremely serious disease of fresh and saltwater fish species. Historically considered one of the most virulent viral diseases of salmonids, the disease has been found in Europe, North America, Japan, and Korea (Meyers and Winton 1995; NOAA 2006). Due to the high morality rate and severe economic consequences resulting from the disease, VHS is listed as a reportable disease by the World Organization for Animal Health (OIE) (USDA APHIS 2006a; OIE 2007a; OIE 2007b).

The recent occurrence and spread of VHS in the Great Lakes area has raised concerns regarding the risk of introduction of the virus to fish farms or additional natural waters in the NCR (USGS 2007). As of mid-2007, VHS has affected over 40 species of finfish and has been found in several inland lakes (USDA APHIS 2006b; NOAA 2006; USGS 2007).

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 (Bebak 1998). Although some fish may survive a disease outbreak, they may have reduced growth rates, lower yields, or reduced product quality, which also impacts their market value. A facility's reputation may also be damaged following identification of a disease on the premises. The occurrence of some aquatic animal diseases may also result in international trade implications, particularly for those listed by the OIE.

Of additional concern is the fact that some diseases, including VHS, can infect fish which may not show signs of illness. These "carrier" fish can then serve as potential sources of the disease to other fish if proper biosecurity and disease prevention precautions are not implemented. In response to the spread of VHS in the Great Lakes area, the U.S. Department of Agriculture (USDA) took steps to minimize the spread of the disease throughout the NCR and the rest of the United States (USDA APHIS 2006c). In October 2006, the agency issued an emergency Federal Order prohibiting the importation and interstate movement of over 30 species of live fish from affected Canadian provinces and U.S. states bordering the Great Lakes. Additional efforts by states and associations were also implemented to help control the spread of the disease. To further protect fish farms, fish producers should also implement prevention or biosecurity efforts. Because very few effective treatments are available for most aquatic animal diseases, effective biosecurity is the key to preventing these diseases. The most effective way to deal with viral infections is to prevent it from occurring (Lee 2003). Increasing awareness and prevention measures for VHS is essential for the early detection and control of the disease.

Controlling the spread of this and other fish diseases will require rapid and well-coordinated efforts. Basic and applied research will be needed to study various biological and ecological aspects of the viral organism. Efforts at educating the general public and various users of public waterways (fishermen, boaters, etc.) in the NCR will be key to slowing the spread of the disease. International collaborations will need to be established given the fact that other strains of VHS have existed for years in other areas of the world, and have been studied in greater detail than the current one afflicting the NCR. No one funding agency is either best suited or has sufficient funds to address all of these issues. Large federal research and granting agencies will certainly become involved, including various divisions of the USDA (Animal and Plant Health Inspection Service, National Research Initiative, Agricultural Research Service, etc.). Federal and state Sea Grant offices play an important role with many issues facing public waterways and have already expressed a willingness to work on the VHS problem. Through its normal review process, the North Central Regional Aquaculture Center (NCRAC) Project Review Committee (PRC) prioritized the evaluation of egg disinfection methods as one specific applied research area. The PRC also prioritized outreach efforts aimed at maximizing biosecurity methods on fish farms in the NCR.

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. Because the current VHS epizootic threat requires increased monitoring and detection, and with new sensitive detection techniques available, 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. Egg disinfection with iodophors has been used in salmonid (coldwater fish) culture to prevent disease transmission for some time. However, the safety (toxicity) and efficacy of iodine disinfection to cool- and warmwater fish eggs have not been evaluated. Commercial fish suppliers and hatchery personnel are concerned that the iodine disinfection process may cause significant egg mortality or cause deformities in fry. Although fish culturists would prefer to disinfect eggs with iodine to reduce the risk of introducing a disease organism to their facility or the wild, there are no data available to assure the safety of iodine disinfection to cool- and warmwater fish. Secondly, there is no information on the effectiveness of iodine to kill the virus in non water hardened and water hardened eggs. This proposed study will provide valuable information to commercial and public fish culturist to make decisions on the safety and efficacy of iodine treatment to eliminate VHS infections on cool- and warmwater fish eggs.

Biosecurity has been defined in a variety of ways, and typically involves practices, policies, or procedures used on a farm to reduce the risk of pathogen introduction into a facility as well as its spread on a facility once introduced (Bebak 1998; Delabbio et al. 2004). Biosecurity programs have been developed and used for a number of livestock species, such as poultry, swine, and cattle (USDA APHIS VS NAHMS 1995; Dargatz 2002; National Pork Board 2002; USDA APHIS VS 2002; U.S. Poultry and Egg Association 2002) and have helped to reduce the incidence and introduction of various diseases. While biosecurity procedures have been described for aquaculture facilities (Bebak 1998; Smith 1998; Goodwin 2002; Lee 2003), the use of these measures in finfish facilities has been neither consistent nor uniform (Delabbio et al. 2004; Delabbio et al. 2005). Increasing awareness of biosecurity principles in the aquaculture industry will help to reduce the risk of VHS introduction and spread to NCR fish farms, and will serve to protect these farms against a number of other fish disease pathogens.

While there are basic biosecurity measures that all fish farms can implement, considerations must also be given to the production method and/or species involved with the aquaculture facility. Fish farms in the NCR are characterized by their diversity with the most prevalent production systems being flow-through, ponds, and recirculating aquaculture systems. Surface and ground water are both used as the water source for fish farms, and water is discharged into public waterways, sanitary sewers, or wetlands. Some fish farms raise a single species and others raise five or more. Some farms market primarily food fish, whereas others primarily sell live fish for bait or stocking into public and private waters. Some farms are dependent on regularly moving fish from various sources onto their facility, and others are not. Without adequate risk assessment of individual operations, regulations could be imposed which would unnecessarily negatively impact the economy of these industries, and/or not effectively reduce the risk of spreading VHS and other fish diseases.

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.

RELATED CURRENT AND PREVIOUS WORK

Egg Iodophor Disinfection (Objective 1)

Previous work has principally focused on the use of iodophor egg disinfection for the culture of salmonids. The use of iodophor solutions (polyvinylpyrrolidone iodine) for the surface disinfection of salmonid eggs is now common place in U.S. and European aquaculture to reduce transmission of susceptible pathogens including VHS. Iodophor egg disinfection has been practiced since the 1970s to reduce the transmission of viral and bacterial pathogens associated with the egg, coelomic fluid, or milt and is an established practice to mitigate the presence of vertically transmitted pathogens. Presently, salmonid eggs are disinfected for 30–60 min at 50–100 mg/L during water-hardening while eyed eggs are disinfected for 10 min at 100 mg/L upon receipt at a hatchery. Although disinfection is most commonly applied in salmonid culture, iodophor egg disinfection increased egg survival of several fish species including sturgeon, grouper, and halibut (Bergh and Jelmert 1996; Bouchard and Aloisi 2002).

Current and recent related work is substantially devoted to improving methods to identify VHS from clinical outbreaks, characterization of virus serotypes, and surveillance of the virus in wild stocks (Elsayed, et al. 2006; Gagné et al. 2007; Lumsden et al. 2007). At present, little (no referenced studies found in the literature) research has been conducted to support the use of iodophor egg disinfection to eliminate viruses on cool- or warmwater eggs. Marking et al. (1994) evaluated iodine as a potential fungicide to control fungus on rainbow trout eggs, however, they determined the chemical was toxic to the eggs at 300 mg/L for 60-min exposures and completed no further research. During a recent (August 2007) conference on VHS sponsored by the Wisconsin Veterinary Diagnostic Laboratory and University of Wisconsin-Stevens Point (UW-Stevens Point) Northern Aquaculture Demonstration Facility (NADF) extension personnel, several research groups reported recent results of the effect of different iodophor treatments on mortality of selected coolwater fish eggs. However, none of these groups conducted studies on the efficacy of disinfection. This proposed research will provide information on the safety and efficacy of iodine to control viral infections on cool- or warmwater eggs that is currently lacking in the literature.

Outreach Efforts on Biosecurity at Fish Farms (Objectives 2-6)

Biosecurity Response Packets

The Iowa State University Center for Food Security and Public Health (CFSPH) has already developed a number of disease specific "response" packets as educational tools for livestock producers as a means of providing essential animal disease and prevention information to this audience. Using these established templates, the CFSPH proposes to create similar educational tools on VHS education for fish producers in the NCR. The response packet will contain a brief overview of the disease, clinical signs, personnel to contact, as well as measures to implement on the farm to prevent the introduction of or spread of the disease.

Biosecurity and HACCP Workshops and Video

NADF extension personnel have been extremely proactive and responsive to the challenges posed by VHS and other fish disease issues in the NCR. In 2007 alone they organized a Veterinary Health Assessment workshop (June 14), two biosecurity workshops (June 14 and September 7), and a VHS workshop (August 9). Each of these workshops was well attended and received excellent reviews from the attendees. In July 2007 Ron Kinnunen worked with the Michigan Wholesale Bait Association and the Michigan Aquaculture Association on coordinating an Aquatic Invasive Species (AIS)-HACCP Training Workshop that focused on practices that the industry can take to deal with new regulations implemented by the State of Michigan on VHS Management Zones for wild baitfish harvest from the Great Lakes. Kinnunen worked with Michigan Baitfish Association leaders on developing model AIS-HACCP and biosecurity plans that would keep their businesses viable and also deal with the new VHS Management Zone regulations. The AIS-HACCP approach was first pilot tested with the Michigan Wholesale Bait Association and the U.S. Fish and Wildlife Service hatchery managers and ecological services personnel in the southwest region. Results of these pilot tests were very positive. Both groups appropriately applied the principles of AIS-HACCP and each felt that the approach was workable from a business/public hatchery management perspective and that it could significantly reduce the risk of spreading AIS. Pilot project participants also provided suggestions to modify the draft manual. Comments from agency, industry, and university reviewers were also incorporated into the training manual (Gunderson and Kinnunen 2001). A second edition of the training manual was completed with an emphasis on assisting natural resource agency personnel prevent the spread of AIS in their work activities (Gunderson and Kinnunen 2004).

ANTICIPATED BENEFITS

Diseases constitute the largest single cause of economic losses in aquaculture (Meyer 1991). There are few treatments available for current and emerging aquaculture diseases. This research on egg disinfection will provide valuable information to commercial and public fish culturists to help them make decisions on the safety and efficacy of iodine treatment to eliminate VHS infections on cool- and warmwater fish eggs. If iodine disinfection can be used to safely eliminate the virus on these eggs, the

direct benefits will include: (1) disinfected eggs could be exchanged between aquaculture facilities without fear of contaminating a facility or spreading the virus to other fish stocks; (2) regulatory agencies may be less restrictive on egg shipments across state and national borders; (3) the profitability of commercial egg production would be maintained or enhanced with a disease free egg product; and (4) the genetic diversity of wild fish populations could be maintained (by facilitating the use of brood fish from wild sources).

The development of methods for treating fish diseases is greatly needed and disease prevention remains the most important and useful strategy for minimizing disease on fish farms. These projects are proposed to develop an integrated set of educational materials and conduct outreach projects targeted to fish farms and farmers in the NCR to help protect the region's fish farms by providing farmers with tools and key information needed to help prevent the spread of VHS and other fish diseases onto farms, between farms, and from farms into natural waters.

The proposed use of the AIS-HACCP approach has many advantages. It can effectively deal with a diverse industry and diverse risk factors associated with a variety of plant, invertebrate, vertebrate, and pathogen AIS. If it develops as it has in the seafood industry, it should prove to be a good partnership between industry and government regulators. It can help avoid overly restrictive regulations, and, if properly applied, can be effective at reducing the risk of spreading AIS via baitfish and aquaculture practices. The HACCP approach concentrates on the points in the process that are critical to the environmental safety of the product, minimizes risks, and stresses communication between regulators and the industry. With proper cooperation between industry representatives, resource management agencies, and other AIS experts, the AIS-HACCP approach will reduce the risk that AIS will be established in new locations while maintaining the economic viability of the baitfish and aquaculture industries. It can provide a mechanism for AIS-free certification, and it can instill confidence in the public that state and federal fish stocking programs are conducting their activities in an environmentally responsible manner.

OBJECTIVES

The overall goal is to minimize the risk of spreading VHS and other fish diseases into fish farms and wild waterways throughout the North Central Region (NCR). Specific objectives are:

1. Determine the safety and efficacy of iodine disinfection on yellow perch and largemouth bass eggs (unhardened and water hardened) 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 websites and information sources where fish farmers can obtain the most current, up-to-date status of the disease.

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4. Utilize the existing Aquatic Invasive Species (AIS) Hazard Analysis Critical Control Point (HACCP) Training Curriculum to develop <u>specific</u> fish disease HACCP plans for each of the six facilities involved in the workshops.

5. Develop and distribute three <u>model</u> fish disease HACCP plans (one each for flow-through, pond, and recirculation systems), relying heavily on the <u>specific</u> plans developed under Objective 3.

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.

PROCEDURES

Egg Iodophor Disinfection (Objective 1)

Upper Midwest Environmental Sciences Center, Genoa National Fish Hatchery, and La Crosse Fish Health Center

Study Location and Partners Responsibility
Adult fish will be obtained from private, state, or federal hatcheries and transferred to the Upper Midwest Environmental Sciences Center (UMESC). The Genoa National Fish Hatchery (GNFH) will assist UMESC in spawning fish, infecting eggs with Viral Hemorrhagic Septicemia virus (VHSv), and iodophor disinfection of eggs. Preparation and culture of VHSv inoculants and confirmation of VHSv presence/absence in eggs or fry will be conducted by the La Crosse Fish Health Center (LFHC). Fish spawning, VHSv challenge, egg disinfection, egg incubation, and fry culture will be conducted at an UMESC isolation facility (Appendix 1). UMESC will record physical (water chemistry) and biological (egg/fry enumeration and characterization [live/dead, normal/deformed]) data during the study. All study personnel will adhere to the UMESC standard operation procedure for biosecurity (Appendix 1; the biosecurity procedures were reviewed by Jill Rolland, Ph.D. [APHIS], Rob Bakal Ph.D. [USWS], and Jim Winton Ph.D. [(USGS] and comments incorporated). All work conducted will be with VHSv Genotype IVb, the strain identified to have caused mortality in Great Lakes fishes (Elsayed et al. 2006). The LFHC already has this strain of VHS and all virus challenges will use this strain.

Time Frame
This will be a two year project. In the first year, emphasis will be on evaluating the safety and efficacy (to control VHS infections) of iodine to eggs of northern pike (*Esox lucius*) and walleye (*Sander vitreum*). A second year will be necessary to finish research not completed in year one and to prepare a final report. A rigid time table is difficult to establish for this research because of the potential variability in brood stock quality, brood stock infection status, spawning success, egg quality, and VHSv challenge success.

Test Animals
The test species selected by the NCRAC PRC were yellow perch and largemouth bass, however, the use of northern pike and walleye is recommended. Walleye and pike are useful surrogate species commonly cultured in aquaculture that are known to be susceptible to VHSv IVb. These species are readily dry-spawned and resultant eggs may be “rolled” during incubation to control fungus. Yellow perch eggs are contained in a gelatinous matrix and largemouth bass are typically substrate spawners. Eggs from both yellow perch and largemouth bass would require special treatments to free the eggs from the matrix or to remove the eggs from the substrate prior to VHSv challenge and incubation. Both activities complicate the study results and could invalidate the study results. In our laboratory experience with incubation of cool- and warmwater eggs, the use of northern pike or walleye will minimize the potential detrimental fungal outbreaks we would expect when not using prophylactic fungal control (formalin or hydrogen peroxide treatments).

Two groups of adult fish of each species will be obtained from private, state, or federal hatcheries and transferred to UMESC. Group 1 will be held at UMESC for ~1 month prior to spawning using a water temperature and photoperiod regime similar to natural conditions. Group 1 females will be VHSv-challenged by intra peritoneal (i.p.) injection with VHSv IVb (see VHSv IVb Challenge). Group 1 males will not be challenged. Group 2 fish will be collected during GNFH or Wisconsin Department of Natural Resources normal spawning operations and will be used to provide eggs for direct VHSv challenge of eggs during spawning (see VHSv IVb Challenge). Group 2 fish will be transported directly to UMESC and used immediately; Group 2 brood stock will not be maintained at UMESC.

Adult fish will be collected, cultured, and dry-spawned according to standard hatchery procedures (Piper et al. 1982; Doug Aloisi, GNFH Manager, personal communication). Eggs will be incubated in a miniature egg hatching system that consists of a headbox, egg hatching jars, and glass aquaria (Rach et al. 1995). Eggs will be incubated at temperatures commonly used in hatchery operations (12°C). Eggs will be measured by volume displacement and each egg jar will contain 25 ± 5 mL of eggs.

All fish used in this study will be euthanized and incinerated at UMESC after spawning or hatch.

VHSv IVb Challenge: Group 1 female brood stock will be challenged by i.p. injection with VHSv IVb. Actual virus titer and injection timing will be determined in preliminary trials; methods used will be similar to those of Al-Hussinee et al. (2007). The i.p. challenge will be timed to produce maximum virus titer to coincide with spawning. Group 1 brood stock will be dry-spawned and eggs iodophor-disinfected (see Egg Disinfection); eggs from two or three Group 1 females will be hand-stripped into stainless steel bowls then milt from three to five Group 1 males will be added. UMESC well water will be added immediately after the addition of milt to activate the sperm; at least 2 sets of Group 1 brood stock will be spawned as previously described and the eggs will be mixed together in approximately equivalent volumes before allocation to treatment group. The presence of VHSv IVb in eggs and milt of Group 1 brood stock will be determined in samples collected during spawning.

Group 2 brood stock will be collected during GNFH or Wisconsin Department of Natural Resources spring spawning operations. Group 2 brood stock will be dry-spawned (Piper et al. 1982) and then challenged by incorporation of VHS IVb during sperm activation. Briefly, eggs from two to three Group 2 females will be hand-stripped into a stainless steel bowl then milt from three to five Group 2 males will be added. Immediately after the addition of milt, VHS IVb inocula and well water will be added; methods used will be similar to those used by Al-Hussinee et al. (2007). At least two sets of Group 2 brood stock will be spawned and the eggs challenged as previously described. The eggs from these two sets of Group 2 brood stock will be mixed together in approximately equivalent volumes after challenge but before allocation to treatment group. A preliminary challenge trial will be conducted to determine appropriate challenge conditions (titer concentration by challenge duration). The challenge duration is expected to be ~5 min; after VHSv IVb immersion challenge, eggs will be rinsed in UMESC well water before being treated according to the methods described in the Egg Incubation and Treatment section.

VHS culture, isolation, challenge preparation, and confirmation will be conducted by LFHC. The LFHC is recognized as a center of excellence in fish disease culture techniques and disease identification. The LFHC is the primary center for the identification and diagnosis of fish disease in the Great Lakes area; LFHC conducts fish health inspections for the U.S. Fish and Wildlife Service and the U.S. Geological Survey and provides certification of fish pathogen status for cultured fish. Only VHSv IVb (the VHSv strain endemic to this region; Elsayed et al. 2006) will be used in this study; LFHC will maintain the VHSv IVb cultures at their facility. Pilot studies will be conducted to define the challenge methods used for i.p. injection of Group 1 brood stock and for challenge of eggs collected from Group 2 brood stock. The VHSv IVb inocula used will be freshly prepared prior to challenge and the titer levels used will be determined according to standard virology procedures.

Egg Incubation and Treatment: Two egg groups will be tested for each fish species – Group 1 eggs (from brood stock challenged prior to spawning by i.p. injection) and Group 2 eggs (eggs challenged by bath immersion in VHSv IVb during fertilization). Within Groups 1 and 2, the eggs will be further divided into five iodophor treatments as defined in Table 1. Immediately after spawning (Group 1 eggs) or VHSv IVb challenge (Group 2 eggs), the eggs will be separated into five equal aliquots (1 aliquot per treatment [Table 1]; each egg aliquot will consist of at least 50 mL of eggs). Each egg aliquot will be further divided into six replicates; each replicate group of eggs will ultimately be placed in an egg jar for incubation.

Table 1. Iodophor-disinfection assignment to treatment group by brood stock group. Iodophor disinfection concentration will be 100 mg/L.

Brood stock group	Treatment group	Iodophor disinfection	Iodophor disinfection timing (min post-fertilization)	Iodophor disinfection duration (min)	Comments
1	1	No	NA	NA	Control eggs for Group 1; Treatment groups 2 and 3
1	2	Yes	~0 (during water hardening)	30	Disinfection initiated immediately after fertilization
1	3	Yes	~0 (during water hardening)	60	Disinfection initiated immediately after fertilization
1	4	No	NA	NA	Control eggs for Group 1; Treatment group 5
1	5	Yes	~90	10	Disinfection initiated after 90 min of water-hardening in UMESC well water
2	1	No	NA	NA	Control eggs for Group 2; Treatment groups 2 and 3
2	2	Yes	~5 (after challenge, during water hardening)	30	Disinfection initiated immediately after fertilization
2	3	Yes	~5 (after challenge, during water hardening)	60	Disinfection initiated immediately after fertilization
2	4	No	NA	NA	Control eggs for Group 2; Treatment group 5
2	5	Yes	~95 (after challenge and after 90 min of water hardening)	10	Disinfection initiated after 90 min of water-hardening in UMESC well water

NA – not applicable

The egg handling and incubation procedures will follow those described by Rach et al. (1997; 1998). Eggs of each treatment group will be acclimated to the appropriate water temperatures. Treatments will be randomly assigned to groups of eggs; egg assignment to incubation jars will be randomized. Each egg jar will be covered to prevent contamination between jars. Water entering the egg incubation headbox will be UV-sterilized water; water will flow by gravity from the headbox to the egg jars and then to glass aquaria positioned below the incubation jars to collect hatched fry. Water flow to each egg jar will be measured and adjusted daily.

Fungus Control

Chemical prophylaxis to prevent or control fungal infestations of incubating eggs will not be used. To reduce potential of fungal infestation, each incubation system will be iodine disinfected, the headbox and egg jars covered, and the incubation water supply will be UV-sterilized. Water flow to the egg jars will be sufficient to gently roll the eggs during the first 48 hours; flow will be increased gradually after the initial 48 hours. Physical removal of fungused eggs by siphoning will be conducted as required; eggs removed will be quantified if possible and the number removed recorded.

Iodophor Concentration Verification
A commercial iodophor solution (Wescodyne, Argentyne, or Betadine) will be used to disinfect eggs as described in Table 1. One water sample will be removed from one replicate of each egg treatment group and analyzed to determine iodophor concentration. Iodophor concentration will be determined by a titrimetric method and will be reported as the free iodine concentration.

Calculations
The mean number of eggs/mL for each species will be determined by enumerating the eggs in three 5 mL egg sample volumes. The total number of eggs per incubation jar will be calculated by multiplying the mean number of eggs/mL by the volume of eggs placed in each incubation jar. The total number of fry (normal or deformed) will be determined by either direct enumeration of hatched fry or by estimating the total number of fry by multiplying the mean fry mass (determined from three 50-fry samples) times the total mass (g) of fry per replicate. The percent hatch will be determined for each replicate by dividing the number of fry by the initial number of eggs.

Egg/Fry Collection and VHS Diagnosis
LFHC personnel will be responsible for collecting egg and fry samples to determine the presence or absence of VHSv IVb. Group 1 egg samples will be collected after fertilization and after iodophor disinfection. Group 2 egg samples will be collected after VHSv IVb challenge and after iodophor disinfection. Fry will be collected from each replicate after egg hatch and at 10 days post hatch. Egg and fry will be pooled (up to 5) and then tested using standard protocols to isolate and identify fish viruses (USFWS/AFS-FHS Standard Procedures for Aquatic Animal Health Inspections, Section 2, Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogen, 2007; and OIE Manual of Diagnostic Tests for Aquatic Animals, 2006 http://www.oie.int/eng/normes/fmanual/a_00022.htm). This sampling will provide 95% confidence that infected samples will be included in the fish sampled, assuming a minimum prevalence of infection equal to or greater that 5%. The pooled samples will be tested using standard tissue cell culture methods for isolation of virus and confirmation by polymerase chain reaction (PCR).

Physical Parameters
Water chemistry (temperature, dissolved oxygen, and pH) measurements will be recorded twice daily in the incubation system headbox.

Data Collected for Analysis
Data collected will consist of the initial number of eggs/jar, the number of live fry/jar, the number of deformed fry/jar and the presence or absence of VHSv IVb in each sample group collected per replicate.

Statistical Analysis
Presence/Absence of VHS IVb - No statistical analyses will be performed on data collected regarding the presence or absence of VHSv IVb in egg or fry samples. Disinfection effectiveness will be determined based solely on a 100% effect level, i.e., a disinfection treatment group will be determined to be effective only if all eggs and fry samples are free of VHSv IVb and VHSv IVb is confirmed present in the concomitant non-disinfected control eggs. A disinfection treatment will be considered ineffective if one or more of the egg or fry samples are confirmed positive for VHSv IVb.

Egg Hatch
Individual eggs can experience one of two outcomes—survival or death. Survival is, therefore, a binary random variable and it is reasonable to assume that both follow a Bernoulli distribution. The survival response data are the numbers of survivors, out of a fixed number of exposed eggs in each jar, and the number of survivors, therefore, follows a binomial distribution. The egg jars are the fundamental experimental units in this study. Eggs held in the same tank will experience identical environmental conditions but those conditions may vary randomly among jars. The study design consists of combinations of four classification variables ([1] VHSv IVb challenge; [2] iodophor disinfection; [3] timing of disinfection; [4] disinfection duration) applied to each of six independently observed replicates, for a total of 60 experimental units. The probability of survival will be modeled using a mixed-effects logistic model fitted using the SAS GLIMMIX procedure based on Wolfinger and O'Connell (1993). Should the data exhibit quasi-complete separation, then comparisons will be conducted using Fisher's exact test.

Appropriate means-comparison tests will be constructed based on overall model significance. The probability of infection following iodine disinfection will be compared using Fisher's exact test. Treatment levels will be judged statistically different if $P < 0.05$.

Isolation Facility and Procedures
All study fish held at UMESC will be cultured in a locked isolation facility whose access will be limited only to personnel working on the study. The facility will have a transition area where employees don/doff protective clothing. All employees working in the isolation facility will be trained in laboratory practices for handling diseased organisms. All personnel involved in this study will adhere to the UMESC standard operation procedure for biosecurity (Appendix 1).

Effluent Treatment and Organism Disposal
All water discharged from the isolation facility will be chlorinated (twice) before discharge into the UMESC activated carbon adsorption units. Water from the activated carbon adsorption units will then be discharged into effluent ponds before discharge to the Black River. All eggs or fish (fry/fish will be euthanized first) will be surface disinfected with chlorine, isopropyl alcohol, or iodophor solution and then incinerated on site. All VHSv IVb material (e.g., challenge solutions) used at UMESC will be autoclaved at UMESC and then incinerated on site. All VHSv IVb materials used at LFHC will be autoclaved then disposed of according to LFHC standard procedures. All GNFH equipment will be chlorine or iodophor-disinfected before departure from UMESC.

Outreach Efforts on Biosecurity at Fish Farms (Objectives 2-6)

Iowa State University Center for Food Security and Public Health, University of Wisconsin-Stevens Point Northern Aquaculture Demonstration Facility, and Michigan State University

In the first three months of this project, the CFSPH will prepare a 6- to 10-page VHS "response" package that specifically targets fish farm producers. The packet will contain several documents in pdf format that provide an overview of VHS and its clinical signs, as well as prevention practices to use on the farm to minimize the risk of introduction or spread of VHS and other fish diseases. The packet will also contain Web sites for up-to-date information on VHS regulations, outbreak status, and prevention advice. The latter is important because the status of VHS in the region will likely change, therefore, access to current and accurate information will be critical.

The VHS response packet will contain the following documents:

1. VHS Fast Fact. This 1-page fact sheet will describe the cause of VHS, how it is spread, what species are currently known to be affected, and general prevention measures to minimize the spread of the disease.

2. Routes of Transmission Handout. This 1-page handout will provide a general overview of the various ways pathogens can spread on aquaculture farms. It will serve to provide producers with insight on the mechanisms of disease spread to help them better understand the importance of the implementation of biosecurity measures to control the spread of disease spread.

3. VHS Prevention Practices. This multiple-page document (approximately 6 pages) will describe prevention measures that can be implemented on a fish farm to help reduce the risk of introduction and spread of VHS and other fish diseases. The document will also include images of clinical signs of VHS to aid producers in recognizing VHS in fish.

4. Information Sources for Current Information. Due to anticipated changes in the status of VHS in the NCR and the U.S., the "response" packet will also contain Web sites and information sources where fish farmers can obtain the most current, up-to-date status of the disease.

The entire "response" packet will be "packaged" in a pdf format for distribution to fish producers, aquaculture associations, extension specialists, fisheries biologists, state agencies, and aquatic veterinarians in the NCR by e-mail. Additionally, the response packet will be distributed during the

proposed regional workshops and posted on the CFSPH, NADF, and NCRAC Web sites for free access and download.

The remainder of the activities will be centered on development and delivery of a set of six on-farm workshops that are proposed to be conducted within a 12-month period. The CFSPH will develop an awareness level PowerPoint presentation (with speaker notes and images) on basic aquaculture biosecurity principles to be implemented on fish farms in the NCR to prevent the introduction and spread of VHS and other fish diseases. Where applicable, specific biosecurity measures for different production systems (e.g., flow-through, pond, and recirculation systems), will also be included. This PowerPoint presentation will be provided to the NADF staff for peer-review and incorporation and delivery during the regional workshops. The presentation will also be posted on the NADF, CFSPH, and NCRAC Web sites for free access and download.

Preliminary contact has been made with a number of fish farms across the region, and it has been determined that the workshops will be conducted at farms in at least five states: Michigan, Ohio, Wisconsin, Iowa or Missouri, and Minnesota or North or South Dakota. The workshops will be conducted at farms using the three system types most commonly used by fish farmers in the region—ponds, flow-through, and recirculating aquaculture systems. The workshops will be organized by NADF extension specialists. No funding for their salaries is being requested from NCRAC. The workshops will be organized in close collaboration with local fish farmers and appropriate state aquaculture associations. Each workshop will include presentations and demonstrations delivered by individuals with diverse areas of expertise in aquaculture. These will include NADF and other regional aquaculture extension specialists, private fish farmers, representatives from state aquaculture associations, regional aquatic veterinarians or fish health specialists, state regulators, and national regulatory, research, and advisory agencies. With regards to the latter, USDA APHIS representatives have already expressed an interest and willingness to become involved in the workshops.

Immediately following the presentations, Kinnunen will lead efforts to develop a fish disease HACCP plan specific for the fish farm hosting the workshop. The fish disease HACCP plans will utilize the AIS-HACCP Training Curriculum that Dr. Kinnunen has co-authored. He will work directly with operators of each fish farm and will be assisted by extension personnel. Persons attending the workshop will be invited to observe the development of the HACCP plans.

After all workshops have been completed, NADF extension specialists will work with Dr. Kinnunen to prepare three generalized model HACCP plans, one for each system type. These model HACCP plans will rely heavily on the specific HACCP plans developed for the six fish farms participating in the project. The model HACCP plans will be made available electronically through the NADF, CFSPH, and NCRAC Web sites, and by hard copy.

NADF extension specialists will also capture all of the proceedings of the workshops and HACCP development efforts on video. Using this footage, a cohesive video will be prepared that will detail all of the key issues involving VHS, fish diseases in general, and fish disease HACCP plan development. This video will be made available on DVD as well as streaming video on the aforementioned Web sites.

FACILITIES

Egg Iodophor Disinfection (Objective 1)

UMESC's state-of-the-art research facility includes numerous laboratories (isolation, wet, and analytical) equipped with technology to conduct fish culture and fish disease assessments. Researchers at the UMESC have conducted research on fish disease model development (fungal and bacterial models for over a decade), fish culture, and evaluations on the efficacy and safety of therapeutic drugs to control fish diseases. All effluent waters can be treated to kill any pathogen in the water. The LFHC is a state-of-the-art fish disease diagnostic laboratory serving the eight-state Great Lakes/Big Rivers Region. The Center's staff perform fish health inspections, diagnostic, and laboratory work for bacterial, parasitic, and viral pathogens in their specially designed facility.

Outreach Efforts on Biosecurity at Fish Farms (Objectives 2-6)

One of the strengths of this project is that much of the work will be done on site at a number of commercial fish farms across the region. This approach will maximize the relevance of the work and facilitate the attendance of as many regional aquaculturists as possible.

The expertise and experience of the project team is considerable. The Wisconsin NADF extension team consists of three full-time outreach specialists. NADF has already taken a lead role in addressing the VHS issue by making VHS the focal point of the Aquaculture Field Day and vendor fair held at NADF June 14-15, 2007. Tom Notton, an expert in video production in the Department of Communicating Arts at the University of Wisconsin-Superior, has agreed to work closely with NADF staff on the production of the proposed video. Wisconsin has taken a leadership role in addressing fish disease issues and its fish disease program is viewed as one of the best in the nation. A core of the program includes a close working relationship between Wisconsin's Department of Agriculture, Trade, and Consumer Protection (the agency responsible for fish disease regulation in the state) and NADF. Kinnunen is a co-author of the Aquatic Invasive Species HACCP Training Curriculum and has been actively involved in aquaculture outreach activities for over two decades. He has conducted AIS-HACCP Training Workshops throughout the U.S. with participants coming from as far away as New Zealand and Germany.

The participation of Iowa State University's CFSPH in this project was actively encouraged by Joe Morris, NCRAC Associate Director at Iowa State University who is responsible for all NCRAC extension activities. Located at Iowa State University's College of Veterinary Medicine, the CFSPH was established in 2002 with the mission to increase national preparedness for the accidental or intentional introduction of disease agents that threaten food production or public health. The Center employs seven veterinary specialists; Drs. Dvorak and Palić have advanced training (M.S. and Ph.D., respectively) in aquaculture and fish health. Dr. Dvorak has also recently become board certified in Veterinary Preventive Medicine. Additionally, the Center has a six person "Creative Services" group (instructional designers, Web management, programmers, and graphic designers) that works with the veterinary specialists to provide a graphic design and layout of the educational materials. The Center has a strong history of developing and providing animal disease and prevention education at the national level to veterinarians, extension specialists, livestock producers, and the general public. Some of the Center's outreach activities on animal health can be viewed at http://www.cfsph.iastate.edu/DiseaseInfo/default.htm.

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PROJECT LEADERS

State	Name/Institution	Area of Specialization
Iowa	Glenda D. Dvorak Iowa State University	Veterinary Medicine, Fisheries biology
Michigan	Ronald E. Kinnunen Michigan State University	Aquaculture/Aquatic Invasive Species/Diseases
Wisconsin	Jeffrey A. Malison University of Wisconsin-Stevens Point	Aquaculture
	Jeffrey J. Rach Upper Midwest Environmental Sciences Center	Fish health, Fish disease

PARTICIPATING INSTITUTIONS AND PRINCIPAL INVESTIGATORS

Iowa State University
Glenda D. Dvorak

Michigan State University – Upper Peninsula
Ronald E. Kinnunen

University of Wisconsin-Stevens Point
Jeffrey A. Malison

Upper Midwest Environmental Sciences Center
Jeffrey J. Rach

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0039
Expires 03/31/2004

BUDGET

ORGANIZATION AND ADDRESS Upper Midwest Environmental Sciences Center 2630 Fanta Reed Road, La Crosse, WI 54603 PROJECT DIRECTOR(S) Jeffrey J. Rach				**USDA AWARD NO.** **Year 1: Objective 1** Duration Proposed Months: 12 **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages	**CSREES FUNDED WORK MONTHS**						
1. No. of Senior Personnel	Calendar	Academic	Summer				
a. 1 (Co)-PD(s)	0.47			$3,240			
b. 1 Senior Associates	4.3			$16,800			
2. No. of Other Personnel (Non-Faculty)							
a. ___ Research Associates-Postdoctorates							
b. ___ Other Professionals							
c. ___ Paraprofessionals							
d. ___ Graduate Students							
e. ___ Prebaccalaureate Students							
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages →				$20,040			
B. Fringe Benefits (If charged as Direct Costs)				$5,429			
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$25,469			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$12,104			
F. Travel				$1,200			
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)				$21,290			
K. Total Direct Costs (C through I) →				$60,063			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K) →				$60,063			
N. Other →							
O. Total Amount of This Request →				$60,063			
P. Carryover -- (If Applicable) . . . . . **Federal Funds: $**				**Non-Federal funds: $**		**Total $**	
Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O) Cash (both Applicant and Third Party) →							
Non-Cash Contributions (both Applicant and Third Party) →							

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		
Signature (for optional use)		

Form CSREES-2004 (12/2000)

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0039
Expires 03/31/2004

BUDGET

ORGANIZATION AND ADDRESS
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road, La Crosse, WI 54603

PROJECT DIRECTOR(S)
Jeffrey J. Rach

USDA AWARD NO. **Year 2: Objective 1**

	CSREES FUNDED WORK MONTHS			Duration Proposed Months: 12 — Funds Requested by Proposer	Duration Proposed Months: ____ — Funds Approved by CSREES (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages							
1. No. of Senior Personnel	Calendar	Academic	Summer				
a. 1 (Co)-PD(s)	0.94			$6,480			
b. 1 Senior Associates	2.5			$12,600			
2. No. of Other Personnel (Non-Faculty)							
a. ___ Research Associates-Postdoctorates							
b. ___ Other Professionals							
c. ___ Paraprofessionals							
d. ___ Graduate Students							
e. ___ Prebaccalaureate Students							
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages →				$19,080			
B. Fringe Benefits (If charged as Direct Costs)				$4,558			
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$23,638			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$5,650			
F. Travel				$1,200			
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)				$21,409			
K. Total Direct Costs (C through I) →				$51,897			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K) →				$51,897			
N. Other →							
O. Total Amount of This Request →				$51,897			

P. Carryover -- (If Applicable) . . . . . **Federal Funds: $** **Non-Federal funds: $** **Total $**

Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)		
Cash (both Applicant and Third Party) →		
Non-Cash Contributions (both Applicant and Third Party) →		

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		
Signature (for optional use)		

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0524-0039. The time required to complete this information collection is estimated to average 1.00 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing the reviewing the collection of information.

Form CSREES-2004 (12/2000)

BUDGET EXPLANATION FOR THE UPPER MIDWEST ENVIRONMENTAL SCIENCES CENTER

(Rach)

Objective 1

A. Salaries and Wages. Years 1 and 2: Support for the PI and a biologist to conduct the research. This research will require a biologist trained in fish propagation, application of therapeutic treatments to eggs, fish disease infection techniques, and biosecurity procedures who will work on the project for 1,176 hours over a two-year period at $25/hour. The PI will direct all aspects of the study, write the completion report and manuscript, and present the data at aquaculture workshops and meetings for approximately 243 hours over a two year period at $40/hour.

B. Fringe Benefits. The fringe benefit rate for the PI will be 12% and for the biologist 30%.

E. Materials and Supplies. Year 1: Supplies needed include: brood stock ($1,175), chemical therapeutants ($350), Lexan pipe ($400), aluminum angle ($425), peristaltic pumps ($976), air pumps ($364), plastic tubing ($250), glass tubing ($250), plumbing materials (pvc and cpvc; $250), temperature regulation valves ($1,650), 3-way valves for plastic tubing ($175), plate glass ($500), titration supplies ($175), analytical reagents ($200), DO meters ($1,734), pH meters ($1,200), effluent sump pumps ($280), chemical pumps ($250), chlorine ($250), iodine ($75), protective clothing ($500), UV-sterilization components ($350), sanitation products ($75), and transfer containers ($250). Year 2: Supplies needed include: brood stock ($1,175), chemical therapeutants ($350), Lexan pipe ($400), aluminum angle ($425), peristaltic pumps ($430), plastic tubing ($250), glass tubing ($250), plumbing materials (pvc and cpvc; $250), 3-way valves for plastic tubing ($175), titration supplies ($175), analytical reagents ($200), effluent sump pumps ($70), chlorine ($250), iodine ($75), protective clothing ($500), UV-sterilization components ($350), sanitation products ($75), and transfer containers ($250).

E. Travel. Years 1 and 2: $1,200/year for transportation, lodging, and meal expenses for the PI to present study results at aquaculture workshops and meetings, locations to be determined.

J. All Other Direct Costs. LFHC is conducting their research as a subcontractor to UMESC. LFHC will conduct VHSv culture, infection, and confirmatory diagnosis. The salaries for Year 1 will be $16,790 and $ 16,909 for Year 2. The cost of supplies will be $3,500/year for PCR, viral culture, and photographic materials, and VHSv diagnostic supplies. LFHC personnel will present study results at aquaculture workshops and meetings ($1,000/year).

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0039
Expires 03/31/2004

BUDGET

ORGANIZATION AND ADDRESS
Center for Food Security and Public Health, Iowa State University
2170 College of Veterinary Medicine, Ames, IA 50011

PROJECT DIRECTOR(S)
Glenda D. Dvorak

USDA AWARD NO. **Year 1: Objectives 2 & 3**

	CSREES FUNDED WORK MONTHS			Duration Proposed Months: 12 Funds Requested by Proposer	Duration Proposed Months: ____ Funds Approved by CSREES (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages							
1. No. of Senior Personnel	Calendar	Academic	Summer				
a. 1 (Co)-PD(s)		3.0		$7,614			
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty)							
a. ___ Research Associates-Postdoctorates							
b. 2 Other Professionals		3.0		$7,096			
c. ___ Paraprofessionals							
d. ___ Graduate Students							
e. ___ Prebaccalaureate Students							
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages →				$14,710			
B. Fringe Benefits (If charged as Direct Costs)				$4,810			
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$19,520			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies							
F. Travel							
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)							
K. Total Direct Costs (C through I) →				$19,520			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K) →				$19,520			
N. Other →							
O. Total Amount of This Request →				$19,520			

P. Carryover -- (If Applicable) . . . . . **Federal Funds: $** **Non-Federal funds: $** **Total $**

Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)		
Cash (both Applicant and Third Party) →		
Non-Cash Contributions (both Applicant and Third Party) →		

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		
Signature (for optional use)		

Form CSREES-2004 (12/2000)

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

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Expires 03/31/2004

BUDGET

ORGANIZATION AND ADDRESS Center for Food Security and Public Health, Iowa State University 2170 College of Veterinary Medicine, Ames, IA 50011 PROJECT DIRECTOR(S) Glenda D. Dvorak				**USDA AWARD NO.** **Year 2: Objectives 2 & 3** Duration Proposed Months: 12 **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	**CSREES FUNDED WORK MONTHS**						
	Calendar	Academic	Summer				
a. 1 (Co)-PD(s)		1.0		$2,745			
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates . . .							
b. 1 Other Professionals		1.0		$1,385			
c. ___ Paraprofessionals							
d. ___ Graduate Students							
e. ___ Prebaccalaureate Students							
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages →				$4,130			
B. Fringe Benefits (If charged as Direct Costs)				$1,350			
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$5,480			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies							
F. Travel							
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)							
K. Total Direct Costs (C through I) →				$5,480			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K) →				$5,480			
N. Other →							
O. Total Amount of This Request →				$5,480			
P. Carryover -- (If Applicable) . . . . . **Federal Funds: $**				**Non-Federal funds: $**		**Total $**	
Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O) Cash (both Applicant and Third Party) →							
Non-Cash Contributions (both Applicant and Third Party) →							

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		
Signature (for optional use)		

Form CSREES-2004 (12/2000)

BUDGET EXPLANATION FOR IOWA STATE UNIVERSITY

(Dvorak)

Objectives 2 & 3

A. Salaries and Wages. Partial salary for Academic and Professional Staff at the Center for Food Security and Public Health at Iowa State University for development and graphical design of the VHS response packet and biosecurity PowerPoint presentation and Webpage development and design.

B. Fringe Benefits. The fringe benefit rate for Academic and Professional Staff at Iowa State University is 32.7%.

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0039
Expires 03/31/2004

BUDGET

ORGANIZATION AND ADDRESS
Michigan Sea Grant, Michigan State University
710 Chippewa Square, Suite 202, Marquette, MI 49855

PROJECT DIRECTOR(S)
Ronald E. Kinnunen

USDA AWARD NO. **Year 1: Objectives 3 - 6**

	CSREES FUNDED WORK MONTHS			Duration Proposed Months: 12 **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages	Calendar	Academic	Summer				
1. No. of Senior Personnel							
a. ___ (Co)-PD(s)							
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty)							
a. ___ Research Associates-Postdoctorates . . .							
b. ___ Other Professionals							
c. ___ Paraprofessionals							
d. ___ Graduate Students							
e. ___ Prebaccalaureate Students							
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages →							
B. Fringe Benefits (If charged as Direct Costs)							
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →							
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies							
F. Travel				$2,100			
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)							
K. Total Direct Costs (C through I) →				$2,100			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K) →				$2,100			
N. Other →							
O. Total Amount of This Request →				$2,100			

P. Carryover -- (If Applicable) . . . . . **Federal Funds: $** **Non-Federal funds: $** **Total $**

Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)		
Cash (both Applicant and Third Party) →		
Non-Cash Contributions (both Applicant and Third Party) →		

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		
Signature (for optional use)		

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0524-0039. The time required to complete this information collection is estimated to average 1.00 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing the reviewing the collection of information.

Form CSREES-2004 (12/2000)

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0039
Expires 03/31/2004

BUDGET

ORGANIZATION AND ADDRESS
Michigan Sea Grant, Michigan State University
710 Chippewa Square, Suite 202, Marquette, MI 49855

PROJECT DIRECTOR(S)
Ronald E. Kinnunen

USDA AWARD NO. **Year 2: Objectives 3 - 6**

	CSREES FUNDED WORK MONTHS			Duration Proposed Months: 12 **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages	Calendar	Academic	Summer				
1. No. of Senior Personnel							
a. ___ (Co)-PD(s)							
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty)							
a. ___ Research Associates-Postdoctorates . . .							
b. ___ Other Professionals							
c. ___ Paraprofessionals							
d. ___ Graduate Students							
e. ___ Prebaccalaureate Students							
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages →							
B. Fringe Benefits (If charged as Direct Costs)							
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →							
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies							
F. Travel				$2,100			
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)							
K. Total Direct Costs (C through I) →				$2,100			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K) →				$2,100			
N. Other →							
O. Total Amount of This Request →				$2,100			

P. Carryover -- (If Applicable) . . . . . **Federal Funds: $** **Non-Federal funds: $** **Total $**

Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)		
Cash (both Applicant and Third Party) →		
Non-Cash Contributions (both Applicant and Third Party) →		

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		
Signature (for optional use)		

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Form CSREES-2004 (12/2000)

BUDGET EXPLANATION FOR MICHIGAN STATE UNIVERSITY

(Kinnunen)

Objectives 3-6

F. Travel. Years 1 and 2: Activities will be centered on a set of six on-farm workshops conducted within the NCR at locations to be determined. Transportation, meals, and lodging expenses for each of the six workshops will average $700 or a total of $4,200.

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0039
Expires 03/31/2004

BUDGET

ORGANIZATION AND ADDRESS
University of Wisconsin-Stevens Point
2100 Main Street, Stevens Point, WI 54481

PROJECT DIRECTOR(S)
Jeffrey A. Malison

USDA AWARD NO. **Year 1: Objectives 3 - 6**

	CSREES FUNDED WORK MONTHS			Duration Proposed Months: 12 **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
	Calendar	Academic	Summer				
A. Salaries and Wages							
1. No. of Senior Personnel							
a. ___ (Co)-PD(s)							
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty)							
a. ___ Research Associates-Postdoctorates . . .							
b. ___ Other Professionals							
c. ___ Paraprofessionals							
d. ___ Graduate Students							
e. ___ Prebaccalaureate Students							
f. ___ Secretarial-Clerical							
g. _1_ Technical, Shop and Other				$11,772			
Total Salaries and Wages →				$11,772			
B. Fringe Benefits (If charged as Direct Costs)				$5,415			
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$17,187			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$4,000			
F. Travel				$14,000			
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)							
K. Total Direct Costs (C through I) →				$35,187			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K) →				$35,187			
N. Other →							
O. Total Amount of This Request →				$35,187			

P. Carryover -- (If Applicable) . . . . . **Federal Funds: $** **Non-Federal funds: $** **Total $**

Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)		
Cash (both Applicant and Third Party) →		
Non-Cash Contributions (both Applicant and Third Party) →		

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		
Signature (for optional use)		

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Form CSREES-2004 (12/2000)

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0039
Expires 03/31/2004

BUDGET

ORGANIZATION AND ADDRESS
University of Wisconsin-Stevens Point
2100 Main Street, Stevens Point, WI 54481

PROJECT DIRECTOR(S)
Jeffrey A. Malison

USDA AWARD NO. **Year 2: Objectives 3 - 6**

	CSREES FUNDED WORK MONTHS			Duration Proposed Months: 12 **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages	Calendar	Academic	Summer				
1. No. of Senior Personnel							
a. ___ (Co)-PD(s)							
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty)							
a. ___ Research Associates-Postdoctorates							
b. ___ Other Professionals							
c. ___ Paraprofessionals							
d. ___ Graduate Students							
e. ___ Prebaccalaureate Students							
f. ___ Secretarial-Clerical							
g. _1_ Technical, Shop and Other				$5,214			
Total Salaries and Wages →							
B. Fringe Benefits (If charged as Direct Costs)				$2,399			
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$7,613			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$2,000			
F. Travel				$7,000			
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)				$5,000			
K. Total Direct Costs (C through I) →				$21,613			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K) →				$21,613			
N. Other →							
O. Total Amount of This Request →				$21,613			

P. Carryover -- (If Applicable) . . . . . **Federal Funds: $** **Non-Federal funds: $** **Total $**

Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)		
Cash (both Applicant and Third Party) →		
Non-Cash Contributions (both Applicant and Third Party) →		

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		
Signature (for optional use)		

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Form CSREES-2004 (12/2000)

BUDGET EXPLANATION FOR UNIVERSITY OF WISCONSIN-STEVENS POINT

(Malison)

Objectives 3-6

A. Salaries and Wages. Services of a limited term employee (LTE) to assist NADF extension personnel with all aspects of organizing and conducting six workshops. This includes helping prepare and distribute schedules, working with the fish farms to help organize meals and lodging for the conferences, and preparing and distributing all tangible outputs of the workshops including the video. Year 1: $11,772 ($20/hour for 588.6 hours) and Year 2: $5,212 ($20/hour for 260.6 hours).

B. Fringe Benefits. The fringe rate charged on LTEs at the University of Wisconsin-Stevens Point is 46%.

E. Materials and Supplies. Year 1 ($4,000) and Year 2 ($2,000) for paper, toner cartridges, folders, photocopy supplies, and mailers to prepare and distribute schedules and handouts for the six workshops.

F. Travel. Year 1: Transportation, meals, and lodging for two NADF extension specialists and three regional and/or national experts to attend four workshops at locations to be determined at $700/person/workshop ($14,000). Year 2: Transportation, meals, and lodging for five persons to attend two workshops at locations to be determined at $700/person/workshop ($7,000).

J. All Other Direct Costs. Year 2: $5,000 to produce the video.

BUDGET SUMMARY FOR EACH PARTICIPATING INSTITUTION

Year 1

	UMESC	ISU	MSU	UW-SP	TOTALS
Salaries and Wages	$20,043	$14,710		$11,772	$46,525
Fringe Benefits	$5,429	$4,810		$5,415	$15,654
Total Salaries, Wages, and Fringe Benefits	$25,472	$19,520		$17,187	$62,179
Nonexpendable Equipment					
Materials and Supplies	$12,101			$4,000	$16,101
Travel	$1,200		$2,100	$14,000	$17,300
All Other Direct Costs	$21,290				$21,290
TOTAL PROJECT COSTS	$60,063	$19,520	$2,100	$35,187	$116,870

Year 2

	UMESC	ISU	MSU	UW-SP	TOTALS
Salaries and Wages	$19,086	$4,130		$5,214	$28,430
Fringe Benefits	$4,558	$1,350		$2,399	$8,307
Total Salaries, Wages, and Fringe Benefits	$23,644	$5,480		$7,613	$36,737
Nonexpendable Equipment					
Materials and Supplies	$5,644			$2,000	$7,644
Travel	$1,200		$2,100	$7,000	$10,300
All Other Direct Costs	$21,409			$5,000	$26,409
TOTAL PROJECT COSTS	$51,897	$5,480	$2,100	$21,613	$81,090

SCHEDULE FOR COMPLETION OF OBJECTIVES

Objective 1: Initiated in Year 1 completed in Year 2.

Objective 2: Initiated in Year 1 and completed in Year 1.

Objective 3: Initiated in Year 1 (4 workshops) and completed in Year 2 (two workshops).

Objective 4: Initiated in Year 1 (4 specific plans) and completed in Year 2 (two specific plans).

Objective 5: Initiated in Year 1 and completed in Year 2.

Objective 6: Initiated in Year 1 and completed in Year 2.

LIST OF PRINCIPAL INVESTIGATORS AND PROJECT LEADERS

Glenda D. Dvorak, Iowa State University
Ronald E. Kinnunen, Michigan State University
Jeffrey A. Malison, UW-Stevens Point Northern Aquaculture Demonstration Facility
Jeffrey J. Rach, Upper Midwest Environmental Sciences Center (USGS)

VITA

Glenda D. Dvorak
Center for Food Security and Public Health
2170 College of Veterinary Medicine
Iowa State University
Ames, Iowa 50011

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FAX: (515) 294-8259
E-mail: gdvorak@iastate.edu

EDUCATION

B.S. Iowa State University, 1995, Zoology; Minor: Animal Ecology
M.S. Iowa State University, 1997, Fisheries Biology-Aquaculture
D.V.M. Iowa State University, 2001, Veterinary Medicine
M.P.H. University of Iowa, 2003, Public Health

POSITIONS

Veterinary Specialist (2003-present), Center for Food Security and Public Health, and Adjunct Instructor, (2002-2003), College of Veterinary Medicine, Iowa State University
Associate Veterinarian (2001-2002), All Creatures Small Animal Hospital, Indianola, Iowa
Research Assistant, (1995-1997) and Program Assistant (1993-1997), North Central Regional Aquaculture Center, Iowa State University

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Veterinary Medical Association
Iowa Public Health Association
Iowa Veterinary Medical Association (Public Health Committee)
Iowa Veterinary Rapid Response Team
National Society of Public Health Educators

SELECTED PUBLICATIONS

Dvorak, G, A. Rovid-Spickler, and J.A. Roth. In press. Handbook for zoonotic diseases for companion animals. Iowa State University, Ames.

Dvorak, G. 2005. Bioterrorism and high consequence pathogen diseases wallchart. Journal of the American Veterinary Medical Association 227(3):supplement to issue.

Bickett-Weddle, D. and G. Dvorak. 2004. Biological risk management: general prevention practices. Center for Food Security and Public Health, Iowa State University, Ames.

Dvorak, G. 2004. Disinfection 101 (Overview document). Center for Food Security and Public Health, Iowa State University, Ames.

Dvorak, G. 2002. Biosecurity on the farm. Center for Food Security and Public Health, Iowa State University, Ames.

Steneroden, K., G. Dvorak, and D. Bickett-Weddle. 2002. Biological risk management for stationary veterinary clinics. Center for Food Security and Public Health. Iowa State University, Ames.

Morris, L., D. Bickett-Weddle, and G. Dvorak. 2002. Biological risk management for mobile veterinary clinics. Center for Food Security and Public Health. Iowa State University, Ames.

Mischke, C.C., G.D. Dvorak, and J.E. Morris. 2001. Growth and survival of hybrid sunfish larvae in the laboratory under different feeding and temperature regimes. North American Journal of Aquaculture. 63:265-71.

VITA

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710 Chippewa Square, Ste. 202
Marquette, MI 49855-4823

Phone: (906) 226-3687
Fax: (906) 226-3687
E-mail: kinnune1@msu.edu

EDUCATION

B.S. Michigan State University, 1976
M.S. Michigan State University, 1979
Ph.D. Michigan Technological University, 1997

POSITIONS

Michigan Sea Grant Extension Agent (1982-present), Upper Peninsula, Michigan State University
Fisheries Pathologist (1981), Rangen Research Laboratory, Hagerman, Idaho
Fisheries Biologist (1979-1980), U.S. Fish and Wildlife Service, Leetown, West Virginia

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society, Salmonid Section
International Association for Great Lakes Research
Alliance for Marine Remote Sensing

SELECTED PUBLICATIONS

Kinnunen, R.E., M.C. Gould, and P. Cambier. 2005. Composting commercial fish processing waste from fish caught in the Michigan waters of the Great Lakes. Michigan State University Technical Bulletin. 40 pgs.

Pangle, K. L., T. M. Sutton, R. E. Kinnunen, and M. H. Hoff. 2005. Effects of body size, condition, and lipid content on the survival of juvenile lake herring during rapid cooling events. Journal of Great Lakes Research 31:360-366.

Pangle, K.L., T.M. Sutton, R.E. Kinnunen, M.H. Hoff. 2004. Overwinter survival of juvenile lake herring in relation to body size, physiological condition, energy stores, and food ration. Trans. Am. Fish Soc. Vol. 133 (5): 1235-1246.

Hinshaw, J.M., G. Fornshell, R.E. Kinnunen. 2004. A profile of the aquaculture of trout in the United States. Report for USDA Risk Management Agency, Federal Crop Insurance Corporation, through Mississippi State University. 46 pgs.

Kinnunen, R.E., editor. 2002. Environmental Strategies for Aquaculture Symposium Proceedings (December 2000). 62nd Midwest Fish and Wildlife Conference, Minneapolis, MN. NCRAC CD Series #101, NCRAC Publications Office, Iowa State University, Ames, IA.

Gunderson, J.L. and R.E. Kinnunen. 2001. Aquatic nuisance species-Hazard analysis and critical control point training curriculum. Michigan Sea Grant Publication No. MSG-00-400.

Kinnunen, R.E. 2000. A white paper on the status and needs of salmonid aquaculture in the North Central Region. North Central Regional Aquaculture Center. Michigan State University.

Kinnunen, R.E. 1996. Walleye fingerling culture in undrainable ponds. Pages 135-145 *in* R.C. Summerfelt, editor. Walleye culture manual. NCRAC Culture Series #101, NCRAC Publications Office, Iowa State University, Ames.

VITA

Jeffrey A. Malison
Dept. of Animal Sciences
1675 Observatory Dr.
University of Wisconsin-Madison
Madison, WI 53706

Phone: (608) 263-1242
Fax: (608) 262-5157
E-mail: jmalison@wisc.edu

EDUCATION

B.S. University of Wisconsin-Stevens Point, 1976
M.S. University of Wisconsin-Madison, 1980
Ph.D. University of Wisconsin-Madison, 1985

POSITIONS

Director (1995-present), Assistant Director (1990-1995), and Associate Researcher (1987-1990), University of Wisconsin Aquaculture Program, University of Wisconsin-Madison; Co-director, UW-Stevens Point Northern Aquaculture Demonstration Facility (2006-present)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society
World Aquaculture Society
Wisconsin Aquaculture Association
Wisconsin Aquaculture Industry Advisory Council

SELECTED PUBLICATIONS

Malison, J.A. and J.A. Held. In press. Farm-based production parameters and breakeven costs for yellow perch grow-out in ponds in southern Wisconsin. NCRAC Fact Sheet Series #115, NCRAC Publications Office, Iowa State University, Ames.

Ng, Y., S. Hanson, J.A. Malison, B.C. Wentworth, and T.P. Barry. 2006. Genistein and other isoflavones in soybeans inhibit estrogen metabolism in salmonid fish. Aquaculture 254:658-665.

Hart, S.D., D.L. Garling, and J.A. Malison. 2006. Yellow perch (*Perca flavescens*) culture guide. NCRAC Culture Series #103, NCRAC Publications Office, Iowa State University, Ames.

Jurgella, G.F., T.P. Barry, A. Marwah, J.A. Malison, and R.E. Peterson. 2006. Effects of xenobiotics and steroids on estrogen metabolism in lake trout. General and Comparative Endocrinology 148:273-281.

Lima, L.C., L.P. Ribeiro, J.A. Malison, T.P. Barry, and J.A. Held. 2006. Effects of temperature on performance parameters and the cortisol stress response of surubim *Pseudoplatystoma* sp. Journal of the World Aquaculture Society 37:89-95.

D'Souza, N., D.I. Skonberg, M.E. Camire, K.E. Guthrie, J. Malison, and L. Lima. 2005. Influence of dietary genistein levels on tissue genistein deposition and on the physical, chemical and sensory quality of rainbow trout, *Oncorhynchus mykiss*. Journal of Agricultural Food Chemistry 53:3631-3636.

Malison, J.A. and C.F. Hartleb, eds. 2005. Best management practices manual for aquaculture in Wisconsin and the Great Lakes region. Publication WISCU-H-05-001. University of Wisconsin Sea Grant Institute, Madison, Wisconsin.

VITA

Jeffrey J. Rach
Upper Midwest Environmental Sciences Center (USGS)
2630 Fanta Reed Road
La Crosse, WI 54603

Phone: (608) 781-6322
E-mail: jrach@usgs.gov

EDUCATION

M.S. University Wisconsin-La Crosse, 1975, Biology/Aquatic Biology

POSITIONS

Research Fishery Biologist

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society

SELECTED PUBLICATIONS

Rach, J.J., T.M. Schreier, S.M. Schleis, and M.P. Gaikowski. 2005. Efficacy of hydrogen peroxide and formalin to control mortality associated with saprolegniasis infections on channel catfish. Journal of North American Aquaculture 65:300-305.

Rach J.J., S. Redman, D. Bast, and M.P. Gaikowski. 2005. A comparison of the efficacy of hydrogen peroxide versus formalin treatments to control mortality associated with saprolegniasis on lake trout eggs (*Salvelinus namaycush*). Journal of North American Aquaculture 67:148-154.

Rach J.J., J.J. Valentine, T.M. Schreier, M.P. Gaikowski, and T.G. Crawford. 2004. Efficacy of hydrogen peroxide to control mortality associated with saprolegniasis infections on channel catfish (*Ictalurus punctatus*) eggs. Aquaculture 238:135-142.

Rach J.J., S.M. Schleis, M.P. Gaikowski, and A. Johnson. 2003. Efficacy of hydrogen peroxide to control mortality associated with external columnaris infections on walleye and channel catfish fingerlings. Journal of North American Aquaculture 65:300-305.

Rach, J.J., M.P. Gaikowski, and R.T. Ramsay. 2001. Efficacy of hydrogen peroxide to control parasitic infestations on hatchery reared fish. Journal of Aquatic Animal Health 12(4):267-273.

Rach, J.J., M.P. Gaikowski, and R.T. Ramsay. 2000. Efficacy of hydrogen peroxide to control mortalities associated with bacterial gill disease infections on hatchery reared salmonids. Journal of Aquatic Animal Health 12:119-127.

Rach, J.J., M.P. Gaikowski, G.E. Howe, and T.M. Schreier. 1998. Evaluation of the toxicity of hydrogen peroxide treatments on warm- and coolwater fish eggs. Aquaculture 165: 11-25.

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Schreier, T.M., J.J. Rach, and G.E. Howe. 1996. Efficacy of formalin, hydrogen peroxide, and sodium chloride on fungal-infected rainbow trout eggs. Aquaculture 140: 323-331.

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Appendix 1

Biosecurity Standard Operating - Upper Midwest Environmental Sciences Center

A. Personnel Training
   1. Personnel working in the Upper Midwest Environmental Sciences Center (UMESC) isolation facility will be required to read this SOP and their compliance with the SOP will be monitored by their supervisor.
   2. Personnel will be provided information (such as: life history of organism, potential health and disease risk to employees or other cultured animals, and potential risk of contamination within the facility) on the organisms they will be culturing or testing. Employees will be required to read all study protocols prior to working on a study.
   3. Access to the isolation facility will be restricted (by keys issued to researchers) to individuals conducting research or culturing organisms in the facility. Only personnel working on projects in the facility will be allowed into the area. If access is required by untrained personnel (e.g., maintenance worker) a team leader will provide access and supervision to the entrant.
   4. All staff will be trained in proper disinfection procedures and will be required to wear appropriate personal protective equipment (PPE) when working in the laboratory.

B. Enter and Egress of Isolation Laboratory
   1. The isolation facility is maintained within Segment B of the UMESC. The UMESC is maintained on federal property with exterior fencing and restricted access. UMESC access doors remain locked during non-business hours and access is restricted to two unlocked access doors during business hours.
   2. Entrances to the isolation laboratory will be locked at all times. Isolation laboratory access is restricted through the use of separate keyed locks.
   3. Any person entering or exiting the isolation facility must step on the disinfection mats located at each door of the isolation laboratory. Employees will sanitize hands with an appropriate hand sanitizing agent when entering and before exiting the isolation laboratory.
   4. Any person that has entered the isolation laboratory will not enter UMESC Center Fish Culture until the following day.

C. Foot Disinfection, Maintenance of Disinfection Mats, and Equipment Disinfection
   1. Boot disinfection baths and hand sanitizers will be placed at the entry/exit of the isolation laboratory.
   2. Boot disinfection baths will contain an appropriate surface disinfectant (e.g., 100 ppm iodine, Virkon® Aquatic 1%); solutions will be changed at a minimum of every 7 days or as directed by the product label.
   3. Equipment/tools used in the isolation laboratory will remain in the isolation laboratory. Items that must be removed must be disinfected with an appropriate surface disinfectant (e.g., 100 ppm iodine for one hour, Virkon® Aquatic at solution and contact time per manufacturer's recommendation).

D. Clean Room and Protective Clothing
   1. There will be a designated clean area where individuals will put on PPE.
   2. The PPE provided will include protective coveralls, gloves, rubber boots (or rubber pull-over boots that fit over shoes), and body aprons appropriate to the risk of pathogen transfer from the isolation laboratory. The need for additional PPE (face shield, respirator, etc.) will be determined based on the pathogen and chemicals used during study.
   3. Employees will wear PPE while working in the isolation laboratory. Upon completion of work, they will remove the PPE, clean as needed, disinfect and store the items in a designated locker. The PPE will be disinfected with an appropriate surface disinfectant or disposed as municipal waste (UMESC municipal waste is incinerated at the Excel Energy French Island power generation plant).

E. Transporting Organisms Into Isolation Laboratory
    1. Organisms transferred into the isolation laboratory from UMESC Center Fish Culture will be transferred in disposable plastic bags. UMESC Center Fish Culture tanks will not enter the isolation facility.
    2. Organisms received from non-UMESC culture facilities (e.g. wild caught or transferred from pathogen-suspect public or private culture facilities) to be housed in the isolation laboratory will enter the facility through the entry doors in lower level A.
    3. Prior to transporting organisms into UMESC, a UMESC employee will meet the individual transporting the organism in the parking lot near building A. The organisms will be placed into a container (e.g., disposable plastic bag or covered plastic bucket) and then placed into a covered polyethylene tank. The organisms will then be transported into the isolation facility. The poly tank, organism holding container, and culture medium will be disinfected with an appropriate disinfectant (e.g., 100 ppm iodine, Virkon® Aquatic, 200 ppm chlorine).

F. Disposal of Organisms Held in Isolation Laboratory
    1. Any organism that enters the isolation laboratory will be will be surface disinfected with an appropriate disinfectant (e.g. 200 ppm iodine, 70% isopropyl alcohol, Virkon® Aquatic, 200 ppm chlorine) after death or euthanasia prior to disposal. Organisms will be euthanized according to UMESC Animal Care and Use Committee (ACUC) SOP. After surface disinfection, organisms will be double bagged in plastic bags with the inner bag labeled according to UMESC ACUC SOP and the outer bag will be disinfected with an appropriate surface disinfectant (e.g. 70% isopropyl alcohol, Virkon® Aquatic) and then incinerated on site. Pathogen cultures (viruses, bacteria etc.) will be deactivated by chemosterilization (e.g. iodine, chlorine, or isopropyl alcohol) for 24 h. Deactivated cultures will be disposed of either by incineration or disposal through the isolation laboratory effluent treatment system.

G. Isolation Facility and Effluent Treatment
    1. Aquatic organisms used in disease research or from suspect waters and select aquatic pathogens will be isolated and tested within the isolation laboratory.
    2. Test and culture water will be UV-sterilized before entering floor troughs.
    3. Isolation laboratory floor trough drains are capped and sealed; water held in the troughs will be chlorinated at 5-10 ppm chlorine by placement of chlorine pellets in the trough. Water held in the trough will be intermittently pumped via sump pumps to a second chlorination system which chlorinates the effluent to a minimum of 50 ppm chlorine (Figure 1).
    4. After the second chlorination, the water will enter the UMESC effluent system where it will pass through an activated carbon adsorption unit before flowing into a detention pond. UV-sterilized and chlorinated effluent water ultimately will be discharged through the UMESC effluent system into the Black River at La Crosse, Wisconsin. The UMESC effluent system is regulated by the Wisconsin Department of Natural Resources (WI DNR) as a wastewater treatment plant; the WI DNR inspected and approved the laboratory depicted in Fig. 1 as an invasive species containment facility.

H. Emergency Procedures and Precautions
    1. In the event of an emergency (power failure, loss of water, suspected disease transmission), essential personnel will be notified.
    2. The sump pumps and chlorine metering pump are connected to the Center's emergency power backup system to ensure operation during a power emergency.
    3. In the event of UMESC water loss, emergency aeration will be provided to each tank. Appropriate disinfection procedures will be implemented as needed.
    4. In the event of a potential disease transmission, UMESC management will be notified and all access to the isolation laboratory will be terminated. The La Crosse Fish Health Center (FHC) will be consulted to determine if a potential disease transmission occurred and to identify the pathogen. Actions taken, including the potential of complete depopulation of all aquatic organisms at UMESC and complete facility disinfection will be made based on the pathogen and risk of transmission elsewhere within the facility. Appropriate disinfection strategies will be selected based on recommendations from the FHC and other aquatic pathogen experts.

Figure 1. Isolation Facility