EFFECTIVENESS RESEARCH LEADING TO APPROVALS FOR CONTROLLING MORTALITY IN COOLWATER AND WARMWATER FINFISH DUE TO AEROMONAD INFECTIONS WITH TERRAMYCIN 200 FOR FISH® (OXYTETRACYCLINE DIHYDRATE) AND AQUAFLOR® (FLORFENICOL)

Chairperson: Mark P. Gaikowski, U.S. Geological Survey Upper Midwest Environmental Sciences Center

Industry Advisory Council Liaison: Mark Willows, Binford, North Dakota

Funding Request: $150,000

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

Objectives:

1. Identify the etiologic agent (*Aeromonas* spp.) from isolates collected from disease outbreaks in the NCR and characterize the disease syndrome before conducting any effectiveness studies.
2. Have active, established Investigational New Animal Drug (INAD) exemptions or work with the sponsors of publicly disclosable INADs for Terramycin 200 for Fish® and Aquaflor®.
3. Develop draft pivotal effectiveness study protocols with the concurrence of the two drug sponsors (Phibro Animal Health=PAH for Terramycin 200 for Fish® and Schering-Plough Animal Health=SPAH for Aquaflor®).
4. Submit the draft pivotal effectiveness study protocols through established INADs for Terramycin 200 for Fish® and Aquaflor® for protocol concurrence from the CVM before beginning the effectiveness studies.
5. Conduct pivotal effectiveness studies on Terramycin 200 for Fish® and Aquaflor® according to Good Clinical Practice and the CVM concurred protocols.
6. Analyze the effectiveness data and prepare draft final study reports for Terramycin 200 for Fish® and Aquaflor® no more than four months after the studies are completed.
7. Submit the respective draft study reports to PAH and SPAH for their review.
8. Submit the final study reports through established INADs for Terramycin 200 for Fish® and Aquaflor® to CVM for acceptance no more than two months after PAH and SPAH have completed their reviews of the draft study reports.
9. Ensure that all questions and concerns about the final study reports are answered no more than one month after receiving comments from CVM.
10. If CVM accepts the data as proving effectiveness for the aeromonad infections encountered in the NCR, provide the acceptance letter and effectiveness studies to PAH and SPAH so that they can pursue supplemental NADA approvals for their respective drug products.

Proposed Budget:

Institution	Principal Investigator	Objec-tives	Year 1	Year 2	Total
U.S. Geological Survey, Upper Midwest Environmental Sciences Center	Mark P. Gaikowski	1-10	**$70,000**	**$80,000**	**$150,000**

Non-funded Collaborators:

Facility	Collaborator
U.S. Fish and Wildlife Survey, La Crosse Fish Health Center	Becky A. Lasee
North American Fish Farmers Cooperative	Mark Willows
Schering-Plough Animal Health Corporation	Richard Endris

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JUSTIFICATION

Cool- and warmwater fish are cultured in large numbers at private, state, and federal hatcheries within the North-Central Region (NCR). Motile aeromonad infections (MAI) cause extensive losses of a variety of coolwater and warmwater finfish (e.g., tilapia, hybrid striped bass, and percids) cultured in the NCR. However, as cool and warmwater fish culture expands, MAI has the potential to cause substantial economic loss within the NCR and across the United States.

The definitive diagnosis for aeromonad infections is confused, primarily because of the historical difficulty in identification of *Aeromonas* isolates through the considerable overlap in response to biochemical test results. Therefore, additional work is needed to identify the etiologic agent(s) (*Aeromonas* spp.) involved in MAI and resultant motile aeromonas septicemia (MAS) disease outbreaks in the production of NCR coolwater and warmwater finfish. Specifically, the use of molecular diagnostic techniques will be required in order to identify the etiologic agent(s) responsible for MAI in the NCR. Development of pivotal effectiveness data are presently constrained by the lack of understanding of the etiological agent(s) of MAS – identification and confirmation of the etiology of MAS would simplify the development of efficacy data which should lead to the approval of effective antibiotic therapies to control MAS in cool or warmwater fish.

Both Terramycin 200 for Fish® (TM-200; oxytetracycline dihydrate) and Aquaflor® (florfenicol) have been shown to be effective against a wide variety of Gram-negative bacterial pathogens of fish including certain *Aeromonas* spp. It is likely that one or both of these antibacterials will effectively reduce mortality associated with MAS in cool and warmwater fish. This research will provide valuable information to commercial and public fish culturists and enable them to effectively reduce production loss in cool- and warmwater fish caused by *Aeromonas* spp.

RELATED CURRENT AND PREVIOUS WORK

Mesophilic aeromonads are common pathogens identified in the aquatic environment and present an important disease risk to a variety of animals from fish to reptiles to mammals, including humans. MAI has been implicated in gastrointestinal infections and death in humans (Janda 1991). The bacteria are important pathogens of fish, causing MAS as well as occurring as secondary pathogens (Kozinska 2007). A substantial body of work has been completed to characterize the etiological agents of MAS in fish. Within the genus *Aeromonas*, two phenotypic groups have been characterized, the psychrophilic and non-motile *Aeromonas salmonicida* and the mesophilic and motile aeromonads of which three phenotypically-distinct species have been recognized: *A. hydrophila, A. caviae*, and *A. sobria* (Popoff 1984; Kozinska et al. 2002). These species are known fish pathogens (Toranzo et al. 1989; Candan et al. 1995; Ogara et al. 1998; Austin and Austin 1999). The genus *Aeromonas* is reported to be represented by at least 14 genetically-distinct species including *A. hydrophila, A. bestarium, A. salmonicida* (non-motile psychrophilic and motile mesophilic biogroups), *A. caviae, A. media, A. eucrenophila, A. sobria, A. veronii* (with biotypes *sobria* and *veronii*), *A. jandaei, A. trota, A. schuberti, A. encheleia, A. allosaccharaphila,* and *A. popoffi* (Janda 1991; Martinez-Murcia et al. 1992; Joseph and Carnahan 1994; Esteve et al. 1995; Ali et al. 1996; Huys et al. 1997a; Huys et al. 1997b). Of these, *A. hydrophila, A. bestarium, A. salmonicida, A. veronii* biotype *sobria, A. caviae*, and *A. jandaei* have been reported as fish pathogens (Torres et al. 1993; Esteve et al. 1995; Ogara et al. 1998; Nielsen et al. 2001; Kozinska et al. 2002; Rahman et al. 2002).

Various authors have categorized the dominant *Aeromonas* species present in a diverse group of healthy and diseased fish species from various aquatic environments around the world. Nine mesophilic *Aeromonas* species were characterized from 131 isolates obtained from common carp (*Cyprinus carpio*) or rainbow trout (*Oncorhynchus mykiss*) cultured in freshwater aquaculture in Poland (Kozinska 2007). Of the mesophilic aeromonads identified, only *A. hydrophila, A. bestarium, A. salmonicida,* and *A. veronii* were classified as pathogenic. The dominant mesophilic *Aeromonas* species in carp were *A. veronii* bt *sobria*, *A. bestarium,* and *A. salmonicida* whereas the dominant species isolated from rainbow trout was *A. hydrophila*. In a second study of common carp, Kozinska et al. (2002) found five *Aeromonas* spp. of which *A. bestarium, A. salmonicida,* and *A. veronii* were pathogenic. Nam and Joh (2007) reported that *A. sobria* was the dominant *Aeromonas* species in rainbow trout from a Korean trout farm. In a study of farmed European perch (*Perca fluviatilis*), *A. sobria* were isolated from lesions of cultured fish. Naïve perch in

experimental challenges with *A. sobria* isolated from farmed fish with clinical signs of MAI developed similar mortality and morbidity to that observed in natural infections (Wahli et al. 2005). *Aeromonas sobria* isolates were haemolytic, autoaggregated, cytotoxic to cultured fish cell lines, and possessed genes for extracellular protein production. In a study of crucian carp (*Carassius carassius*) and Wuchang bream (*Megalobrama amblycephala*) with MAS, *A. hydrophila* only represented ~50% of the isolated motile aeromonads (Nielsen et al. 2001); other *Aeromonas* species were not speciated. Sugita et al. (1995) identified *A. veronii, A. caviae, A. hydrophila, A. sobria, A. jandaei*, and other unspeciated Aeromonas spp. in intestines of healthy fish (common and crucian carp, gray mullet [*Mugil cephalus*]) angled from the Hikiji River, Japan.

Descriptions of *Aeromonas* spp. infections in North America include a variety of aeromonads isolated from both wild and farmed fish. Nawaz et al. (2006) obtained 81 aeromonad isolates from farmed channel catfish (*Ictalurus punctatus*). *Aeromonas hydrophila, A. trota, A. caviae, A. veronii*, and *A. jandaei* were identified with *A. veronii* representing the dominant isolate. A combination infection of *A. hydrophila* and *Flavobacterium spp.* was implicated in a mass mortality of common carp in the St. Lawrence River in 2001 (Monette et al. 2006). Although both *A. hydrophila* and *Flavobacterium spp.* were identified, pure *A. hydrophila* isolates were obtained from internal organs and lesions consistent with MAS. *Aeromonas hydrophila* were isolated from tournament-caught and electrofished largemouth bass *Micropterus salmoides* in a southern reservoir, along with *Pseudomonas spp., Edwardsiella tarda*, and *Flavobacterium columnare* (Steeger et al. 1994). The latter two studies lacked the specific genomic testing completed in the former study and those described from Europe and Asia. Literature was not identified that characterized the *Aeromonas* phenotypically- or genetically-distinct species present during MAI outbreaks in the NCR.

ANTICIPATED BENEFITS

Mesophilic or motile *Aeromonas* infections are extremely relevant to the aquaculture industry in the NCR as the industry has experienced a loss of income in both commercially important food fish species and baitfish. These economic losses result directly from fish mortality due to MAI and from opportunistic secondary infections, and indirectly because of unappealing visual appearance of food fish with gross external lesions. As a result, North Central Regional Aquaculture Center (NCRAC) Board of Directors authorized up to $150,000 for a project on drug approval efforts on controlling mortality in coolwater and warmwater finfish due to aeromonad infections with the medicated feeds TM-200 and Aquaflor®. The NCRAC Board made it clear that the intent of these monies would be to ensure that all the needed effectiveness studies for this label claim could be completed so that the drug sponsors could submit supplemental New Animal Drug Applications (NADA) to the Food and Drug Administration's Center for Veterinary Medicine (CVM) for approval.

From the proposed investigations, the phenotypically and genetically-distinct *Aeromonas* species typically involved in MAI in NCR cultured fish will be identified. Additionally, the clinical signs associated with MAI in the NCR will be confirmed.

OBJECTIVES

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PROCEDURES

Identify the Etiologic Agent (Objective 1)

This study will be initiated with a thorough review of the literature and interviews with fish health specialists currently diagnosing MAI within the NCR and across the United States. Observations of the clinical signs and gross necropsy of at least two fish species exhibiting MAI from at least five NCR culture facilities will be completed (Austin and Austin 1987; Noga 1996). Microbiological samples will be aseptically collected from lesions and kidney on Rimler-Shotts agar (Shotts and Rimler 1973) or other appropriate non-selective or selective media as needed. Creamy to tan, round, raised, shiny colonies 2–3 mm in diameter will be selectively identified to genus by standard biochemical tests (Austin and Austin 1987). β-hemolysin, gelatinase, and caseinase activity of *Aeromonas* isolates will be determined (Hsu et al. 1981). Isolates exhibiting a haemolytic activity level as measured by zone ratio (R) of at least $\geq$4 and one proteolytic activity will be selected for identification to species (*A. hydrophila, A. bestarium, A. veronii* bt *sobria, A. caviae,* and *A. jandaei*) and challenge trials. Polymerase-chain reaction of 16S-rDNA will be completed on *Aeromonas* spp. isolates to identify to species (Borrel et al. 1997); isolates which are determined to not be one of the species in the preceding list will be reported as *Aeromonas* spp.

Isolates selected for challenge experiments will be cultured on tryptic soy agar and harvested after incubation (24-h, 27°C). Harvested bacteria will be concentrated by centrifugation (~900 $\times$ g) then the pellet resuspended in phosphate-buffered saline (PBS) to a final concentration of approximately 10^7 colony-forming units (CFU)/mL. Five naïve fish (two NCR-cultured species; one coolwater, and one warmwater species; selection to be determined from MAI infection data from NCR culture facilities) will be injected with 0.1 mL of the bacterial suspension. As appropriate, two or more *Aeromonas* species may be combined if co-cultured from fish with MAI. However, when combination challenges are conducted, pure isolate challenges will similarly be conducted and the total bacterial challenge suspension will not exceed 10^7 CFU/mL. Five control fish per experimental challenge will be sham-challenged with sterile PBS. Fish will be anesthetized by immersion in a bath of tricaine-methanesulfonate (MS-222) at ~100 mg/L. Fish will be maintained at 17°C (coolwater species) or 25°C (warmwater species) in flow-through systems at densities appropriate for the species and culture unit (Piper 1982); water flow will be sufficient to provide $\geq$ one tank-volume exchange per hour. Clinical signs, morbidity, and mortality will be monitored for a minimum of 14 days post-challenge. Bacterial re-isolation will be attempted on all challenged fish from

kidney and lesions. Virulence level will be estimated using the categories reported by Kozinska (2007): strongly virulent – ≥ 3 fish with MAI signs and ≥ 3 mortalities; virulent – ≥ 3 fish with MAI signs and 1–2 mortalities; weakly virulent – ≥ 2 fish with MAI signs and no mortality; avirulent – 1–2 fish with slight or no MAI signs and no mortality.

Have Active, Established INAD (Objective 2)

The Upper Midwest Environmental Sciences Center (UMESC) presently has a publicly disclosable INAD for Terramycin 200 for Fish® (INAD 11-366) into which UMESC has submitted the pivotal animal safety, human food safety, and environmental safety studies required to support approval in cool and warmwater fish cultured within the NCR. All protocols, data, and final study reports submitted to CVM will be submitted by UMESC to INAD 11-366.

UMESC will submit a written request to CVM requesting establishment of a fully-disclosable public INAD for Aquaflor®. The CVM indicated prior to UMESC preparation of the letter of intent for this proposal that UMESC would be able to obtain an INAD file for Aquaflor®. Previous work completed by UMESC with Aquaflor® has been submitted either through the sponsor (Schering-Plough Animal Health Corporation) or through the U.S. Fish and Wildlife Service's INAD. All protocols, data, and final study reports developed by UMESC as a result of this project will be submitted to CVM within the INAD granted to UMESC.

Develop Draft Pivotal Effectiveness Study Protocols (Objective 3)

Concurrent with the completion of Objective 1, draft pivotal efficacy protocols will be collaboratively established by UMESC and the La Crosse Fish Health Center (LFHC). The pivotal efficacy protocols will conform to appropriate CVM guidance documents. The pivotal efficacy protocols will be tailored to accommodate the specific NCR culture facility at which the study will be conducted. Selection of the NCR facility (or facilities) at which to conduct the pivotal studies will be based on (1) available infected cool or warmwater fish, (2) facilities and space appropriate to conduct a pivotal efficacy trial, and (3) available staff to support the study conduct (e.g. collection of pre-study mortality data, collection of fish during distribution to test tanks, etc.). Briefly, dose-confirmation protocols will be developed which will include two equally sized groups, a non-medicated control and an active treatment group. Each group will contain 5–10 tanks, depending on the available space and facilities at the NCR culture facility. Fish loading density within the tanks will be maintained at 125–150% of the loading density of the fish source tank. All tanks will be individually plumbed and will receive water from the NCR culture facility at which the trial is conducted at a rate sufficient to provide one-volume exchange per hour. Effluent from the test tanks will be directed into the facility's waste stream and will be excluded from recirculation within the NCR culture facility.

Fish inclusion criteria will be daily mortality of ≥0.5% of the culture tank population and the presence of clinical signs of MAI. Presumptive diagnosis will be based on confirmation of clinical signs and the presence of motile, Gram-negative rods collected from surface lesions and kidney or liver. Confirmatory diagnosis will be based on appropriate biochemical assays of aseptically collected samples of lesions and kidney on Rimler-Shotts agar (Shotts and Rimler 1973). Yellow-pigmented colonies will be selectively identified to genus by standard biochemical tests (Popoff 1984). Polymerase-chain reaction of 16S-rDNA will be completed on *Aeromonas* spp. isolates to identify to species (Borrell et al. 1997).

Following a presumptive diagnosis of MAI, fish will be randomly assigned to tanks in groups of ≤10 fish until the desired loading is achieved. Fish will then be offered either the medicated ration or the nonmedicated ration for a period of 10 days (dosing period) followed by a 14-day post-dosing observation period during which non-medicated ration will be the only feed offered. Medicated rations (authorized feed mills, drug concentration and dosing regimen) will be obtained in consultation with the drug sponsor. Mortality/morbidity and clinical signs will be recorded daily in each tank during the dosing and post-dosing period. Mortalities will be removed, weighed, and total length measured and recorded. Gross necropsy will be completed on all mortalities during the study. Microbiological samples will be collected from ≤5 fish per tank during the dosing and post-dosing period to confirm continuation of the infection. Fish will be enumerated at the trial termination and final weight and total length determined. Control fish will be

returned to the NCR culture facility for grow out. Treated fish surviving to test termination will be euthanized. All mortalities and euthanized fish will be disposed of according to the NCR culture facility standard operating procedure or will be incinerated at UMESC. Water chemistry (temperature, pH, and dissolved oxygen) will be monitored daily throughout the dosing and post-dosing period.

Each trial will be a separately blinded trial in which the technician collecting daily observations will have no knowledge of the treatment assignment. A qualified Study Monitor will conduct appropriate on-site inspections of each trial and prepare written audits of the findings. Efficacy will be evaluated based on the drugs (TM-200 or Aquaflor®) ability to reduce mortality relative to nonmedicated control fish. Mortality (binomial data) will be analyzed by Logistic Regression or general linear mixed model using fish nested within tank with treatment as a fixed effect. Tank (treatment) will be treated as a random effect and statistical significance will be declared at $p < 0.05$.

Upon completion of the draft protocols, each draft protocol will be submitted to the appropriate sponsor for review and concurrence. The sponsors will be consulted during protocol preparation to incorporate the sponsor's proposed treatment regimen and potential modifications of the drug formulation. Appropriate CVM staff will be consulted during protocol preparation to ensure the protocols meet CVM expectations and data requirements.

Submit Draft Pivotal Effectiveness Study Protocols (Objective 4)

UMESC routinely provides CVM with draft protocols to obtain protocol concurrence prior to conducting the actual study. UMESC will submit the draft protocols that have been reviewed by the sponsors to CVM though UMESC's INAD files. The CVM concurrence letters will be reviewed and appropriate modifications made to the study protocols before initiation of clinical efficacy trials.

Conduct Pivotal Effectiveness Studies on Terramycin 200 for Fish® (Objective 5)

Pending CVM concurrence with the pivotal efficacy protocols, UMESC and LFHC will initiate pivotal field efficacy trials on-site at identified NCR aquaculture facilities. The species selected (one coolwater and one warmwater species) will be determined in consultation with NCR industry representatives and after characterization of clinical field outbreaks. LFHC will confirm the presence/absence of *Aeromonas* spp. in (1) samples collected from test fish to confirm that mortality in fish selected for inclusion in a trial is associated with MAS and (2) samples collected from selected mortalities during the efficacy trial. UMESC staff will remain on-site for the duration of the pivotal efficacy trial to conduct the efficacy trial and to ensure adequate control and data collection. Any adverse reaction observed within the dosing group will immediately be reported to the drug sponsor. The drug sponsor will be responsible for CVM notification that an adverse event has occurred with the use of their product. Each trial will have a minimum of one inspection by UMESC's Quality Assurance Officer (QAO) to ensure the integrity of the study data. Drug concentration will be confirmed at the initiation and termination of dosing for each trial. A minimum of four pivotal efficacy trials (two fish species [one coolwater species and one warmwater species] $\times$ two drugs) will be conducted according to the pivotal efficacy protocols.

Analyze Effectiveness Data and Prepare Draft Final Study Reports (Objective 6)

Data analysis will be accomplished according to the approved study protocol and a final report will be prepared for each trial within 60 days of trial completion. Statistical analysis of mortality will be completed according to the study protocol for each trial; analysis of water chemistry, feed consumption, and other study data will be limited to simple summary statistics as appropriate for the data.

Submit the Respective Draft Study Reports (Objective 7)

Final reports will be prepared for each trial. Each final report and its associated data will be audited by the UMESC QAO before review and acceptance by UMESC management. UMESC will submit the reviewed and data-audited final reports to the appropriate drug sponsor for review prior to submission to CVM. The

drug sponsors will have a minimum of 60 days to provide review comments to UMESC before final report submission to CVM.

Submit Final Study Reports (Objective 8)

Sponsor review comments to the final study reports will be incorporated and the final study reports completed for each trial. The completed final report and all trial data will be archived according to UMESC Standard Operating Procedures. UMESC will submit the final reports to CVM within 60 days of receipt of Sponsor review comments.

Ensure That All Questions and Concerns Are Answered (Objective 9)

UMESC will coordinate with the CVM reviewer to address specific questions during the CVM review of the final study reports as needed. UMESC will address specific study related issues identified in the review letter with an amended final report if needed. If additional data are required that are beyond the scope of this project, UMESC will notify the NCRAC Board of Directors in writing within 30 days of receipt of the CVM response letter.

Provide Acceptance Letter and Effectiveness Studies, If Needed (Objective 10)

UMESC will provide the CVM response letter to the drug sponsors and will provide draft freedom of information summaries to the drug sponsor for inclusion in a supplemental NADA within 30 days of receipt of the CVM review letter. UMESC will provide access to the study raw data as needed to allow the drug sponsor to prepare the supplemental NADA package.

Extension Plan

Results of the experiments, where appropriate, will be presented at scientific meetings and extension workshops and may be published in scientific journals, extension bulletins, or NCRAC fact sheets and bulletins. Research results will also be disseminated through the NCRAC Annual Progress Reports. These reports are available on the NCRAC Web site (http://www.ncrac.org).

FACILITIES

UMESC

UMESC has a proven expertise in the evaluation of drugs for use in fish culture. UMESC scientists have submitted numerous reports summarizing their research to the U.S. Food and Drug Administration (FDA); these reports have led to the approval of several drugs to control diseases of fish and their eggs. The assigned investigator has led numerous regulated studies which were accepted by FDA and his body of work includes successfully completing several efficacy studies and developing a columnaris infection model useful in a diverse set of freshwater fish. UMESC's state-of-the art research facility includes numerous laboratories (isolation, wet, and analytical laboratories) equipped with technology to conduct fish culture and fish disease assessments.

UMESC has developed a mobile efficacy test system complete with up to 40 individually-plumbed test tanks. The flexible plumbing requirements of this mobile test system enable it to be used in nearly any situation provided appropriate environmental and physical security is provided. UMESC also has a mobile efficacy laboratory equipped to support on-site efficacy testing including analytical balances, dissecting and necropsy space, dedicated compound microscope with image-capture system, and secure sample storage areas.

LFHC

The LFHC is a state of art fish disease diagnostic laboratory serving the eight-state Great Lakes/Big Rivers Region. The center's staff performs fish health inspections, diagnostic, and laboratory work for

bacterial, parasitic, and viral pathogens. The assigned investigator is the senior diagnostician at the Center and has a history of supporting clinical efficacy studies including a long collaborative history with UMESC.

NCR Culture Facilities

The NCR culture facilities selected will have adequate space in which to safely and effectively conduct the proposed efficacy trials. The selected NCR facilities will have a history of MAI and associated MAS-related mortality. The specific NCR facilities used in this study will be identified in collaboration with the North American Fish Farmers Cooperative.

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PROJECT LEADER

State	Name/Institution	Area of Specialization
Wisconsin	Mark P. Gaikowski U.S. Geological Survey, Upper Midwest Environmental Sciences Center	Aquaculture/Drug Approval

PARTICIPATING INSTITUTION AND PRINCIPAL INVESTIGATOR

U.S. Geological Survey Upper Midwest Environmental Sciences Center
Mark P. Gaikowski

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

OMB Approved 0524-0039
Expires 03/31/2004

BUDGET

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

PROJECT DIRECTOR(S)
Mark P. Gaikowski

USDA AWARD NO. **Year 1: Objectives 1-10**

	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. ___ (Co)-PD(s)							
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty)	12			$36,687			
a. 1 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 →				$36,687			
B. Fringe Benefits (If charged as Direct Costs)				$11,006			
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$47,693			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$8,000			
F. Travel				$2,500			
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.)				$11,807			
K. Total Direct Costs (C through I) →				$70,000			
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) →							
N. Other →							
O. Total Amount of This Request →				$70,000			

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
US Geological Survey
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road, La Crosse, WI 54603

PROJECT DIRECTOR(S)
Mark P. Gaikowski

USDA AWARD NO. **Year 2: Objectives 1-10**

	CSREES FUNDED WORK MONTHS			Duration Proposed Months: 12 Year 2 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)	12			$37,787			
a. __1__ 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.....→				$37,787			
B. Fringe Benefits (If charged as Direct Costs)				$11,336			
C.Total Salaries, Wages, and Fringe Benefits (A plus B) →				$49,123			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$6,000			
F. Travel				$7,500			
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.)				$17,377			
K. .....Total Direct Costs (C through I) →				$80,000			
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) . →							
N. .....Other →							
O. ..... Total Amount of This Request →				$80,000			

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 U.S. GEOLOGICAL SURVEY UPPER MIDWEST ENVIRONMENTAL SCIENCES CENTER

(Gaikowski)

Objectives 1-10

A. Salaries and Wages. Year 1: Salaries are requested for one 100% FTE research associate to record MAI signs in fish reported to have MAI, and collect isolates and identify potential etiological agents in fish with possible MAI from NCR facilities. The research associate will develop a research protocol to evaluate the efficacy of TM-200 and Aquaflor to control mortality associated with MAS in NCR fish. Year 2: Salaries are requested for one 100% FTE research associate to conduct the on-site effectiveness studies, complete the microbiological identification of isolated *Aeromonas* spp. and prepare the final study reports.

B. **Fringe Benefits.** Years 1 and 2: The fringe benefit rate is approximately 30%.

E. Materials and Supplies. Year 1: microbiological culture and biochemical test supplies ($2,500); Polymerase-Chain Reaction (PCR) primers, buffers, beads, agarose gels, pipette tips ($4,500); isopropyl alcohol and DNA-away ($150); general wet-laboratory supplies ($325); office and study record keeping supplies ($525). Year 2: microbiological culture and biochemical test supplies ($1,500); polymerase-chain reaction (PCR) primers, buffers, beads, agarose gels, pipette tips ($2,500); plumbing supplies ($600); air stones and tubing ($125); submersible water pumps ($300); isopropyl alcohol and DNA-away ($200); general wet-laboratory supplies ($350); office and study record keeping supplies ($425).

F. Travel. Year 1: $2,500 is requested for transportation, lodging, and meal expenses to collect isolates from NCR facilities, locations to be determined (10 trips at an average of $250 per trip). Year 2: $7,500 is requested for transportation, lodging, and meal expenses to conduct two pivotal effectiveness trials at NCR facilities, locations to be determined (two trials at an average of $3,750 per trip).

J. All Other Direct Costs. Year 1: $11,807 to support contract laboratory biochemical- and PCR-confirmation of isolates collected from NCR facilities. Year 2: $17,377 to support contract laboratory biochemical and PCR identification of isolates collected during disease trials conducted at NCR facilities.

SCHEDULE FOR COMPLETION OF OBJECTIVES

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

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

Objective 3: Initiated in Year 1 completed in Year 1.

Objective 4: Initiated in Year 1 completed in Year 2.

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

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

Objective 7: Initiated in Year 2 completed in Year 2.

Objective 8: Initiated in Year 2 completed in Year 2.

Objective 9: Initiated in Year 2 completed in Year 2.

Objective 10: Initiated in Year 2 completed in Year 2.

PRINCIPAL INVESTIGATOR

Mark P. Gaikowski, U.S. Geological Survey Upper Midwest Environmental Sciences Center

VITA

Mark P. Gaikowski Phone: (608) 781-6284
U.S. Geological Survey Upper Midwest Environmental Sciences Center E-mail: mgaikowski@usgs.gov
2630 Fanta Reed Road
La Crosse, WI. 54603

EDUCATION

B.S. University of South Dakota, 1991, Biology
M.A. University of South Dakota, 1994, Biology

POSITION

Research Physiologist

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society
Phi Sigma Biological Honor Society

SELECTED PUBLICATIONS

Gaikowski, M.P., W.J. Larson, and W.H. Gingerich. 2008. Survival of cool and warm freshwater fish following chloramine-T exposure. Aquaculture 275:20-25.

Meinertz, J.R., S.L. Greseth, M.P. Gaikowski, and L.J. Schmidt. 2008. Chronic toxicity of hydrogen peroxide to *Daphnia magna* in a continuous exposure, flow-through test system. Science of the Total Environment 392:225-232.

Ronan, P.J., M.P. Gaikowski, S.J. Hamilton, K.J. Buhl, and C.H. Summers. 2007. Ammonia causes decreased brain monoamines in fathead minnows (*Pimephales promelas*). Brain Research 1147:184-181.

Rach, J.J., S.D. Redman, D. Bast, and M.P. Gaikowski. 2005. Efficacy of hydrogen peroxide versus formalin treatments to mortality associated with saprolegniasis on lake trout eggs. North American Journal of Aquaculture 67:148-154.

Barnes, M.E. and M.P. Gaikowski. 2004. Use of hydrogen peroxide during incubation of landlocked fall chinook salmon eggs in vertical-flow incubators. North American Journal of Aquaculture 66:29-34.

Gaikowski, M.P., J.C. Wolf, S.M. Schleis, and W.H. Gingerich. 2003. Safety of oxytetracycline (Terramycin, TM-100F) administered in feed to hybrid striped bass, walleye, and yellow perch. Journal of Aquatic Animal Health 15:274-286.

Gaikowski, M.P., J.C. Wolf, R.G. Endris, and W.H. Gingerich. 2003. Safety of Aquaflor® (Florfenicol, 50% Type A Medicated Article), Administered in Feed to Channel Catfish, *Ictalurus punctatus*. Toxicologic Pathology 31:689-697.

Gaikowski, M.P., J.J. Rach, M. Drobish, J. Hamilton, T. Harder, L.A. Lee, C. Moen, and A. Moore. 2003. Efficacy of hydrogen peroxide to control mortality associated with saprolegniasis on walleye, white sucker, and paddlefish eggs. North American Journal of Aquaculture 65:349-355.

Rach, J.J., M.P. Gaikowski, 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.

Gaikowski, M.P., J.J. Rach, and R.T. Ramsay. 1999. Acute toxicity of hydrogen peroxide treatments to selected life stages of cold-, cool-, and warmwater fishes. Aquaculture 178:191-207.