EFFICACY OF EUGENOL TO REDUCE TRANSPORT STRESS AND MORTALITY OF TILAPIA AND YELLOW PERCH

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

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

Funding Request: $100,000

Duration: 2 Years (September 1, 2011 - August 31, 2013)

Objectives:

1. Interact with CVM to determine the study design and protocol needed to develop the effectiveness data to support a transport sedative claim for eugenol for selected finfish species. The protocol must comply with current CVM Guidance For Industry for the development of pivotal effectiveness data and the study data collection must with CVM Good Clinical Practices regulations.
2. Obtain fully disclosable Investigational New Animal Drug (INAD) exemptions for the selected sedative to be tested from CVM.
3. Obtain Categorical Exclusions from the requirement to complete an Environmental Assessment or complete an Environmental Assessment for the selected sedative prior to its use and receive concurrence from CVM Environmental Safety Team.
4. Submit the pivotal effectiveness protocol to CVM for concurrence.
5. Conduct pivotal effectiveness studies using the selected sedative on finfish species according to the CVM-concurred protocol and in compliance with CVM Good Clinical Practices regulations.
6. Summarize the study data into a Final Study Report (FSR) and archive all study data in publicly accessible archives
7. Submit the FSR to the publicly disclosable INAD file provided by CVM and request CVM review of the FSR and concur that the effectiveness technical section is complete for the selected sedative.
8. Respond to CVM comments on the FSR to ultimately obtain concurrence that the effectiveness technical section is complete for the use of the selected sedative as a transport sedative for the selected species
9. Prepare a Freedom Of Information summary of the submitted data and provide it to CVM.

Proposed Budget:

Institution	Principal Investigator	Objec -tives	Year 1	Year 2	Total
U.S. Geological Survey, Upper Midwest Environmental Sciences Center	Mark P. Gaikowski	1-9	**$23,684**	**$23,324**	**$47,008**
University of Wisconsin-Stevens Point	Christopher F. Hartleb	5-9	**$26,316**	**$26,676**	**$52,992**
		TOTALS	**$50,000**	**$50,000**	**$100,000**

Non-funded Collaborators:

Facility	Collaborator
Viterbo University	Kim A. Fredricks
North American Fish Farmers Cooperative, Binford Eagle Fisheries, LLC.	Mark Willows

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JUSTIFICATION

Private finfish producers have a great need for a sedative to reduce stress and mortality associated with fish transport of all finfish species whether the fish are destined for live market, immediate slaughter, or other uses. Fish are generally transported for one of two activities within private aquaculture, either transport to live/slaughter markets or transport of baitfish or food fish stockers. Market-size fish, either aquaculture-reared (e.g. tilapia, hybrid striped bass, largemouth bass, grass carp, channel catfish) or wild-caught (e.g. common carp, bigmouth buffalo, and smallmouth buffalo) species, are transported to live or slaughter markets. The slaughter market (i.e., food-sized fish) is the biggest market in the United States, using 475 million fish and 723 million pounds as of 2005. A sedative that increases fish loading density during transport and decreases stress and mortality associated with transport could significantly decrease transport costs and increase production efficiency. Similar to the need for market-sized fish, a sedative is needed to increase transport efficiency of finfish not destined for live market (e.g., food finfish stockers, baitfish) to increase fish loading density during transport and reduce transport stress that may result in mortality or weakened fish. In either case, the development of a sedative that increases hauling capacities (i.e., greater stocking densities in transport tanks or lengthens safe hauling times) while reducing the stress and mortality associated with transport would improve profitability of private aquaculture.

Two candidate sedatives, eugenol and benzocaine, have potential to meet the needs of private aquaculture as sedatives to increase fish transport efficiency. These sedatives (1) are presently being evaluated by the Association of Fish and Wildlife Agencies Drug Approval Working Group (DAWG), (2) have been proven to be effective in preliminary studies to induce sedation in a number of finfish species, (3) have active sponsors who will support data generation for drug approval if their drug (eugenol or benzocaine) is selected for development as an immediate-release sedative by the DAWG, and (4) are designated under the U.S. Food and Drug Administration Center for Veterinary Medicine (CVM) Office of Minor Use/Minor Species (OMUMS) for these label claims and thus eligible for OMUMS grant funding to complete some drug approval data requirements.

The transport label claim developed is expected to include the use of either sedative at a low concentration over a long period of time ($\geq$12 h). The proposed species for study are tilapia (*Oreochromis* sp.) and yellow perch (*Perca flavescens)*, however, the exact species selected for study will be finalized based on protocol concurrence by the CVM and the need to address one or more temperature grouping of finfish species (e.g., cool water or warm water finfish). The proposed research represents the initial step to obtain approval of a sedative to reduce transport-associated stress, increase post-transport survival and increase safe loading densities during transport. Additional data to describe animal safety, human food safety and environmental safety will be required in order to obtain approval of this drug for use as a sedative during transport.

RELATED CURRENT AND PREVIOUS WORK

The availability of safe and effective sedatives is critical to private and public aquaculture as well as to fisheries researchers and managers. The use of sedatives to ease fish handling and reduce physical injury to both fish and personnel is a long-studied area of fishery research; in a recent literature search, 500 peer-reviewed journal articles were found summarizing some aspect of the use of chemical sedation in fish. The need for and uses of sedation in aquatic animals has been well summarized by a number of authors like Ross and Ross (2008). The use of chemical sedation during transport of fish has been studied in freshwater and marine species including Atlantic salmon (*Salmo salar)* (AQUI-S®, Iversen and Eliassen, 2009; clove oil, Iversen et al., 2009; metomidate, Sandodden et al. 2001), American shad (*Alosa sapidissima)* (benzocaine, clove oil, tricaine methanesulfonate, Du et al. 2007), Convict cichlid (*Cichlasoma nigrofasciatum)* (metomidate, Kilgore et al. 2009), freshwater prawn (*Machrobrachiurn rosenbergii)* (AQUI-S®, clove oil, Coyle et al. 2005), Lake Victoria cichlid (*Haplochromis obliquidens)* (clove oil, Kaiser et al. 2006), largemouth bass (*Micropterus salmoides)* (clove oil, Cooke et al. 2004), matrixna (*Bryon cephalus)* (clove oil, Inoue et al. 2005), (*Pontius filamentosus)* (benzocaine, tricaine methanesulfonate, Pramod et al. 2010), whitefish (*Chirostoma estor)* (benzocaine, Ross and Ross 2008),

winter flounder (*Pleuronectes americanus)* (lidocaine hydrochloride, Park et al. 2009). In general sedatives, especially benzocaine, eugenol (clove oil), isoeugenol (AQUI-S®), and tricaine methanesulfonate were effective at reducing mortality during and after stress and decreasing transport-related physiological indicators of transport-mediated stress.

ANTICIPATED BENEFITS

Fish transport costs are a substantial portion of the operational expenses of the aquaculture industry in the North Central Region (NCR), especially as fuel costs continue to increase. Increasing fish loading density during transport could substantially increase the efficiency of NCR aquaculture operations by enabling the transport of more fish per gallon of fuel. Also, gains in operator efficiency may be seen as fewer staff days may be required for transport and hauling with increased loading density. Reducing transport-mediated stress in fish could also improve market sales, especially at live market (either for food fish or baitfish) by improving fish quality and appearance by reducing physical damage of fish during transport and decreasing post-transport disease occurrence. Reducing transport-mediated fish stress may also enhance fillet quality in fish transported to slaughter markets by reducing aerobic metabolism during transport, potentially improving fillet quality by maximizing residual energy stores in the fillet. When hauling juvenile fish for stocking, potential benefits would be realized by increasing loading density during transportation and increasing post-transport survival. The potential economic gain from increasing fish density during transport and increasing fish survival post-transport motivated the North Central Regional Aquaculture Center's (NCRAC's) Board of Directors to authorize up to $100,000 for a project to develop pivotal effectiveness data to support the potential approval of eugenol (AQUI-S 20E®). The NCRAC Board made it clear that the intent of these monies would be to complete the needed effectiveness studies for one fish grouping to support a drug sponsor's New Animal Drug Application (NADA) to CVM for approval.

From the proposed investigations, the safe transport regimens will be developed for eugenol to increase fish loading density during transport and increase post-transport survival of NCR cultured fish.

OBJECTIVES

1. Interact with CVM to determine the study design and protocol needed to develop the effectiveness data to support a transport sedative claim for eugenol for selected finfish species. The protocol must comply with current CVM Guidance For Industry for the development of pivotal effectiveness data and the study data collection must with CVM Good Clinical Practices regulations.
2. Obtain fully disclosable Investigational New Animal Drug (INAD) exemptions for the selected sedative to be tested from CVM.
3. Obtain Categorical Exclusions from the requirement to complete an Environmental Assessment or complete an Environmental Assessment for the selected sedative prior to its use and receive concurrence from CVM Environmental Safety Team.
4. Submit the pivotal effectiveness protocol to CVM for concurrence.
5. Conduct pivotal effectiveness studies using the selected sedative on finfish species according to the CVM-concurred protocol and in compliance with CVM Good Clinical Practices regulations.
6. Summarize the study data into a Final Study Report (FSR) and archive all study data in publicly accessible archives
7. Submit the FSR to the publicly disclosable INAD file provided by CVM and request CVM review of the FSR and concur that the effectiveness technical section is complete for the selected sedative.
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9. Prepare a Freedom Of Information summary of the submitted data and provide it to CVM.

PROCEDURES

Interact with CVM to Determine the Study Design and Protocol (Objective 1)
This project will assess the use of eugenol to enhance post-transport survival and increase loading density during long duration (≥12 h) transport events. This study will be initiated with a thorough review of the literature to compile existing data on the use of sedatives during fish transport. The Upper Midwest Environmental Sciences Center (UMESC) will request and schedule a pre-submission conference with CVM to discuss needed study parameters to be included into the final study protocols prepared for the project. UMESC and the University of Wisconsin-Stevens Point (UWSP) will collaboratively prepare the protocols and then UMESC will submit the protocols to CVM through the UMESC publicly-disclosable Investigational New Animal Drug (INAD) permits for eugenol and request CVM review and protocol concurrence prior to conducting the study. Additionally, UMESC maintains a Quality Assurance Unit with a full-time Quality Assurance Officer who will assist research scientists conducting this project to comply with the CVM Good Clinical Practice regulations.

Obtain Fully Disclosable Investigational New Animal Drug (INAD) Exemptions (Objective 2)
UMESC presently has publicly disclosable INADs for benzocaine (INAD 9413) and eugenol (INAD 011-766) into which UMESC has submitted various data sets relative to the potential approval of these compounds as fish sedatives. All protocols, data, and final study reports submitted to CVM will be submitted by UMESC to either INAD 9413 or INAD 011-766.

Obtain Categorical Exclusions from the Requirement to Complete an Environmental Assessment or Complete an Environmental Assessment (Objective 3)
UMESC has completed three Environmental Assessments for aquaculture drugs (chloramine-T, hydrogen peroxide, and oxytetracycline dihydrate) and routinely interacts with the CVM Environmental Safety Team. UMESC will coordinate with the CVM Environmental Safety Team to determine the data requirements (if any) to request a Categorical Exclusion from the requirement to complete an Environmental Assessment to conduct the required effectiveness studies. Based on UMESC experience with submitting categorical exclusions to CVM to conduct pivotal effectiveness studies, there should be no impediments to obtaining CVM concurrence on the request for the categorical exclusion.

Submit the Pivotal Effectiveness Protocol to CVM for Concurrence (Objective 4)
UMESC routinely submits draft protocols to CVM to obtain concurrence on proposed experimental design and procedures prior to conducting the actual study. UMESC will submit the draft protocols that have been reviewed by the drug sponsors to CVM though UMESC's active INAD files. The CVM concurrence letters will be reviewed and appropriate modifications made to the study protocols before initiation of the effectiveness trials.

Conduct Pivotal Effectiveness Studies (Objective 5)
The overall goal of this part of the study is to determine the optimal sedative concentration that will allow an increased stocking density during transport. Two species of fish that are commonly cultured and transported in the NCR will be tested: tilapia (*Oreochromis niloticus)* and yellow perch (*Perca flavescens)*. Exact species selected will be based on the pre-submission conference with CVM. Fish used in the study will be obtained from specific pathogen-free stocks. The age of the fish tested will be typical for the age at transport. For example, sexually immature yellow perch are commonly transported as fingerlings (1- 4 months old), thus that size (age) will be used in the experiments. Common procedures will be used when testing eugenol. For eugenol, the commercially-available formulation AQUIS-20E® will be used in all experiments. All experiments will be conducted at the UMESC.

Initial exposures will determine the sedative concentration required to achieve sedation to selected anesthesia planes (Palic et al. 2006; selected during pre-submission conference with CVM) during short (~2- to 15-min) or long (12-, 24-, or 48-h) exposure. To determine the sedative efficacy and optimal dose of eugenol, the fish will be exposed to different sedative concentrations and time to induction to selected anesthesia planes, recovery time, and survival measured. Six dose levels of the sedative (to be determined based on literature-derived values and preliminary tests to determine the concentrations that cause fish to become handleable within 2 min) and a control group (0 mg/L) will be tested. Fifty fish will be randomly placed into ten static, aerated, sedative baths (five fish each) resulting in 10 replicates per

dose level. Fish will be exposed to a sedative for the assigned exposure period then moved to aquaria with continuous-flow, aerated, sedative-free water for recovery and observed daily for any abnormal behavior and mortalities for 14 days. The percent of fish at selected anesthesia planes after induction, average recovery time for individual fish, and percent survival at the time of recovery will be determined for each concentration. Least-square regression analysis will be applied to test if recovery time was dependent on eugenol concentration (Kaiser et al. 2006).

Based on the dose response study, a concentration will be selected for use in developing the pivotal effectiveness protocol. The concentration selected will be based on fish survival during preliminary assays. The overall goal of this part of the study is to establish recovery and survival of fish following sedation for 12, 24, or 48 hours. A 2×3×3 factorial design will be used with three replicates per sedative treatment (control and eugenol) at each of three loading densities replicated at each of three transport durations (12, 24, and 48 hours); the three transport durations will be tested consecutively (e.g. sedation by loading density will be tested first 12 h, then 24 h, then 48 h). The experimental variables will be two sedative methods (control-no sedation or eugenol) three loading densities (typical, 1.5 × typical, or 2 × typical) and three transport durations (12, 24, and 48 hours). Twenty fingerlings (360 fish per transport duration; 1,080 fish total per species) will be indiscriminately placed into each of eighteen 20.0-L (5.3-gal) static, aerated baths. Each tank will be oxygenated at rates similar to those used during transport. To mimic a "real-life" fish transport, fish will be crowded by rapidly reducing the water levels to the desired loading density (e.g. typical loading densities for yellow perch are ~60 g/L [0.5 lb/gal] at <15°C [59°F] with a dissolved oxygen level of >7 mg/L [>7 ppm]) with water pumps (Iversen et al. 2009).

Fish mortality and plasma cortisol levels will be measured as indicators of stress. Blood will be sampled one week before the onset of the experiment (pre-stress), after loading, and at selected sampling times after exposure to conditions that mimic transport. Fish will be removed from the sedative bath and blood samples collected from the caudal peduncle. If necessary, blood from 3-5 fish may be pooled prior to centrifugation (Palic et al. 2006). The blood samples will be allowed to clot overnight at 4°C (39°F) then centrifuged at 4000 × g for 15 min. The serum will be collected and stored at ≤-35 °C (-95°F) until it analyzed for cortisol (Barry et al. 1993). Fish will be returned to an aquarium receiving continuous-flow sedative-free water (single-pass) and monitored for 14 days. The time taken for the fish to adopt upright posture and resume normal swimming, and recovery time, will also be recorded (Hasan and Bart 2007). Upon conclusion of the experiment, the fish will be humanely euthanized and measured for total length and weight. Fish will be monitored during the exposure and through the post-exposure period. Any fish that do not survive will be promptly removed and the mortality recorded. Dead fingerlings, fish with no opercular beats for a 15 min period of continuous observation, will be removed after 1, 6, 12, 24, and 48 h from all tanks to determine mortality rates. Dissolved oxygen will be monitored continuously through the pivotal effectiveness study and be maintained at a minimal concentration of 7 mg/L (7 ppm). At the completion of the transport simulation, the treated fingerlings from each experimental unit will be stocked into recovery tanks to observe post-exposure mortality.

All datasets will be tested for normality using a Kolmogorov-Smirnov test and for homogeneity of variance using a Levene test. A two-way ANOVA test will thereafter be performed at each sampling time to test for differences between the two sedative groups. If the F-values are significant, a Bonferroni post hoc test will be used to determine which groups differed (Sokal & Rohlf 1987). A one-way ANOVA with Bonferroni post hoc within-group will also be used to identify differences among all sampling times for each physiological parameter. A Kruskal-Wallis ANOVA (non-parametric) and a Mann-Whitney U-test with a Bonferroni-adjusted significance level will be used when requirements for parametric statistics are not met. Significance will be declared if $P \leq 0.05$.

Summarize the Study Data into a Final Study Report (FSR) and Archive All Study Data in Publicly Accessible Archives (Objective 6)

Final study reports will be prepared for each trial conducted. Each final study report and its associated data will be audited by the UMESC Quality Assurance Officer before review and acceptance by UMESC management. Final study reports will be provided to the appropriate drug sponsor for review prior to submission to CVM. The drug sponsors will have a maximum of 60 days to provide review comments to UMESC before the complete final study report and all trial data are archived according to UMESC

Standard Operating Procedures. The final study report will then be submitted to CVM through the UMESC publicly disclosable appropriate INAD file.

Submit the FSR to the Publicly Disclosable INAD File and Request CVM Review of the FSR (Objective 7)
UMESC routinely submits FSRs to CVM for review and acceptance. UMESC will prepare triplicate copies of the FSRs and associated data and submit the FSRs to CVM through UMESC's publicly disclosable INAD file. Included with the submission will be appropriate correspondence and CVM-mandated forms to request CVM review to determine whether the submitted data support the potential approval of eugenol as a sedative to improve fish transport loading density and reduce post-transport mortality.

Respond to CVM Comments on the FSR (Objective 8)
UMESC will coordinate with the CVM reviewer to address specific questions during the CVM review of the FSRs 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.

Prepare a Freedom Of Information Summary of the Submitted Data and Provide It to CVM (Objective 9)
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 chemicals for use to control aquatic invasive species and drugs for use in fish rearing operations. UMESC scientists have submitted numerous reports summarizing their research to the U.S. Environmental Protection Agency (EPA) and the U.S. Food and Drug Administration (FDA); these reports have led to the registration of the general piscicides rotenone and antimycin A and the continued registration for the lampricides 3-trifluoromethyl-4-nitrophenol (TFM) and niclosamide and the approval of several drugs to control diseases of fish and their eggs. UMESC maintains a full-time on-site quality assurance officer to manage its Good Laboratory Practices (GLP) compliance program. The UMESC GLP program is routinely inspected by FDA or EPA auditors. A recent (2009) audit by FDA had no reported findings. The assigned investigator has led numerous regulated studies and the resulting data were accepted by the FDA. His body of work includes several successfully completed drug toxicity studies as well as three completed environmental assessments of drugs proposed for use in fish culture facilities. Staff within the assigned investigator's research branch have a long history of work on the use of sedatives to enhance fish handling operations. UMESC's state-of-the-art research facility includes numerous laboratories (isolation, wet, and analytical laboratories) equipped with technology to rear test animals and to conduct laboratory and field assessments.

UMSP
UWSP operates a state-of-the-art, commercial-scale aquaculture demonstration, applied research, and extension facility (Northern Aquaculture Demonstration Facility [NADF]) serving the aquaculture industries of the northern Midwest United States. The facility conducts projects involving production, transport, disease prevention, and new technologies in aquaculture and manages a statewide extension program that emphasizes delivering research results directly to fish farmers. The assigned investigator is a Co-

Director of the NADF and is the coordinator of the aquaculture program at UWSP serving undergraduate and graduate students in Biology and Fisheries.

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PROJECT LEADERS

State	Name/Institution	Area of Specialization
Wisconsin	Mark P. Gaikowski US Geological Survey, Upper Midwest Environmental Sciences Center	Aquaculture/Drug Approval
	Christopher F. Hartleb University of Wisconsin-Stevens Point	Aquaculture/Fishery Biology

PARTICIPATING INSTITUTIONS AND CO-PRINCIPAL INVESTIGATORS

US Geological Survey Upper Midwest Environmental Sciences Center
Mark P. Gaikowski

University of Wisconsin-Stevens Point
Christopher F. Hartleb

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 1-9**

	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. _1_ Research Associates-Postdoctorates . . .	12.0			$20,166			
b. ___ Other Professionals							
c. ___ Paraprofessionals.....							
d. ___ Graduate Students.....							
e. ___ Prebaccalaureate Students.....							
f. ___ Secretarial-Clerical.....							
g. ___ Technical, Shop and Other							
Total Salaries and Wages.....→				$20,166			
B. Fringe Benefits (If charged as Direct Costs)				$1,518			
C.Total Salaries, Wages, and Fringe Benefits (A plus B) →				$21,684			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$1,000			
F. Travel				$1,000			
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) →				$23,684			
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 →				$23,684			

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 1-9**

	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. _1_ Research Associates-Postdoctorates . . .	12.0			$19,701			
b. ___ Other Professionals							
c. ___ Paraprofessionals.....							
d. ___ Graduate Students.....							
e. ___ Prebaccalaureate Students.....							
f. ___ Secretarial-Clerical.....							
g. ___ Technical, Shop and Other							
Total Salaries and Wages.....→				$19,701			
B. Fringe Benefits (If charged as Direct Costs)				$1,483			
C.Total Salaries, Wages, and Fringe Benefits (A plus B) →				$21,184			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$1,000			
F. Travel				$1,140			
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) →				$23,324			
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 →				$23,324			

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)		

BUDGET AND BUDGET EXPLANATION FOR EACH PARTICIPATING INSTITUTION

(Gaikowski)

Objectives 1-9

A. Salaries and Wages. Year 1: Salary ($20,166) is requested for one 100% FTE research associate to support sedation studies, culture fish and conduct chemical analyses. The research associate will collaborate with the UWSP graduate student to develop a research protocol to evaluate the efficacy of eugenol in NCR fish. The research associate will be trained in Good Clinical Practices to ensure research is conducted in compliance with CVM requirements. Year 2: Salary ($19,701) is requested for one 100% FTE research associate to support sedation studies, culture fish and conduct chemical analyses. The research associate will collaborate with the UWSP graduate student to develop the comprehensive FSR for submission to CVM. The research associate will be trained in Good Clinical Practices to ensure research is conducted in compliance with CVM requirements.

B. Fringe Benefits. Year 1: 7.53% of salary ($1,518); Year 2: 7.53% of salary ($1,483).

E. Materials and Supplies. Year 1: General wet laboratory supplies ($400); general analytical laboratory supplies ($400); office and study record keeping supplies ($200). Year 2: General wet laboratory supplies ($400); general analytical laboratory supplies ($400); office and study record keeping supplies ($200).

F. Travel. Year 1: $1,000 is requested for transportation, lodging, and meal expenses to conduct pre-submission conference with CVM. Year 2: $1,140 is requested for transportation, lodging, and meal expenses to attend a national scientific meeting to present results.

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Biological Resources Division
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

April 25, 2011

Dr. Ted R. Batterson, Director
North Central Regional Aquaculture Center
Michigan State University
13 Natural Resources Building
East Lansing, Michigan 48842

SUBJECT: Project entitled "EFFICACY OF EUGENOL TO REDUCE TRANSPORT STRESS AND MORTALITY OF TILAPIA AND YELLOW PERCH"

Dear Dr. Batterson:

As the Authorized Organizational Representative (AOR) I would like to inform you the U.S. Geological Survey Upper Midwest Environmental Sciences Center (UMESC) wishes to participate in the above referenced project as a collaborator with Michigan State University. Mr. Mark P. Gaikowski will serve as the Principal Investigator of the collaborative agreement and has access to all of the necessary equipment, laboratory, and office space to successfully undertake this project. I also approve the budget as submitted for Mr. Gaikowski's involvement in this project. Upon issuance of approval to the North Central Regional Aquaculture Center for this project, UMESC will enter into a formal agreement with your institution.

Sincerely,

Michael Jawson

Michael D. Jawson
Center Director
UMESC

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
Northern Aquaculture Demonstration Facility
800 Reserve Street, Stevens Point, WI 54481

PROJECT DIRECTOR(S)
Christopher F. Hartleb

USDA AWARD NO. **Year 1: Objectives 5-9**

	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. _1_ Graduate Students.....				$18,000			
e. ___ Prebaccalaureate Students.....							
f. ___ Secretarial-Clerical.....							
g. ___ Technical, Shop and Other							
Total Salaries and Wages.....→				$18,000			
B. Fringe Benefits (If charged as Direct Costs)				$8,316			
C.Total Salaries, Wages, and Fringe Benefits (A plus B) →				$26, 316			
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) →				$26,316			
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 →				$26,316			

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)		

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

OMB Approved 0524-0039
Expires 03/31/2004

BUDGET

ORGANIZATION AND ADDRESS	USDA AWARD NO. Year 2: Objectives 5-9			
University of Wisconsin-Stevens Point Northern Aquaculture Demonstration Facility 800 Reserve Street, Stevens Point, WI 54481	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)
PROJECT DIRECTOR(S) Christopher F. Hartleb				

A. Salaries and Wages	CSREES FUNDED WORK MONTHS						
1. No. of Senior Personnel	Calendar	Academic	Summer				
a. ___ (Co)-PD(s)							
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty)							
a. __ Research Associates-Postdoctorates . . .							
b. ___ Other Professionals							
c. ___ Paraprofessionals.....							
d. _1_ Graduate Students.....				$18,000			
e. ___ Prebaccalaureate Students.....							
f. ___ Secretarial-Clerical.....							
g. ___ Technical, Shop and Other							
Total Salaries and Wages.....→				$18,000			
B. Fringe Benefits (If charged as Direct Costs)				$8,676			
C.Total Salaries, Wages, and Fringe Benefits (A plus B) →				$26,676			
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) →				$26,676			
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 →				$26,676			

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 AND BUDGET EXPLANATION FOR EACH PARTICIPATING INSTITUTION

(Hartleb)

Objectives 5-9

A. Salaries and Wages. Year 1: Salary ($18,000) is requested for one graduate student to determine study design and protocols for effectiveness studies; interact with agencies to acquire exemptions, exclusions, and concurrence. The graduate student will collaborate with UMESC to develop a research protocol to evaluate the efficacy of eugenol in NCR fish. Year 2: Salary ($18,000) is requested for one graduate student to conduct sedation effectiveness studies, culture fish, conduct chemical analyses, and summarize data for the CVM.

B. Fringe Benefits. Year 1: 46.2% of salary ($8,316); Year 2: 48.2% of salary ($8,676).

University of Wisconsin-Stevens Point
College of Letters and Science
Department of Biology

Stevens Point WI 54481-3897
715-346-2159; Fax 715-346-3624
E-mail: biology@uwsp.edu
www.uwsp.edu/biology

April 5, 2011

Dr. Ted R. Batterson, Director
North Central Regional Aquaculture Center
Michigan State University
13 Natural Resources Building
East Lansing, Michigan 48842

SUBJECT: Project entitled "Efficacy of Eugenol to Reduce Transport Stress and Mortality of Tilapia and Yellow Perch"

Dear Dr. Batterson:

As the Authorized Organizational Representative (AOR) I would like to inform you that the University of Wisconsin-Stevens Point (UW-Stevens Point) wishes to participate in the above referenced project as a subcontractor to Michigan State University. Dr. Christopher Hartleb will serve as the Principal Investigator of the subcontract and he has access to all of the necessary equipment, laboratory, and office space to successfully undertake this project. I also approve the budget as submitted for Dr. Christopher Hartleb's involvement in this project. Upon issuance of approval to the North Central Regional Aquaculture Center for this project, the University of Wisconsin-Stevens Point (UW-Stevens Point) will enter into a formal agreement with your institution.

Sincerely,

Dr. Katherine Jore
Associate Vice Chancellor for Personnel, Budget and Grants

BUDGET SUMMARY FOR EACH YEAR FOR EACH PARTICIPATING INSTITUTION

Year 1

	UMESC	UWSP	Totals
Salaries and Wages	$20,166	$18,000	$38,166
Fringe Benefits	$1,518	$8,316	$9,834
Total Salaries, Wages, and Fringe Benefits	$21,684	$26,316	$48,000
Nonexpendable Equipment			
Materials and Supplies	$1,000		$1,000
Travel	$1,000		$1,000
All Other Direct Costs			
TOTAL PROJECT COSTS	$23,684	$26,316	$50,000

Year 2

	UMESC	UWSP	Totals
Salaries and Wages	$19,701	$18,000	$37,701
Fringe Benefits	$1,483	$8,676	$10,159
Total Salaries, Wages, and Fringe Benefits	$21,184	$26,676	$47,860
Nonexpendable Equipment			
Materials and Supplies	$1,000		$1,000
Travel	$1,140		$1,140
All Other Direct Costs			
TOTAL PROJECT COSTS	$23,324	$26,676	$50,000

SCHEDULE FOR COMPLETION OF OBJECTIVES

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

LIST OF PRINCIPAL INVESTIGATORS

Mark P. Gaikowski, U.S. Geological Survey Upper Midwest Environmental Sciences Center
Christopher F. Hartleb, University of Wisconsin-Stevens Point

VITA

Mark P. Gaikowski
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, WI 54603

Phone: (608) 781-6284
E-mail: mgaikowski@usgs.gov

EDUCATION

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

POSITION

Supervisory Biologist (1993-present), USGS, UMESC

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society
Phi Sigma Biological Honor Society

SELECTED PUBLICATIONS

Gaikowski, M.P., M. Mushtaq, P. Cassidy, J.R. Meinertz, S.M. Schleis, D. Sweeney, R.G. Endris. 2010. Depletion of florfenicol amine, marker residue of florfenicol, from the edible fillet of tilapia (*Oreochromis niloticus* × *O. niloticus* and *O. niloticus* × *O. aureus*) following florfenicol administration in feed. Aquaculture 301:1-6.

Tuttle-Lau, M.T., K.A. Phillips, and M.P. Gaikowski. 2010. Evaluation of iodophor disinfection of walleye and northern pike eggs to eliminate viral hemorrhagic septicemia virus. USGS Fact Sheet 2009-3107.

Rach, J.J, G.G. Sass, J.A. Luoma, and M.P. Gaikowski. 2010. Effects of water hardness on size and hatching success of silver carp eggs. North American Journal of Fisheries Management 30:230-237.

Gaikowski, M.P., C.L. Densmore, and V.S. Blazer. 2009. Histopathology of repeated, intermittent exposure of chloramine-T to walleye (*Sander vitreum*) and (*Ictalurus punctatus*) channel catfish. Aquaculture 287:28-34.

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 1147:184-191.

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. North American Journal of Aquaculture 65:300-305.

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.

Gaikowski, M.P., W.J. Larson, J.J. Steuer, and W.H. Gingerich. 2004. Validation of two dilution models to predict chloramine-T concentrations in aquaculture facility effluent. Aquaculture Engineering 30:127-140.

VITA

Christopher F. Hartleb
Northern Aquaculture Demonstration Facility
Department of Biology
University of Wisconsin-Stevens Point
800 Reserve Street
Stevens Point, WI 54481

Phone: (715) 346-3228
Fax: (715) 346-3624
E-mail: chartleb@uwsp.edu

EDUCATION

B.S. Rensselaer Polytechnic Institute, 1990, Biology
M.S. University of New Hampshire, 1992, Zoology (Limnology)
Ph.D. University of Maine, Maine Cooperative Fish & Wildlife Research Unit, 1996, Fisheries Biology

POSITIONS

Professor of Fisheries Biology & Aquaculture (2006-present), Associate Professor of Fisheries Biology & Aquaculture (2002-2006), and Assistant Professor of Biology & Water Resources (1996-2002), Department of Biology and Co-Director, Northern Aquaculture Demonstration Facility (2006-present); University of Wisconsin-Stevens Point
Researcher Assistant (1992-1996), Maine Cooperative Fish & Wildlife Research Unit, University of Maine
Research Assistant (1990-1992), Lakes Fish Condition Program, University of New Hampshire
Research Assistant (1988-1990), Rensselaer Fresh Water Institute, Rensselaer Polytechnic Institute

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society, Fish Culture & Education Sections
Wisconsin Aquaculture Association
Wisconsin Aquaculture Industry Advisory Council
World Aquaculture Society / U.S. Aquaculture Society

SELECTED PUBLICATIONS

Koehler, R.A., B. Sloss and C.F. Hartleb. In preparation. Population distribution of North American yellow perch (*Perca flavescens*) analyzed with microsatellite loci.

Fischer, G.J., C.F. Hartleb, J.A. Held, K. Holmes, and J. Malison. 2009. Evaluation of brook trout in a coldwater recycle aquaculture system. Aquacultural Engineering 41:109-113.

Malison, J.A., and C.F. Hartleb (eds.). 2005. A manual of best management practices for aquaculture in Wisconsin and the Great Lakes Region. University of Wisconsin Sea Grant Institute, Madison.

Hartleb, C.F. 2004. Floating raceways to raise yellow perch at cranberry farms. Aquaculture Magazine Jan/Feb.

Hartleb. C.F. 2003. Food chain dynamics and diets of larval and post-larval yellow perch in culture ponds. *In* Barry, T.P. and J.A. Malison, eds., Proceedings of Percis III: The Third International Percid Fish Symposium, University of Wisconsin Sea Grant Institute, Madison.

Hartleb, C.F., and J.F. Haney. 1998. Use of a thermal and light refugium by *Daphnia* and its effects on foraging pumpkinseeds. Environmental Biology of Fishes 51: 339-349.

Hartleb, C.F., and J.R. Moring. 1995. An improved gastric lavage device for removing stomach contents from live fish. Journal of Fisheries Research 24: 261-266.