ASSESSMENT OF CARBON DIOXIDE (CO_2) AND INORGANIC NITROGEN COMPOUNDS TO ENHANCE WINTER KILL IN NATURAL REARING PONDS USED FOR FISH PRODUCTION IN THE NORTH CENTRAL REGION

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

Industry Advisory Council Liaison: Gregory Oswald, Ellendale, Minnesota

Funding request: $175,000

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

Objectives:

1. Conduct a literature review to summarize the toxic effects of carbon dioxide (CO_2) and inorganic nitrogen compounds (e.g. N_2, NO_2^-, NH_3, etc.) on fish with an emphasis on common carp *Cyprinus carpio*, black bullhead *Ameiurus melas* and walleye *Sander vitreum*.
2. Estimate the cost per acre of pond treatment using either CO_2 or inorganic nitrogen compounds to enhance winter kill conditions during late winter periods in the North Central Region (NCR).
3. Consult with EPA to determine the registration eligibility and requirements for the use of CO_2 or inorganic nitrogen compounds to enhance winter kill conditions.
4. Determine, through laboratory study, application rates required of CO_2 or inorganic nitrogen compounds to enhance winter kill conditions to remove unwanted fish from natural rearing ponds. Studies required for the registration of CO_2 or inorganic nitrogen compounds to enhance winter kill conditions will be conducted according to GLP regulations (40CFR160).
5. Evaluate, through laboratory pond experiments, the efficacy of laboratory-derived application rate data for CO_2 or inorganic nitrogen compounds to enhance winter kill conditions.
6. Collect late winter water chemistry condition data in representative NCR natural rearing ponds
7. Obtain an experimental use permit (EUP) from the EPA and appropriate state regulatory agencies to conduct experimental applications of CO_2 or inorganic nitrogen compounds, singularly or in combination, to enhance winter kill conditions in natural rearing ponds to remove populations of unwanted fish.
8. Compile data into final study reports suitable for submission to the EPA to support potential approval of CO_2 or inorganic nitrogen compounds to enhance winter kill conditions.
9. Summarize results into appropriate extension materials for dissemination to NCR aquaculturists.

Proposed Budgets:

Institution	Principal Investigator	Objec-tives	Year 1	Year 2	Total
U.S. Geological Survey UMESC	Mark P. Gaikowski	3, 7-9	$11,000	$11,000	$22,000
Ohio State University (OSU)	Konrad Dabrowski	1-9	$38,250	$38,250	$76,500
U.S. Geological Survey. Northern Rocky Mountain Science Center (NOROCK)	Jason Gross	1-9	$38,250	$38,250	$76,500
		TOTALS	**$87,500**	**$87,500**	**$175,000**

Non-funded Collaborators:

Facility	Collaborator
Minnesota Sea Grant College Program	Jeffrey L. Gunderson
Oswald Fisheries	Gregory Oswald
U.S. Geological Survey, Leetown Science Center	Barnaby J. Watten

TABLE OF CONTENTS

JUSTIFICATION

Natural winter kills effectively eliminate unwanted fish species from commercial rearing ponds, saving producers time and money. However, winter kill conditions occur sporadically and are difficult to predict. Eliminating unwanted species (e.g., bullhead, carp) by enhancing natural winter kill conditions can increase harvest and productivity of desired cultured species. Identification of methods that consistently and inexpensively enhance natural winter kill conditions is needed to enhance this natural process and ensure winter kill conditions are reliably achieved in natural rearing ponds.

RELATED CURRENT AND PREVIOUS WORK

Evidence and conditions of winterkill in fish ponds and lakes

Elimination of unwanted fish species in water temperatures of 2 to 4°C, characteristic for winter conditions in ice and snow covered ponds, represents a challenge as the tolerance for low oxygen concentrations is as low as 0.25 - 0.5 mg/L in fish species such as yellow perch, northern pike and bluegill (Petrosky and Magnuson 1973). A decrease in dissolved oxygen level during winter when photosynthesis is minimal and no water mixing takes place is considered a major cause of fish mortality (Scidmore 1957; Johnson 1965). Because fish differ in their tolerance of low oxygen level (hypoxia) and respiratory and decomposition products (carbon dioxide, ammonia, hydrogen sulfide), all those factors need to be considered to better understand and predict winter mortality. Cooper and Washburn (1949) noticed severe winter mortality occurring only in lakes in which hypoxia reached 0.6 mg O_2/L under ice and snow conditions for several months. Bluegill and largemouth bass suffered great mortality whereas northern pike and bullheads survived oxygen depletion down to 0.2-0.3 mg/L. Johnson (1965) examined fertile ponds in Ontario devoted to largemouth bass production that were 0.04-1.0 ha in size and 1.5-4 m in depth. Ponds with total winterkill had 0.4-2.6 mg O_2/L at the surface and 0.2-1.1 mg O_2/L at the bottom. This work is one of very few that examined ammonia concentrations; NH_3-N increased from 0.1 in December to 0.76 mg/L by the end of February. The authors, however, concluded that ammonia was present at high levels in ponds that had winterkill and those in which fish survived. Scidmore (1957) concluded that “ammonia was an accessory factor to winterkills”, particularly when oxygen level reached 0.2 mg/L; ammonia higher than 2.5 mg/L was considered detrimental or lethal to fish.

In larger lakes Mathias and Barica (1980) recorded a strong negative correlation between oxygen depletion (extent of hypoxia) and the mean depth of the ice-covered lakes. Oxygen depletion rate during winter increased 10 fold in lakes where mean depth decreased from 10 to 1 m. Importantly, Cong et al. (2009) demonstrated that ammonia release rate from the sediments (the major oxygen consumer) increased linearly 2.5 fold as oxygen concentration dropped from 4 to 0-1 mg/L. The same authors also showed that ammonia-N increased from surface (0.05) to the bottom (0.18 mg/L) as oxygen level decreased from 10 to 1 mg/L in a 14-m deep lake in winter. Earlier, Lathrop (1992a) indicated that hypolimnetic NH_4-N at 20 m depth in September in Lake Mendota (Wisconsin) between 1970 and 1990 varied between 1.2 and 2.2 mg/L. These concentrations were associated with essentially hypoxic conditions between July and October below 16 m depth (below 2 mg O_2/L) at water temperatures of 12-14°C. However, Lathrop (1992b) concluded that although hypolimnetic hypoxia (below 1 mg O_2/L) has been recorded in Lake Mendota since 1900, ammonia and hydrogen sulfite (3 mg/L) levels increased dramatically only in the 1970s. No connection was made between increased toxicity of ammonia in hypoxic water to fish.

In summary, there is no well-presented case of environmental hypoxia and ammonia toxicity in fish although the frequency of co-occurrence of these events in aquatic systems is striking.

Ammonia toxicity and hypoxia tolerance in individual fish species

Channel catfish – extensive studies on ammonia toxicity to catfish were conducted (Tomasso et al 1980; Arthur, et al. 1987, Sheehan and Lewis, 1986), however, among factors that were considered accessory to its toxicity were temperature, pH, K^+, Ca^{++}, Na^+ concentrations, and ammonium compounds. Oxygen level was not among them. Contrary to the assertion of Arthur et al. (1987), the 96-h LC_{50} determined for catfish following acute exposure to ammonia decreased from 1.29 to 0.98 and 0.5 mg NH_3/L as water temperature decreased from 19.6 to 14.6 and 3.5°C, respectively. According to Sheehan and Lewis (1986), the 24-h

LC_{50} at 2.1°C decreased from 2.1 to 1.6 and 1.1 to 0.78 mg/L when the pH decreased gradually from 8.8 to 8.0 and 7.2 to 6.0.

Watenpaugh and Beitinger (1986) demonstrated that sublethal exposure of catfish to nitrite that results in severe methemoglobinemia (43 – 78% of hemoglobin rendered nonfunctional) reduces their ability to survive subsequent hypoxia of 0.65 mg/L at 30°C. The survival of fish exposed to sublethal NO_2 (or NH_3) in aquatic systems could occur simultaneously with, before, or after oxygen depletion but is likely to induce increased mortality.

Survival of catfish embryos and larvae was reduced by half at 50% oxygen saturation (survival of 25 - 28%) with nearly complete mortality at 30% oxygen saturation (Carlson et al. 1974). Scott and Rogers (1981) evaluated the response of winter-acclimated catfish (10 - 15°C) to hypoxia (sublethal exposure of 1.5 mg O_2/L for 14 to 72 h) and noticed gradual increases in hemoglobin concentration from 7.74 to 10.42 g/L accompanied by lactic acid increases from 9.9 to 64.2 mg/L, indicative of highly stressful anaerobic conditions. Gills were the most affected organ by hypoxic conditions in catfish with hemorrhagic and edematous gill lamellae and epithelial cell hypertrophy and hyperplasia (Scott and Rogers 1980).

Rainbow trout and salmonids – the effect of hypoxia on toxicity of ammonia to rainbow trout was recognized by Lloyd (1961), although mistakenly increases in severity were attributed to decreases in CO_2 concentration rather than an increase in pH and the concomitant increase in the proportion of un-ionized ammonia. A decrease in oxygen saturation to 37% increased ammonia toxicity by factor of 2.5 in comparison to normoxic conditions. Thurston et al. (1981) evaluated the effect of dissolved oxygen (2.6-8.6 mg/L) on ammonia toxicity and confirmed early reports of the association between ammonia toxicity and hypoxia. They identified a linear relationship between the ammonia LC_{50} (0.32-0.81mg NH_3 /L) and oxygen saturation. However, these studies were carried out at 17.5 and 12.5°C; the relationship between ammonia toxicity and hypoxia at temperatures relevant to winterkill conditions has not been determined.

Alabaster et al. (1979a) reported 72-h LC_{50}s for Atlantic salmon smolts depend on salinity and acclimation period but in general varied between 2.5-3 mg O_2/L at 13-15.2°C. When evaluating the combined effect of hypoxia (3.5 mg O_2/L) and ammonia toxicity, 24-h LC_{50} values were 0.15 mg NH_3/L when tested under normoxia and 0.09 mg NH_3/L in hypoxia (Alabaster et al. 1979b). Knoph (1992) examined ammonia toxicity in Atlantic salmon at temperatures ranging from 2 to 17.1°C and pH 6-6.4, conditions relevant to winterkill. Knoph found that the 96-h LC_{50} for un-ionized ammonia increased with temperature from 0.03 to 0.111 mg NH_3-N/L. Magaud et al. (1997) confirmed this when they examined resistance to ammonia toxicity up to 0.5 mg/L in rainbow trout exposed to hypoxia (1.7-2.9 mg/L dissolved oxygen). They concluded that survival of trout is always lower than predicted when exposed to ammonia and hypoxia simultaneously based on the simple addition of individual effects. Linton et al. (1998) elaborated further on the conditions of ammonia exposure and concluded that pre-exposure to sublethal ammonia (0.2 mg NH_3/L) does not prolong survival of rainbow trout when exposed to lethal ammonia concentrations.

Common carp and cyprinids – critical oxygen level (measured as a drop in metabolic rate) in early life history of small cyprinid fish, zebrafish, decreased linearly from 75% saturation at the embryonic stage to 15% saturation in 100-d old fish (Barrionuevo et al. 2010). Common carp are known for making multiple metabolic adjustments in severe hypoxic conditions (0.5 mg/L) followed by a fast return to normal metabolic characteristics upon return to normoxic conditions (Zhou et al. 2000). The major adjustment of carp in severe hypoxic conditions (20% saturation) was >10-fold increase in gill ventilation volume (Itazawa and Takeda 1978). Carp exposed to hypoxic conditions (0.5 mg O_2/L) for 42 days at 20°C did not exhibit decreased liver cell proliferation or increase in apoptosis, indicating that after an initial adjustment (1 week) period the liver cells adapted to hypoxic conditions and established a new homeostatic level (Poon et al. 2007).

Ammonia toxicity in juvenile common carp was examined at 28°C with 168-h LC_{50}s of 1.6-1.78 mg NH_3-N/L reported (Hasan and MacIntosh, 1986). The authors concluded that acute toxicity of ammonia was not altered after 48 h, that fish that survive the initial 48-h of exposure will survive the balance of the exposure. To the contrary, crucian carp exposed to ammonia at 25°C and at an oxygen concentration of 5 mg/L had a noticeable decrease in LC_{50} from 0.7 to 0.58 and 0.52 mg NH_3-N/L at 24, 48 and 96 h, respectively (Yang et al. 2010). In another cyprinid, golden shiner, the toxicity of ammonia increased with increasing pH with LC_{50} values of 1.26, 0.75 and 0.71 mg/L at pH 7, 8, and 9, respectively; addition of calcium chloride decreased ammonia toxicity by 21% (Sink 2010).

Carbon dioxide

There have been many studies to address the physiological effects of CO_2 on a variety of freshwater and marine fishes; however, fewer than a dozen of the 40,000+ species of fish have been exposed to CO_2 levels with an attempt to define lethal concentrations (e.g., Ishimatsu et al. 2004). A considerable amount of available data is focused on adults, while little data exists on the effects of CO_2 on earlier life history stages of either freshwater or saltwater species (Ishimatsu and Kita, 1999). Studies investigating freshwater acidification by gasses demonstrate increased sensitivity to acute acid stress in early life history stages (Morris et al. 1989; Sayer et al. 1993; Heath 1995).

Once introduced to a biological system, CO_2 dissociates into bicarbonate (HCO_3^-) and hydrogen (H^+) ions as pH is reduced. From studies on freshwater fish it is known that carbon dioxide may be more toxic at lower pH levels, especially below pH 6.0 (Alabaster and Lloyd 1982). Therefore, the total CO_2 content of the water needed to achieve a certain partial pressure is directly affected by the pH of that solution (Baker and Brauner 2009). When a fish is exposed, body fluid pH rapidly decreases due to the increased partial pressure of CO_2 (pCO_2) in body fluid, but is subsequently compensated for by the accumulation of bicarbonate ions in body fluids, provided pCO_2 remains at sub-lethal levels. Within the fish, pCO_2 quickly equilibrates between plasma and blood cells within the animal, inducing a reduction in blood pH (acidosis) (Baker et al. In revision). This acidosis results in the limited ability of the hemoglobin in the blood to carry oxygen. Other short term effects include disturbance of respiration, blood circulation and nervous activities of exposed animals, while long term effects include reduced growth rate and reproduction, and nephrocalcinosis or calcareous precipitates in the lumen of kidney tubules and ducts (Smart et al. 1979; Ishimatsu and Kita 1999; Fivelstad et al. 1999, 2003).

In a study conducted by Ishimatsu et al. (2004), larvae from silver sea bream and Japanese whiting had similar lethal tolerance when exposed to carbon dioxide. Median lethal pCO_2 peaked in the pre-flexion stage or one day after, i.e. the flexion stage, with CO_2 sensitivity much higher in the preceding and following stages. The gradual fall in CO_2 tolerance from the larval to juvenile stage may result from the development of gill lamellae in the pre-flexion stage of cell development (Oozeki et al. 1992; Oikawa et al. 1999), which dramatically increases the surface area available for diffusion.

Studies evaluating the toxicity of CO_2 were conducted by the USGS Northern Rocky Mountain Science Center on embryonic and larval rainbow trout to establish a dose response and determine critical periods of susceptibility in early life history stages of rainbow trout. Immediate post hatch larvae, or embryonic egg stage, age 648 to 729 temperature units (TU, 59°F), were exposed to 160.38, 200.23, and 240.41 grams of CO_2 (solid) which had no effect on survival. However, the critical period of susceptibility, 100% mortality, was identified at 783 TU; approximately 5 week old sac fry larvae that are associated with tail movement and increased swimming activity. It was found that this sensitivity to CO_2 was not altered in larvae through 1,134 TU's and that there was no significant difference in susceptibility to CO_2 (100% mortality in all treatments above 150 mg/L CO_2). These results were also consistent (100% mortality) with 3-month old rainbow trout fry exposed to CO_2 concentrations above 150 mg/L (Barrionuevo et al. 2009).

While rainbow trout are fairly sensitive to CO_2 and exhibit mortality at pCO_2 as low as 30 mmHg (~4% CO_2 or 4 kPa), exposure to lower levels for extended periods results in other measurable physiological changes (e.g., Brauner et al., 2000; Baker et al. 2009). One study analyzed the effects of chronic exposure (7-mm Hg pCO_2) in adult rainbow trout and found fish had lower muscle potassium concentrations than controls and that those with observable nephrocalcinosis had elevated urine acidity and bicarbonate (Eddy et al. 1979). Baker and Brauner (2009) also found that pumpkinseed, smallmouth bass, and yellow perch all exhibited 100% morbidity within 12 h at all CO_2 concentrations tested above 80 mmHg.

ANTICIPATED BENEFITS

The proposed studies include determining thresholds of the combined effects of CO_2/inorganic nitrogen compounds and oxygen concentrations for three fish species which will generate, for the first time, results relevant to winter conditions. There are no direct data in the literature on this subject. These data will also be extremely useful to predict constraints on fish survival related to winterkill conditions in productive ponds and lakes and may be used in simulation of thermal/dissolved oxygen/ammonia conditions in habitat for fishes under different climate scenarios including severity and duration of winter.

Diffused gases and inorganic nitrogen compounds offer significant piscicidal alternatives for aquaculture. Commonly applied chemical treatments for fish control include antimycin and rotenone, compounds that have traditionally been used but which are receiving greater public scrutiny and which may leave undesired residues in pond sediments, especially when applied in cold water. Most gases are readily available commercially, are inexpensive, have short half-lives, and off-gas from water leaves little residual environmental impact. Carbon dioxide gas, for example, is Generally Regarded as Safe (GRAS) by the Food and Drug Administration (FDA) and is currently used as a humane method of euthanasia in laboratory animals in research, as well as in the aquaculture industry with fish (Pirhonen and Schreck 2003). Many gases have greater binding affinity for hemoglobin than oxygen, providing rapid biological uptake with little bioaccumulation. Determination of appropriate application rates and times to enhance natural winter kill conditions in natural rearing ponds has the potential to substantially reduce fish production costs and reduce dependence on other chemical toxicants like rotenone or antimycin.

OBJECTIVES

1. Conduct a literature review to summarize the toxic effects of carbon dioxide (CO_2) and inorganic nitrogen compounds (e.g. N_2, NO_2^-, NH_3, etc.) on fish with an emphasis on common carp *Cyprinus carpio*, black bullhead *Ameiurus melas* and walleye *Sander vitreum*.
2. Estimate the cost per acre of pond treatment using either CO_2 or inorganic nitrogen compounds to enhance winter kill conditions during late winter periods in the North Central Region (NCR).
3. Consult with EPA to determine the registration eligibility and requirements for the use of CO_2 or inorganic nitrogen compounds to enhance winter kill conditions.
4. Determine, through laboratory study, application rates required of CO_2 or inorganic nitrogen compounds to enhance winter kill conditions to remove unwanted fish from natural rearing ponds. Studies required for the registration of CO_2 or inorganic nitrogen compounds to enhance winter kill conditions will be conducted according to GLP regulations (40CFR160).
5. Evaluate, through laboratory pond experiments, the efficacy of laboratory-derived application rate data for CO_2 or inorganic nitrogen compounds to enhance winter kill conditions.
6. Collect late winter water chemistry condition data in representative NCR natural rearing ponds
7. Obtain an experimental use permit (EUP) from the EPA and appropriate state regulatory agencies to conduct experimental applications of CO_2 or inorganic nitrogen compounds, singularly or in combination, to enhance winter kill conditions in natural rearing ponds to remove populations of unwanted fish.
8. Compile data into final study reports suitable for submission to the EPA to support potential approval of CO_2 or inorganic nitrogen compounds to enhance winter kill conditions.
9. Summarize results into appropriate extension materials for dissemination to NCR aquaculturists.

PROCEDURES

Conduct a Literature Review (Objective 1)

OSU and NOROCK will collaboratively complete a comprehensive literature review of the effects of CO_2 and inorganic compounds on fish. The outcome of the literature review will be combined with results from this study into a white paper synopsis of the effects of these compounds on target fish species and potential minimum toxic concentrations for use in future research. This white paper will be reported to the North Central Regional Aquaculture Center (NCRAC) and prepared for publication in a peer-reviewed journal.

Estimate the Cost Per Acre of Pond Treatment (Objective 2)

OSU and NOROCK will collaboratively develop per acre cost estimates for applications of CO_2 or inorganic nitrogen compounds to enhance winter kill conditions. Cost estimates will include, at a minimum: personnel time to apply the compounds, potential regulatory costs, product costs, and transportation costs. The cost estimates will focus on application of proposed components to enhance winter kill, not for removal of unwanted fish at other times of the year.

Consult with EPA to Determine the Registration Eligibility and Requirements (Objective 3)

UMESC is in regular communication with the EPA Office of Pesticide Programs (OPP) to support the registrations of the chemicals used to control the invasive sea lamprey (*Petromyzon marinus)* in tributaries of the Great Lakes. UMESC will request a pre-submission conference with the EPA OPP to determine the registration eligibility of CO_2 or inorganic nitrogen compounds to enhance winter kill conditions. This meeting will determine the additional data requirements to support a potential registration of one or more of these compounds and will help guide future toxicity studies.

Determine, Through Laboratory Study, Application Rates Required (Objective 4)

Based on the data gaps identified during the comprehensive literature review, laboratory toxicity studies will be conducted using rainbow trout (*Oncorhynchus mykiss)*, a model species used in toxicology research (data for rainbow trout are generally required to support regulatory agency evaluation), as well as common carp (*Cyprinus carpio)* and channel catfish (*Ictalurus punctatus)* to assess the concentration x exposure time required for dissolved CO_2 or inorganic nitrogen compounds to induce mortality. Standardized laboratory studies will be conducted at water temperatures, dissolved oxygen concentrations (to be determined), and pH (7.5-9) representative of water chemistries during late winter (e.g., near winter kill) with a minimum of 10 juvenile fish (5-50 g) per replicate and a minimum of three replicates per chemical concentration. The experimental treatments will follow the line of testing single species with a single compound in normoxic (control) and hypoxic conditions. The effect of pH will be evaluated separately using a similar experimental process. Acclimation to hypoxic conditions will be completed as appropriate for the species being tested (e.g., rainbow trout are more sensitive to hypoxia than carp and will thus require longer acclimation), further, the hypoxic conditions selected for evaluation will be based on species sensitivity (e.g., thresholds for rainbow trout, common carp, and channel catfish will be 1.5, 0.5, and 0.25 mg O_2/L, respectively). Concentration (i.e., CO_2 or inorganic nitrogen compounds) x exposure time x water chemistry predictive charts will be developed to identify those conditions which would cause >90% mortality. Toxicity experiments will be conducted according standardized methods and data collection will be in accordance with Good Laboratory Practices (GLP) regulations. Studies conducted by NOROCK will be conducted using the GLP procedures presently in place at the Aquatic Animal Drug Approval Partnership program (U.S. Fish and Wildlife Bozeman Fish Technology Center [BFTC], Bozeman, Montana). Studies conducted by OSU will be conducted under the auspices of the OSU Animal Care and Use Committee and UMESC's GLP program. Study protocols prepared by both NOROCK and OSU will be reviewed by UMESC for compliance with EPA GLP requirements. Studies conducted by NOROCK will focus on the assessment of the toxicity of dissolved CO_2 whereas studies conducted by OSU will focus on the assessment of inorganic nitrogenous compounds.

Evaluate, Through Laboratory Pond Experiments, the Efficacy of Laboratory-derived Application Rate Data (Objective 5)

NOROCK, OSU, and UMESC will collaborate to assess the efficacy of effective compounds at appropriate application rates through the conduct of experimental pond-scale exposures in the experimental pond systems located at UMESC. Six to nine UMESC earthen ponds (0.1 acre) will be filled and stocked with appropriately-sized channel catfish and walleye in late fall; the number of fish stocked per pond will be determined according to fish size at placement into the pond. Fish may or may not be placed in cages.

Appropriate forage will also be provided. Ponds will be allowed to freeze over – pond water levels will be maintained as needed by addition of UMESC well water. Pond water chemistry (dissolved oxygen, temperature, pH, and alkalinity) will be monitored monthly after preparation then weekly or daily as needed during application of CO_2 or inorganic nitrogen compounds. Chemical applications will be timed to coincide with low dissolved oxygen levels. Depending on laboratory trial results, one or two active chemical application groups will be selected along with one control group. Each group will have a minimum of three ponds randomly assigned to the treatment group. A method to apply the chemicals under ice that ensures uniform distribution will be identified prior to use. The number of surviving fish will be compared between the active and control treatment groups using appropriate survival analysis techniques.

Collect Late Winter Water Chemistry Condition Data in Representative NCR Natural Rearing Ponds (Objective 6)

OSU will collaborate with NCR industry personnel to collect water chemistry (temperature, pH, dissolved oxygen, alkalinity, hardness, dissolved carbon dioxide, ammonia, and nitrate levels) during late winter in multiple natural rearing ponds located throughout the NCR. Natural rearing pond locations will be selected based on access and ability of NCR partners to collect these data.

Obtain an Experimental Use Permit (EUP) from the EPA and Appropriate State Regulatory Agencies (Objective 7)

Based on the laboratory and experimental toxicity data and the late winter water chemistry information collected, one to two active chemical treatments will be selected for field evaluation under EPA EUP in NCR natural rearing ponds determined by OSU using data obtained above (See Objective 6). UMESC, in collaboration with NOROCK and OSU, will submit the appropriate regulatory documents to request EUP approval from EPA. Once EUP approval is obtained, NOROCK and OSU, with GLP oversight provided by UMESC, in collaboration with NCR natural rearing pond owners, will conduct late winter field applications of the selected active treatment. Application will be limited to no more than two natural rearing pond locations. Natural rearing pond selection will be based on: availability of pond owner personnel to support chemical application and assist in assessing fish population present before and after application; pond owner funding to purchase the appropriate chemical; limited or no water outflow from the rearing pond to public surface waters; and late winter access to the rearing pond.

Compile Data into Final Study Reports (Objective 8)

NOROCK and OSU will prepare comprehensive final study reports, with GLP oversight provided by UMESC, for all laboratory and field studies completed. UMESC will archive these reports in its public archives then submit the reports to EPA for review to support a potential registration of one or more potential active chemical applications (CO_2 or inorganic nitrogen compounds).

Summarize Results into Appropriate Extension Materials (Objective 9)

NOROCK and OSU, in collaboration with Minnesota Sea Grant, will present study results at scientific workshops and extension meetings and publish results through journals, bulletins, or fact sheets. Application methods and rates will be summarized and presented.

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. 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. EPA and the U.S. 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 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 successfully completing several drug toxicity studies as well as completing three 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) equipped with technology to rear test animals and to conduct laboratory and field assessments.

UMESC has an extensive indoor fish culture facility that includes the ability to reconstitute water and provide rearing conditions nearly identical to those expected in ponds found throughout the NCR. Additionally, UMESC has a wide variety of outdoor rearing ponds that includes: 18 10-acre ponds, one 0.25-acre pond, and two 0.5-acre ponds.

NOROCK

The role of the Invasive Species Control and Eradication Center at the UBFTC and NOROCK is to develop innovative conservation technologies for the control and suppression of aquatic invasive species. Both facilities have a long history of conducting research on aquatic invasive species. Additional expertise also exists with the Aquatic Animal Drug Approval Program (AADAP) at the BFTC as AADAP has submitted numerous reports collecting data and summarizing research to the EPA and the FDA leading to the registration, evaluation, and assessment of drugs for aquaculture. State-of-the- art facilities exist at the BFTC to conduct research and provide assistance in the fields of traditional fish culture and water treatment systems, fish nutrition and diet development, reproductive physiology and ecology, sensitive aquatic species conservation, and aquatic nuisance species and ecosystem health.

OSU

Ohio State University is a world-renowned institution with a proven research history. The assigned investigator has led numerous studies focused on understanding fish nutritional physiology and biochemistry with an emphasis on enhancing aquaculture. His body of work includes many pivotal studies spanning a diverse array of important fish physiological questions. OSU's state-of-the art research facility includes three research laboratories (analytical, histology/histochemistry, and radioactive) and two experimental fish facilities. The experimental fish facilities are equipped with technology to support fish rearing and exposures of fish to compounds during hypoxic, normoxic, and hyperoxic conditions.

REFERENCES

Alabaster, J.S., and R. Lloyd. 1982. Water Quality Criteria for Freshwater Fish. Food and Agriculture Organization of the United Nations. Butterworth, London.

Alabaster, J.S., D.G. Shurben, and G. Knowles. 1979a. The effect of dissolved oxygen and salinity on the toxicity of ammonia to smolts of salmon, *Salmo salar* L. Journal of Fish Biology 15: 705-712.

Alabaster, J.S., D.G. Shurben, and M.J. Mallett. 1979b. The survival of smolts of salmon *Salmo salar* L. at low concentrations of dissolved oxygen. Journal of Fish Biology 15: 1-8.

Arthur, J. W., C.W. West, K.N. Allen, and S.F. Hedtke. 1987. Seasonal toxicity of ammonia to five fish and nine invertebrate species. Bulletin of Environmental Contamination and Toxicology 38:324-331.

Baker, D.W., and C.J. Brauner. 2009. Assessing the feasibility of using CO_2 in controlling invasive fish species in BC; Final Report. Effects of CO_2 on invasive fish species, and characterization of Miller Lake water at different CO_2 levels. Department of Zoology, University of British Columbia, Vancouver BC, Canada.

Baker, D. W., T. May, and C.J. Brauner. In revision. A validation of intracellular pH measurements in fish exposed to hypercarbia: The effect of duration of tissue storage and efficacy of the metabolic inhibitor tissue homogenate method. Journal of Fish Biology.

Baldes, J., J.A. Gross, M.W. Webb, B. Gresswell. 2009. Effects of Carbon Dioxide on Rainbow Trout (*Oncorhynchus mykiss*) Larvae: Applications for Invasive Fish Eradication. Poster. SETAC, New Orleans, Louisiana.

Barrionuevo, W.R., M.N. Fernandes, and O. Rocha. 2010. Aerobic and anaerobic metabolism for the zebrafish, Danio rerio, reared under normoxic and hypoxic conditions and exposed to acute hypoxia during development. Brazilian Journal of Biology 70:425-434.

Brauner, C.J., H. Rhorarensen, P. Gallaugher, A.P. Farrell, and D.J. Randall. 2000. CO_2 transport and excretion in rainbow trout (*Oncorhynchus mykiss*) during graded sustained exercise. Respiration Physiology 119:69-82.

Carlson, A.R., R.E. Siefert, and L.J. Herman. 1974. Effects of lowered dissolved oxygen concentrations on channel catfish (*Ictalurus punctatus*) embryos and larvae. Transactions of the American Fisheries Society 103:623-626.

Cong, H.B., T-L.Huang, B.B. Chai, and J-W Zhao. 2009. A new mixing-oxygenating technology for water quality improvement of urban water source and its implication in a reservoir. Renewable Energy 34:2054-2060.

Cooper, G.P. and G.N. Washburn. 1949. Relation of dissolved oxygen to winter mortality of fish in Michigan lakes. Transactions of the American Fisheries Society 76:23-33.

Eddy, F.B., G.R. Smart, and R.N. Bath. 1979. Ionic content of muscle and urine in rainbow trout *Salmo gairdneri* kept in water of high CO_2 content. Journal of Fish Diseases 2:105-110.

Fivelstad, S., A.B. Olsen, H. Kloften, H. Ski, and S. Stefansson. 1999 Effects of carbon dioxide on Atlantic salmon (*Salmo salar* l.) smolts at constant pH in bicarbonate rich freshwater. Aquaculture 178:171-187.

Fivelstad, S., A.B. Olsen, T. Asgard, G. Baevoerfjord, T. Rasmussen, T. Vindheim, and S. Stefansson. 2003. Long-term sublethal effects of carbon dioxide on Atlantic salmon smolts (*Salmon salar* L.); ion regulation, haematology, element composition, nephrocalcinosis and growth parameters. Aquaculture 215:301-319.

Hasan, M.R., and D.J. Macintosh. 1986. Acute toxicity of ammonia to common carp fry. Aquaculture 54:97-107.

Heath, A. 1995. Water pollution and fish physiology, 2nd edition. CRC Press, Boca Raton, Florida.

Ishimatsu A. and J. Kita. 1999. Effects of environmental hypercapnia on fish. Japanese Journal of Ichthyology 46:1-13.

Ishimatsu, A., T. Kikkawa, M. Hayashi, K.S. Lee, and J. Kita. 2004. Effects of CO_2 on marine fish: larvae and adults. Journal of Oceanography 60:731-741.

Itazawa, Y., and T. Takeda. 1978. Gas exchange in the carp gills in normoxic and hypoxic conditions. Respiratory Physiology 35:263-269.

Johnson, M.G., 1965. Limnology of Ontario ponds in relations to winterkill of largemouth bass. Progressive Fish Culturist 27:193-198.

Knoph, M.B. 1992. Acute toxicity of ammonia to Atlantic salmon. (*Salmo salar*) parr. Comparative Biochemistry and Physiology 101C:275-282.

Lathrop RC. 1992a. Nutrient loadings, lake nutrients, and water clarity. Pages 71–98 *in* J.F. Kitchell, editor. Food Web Management: A Case Study of Lake Mendota. Springer. New York.

Lathrop, R. C. 1992b. Decline in zoobenthos densities in the profundal sediments of Lake Mendota (Wisconsin, USA). Hydrobiologia 235/236:353-361.

Linton, T., I.J. Morgan, S.D. Reid, and C.M. Wood. 1998. Long-term exposure to small temperature increase and sublethal ammonia in hardwater acclimated rainbow trout: does acclimation occur? Aquatic Toxicology 40:171-191.

Lloyd, R. 1961. Effect of dissolved oxygen concentrations on the toxicity of several poisons to rainbow trout (*Salmo gairdneri* Richardson). Journal of Experimental Biology 38:447-455.

Magaud, H., B. Migeon, P. Morfin, Garric, and E. Vindimian. 1997. Modelling fish mortality due to urban storm run-off: Interaction effects of hypoxia and un-ionized ammonia. Water Research 31:211-218.

Mathias, J.A., and J. Barica. 1980. Factors controlling oxygen depletion in ice-covered lakes. Canadian Journal of Fisheries and Aquatic Sciences 37:185-193.

Morris R., E.W. Taylor, K.J.A. Brown, and J.A. Brown. 1989. Acid toxicity and aquatic animals. Cambridge University Press, Cambridge.

Oikawa S., M. Hirata, J. Kita, and Y. Itazawa. 1999. Ontogeny of respiratory area of a marine teleost, porgy, *Pagrus major*. Ichthyology Research 46:233-244.

Oozeki Y., P-P. Hwang, and R. Hirano. 1992. Larval development of the Japanese whiting, *Sillago japonica*. Japanese Journal of Ichthyology 39:59-669.

Petrosky, B.R., and J.J. Magnuson. 1973. Behavioral responses of northern pike, yellow perch and bluegill to oxygen concentrations under simulated winterkill conditions. Copeia 1973:124-133.

Pirhonen, J., and C.B. Schreck. 2003. Effects of anesthesia with MS-222, clove oil and CO_2 on feed intake and plasma cortisol in steelhead trout (*Oncorhynchus mykiss*). Aquaculture 220:507-514.

Poon, W.L., C.Y. Hung, K. Nakano, and D.J. Randall. 2007. An in vivo study of common carp (Cyprinus carpio L.) liver during prolonged hypoxia. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics 2:295-302.

Sayer, M.D.J., J.P. Reader, and T.R.L. Dalziel. 1993 Freshwater acidification: effects of the early life sages of fish. Reviews in Fish Biology 3:95-132.

Scidmore, W.J. 1957. An investigation of carbon dioxide, ammonia, and hydrogen sulfide as factors contributing to fish kills in ice-covered lakes. Progressive Fish Culturist 19:124-127.

Scott, A. L. and W.A. Rogers.1980. Histological effects of prolonged sublethal hypoxia on channel catfish *Ictalurus punctatus* (Rafinesque). Journal of Fish Diseases 3:305-316.

Scott, A.L., and W.A. Rogers. 1981. Haematological effects of prolonged sublethal hypoxia on channel catfish, *Ictalurus punctatus* (Rafinesque). Journal of Fisheries Biology 18:591-601.

Sheehan, R.J., and W.M. Lewis. 1986. Influence of pH and ammonia salts on ammonia toxicity and water balance in young channel catfish. Transactions of the American Fisheries Society 115:891-899.

Sink, T.D. 2010. Influence of pH, salinity, calcium, and ammonia source on acute ammonia toxicity to golden shiners, *Notemigonus crysoleucas*. Journal of the World Aquaculture Society 41:411-419.

Smart, G., D. Knox, J. Harrison, J. Ralph, R. Richards, and C. Cowey. 1979. Nephrocalcinosis in rainbow trout *Salmo gairdneri* Richardson; the effect of exposure to elevated CO_2 concentration. Journal of Fish Disease 2:278-289.

Thurston, R.V., G.R. Phillips, and R.C. Russo. 1981. Increased toxicity of ammonia to rainbow trout (*Salmo gairdneri*) resulting from reduced concentrations of dissolved oxygen. Canadian Journal of Fisheries and Aquatic Sciences 38:983-988.

Tomasso, J.R., C.A. Goudie, B.A. Simco, and K.B. Davis. 1980. Effects of environmental pH and calcium on ammonia toxicity in channel catfish. Transactions of the American Fisheries Society 109:229-234.

Watenpaugh, D.E., and T.L. Beitinger. 1986. Resistance of nitrite-exposed channel catfish, *Ictalurus punctatus*, to hypoxia. Bulletin of Environmental Contamination and Toxicology 37:802-807.

Yang, W., F. Xiang, L. Liang, and Z. Yang. 2010. Toxicity of ammonia and its effects on oxidative stress mechanisms of juvenile crucian carp (Carassius auratus). Journal of Freshwater Ecology 25:297-302.

Zhou, B.S., R.S.S. Wu, D.J. Randall, P.K.S. Lam, Y.K. Ip, and S.F. Chew. 2000. Metabolic adjustments in the common carp during prolonged hypoxia. Journal of Fish Biology 57:1160-1171.

PROJECT LEADERS

State	Name/Institution	Area of Specialization
Ohio	Konrad Dabrowski Ohio State University	Aquaculture/Aquatic Toxicology
Montana	Jason “Jackson” Gross US Geological Survey, NOROCK	Environmental Toxicology
West Virginia	Barnaby J. Watten US Geological Survey, LSC	Gas Transfer in Aquatic Systems/Instrumentation Design and Engineering/Aquaculture Engineering
Wisconsin	Mark P. Gaikowski US Geological Survey, UMESC	Aquaculture/Drug Approval

PARTICIPATING INSTITUTIONS AND CO-PRINCIPAL INVESTIGATORS

Ohio State University
Konrad Dabrowski

U.S. Geological Survey, Leetwon Science Center
Barnaby J. Watten

U.S. Geological Survey, Northern Rocky Mountain Science Center
Jackson Gross

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 U.S. 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 3, 7-9 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. ___ (Co)-PD(s)							
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty) a. _1_ Research Associates-Postdoctorates . . .	2.0			$7,000			
b. ___ Other Professionals							
c. ___ Paraprofessionals.....................................							
d. __ Graduate Students							
e. __ Prebaccalaureate Students........................							
f. ___ Secretarial-Clerical....................................							
g. ___ Technical, Shop and Other							
Total Salaries and Wages......................→				$7,000			
B. Fringe Benefits (If charged as Direct Costs)				$3,000			
C.Total Salaries, Wages, and Fringe Benefits (A plus B) →				$10,000			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies							
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) →				$11,000			
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 →				$11,000			

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)

OMB Approved 0524-0039
Expires 03/31/2004

BUDGET

ORGANIZATION AND ADDRESS	USDA AWARD NO. Year 2: Objectives 3, 7-9			
U.S. Geological Survey Upper Midwest Environmental Sciences Center 2630 Fanta Reed Road, La Crosse, WI 54603 PROJECT DIRECTOR(S) Mark P. Gaikowski	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)

Item	CSREES FUNDED WORK MONTHS: Calendar	Academic	Summer	Funds Requested by Proposer	Funds Approved by CSREES	Non-Federal Proposed	Non-Federal Approved
A. Salaries and Wages 1. No. of Senior Personnel							
a. ___ (Co)-PD(s)							
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty) a. 1 Research Associates-Postdoctorates . . .	2.0			$7,000			
b. ___ Other Professionals							
c. ___ Paraprofessionals.....................................							
d. __ Graduate Students							
e. __ Prebaccalaureate Students........................							
f. ___ Secretarial-Clerical....................................							
g. ___ Technical, Shop and Other							
Total Salaries and Wages......................→				$7,000			
B. Fringe Benefits (If charged as Direct Costs)				$3,000			
C.Total Salaries, Wages, and Fringe Benefits (A plus B) →				$10,000			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies							
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) →				$11,000			
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 →				$11,000			

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 AND BUDGET EXPLANATION FOR EACH PARTICIPATING INSTITUTION

(Gaikowski)

Objectives 3, 7-9

A. **Salaries and Wages.** Year 1: Salary ($7,000) is requested for one 17% FTE research associate to support EPA consultation, provide GLP oversight, and support pond studies. The research associate will be trained in Good Laboratory Practice regulations to ensure research is conducted in compliance with EPA requirements. Year 2: Salary ($7,000) is requested for one 17% FTE research associate to support EPA consultation, prepare EUP documentation, provide GLP oversight, and support experimental field applications. The research associate will be trained in Good Laboratory Practice regulations to ensure research is conducted in compliance with EPA requirements.

B. **Fringe Benefits.** Year 1: 42.85% of salary ($3,000); Year 2: 42.85% of salary ($3,000).

F. **Travel.** Year 1: $1,000 is requested for transportation, lodging, and meal expenses to conduct pre-submission conference with EPA. Year 2: $1,000 is requested for transportation, lodging, and meal expenses to support field application studies at locations to be determined.

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 "ASSESSMENT OF CARBON DIOXIDE (CO2) AND INORGANIC NITROGEN COMPOUNDS TO ENHANCE WINTER KILL IN NATURAL REARING PONDS USED FOR FISH PRODUCTION IN THE NORTHCENTRAL REGION"

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
U.S. Geological Survey
Northern Rocky Mountain Science Center
2327 University Way, Bozeman, MT 59715

PROJECT DIRECTOR(S)
Jackson Gross

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

	CSREES FUNDED WORK MONTHS: Calendar	Academic	Summer	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							
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...................................				$27,243			
e. ___ Prebaccalaureate Students......................							
f. ___ Secretarial-Clerical....................................							
g. ___ Technical, Shop and Other							
Total Salaries and Wages......................→				$27,243			
B. Fringe Benefits (If charged as Direct Costs)				$2,257			
C.Total Salaries, Wages, and Fringe Benefits (A plus B) →				$29,500			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$7,000			
F. Travel				$1,750			
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) →				$38,250			
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 →				$38,250			

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
U.S. Geological Survey
Northern Rocky Mountain Science Center
2327 University Way, Bozeman, MT 59715

PROJECT DIRECTOR(S)
Jackson Gross

USDA AWARD NO. **Year 2: Objectives 1-9**

	CSREES FUNDED WORK MONTHS: Calendar	Academic	Summer	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							
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...................................				$27,243			
e. ___ Prebaccalaureate Students......................							
f. ___ Secretarial-Clerical....................................							
g. ___ Technical, Shop and Other							
Total Salaries and Wages......................→				$27,243			
B. Fringe Benefits (If charged as Direct Costs)				$2,257			
C.Total Salaries, Wages, and Fringe Benefits (A plus B) →				$29,500			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$5,250			
F. Travel				$3,500			
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.)							
K. ....**Total Direct Costs (C through I)** →				$38,250			
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** →				$38,250			

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 AND BUDGET EXPLANATION FOR EACH PARTICIPATING INSTITUTION

(Gross)

Objectives 1-9

A. Salaries and Wages. Year 1: Salary ($27,243) is requested for one graduate student to complete literature review, collect rearing pond water chemistry data, and conduct laboratory toxicity studies. The graduate student will collaborate with OSU and UMESC to develop research protocols. Year 2: Salary ($27,243) is requested for one graduate student to conduct field toxicity studies and prepare final study reports in collaboration with OSU and UMESC.

B. Fringe Benefits. Year 1: 8.28% of salary ($2,257); Year 2: 8.28% of salary ($2,257).

E. Materials and Supplies. Year 1: General wet laboratory supplies ($3,673); General analytical laboratory supplies ($2,000); office and study automation and record keeping supplies ($1,500); Year 2: General wet laboratory supplies ($3,173); General analytical laboratory supplies ($2,000); office and study automation and record keeping supplies ($250).

F. Travel. Year 1: $1,750 is requested for transportation, lodging, and meal expenses to conduct pond study at UMESC. Year 2: $3,500 is requested for transportation, lodging, and meal expenses to conduct natural rearing pond experimental exposures at locations to be determined.

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Northern Rocky Mountain Science Center
2327 University Way, Suite 2
Bozeman, MT 59715

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 "*ASSESSMENT OF CARBON DIOXIDE (CO_2) AND INORGANIC NITROGEN COMPOUNDS TO ENHANCE WINTER KILL IN NATURAL REARING PONDS USED FOR FISH PRODUCTION IN THE NORTHCENTRAL REGION*"

Dear Dr. Batterson:

As the Authorized Organizational Representative (AOR) I would like to inform you that the U.S. Geological Survey's Northern Rocky Mountain Science Center (USGS NOROCK) wishes to participate in the above referenced project as a subcontractor to Michigan State University. Dr. Jackson Gross will serve as a co-Principal Investigator of the subcontract and because of his collaborations with other named investigators, 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. Jackson Gross for his involvement in this project. Upon issuance of approval to the North Central Regional Aquaculture Center for this project, USGS NOROCK will enter into a formal agreement with your institution.

Sincerely,

Dr. Jeffrey Kershner
Center Director
Northern Rocky Mountain Science Center
2327 University Way, Suite 2
Bozeman, MT 59715

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

OMB Approved 0524-0039
Expires 03/31/2004

BUDGET

ORGANIZATION AND ADDRESS Ohio State University 2021 Coffey Rd. Columbus, OH 43210 PROJECT DIRECTOR(S) Konrad Dabrowski				USDA AWARD NO. Year 1: Objectives 1-9			
				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. ___ (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...................................				$19,200			
e. ___ Prebaccalaureate Students.......................				$4,200			
f. ___ Secretarial-Clerical....................................							
g. ___ Technical, Shop and Other							
Total Salaries and Wages......................→				$23,400			
B. Fringe Benefits (If charged as Direct Costs)				$2,270			
C.Total Salaries, Wages, and Fringe Benefits (A plus B) →				$25,670			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$10,914			
F. Travel				$1,666			
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) →				$38,250			
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 →				$38,250			

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 Ohio State University 2021 Coffey Rd. Columbus, OH 43210 PROJECT DIRECTOR(S) Konrad Dabrowski	USDA AWARD NO. Year 2: Objectives 1-9 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: 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	$20,400			
e. ___ Prebaccalaureate Students	$4,600			
f. ___ Secretarial-Clerical				
g. ___ Technical, Shop and Other				
Total Salaries and Wages →	$25,000			
B. Fringe Benefits (If charged as Direct Costs)	$2,675			
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →	$27,675			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)				
E. Materials and Supplies	$9,025			
F. Travel	$1,550			
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) →	$38,250			
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 →	$38,250			

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

(Dabrowski)

Objectives 1-9

A. Salaries and Wages. Year 1: Salary ($23,400) is requested for one graduate student and one prebaccalaureate student to complete literature review, collect rearing pond water chemistry data, and conduct laboratory toxicity studies. The graduate student will collaborate with NOROCK and UMESC to develop research protocols. Year 2: Salary ($25,000) is requested for one graduate student and one prebaccalaureate student to conduct field toxicity studies and prepare final study reports in collaboration with NOROCK and UMESC.

B. Fringe Benefits. Year 1: 9.7% of salary ($2,270);Year 2: 10.7% of salary ($2,675).

E. Materials and Supplies. Year 1: General wet laboratory supplies ($5,591); general analytical laboratory supplies ($2,659) including purchase of the saturometer for N2 analysis ($1,750); office and study automation and record keeping supplies ($914); Year 2: General wet laboratory supplies ($6,616); general analytical laboratory supplies ($1,500); office and study automation and record keeping supplies ($909).

F. Travel. Year 1: $1,666 is requested for transportation, lodging, and meal expenses to conduct pond study at UMESC. Year 2: $1,550 is requested for transportation, lodging, and meal expenses to conduct natural rearing pond experimental exposures at locations to be determined.

THE OHIO STATE UNIVERSITY

Research Foundation

Food, Agricultural, and Environmental Sciences Office
590 Woody Hayes Drive
Suite 112
Columbus, OH 43210-1057

Phone (614) 292-3721
Fax (614) 292-8555

April 20, 2011

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

Re: Collaborative **USGS/NCRAC** Proposal titled: ***"Assessment of Carbon Dioxide (CO2) and inorganic nitrogen compounds to enhance winter kill in natural rearing ponds used for fish production in the North Central Region."***

Dear Colleagues:

The Ohio State University (Office of Sponsored Programs) is pleased to indicate our institution's willingness to participate with the United States Geological Survey on the above referenced ***North Central Regional Aquaculture Center*** research proposal. The budget has been reviewed and approved in the amount of $76,500. The portion of the project to be conducted at this institution will be under the direction of Dr. Konrad Dabrowski, Professor in the School of Environment and Natural Resources with The Ohio State University. The lead principle investigator is Dr. Mark P. Gaikowski.

The Ohio State University (Office of Sponsored Programs) administers all grants and contracts on behalf of the faculty of the University. Therefore, any awards should be addressed to The Ohio State University (tax I.D. #31-6025986) and directed to my attention at the following address:

The Ohio State University Office of Sponsored Programs
1960 Kenny Rd.
Columbus, Ohio 43210

We look forward to a productive collaboration in this program. Please contact Dr. Dabrowski at (614) 292-4555 or e-mail: dabrowski.1@osu.edu for technical information regarding the proposal. Questions administrative in nature should be directed to the undersigned at (614) 292-3721, or e-mail: finch.24@osu.edu.

Respectfully,

THE OHIO STATE UNIVERSITY
Office of Sponsored Programs

Marta L. Morris
Director, Research Support

Cc: KD

BUDGET SUMMARY FOR EACH PARTICIPATING INSTITUTION

Year 1

	UMESC	NOROCK	OSU	Totals
Salaries and Wages	$7,000	$27,243	$23,400	$57,643
Fringe Benefits	$3,000	$2,257	$2,270	$7,527
Total Salaries, Wages, and Fringe Benefits	$10,000	$29,500	$25,670	$65,170
Nonexpendable Equipment				
Materials and Supplies		$7,000	$10,914	$17,914
Travel	$1,000	$1,750	$1,666	$4,416
All Other Direct Costs				
TOTAL PROJECT COSTS	$11,000	$38,250	$38,250	$87,500

Year 2

	UMESC	NOROCK	OSU	Totals
Salaries and Wages	$7,000	$27,243	$25,000	$59,243
Fringe Benefits	$3,000	$2,257	$2,675	$7,932
Total Salaries, Wages, and Fringe Benefits	$10,000	$29,500	$27,675	$67,175
Nonexpendable Equipment				
Materials and Supplies		$5,250	$9,025	$14,275
Travel	$1,000	$3,500	$1,550	$6,050
All Other Direct Costs				
TOTAL PROJECT COSTS	$11,000	$38,250	$38,250	$87,500

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 1 completed in Year 2.
Objective 7: Initiated in Year 1 completed in Year 2.
Objective 8: Initiated in Year 2 completed in Year 2.
Objective 9: Initiated in Year 2 completed in Year 2.

PRINCIPAL INVESTIGATORS

Mark P. Gaikowski, U.S. Geological Survey Upper Midwest Environmental Sciences Center
Jackson A. Gross, U.S. Geological Survey Northern Rocky Mountain Science Center
Konrad Dabrowski, Ohio State University
Barnaby J. Watten, U.S. Geological Survey Leetown Science Center

VITA

Konrad Dabrowski
School of Natural Resources
Ohio State University
2021 Coffey Rd.
Columbus, OH 43210

Phone: (614) 292-4555
FAX: (614) 292-7432
E-mail: dabrowski.1@osu.edu

EDUCATION

M.Sc. Agriculture University, Olsztyn, Poland, 1972, Inland Fisheries
Ph.D. Agriculture University, Olsztyn, Poland, 1976, Fisheries
D.Sc. Agricultural University, Szczecin, Poland, 1984, Fish Physiology

POSITIONS

Professor (1989-present), School of Natural Resources, Ohio State University, Columbus
Visiting Professor (2009), University of Ghent, Belgium
Visiting Professor (1987-1989), Institute of Zoology, University of Innsbruck, Innsbruck, Austria
Visiting Professor (1985), Department of Biology, University of Paris VII, Orsey, France
Visiting Professor (1984-1985), Department of Aquaculture, University of Fisheries, Tokyo
Assistant/Associate Professor (1972-1987), Inland Fisheries and Water Protection, Agriculture University, Olsztyn, Poland

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society
World Aquaculture Society

SELECTED PUBLICATIONS

Jaroszewska, M., B.J. Lee, K. Dabrowski, S. Czesny, J. Rinchard, P. Trzeciak, and B. Wilczyńska. 2009. Effects of vitamin B_1 (thiamine) deficiency in lake trout (*Salvelinus namaycush*) alevins at hatching stage. Comparative Physiology and Biochemistry 154A:255-262.

Dabrowski, K., K. Ware, M. Jaroszewska, and K. Kwasek. 2009: Evaluation of walleye embryo survival and larval viability following iodine treatment. North American Journal of Aquaculture 71:122-129.

Czesny, S., J.M. Dettmers, J. Rinchard, and K. Dabrowski. 2009. Linking egg thiamine and fatty acid concentrations of Lake Michigan lake trout with early life stage mortality. Journal of Aquatic Animal Health 21:262-271.

Jaroszewska, M., and K. Dabrowski. 2009: The nature of exocytosis in the yolk trophoblastic layer of silver arowana (*Osteoglossum bicirrhosum*) juvenile, the representative of ancient teleost fishes. Anatomical Record-Advances in Integrative Anatomy and Evolutionary Biology 292:1745-1755.

Rodriguez de Oca, G.A.R.M., K. Dabrowski, K. Park, K.J. Lee, and M. Abiado. 2009. Interaction of phytochemical-quercetin with the other antioxidant, ascorbic acid and their protective effect in tilapia after ultraviolet irradiation. Journal of the World Aquaculture Society 40:586-600.

Dabrowski, K., M. Arslan, J. Rinchard, and M.E. Palacios. 2008. Growth, maturation, induced spawning, and production of the first generation of South American catfish (*Pseudoplatystoma* sp.) in the North America. Journal of the World Aquaculture Society 39:174-183.

Tratner, S., J. Pickova, K.H. Park, J. Rinchard, and K. Dabrowski. 2007. Effects of alfa-lipoic and ascorbic acid on the muscle and brain fatty acids and antioxidant profile of the South American pacu *Piaractus mesopotamicus*. Aquaculture 273:158-164.

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, and R.G. Endris. 2010. Depletion of florfenicol amine, marker residue of florfenicol, from the edible fillet of tilapia (*Oreochromis niloticus x O. niloticus* and *O. niloticus x 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, 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

Jason "Jackson" A. Gross
Northern Rocky Mountain Science Center (USGS)
2327 University Way, Suite 2
Bozeman, MT 59715

Phone: (406) 994-7408
FAX: (406) 994-6556
E-mail: jgross@usgs.gov

EDUCATION

B.S. San Diego State University, 1990, Biology
M.S. San Diego State University, 2004, Public Health/Toxicology
Ph.D. University of Wisconsin-Madison, 2006, Animal Sciences

POSITION

Biologist

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

Society of Environmental Toxicology and Chemistry
Society of Toxicology

SELECTED PUBLICATIONS

Gross, J. A., P.T.J. Johnson, L.K. Prahl, and W.H. Karasov. 2009. Critical period for effects of chronic cadmium exposure on growth and development in northern leopard frog (Rana pipiens) tadpoles. Environmental Toxicology and Chemistry 28:1227-1232.

Chen, T-H, J.A. Gross, and W.H. Karasov. 2009. Chronic exposure to pentavalent arsenic of larval leopard frogs (*Rana pipiens*): bioaccumulation and reduced swimming performance. Ecotoxicology 18(5):587-593.

Johnson, P.T.J., J.M. Chase, K.L. Dosch, R.B. Hartson, J.A. Gross, D. Larson, D.R. Sutherland, and S.R. Carpenter. 2007. Aquatic eutrophication promotes pathogenic disease in amphibians. Proceedings of the National Academy of Science 104(40):15781-15786.

Gross, J.A., T-H. Chen, and W.H. Karasov. 2007. Effects of cadmium on development in northern leopard frog (*Rana pipiens*) tadpoles. Environmental Toxicology and Chemistry 26:1192-1197.

Chen, T-H, J.A. Gross, and W.H. Karasov. 2007. Adverse effects of chronic copper exposure in larval northern leopard frogs (*Rana pipiens*). Environmental Toxicology and Chemistry 26:1470-1475

Chen, T-H, J.A. Gross, and W.H. Karasov. 2005. Sublethal effects of lead on northern leopard frog (*Rana pipiens*) tadpoles. Environmental Toxicology and Chemistry 25:1383-2389.

VITA

Barnaby J. Watten
Leetown Town Science Center (USGS)
11649 Leetown Road
Kearneysville, WV 25430

Phone: (304) 724-4425
E-mail: barnaby_watten@usgs.gov

EDUCATION

B.S. Bemidji State University, 1976, Aquatic Biology
M.Ag. Oregon State University, 1982, Agricultural Engineering
Ph.D. Auburn University, 1989, Fisheries and Allied Aquacultures

POSITION

Chief, Restoration Technologies Branch

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society
Aquacultural Engineering Society

PATENTS

Multiple stage gas absorber (1989), U.S. Patent No. 4,880,445; Process and apparatus for carbon dioxide pretreatment and accelerated limestone dissolution for treatment of acidified water (1999), U.S. Patent No. 59114046; Apparatus for carbon dioxide pretreatment and accelerated limestone dissolution for treatment of acidified water (1999), U.S. Patent No. 5863422.

SELECTED PUBLICATIONS

Pfeiffer, T.F., S.T. Summerfelt, and B.J. Watten. 2011. Comparative performance of CO_2 measuring methods: marine aquatic recirculation system application. Aquacultural Engineering 44:1-9.

Colt, J., B. Watten, and M. Rust. 2009. Modeling carbon dioxide, pH and un-ionized ammonia relationships in serial reuse systems. Aquacultural Engineering 40:28-44.

Watten, B.J., Sibrell, P.L., Montgomery, G.A., and S.M. Tsukuda. 2004. Modification of pure oxygen absorption equipment for concurrent stripping of carbon dioxide. Aquacultural Engineering 32:183-208.

Watten, B.J., C.E. Boyd, M.F. Schwartz, S.T. Summerfelt, and B.L. Brazil. 2004. Feasibility of measuring dissolved carbon dioxide based on head space partial pressures. Aquacultural Engineering 30:83-101.

Cole, M.B., D.E. Arnold, B.J. Watten, and W.F. Krise. 2001. Haematological and physiological responses of brook charr, to untreated and limestone-neutralized acid mine drainage. Journal of Fish Biology 59:79-91.

Watten, B.J., D.C. Honeyfield, and M.F. Schwartz. 2000. Hydraulic characteristics of a rectangular mixed-cell rearing unit. Aquacultural Engineering 24:59-73.

Summerfelt, S.T., B.J. Vinci, M.B. Timmons, and B.J. Watten. 1999. Stripping columns remove carbon dioxide from recirc systems. Recirc Today 1:24-26.

Watten, B.J., D.R. Smith, and W.J. Ridge. 1997. Continuous monitoring of dissolved oxygen and total dissolved gas pressure based on head-space partial pressures. Journal of the World Aquaculture Society 28:316-333.

Vinci, B.J. B.J. Watten, and M.B. Timmons. 1997. Modeling gas transfer in a spray tower oxygen absorber. Aquacultural Engineering 16:91-105.