Trust Fund 2009 Work Program

Date of Report: 5/22/2009
Date of Next Progress Report: 12/31/2009
Date of Work Program Approval: Pending
Project Completion Date: 6/30/2012

I. PROJECT TITLE: Vulnerability of Lakes to Endocrine Disruption

Project Manager:	Richard L. Kiesling
Affiliation:	U.S. Geological Survey
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Location: Ramsey County, MN; Winona County, MN

Total Trust Fund Project Budget:	**Trust Fund Appropriation**	**$297,000**
	Minus Amount Spent:	**$ 0**
	Equal Balance:	**$297,000**

Legal Citation: ML 2009, Chap.142, Sec. 2, Subd. 5b
Appropriation Language:
$297,000 is from the trust fund to the commissioner of natural resources for an agreement with the United States Geologic Survey and St. Cloud State University to develop quantitative data on juvenile and adult fish vulnerability to endocrine-active emerging contaminants found in Minnesota lakes. This appropriation is available until June 30, 2012, at which time the project must be completed and final products delivered, unless an earlier date is specified in the work program.

II. PROJECT SUMMARY AND RESULTS:
The project will develop quantitative data on juvenile and adult fish vulnerability to endocrine-active emerging contaminants (EACs) found in Minnesota lakes using mesocosms enclosures. Effects of EACs will be measured in a balanced design using four-cubic meter mesocosms located at the USGS Upper Mississippi Ecosystem Science Center (UMESC). Three species of juvenile fish reared at the UMESC facility (fathead minnow *Pimephales promelas*, bluegill (*Lepomis macrochirus*), and walleye (*Stizostedion vitreum*) will each be exposed to a known EAC for six weeks. Water and sediment concentrations will be monitored during the experiment, and fish will be evaluated using blood-chemistry biomarkers (e.g., plasma vitellogenin) and histo-pathological indices of abnormal development (e.g., intersex).

Effects of EACs on reproductive success will be measured in a balanced design using four-cubic meter mesocosms. Half of the juvenile fathead minnows and bluegills exposed in this experiment will be allowed to grow to sexual maturity and will be evaluated for adult reproductive success along with adult walleye. Juvenile fathead minnows and bluegills reared will be allowed to spawn under controlled conditions and evaluated for behavioral endpoints, spawning success, and reproductive output.

III. PROGRESS SUMMARY AS OF *[Insert date of WP Update]***:**

IV. OUTLINE OF PROJECT RESULTS*:*

Result 1: Developmental impacts of juvenile exposure to water and sediment concentrations of EACs. Budget: $176,000

Description:
Effects of EACs will be measured in a three-way, balanced design using mesocosms. Mesocosms will be installed at the USGS Upper Mississippi Ecosystem Science Center (UMESC). Three species of juvenile fish reared at the UMESC facility [fathead minnow (*Pimephales promelas)*, bluegill (*Lepomis macrochirus*), and walleye (*Stizostedion vitreum*)] will each be exposed to one of three EACs (ethynylestradiol, 4-nonylphenol (4-NP), or nonylphenol ethoxylate (NPEO)) for six weeks. Water and sediment concentrations will be monitored four times during the experiment, and fish will be evaluated using blood-chemistry biomarkers (e.g., plasma vitellogenin) and histo-pathological indices of abnormal development (e.g., intersex). Half of the fathead minnows and bluegills exposed in this experiment will be allowed to grow to sexual maturity and will be evaluated for reproductive success as part of Result 2 below.

Summary Budget Information for Result 1: **Trust Fund Budget:** $176,000
Amount Spent: $ 0
Balance: **$** 176,000

Deliverable/Milestone	Completion Date	Budget
SCSU sub-contract for their work on this result	July 31 2009	$73,000
Build and test mesocosms	September 30 2009	$40,000
Establish captive fish stock	September 30 2009	$13,000
Run F0 generation experiments	June 30 2010	$40,000
Final Report for Result 1	June 30 2012	$10,000

Result Status as of 12/31/09:

Result Status as of 06/30/10:

Result Status as of 12/31/10:

Result Status as of 06/30/11:

Result Status as of 12/31/11:

M.L. 2009 Final Report Summary 06/30/12:

Result 2: Reproductive impacts of juvenile and adult exposure to water and sediment concentrations of EACs. Budget: $121,000

Effects of EACs on reproductive success (i.e, mating behavior and spawning success) will be measured in a two-way, balanced design using mesocosms. Juvenile fathead minnows and bluegills reared under Result 1 above will be allowed to spawn under controlled conditions and evaluated for reproductive output. Sexually mature adults of walleye at UMESC (and bluegill if necessary) will each be exposed to ethynylestradiol through water or sediment exposure for six weeks prior to spawning. Water and sediment concentrations will be monitored four times during the experiment, and fish will be evaluated using blood-chemistry biomarkers (e.g., plasma vitellogenin) and histo-pathological indices of abnormal reproductive physiology before and after spawning as appropriate.

Summary Budget Information for Results:

Trust Fund Budget:	$ 121,000
Amount Spent:	$ 0
Balance:	**$** 121,000

Deliverable/Milestone	Completion Date	Budget
SCSU sub-contract for their work on this result	July 31 2009	$34,600
Rear F1 generation	December 31 2010	$30,000
Run F1 generation experiments	June 30 2011	$40,000
Complete chemical analysis	December 31 2011	$8,000
Final Report for Result 2	June 30 2012	$8,400

Result Status as of 12/31/09:

Result Status as of 06/30/10:

Result Status as of 12/31/10:

Result Status as of 06/30/11:

Result Status as of 12/31/11:

M.L. 2009 Final Report Summary 06/30/12:

V. TOTAL TRUST FUND PROJECT BUDGET:

Personnel: $ 159,000
Contracts: $ 107,900 [with St. Cloud State University]
Equipment/Tools/Supplies: $ 54,700

[Turbo-evaporator for processing chemical extractions from mesocosm sediment samples = $3,200]

[Tanks, plumbing, air-lifts, and effluent treatment filters for mesocosms = $12,000]

[Supplies for Enzyme-linked immunosorbent (ELISA) chemical assays of EDC compounds in water and fish from mesocosms = $31,500 (SCSU sub-contract)]

[Laboratory supplies and analysis kits for chemical determinations of EACs in sediment extracts = $8,000]

Travel (in-state)**:** $ 7900
Other (Printing and Shipping)**:** $ 3500
TOTAL TRUST FUND PROJECT BUDGET: $297,000

VI. PROJECT STRATEGY:

A. Project Partners: This project is a continuing partnership between the United States Geological Survey (USGS) and Dr. Heiko L. Schoenfuss, Professor and Director of the Aquatic Toxicology Laboratory, Department of Biological Sciences, St. Cloud State University. Team members from the USGS include Dr. Richard Kiesling (project Leader) and Mark Gaikowski (USGS-UMESC Mesocosm Facility Director). Team members from the USGS will manage mesocosm installation and experimental protocols, exposure experiments, chemical analysis, and spawning experiments. Team members from St. Cloud State University will manage histopathology, behavioral experiments and spawning experiments. All team members will participate in writing the final report and communicating results to state user groups.

Funding by Partner	Budget
1. USGS Cooperative Water Program	$189,100
2. St. Cloud State University (sub-contract)	$107,900
TOTAL	$297,000

B. Project Impact and Long-term Strategy:

1. Project provides direct estimate of how juvenile fish of common lake species respond to environmentally meaningful EAC exposures in a lake setting. Most EACs are found at very low concentrations in water (Kolpin and others; 2002; Lee and others; 2004) but reach higher concentrations in sediment (e.g., Mayer and others, 2006; Pojana and others 2007; Kim and Carlson, 2007). Despite these low concentrations, research has identified developmental and reproductive effects on fish species at environmentally relevant concentrations. The proposed work will extend this work to include important lake species under chronic exposure through either water or sediment exposure pathways.

2. Project provides an estimate of the importance of longevity and other reproductive characteristics in the magnitude of fish response to EAC exposure during different growth stages/ages.

In Minnesota, endocrine disruption has been observed in short- and long-lived fish species including vitellogenin induction in male fathead minnows, male carp, and walleye (Folmar and others, 1996, 2001; Lee and others, 2000). Vitellogenin in male carp was also observed at numerous sites downstream of WWTP discharges throughout central Minnesota (Lee and others, 2000). Two ongoing studies in Minnesota have recently identified additional fish species affected by EACs in tributaries of the Mississippi and the St. Croix Rivers (Jahns and others in prep; Lee and others in review) as well as urban lakes (Schoenfuss and others – unpublished data). Taken as a whole, these results indicate that Minnesota fish communities are vulnerable to reproductive impacts. This study helps answer how vulnerable adult fish are to EAC exposure and whether life-history characteristics mitigate EAC exposure.

C. Other Funds Proposed to be Spent during the Project Period:

Budget Source	Budget
1. USGS Cooperative Water Program Funding	$133,000
2. USGS In-kind Contribution	$28,000

In order for USGS match

D. Spending History:

No funds have been spent to date.

VII. DISSEMINATION: Results will be communicated to local groups, state agencies and national peer groups through presentations at regional and national meetings including state resource management meetings. Details of results will be available as a final project report to LCCMR, fact-sheet summaries, and scientific journal articles.

VIII. REPORTING REQUIREMENTS: Periodic work program progress reports will be submitted every six months not later than 12/31/09, 06/30/10, 12/31/10, 06/30/11, and 12/31/11. A final work program report and associated products will be submitted between June 30 and August 1, 2012 as requested by the LCCMR.

IX. RESEARCH PROJECT ADDENDUM:

2009-2012 LCCMR Proposal – Assessing the Vulnerability of Lake Fish Communities to Endocrine Disruption from Water and Sediment Using Pond Mesocosms

1. Overview of the Study

Fathead minnows and bluegill sunfish will be exposed for six weeks to two environmentally realistic concentration of the endocrine active compounds 17b Estradiol (E2) during two sensitive ontogenetic stages: during larval development and during reproduction. F0 adults will be exposed and assessed for the reproductive output and physiological alterations consistent with EAC exposure. F1 generation offspring will be exposed from prior of hatching to six weeks post-hatch. A subset of exposed F1 generation fish will be assessed for the induction of vitellogenin or their ability to perform C-start predator avoidance behaviors. The remaining larvae will be reared to adulthood (one year for fatheads, two years for bluegill). Adult F1 generation fish will be placed into reproductive scenarios for six weeks and reproductive output will be assessed. During this reproductive period, a subset of fish will be exposed for a second time to establish three F1 generation adult treatments: (1) control-never exposed; (2) control exposed as larvae only; (3) exposed as larvae and as adults. Following exposure, fish will be processed for vitellogenin and histopathology.
In addition, walleye or other top predator will be exposed for six weeks in the same exposure scenario and assessed for reproductive, physiological and behavioral endpoints during year 2 of the study.

2. Experimental Design

2.1. Sub-system Design

Each of the six replicate exposure sub-systems will consist of four 200 gallon treatment / exposure tanks connected to a central 200 gallon balancing reservoir. The over all design is for a recycling static-renewal exposure with daily replacement of 5% of total tank volume. Daily water renewal will be necessary to prevent accumulation of waste products from fish metabolism. In addition to the daily water exchange, the balancing reservoir will be equipped with a bio-filter to help process accumulated nitrogenous wastes. The balancing reservoir will receive a constant dose of 17-B estradiol (E2) dissolved in ethanol and delivered by peristaltic pump at a constant flow rate.

Concentrations of E2 will be maintained at 10 or 30 ng/L for six weeks. Culture water will be recycled between all four treatment tanks in a sub-system and the central balancing reservoir. The four exposure tanks will be equipped with center-mounted outflow connections positioned approximately 20 cm below the water surface. Outlets will be double screened to prevent exchange of fish between treatment tanks and the balancing reservoir. Each treatment tank will be equipped with an airlift standpipe that delivers recycled water from the balancing reservoir to each tank. A

single air compressor will run the four airlift standpipes that deliver recycling water from the balancing reservoir to the individual treatment tanks. Water will return to the balancing reservoir by gravity equalization of water level through the center-mounted outflow connections.

Effluent generated from the 5% daily renewal rate of each sub-system will be processes using a flow-through UV exposure unit rated at 20 gallons per minute. The combined effluent flow rate for all six sub-systems is estimated to be less than 15 gallons per hour. Following UV exposure, effluent will be passed through an ozonation system prior to discharge to the UMESC holding pond. Treatment efficiency will be tested in an off-site facility prior to field deployment.

The sub-systems will be assembled in one of two 0.01 acre concrete ponds enclosed in wire fencing to prevent predation on the fish. Three sub-system will be placed in each of two concrete ponds, and replicate sub-systems will be evenly split between the two concrete ponds. Each sub-system will be rinsed three times with well water from the UMESC supply wells. Once they have been drained, all sub-system tanks will be filled with ambient water from one of the 0.10 acre fish ponds at the UMESC facility. These source ponds are filled with well water and allowed to develop a natural planktonic community. Once the tanks are filled, the surrounding concrete pond will be filled with well water to provide a thermal buffer. The surrounding pond water will be continuously mixed with four air-lift standpipes and temperature will be regulated with additions of well water.

Treatment systems will be allowed to equilibrate with the continuous E2 addition and to develop stable physical and biological conditions prior to addition of fish. Efforts will made to establish acceptable food levels in the treatment tanks. Plankton dynamics will be monitored in the sub-systems

2.2. Fathead Minnow Exposures

F0 generation adult fathead minnows and F1 generation eggs are being placed randomly into two of the four exposure tanks of each of the six identical systems. Two systems have been exposed prior to fish placement with the carrier control ethanol, two have been exposed to 10 ng/L E2, and two have been exposed to 30 ng/L E2. Fish are maintained in the exposure scenarios for six weeks. F1 generation larvae (which will hatch within the first three days of exposure) will receive supplemental fed of newly hatched brine shrimp. Adult fathead minnows (10 males, 10 females per tank) receive supplemental frozen brine shrimp daily. Adult fathead minnow tanks will contain 10 nest sites which will be exchanged daily (eggs are counted daily and the discarded). After six weeks exposure, all adult fathead minnows are sacrificed in MS-222, a blood sample is taken, fish are measured for weight and length, and livers and gonads are excised, weight and preserved in 10% buffered formalin for later histopathological analysis. A subset of ten larvae per tank (n=20 per exposure scenario, n=40 per treatment) are being assessed for their ability to perform C-start predator avoidance behaviors. Following the behavioral observation, these larvae and ten additional larvae(n=2- per tank, n=80 per

treatment) are sacrificed in MS-222 and preserved for later vitellogenin analysis of the homogenate.

The remaining larval fish are transferred to rearing facilities at UMESC and reared to adulthood. Upon reaching maturity (Year 2), F1 generation adults are split into two groups, one group of ten males, ten females per tank will be sacrificed and assessed for vitellogenin and histopathology. A second set of ten males and ten females per tank are reintroduced to the exposure scenarios and allowed to spawn (similar to F0 generation fish in Year1). Three treatments are prepared based on the results of the prior analysis of Year 1 effects:

(1) Control – F1 generation fish that were never exposed;
(2) F1 generation fish exposed as larvae only; and
(3) F1 generation fish exposed as larvae and now again as adults.

The lowest effects concentration determined in Year 1 of the study (10 or 30 ng/L) will be used for the Year 2 exposures. Reproductive output will be quantified through daily counting of eggs on all spawning tiles similar to Year 1. Following the six week exposure all fish will be sacrificed and assessed for vitellogenin and histopathology.

2.3. Bluegill Sunfish Exposures

In Year 1 of the study, bluegill sunfish eggs and adult F0 generation bluegill sunfish will be exposed for six weeks to 10 or 30 ng/L E2 for six weeks. Exposures will utilize the same setup previously used for fathead minnows (see above). Two pairs of mature male and female bluegill will be placed into two of every four tanks in each exposure scenario (n=2 pairs per tank, 4 pairs per scenario, 8 pairs per treatment). These pairs will be separated by a stainless steel mesh barrier to avoid territorial interactions among the male fish. Bluegill reproductive output will be monitored daily for the six week exposure duration. F1 generation larvae (hatched during the first few days of the exposure) in the other two tanks of each exposure scenario will be exposed for six weeks as well. Following the six week exposure, all F0 generation adults will be sacrificed and assessed for plasma vitellogenin concentrations and histopathology. A subset of 10 F1 generation larvae per tank (n=40 per treatment) will be assessed for their ability to perform a predator avoidance C-starts. These larvae and ten more (n=20 per tank, n=80 per treatment) will be preserved after observation for later determination of vitellogenin concentration in whole larvae homogenate. The remaining larvae are reared to adult hood (two year rearing time to Year 3). Upon reaching maturity (Year 3), F1 generation adults are split into two groups, one group of ten males, ten females per tank will be sacrificed and assessed for vitellogenin and histopathology. A second set of two pairs of fish per tank are reintroduced to the exposure scenarios and allowed to spawn (similar to F0 generation fish in Year1). Three treatments (n=16 spawning pairs per treatment) are prepared based on the results of the prior analysis of Year 1 effects: (

1) Control – F1 generation fish that were never exposed;
(2) F1 generation fish exposed as larvae only; and (

3) F1 generation fish exposed as larvae and now again as adults.

The lowest effects concentration determined in Year 1 of the study (10 or 30 ng/L) will be used for the Year 3 exposures. Reproductive output will be quantified through daily counting of eggs in the nest of each spawning pair (similar to Year 1). Following the six week exposure all fish will be sacrificed and assessed for vitellogenin and histopathology.

2.4. Walleye Exposures

During year 2 of the study (2011), we will expose adult walleye (F0 generation) and eggs/newly hatched larvae (F1 generation) to two concentrations of E2 for six weeks following the same exposure protocol outlined in 2.2 and 2.3 and assessing the same endpoints as outlined in 2.5. This exposure will be conducted early in the season (March) and no fish will be reared to adulthood from this experiment. All fish will be assessed at the end of the six week exposure period for plasma or homogenate vitellogenin concentrations, histopathological endpoints and, in the case of the larvae, C-start predator avoidance performance.

2.5. Endpoints & Total Fish Sample Numbers

I. **Vitellogenin (vtg) analysis**. VTG analysis for fathead minnows will utilize purified fathead minnow vitellogenin and an in-house developed monoclonal vtg antibody. This ELISA has been used on several thousand fathead minnows and has been peer reviewed extensively. For bluegill sunfish vtg analysis, we will first purify vtg form a gravid female bluegill sunfish, purify the vtg and then develop a polyclonal vtg antibody specific for the bluegill sunfish. To assess vtg concentrations in larval fish, we will produce homogenates of 2 larval fish each, centrifuge the homogenate and the conduct the ELISA analysis on the supanate. For adult fish, we will measure plasma vtg concentrations separately for each fish.

   i. YEAR 1.

      a. Fathead minnow larvae: 80 larvae per treatment x 3 treatments = 240 larvae/2 (two larvae per homogenate) = 120 vtg analyses

      b. Fathead minnow adults: 10 males/10 females per tank, 40 males/40 females per treatment x 3 treatments = 120 males; 120 females → 240 vtg analyses of adult fathead minnows

      c. Bluegill sunfish larvae: 80 larvae per treatment x 3 treatments = 240 larvae/2 (two larvae per homogenate) = 120 vtg analyses

      d. Bluegill sunfish adults: 2 males/2 females per tank, 8 males/8 females per treatment x 3 treatments = 24 males; 24 females → 48 vtg analyses of adult bluegill sunfish

   ii. YEAR 2

a. F0 generation adult walleye exposed for 6 weeks: 48 vtg analyses
b. F1 generation juvenile walleye exposed for six week: 120 vtg analyses
c. F1 generation adult fathead minnows (no further exposure): 240 vtg analyses
d. F1 generation adult fathead minnows (secondary exposure experiment): 240 vtg analyses

iii. YEAR 3
a. F1 generation adult bluegill sunfish (no further exposure): 48 vtg analyses
b. F1 generation adult bluegill sunfish (secondary exposure experiment): 96 vtg analyses

iv. TOTAL:
a. Larval fathead minnow vtg: 120
b. Larval bluegill sunfish vtg: 120
c. Laval walleye vtg: 120
d. Adult fathead minnow vtg: 720
e. Adult Bluegill sunfish vtg: 240
f. Adult walleye vtg: 48

II. Histopathology

Histopathological analysis will follow well established protocols developed at the US EPA (2008 *Histology and histopathological guidelines for phase 1b of the OECD fish screening assay for EDCs*. EPL Project No. 481-017.) and applied extensively at the St. Cloud State University Aquatic Toxicology Laboratory. Briefly, tissue of interest (liver, testis, ovaries) are excised from the animals immediately following their sacrifice with MS-222. Tissues from each animal are placed together in a individually labeled histo-cassette and immersed in 10% buffered formalin for later processing. Upon return to the laboratory, tissues are dehydrated and paraffin embedded using a Leica automated tissue processor. After embedding, tissues are sectioned at 5µm using a Leica motorized microtome. Tissue sections are stained following established staining protocols for Haematoxylin & Eosin stains or for other stains (i.e., reticular stain) as appropriate. After coverslipping and drying, tissues parameters are ranked on a 0-4 scale based on the presence and abundance of cell types in livers, ovaries, and testis following standardized procedures outlined by the US EPA (2008). The analysis of tissues will be conducted by a trained histologist with years of experience analyzing fish tissue sections.

i. YEAR 1
   a. Fathead minnows: 240
   b. Bluegill sunfish: 48
ii. YEAR 2
   a. Walleye: 48
   b. Fathead minnows:480
iii. YEAR 3
   a. Bluegill sunfish:144

III. C-START PREDATOR AVOIDANCE PERFORMANCE

The C-start predator avoidance performance of exposed larvae will be measured using a trigger-activated system with a small light-emitting diode (LED) and a vibrating electronic chip attached to the base of the filming arena to provide a stimulus. When activated, the system causes a short vibrational stimulus (< 1 s) marked in the field of view by the appearance of the LED light used to determine time zero for data analyses. The filming arena consists of a 5-cm diameter glass Petri dish positioned on top of a 1-mm grid. The larval escape behavior in the filming arena will be recorded using a high-speed digital video cameras (Redlake MotionScope M1, Tucson, AZ) at 1,000 frames per second. Larvae will be fed 30 min prior to testing and a resultant time limit of 6 h will be set for data collection to avoid an observed drift in response due to time since last feeding. Individuals will be placed singularly and in random order into the filming arena and allowed to acclimate. Once a larval fish swims into the center portion of the grid (marked with a square), the trigger is depressed and the vibrational stimulus provokes a predator avoidance response (Fig. 3). Each larval fish is used for only one performance recording. High-speed video sequences of predator avoidance behaviors (Fig. 3.) are used to calculate the time to induction of behavior (latency period), escape velocity (velocity during the first 40 ms after the initiation of an evasive maneuver; body length/ms to exclude any size differences as confounding factors among individual fish), and total escape response [body length/(latency in ms+40 ms)]. Videos will not be considered if the latency response is <5 ms (false start).

i. YEAR 1
   a. Fathead minnows: 120
   b. Bluegill sunfish: 120
ii. YEAR 2
   a. Walleye: 120
   b. Fathead minnows: 120
iii. YEAR 3
   a. Bluegill sunfish: 120

IV. ESTRADIOL ANALYSIS

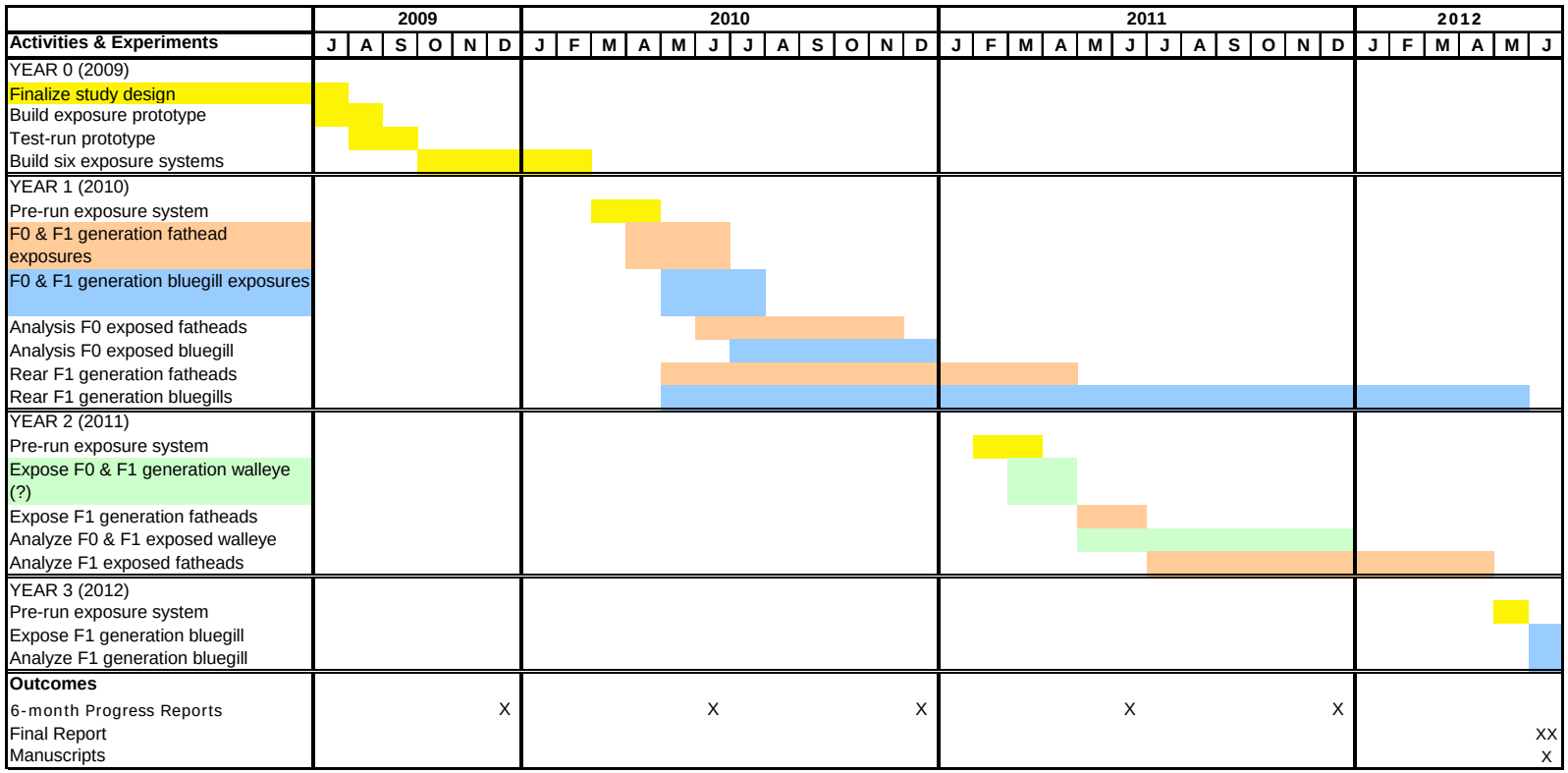
Estradiol analysis utilizes a commercially available 17ß-Estradiol Kit (Cayman Chemicals, Ann Arbor, MI) based on enzyme-linked immunosorbent assay (ELISA) methodology. Assays will be conducted by a research fellow (S. Bartell) with 15+

years in ELISA development and application. Briefly, water samples will be added to a 96 well plate, exposed to a E2 specific antibody and after several washed, challenged with a second antibody tagged with a fluorescent tag. Fluorescence of each sample well will be assessed against a standard curve of known E2 values to determine final E2 concentration in the sample water. This technique has allowed for sample analysis to a detection limit of roughly 2 ng/L - 5 times below the lower nominal values expected in this study.

i. YEAR 0: 100 analyses
ii. YEAR 1: 672
iii. YEAR 2: 336
iv. YEAR 3: 336

Sediment samples will be collected at the beginning and the end of each mesocosms deployment, extracted and concentrated using N_2 gas evaporation or solid-phase extraction (SPE), and analyzed using the ELISA as outlined above for the water samples. Other components of the mesocosm food-webs (e.g., algae and invertebrates) will also be analyzed for 17ß-Estradiol at the end of each mesocosms experiment.

Result Completion Timeline with Deliverables:



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Attachment A: Budget Detail for 2009 Projects - Summary and a Budget page for USGS

Project Title: Vulnerability of Lakes to Endocrine Disruption

Project Manager Name: Richard L. Kiesling

Trust Fund Appropriation: $ *297,000*

2009 Trust Fund Budget	**Result 1 Budget:**	**Amount Spent** *(date)*	**Balance** *(date)*	**Result 2 Budget:**	**Amount Spent** *(date)*	**Balance** *(date)*	**TOTAL BUDGET**	**TOTAL BALANCE**
	Developmental impacts of juvenile exposure to water and sediment concentrations of EACs. Budget: $80,000			*Reproductive impacts of juvenile and adult exposure to water and sediment concentrations of EACs. Budget: $79,000*				
BUDGET ITEM								
PERSONNEL: wages (%FTE) and benefits	80,000			79,000			159,000	159,000
Richard Kiesling (6%) - Project Manager: USGS								
Steve Redmman (8%) - Fish Culturalist: USGS								
Sue Schleis (12%) - Technician: USGS								
Research Intern (90%): USGS								
Contracts	73,300			34,600			107,900	107,900
Professional/technical: *with St. Cloud State University for Dr. Heiko Schoenfuss - $107,900*								
Other direct operating costs:								
Non-capital Equipment / Tools								
Turbo-evaporator for processing chemical extractions from mesocosm sediment samples	3,200						3,200	3,200
Printing	1,100			1,400			2,500	2,500
Supplies								
Tanks, plumbing, air-lifts, and effluent treatment filters for mesocosms	12,000						12,000	12,000
Laboratory supplies and analysis kits for chemical determinations of sediment extractions	4,200			3,800			8,000	8,000
Travel expenses in Minnesota								
USGS travel	1,600			1,800			3,400	3,400
Shipping	600			400			1,000	1,000
COLUMN TOTAL	**$176,000**	**$0**	**$176,000**	**$121,000**	**$0**	**$121,000**	**$297,000**	**$297,000**

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