Developing Pheromones for use in Carp Control.

Subd.5h $100,000
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07 - 0063

Overall Project Outcome and Results

The common carp (*Cyprinus carpio*) was introduced to Minnesota waters from eastern Europe just over a century ago and has been a problem ever since. This species of fish reproduces in great numbers, is robust, and has the habit of rooting in the bottom for food, thereby degrading water quality in shallow lakes and wetlands. The only technique presently available to control carp is a non-specific poison and barriers, both of which are expensive and ecologically damaging. This project sought to determine whether carp employ specific-specific odors (pheromones) to locate each other and if so, whether these cues might be comprised of bile acids, a class of compounds implicated in pheromonal attraction. Our ultimate goal is develop pheromonal attractants that can be used to catch and remove carp. Both carp and goldfish were used in this laboratory study with the later being used for initial work because it is closely related to carp and more easily tested. We found that immature goldfish are highly attracted to odors released by their own species but not to odors released by six other species of fish we tested. Studies with juvenile carp showed them to also exhibit very strong, specific-specific attraction to conspecific washings. Biochemical studies next found goldfish and carp to both release cyprinol sulfate (CS), taurocholic acid (TCA), taurochenodeoxycholic acid (TCDC), suggesting that while these stimuli may be active they cannot account for the specificity of the cue. Finally, two behavioral studies found that while neither CS nor TCDC is behaviorally active, TCA is weakly attractive to mature fish (especially female goldfish) and stimulates weak food-sampling behavior. We conclude that carp

and goldfish release a potent pheromone which has great potential for use in control which contains non-bile acid components. A new LCMR project is now attempting to identify these component(s).

This project was completed: 6/30/2006

FINAL REPORT

Date of Report: June 30, 2006
LCMR FINAL Work Program REPORT
Date of Work program Approval: November 19, 2003
Project Completion Date: June 30, 2006

I. PROJECT TITLE: Developing Pheromones for use in Carp Control.

Project Manager: Peter W. Sorensen
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Total Biennial LCMR Project Budget:	**LCMR Appropriation:**	**$100,000**
	Minus Amount Spent:	**$100,000**
	Equal Balance:	**$0**

Legal Citation: ML 2003, [Chap. 128], Art 1, Sec.[14], Subd.5h.
$50,000 the first year and $50,000 the second year are from the trust fund to the University of Minnesota for research on new options for controlling carp. This appropriation is available until June 30, 2006, at which time the project must be completed and final products delivered, unless an earlier date is specified in the work program

II. FINAL PROJECT SUMMARY.

The common carp (*Cyprinus carpio*) was introduced to Minnesota waters from eastern Europe just over a century ago and has been a problem ever since. This species of fish reproduces in great numbers, is robust, and has the habit of rooting in the bottom for food, thereby degrading water quality in shallow lakes and wetlands. The only technique presently available to control carp is a non-specific poison and barriers, both of which are expensive and ecologically damaging. This project sought to determine whether carp employ specific-specific odors (pheromones) to locate each other and if so, whether these cues might be comprised of bile acids, a class of compounds implicated in pheromonal attraction. Our ultimate goal is

develop pheromonal attractants that can be used to catch and remove carp. Both carp and goldfish were used in this laboratory study with the later being used for initial work because it is closely related to carp and more easily tested. We found that immature goldfish are highly attracted to odors released by their own species but not to odors released by six other species of fish we tested. Studies with juvenile carp showed them to also exhibit very strong, specific-specific attraction to conspecific washings. Biochemical studies next found goldfish and carp to both release cyprinol sulfate (CS), taurocholic acid (TCA), taurochenodeoxycholic acid (TCDC), suggesting that while these stimuli may be active they cannot account for the specificity of the cue. Finally, two behavioral studies found that while neither CS nor TCDC is behaviorally active, TCA is weakly attractive to mature fish (especially female goldfish) and stimulates weak food-sampling behavior. We conclude that carp and goldfish release a potent pheromone which has great potential for use in control which contains non-bile acid components. A new LCMR project is now attempting to identify these component(s).

IV. OUTLINE OF PROJECT RESULTS:

Result 1: To determine if goldfish and common carp use a taxon-specific attractant.

Two sets of experiments were conducted to determine if goldfish and/or carp are attracted to the odor of conspecifics (individuals of the same species) and/or a variety of other fishes. Experiments employed a matched set of 1.4-meter circular tanks which were partitioned to function as two-choice mazes. Odors were added to one end of these mazes while fish distribution was noted with an overhead video camera and recorded every 15-seconds. Each experiment had at least one 15-minute pre-test period during which time no odor was added which was followed by a 15-minute test period during which time a fish odor was added. Initial experiments employed small groups of three immature goldfish because we found that small groups behaved more naturally than isolated, individual fish. We described responses of these goldfish in the presence of the odor of other goldfish (conspecifics) as well as the odor of 5 other species of fish (common carp, fathead minnows (*Pimpephales promelas*), white sucker (*Catostomus commersoni*), channel catfish (*Ictalurus punctatus*), yellow perch (*Perca flavescens*), and northern pike (*Esox lucius*). Fish odors were made by placing 200 grams of fish into 10 liters of well water for one hour. Food odor (made by placing 10 grams of food into 1 liter of water for 1 hour) was also tested as positive control. Well water (alone) was also tested as a control. To describe and evaluate fish distribution we devised an 'attraction index' which represented changes in the number of times fish were

observed in the side of the maze to which odor was added, before and then after odor addition. Data were analyzed using one way analysis of variance (ANOVA), followed by appropriate post-hoc tests. Goldfish showed no inherent bias in the mazes and while their behavior was not affected by adding well water (control; $p>0.05$), they were strongly attracted to odor of other goldfish ($p<0.0001$) (Figure 1). In contrast, only weak attraction was noted to both the odor of common carp and fathead minnows ($p<0.05$). None of the other fish odors had any apparent effect unresponsive to the odors of the five other species of fish (Figure 1).

Responses of groups of three immature goldfish were also evaluated to the odors of other goldfish, carp, fathead minnows, perch, food, and well water control in 70-liter glass aquaria. In these experiments we sought to determine whether these odors they stimulate social interaction (touching, chasing, swimming rate) or feeding responses. When tested in this manner, goldfish did not respond to any fish cue although they did respond to food odor. The extreme specificity of the attraction response to goldfish odor and its failure to stimulate other behaviors such as feeding leads us to conclude that this odor is an aggregation pheromone.

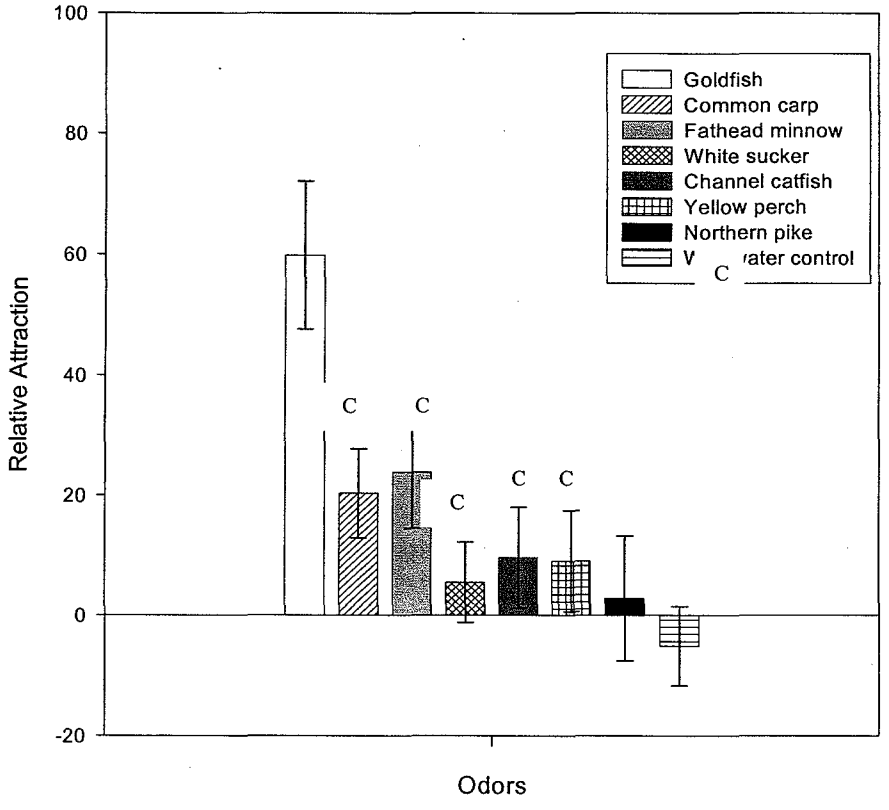

Figure 1. Preferences of goldfish to different fish odors and well water control. Fish odors are arranged relative to their taxonomic relationship to goldfish (more distant relatives are further away). Bars with the different letters differ at $p<0.05$ while those with the same letter do not.

In a second set of experiments we examined responses of juvenile common carp to the washings of common carp, goldfish, fathead minnow, and yellow perch

(the variety of test odors was reduced because of the difficulty of testing carp in the laboratory). Ten groups of four common carp were tested in each. Carp showed no inherent bias in the mazes, were not effected by the addition of well water control ,but were strongly attracted to odor of other common carp ($p<0.0001$; Figure 2). Once again, the odor of the other fish species was without apparent effect while food odor was highly attractive. Together, these results demonstrate that common carp are also attracted to odor of juveniles of their own species and that an aggregation pheromone which differs slightly from that of the goldfish is responsible.

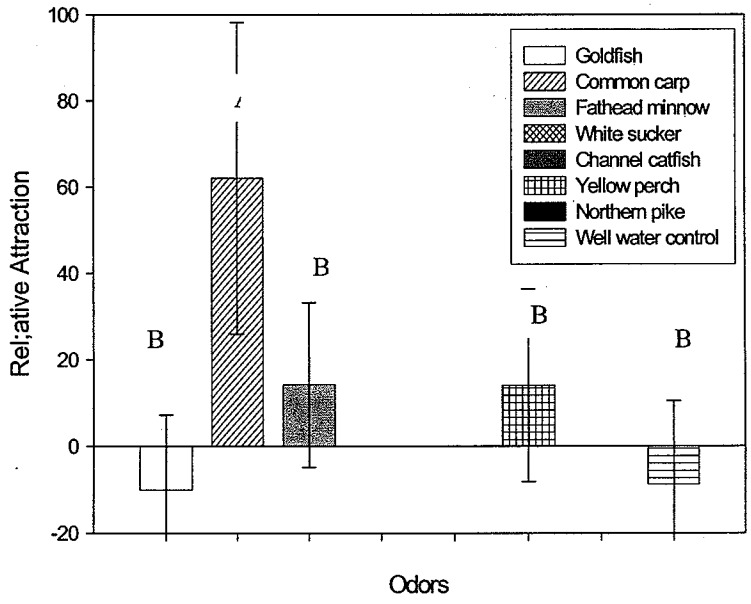

Figure 2: Preferences of common carp to the odor of other common carp, 3 other fish odors and well water control. Fish odors are arranged according to taxonomic status (distance from carp denotes a more distant relationship). Bars with the different letters are statistically different ($p<0.05$) while those with the same letter are not different.

Summary Budget Information for Result 1:	**LCMR Budget**	**$ 42,985**
	Balance	**$ 0**

Result 2: To determine what types of behaviors bile acids stimulate in small groups of fish, thereby establishing the basic behavioral function of bile acids before conducting more time consuming tests of attraction.

This experiment sought to determine the identities of bile acids released by goldfish and carp to determine if these compounds might be the reason that conspecific fish

odor is attractive. This hypothesis was based on half-dozen published accounts that have proposed this based on the unique chemistries of these compounds and their olfactory abilities but have not tested whether they might actually be attractive. We analyzed release waters of both carp and goldfish and then in an initial behavioral test evaluated the primary bile acids stimulate social and/ or feeding behavior of small groups of fish in glass aquaria to these compounds. Thus, this test served as both a prelude to more time-consuming attraction tests (Result #3) and to see whether bile acids alone elicited to same responses noted to whole odors in Result #1.

Fish waters were collected by holding fish in 2 liters of well water for 24 hours, and then extracting them by passing them from activated C18 columns and eluting the columns with methanol (a well established protocol). Extracts were then analyzed using high performance liquid chromatography (HPLC) in conjunction with ion spray mass-spectrometry (ESI-MS). We discovered that goldfish and carp release substantial quantities of the same three bile acids in similar ratios: cyprinol sulfate (CS) and taurocholic acid (TCA), and trace amounts of taurochenodexyocholic acid (TCDC) (Table 1). Approximately ten times more CS was released than any other bile acid by both species, an especially notable finding because we had previously shown this compound to have strong olfactory activity (Sorensen, unpublished results). Although the large quantities of bile acid release suggested that these compounds could have biological/ pheromonal activity, the fact that they were released in similar ratios also suggested that they alone might not be able to explain the activity of conspecific odor because our behavior tests (Result #1) had shown that goldfish and carp and discriminate between each other's odors.

TABLE 1. Composition and quantification (ng/g/hr) of bile acids in fish release water (mean±SE)

					Bile acid release ratio
Species	***n***	***TCA***	***TCDC***	***CS***	***TCA:TCDC:CS***
Carp	4	7.88±2.95	1.63±0.73	68.55±34.11	10: 2: 88
Goldfish	5	1.02±0.47	0.71±0.21	7.51±3.31	11: 8: 81

Three experiments using small groups of goldfish (immature, mature male, and mature female) were conducted to test the behavioral responses of these fish to well water control, CS, TCA, a known sex pheromone (Prostaglandin F2α [PGF2α]; a positive control), and food odor (another positive control). Bile acids were added at a concentration of 5×10^{-7} Molar (M) to these aquaria so that upon final dilution fish were exposed to 5×10^{-9} M. Experiments were conducted in 70 liter aquaria using groups of three male, female or immature fish. We noted changes In various behaviors during a 10 minute pre-test period and then for 20 minutes while an odor were added using a pump. Swimming activity (line crossing), food-sampling, nudging, chasing were noted. Changes in behavioral activity were evaluated using ANOVA. Exposure to TCA stimulated small increases in feeding behavior amongst groups of mature male and female goldfish ($p<0.05$) but had no apparent effect on immature fish (Figure 3). No other behavioral changes (line crossing, pushing, nudging) were noted to either TCA acid or any of the other bile acids in goldfish except for food odor which stimulated increased activity and food-

sampling. Mature males also responded to the sex pheromone with increased activity, chasing and nudging ($p<0.001$; data not shown).

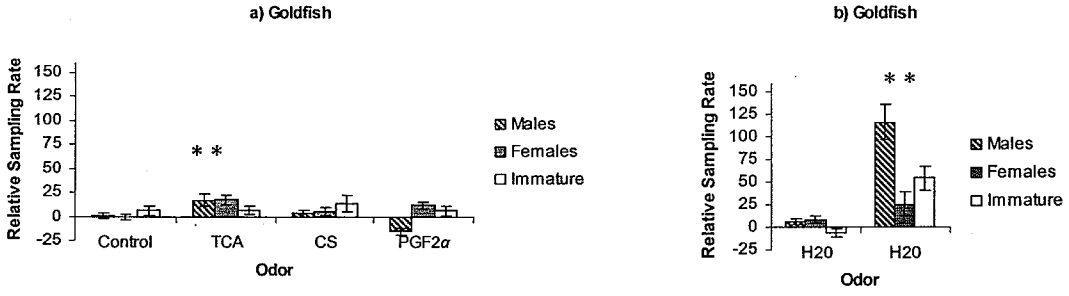

Figure 3: Behavioral responses of male, female, and immature goldfish to: a) well water control, the two most abundant bile acids (TCA, CS) and a sex pheromone (PGF2α); and b) a control and then food odor. This particular figure shows food-sampling behavior, the only responses which were significant. * $P<0.05$

Groups immature carp were tested in a similar manner as goldfish and yielded similar but less pronounced effects. Only mature female carp and immature fish were tested because we only had a few males and the male goldfish had not responded to these cues. Female fish were showed small but non-significant increases in food-sampling behavior to TCA and large changes ($p<0.05$) to food odor alone. No other behavioral changes were noted. Although these results were not inconsistent with the possibility that bile acids function as the primary constituents of the aggregation pheromone, they also provided little evidence about their specific function.

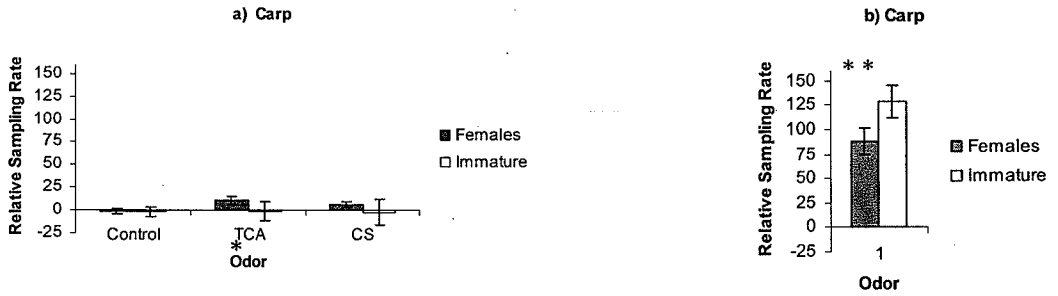

Figure 4. Behavioral responses of mature female, and immature goldfish to: a) well water control, the two most abundance bile acids (TCA, CS); and b) a food odor. This particular figure shows food-sampling behavior, the only responses which were significant. * $P<0.05$

Summary Budget Information for Result 2: **LCMR Budget** **$16,025**
Balance **$0**

Result 3: To determine if particular bile acids are attractive to goldfish and carp

This experiment determined whether the bile acids identified in fish release water were attractive (i.e. functions like crude holding waters). Small groups of goldfish and carp were tested in the same 2.5 Meter circular mazes used in Result #1. We tested cyprinol sulfate (CS), taurocholic acid (TCA), and a mixture of all three bile acids. These compounds were tested at a concentration of 5×10^{-9}M, the same as tested in Result #2 and a concentration thought to be biologically relevant. Positions of fish were noted every 15 seconds before, and then during odor addition, and then compared afterwards ANOVA and if appropriate, paired-t tests. We discovered that mature female goldfish (gonadosomatic index [GSI] = 2.97±1.03) are attracted to TCA ($p<0.05$) and the mixture of all three compounds, but neither CS or TCDC (Fig. 5a). Neither sexually immature (GSI = 0.46±0.20), nor male goldfish (GSI =1.62±0.20) were attracted to CS, TCA, TCDC any mixture thereof. Food odor was strongly attractive to all three groups of goldfish ($p<0.05$; Fig. 5b).

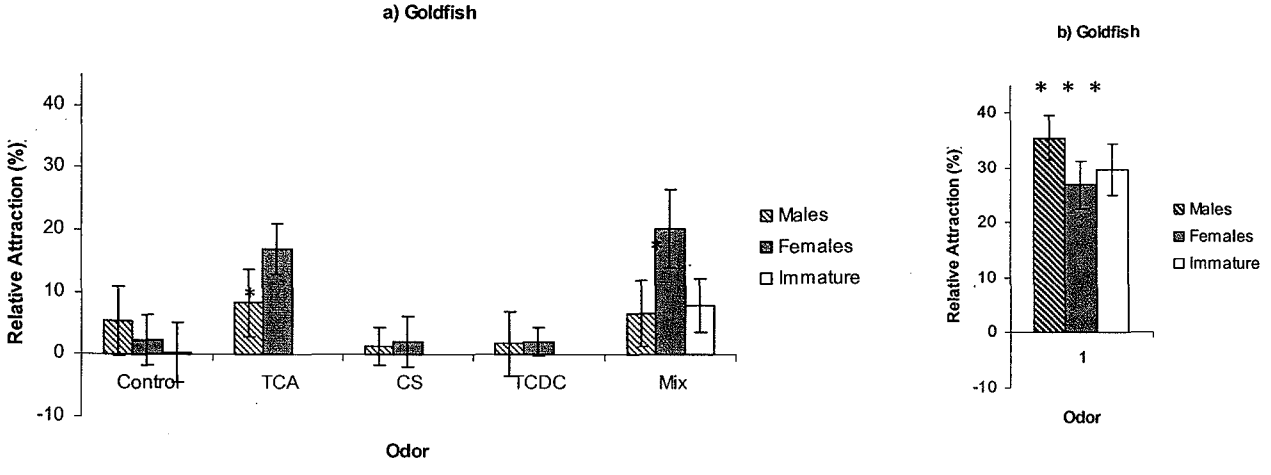

Figure 5. Behavioral responses of mature female, mature male, and immature goldfish to odorants: a) tests to the three bile acids, a mixture of the three, and well water control. b) tests of food odor or control . N= 9 for each; * $p<0.05$.

Groups of five mature common carp (GSI= 4.0±0.01) or three immature carp (GSI= 0.41±0.14) were also tested in the same set of circular mazes used for the goldfish

experiments. Mature male carp were not tested because goldfish experiments showed only females to be attracted. Behavioral responses of these fish were measured to the same stimuli tested for goldfish. Analysis of variance failed to find any significant attraction or repulsion to the bile acids we tested for either mature or immature carp although there were some indications of very weak attraction to TCA (Fig. 6a). This lack of response might be explained either by laboratory stress and/or specific-specific differences in olfactory behavior. However, the strong attraction to food odor ($p<0.05$; Fig. 6b), indicates the latter explanation is more likely. Thus, it appears that bile acids have little, if any, role as pheromones or attractants on common carp behavior. We conclude that while bile acids may function as minor components of the carp aggregation pheromone, they are unlikely to be of major importance. Because bile acids had been a leading candidate for pheromones, our finding is of notable significance, publishable, and will be helpful in ongoing efforts (LCMR 2005 appropriation) which focus on attempting to identify the novel components which must comprise the bulk of the carp aggregation pheromone.

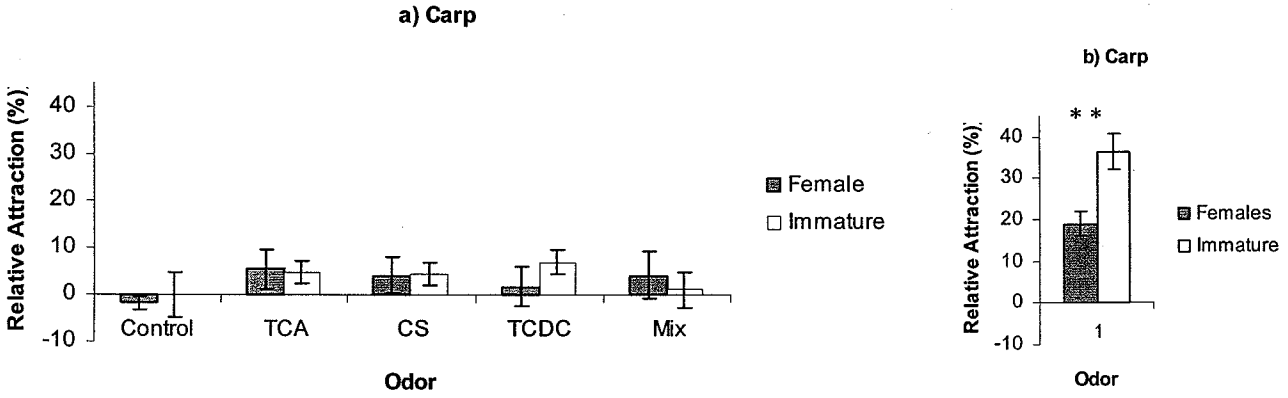

Figure 6. Behavioral responses of mature female and immature carp to bile acid odorants: a) tests to the three bile acids, a mixture of the three, and well water control. b) tests of food odor or control . N= 9 for each; * $p<0.05$.

Summary Budget Information for Result 3:	**LCMR Budget**	**$40,990**
	Balance	**$ 0**

V. TOTAL LCMR PROJECT BUDGET:

All Results: Personnel: $83,605 re-budgeted
All Results: Equipment: $0
All Results: Development: $0
All Results: Acquisition: $0

All Results: Other: $16,395 (office and lab supplies, fish, travel)

TOTAL LCMR PROJECT BUDGET: $100,000

Explanation of Capital Expenditures Greater Than $3,500: none

VI. PAST, PRESENT AND FUTURE SPENDING:

A. Past Spending: none

B. Current Spending: none

C. Required Match (if applicable): none

D. Future Spending: *NEW LCMR*

VII. Project Partners: none

A. Partners Receiving LCMR Funds: none

B. Project Cooperators: The Minnesota DNR is assisting us by providing advise and $45,000 of 'pass through' funds from a U.S. Fish and Wildlife Service grant for exotic species control. These funds are administered as a separate contract to support related, complimentary work on the carp sex pheromones. In addition, we received several donations from lake owners associations and private citizens which have been placed in the University Foundation to help pay for small, unanticipated expenses (ex. undergraduate studies, equipment repair) associated with carp control research.
A note

VIII. DISSEMINATION:

Publications:

1. Sisler, S.P. 2005. Behavioral evidence of aggregation pheromones in goldfish (*Carassius auratus*) and common carp (*Cyprinus carpio*). Masters Thesis, University of Minnesota, St. Paul. MN
2. Sharpe, L. 2006. The production of bile acids by goldfish and carp and their effects of behavior. Masters Thesis, University of Minnesota, St. Paul. MN (scheduled defense date August 25, 2006)
3. Sisler, S, and Sorensen, P.W. in preparation. An aggregation pheromone in the common carp. Environmental Biology of Fishes.

Presentations at Meetings:

1. Sisler, S.P. 2004. Behavioral evidence of aggregation pheromones in goldfish (*Carassius auratus*) and common carp (*Cyprinus carpio*). Midwestern Fish and Wildlife Society meeting, Indianapolis. (Best student poster award)
2. Sisler, S.P. and Sorensen, P.W. 2005. Behavioral evidence of aggregation pheromones in goldfish (*Carassius auratus*) and common carp (*Cyprinus carpio*). Minnesota Chapter of the American Fisheries Society, Bemidji, MN.
3. Sharpe, L. Sherman, M., Fine, J. and Sorensen, P.W. 2006. Bile acids as social cues that could be used to attract and remove an invasive species, the common carp. Minnesota Chapter of the American Fisheries Society, Bemidji, MN.

IX. LOCATION:

St. Paul Campus, University of Minnesota

X. REPORTING REQUIREMENTS:

Periodic work program progress reports will be submitted no later than twice a year, June 30 and December 31.

A final work program report and associated products will be submitted by June 30, 2006.

XI. RESEARCH PROJECTS:

Research Addendum as Attachment B.

Attachment A: Budget Detail for 2003 Projects - Summary and a Budget page for each partner

Proposal Title: Developing pheromones for use in carp control

Project Manager Name: Sorensen.

LCMR Requested Dollars: $ *100,000.*

2003 LCMR Proposal Budget	Result 1 Budget:	Amount Spent *(6/05)*	Balance *(12/05)*	Result 2 Budget:	Amount Spent *(12/05)*	Balance *(12/05)*	Result 3 Budget: *revised 6/15/06*	Amount Spent *(6/13006)*	Balance *(6/13006)*	
	Testing crude odors as attractants			*Testing bile acids as social stimuli*			*Testin bile acids as attractants*			
BUDGET ITEM										TOTAL FOR BUDGET ITEM
PERSONNEL: Staff Expenses, wages, salaries	Grad std: $14,762; P.I.: $4,173; Undergrad: $5,000	23,935	0	Grad std: $7,385; Undergrad: $960	8,345	0	PI, Res Assist, Grad Student 33,550	33,550	0	65,830
PERSONNEL: Staff benefits – *standard benefits for University employees*	Grad std: $9,860; P.I.: $1,335; Undergrad: $105	11,300	0	Grad std: $5,180	5,180	0	PI, Res Assist, Grad Student 4,628	4,628	0	21,108
Contracts										
Professional/technical										
Other contracts										
Space rental:	X			X			X			X
Other direct operating costs *(lab and fish supplies to conduct experiments)*	fish, fish food, and lab supplies: $2,750	2,750	0	fish, fish food, and lab supplies: $2,000	2,000	0	fish, fish food, and lab supplies: $1,776	1,776	0	6,526
Equipment / Tools (small scale electrical devises needed to run behavioral expriments)	pumps: $1,350; cameras: $2,400	3,750	0	0	0	0		0	0	3,750
Other Capital equipment										
Land acquisition										
Land rights acquisition										
Printing							Publication: $0	0	0	0
Advertising										
Communications, telephone, mail, etc.										
Office Supplies *(CDs, paper, printer ink, notebooks, computer software)*	500	500	0	500	500	0	500	500	0	1,500
Other Supplies *(list specific categories)*										
Travel expenses in Minnesota *(travel to collect test fish and related supplies)*	750	750	0	0	0	0	536	536	0	1,286
Travel outside Minnesota *(To attend midwest and national scientific fisheries meetings)*							sci meeting: 0			0
Construction										
Other land improvement *(for what?)*										
Other *(Describe the activity and cost)*										
COLUMN TOTAL	42,985	42,985	0	16,025	16,025	0	40,990	40,990	0	100,000

Notes: June 30 05. PWS The University accounting system does not beak cost down by 'result ' (we have a cumulative estimate) so I have estimated. Also, costs for this summer are not in the system yet so I have estimated them and placed them all into Result 2.
Notes: June 6, 2006 Accounts adjusted to clear up remaining funds following recommendations of Susan Thornton