GREAT LAKES FISHERY COMMISSION

2002 Project Completion Report[1]

Experimental examination of factors affecting polygyny and polyandry of sea lampreys (*petromyzon marinus*) as a means of optimizing sterile female releases

by:

Weiming Li[2], Kim Scribner[2], Mike Twohey[3], and Roger Bergstedt[4]

[2]Department of Fisheries and Wildlife
Michigan State University
East Lansing, MI

[3]US Fish and Wildlife Service
Marquette Biological Station
Marquette, MI

[4]US Geological Survey
Great Lakes Science Center
Ann Arbor, MI

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Completion Report of GLFC Research Project

Title of Project

Experimental Examination of Factors Affecting Polygyny and Polyandry of Sea Lampreys (*Petromyzon marinus*) as a means of optimizing sterile female releases

Principal Investigators and Affiliations

Weiming Li
PERM Scientist
Department of Fisheries and Wildlife
Michigan State University

Kim Scribner
Department of Fisheries and Wildlife
Michigan State University

Mike Twohey
US Fish and Wildlife Service
Marquette Biological Station

Roger Bergstedt
US Geological Survey
Great Lakes Science Center

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Summary

The goal of this study was to determine the feasibility of utilizing sterilized females in the control of sea lampreys, *Petromyzon marinus*. Our primary hypothesis was that male lampreys will expend at least part of their energy, time, and gametes by mating with introduced sterile females, resulting in a reduction in the number of viable progeny produced by resident females. It was further postulated that the extent to which the offspring numbers would be reduced should depend on the density and sex ratio of spawning populations after sterile females are introduced.

To test this hypothesis, we proposed to integrate a series of behavioral experiments in conjunction with innovative molecular techniques to accomplish our objectives. In particular, two sets of experiments were to be conducted. The first step was to develop microsatellite markers to facilitate identification of lineage, thus enabling estimation of means and variance of levels of polygamy and polyandry in spawning populations. The second step was to develop a practical protocol or technique that would allow for observations of lamprey behavior and reproductive fitness under different levels of treatment, thus determining the influence of density and sex ratio on lamprey mating behavior in a quasi-natural condition. Based on the anticipated results, we would determine whether the relative number of offspring produced per non-sterile female is reduced in response to the introduction of sterilized females.

We have succeeded in developing genetic markers useful for both construction of lamprey genealogy and studies of population dynamics, and the results have been accepted for publication in Conservation Biology. In the past three years, we have attempted to establish protocols for the second set of experiments, and have been able to successfully contain lampreys in netted enclosures, or sections in streams. However, extensive mortality caused by stress, predation by wildlife, and unfortunate weather conditions has limited our success in colleting samples to complete this set of experiments. The limited data obtained in this study suggest that male sea lampreys may mate with numerous females when sex ratio is skewed toward females. Here we will provide a brief introduction to describe the rationale for this project, a description of the development of the molecular markers, a chronicle of attempts to observe lamprey behavior in enclosures and sectioned streams, and a discussion of collateral benefits and lessons learned from this project.

I. Introduction

In the past two decades, chemical sterilization techniques have been developed for adult sea lampreys (Hanson and Manion 1980) and applied in a sterile male release program (GLFC 1990). In this program, male lampreys are captured and sterilized with an intraperitoneal injection of bisazir (P,P-bis(1-azirindinyl)-N-methylphosphinothioic amide) and released into lamprey spawning streams. Initial studies showed that sterilized males competed successfully with resident males in mating, thus reducing the number of viable eggs (Hanson and Manion 1978). In 1991, the GLFC initiated an experimental program to determine the utility of this technique in an integrated management approach for sea lamprey. Preliminary data suggests that sterile male release is an environmentally safe and technologically feasible method that does reduce the number of viable offspring by the time sea lamprey eggs hatch (Schleen et al. 1996).

Currently, the limited number of males available for sterilization is a major factor limiting the effectiveness of the sterile male release technique (Sterile Male Release Technique Task Force 1998). To further enhance the effectiveness of this program, and to extend its application to a greater number of streams, the Sterile Male Release Technique Task Force recommended exploring the feasibility of implementing a sterile female release program. The task force further recommended that studies be conducted to determine the extent of polygamous mating in sea lampreys and the influence of polygamous behavior on the reduction of progeny number in the context of a sterile female release program.

The use of sterile females was suggested as a possible means to decrease the reproductive success of resident females under the assumption that males will expend part or all of their energy, time, and gametes by mating with introduced sterile females. There are two primary requirements for the use of sterilized animals before the strategy can be successfully implemented. The first requirement is a sterilization technique that does not reduce mating competitiveness (Knipling 1964). This requirement can be met because female lampreys sterilized with a protocol identical to that for male lampreys have been found to compete successfully with non-sterile females in mating (Hanson and Manion 1978; 1980). The second requirement is that the introduction of sterilized animals must result in an actual reduction of viable offspring. This requirement has not been addressed in the context of the proposed sterile female lamprey release program (The Sterile Male Release Technique Task Force 1998). Introductions of sterile females have an increased chance in resulting in lower numbers of offspring because sea lampreys allocate all their reproductive effort to a single bout of reproduction and then expire (Hardisty and Potter 1974). Spermatogenesis is a single synchronous event in this species. Since released sterile females compete for mates as well as non-sterile females (Hanson and Manion 1978), male lampreys likely will expend part of their energy, time, and gametes by mating with introduced sterile females. Whether the observed behavior of males will result in a reduction in the number of viable progeny produced by non-sterile females, however, requires experimental testing. It is essential to understand the mating behavior of sea lampreys when large numbers of potential mates are introduced. The goal of this study was to establish a model that predicts the optimum strategy and tactics for release of sterile female lampreys.

We proposed a two-step approach to address our objectives. The first step was to develop microsattelite markers to facilitate identification of lineage, thus enabling estimation of mean and variance of levels of polygamy and polyandry from samples of spawning populations.

The second step was to develop a practical protocol or technique that would allow for observation of lamprey behavior under various treatments in a controlled setting, and use this protocol to determine the variables that influence lamprey mating behavior in a quasi-natural condition. We would then determine whether the relative number of offspring produced per resident female is reduced in response to the introduction of sterilized females and whether this reduction is consistent with that predicted from results of enclosure experiments.

II. Development of microsatellite markers

In order to complete our objectives, it was first necessary to have a method to identify parentage of prolarvae in an experimental arena, which would allow an estimation of the mating and reproductive success of subjects. Genetic markers were developed based on conserved core sequences within tandem-repetitive regions of DNA (microsatellites) dispersed in the genome. Our effort resulted in development of ten markers; six based on novel microsatellite loci from *P. marinus* and four based on optimized PCR amplification using hetero-specific primers.

Novel markers were developed using modifications of the library enhancement method of Ostrander et al. (1992). The DNA from positive clones was sequenced using a di-deoxy random termination method using T3 and T7 fluorescently labeled primers (Epicentre). The fragments were separated on a 6% denaturing polyacrylamide gel and visualized using a FM-BIO II machine (Hitachi Corporation). Additionally, libraries enriched for repeat motifs of [AAG], [AAT], [ATG], [TAGA], [CATC], and [TACA] were synthesized and positive clones were sequenced by Genetic Identification Services (Chatsworth, CA). Inserts containing suitable repeat sequences were selected and appropriate primers were designed using OLIGO version 2.0 (Rychlik and Rhoads, 1989). Hetero-specific primers previously described in teleost fishes and amphibians were randomly searched (N = 39 primer pairs). Loci found to amplify *P. marinus* DNA were tested for variability.

DNA was extracted from fin clips and prolarvae stored in 95% ethanol using Qiagen DNeasy kits (Valencia, CA), as it was determined that DNA purity is important for good gel resolution. Microsatellite loci cloned from *P. marinus* and other hetero-specific loci were amplified by polymerase chain reaction (PCR) in 25 µL reaction volumes containing 100 ng of template DNA, 0.25 units of Taq polymerase, 400 pM each forward and reverse primer, 80 µM dNTPs, and PCR buffer containing (final concentrations) 10 mM Tris-Cl pH 8.3, 50 mM KCl, 100 µg/mL gelatin, 0.01% NP-40, and 0.01% Triton-X 100. The exceptions to these conditions were Pmaµ 4, which contained 120 µM dNTPs and 600 pM primers, and Pmaµ 1, which contained 160 µM dNTPs and included 2% DMSO. The magnesium concentrations in the reaction mix and the annealing temperatures differed for each locus (Table 1, in Appendix).

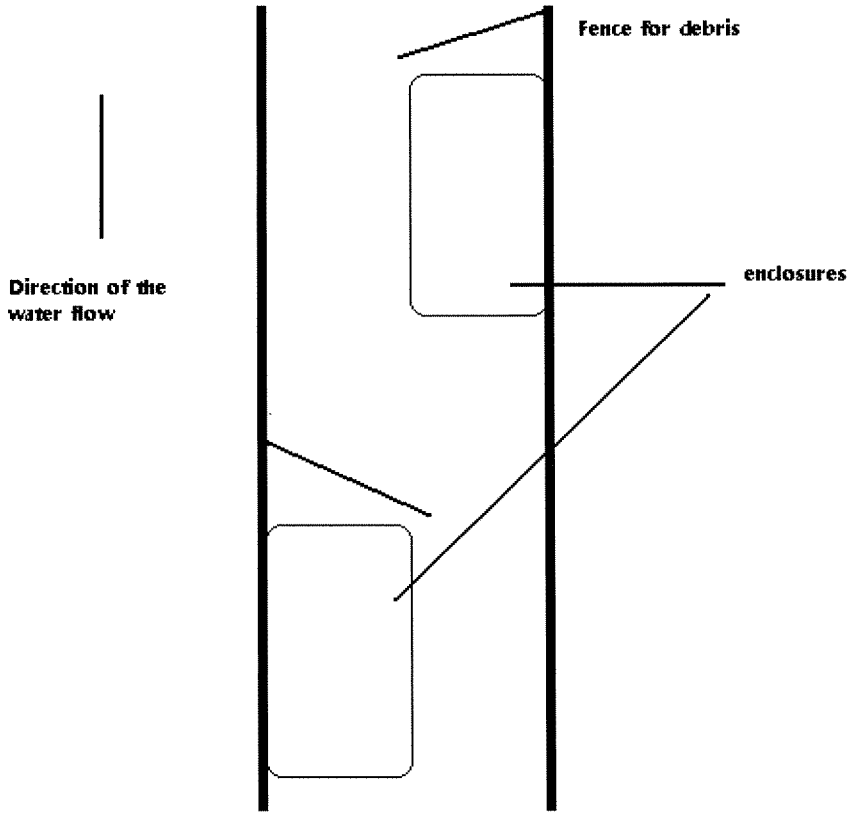
Mendelian inheritance was confirmed for all ten loci using parents and progeny from controlled matings. A minimum of two full-sibling families of more than 30 progeny was employed for all loci. Pair combinations of loci were tested for genetic disequilibrium using Genepop version 3.1b (Raymond and Rousset 1995). After applying a Bonferroni correction for multiple comparisons (Rice 1989), there was no evidence for statistically significant linkage between loci. Estimates of genetic diversity and tests for deviations from Hardy-Weinberg equilibrium were determined for each locus using Cervus version 2.0 (Marshall et al. 1998) using 82 unrelated individuals. Over all loci, the estimates of the mean number of alleles per locus (A) was 3.3, mean expected heterozygosity (H_e) was 0.508, and the mean polymorphic information

content (PIC) was 0.436. The tests for deviations from Hardy-Weinberg equilibrium were conducted using a chi-square goodness-of-fit test, and none of the loci exhibited significant deviations. Some primer pairs amplified DNA from native lamprey species from the Great Lakes region, such as northern brook lamprey (*Ichthyomyzon fossor*); silver lamprey (*Ichthyomyzon unicuspis*); American brook lamprey (*Lethenteron appendix*). A detailed description of characteristics of individual loci is available in Bryan et al. (2002).

III. Efforts to observe sea lamprey behavior in enclosures and sections in spawning streams

Experiments in 1999

In the summer of 1999, we attempted to observe spawning behaviors of sea lampreys contained in enclosures illustrated in Figure 1, constructed in the Rock River, Michigan. Plastic mesh was attached to fence posts and formed three walls of the enclosure, with the bank forming the fourth



The first two attempts to contain sea lampreys in these enclosures were not successful because a storm resulted in severe flood conditions in the stream. The water level rose

approximately 2 feet above the top of the enclosures. Later in the season, when the water levels stabilized, we noticed that, as the bottom of the stream is very rocky, it would be impossible to contain lampreys efficiently given our current design. It became apparent that, if there was a hole in any part of the mesh, or if the mesh was not sealed completely to the stream bottom, lamprey would manage to find the gap and escape. We also noticed that predation by mammalian wildlife was severe.

In the fall of 1999, we focused on improving the project by selecting a more suitable study site and designing a better enclosure. We found our previous site, the Rock River, to be too deep, fast, and rocky, all of which were contributing factors in our inability to hold the lampreys in our enclosures. After meetings with lamprey control agencies and consulting with experienced lamprey biologists, we produced a list of candidate streams, and walked through each of these streams to select a new site. The North Branch of the White River was selected because it was a historic lamprey spawning ground, but was shallower and slower than the Rock River, and the bottom substrate is primarily gravel and sand.

Furthermore, we thoroughly redesigned our enclosures. After working out several designs on paper, we field-tested two different enclosure designs in streams; one enclosure type is more natural while the other is more secure. The first (referred to as the "net enclosure"; Figure 2) consists of nylon net supported by fence posts. Heavy chain is tied to the bottom of the net, which is jet-pumped five inches underground using a water pump. The bottom of the enclosure is further reinforced using screw anchors and tent stakes. This enclosure surrounded approximately 250 square feet of spawning habitat. Refugia, such as cement blocks, pipes, and plywood covers, were placed in the enclosure. The top is covered with mesh netting to deter predation. This type of enclosure allows for the most natural environment.

Figure 2. The net enclosure.

The second type of enclosure (referred to as the "cage enclosure"; Figure 3) consisted of a cage with an open top constructed of plastic mesh with an angle iron frame. The bottom (also constructed to mesh) was covered with a heavy cloth to prevent eggs from falling through the bottom. The cage enclosure was placed in the stream and the bottom is filled with 5-6 inches of gravel. Lampreys should spawn in this gravel as they have been found spawning in gravel added to streams during the construction of highways or railroads. The sides were supported with fence posts. This structure contained 120 square feet of spawning habitat. Again, refugia were provided and the top is covered with mesh to prevent predation. This type of enclosure is more secure, but is a less natural environment. Both types of

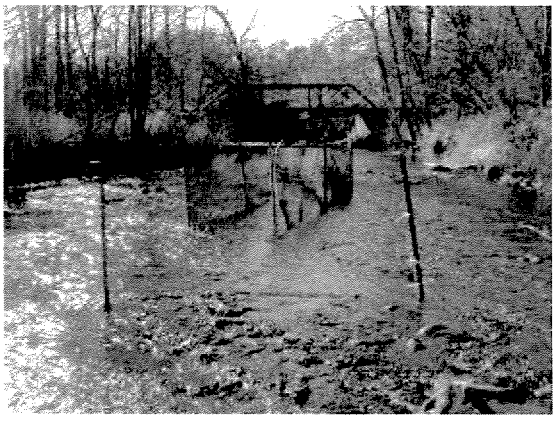


Figure 3. The cage enclosure.

new enclosures were tested for a week in the fall of 1999 to insure that both structures would maintain their integrity in a field situation before using them in the coming summer.

There were a few main differences between the enclosures used in the summer of 1999 and the enclosures we designed for the summer 2000. First, the new enclosures had walls sealed to the ground to prevent the lampreys from escaping as they had done the previous year. Second, the new enclosures were four-sided. The 1999 enclosures had three sides and used the bank as a fourth, which may have contributed to the predation problems and to escapes at high water levels. Finally, the new enclosures were structurally more secure than the older ones.

Experiments in 2000

In the summer of 2000, we began transporting materials to the field site at the end of April. Construction of the enclosures began in the week of May 8. The 12 net enclosures contained lampreys efficiently; close to 100 % of stocked animals were accounted by the end of each experiment. They also maintained structural integrity through flood conditions. Lampreys were stocked in all twelve enclosures, with 24 lampreys per enclosure, on June 8. We began finding a large number of dead lampreys in the enclosures seven days after stocking. On June 16 we removed the remaining lampreys from the first stocking attempt. After consulting with control agencies (USFWS and DFO Canada), we thought that the most probable reason for the high mortality was stress. On the same day DFO personnel delivered lampreys captured in the St. Mary's River, and they were stocked immediately. For this stocking attempt, new refuge was added to the enclosure, and the animals were handled in a less stressful manner. They were placed in oxygenated water throughout the stocking procedure. By June 22 there were massive mortalities, and by June 27 there were only a few animals left in each enclosure.

On June 27 we restocked six of the enclosures, again with 24 lampreys per enclosure. For this stocking attempt, we tried to reduce the stress level of the lampreys even further. We did not tag, weigh or measure the animals; the only handling involved taking a fin clip and transporting the animals to the enclosures in oxygenated water. We also added more types of refuge. By July 3, we again began to see a substantial number of mortalities.

After discussing our options on June 30, we decided to remove the lampreys currently in the enclosures, and restock with new animals. We received lamprey from the St. Mary's River on July 5 and began restocking six enclosures immediately. We injected each lamprey with GnRH in order to induce reproductive maturity. The animals were tagged, but we attempted to keep their stress level minimized otherwise. The refugia added to the enclosures during past stocking attempts were maintained. By July 16 less than one-third of the animals were still alive.

Throughout the duration of the project, we observed other fish in the river. Two native lampreys were observed on one occasion at the beginning of June. The lampreys in the enclosures were never seen exhibiting nesting or mating behavior.

Experiments in 2001

In light of the difficulties we had observing spawning behavior and collecting embryos in enclosures, we held a meeting at USGS Lake Huron Biological Station in December 2000 to explore options for conducting this research in a more natural setting. The attendees for the meeting included all principal investigators and several biologists from USFWS. Numerous

ideas were discussed and the merits and potential disadvantages of each were compared. These ideas were:

- Use whole streams for treatments. This affords less control, but it is more natural and has a good chance of success. Finding enough streams within a workable distance, however, is difficult. The possibility of using long term study streams was discussed (Stokely, Carp, Big Carp, Rock, Salmon Trout, Falls, Silver, and the two branches of the Huron) but again the distances among these streams are too great for our summer crew.
- Conduct the study in hatcheries. There may be some hatcheries with available raceways. Possibilities include hatcheries in Minneapolis, Wolverine, Oden, Bear Creek, and Pere Marquette. This may require a large effort to modify raceways, and the setting is not natural.
- Divide the Garlic River into sections, and use each section to host a stocking treatment.

The last option was deemed most favorable because each section is large enough to allow a natural setting for spawning lamprey. This stream was the initial stream used for the evaluation of sterile male releases (Hanson and Manion 1980) and thus has proven to have a reasonable chance of success. We essentially adopted the design that Hanson and Manion used for their study of sterile male release. For each of the river sections, a waterfall prevented upstream migration, and fyke nets and lampreys traps were used to prevent downstream migration. Behavioral observations, nest monitoring and sampling, and genetic identification of offspring were conducted as described in the original proposal. At the meeting we also proposed that Lee Hanson be hired as a consultant to assist in conducting the summer field experiments. Hanson developed expertise in field observation of lamprey reproductive behaviors during his pioneer study of sterile male release techniques. This proposed amendment was approved by the GLFC.

In April 2001, Lee Hanson participated in a three-day site evaluation trip to the Big Garlic River. Then, from June 1 to July 15, he helped organize experiments in the Big Garlic River. During this period of time, he was responsible for the aiding with daily operations in the Big Garlic River, including constructing study sites, stocking animals, observing spawning behavior, collecting embryos, etc. He trained and supervised student interns on the methods needed to conduct the study, and consulted with Mara Bryan, the graduate student who implemented this project.

The objective for the summer of 2001 was to examine how increased female to male sex ratios affect reproductive success in the sea lamprey. The Big Garlic River was divided into sections, and stocked with 100 adults at female: male ratios of 1:1, 4: 1, 8:1, and 16:1. Daily behavioral observations were taken, and nests were evacuated when the prolarvae reached stages 13-14. Adult fin clips and prolarvae were brought back to Michigan State University for genetic analysis, so that the parentage of a random sample of prolarvae from each nest could be determined.

Unfortunately, we again experienced several weather-related difficulties. In the first half of June, the Big Garlic rose by over a foot on two separated occasions. Each time, all of the fyke nets and barriers that had been erected were severely damaged and needed repairs. By the time the repairs from the second flood had been completed, there were no lampreys available in good condition, so stocking was delayed for a week to wait for lamprey from the St. Mary's River to become available. As a result, the stream was not stocked until June 22; at this time the stream

temperature was 10° C. On June 27 we began to notice very high mortalities. After checking the thermograph, it was determined that the river temperature peaked at 23° C on June 26, and therefore the temperature change was probably the cause of the mass mortalities.

As a result of these difficulties, the anticipated results were not obtained. Out of the four sections in the river, we only found nests in the upstream two sections. It is believed that the reason for this is that upstream areas of the Big Garlic have historically been 1-2° C cooler than the downstream areas; therefore, the mortality rates were lower in the upstream sections. We found three nests in the 8:1 female-to-male ratio section (second from upstream) and two nests in the 16:1 female-to-male ratio section (farthest upstream). Animals were only observed on two of these nests even though the river was monitored daily; three of the nests were apparently built, spawned in, and abandoned in one night.

Microsatellite markers were used to determine parentage. To obtain preliminary results, DNA was extracted from fifteen pro-larvae from each nest, but because of the low amounts of DNA in pro-larvae not all of the selected progeny could be analyzed. In the table below parents identified with 50% confidence or greater are listed.

Nest	Sex Ratio	Prolarvae analyzed	Adults observed on nests		Genetically determined parents	
			Males	Females	Males	Females
4-1	8:1 F	15	1	2	2	3
4-2		15	0	0	Unable to determine	6
4-3		15	0	0	2	2
5-1	16:1 F	13	1	9	2	4
5-2		15	0	0	2	4

At the time the first nest was built, in section 4 there were 4 males and 48 females in and in section 5 there were 4 males and 64 females that had not been found dead, and could therefore be considered as possible parents. Unfortunately, due to the low levels of genetic variation found thus far in landlocked sea lamprey, the markers are not powerful enough to distinguish definitively among high numbers of possible parents, so the data presented is only preliminary. Of the two nests for which spawning was observed, both had polygamous groups. The first nest observed in the 8:1 F section had one male and two females actively spawning, and the first nest observed in the 16:1 F section had one male and nine females actively spawning. However, our molecular studies indicate that there is a good chance that a second male participated in both nests, and that different numbers of females participated than observed.

These results collaborate with those from a recently completed GLFC project lead by Kim Scribner and Mike Jones. The aim of that project was to characterize larval dispersal and adult reproductive success. In that study, adult sea lampreys were released into spawning streams at a female to male ratio of 1:1. It was evident that polygyny and polyandry were common among adults. Most adults spawn with more than one mates, and some with more than three or four mates. From the limited data we collected, it is likely that multiple males and females participate in spawning activities in most nests. Further, one male mated with up to nine females at a sex ratio extremely skewed toward females. It is possible that when large numbers of sterilized females are introduced into a stream, the average number of mates for males would increase substantially.

IV Discussion

This project supported our effort to develop genetic markers that are useful for constructing genealogical relationships in the sea lamprey. These markers have been applied in studies of mating systems of the sea lamprey. One characteristic of these microsatellite loci, which we noticed very early in our study, is that they have unusually low numbers of alleles in samples collected from the Great Lakes. Although this feature limited the utility of these markers in construction of parentage relationships among large numbers of potential adults, it prompted us to wonder whether this low level of genetic diversity observed in our sea lamprey samples is unique to the Great Lakes, or is a characteristic of all the sea lampreys around the world. In the past two years, we have collected tissues samples from the Great Lakes region (17 streams), Lake Champlain (14 streams), the finger lakes region (1 stream) the Atlantic coast of North America (5 streams), and from Portugal (1 stream). We are in the process of estimating allelic frequencies of microsatellite loci from all these samples. The results from this analysis will be a collateral benefit that may allow us to provide very useful information on the initial invasion of the Great Lakes by the sea lamprey.

During the three years when field experiments for this project were conducted, we experienced difficulties caused by factors beyond our control, such as heavy rains, severe floods, water temperatures close to the lethal temperature for the sea lamprey, and predation of test subjects by wildlife. It appears that, in planning of experiments that require well-controlled treatment levels of animals in natural or quasi-natural settings, sufficient time should be allowed to account difficulties from unpredictable factors. It also became apparent to us that this type of project requires close collaboration between universities and lamprey control agencies. We received extensive support from both USFWS Marquette Biological Station and Canada DFO sea lamprey control station at Sault Ste. Marie. They provided extensive logistic support, shared information, advised on methods of transporting sea lampreys, and trained our students on collection of pre-larval samples. Their support enabled us to collect the data reported here. In the future, when this type of project, which demands the research team to integrate extensive experience in field work and laboratory skills for molecular biology, are proposed, it might be prudent to design the project into two parts; the field experiments lead by lamprey control agencies and laboratory work lead by university personnel.

In this project we attempted to construct enclosures to allow observation of lamprey behavior and mating strategy in a quasi-natural environment and, for our objectives, test the effects of different treatment levels of well-controlled sex ratios and density. If successful, this approach would enable collection of very useful information for applications of sterile male and sterile female release techniques. Through trial and error, we have developed designs of enclosures that contain lampreys efficiently and maintain structural integrity in floods. However, we have not been able to reduce the mortality of stocked subjects to a sufficiently low level that allows us observe behaviors and collect embryo samples. Although this excessive mortality may be due to stress, we have no method to confirm this possibility because the stress response and its consequences in the sea lampreys are virtually unknown. It would be useful to develop a basic understanding of lamprey stress hormones and stress reactions. This information would be useful to monitor the stress levels of the sea lamprey during migration, encountering barriers, captivity, and handling for sterilization and release.

References Cited

Bryan, M.B., S.V. Libants, J.A. Warrillow, W. Li, and K.T. Scribner. Polymorphic microsatellite markers for the sea lamprey, *Petromyzon marinus*. Conservation Genetics, in press.

Great Lakes Fishery Commission. 1990. Integrated Management of Sea Lamprey Vision Statement. Ann Arbor, Michigan.

Hanson LH, Manion PJ (1980) Sterility method of pest control and its potential role in an integrated sea lamprey (*Petromyzon marinus*) control program. *Can. J. Fish. Aquat. Sci.*, **37**, 2108-2117.

Hanson, L. H. and Manion, P. J. 1980. Sterility method of pest control and its potential role in an integrated sea lamprey (*Petromyzon marinus*) control program. Can. J. Fish. Aquat. Sci 37: 2108-2117.

Hanson, L. H. and P. J. Manion. 1978. Chemosterilization of the sea lamprey (Petromyzon marinus). Great Lakes Fis. Comm. Tech. Rep. 29: 15p.

Hardistry, M. W. and Potter, I. C. 1971. The general biology of adult lampreys. In: The Biology of Lampreys, vol. 1, edited by: Hardistry, M. W. and I. C. Potter, Academic Press, New York, pp.127-206.

Knipling, E. F. 1964. The potential role of the sterility method for insect population control with special reference to combining this method with conventional methods. U.S. Department of Agriculture, Agricultural Research Service Report ARS-33-98.

Manion, P. J. and Hanson, L. H. 1980. Spawning behavior and fecundity of lampreys from the upper three Great Lakes. Can. J. Fish. Aquati. Sci 37: 1635-1640.

Ostrander, E., P.M. Jong, J. Rine, and G. Duyk. 1992. Construction of small-insert genomic DNA libraries highly enriched for microsatellite repeat sequences. Proceedings National Academy of Sciences (USA). 89:3419-3423.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223-225.

Raymond M, Rousset F (1995) GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenicism. *J. Heredity*, **86**, 248-249.

Rychlik W, Rhoads RE (1989) A computer program for choosing optimal oligonucleotides for filter hybridization, sequencing and in vitro amplification of DNA. *Nucleic Acids Research*, **17**, 8543-8551.

Schleen, L. P. , R.S. Young, and G.T. Klar (editors). 1996. Annual Report to the Great Lakes Fishery Commission, June 4-6, 1996. In . Integrated Management of Sea Lampreys in the Great Lakes 1995. Pp54-57.

Sterile male release technique task force. 1998. Potential for use of female lampreys in the sterility programs.

Appendix 1: Information regarding microsatellite loci

Table 1. Characteristics of sea lamprey and hetero-specific microsatellite loci including repeat motif, primer sequences, annealing temperature (T_m, °C), magnesium concentration ($MgCl_2$, mM), expected size of amplified fragments (bp), number of alleles (A), observed heterozygosity (H_o), expected heterozygosity (H_e), polymorphic information content (PIC), the number of individuals used in these analyses (N), and the GenBank Accession number. The repeat motifs for the hetero-specific loci have not been listed explicitly, as it is unknown how many repeats the fragments contain in sea lamprey. Atr14 was originally developed for use in tailed frog, *Ascaphus truei* (Scribner, unpublished data), FGT3 was developed for use in rainbow trout, *Oncorhynkus mykiss* (Sakamoto et. al 1994), Gac9 was developed for threespine stickleback, *Gasterosteus aculeatus* (Taylor 1998), and Spl120 was developed for shovelnose sturgeon, *Scaphirhynchus platorhynchus* (McQuown et. al 2000).

Locus[a]	Repeat motif	Primer Sequences (5'-3')	T_m	$MgCl_2$	Exp. Size	A	H_o	H_e
***P. marinus*-specific**								
Pmaμ 1	$[GA]_3GG[GA]_8$	F: TAG CAC GAC GTA AAG AAT GAC C R: GAA CTC CTC GTA GCC AGC AC	50	1.5	129-134	5	0.646	0.640
Pmaμ 2	$[GA]_9$	F: GGG TGG CAG TGT GGT AAT R: GCT ATC TTT TCT CCA CCC A	48	2.0	99-101	2	0.476	0.503
Pmaμ 3	$[GA]_7$	F: CCG AGA AGG GAG AGG CAG R: CCC TGT CCA TCA CTC TCA CT	52	1.5	224-229	2	0.235	0.280
Pmaμ 4	$[CA]_4$ $[CCA]_2[CA]_7$	F: TGG GAA TCA AAC TCG GGT R: TTT AGG CGC TGC ATT TTC	56	1.5	154-160	4	0.704	0.619
Pmaμ 5	$[TC]_7AC[TC]_3$	F: CAA CGT CAA GAG GGG AAT R: GGT GAG ATA GCG TAA AAT AAA GAG	54	1.5	136-141	2	0.378	0.377
Pmaμ 6	Highly interrupted $[CA]_N$	F: GGT CGC CAG GTT CAT AG R: CAC TTC ACA TGG CCT CAA	62	1.5	229-271	8	0.747	0.807
Hetero-specific								
Atr14	$[GA]_N$	F: TAA TCA TTC TCT GTG TTG TTG G R: ACA ATA CAC AAC AAG TCT CTC AGT	46	2.5	136-139	2	0.439	0.460
FGT3	$[CA]_N$	F: CAA GAA ATT TGT GGA GCG G R: GAA GCC CTG TTT CAG TTT TAG C	46	3.5	254-257	2	0.293	0.268
Gac9	$[CA]_N$	F: GAA ACT GGT TTG ATG ACC GGA G R: CAG ATT GTC AGA TCA CGG AGA TA	46	2.5	249-258	2	0.654	0.650
Spl120	$[TATC]_N$	F: ATT CCA TGA GCA ACA CCA CA R: TGA TGG TCT GAT GAG ATC GG	46	3.5	178-180	2	0.481	0.478

[a]When used as heterologus primers in other lamprey species (northern brook lamprey, *Ichthyomyzon fossor*=1; silver lamprey, *Ichthyomyzon unicuspis*=2; American brook lamprey, *Lethenteron appendix*=3; a=number of alleles, sample sizes for each species provided in the text) amplification products of size consistent with *P. marinus* were observed. Pmaμ1: 1 (a=4), 2 (a=3), 3 (a=3); Pmaμ2: 1 (a=3), 2 (a=1), 3 (a=1); Pmaμ4: 1, 3 (all a=1); Pmaμ5: 1, 2, 3 (all a=2); Atr14: 1 (a=1), 2 (a=1), 3 (a=2); Gac9: 1 (a=2), 2 (a=1), 3 (a=1).