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Research to Support Sterile-male-release and Genetic Alteration Techniques for Sea Lamprey Control

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ABSTRACT. *Integrated pest management of sea lampreys in the Laurentian Great Lakes has recently been enhanced by addition of a sterile-male-release program, and future developments in genetic approaches may lead to additional methods for reducing sea lamprey reproduction. We review the development, implementation, and evaluation of the sterile-male-release technique (SMRT) as it is being applied against sea lampreys in the Great Lakes, review the current understanding of SMRT efficacy, and identify additional research areas and topics that would increase either the efficacy of the SMRT or expand its geographic potential for application. Key areas for additional research are in the sterilization process, effects of skewed sex ratios on mating behavior, enhancing attractiveness of sterilized males, techniques for genetic alteration of sea lampreys, and sources of animals to enhance or expand the use of sterile lampreys.*

INDEX WORDS: *Sea lamprey, sterile male, genetic, pest management.*

INTRODUCTION

The parasitic sea lamprey (*Petromyzon marinus*) has been a serious pest since its introduction into the Great Lakes, where it contributed to severe imbalances in the fish communities by selectively removing large predators (Smith 1968, Christie 1974, Schneider *et al.* 1996). Since the 1950s, restoration and maintenance of predator-prey balance has depended on the Great Lakes Fishery Commission (GLFC) sea lamprey management program. Initially, management relied primarily on stream treatments with a selective lampricide to kill larvae, barriers to migration, and trapping to remove potential spawners (Smith and Tibbles 1980). By the late 1970s, however, the future of sea lamprey management clearly lay in development of a larger array of control strategies, including alternatives to lampricide applications (Sawyer 1980).

The only new alternative to chemical control to reach operational status is the release of sterilized male sea lampreys. Research on the concept began at the U. S. Geological Survey (USGS), Hammond Bay Biological Station in Millersburg, MI (HBBS) during the 1970s (Hanson and Manion 1980). Development and evaluation continued through the 1980s, and led to the release of sterilized males in Great Lakes tributaries since 1991 (Twohey *et al.* 2003a).

The purpose of this paper is 1) to review the implementation and evaluations of sterile-male-release technique (SMRT) as it is being applied against sea lampreys in the Great Lakes, 2) to review our current understanding of its efficacy, and 3) to identify additional research areas and topics that would increase either the efficacy of SMRT or expand its geographic potential for application.

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STERILIZATION TECHNIQUE

Theory

Edward Knipling proposed using sterilization to control or eradicate insect pests in 1937 (Baumhover 1966, Knipling 1968). When integrated with a pesticide control program, the combination was more effective than either approach alone, and his models demonstrated that the sterilized animals become increasingly effective as the natural population declines (Knipling 1965). The first successful use of the method was in 1954 to eradicate the screwworm fly (*Cochliomyai hominivorax*) from the island of Curacao in the West Indies (Baumhover *et al*. 1955). The screw-worm fly was subsequently eradicated from Florida and the southeastern United States (Knipling 1960). Sterile insect release has since been used to control insect pests in a number of instances worldwide (Curtis 1985).

Success of sterile-male releases depends on two requirements. The first is development of a sterilization technique that does not reduce survival or mating competitiveness of the sterilized animals (Knipling 1965). Second, density-dependent changes in growth or survival (compensation) must not offset reductions in the number of offspring achieved by SMRT (Knipling 1965, Jones *et al*. 2003). While focusing on the potential of SMRT for sea lamprey management, the GLFC's SMRT Task Force constructed a list of key conditions that must be met to reduce sea lamprey damage to the fish community (Twohey *et al*. 2003a). Although SMRT might be successful if several of the conditions were only partially met, complete failure to meet any one would mean failure of the program. Those conditions are:

- H1. Male sea lampreys are successfully sterilized;
- H2. Sterilized males survive to reach the spawning grounds and construct nests in proportion to their presence in the male population;
- H3. Sterilized males attract females to nests and mate normally;
- H4. Sterility persists through mating and survival of embryos at hatch is reduced in individual nests;
- H5. Survival of embryos at hatch is reduced in individual streams;
- H6. The abundance of burrowed larvae in each year class (after leaving the nest) is reduced in individual streams;
- H7. Reductions in abundance of larvae persist through the larval life stage and result in reductions in the number of metamorphosing sea lampreys in individual streams (H6 and H7 require density independence and lack of compensation in growth or survival);
- H8. The number of parasitic-phase sea lampreys in the lake is reduced;
- H9. Damage to fish in the lake is reduced.

These conditions are easily converted to testable null hypotheses (hence labeling as H1–H9) and in later sections we describe research already conducted to test a number of them.

Development of the Sterilization Method

Research to identify an effective chemosterilant for sea lampreys spanned a 15-year period from 1971 through 1985. Experimental field and laboratory tests conducted from 1971 to 1978 showed that intra-peritoneal injections of P, P-bis (1-aziridinyl)-N-methylphosphinothioic amide (bisazir, Chang *et al*. 1970) effectively sterilized adult male sea lampreys (Hanson and Manion 1978, 1980).

Additional sterilants and methods of sterilization were investigated because of concerns over the cost and the potential human health hazards of bisazir (Borkovec 1972, Rudrama and Reddy 1985). Most methods tested (other chemicals, hormones, and immunology) showed little promise and were abandoned. The most promising alternatives were ionizing radiation, use of a lamprey GnRH antagonist, and gossypol (an anticancer drug extracted from the cotton plant). Radiation was effective, but damaged the immune system and treated sea lampreys were too susceptible to disease to survive and be competitive spawners (Manion *et al*. 1988, Hanson 1990). The structure of sea lamprey GnRH is known and timed release microsphere formulations of potential antagonists to GnRH were evaluated (Sower 2003). An effective antagonist has not yet been found, but the intramuscular injection of microspheres was a viable method of controlled release and a GnRH antagonist remains a viable concept. Further, doses from 50 to 200 mg/kg of gossypol resulted in increased mortality, which suggested that an application method other than IP injection was required (Rinchard *et al*. 2000).

The Chemosterilant Bisazir

Bisazir is not readily available and is obtained from commercial laboratories that synthesize the compound. The cost has been about $20/g (or $0.50 per lamprey). Bisazir is difficult to put into solution and stock solutions must be filtered during preparation to remove any remaining particulates that can interfere with syringe operation. Analysis of a 2-L preparation of bisazir stock solution indicated that concentration dropped from 11,271 mg/L before filtration to 10,064 mg/L after filtration (11% loss), likely because particulates of undissolved bisazir were filtered.

Bisazir has toxic and mutagenic properties (Rudrama and Reddy 1985) and requires special procedures and facilities for its use (Borkovec 1972). However, bisazir is also highly reactive and sterilized sea lampreys held for 48 h after sterilization no longer contain parent bisazir (Allen and Dawson 1987).

OPERATIONS

Facilities

Because of the need to keep bisazir-injected males in isolation for 48 h, sterilization occurs in a specialized facility constructed in 1991 at the HBBS (Twohey *et al.* 2003a). The facility was designed to prevent the release of bisazir to the environment and to protect the health of workers. Air and water passing through the facility are carbon filtered before release to the environment. Personnel wear protective gear including hooded, powered air-purifying respirators, Tyvek® coveralls, and rubber gloves and boots.

Sea lampreys are injected by a robotic device that weighs and measures each animal and administers an accurate dose through IP injections made along the ventral midline at 46% of the animal's total length (range, 35 to 54%) from the anterior end. Quality assurance checks are routinely made to verify accuracy of the injection point and dosage (Twohey *et al.* 2003a).

Sterilized males are held for 48 h in flowing Lake Huron water before leaving the facility to ensure that all bisazir has been metabolized or excreted (Allen and Dawson 1987). Effluent from tanks holding injected sea lampreys during that period is filtered through carbon to remove any excreted bisazir before the water is returned to Lake Huron. Drains receiving the effluent are monitored in compliance with an NPDES discharge permit issued by the State of Michigan. Bisazir has never been detected in the effluent from the facility. Additional operational details are available in Twohey *et al.* (2003a, 2004), and Klar and Young (2004).

Supply of Sea Lampreys for Sterilization

Access to a dependable supply of male sea lampreys for sterilization is vital for success of SMRT and the number of available males ultimately limits how much reproduction can be suppressed and the geographic extent to which the technique can be expanded. Male sea lampreys are trapped each spring during their spawning migration into Great Lakes tributaries as part of an ongoing, basin-wide sea lamprey assessment program (Schuldt and Heinrich 1982, Mullett *et al.* 2003, Twohey *et al.* 2003a). An average of 26,000 males (range 19,000–36,000) was harvested annually for SMRT from up to 17 tributaries on four Great Lakes during 1991–1999.

Captured animals are sorted by gender and transported to the sterilization facility (Twohey *et al.* 2003a). Transport occurs daily from sites within 50 km of the sterilization facility. Males from more distant sites are accumulated in cages until enough are available for transport. About 85% of the males sterilized come from five sites located within 250 km from the facility. The longest transport distance has been about 750 km from Duffins Creek, Lake Ontario. Transport and handling methods are described in Twohey *et al.* (2003a).

Recently, the Fish Health Committee (FHC) of the GLFC recommended that fish movement from Lake Ontario be minimized to prevent the spread of the microsporidian parasite *Heterosporis* from Lake Ontario. Further, the FHC has recommended that sea lampreys moved among the lakes be screened for restricted diseases using its model program. Sea lampreys have been found to harbor a number of diseases that are common to other Great Lakes fishes, including bacterial kidney disease (BKD) and enteric redmouth. The screening seeks to restrict movement of diseases that have a limited geographic range in the lakes. Along with *Heterosporis*, members of the FHC are concerned about movement of the following geographically isolated or non-evident diseases: whirling disease, anti-biotic resistant furunculosis, and Epizootic Epitheliotropic Disease (EED).

In compliance with the FHC recommendation, adult sea lampreys captured in Lake Ontario have been screened for diseases of concern and for emergency and restricted diseases from the model program before transport to the sterilization facility. Heterosporis has not been detected in sea lampreys, and the strains of BKD and furunculosis detected were already common to the upper lakes. No diseases have been found that precluded transfers of lampreys.

All logistically and economically feasible sources are already being exploited. A 1997 analysis determined the average cost to harvest a male for sterilization was about \$6 (U.S.). About 6,500 more males could be obtained from 14 additional sites in the Great Lakes at a cost of about \$25 (U.S.) per male. The higher cost is because each additional site potentially provides only a small number of males, some are a long distance from the facility, and some require construction of expensive weirs to make trapping effective.

IMPLEMENTATION

Lake Superior

The GLFC approved Lake Superior as the initial site for an experimental application of the sterile-male-release technique in 1987. The experiment would test the effect on a whole lake population. Continuation of current lampricide control effort plus application of sterile males could, in theory, reduce lamprey populations in successive generations to near eradication. Lake Superior met the requirements of a suitable study site because of its relatively low sea lamprey numbers, hydrologic isolation, and regular assessments of fish and sea lamprey abundance to evaluate the technique, and sterilized males were released there during 1991–1996.

Sterilized males were released into a subset of streams selected by the SMRT task force that were believed to collectively be the primary source of sea lampreys residual to chemical treatments in Lake Superior. The number varied annually from 10 to 27 streams. The primary criterion for stream selection was a history of regular treatments that were difficult or prone to be ineffective (Hanson and Manion 1980). The number of males released in each stream depended on numbers available for sterilization and the predicted number of resident spawning sea lampreys based on historical data. The average annual release of sterile males was about 16,100, the average predicted number of resident males was about 10,600, and the average ratio of sterilized to untreated males was 1.5:1, which resulted in a theoretical reduction in larval production of 59%. The theoretical reduction was calculated as:

$$\left[r = \frac{1-t}{s:n+1} \right]$$

where *r* is the theoretical reduction in reproduction from sterile males and trapping, t is the proportion of animals trapped and *s:n* is the ratio of sterile to normal males based on M-R estimates of the number of males remaining after trapping and the number of sterilized males released.

The experimental application in Lake Superior was discontinued in 1997 because the sea lamprey population in Lake Huron was not under control and SMRT seemed the best hope for control in that lake (Schleen *et al.* 2003). Beginning in 1997, the entire supply of males was reallocated to the St. Marys River (the outflow from Lake Superior to Lake Huron). The new control initiative was deemed more important than further experimental application in Lake Superior. Also, 6 years of experimental releases in Lake Superior (1991–1996) were considered sufficient to evaluate effectiveness of the technique as it had been applied.

St. Marys River

The population of larval sea lampreys in the St. Marys River had been growing and by the mid 1990s was believed to be producing over 80% of the parasitic-phase sea lampreys in Lake Huron. Because conventional control techniques were not considered feasible (Schleen *et al*. 2003), the GLFC concluded that a combination of trapping and sterile-male releases would be the best hope for control in the river. Application of these techniques was intended to achieve a sustainable reduction in recruitment in one very important and untreatable river rather than lake-wide suppression of an isolated population as implemented in Lake Superior.

Spawning males captured in the St. Marys River could not be used for sterile-male releases elsewhere in the Great Lakes because the cold waters flowing from Lake Superior delays spawning from 6 to 8 weeks. Therefore, most males captured in the St. Marys River have been sterilized and returned to the river since 1991.

The average number of sterilized males released annually into the St. Marys River during 1991–1996 was 4,600 and the average ratio of sterile to untreated males was 0.6:1 (Table 1, Twohey *et al*. 2003a). The average theoretical reduction in reproduction (when combined with trapping) was 58%, which should have reduced the average number of reproducing females in the river from 11,100 to 5,000.

A new strategy to enhance control in the St. Marys River (Schleen *et al*. 2003) was adopted in

TABLE 1. Theoretical effects of trapping and sterile-male release, and theoretical suppression of reproduction in the estimated population of sea lampreys in the St. Marys River during 1991–2004.

	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004
Population estimate	35,582	19,508	45,620	10,624	19,608	22,255	8,162	20,235	19,860	38,829	25,311	13,619	27,011	19,864
Percent males	53	58	56	57	55	63	56	57	60	64	63	63	66	70
Percentage of population trapped	42	39	22	53	44	20	30	35	53	48	45	59	33	27
Sterile males released	7,516	4,508	4,832	2,667	4,238	3,650	17,181	16,743	26,285	43,184	31,459	22,684	27,963	26,472
Estimated ratio sterile to untreated males	0.7:1	0.7:1	0.2:1	1.0:1	0.7:1	0.3:1	5.4:1	2.2:1	4.7:1	3.3:1	3.6:1	6.4:1	2.3:1	2.6:1
Theoretical percent reduction in reproduction[1]	65	63	38	76	67	39	89	80	92	88	88	94	80	80
Theoretical reproducing females[2]	5,805	3,029	12,534	1,091	2,873	4,922	402	1,771	638	1,670	1,113	289	1,860	1,203

[1] Percent reduction was calculated as $\left[r = \frac{1-t}{s:n+1} \right]$: where r is the theoretical reduction in reproduction from sterile males and trapping, t is the proportion of animals trapped and s:n is the ratio of sterile to normal males based on M-R estimates of the number of males remaining after trapping and the number of sterilized males released.
[2] Theoretical reproducing females = the theoretical percent reduction in reproduction (r) × female population estimate.

1997 and had two objectives: 1) reduce the population of existing larvae through application of a granular bottom release pesticide (Fodale *et al.* 2003); and 2) reduce reproductive success and recruitment to the larval population annually through trapping of spawning-phase sea lampreys and enhanced release of sterile males (Twohey *et al.* 2003a). As noted above, releases were discontinued in Lake Superior after 1996 to make all captured male sea lampreys available for use in the St. Marys River. A 50% reduction from trapping and the redirection of all sterile males to the St. Marys River was predicted to achieve a 90% reduction in spawning success. Because of potential compensation in growth and mortality if larval population density was reduced, a more conservative goal of a 75% reduction was adopted.

The average number of sterile males released in the St. Marys River during 1997 to 2004 was 26,500 and the average ratio of sterile to untreated males was 3.8:1. The average theoretical reduction in reproduction from SMRT and trapping (Table 1, footnote 1) was 86%, which would leave only 1,100 reproducing females in the river, rather than 7,900 in the absence of trapping and SMRT.

EVALUATION

Efficacy of Bisazir Injections (H1)

Measuring sterility of injected male sea lampreys requires that they be brought to maturation and the proportion of eggs they fertilize measured just before hatch (Hanson and Manion 1978). With slight variations in details among studies, injected males were placed in either a raceway (Hanson and Manion 1978) or an indoor spawning stream (Fredricks and Seelye 1995) along with untreated males and females and observed daily for maturation. Each replicate required a spermiated treated male, a spermiated untreated male, and an ovulated female. Eggs were stripped from the female and divided into two lots. The treated and untreated males were then randomly assigned to fertilize one lot. Embryos were incubated at 18 ± 1°C and embryological development followed for 21 days. An embryo was considered viable if it reached stage 17 (Piavis 1961) with no visible deformities.

Studies by Hanson and Manion (1978, 1980) indicated that a dose of 100 mg/kg was effective, but suggested a lower dose might be effective. For field studies that led to the current operational program, they conservatively chose a dose of 100 mg/kg, where six males had produced from 0.0 to 0.1% viable embryos. Doses of 50 and 25 mg/kg produced a maximum of 0.7% viable embryos, but only two injected males were tested at each dose. Additional tests were conducted to further evaluate the potential for using less bisazir in 1994 and 1995 (Twohey *et al.* 2003a), but results did not conclusively support a reduction in dose, which was left at 100 mg/kg.

The automated sterilization process was also evaluated in 1994 at a putative dosage of 100 mg/kg (Twohey *et al.* 2003a). Males for testing were sterilized at the sterilization facility using standard operating procedures. A total of 18 paired samples of eggs were successfully fertilized and cultured. Mean survival of embryos in the control egg lots was 67% (SE 5.0%, range 17 to 95%) and in the treated lots was 1% (SE 0.8% range 0 to 15%). The auto-injector successfully delivered an effective dose of bisazir to males, which significantly reduced the survival of embryos in the treated group compared to the control group (t = 13.0, df = 18, P < 0.001). Twohey *et al.* (2003a) reviewed the 1994 and 1995 studies in more detail. Based on the results of all of these studies, sterilization of male sea lampreys by current procedures is nearly 100% effective (H1).

The Proportion of Sterilized Males on Nests (H2)

To determine if the proportion of sterilized males observed on nests differed from their expected proportion in the population (H2), mean observed and expected proportions for each river-year combination where data were available were compared using a paired t-test. This comparison was first made using all available data and then with only data from the St. Marys River. In all comparisons, significance was assumed at $\alpha = 0.05$. More details on these comparisons are available in Bergstedt *et al.* (2003).

The expected proportion of sterilized males could only be established in two of the Lake Superior study streams and the St. Marys River and in 13 stream-years (Fig. 1). Other stream-year combinations had either trap sites with too few captures and recaptures, or in several cases, spring floods made trapping impossible. The Rock and Misery rivers in Michigan had known numbers of sea lampreys placed above a barrier and the St. Marys River, with its controlled flow, permitted consistent trapping efficiency and reliable mark and recapture estimates each year from 1993 through 2000, except for 1997 when record water levels interfered with trapping.

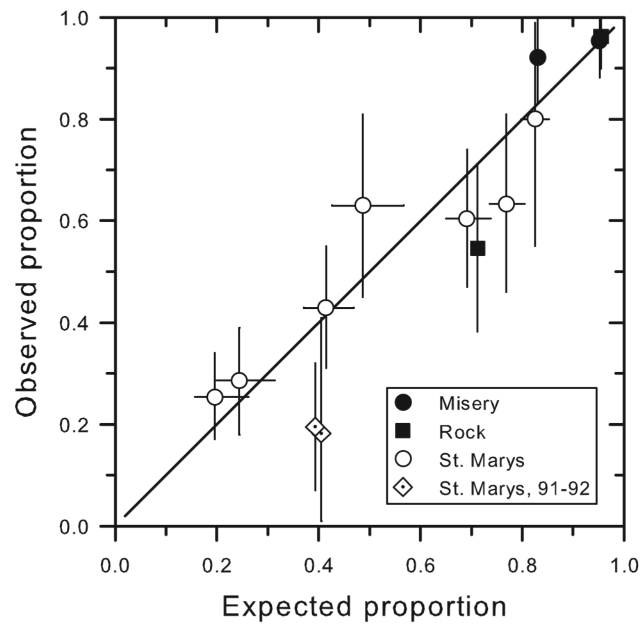

FIG. 1. Expected and observed proportions of sterilized males in the male spawning population of three rivers. The vertical and horizontal lines are 95% confidence intervals. The expected proportions for the Misery and Rock rivers in 1994–95 were known and the confidence intervals for the St. Marys River in 1991–92 were not available. The diagonal line is the locus of equal proportions (from Bergstedt* et al. *2003).

The proportions of sterilized males observed on nests were typically near expected values (Fig. 1). Deviations, expressed as percent deviation from expected, ranged from –55% to 29% and averaged –5% across all streams and years. Expected and observed proportions did not differ significantly (t = 1.15, df = 12, p = 0.27). For the St. Marys River alone, the average percent deviation was –7%, and expected and observed proportions did not differ significantly (t = 1.11, df = 8, p = 0.30). Based on this result, it was concluded that sterilized males released into the St. Marys River survived and appeared on the spawning grounds near the expected proportion (H2, Bergstedt *et al.* 2003).

Egg Viability and Reductions in Reproductive Potential (H3–H5)

The overall reduction in egg viability in individual nests (H3 and H4) was assessed by comparing viabilities in nests with untreated and sterilized male parents (Bergstedt *et al.* 2003). In an analysis of variance of rank-transformed data from 11 stream-year combinations, and including nest class and stream-year as independent variables, viability in sterilized nests was significantly lower than in untreated nests (F = 40.1; df = 1,10; p < 0.001). Although confidence intervals often overlapped, average viability in sterile nests was lower in 10 of 11 stream-years (Fig. 2). Based on this test result, sterile males appeared on spawning grounds, remained sterile and subsequently attracted and mated with females (H3 and H4).

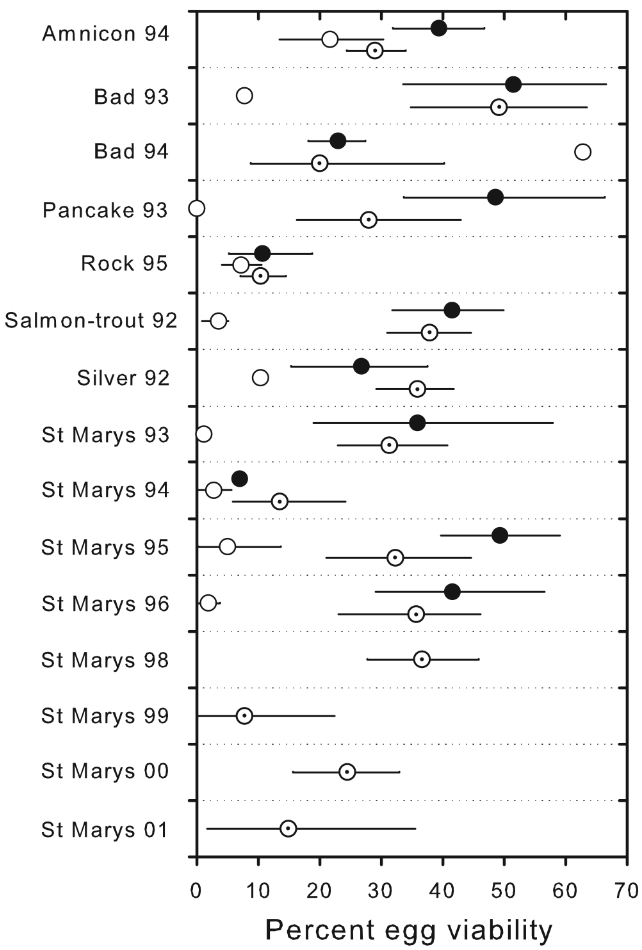

FIG. 2. Mean percent viability at hatch in samples of eggs from sea lamprey nests in 15 stream year combinations in tributaries to Lake Superior and in the St. Marys River, Lake Huron. Symbols denote whether the male parent was untreated (filled circles), sterilized (open circles), or unobserved (dotted circles). In the St. Marys River, nest classes were not separated after 1998. Horizontal lines are 95%, bias corrected, bootstrap confidence intervals for samples where N was three or more.

To determine if the average viability at hatch was related to the observed proportion of sterile males (H5), the unweighted average viability in nests where the male parent was unobserved (as a representative sample of all matings) was correlated to the observed proportion of sterilized males on nests in the St. Marys River (Bergstedt *et al.* 2003). In the St. Marys River from 1993 to 2001, mean egg viability in nests where the male parent was not observed was negatively correlated to the observed proportion of sterile males (r = 0.77, df = 6, p = 0.026, Fig. 3). However, when compared to the theoretical viability calculated using a baseline viability of 0.434 (the average proportion of viable eggs in all nests of untreated males sampled in the St. Marys River) and assuming 100% sterility in sterilized males, the observed viabilities were either close to or above the expected values with none substantially lower (Fig. 3). Based on this test result, the average viability at hatch was negatively related to the observed proportion of sterilized males (H5), although the observed viability was slightly greater than expected. The close proximity of observed to expected reductions also supported the conclusion that bisazir injections effectively sterilized male sea lampreys (H1). The predominance of positive deviations from expected egg viability could be due either to incomplete sterility or to undocumented spawning in nests of untreated males, which would cause underestimation of both baseline viability and expected viability.

Expressing the observed viabilities in the St. Marys River (Table 2, class UO) as a proportional decrease from a baseline viability of 0.434, the average decrease (and range) was 0.43 (0.16 to 0.82) across all years, and 0.64 (0.43 to 0.82) during 1999–2001, when the current program on the St. Marys River appeared to stabilize (Table 2). When reductions due to removal of females by trapping were also considered, the overall percent reduction in reproductive potential in the St. Marys River averaged 64% (range = 34 to 92%) during 1993–2001 and 81% (range = 71–92%) during 1999–2001 (Table 2).

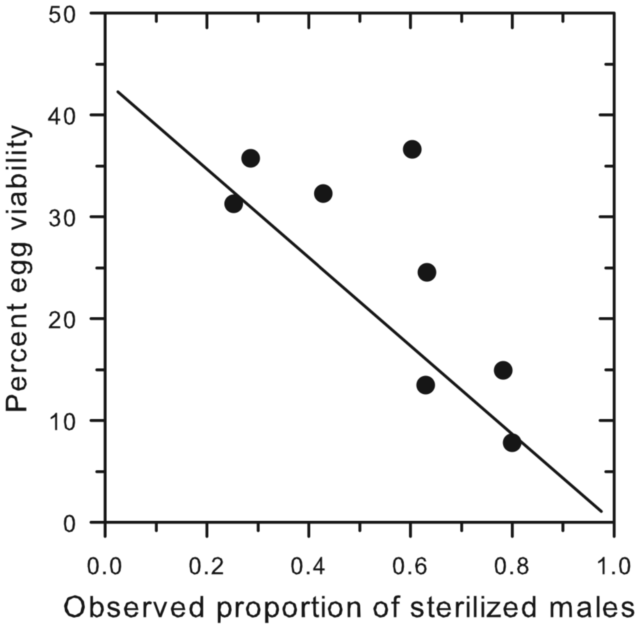

FIG. 3. Mean percent viability at hatch in samples of eggs from sea lamprey nests in the St. Marys River, where the male parent was unobserved, versus the proportion of sterilized male sea lampreys observed on nests. The line shows the theoretical relation if the baseline percent viability without release of sterilized males was 43.4% (the average for nests in the St. Marys River where only an untreated male parent was observed).

Field Evaluations of Demographic Responses to Reductions in Reproduction (H6-H7)

Manipulations of Spawning Populations

In 1996, field studies were initiated to evaluate whether larval intra-specific competition was important, and whether the required condition of density independence and lack of compensation in growth or survival was being met. The objectives of the new studies were to determine if the sterile-male-release technique reduced production of yearling larvae, and eventually, metamorphosed juveniles (H6 and H7). These hypotheses are more difficult to assess because (1) production of age-0 larvae within streams is highly variable, (2) assignment of age classes to larvae can be inaccurate, and (3) numerous stream- and site-specific factors determine the survival, growth, and transformation rates of larvae. One of those studies included estimation of recruitment of yearling larvae per adult female in streams (Jones *et al.* 2003). Known numbers of normal adult male and female sea lampreys were released upstream of barriers in 11 tributaries during 1996–1999. Additionally, normal adult male and female numbers were estimated by mark-recapture from additional Great Lakes tributaries. Sterilized males were released into some of the streams at varying ratios of sterilized to untreated males, and the theoretical number of effective females (number of normal females reduced by the theoretical effect

TABLE 2. Effects of trapping sea lampreys and releasing sterilized males in the St. Marys River on reproductive potential (1993 to 2001). Mean viability was for nests where the male parents were unobserved (assumed to be a random sample of all matings). Mean viability was also expressed as a proportion of baseline viability (viability/baseline), where the baseline (0.434) was the mean of nests where the male parent was observed to be untreated; a value of 0.75 indicates that 75% of the reproductive potential remains after release of sterilized males. Trap efficiency was calculated from recaptures of marked animals. The effect on reproductive potential (RP) was given as the percent remaining after trapping and after trapping and release of sterilized males (SMR) combined, and as the percent reduction in RP from trapping and SMR combined.

Year	Mean viability (proportion of viable eggs)	Mean viability as a proportion of baseline viability	Trap efficiency (%)	Remaining RP after trapping (%)	Remaining RP after trapping and SMR (%)	Reduction in RP from trapping and SMR (%)
1993	0.313	0.721	22	78	56	44
1994	0.135	0.310	54	47	14	86
1995	0.323	0.744	44	56	41	59
1996	0.357	0.823	20	80	66	34
1998	0.366	0.843	35	65	55	45
1999	0.078	0.180	53	47	8	92
2000	0.245	0.565	48	52	29	71
2001	0.149	0.343	45	55	19	81

of sterile males, Knipling 1968) was calculated. Recruitment of yearling larvae was estimated the following year and compared across streams and years. Analysis of all data from all streams and years suggested significant density-dependant compensation occurred in the range of 0 to 50 reproducing females per 100 m^2 of larval habitat, and substantial density-independent variation resulted in a wide range of recruitment for any given stock size. At densities of less than 0.5 reproducing females per 100 m^2, recruitment variation was much reduced and recruitment appeared to decline as females per 100 m^2 declined. Because densities in the St. Marys River are among the lowest in the Great Lakes, at about 0.002 reproducing females per 100 m^2 (calculated for 1997–1999 from substrate samples collected by Fodale *et al.* 2003), recruitment would also likely be independent of density there.

Measures of Recruitment in the St. Marys River

Observations of larval abundance in the St. Marys River support the conclusion that SMRT and trapping have reduced recruitment in the river. The

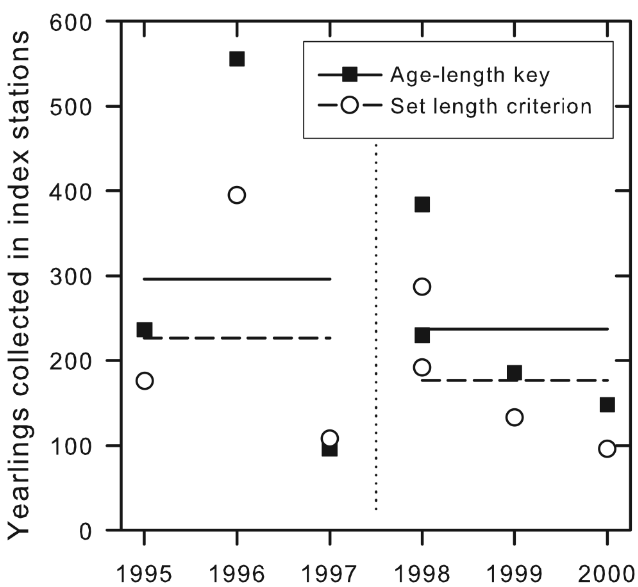

FIG. 4. Observed mean yearling abundance attributable to spawning before and after SMRT and trapping enhancement (vertical dashed line); yearlings were identified using either an age-length key by Haeseker* et al. *(2003; filled squares and solid lines) or a set length criterion determined each year based on length frequency (GLFC 2001a; open circles and dashed lines).

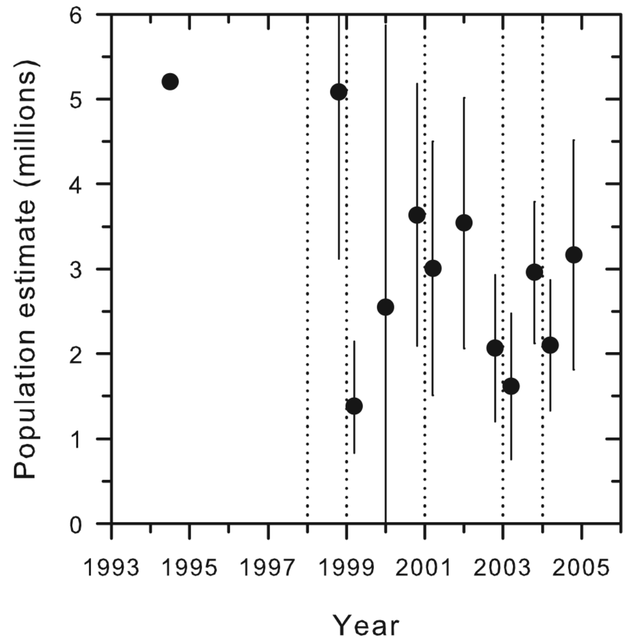

FIG. 5. Estimates of the abundance of larval sea lamprey in the St. Marys River. The estimate for 1993–1996 is a spatially based estimate from the whole river mapping effort and lacking a measure of error; estimates and 95% confidence intervals in 1999–2001 are based on a stratified random design, with adaptive sampling added in 2002–2006.

trend of larval recruitment appeared to decrease after the advent of the enhanced program in 1997 (Fig. 4). Also, since the major Bayluscide spot treatments of 1999 (1,800 acres), and after periodic applications in 2001, 2003, and 2004, 2005, and 2006 the larval population has not rebounded at a typical rate (Fig. 5). In other lamprey producing rivers in the upper Great Lakes, larval abundance always increases to pre treatment levels within 3–4 years following lampricide treatment. The separate effects of individual elements of the control strategy cannot be determined, but recruitment has remained low and periodic Bayluscide applications have been required at a lower rate than from the original application in 1999. Combined with the model comparison described above, the evidence of suppressed recruitment is strong.

Modeling of Demographic Responses to Reductions in Reproduction (H6-H7)

This subject has been studied more extensively in relation to sterile insect release. A valuable set of current reviews are provided in "Sterile Insect Technique; Principles and Practice in Area-Wide Integrated Pest Management," edited by Dyck, Hendrichs and Robinson (2005). The rich literature in this area should be useful in suggesting ways to explore demographic responses to reducing reproduction in sea lampreys. However, extending models and field approaches to sea lampreys is complicated by their multi-year larval stage, the aquatic environment, and the current imprecision of aging, as discussed below.

A Ricker-type stock-recruitment model was developed for the St. Marys River by Haeseker *et al.* (2003) from available life history and assessment data (Fig. 6). The model predicts that reductions in reproduction that do not reduce the number of reproducing females below 5,000 will actually increase recruitment, but that below 5,000 reproducing females, reductions become increasingly efficient as the number of females is further reduced. For example, during 1996, the estimated population of 8,100 reproducing females was theoretically reduced to 4,900 (40% reduction) from trapping and sterile-male releases (Table 1), but the model suggests that this reduction would increase recruitment of parasites by 15%. Next, during 1998, an estimated population of 8,800 reproducing females was theoretically reduced to 1,800 (80% reduction) from trapping and sterile-male releases, but the model suggests that recruitment of parasites only decreased by 17%. Finally, during 2002 an estimated population of 5,000 reproducing females was reduced to about 300 (94% reduction) from trapping and sterile-male releases and the model suggests that recruitment was reduced by 85%.

Jones *et al.* (2003) also developed simulation models using observed recruitment variation and concluded that recruitment during any given year could vary substantially from the average. Haeseker *et al.* (2003) predicted similar effects using a stock-recruitment relationship developed for the St. Marys River. Both models suggested that reduction in reproducing females would result in fewer recruits, but that there was risk of periodic high recruitment. These studies suggest that strategies to reduce reproduction will, on average, reduce abundance of yearling larvae (H6) and will be most reliable when low densities of reproducing females are achieved.

Variation in observed year-class strength will affect the power of comparisons of changes in abundance over time. Jones compared observed

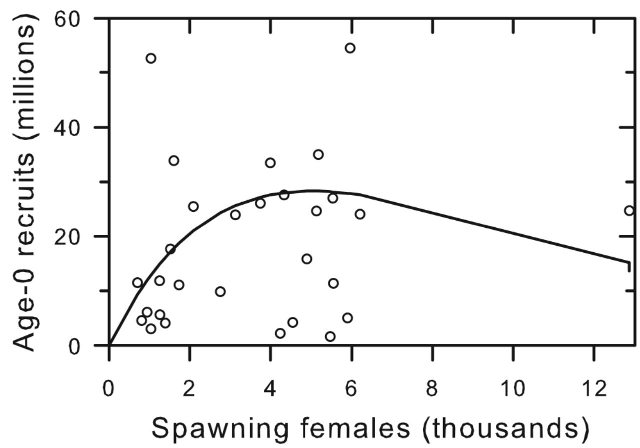

FIG. 6. Stock recruitment model for the St. Marys River developed by Haeseker* et al. *(2003).

recruitment at age one from larval assessment in the St. Marys River to distributions of predictions from a simulation model using a stock-recruitment relationship (GLFC 2002, Fig. 6). Observed recruitment estimates are closer to predicted effects of sterile-male release than the effect of trapping alone. Inferences from these results are weak, however, because of the wide confidence limits on model predictions (Fig. 7). Additional observations, particularly if numbers of effective females can be reduced to very low numbers, would increase the power of these conclusions.

A decision-analysis simulation of the effects of control options on numbers of parasitic sea lampreys in Lake Huron suggests that SMRT will provide a significant amount of suppression in the St. Marys River. The simulation model includes variation in the stock-recruitment relationship described above and additional sources of variation. The model was used to simulate the effects of Bayluscide treatments alone and combined with SMRT and trapping. With repeated treatments with Bayluscide alone, sea lamprey abundance is predicted to increase from current levels (Fig. 8). When combined with SMRT and trapping, numbers of sea lampreys are reduced over time. The long-term average effect of SMRT and trapping at the current level of intensity (40%, 4:1) is a reduction of 160,000 parasitic sea lampreys from the lake. Achieving an enhanced level of SMRT and trapping is predicted to have an even greater additional suppression of 190,000 parasitic sea lampreys.

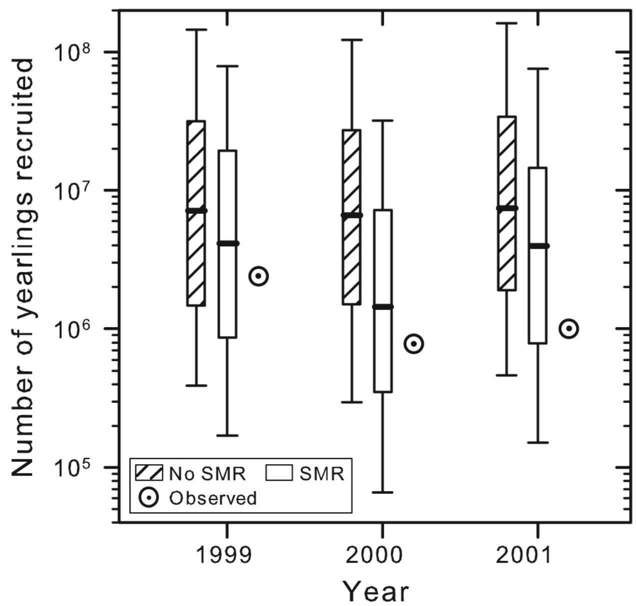

FIG. 7. Predicted and observed sea lamprey recruitment at age one for the St. Marys River, 1999–2001. The median and the 10th, 25th, 75th, and 90th percentiles of the distributions of simulation results are shown both with and without the adjustment for the theoretical SMRT reduction. (M. Jones, unpublished data).

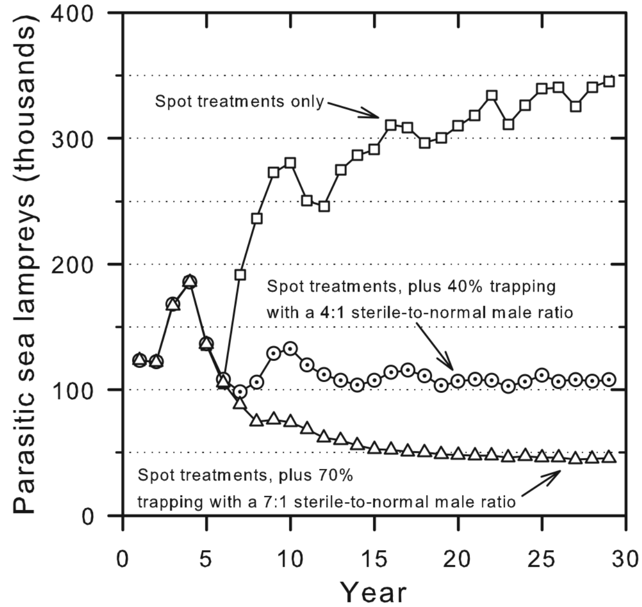

FIG. 8. Decision analysis simulation model projections of the effects on production of parasitic-phase sea lampreys of the application of Bayluscide only compared a combination of Bayluscide with trapping and SMRT at the current level (40% trapped, 4:1 sterile to resident ratio) and an enhanced level (70%, 7:1), from results by Haeseker **et al.** ***(in review.)***

Effects on Parasitic-phase Abundance and on Damage to Fish H8-H9

Long-term abundance of spawning sea lampreys and damage to fish from the experimental application in Lake Superior did not change significantly during 1991–1996 (Heinrich *et al.* 2003). The application of sterile males in Lake Superior was not optimally designed to affect these indices. Reasons are described further in Twohey *et al.* (2003a).

The effects of individual components of the St. Marys River control strategy cannot be quantified. Nevertheless, the entire strategy has been successful. Since the enhanced program began in 1997, spawning-phase sea lampreys in northern Lake Huron declined 38%, wounding rates on lake trout declined 61%, and lake trout mortality declined 66%.

Summary of Evaluations Addressing the Nine Hypotheses

H1—Males are Sterilized

Sea lampreys were effectively sterilized in the laboratory by an intra-peritoneal injection of 100 $mg \cdot kg^{-1}$ of bisazir (Hanson and Manion 1978). Sterility was also confirmed in field studies (Hanson and Manion 1978, 1980). In current operations, the accuracy of the bisazir dosage administered by the auto-injector and the effectiveness of sterilization were examined (Twohey et al. 2003a).

H2—Sterilized Males Survive and Nest

Kelso and Gardener (2000) found that sterilization did not alter sea lamprey emigration rates, daily upstream movement, or habitat selection prior to spawning. Hanson and Manion (1978, 1980) and Bergstedt *et al.* (2003) both confirmed that sterilized males reached spawning grounds and constructed nests at the expected ratios of sterilized to resident males.

H3 and H4—Sterilized Males Attract Females and Mate Normally, Reducing Hatch in Individual Nests

Ratios of sterilized to resident males on nests and subsequent reductions in egg viability in nests were shown by Hanson and Manion (1978, 1980) and Bergstedt *et al.* (2003) to be near expected values.

H5—Hatch is Reduced in Individual Streams

Hanson and Manion (1978, 1980) and Bergstedt *et al.* (2003) demonstrated that survival of embryos at hatch is reduced in individual streams.

H6—Year Classes are Reduced in Individual Streams

Jones *et al.* (2003) found little evidence of compensation at current larval densities. Analyses further suggested that reductions in reproducing females through trapping and SMRT should result in fewer recruits, on average, but at risk of high recruitment events (Jones *et al.* 2003, and Haeseker *et al.* 2003). Evaluations of larval collections at index sites in the St. Marys River during 1994-1999 lacked power to discern year class reductions because of large interannual variability in recruitment (GLFC 2001b). However, recruitment of yearling larvae in the St. Marys River during 1999–2001 more closely matched the modeled yearling abundances with rather than without SMRT (GLFC 2002).

H7—Reductions Persist through Metamorphosis in Individual Streams

Larval populations in the St. Marys River have been monitored for trends since 1994 (Adams *et al.* 2003). Modeling by Haeseker *et al.* (2003) suggests that reductions in reproducing females in the St. Marys River below 5,000 will reduce recruitment.

H8—Parasitic Abundance in the Lake is Reduced

The effect of sterile-male releases on the abundance of adult sea lampreys in Lake Superior during 1986–1999 was not apparent (Heinrich *et al.* 2003). Following transfer of SMRT to the St. Marys River populations of adult sea lampreys in lakes Huron (since 1995) and Michigan (since 1996) continue to be monitored for long-term trends (Adams *et al.* 2003).

H9—Damage to Fish is Reduced

The expected reduction in wounding rates due to sterile-male releases was not observed on Lake Superior fish during 1998 or 1999 (Heinrich *et al.* 2003). A statistical catch-at-age model shows that survival of lake trout during 2002 to 2004 in northern Lake Huron approached goals—primarily due to a reduction in sea lamprey attacks (Personal Communication, James Johnson and Ji He, Michigan Department of Natural Resources, Alpena, MI). Wounding rates on Lake Huron continue to be monitored by management agencies and will contribute to the long-term evaluation of SMRT in the St. Marys River (Adams *et al.* 2003).

RESEARCH NEEDS

In this section we describe research needs as identified by GLFC task forces, the internal research program, other cooperating scientists, SLIS committees, and a recent program review. Sentences highlighting identified research issues or questions are *italicized* and summarized in Table 3.

Sterilization: Methods: Dosage, Synthesis, and Evaluation

The current method of IP injection of bisazir has proven to be an effective sterilant (Twohey *et al.* 2003a, Hanson and Manion 1978), to be effective in the field (Bergstedt *et al*. 2003), and is safe and cost effective (Koonce *et al.* 2003). *Research into a new method of sterilization would be welcome, but only if the new method would be safe enough that protective clothing and a containment facility were not needed or if field application were possible.*

The lowest possible dose of bisazir is desirable to minimize adverse effects on the treated sea lampreys, to reduce cost, and to minimize use of the chemosterilant. Twohey *et al*. (2003a) suggested a lower dose may be possible but that *additional research is needed to confirm the minimum dose of bisazir that can be relied upon to create 99% or better sterility*.

Evidence suggests that efficacy depends on having enough volume of bisazir solution injected to effectively bathe the gonads. *The efficacy at differing concentrations of stock solution administered at the recommended dose (which affects the volume injected) should be investigated.*

Research in two areas could reduce the long-term cost of sterilant. The cost has been about $0.50 per lamprey, partly because synthesis is difficult. If 30,000 males are sterilized, about $15,000 is spent on bisazir. *A more efficient method of synthesis needs to be developed to reduce the cost of bisazir*. A portion of bisazir does not go into solution, so is wasted when filtered out. *Development of a method to put all bisazir into solution could save about $0.05 per sterilized lamprey, and would reduce de-*

TABLE 3. Summary of research needs related to sterile-male release and genetic alteration of sea lamprey.

STERILIZATION
1. Develop new methods of sterilization that are safe enough that protective clothing and a containment facility are not needed or that will permit field application.
2. Determine the minimum dose of bisazir that can be relied upon to create 99% or better sterility.
3. Determine the sterilizing effect of differing concentrations of bisazir stock solution administered at the recommended dose (as it affects the volume injected).
4. Develop a more efficient method of synthesis to reduce the cost of bisazir.
5. Develop a method to completely dissolve bisazir into solution.
6. Develop a quicker and less costly method that can assay the degree of genetic disruption and the likelihood of sterility without the need for holding until maturity and incubating embryos to hatch.
THE EFFECTS OF SKEWED SEX RATIOS ON MATING BEHAVIOR
7. Determine the effect of skewed sea lamprey sex ratios on intensity of mate selection and how it affects the efficacy of sterile-male release?
8. Determine the effect of skewed sea lamprey sex ratios on the degree of polygyny.
ENHANCING THE ATTRACTIVENESS OF STERILIZED MALES
9. Determine if sterile-male lampreys can be treated to increase male sex pheromone production and if treated males would be more competitive and provide greater suppression of recruitment.
10. Identify visual and other sensory cues that contribute to mate selection in sea lamprey.
DEVELOPMENT OF TECHNIQUES FOR GENETIC ALTERATION OF SEA LAMPREY
11. Develop a daughterless technology that could be used in conjunction with SMRT to enhance control or possibly eradicate sea lampreys in the Great Lakes, and assess associated risks in the Great Lakes.
12. Develop models to predict the potential effect of “daughterless” males spreading into the native population on the Atlantic Coast.
SOURCES OF ANIMALS TO ENHANCE OR TO EXPAND THE USE STERILE LAMPREYS
13. Determine the number of male sea lampreys potentially available from Atlantic Coast streams.
14. Assess the types and prevalence of diseases carried by anadromous sea lampreys along the Atlantic Coast and the likelihood of transmission to Great Lakes fish.
15. Determine the genetic contribution to the observed phenotypic size difference between Great Lakes and Atlantic lampreys.
16. Determine the sterility of bisazir-injected females throughout the spawning run.
17. Determine the effect of time of capture and sterilization on survival of females to spawning at current and lower doses of bisazir.
18. Determine the number of females a male can service (male mating potential) and how many sterilized females are required per male to produce a reduction in reproductive success?
19. Develop trapping techniques for streams that lack barriers.
20. Develop a practical method of raising male sea lampreys through the parasitic phase for sterilization and release.

lays caused by the particulate matter clogging valves or the syringe.

Quality assurance testing for sterility of treated males is difficult and time consuming (see section on evaluating the efficacy of bisazir injections) and has therefore been conducted only a few times. Bizazir produces sterility by inducing lethal mutations in the developing sperm cells. *We need to test for sterility on a more regular basis and, therefore, need to develop a quicker and less costly method that can assay the degree of genetic disruption and likelihood of sterility without the need for holding males and females until spermiation or ovulation and incubating embryos to hatch.*

Mating Behavior: Effects of Skewed Sex Ratios

In any fish species, mating involves a complex set of cues and behaviors. The fish's perceptions of sex ratio and density of spawners are among these cues. Evidence from SMRT evaluations suggests that a skewed sex ratio only minimally affects the achieved reduction in reproduction. However, if release of sterile females is considered, altering density and sex ratio may have a greater effect for reasons described under "Female Sea Lampreys" further in the article. The effect of altering density and sex ratio on mating behaviors is a potentially difficult field research problem, but we would benefit from a greater understanding of those issues.

As the sex ratio becomes increasingly biased toward one sex, the selectivity of one sex for mates can also increase (Emlen and Oring 1977). If this were true, then adding large numbers of males or females could result in the opposite sex becoming choosier about their mates. This would tend to accentuate the effect of any reduction in competitiveness of sterilized animals. Changing selectivity for mates in response to skewed sex ratios had been noted with pipefish (Berglund 1994, Vincent *et al.* 1994) and with guppies Jirotkul (1999). *To better predict the outcome of sterile releases, we should understand the effect of skewed sea lamprey sex ratios on mate selection.*

Hanson and Manion (1980) suggested that sex ratio regulates whether lampreys are monogamous and how many females are found on polygamous nests. Sea lampreys tend to be more monogamous early in the spawning season, but as the season progresses, females become more numerous than males and the frequency of polyandry increases (Applegate and Smith 1950). With SMRT, polygyny is less important, because mating with a sterile male immediately wastes reproductive potential. With the release of sterile females, however, a more complex issue is related to how many females and eggs a male can mate with or fertilize (see below under female sea lampreys). *To better predict the outcome of sterile releases, we should understand the effect of skewed sea lamprey sex ratios on the degree of polygyny.*

Enhancing the Attractiveness of Sterilized Males

The amount of reproduction wasted by sterile-male sea lampreys could be increased if their competitiveness in attracting females were enhanced, particularly if their competitiveness could be raised above that of typical, untreated males. Two approaches to this have been discussed.

The first approach would be to enhance attractiveness by increasing the output of a pheromone. This concept was first suggested by Knipling (1979), who visualized coating sterile insects with a pheromonal attractant. Similarly, Klassen *et al.* (2005) proposed attaching slow-release capsules of the sea lamprey male sex pheromone (Li *et al.* 2002, 2003) to sterile males to increase their success (see Pheromone Theme). Li *et al.* (2003) suggested that up-regulating production of the male sex pheromone could make sterile males more attractive. Sterilization does not suppress production of that pheromone (Siefkes *et al.* 2003), so such an enhancement is theoretically possible. An effective and feasible approach to either increase production of the male sex pheromone or to augment the amount released should be developed (Twohey *et al.* 2003b). *Can sterile-male lampreys be treated to increase natural production of male sex pheromone, and would treated animals provide greater suppression of recruitment than non-enhanced sterile males?*

The second approach would be to enhance secondary sex characteristics that provide visual (or other sensory) cues identifying desirable male mates. The only clear sexual dimorphism is the rope-like dorsal ridge anterior to the first dorsal fin on mature males (Hardisty and Potter 1971). However, no extensive behavioral research has focused on more subtle morphological cues. *Research is needed to identify visual or other sensory cues that contribute to mate selection by females.*

Development of Techniques for Genetic Alteration of Sea Lamprey

"Genetic control of insect pests: growth industry or lead balloon?"—This quote is the title of a review paper by Curtis (1985). Discussions of the potential of genetic approaches began with Knipling and colleagues in the 1960s and each new leap in genetics and molecular biology has generated new interest. However, real progress in deployment of genetic controls has been slow, as evidenced by the quote above penned several decades later. Advances in genetic techniques in the last decade have been much more rapid and it seems likely that the promise of genetic approaches may begin to be realized. Selection of the sea lamprey for genome sequencing by NIH (sequencing of their genome is currently underway) could help to identify genes controlling processes with potential for control. We do not have the expertise to provide a meaningful review of progress in this area, but hope that the remainder of this document provides enough background on life history and ongoing approaches to reducing reproduction that experts in genetic pest control will be able to see opportunities. Below, we discuss the two issues that have received internal discussion, but have not progressed past that stage.

A possible alteration would shift the parasitic landlocked sea lamprey in the Great Lakes to a non-parasitic form. Most lamprey species occur in pairs, where one has parasitic phase and one does not (Potter 1980). Control of the hormonal or biochemical cue to metamorphosis could result in creation of a non-parasitic form. Factors controlling metamorphosis and the concept of block onset of the process are described in more detail by Youson (2003).

A promising recent development is to insert sex-linked, fatal genes that result in mortality of all females while allowing males to survive and pass on the lethal genes (hence "daughterless" technology). Lethal gene insertion has been accomplished in the laboratory with the fruit fly *Drosophila melanogaster* (Thomas *et al.* 2000), and suitable strains are being developed for mosquito control (Benedict and Robinson 2003). In Australia, progress has been made in developing a daughterless approach for carp control (Ron Thresher, CSIRO Marine Laboratory, Hobart, Tasmania, personal communication). This technique potentially jumps the important hurdle of how to pass a fatal genetic trait on against the selection forces that would quickly remove the trait from a wild population. For more information we suggest consulting Schliekelman and Gould (2000a, 2000b) and Thomas *et al.* (2000).

As discussed in a later section, sea lampreys cannot feasibly be reared through the juvenile, parasitic phase, but we do believe that we could raise larvae from fertilized eggs to a stage where they could survive in a stream and possibly to a stage where they could be marked with a coded-wire tag. If a sex-linked fatal gene could be inserted into sea lampreys, altered males could be placed in areas not treated with TFM, where they could grow, metamorphose, enter the lake, and eventually spawn and spread the lethal gene. Again, the sea lamprey genome project could be a key to feasibility of finding such a gene. If a sex-linked lethal trait could be combined with an easily-identified marker, only animals bearing the trait could be released. If recently metamorphosed, treated males tagged with coded-wire tags before migration to the lake could be recovered as adults, they could be used to breed more treated larvae. The concept might also be applied to the concept of blocking metamorphosis. *In conjunction with SMRT, daughterless technology could be a powerful tool potentially leading to eradication in the Great Lakes, so its potential and risks should be explored.*

An objection to any genetic control technique in the Great Lakes is that we do not work with an isolated population, so some animals could move downstream to the Atlantic Ocean. No evidence supports this concern, but no obstacle in the St. Lawrence drainage would prevent it. Therefore, we must assume that escape could occur. Any research in this area should consider and address that possibility. Any genetic technique with potential for approval would not be capable of eliminating sea lampreys from their native range through the escape of a limited number of animals. This would only be true if the approach was only partially effective and continued insertion of engineered animals was required to achieve eradication. *Models of the limits to the potential unaided spread of "daughterless" males in a wild population are needed to evaluate the ethical acceptability of this technology in the Great Lakes.* Models by Schliekelman and Gould (2000a, 2000b) illustrate an approach to such models.

Sources of Animals to Enhance or to Expand the Use of Sterile Lampreys

We are convinced that SMRT is helping to achieve control in the St. Marys River and Lake

Huron, but additional sterilized animals would allow us to either do a better job there or to expand SMRT geographically to other problem areas. Much thought and effort has gone into obtaining the maximum number of males feasible under current funding and any research leading to new sources would be of considerable value to the GLFC. Below, we list and discuss approaches to providing additional animals that the SMRT Task Force has considered, and where appropriate, direct you to other sea lamprey research theme papers.

Male Sea Lampreys from Atlantic Coast Tributaries

Anadromous male sea lampreys from Atlantic coastal tributaries (Atlantic males) could augment the supply of males for SMRT in the Great Lakes (Hanson and Manion 1980). Male sea lampreys from the Great Lakes can be effectively sterilized with a 100 mg/kg dose of bisazir, and the same dose would likely be effective on Atlantic males. A 1988 planning report suggested that about 20,000 Atlantic males could be available (Sterile Male Release Technique Task Force 1988). A subsequent survey sent to fishery professionals in 15 states and four provinces (Swink 1997) and field observations in 1997 confirmed that up to 20,000 Atlantic males were available at four sites in Connecticut, Massachusetts, and Nova Scotia. Abundance of the sea lamprey population in the Connecticut River at Holyoke MA was estimated by mark-recapture in 2000, and methods of capturing the animals were explored. Because of concerns about disease and importation of new genetic material, the GLFC decided not to pursue importation of Atlantic males. This decision was made based on current knowledge and could change as more facts become known.

Like many anadromous species on the East Coast, sea lamprey populations have declined due to restrictions of habitat and the sea lamprey may have become a species of concern in some watersheds originally surveyed. If the East Coast is reconsidered as a source of males for sterilization, a new survey would be required to determine whether 20,000 is still a viable estimate of what could be obtained.

Two prevalent concerns exist that prevented us from immediately importing Atlantic-origin males. At the request of the GLFC, we investigated the potential for importing diseases harmful to Great Lakes fishes or for importing new genetic material that might result in larger parasitic sea lampreys or promote hybrid vigor.

The risk of importing unwanted diseases with Atlantic-origin sea lampreys was investigated by the SMRT task force. The U.S. Fish and Wildlife Service conducted disease inspections on 60 Atlantic-run sea lampreys annually in 1992, 1997, 1999 (Northeast Fishery Center, Fish Health Section, Lamar, PA), 1993 (Fish Health Center, LaCrosse, WI), and 1996 (Leetown Science Center, Leetown, WV). Specimens were tested for presence of emergency and restricted diseases (Hnath 1985) using procedures of Thoesen (1994). A single lamprey was infected with *Aeromonas salmonicida*, but showed no overt signs of disease. All other lampreys were free of restricted disease organisms. The SMRT task force and the Fish Health Committee of the GLFC implemented a 4-year protocol (1999 to 2002) to develop a disease profile of sea lampreys in three Atlantic coastal tributaries. No diseases were found that would have precluded importation. If the east coast were to be reconsidered as a source, additional research into the types and prevalence of diseases carried by anadromous sea lampreys and the likelihood of transmission would be needed before large numbers would be imported.

The question of whether landlocked sea lampreys are a genetically distinct form would also be an important consideration in a policy-level decision to import Atlantic-run males. Sea lampreys in the Great Lakes reach a smaller size (Bergstedt and Swink 1995) than in the Atlantic Ocean (Beamish 1980). Great Lakes animals also have been shown to spend only one feeding season in the parasitic stage (Bergstedt and Swink 1995), whereas marine animals may spend two feeding seasons as parasites (Beamish 1980). If Great Lakes sea lampreys are a distinct form, then release of Atlantic-run males could introduce genetic material into a unique landlocked population and could also result in production of larger parasitic sea lampreys that could cause more damage to fish stocks.

Landlocked forms of fish species can have heritable differences in growth potential and maturity schedules (e.g., Sutterlin and MacLean 1984). However, size differences are not necessarily evidence of genetic separation because growth is governed by both heredity and environment. Individuals of other introduced anadromous species in the Great Lakes, such as Chinook salmon (*Oncorhynchus tshawytscha*), pink salmon (*O. gorbuscha*), and alewife (*Alosa pseudoharengus*), consistently reach smaller sizes than individuals from their parent marine stocks. Lower productivity and food availability in the Great Lakes is almost certainly a factor with those species. A portion of this growth difference has also been generally attributed to lower salinity and greater osmoregulation costs, a concept probably dating back to work by Canagaratnam (1959) with Chinook salmon. More recent work suggests that this is not the case (Morgan and Iwama (1991). Similarly, sea lampreys in the Great lakes have access to much smaller hosts. The simplest explanation of the smaller size of sea lampreys in the Great Lakes is that their growth potential is not realized because of the freshwater environment with fewer hosts, smaller hosts, and a shorter growing season.

A competing explanation is that Great Lakes sea lampreys have been separated long enough for significant differences to evolve or the initial colonizers were few in number. Unfortunately, the question of the endemicity of sea lampreys in Lake Ontario and the New York Finger Lakes has been a lively and unresolved debate. Some, as summarized by Christie (1973, 1974), maintain that sea lampreys were present since the last glaciation and only became abundant after human alteration of the environment favored production of their larvae. More recently, Smith (1995) argued that the source was the New York canal system following linkage of the Great Lakes and Atlantic drainages in 1819. If Smith's (1995) explanation is correct, substantial genetic shifts seem unlikely for this slowly-changing animal over the ensuing 180 years. However, a strong founder effect from the introduction of relatively few animals through the canals is possible.

The literature on possible genetic differentiation of landlocked and anadromous sea lampreys is limited and somewhat conflicting. Wright *et al.* (1985) compared genotypic frequencies from 3,253 individuals at four polymorphic loci sampled at 53 sites in northeastern North America and the British Isles. They found only 1% of genetic variation was between freshwater and anadromous systems and that Lake Ontario and the Finger Lakes (where a landlocked form would have evolved) were more closely related to anadromous sites than to the upper Great Lakes. Although they believed the endemicity issue in New York is not completely resolved, introduction into the eastern Great Lakes appeared quite recent unless the rate of differentiation was strikingly different in the upper and lower Great Lakes. Taken as a whole, these studies do not show the level of difference that might be expected between two groups differing so greatly in size, if the cause of the size difference was genetic. No genetic differences were reported that would be considered as evidence of sub-specific or specific status.

Krueger (1980) detected significant differences at one or more loci among sea lampreys collected from different localities, but genetic distances (Rogers 1972) were too small to suggest interpopulation genetic divergence. Krueger and Spangler (1981) found large enough genetic distances to suggest separate populations existed in Lake Superior, but warned these differences could also be a continuous expression of founder effects related to TFM treatment and subsequent recolonization. Brussard *et al.* (1981) grouped samples into three clusters: (1) anadromous, (2) Lake Ontario and the interior New York lakes, and (3) Lakes Erie and Superior. Jacobson *et al.* (1984) presented evidence that allelic variation was higher among samples within a drainage than among drainages and speculated that ammocoetes from a collection site might be derived from a relatively small spawning population. This would seemingly support the alternative explanation of differences suggested by Krueger and Spangler (1981). Based on studies of homing (Bergstedt and Seelye 1995) and on studies of bile salts that serve as a migratory pheromone directing stream selection (Li *et al.* 2002, Sorensen and Vrieze 2003, Sorensen *et al.* 2005), we currently believe that sea lampreys do not home and that allelic differences within a lake basin where juveniles mix freely are artifacts of the treatment program or of small numbers of spawners colonizing streams following treatment.

A study at the Hammond Bay Biological Station in 1999 attempted to compare the phenotypic expression of growth in juvenile sea lampreys of Atlantic and Great Lakes origins when held in fresh water and fed on freshwater fish hosts. This study was terminated before completion, but in the first year of the study, we only succeeded in raising parasitic animals partway though the feeding cycle (6-7 months) when all animals of both origins died. However, growth trajectories of Atlantic-origin animals that fed and grew were no higher than those observed for the landlocked form (Hammond Bay Biological Station, unpublished data). This suggests that growth differences between sea lampreys from the Great Lakes and Atlantic Ocean are largely driven by environmental differences. *If the East Coast were to be reconsidered as a source of males for sterilization, a measure of the genetic contribution to the observed phenotypic size difference would be* *an important factor in a decision to import sea lampreys.*

Our discussions of the use of anadromous males have also included issues related to the much greater size of those males. Landlocked sea lampreys rarely exceed 550 mm in length at spawning (Bergstedt and Swink 1995) whereas anadromous males can average over 800 mm (Beamish 1980). One view has been that the larger anadromous males would outcompete the smaller landlocked males. A competing concern is that the larger males would not mate effectively with smaller females. Studies with brook lampreys (*Lampetra planeri*) suggested that females preferred larger mates, but that fertilization rate dropped as the size discrepancy increased (Malmqvist 1983). As long as mating occurred, this last aspect would not affect SMRT. If the East Coast were to be reconsidered as a source of males for sterilization, *the effect of male size on competitiveness and female mate selection needs to be investigated.*

Female Sea Lampreys

Sterilization of female lampreys has been proposed as a means to supplement the supply of sterile lampreys (Koonce *et al.* 2003). A modeling exercise by Klassen *et al.* (2004) suggested that a sterile-female release technique could reduce reproduction, but would not be as effective as a sterile-male release technique. Because the addition of sterile females to a stream already receiving sterile males offers no additional benefit (Knipling 1979), sterile females would not be used in the St. Marys River. About 20,000–30,000 female lampreys could be available annually for sterilization and use in other areas in lieu of males.

To evaluate female sterilization, we also need to ask some of the same basic questions we did for males. Hanson and Manion (1978) demonstrated that female lampreys were sterilized in the laboratory by an intraperitoneal injection of 100 mg/kg bisazir. However, a subsequent field trial (Hanson and Manion 1978) failed to provide conclusive evidence that females were completely sterile. Some sterilization methods tested during 1981–1986 failed to completely sterilize early-run males and Hanson (unpublished 1985) conducted additional tests to confirm that bisazir was effective at this stage. Recent observations by Dabrowski *et al.* (2004) showed average egg viability from bisazir injected females to be 21% ± 31 % at pre-hatching. However, eight batches of eggs from sterilized females incubated at HBBS in 2004 had a mean survival of 1.4% and a maximum survival of 4.3%. *Additional tests are needed to solidify estimates of the sterility of females injected with bisazir throughout the run.*

More important issues may be mortality of females injected with bisazir. Sterilized females died in the laboratory sooner than untreated females (personal communication, Konrad Dabrowski, Ohio State University, Bill Swink, U.S.G.S., Hammond Bay Biological Station, Millersburg, MI), although we do not see this pattern with sterilized males in the field. Males released a month or more before spawning are observed in expected ratios on nests in the St. Marys River (Bergstedt *et al.* 2003). *The effect of time of capture and sterilization on survival of females until spawning at current and lower doses needs to be investigated.*

Evaluation of the likely efficacy of the release of sterile females is complicated by the fact their release reduces reproduction in a different manner than the release of sterile males. Knipling (1979) reported that monogamous mating of the female is not a basic requirement of the technique, nor is it necessary that the animals are completely sterile, though some fertility will reduce effectiveness. Where sterile-male release will cause a portion of the female population to immediately waste their eggs when mating with sterilized males, sterile-females release only causes untreated females to waste their eggs to the extent that males cannot mate with all available females and fertilize all eggs deposited (Weidhaas 1968, Knipling 1979). Efficacy of a sterile-female technique is affected by the number of eggs that a male can fertilize (male mating potential). Scribner and Jones (2002) found evidence of polygamous mating, but male mating potential remains unknown. *To evaluate the potential for sterile-female release, we need to know the number of females a male can service and how many sterile females are required per male before we can observe a reduction in reproductive success.* We warn that past efforts to study the sea lamprey mating system by following activities of individuals in enclosures in field tests has proven very difficult. Future studies should consider a broader approach that looks at overall effects on the population scale.

Trapping Additional Animals

Additional sea lampreys can be obtained in several ways. The most obvious is to trap additional rivers. This has been considered and is discussed above under the section on "collection of sea lampreys for sterilization." Minor advances can be made in this area, but most of the economically and logistically viable sources are already being exploited.

Improving trapping technologies is an area of research with greater potential. Some fixed traps that provide attractant water flows at barriers to sea lamprey spawning migrations have efficiencies approaching 80%, but these are exceptions. Behavioral research to help develop more efficient traps in other situations is still needed. *Better techniques to trap animals in streams that lack barriers would be of particular interest*. This issue is discussed in greater length in the GLFC trapping theme paper (under sea lamprey research at glfc.org). Please also see the pheromone theme paper for information on two sea lamprey pheromones that can potentially serve as attractants to increase trap effectiveness (under sea lamprey research at glfc.org).

Culture of Males for SMRT or Genetic Techniques

Expansion of the sterile-male-release technique beyond current levels requires additional males for sterilization. Most scenarios assumed that additional sea lamprey spawners would be imported from outside the Great Lakes basin (i.e., Atlantic Ocean tributaries), but growing sea lampreys from metamorphosis to spawning phase in hatcheries has also been suggested. The previously mentioned failure to raise Great Lakes and Atlantic sea lampreys through the feeding season and two earlier laboratory research projects that tracked feeding and growth of sea lampreys through their parasitic phase (Parker and Lennon 1956, Swink 2003) provide some insight into the difficulties of raising sea lampreys in captivity. In three batches of newly-metamorphosed sea lampreys collected by Parker and Lennon (1956) only 9 of 350 (2.6%), 6 of 40 (15.0%), and 16 of 60 (26.7%) survived and reached maturity. Additionally, the mean weight of surviving sea lampreys (52 g) was much less than for spawning-phase sea lampreys from Lake Huron (125 g).

In five laboratory studies conducted over 7 years by Swink (2003), 292 metamorphosed sea lampreys (total) fed on hosts. Initial weight on the six lampreys that survived through the study ranged from 9.7 to 46.1 g, but all were somewhat larger than newly-metamorphosed sea lampreys when first used. None of the newly metamorphosed sea lampreys and only 4.3% of those that began feeding early in the season survived to the end of a study. Farmer (1980) summarizes a series of studies he conducted that involved feeding sea lampreys on teleost hosts. Estimates of instantaneous growth rate and numbers of hosts killed would have required survival of sea lampreys over time and across hosts. However the fate of individual sea lampreys is not summarized.

With current knowledge, rearing large numbers of spawning-phase male sea lampreys for sterilization does not appear to be practical. Generally less than 25% of laboratory-reared animals survived to spawning phase and growth was often less than for sea lampreys collected from the wild. The mass of live fish required to feed a substantial number of lampreys and potential public reaction to that activity are also daunting. Novel approaches such as a nutritional medium provided through a semi-permeable surface and force feeding have been suggested but never pursued. *Development of a practical method of raising sea lampreys through the parasitic phase would benefit sterile-male release and research into any genetic-based controls.*

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