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2   Evaluating active genetic options for the control of Sea Lampreys (*Petromyzon*

3   *marinus*) in the Laurentian Great Lakes

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26

27   Abstract

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29   For more than two decades the Great Lakes Fishery Commission has sought

30   tactics to complement, and potentially replace, the use of barriers and lampricides to

31   control Sea Lampreys in the Great Lakes, but thus far without success. This paper

32   examines the potential of modern genetic technology to suppress these invasive

33   populations. We identified six recombinant options that appeared to be moderately to

34   highly feasible, most of which were judged by an expert panel as extremely low or

35   low risk, and for which R&D was broadly supported by stakeholders. The two

36   options judged to overall best combine high efficacy and low risks were a Mendelian

37   "sex ratio drive" and genetically modifying a prey species as to kill or sterilize Sea

38   Lampreys that fed on it. Core issues regarding use of genetic biocontrol in the Great

39   Lakes include technical problems associated with maintaining a Sea Lamprey brood

40   line, information gaps for most options, the extent of broader public support, and the

41   extent and nature of national and international consultation required in making

42   decisions about control options.

Introduction

43   **Introduction**

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45   The Sea Lamprey (*Petromyzon marinus*) is one of the world's most

46   destructive environmental species. After invading the upper Laurentian Great Lakes

47   (GL) in the 1930s, the Sea Lamprey destroyed commercial fisheries worth millions of

48   dollars and fundamentally altered the lake ecosystems. Currently, the species is

49   controlled by a joint U.S. and Canadian program, managed by the Great Lakes

50   Fishery Commission (GLFC), that is based principally on trapping, barriers to prevent

51   access to spawning sites, and biocidal treatment of spawning and nursery tributaries.

52   The annual cost exceeds US $20 million. Alternative approaches, such as

53   chemosterilization/sterile male release programs (Twohey et al., 2003; Bergstedt and

54   Twohey 2007) and pheromone-based attractants and repellents (Li et al., 2003;

55   Johnson et al., 2009b), have yet to demonstrate efficacy as management tools (e.g.,

56   Johnson et al., 2013, Dawson et al., 2016). Over the next several years, the costs of

57   control could increase markedly due to stakeholder pressure to remove barriers that

58   currently prevent Sea Lamprey access to large areas of productive habitat (Lavis et

59   al., 2003) and possibly the development of resistance to lampricides (Dunlop et al.,

60   2017). Further, significant Sea Lamprey production arises from connecting

61   channels, such as the St. Clair River between Lake Huron and Lake St. Clair, that are

62   poorly suited for cost-effective management using existing control tactics. These

63   issues reinforce the need for alternative long-term, cost-effective options for

64   managing Sea Lampreys in the Great Lakes (Dunlop et al., 2017).

65   Modern genetic technology could help satisfy this need. The concept of

66   actively using genetic techniques to suppress invasive pest populations is not new. As

67   early as the 1960s, entomologists speculated that genetics could be used to manage

68   insect pests, based on the observation that meiotic drive could distort sex-ratios in

69   insect populations to the point of extinction (Hamilton, 1967). However, the idea

70   languished in the absence of practical genetic tools to effectively manipulate inherited

71   sex ratios. Since then, modern recombinant genetics has provided a range of options

72   for manipulating phenotype. A recombinant lethal construct - the genetic equivalent

73   of the traditional Sterile Insect Technique (SIT) widely used to control insect pests

74   (Krafsur, 1998) - has been extensively tested in the laboratory and in cage trials

75   (Thomas et al., 2000; Phuc et al., 2007; Klein et al., 2012), and has been used

76   successfully to suppress mosquito populations in Brazil, Malaysia and the Cayman

77   Islands (Harris et al. 2012; Lacroix et al., 2012). Several variants of a female-specific

78   lethal gene, a form of inherited sex ratio distortion, have also been demonstrated in

79   insects (Heinrich and Scott, 2000; Thomas et al. 2000; Fu et al. 2007, 2010;

80   Windbichler et al., 2007; Ant et al. 2012; Galizi et al. 2014) and in fish (Thresher et

81   al., 2014b). In part because of these developments, and in part because most well-

82   established invasive species still cannot be effectively controlled using conventional

83   techniques, interest in "genetic biocontrol" has surged (e.g., Saey, 2015; Hall, 2017;

84   Owens, 2017; Prowse et al., 2017). Over this same period, a considerable amount of

85   work has been done on lamprey genetics, including full sequencing of the Sea

86   Lamprey genome (Smith et al., 2013), analysis of gene functions (e.g., Parker et al.,

87   2014), the development of tools for manipulating gene expression (Heath et al., 2014;

88   Romasek et al., 2015) and transgenic gene insertion (Kusakabe et al., 2003). These

89   developments greatly increase the possibility of identifying and manipulating genes

90   that could potentially affect the survival, fertility, and sexual differentiation of GL Sea

91   Lampreys.

92   In this paper, we review and evaluate genetic approaches that could be

93   used to control Sea Lampreys in the Great Lakes. Specifically, we canvas the

94   range of genetic options that could be used against the species, assess the

95   likelihood that any will suppress their abundance (and therefore, their

96   ecological and social impacts) substantially and cost effectively, review the

97   technical feasibility and the risks involved in the deployment of the more

98   promising options, and in a preliminary way, gauge the level of support (or

99   opposition) to research and development of these options and to beginning a

100   wider consultative process that might ultimately lead to their implementation.

101   Specifically, two overarching questions were tested by our analyses:

102   1. Can GL Sea Lamprey abundance be controlled to target levels with a

103   combination of genetic control tactics and other existing tactics using a

104   program that is cost-effective compared to other known alternatives; and

105   2. Can any genetic control tactic for Sea Lamprey management be implemented

106   at levels of risks of unintended consequences that would be judged acceptable

107   by GL stakeholders.

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111   **Methods**

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113   Four approaches were taken to inform the analysis.

114   <u>Consultation with experts</u> – Over an 18-month period, the authors

115   individually or as a group met with expert Sea Lamprey geneticists and

116   ecologists to discuss the state of genetic research on Sea Lampreys, aspects of

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117   their ecology or behaviour that might be exploited using genetic techniques (the

118   "Achilles heel" approach), conservation and management concerns, and

119   suggested genetic options that might be used against the species. Overall, 22

120   experts contributed to the dialogue. As well, the authors met on two occasions

121   with Sea Lamprey management staff of the GLFC to discuss ideas as they

122   developed, and presented status reports and received feedback at the 2016

123   autumn meeting of the GLFC Sea Lamprey Control Board and the 2017 annual

124   meeting of the GLFC.

125   Population modelling - A model of Sea Lamprey population ecology/genetics

126   was used to explore the potential efficacy of different genetic biocontrol options.

127   Model details are provided in the Supplemental Material. Key features of the model

128   are that it simulates two age-structured Sea Lamprey populations, one for the Great

129   Lakes and one "other" (i.e., non-GL) population, which are connected by a small

130   amount of unidirectional parasitic phase migration from the GL population to the

131   other population. The structure is based on the concept of a semi-constrained GL

132   population weakly and intermittently connected to a downstream population, which

133   was incorporated to explore potential non-target effects of genetic control on Sea

134   Lamprey populations beyond the Great Lakes basin (e.g., Finger Lakes or North

135   Atlantic populations). The model is age-structured and assumes constant survival and

136   metamorphosis rates, a Ricker stock-recruitment relationship to predict age 0

137   recruitment and the ability to track the independent genetic dynamics for wild type

138   and genetically modified lampreys, as well as their interaction following biocontrol.

139   Model parameters were informed by prior empirical work on recruitment dynamics

140   (Dawson and Jones 2009) and by a more detailed operating model of Sea Lamprey

141   management (Jones et al. 2009). The model operates at the spatial scale of an entire

142 Great Lake and a single, separate downstream population. The Great Lake population

143 was initialized at a low population level where density dependence is very limited (to

144 reflect the current situation with GL Sea Lamprey given the existing control program).

145 The model was originally developed to explore the expected performance of a

146 Mendelian sex ratio drive tactic (aka a "daughterless construct") and was adapted for

147 this project to consider four other genetic biocontrol options.

148   Risk Assessment - On 13 and 14 October 2016, the authors hosted a risk

149 assessment workshop at the Quantitative Fisheries Center, Michigan State University,

150 on possible genetic options to manage Sea Lampreys in the Great Lakes. Eight

151 experts attended, along with authors, representing a wide range of specialist skills.

152 These included GL invasion biology, genetic technology and biocontrol, the human

153 health implications of genetic techniques, risks and processes underlying horizontal

154 and vertical gene transfer, lamprey developmental genetics, physiology, ecology and

155 control, Sea lamprey reproductive biology and population structure, and Sea Lamprey

156 management in the Great Lakes. An agenda and background document on the genetic

157 options was circulated to panellists prior to the workshop. The workshop was

158 structured around a) an overview of qualitative and semi-quantitative risk assessment

159 methodologies, b) discussion about and evaluation of genetic approaches overall (e.g.,

160 genetic transformation and delivery methods and potential application to Sea Lamprey

161 control), and then c) detailed analysis of six "focal" options (see below), followed by

162 d) a broader discussion of overarching elements associated with the use of genetics to

163 control GL Sea Lamprey. Each focal option was briefly summarized by the authors,

164 including salient modelling results where appropriate, and discussed by the panel.

165 Participants then individually scored and commented on potential adverse effects on:

166   1. Native (non-Sea Lamprey) lamprey species in the Great Lakes (encompasses

167   all native species including those with conservation concern, such as Silver

168   Lamprey and Northern Brook Lamprey)

169   2. The natural (and valued) Sea Lamprey population in the North Atlantic (and

170   possibly those in non-GL freshwater bodies, some of which may be natural)

171   3. Other fish and non-fish species in the Great Lakes (and possibly wider) region,

172   4. Human health

173   5. Other social and ecological endpoints, e.g., cultural issues, recreational uses,

174   water use, ecosystem function

175 Risk estimates were scored by panellists as 1 through 7, where 1 (extremely low) =

176 probability of occurrence during the control program < 1 in 1,000,000; 2 (very low) =

177 > 1 in 1,000,000 to < 1 in 10,000; 3 (low) = > 1 in 10,000 to < 1 in 100; 4 (moderate)

178 = 1-10%; 5 (high) = 10-50%; 6 (very high) = 50-95% and 7 (extremely high) = > 95%

179 probability of occurrence. Following initial scoring for each option, panellists

180 revealed their scores, participants with the highest and lowest scores explained their

181 logic and all panellists were given the option of changing their scores in light of the

182 discussions. Finally, panellists were asked to score each option by their overall

183 assessment of the risk of that option. Given their collective expertise, panellists were

184 also asked to comment on and score the logistical and technical feasibility of each

185 focal option.

186   <u>Stakeholder and community consultation</u> – Along with informal feedback at

187 the meetings of the Sea Lamprey Control Board and Great Lakes Fishery

188 Commission, fisheries managers, scientists and members of the Lake Superior and

189 Lake Huron fishing communities from Canada and the U.S. were formally consulted

190 about the acceptability of using genetics actively to control GL Sea Lampreys. Based

191   on the Committee on Gene Drive Research in Non-Human Organisms (2016), the

192   respondents were categorized as "stakeholders", which included professional state,

193   provincial, federal, regional and tribal biologists, fishery managers, the GLFC

194   Commissioners and staff, academic and government scientists, and Canadian and U.S.

195   members of the GLFC citizen advisory groups, all of whom have some involvement

196   in managing Sea Lamprey, and the "fishing community", which included avid

197   recreational fishers who would be broadly familiar Sea Lamprey impacts, the ecology

198   of the lakes, and on-going efforts to manage the problem, but not directly connected

199   with the GLFC and its management activities. With the exception of a few scientists,

200   none of the survey participants had apparent backgrounds in biotechnology beyond

201   basic information gleaned from the media.

202   Two on-line surveys were conducted (SurveyMonkey Inc., San Mateo, CA,

203   USA, www.surveymonkey.com). The stakeholder survey was conducted in

204   March/April, 2017 with a response rate of 73% (95 returns from 131 individuals

205   contacted). The fishing community survey was conducted in August/Sept. 2017, with

206   a response rate of 51% (49 of out 96 individuals contacted). All responses were

207   anonymous and the same questionnaire was used for both surveys. It consisted of

208   three sections: (1) questions about the respondent's background and their perception

209   of the importance of managing Sea Lamprey, (2) a brief description of each focal

210   option, including a summary of risks as determined by the expert panel, followed by

211   a) questions regarding level of support for research and development (R&D) on that

212   option and for beginning a consultative process that could lead to its' implementation,

213   and b) a checklist of generic objections (e.g., cost, unethical, other options available,

214   human health risks), along with a space to list "other" objections, and (3) a free-form

215   section soliciting comments on any subject relevant to the issue, but in particular

216   reasons why the respondent favoured or opposed the use of biotechnology to manage

217   Sea Lamprey in the Great Lakes.

218   Non-parametric statistics were used to analyse the data, using Staview.

219   Regression tree analysis (De'ath and Fabricius 2000), using the R-package 'tree'

220   (Ripley 2016), was used to identify key concerns that distinguished between those

221   supporting and those opposing the use of each focal option.

222

223   **Results**

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225   The initial scope of the project was deliberately broad (Table 1),

226   spanning concepts suggested in the literature and ideas solicited from experts

227   and the wider community. Two approaches were subsequently excluded from

228   further consideration. One line of genetic biocontrol research focuses on using

229   modified pathogens or parasites to suppress pest populations (Cowan, 1996;

230   Hardy et al., 2006), including disease-vectoring mosquitoes (Hoffmann et al.,

231   2011). Based on initial discussions with stakeholders and the GLFC, and

232   adverse public reaction to this approach elsewhere when applied to vertebrates

233   (Hardy et al., 2006), we did not evaluate it for future Sea Lamprey applications.

234   An alternative approach, broadly referred to as "autocidal" (Gould and

235   Schliekelman, 2004; Thresher, 2008), involves genetically modifying the pest

236   itself such as to reduce its impacts. One such set of autocidal options involves

237   manipulating parental chromosomes (e.g., triploidy and YY males) to reduce

238   population fecundity or distort population sex ratios (Gutierrez and Teem, 2006;

239   Erickson et al., 2017; reviewed by Thresher et al., 2014a). These options require

240   obligate chromosomal sex determination with few, if any, autosomal sex

241   modifiers. Available information, though not definitive, suggests weak

242   chromosomal sex determination in Sea Lampreys, with an individual's sex

243   determined by environmental cues or growth rates (Docker and Beamish, 1994;

244   Johnson et al., 2017). Consequently, we have also excluded chromosomal

245   approaches from our evaluation.

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247   **Evaluation of genetic options**

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249   Modelling, plus the initial discussions with lamprey and GL biologists and

250   managers, resulted in the diverse genetic options initially considered being narrowed

251   down to six "focal" options. All appear to be logistically and technically feasible, and

252   all could potentially suppress Sea Lamprey population abundance and associated

253   impacts in the Great Lakes.

254

255   Heritable (Mendelian) sex ratio drive – Among the numerous genetic

256   biocontrol options being considered globally, one that heritably distorts offspring, and

257   hence population, sex ratios is considered the most effective (Bax and Thresher,

258   2009). Specifically, the approach is based on a genetic construct that is carried by

259   individuals of one sex with no adverse effects but when passed on to offspring

260   sterilizes or kills members of the other sex, or that results in obligate male or female

261   development, irrespective of genotype. Theory and modelling indicate that for most

262   species, a construct that is lethal to females or that causes females to develop as

263   phenotypic males ("daughterless") is more effective than a "sonless" construct, as the

264   number of females typically constrains population fecundity (Hamilton, 1967).

265   Application of this technique requires identification of genes that are either sex-

266 specific or that determine phenotypic sex. Sexual differentiation in lampreys may be

267 polygenic, in which individuals with high numbers of "sex determining genes"

268 become one sex and those with fewer such genes become the other (M. Docker,

269 University of Manitoba, Winnipeg, Manitoba, personal communication, 2016). If so,

270 then a knockout of a mammalian SRY-equivalent (Sekido and Lovell-Badge, 2009) in

271 lampreys may not be possible. However, targeting key molecules further along the

272 differentiation pathway, perhaps equivalent to aromatase in teleosts, may be feasible.

273 Aromatase converts androgens to estrogens in teleosts and, when blocked chemically

274 (Piferrer et al., 1994; Kwon et al., 2000) or genetically (Thresher et al., in prep.), can

275 cause individuals to develop as males irrespective of genetic sex. Aromatase is absent

276 in lampreys (M. Docker, University of Manitoba, Winnipeg, Manitoba, personal

277 communication, 2016; W. Li, Michigan State University, Lansing, Michigan, personal

278 communication, 2016) but there may be analogues. The genes involved in sexual

279 differentiation in Sea Lamprey have not yet been identified, but considerable work

280 has been done on their reproductive physiology (reviewed by Sower, 2015). The

281 complex set of steroidogenic pathways in the lamprey reproductive system differs

282 chemically from those of jawed vertebrates and could offer options for manipulating

283 sex ratios in an agnathan-specific fashion. Alternatively, field data suggests that

284 gender in Sea Lamprey is often growth rate dependent (see below), suggesting that

285 offspring sex ratios could be manipulated indirectly by genetically down- or up-

286 regulating rates of larval growth using, for example, CRISPR-Cas9 knock-outs or

287 knock-ins (Square et al., 2015; Zu et al., 2016) of growth hormones.

288   The use of a sex-biasing construct inherited via Mendelian processes to

289 suppress invasive species would require high stocking rates of individuals with many

290 independently segregating copies of the blocking construct (Schliekelman et al., 2005;

291   Bax and Thresher, 2009; Thresher et al., 2014a). However, this strategy assumes

292   strong chromosomal sex determination and an equal sex ratio. Neither assumption

293   may hold for Sea Lamprey in the Great Lakes. Laboratory and field data provide

294   evidence for environmental sex determination in lampreys (Beamish, 1993; Docker

295   and Beamish, 1994), possibly driven by population density and consequent variations

296   in larval growth rates. Faster growing, earlier maturing individuals are predominantly

297   female (Johnson et al., 2017). As a likely consequence, long term data from the Great

298   Lakes indicate a shift from an initial pronounced male bias among Sea Lamprey

299   populations at the start of control efforts using lampricides to one dominated by

300   females after effective control had been achieved (Purvis, 1979). By the late 1970s,

301   females constituted an average of about 70% of the adult Sea Lamprey population in

302   the upper Great Lakes. More recent work suggests populations may have shifted back

303   to a roughly 50:50 male-to-female sex ratio (Hanson et al., 2016), though the authors

304   note the inherent difficulties of sampling sex ratios accurately. Data on sex ratios of

305   Sea Lamprey populations native to the North Atlantic are sparse. Beamish (1980a)

306   reports males outnumbering females in Canadian Sea Lamprey populations. Recent

307   qualitative estimates suggest roughly equal sex ratios in small samples of Sea

308   Lampreys collected in the north-eastern US (S. Sower, University of New Hampshire,

309   Durham, New Hampshire, personal communication, 2016). In contrast, Beaulaton et

310   al. (2008) reports a female bias in Sea Lamprey populations in French rivers.

311   Our modelling indicates that release of individuals carrying conventionally

312   (Mendelian) inherited constructs that dictate the male phenotype or are lethal to

313   females can effectively suppress GL Sea Lamprey populations, consistent with

314   models based on other taxa (Fig. 1A, Table 2). In contrast with other studies (Bax &

315   Thresher, 2009) we found that a female-lethal construct was somewhat less effective

316   than a male-specifying (daughterless) construct. For a given stocking rate of

317   genetically modified (GM) individuals, we identified two other factors that affect the

318   efficacy of a sex-ratio-distorting program. First, effective suppression depended on

319   Sea Lamprey abundance already being at low levels as a result of other, coincident

320   control actions (e.g., lampricides or barriers). At higher abundances, compensatory

321   processes severely limit the effect of sex ratio distortion on future recruitment.

322   Second, the sex-ratio of the target population had a large impact on suppression (Fig.

323   1B, Table 2). If sex ratios are biased towards females in low density populations, then

324   female numbers, and hence population fecundity, will decline as genetically modified

325   individuals that would otherwise have been females develop as phenotypic males.

326   The smaller the target population, the more the daughterless carriers dominate the

327   male population and the smaller the remaining number of females, resulting in a

328   ratchet that will eventually drive the population to extinction. The more female-

329   biased the wild type sex-ratio, the stronger the impact of a daughterless construct. For

330   populations in which sex ratios are roughly equal, the impact of stocking daughterless

331   carriers is far less, and is reversible if stocking is discontinued.

332

333   Trojan Gene – A Trojan Gene is a construct that pleiotropically has a positive

334   effect on one or more fitness components, and negative effects on others. An example

335   would be a gene that increases mating advantage or attractiveness while decreasing

336   the viability of genetically modified offspring. The concept was developed and

337   modelled by Muir and Howard (1999, 2002), who noted that the use of genetic

338   sterility to contain genetically (or otherwise) modified animals could have a severe

339   adverse effect on wild type populations if the construct also increased carrier

340   competitiveness for mates. However, they also noted that a Trojan Gene could a

341   useful tool for managing invasive pests (Muir and Howard, 2004). Bax and Thresher

342   (2009) further modelled Trojan Genes as a method of pest control, confirming

343   predictions by Muir and Howard (1999, 2002), but also noted that there were tight

344   constraints on the combinations of partial sterility and mate attractiveness that could

345   result in eradication of a wild type population.

346   The use of technology analogous to a Trojan Gene to control GL Sea Lamprey

347   populations was suggested by Li et al. (2003b), based on genetically increasing the

348   attractiveness of males that had been independently, artificially sterilized using

349   Bisazir (Siefkes et al., 2003; Twohey et al., 2003). Diverse genes can potentially be

350   targeted to induce sterility in Sea Lamprey, though none have been described thus far.

351   In other taxa, including teleosts, disrupting expression of genes associated with

352   spermatogenesis and oogenesis constitute tissue- and stage-specific targets for

353   reducing fertility or causing full sterility (Hardy et al., 2006; Klein et al., 2012).

354   Analogues in Sea Lamprey are highly likely. As well, the 15alpha hydroxylated

355   steroids, apparently unique to lampreys (Sower, 2015), constitute appealing targets for

356   inducing lamprey-specific sterility if other effects on sexual development and

357   reproduction are minimal. Increasing mating success in male individuals could be

358   accomplished by genetically up-regulating the production of pheromones that attract

359   females to nest-tending males (Li et al., 2003a). The physiological factors that limit

360   pheromone production are not yet clear, but there are a number of possibilities that

361   could be explored (W. Li, Michigan State University, Lansing, Michigan, personal

362   communication, 2016). Whether pheromone production can be increased to the point

363   of substantially increasing the mating success of individual males in the complex

364   topography of nesting males and migrating females in streams is also not yet clear.

365   We modelled this option assuming Trojan males are sterile. Allowing the

366   males to be partially fertile results in an on-going, non-hatchery production of Trojan

367   males, but the benefits are relatively small (Bax and Thresher, 2009). The effect of

368   releasing sterile males depends on the number released, their mating competitiveness

369   and the duration of the release program (Fig. 1C and D). If there is no increase in

370   mating success, the program is the GM equivalent of the previous sterile male release

371   program that relied on chemosterilization (Twohey et al., 2003), although with

372   differing costs and bio-hazard risks. Under such conditions, a 98% reduction in GL

373   Sea Lamprey abundance within 50 years requires an annual release of approximately

374   1 million sterile male larvae (Table 1). Increasing the mating competitiveness of the

375   released males threefold reduces the required stocking rate to 200,000 males/yr (Table

376   2).

377

378   Development of a non-parasitic Sea Lamprey – Lampreys frequently occur as

379   species pairs, one of which is anadromous and parasitic and one of which is non-

380   migratory and non-parasitic (Docker, 2009). The taxonomic status of such species

381   pairs, which typically are identical as larvae and differ only as adults (parasitic forms

382   typically larger) is ambiguous, though recent work by Mateus et al. (2013) finds

383   significant differences in allelic frequencies in a European species pair. The

384   differences appear to relate to genes associated with the migratory ability of the

385   parasitic form.

386   The ubiquity and apparently small genetic differences between parasitic and

387   non-parasitic forms suggest a relatively simple genetic "switch" between the

388   phenotypes, possibly related to age at sexual maturation (M. Docker, University of

389   Manitoba, Winnipeg, Manitoba, personal communication, 2016). Whether or not

390   such a "switch" occurs in Sea Lampreys is not known, but if so, it may be possible to

391   trigger it prior to metamorphosis either in a genetically modified integrated line or

392   through mass transformation. Further, if parasite population sizes are constrained by

393   density-dependent competition at the ammocoete stage, which is not certain, then

394   release of a heritable non-parasitic form could sustainably reduce numbers of

395   metamorphs of the parasitic form. The magnitude of the reduction would depend on

396   the relative competitiveness of the parasitic and non-parasitic forms and the extent to

397   which the latter can build up high densities in individual drainages. Whether the non-

398   parasitic form could displace the parasite within the Great Lakes in general would

399   depend on 1) hybridization between the two phenotypes, which might be low given

400   likely size differences between adults of the parasitic and non-parasitic forms and a

401   tendency for size-selective mating in lampreys (Beamish and Neville, 1992); 2) the

402   construct being dominant (haplosufficient); and 3) the construct conferring a fitness

403   gain high enough to offset the likely much higher individual fecundity of the larger

404   parasitic females.

405   We did not model this approach, given the large uncertainties in larval ecology

406   and the effects of competition not only among Sea Lamprey larvae, but potentially

407   with the larvae of sympatric native lampreys and other stream taxa. Dawson and

408   Jones (2009) did not find evidence for negative effects of high competitor density on

409   Sea Lamprey recruitment, and concluded that higher competitor density was

410   indicative of better rearing habitat in the streams being investigated. Nevertheless, if

411   GL Sea Lamprey ammocoetes compete with those of native species, then it might be

412   possible to reduce population abundance without genetic manipulation by augmenting

413   the abundance of native non-parasitic taxa.

414

415   <u>Sustained release of sterilizing or lethal molecules in nursery drainages</u> –

416   Molecular techniques hold promise for developing cost-effective slow release,

417   species-specific biocides, sterilizing agents or reproductive disrupters. A range of

418   molecular blockers (zincfinger nucleases, morphelinos, microRNAs, long hairpin

419   RNAs) are well documented to block gene expression *in vivo* and have been widely

420   used experimentally to determine gene function (e.g., Meng et al., 2008; Bill et al.,

421   2009). However, the most widely applicable and effective molecular means of gene

422   knockdown is short interfering RNA (siRNA), which is widely used for therapeutic

423   applications in human health (Kim and Rossi, 2007) and, in agriculture, to suppress

424   pest impacts through endogenous production in plant tissues of siRNAs that are toxic

425   to pest insects (Kim et al., 2015). Similar approaches appear to be applicable to Sea

426   Lamprey. Short interfering RNAs delivered orally in a mixture of a transfection agent

427   and yeast cells (normal food for cultured ammocoetes) not only delivered the siRNAs

428   to feeding Sea Lamprey larvae, but also resulted in significant larval mortality (Heath

429   et al., 2014). The proof-of-concept study suggests that oral delivery of suitable

430   siRNAs could be used for a species-specific knockdown of Sea Lamprey larvae in

431   nursery drainages throughout the Great Lakes.

432   Four factors need to be addressed before siRNAs can be used in the field.

433   First, although widely cited as sequence-specific (and hence species-specific), partial

434   matching of the siRNA nucleotide sequence can result in off-target effects (e.g.,

435   Jackson et al., 2003; Lundgren and Duan, 2013). Off-target effects, in the form of full

436   or partial suppression of non-targeted genes, could be irrelevant or even useful if the

437   intent of the siRNA treatment is high levels of larval mortality. However, expression

438   due to partial sequence matching (as few as 6 nucleotides) could reduce the degree of

439   species-specificity and hence result in impacts on taxa other than Sea Lamprey. This

18

440   risk can be minimized in the design of the construct (e.g., Kamola et al., 2015).

441   Second, disrupting genes, such as those involved in sexual differentiation, require

442   organism-wide (systemic) effects of the siRNA molecules. Whether this is readily

443   achievable when the molecules are delivered in the larval diet is not clear. If impacts

444   are localized only in gut tissues (Heath et al., 2014), suitable targets might be limited

445   to gut-specific genes, many of which are likely to be lamprey specific (e.g., Conlon et

446   al., 1993). Third, the persistence of naked siRNA molecules in the aquatic

447   environment is not known, but could be on the order of hours (Dubelman et al., 2014).

448   If so, effective delivery could need to be in the form of microencapsulated particles

449   (Peanparkdee et al., 2016; for a fish example, see Yufera et al., 1999) of the correct

450   size to be ingested by filter-feeding ammocoetes. The particles could be dispersed

451   manually, or slow-released in the form of slowly dissolving blocks placed into

452   drainage headwaters (S. Whyard, University of Manitoba, Winnipeg, Manitoba,

453   personal communication, 2016.).   The latter option would result in long-term

454   accumulation of a lethal, sex determining or sterilizing agent in ammocoetes with a

455   large and potentially cost-effective flow-on effect to parasite population numbers.

456   Fourth, such a strategy requires large scale production of siRNAs. Therapeutic

457   applications of siRNAs involve minute amounts of the molecules. Larger amounts of

458   siRNAs can be produced using bacterial and viral "biofactories" (e.g., Aalto et al.,

459   2007), possibly at relatively low cost.

460   An alternative approach, which could achieve permanent reduction in GL Sea

461   Lampreys at negligible sustained cost, would involve genetically engineering an alga

462   or bacteria that is naturally fed upon by the ammocoetes (Dawson et al., 2015) to

463   produce the siRNA. The molecule could be delivered either in the bacteria or alga

464   themselves or in the organic detritus derived from such sources. The approach is

465   analogous to the *in vivo* production of siRNAs in crop plants to suppress insect pest

466   damage, noted above and reviewed by Kim et al. (2015). Methods for genetically

467   modifying algae, bacteria and viruses are very well developed (reviewed by Mendoza

468   et al., 2016) and recently one genetically modified alga was successfully field trialled

469   (Szyjka et al., 2017). Species-specificity, as well as efficacy, would need to be

470   rigorously confirmed and, if an algal or bacterial host was used to express and

471   permanently distribute the agent, it would be critical to use a species found only in

472   Great Lakes drainages as a vector.

473   The magnitude of the impacts of a synthetic blocking molecule on parasite

474   populations depends on the efficacy of the blocking and the number of drainages into

475   which it is applied. Modelling suggests that even a sustained 10% increase in rates of

476   ammocoete mortality over those currently achieved using existing biocidal treatments

477   could substantially depress parasite abundance; a 20% increase essentially results in

478   virtual eradication of the GL Sea Lamprey population within 50-75 years (Fig. 2A,

479   Table 2). Direct impacts on non-target populations would be nil if the construct was

480   species-specific and delivery was in the form of manual dispersal or slow-release

481   blocks in nursery area headwaters. Impacts could be more significant if an alga or

482   bacteria was modified to produce and deliver the agent, depending on the distribution

483   of the vector.

484

485   Vaccinated prey – Parasitism by Sea Lamprey involves post-metamorphic pre-

486   adults attaching to a host fish, inflicting a non-lethal wound, and consuming blood

487   and other body fluids. Smaller hosts frequently do not survive the attack due to loss

488   of host fluids, whereas larger ones frequently survive (Jorgenson and Kitchell, 2005)

489   and, on the basis of multiple scars, can be attacked again. Fatty acid analysis of

490 lampreys (Happel et al., 2016) and their activity cycles (Bergstedt and Swink, 1995)

491 suggest each individual feeds on multiple hosts, but the number of hosts is not known

492 with certainty. For our analysis, we use ten as a plausible first estimate, based on

493 Table 4 in Bence et al. (2003).

494   The ectoparasitic feeding mode suggests that Sea Lamprey could be

495 vulnerable to molecules in the blood stream of their fish hosts that are lethal to,

496 sterilize or cause morbidity in the parasite. As noted above, a likely candidate set are

497 short interfering RNAs (siRNAs), which have already been demonstrated to affect

498 lampreys when consumed (Heath et al., 2014). Potential targets for siRNAs include

499 lamprey-specific anti-coagulants injected by lampreys into their hosts to facilitate

500 feeding (Ito et al., 2007), and gut-specific genes, many of which are also likely to be

501 lamprey specific given their unusual feeding ecology (e.g., Conlon et al., 1993).

502 Down-regulation of either target could reduce lamprey feeding efficacy and lead to

503 parasite morbidity, increased mortality, or reduced fecundity and/or mating success as

504 adults. If systemic up-take is feasible, mortality may be achievable. Zu et al. (2016)

505 showed that disrupting expression of the gene kctd10 in Sea Lampreys resulted in 55-

506 85% of the injected animals showing severe heart defects, which presumably would

507 lead to mortality in field situations. More broadly, two gonadotropic releasing

508 hormones (GnRH-I and III) in Sea Lampreys not only differ in protein structure from

509 those of gnathostome vertebrates, but when blocked experimentally result in

510 approximately 60% of males being sterile (Sower, 2003). The experiments were not

511 "optimized" in terms of the experimental GnRH antagonists involved, which suggests

512 that inducing higher rates of sterility is possible (S. Sower, University of New

513 Hampshire, Durham, New Hampshire, personal communication, 2016). Agnathan-

514 specific hormones critical to sexual maturation appear to be promising targets for

515   orally sterilizing the parasites with no adverse effects on the construct-carrying hosts

516   and on other GL vertebrates that might consume the hosts, including humans. The

517   DNA sequences of GnRH-I and III have been published (Suzuki et al., 2000; Silver et

518   al., 2004), facilitating design of siRNA blockers. Alternatively, high rates of lamprey

519   sterility could be induced by up-regulating, for example, thiaminase production on

520   one or more host species. Recent observations suggest that a high proportion of

521   female Sea Lamprey collected in Lake Michigan are functionally sterile, producing

522   low quality eggs and larvae (D. Medeiros, University of Colorado, Boulder, Colorado,

523   personal communication, 2016; D. McCauley, University of Oklahoma, Norman,

524   Oklahoma, 2017) reminiscent of the effects of orally consumed thiaminase on Lake

525   Trout reproduction (Jarszewska et al., 2009). The cause of the low fertility is not

526   known, but that Sea Lamprey consume bodily fluids of Lake Trout and other

527   salmonids that can be high in thiaminase is perhaps worth examining in more detail

528   (Dabrowski et al., 2004).   The effects of injected molecules on the survival and

529   fertility of Sea Lamprey could be tested experimentally either using an artificial

530   feeding method ("blood bags", originally suggested for lamprey trials in the 1990s,

531   but not subsequently developed) or injected into hosts that are subsequently fed to the

532   parasites.

533   Methods for genetically modifying some host species (e.g., Rainbow Trout,

534   Carp) are well developed and in routine use (Iyengar et al., 1996), including mass

535   transformation techniques (Powers et al., 1992). It is highly likely these techniques

536   could be readily adapted for use in other host species, e.g., Lake Trout or Lake

537   Whitefish. Host species selection is likely to be highly critical for this technique to be

538   successful, both in terms of maximizing the likelihood of a Sea Lamprey attacking a

539   molecule-carrying target and in terms of public acceptability (see below). An ideal

540   host would be one routinely attacked by Sea Lamprey, but not native parasitic species

541   (e.g., Silver Lamprey) and not consumed by humans, due to perceptions of possible

542   health risks. Although Sea Lamprey attack a range of host species (Happel et al.,

543   2016), Lake Trout appear to be a preferred host. Lake Trout are also hatchery

544   produced and stocked in large numbers into most of the Great Lakes, such that the

545   infrastructure required to produce and release large numbers of carriers is mostly

546   already in place. Wounds on the fish suggests that the siscowet Lake Trout of Lake

547   Superior may be a useful possibility, in that the form is relatively sluggish, inhabits

548   deep-water, may be a preferred target for parasitism (Zhuikov, 2016) and, being high

549   in fat, is not preferred by recreational and commercial fishers. The deep-water habitat

550   of the fish overlaps that of Sea Lamprey, but not that of Silver Lamprey, which prefer

551   warmer water and apparently feed inshore (Cochran and Marks, 1995). Siscowet are

552   also morphologically different from other Lake Trout strains (smaller head, deeper

553   body, blunter convex snout, shorter, thicker caudal peduncle) which could facilitate

554   avoidance of the carriers by fishers, if desired, in lakes where the strain is not native.

555   Depending on the stocking strategy (see below), stocked carriers could also be tagged.

556   We modelled the potential effect of vaccinated hosts by adjusting downward

557   Sea Lamprey survival rates at the parasitic life stage to reflect plausible levels of

558   additional mortality due to the genetically modified hosts (Fig. 2B). Assuming 10

559   attacks per parasite, the probability of a parasite attacking a vaccinated host if 4% of

560   hosts were GM type (i.e., 200,000 GM hosts in a population of 5,000,000) would be

561   34%. Assuming a single feed is lethal to or sterilizes a parasite, this would translate

562   into an equivalent reduction in parasitic survival (ignoring possible compensatory

563   effects). Simulating a 34% reduction in parasitic survival, together with continuation

564   of existing control measures, resulted in a 92% population reduction in 50 years

565   (Table 2). If parasites on average attack fewer than 10 hosts, the proportion of GM

566   hosts would need to be higher to achieve the same outcome. A positive aspect of the

567   vaccinated prey option is that if the GM host is a freshwater species there is no risk to

568   native North Atlantic Sea Lamprey populations.

569   The combination of the relatively small number of carriers required to achieve

570   substantial GL Sea Lamprey control, existing hatchery facilities and available

571   expertise suggest it may not be necessary to create a lineage of genetically modified

572   hosts to achieve control objectives. Mass transformation techniques, such as

573   electroporation (Powers et al., 1992) and biolistics (Zelinin et al., 1991) applied to

574   eggs and early stage larvae, can produce very high numbers of carrier phenotypes

575   quickly and at low cost. Intramuscular injection of constructs, though more labor

576   intensive, can also produce salmonids capable of expressing a construct for up to 1.5

577   years post-injection, though this is primarily realized only near the point of injection

578   (Anderson et al., 1996; Seternes et al., 2016) without integration (i.e., germ line

579   transformation). Potentially, hosts could be sterilized using heat or pressure-shock

580   induced triploidy, then transformed *en masse* using electroporation for subsequent

581   grow out, tagging and release. Given existing hatchery capability and stocking efforts

582   for, e.g., Lake Trout, even if only a proportion of the treated fish proved to be

583   effective carriers, it should be possible to achieve the 6-7% carrier stocking rate

584   required to suppress parasite abundance without genetically contaminating existing

585   host stocks. Such an approach would also ensure that the carrier phenotype

586   disappears at the end of the control program, because they are no longer stocked.

587

588   Gene-driven sex ratio distortion – As noted above, the release of a normally

589   (Mendelian) inherited gene construct that causes male or female-specific lethality,

590   sterility or obligate phenotypic development can result in long-term population

591   decline, irrespective of native population sex ratio. The magnitude and efficiency of

592   the approach depends on the number of independently segregating copies of the

593   construct and the rate at which carriers are stocked into the targeted population

594   (Schliekelman et al., 2005; Bax and Thresher, 2009). Achieving high copy numbers

595   and high stocking rates can be technically and logistically challenging. Burt (2003)

596   suggested an alternative approach, based on "selfish genes". Homing endonucleases

597   are a class of genes that when inherited as a single copy (heterozygousity) duplicate

598   themselves onto the complementary chromosome, thereby becoming homozygous

599   (Gimble and Thorner, 1992). As a result, homing endonucleases are potentially

600   inherited by all of an individual's offspring, rather than only half, and the construct

601   spreads rapidly in the population so long as any fitness cost due to the construct is less

602   than the gain due to producing more than the normal number of carrying offspring

603   (Burt, 2003; Deredec et al., 2008; 2011). Burt (2003) suggested than such "gene

604   drives" could be used to efficiently engineer natural populations to, for example,

605   render than incapable of transmitting disease-causing parasites or, in the case of an

606   invasive species, distort sex ratios to suppress pest populations. In theory, the release

607   of a single individual carrying a gene drive could permanently alter or suppress a

608   targeted population (Saey, 2015; Webber et al., 2015). The logistical advantages

609   gained by use of a gene drive for pest population suppression are substantial, as are,

610   potentially, the risk to non-target populations and species if the construct spreads from

611   the targeted population (Webber et al., 2015; Esvelt and Gemmel, 2017), either

612   through normal vertical transmission or horizontal gene transfer (Kuraku et al., 2012).

613   Whether or not this risk is realized is uncertain at this stage, due to strong selection

614   for resistant individuals, and could depend on the design of the gene-drive construct

615   and the sequence targeted by it (Hammond et al., 2017). Strong counter-selection

616   could also dilute and possibly fully negate the gain in efficacy and the ultimate impact

617   of a gene-driven system on targeted populations

618   Burt and colleagues have been developing the concept for the control of

619   Anopheline mosquitoes, using a modified naturally occurring homing endonuclease

620   (Windbichler et al., 2011; Klein et al., 2012). The need to use naturally occurring

621   drive elements limits the scope of potential targets and also the efficacy of the

622   approach, as the rate of gene duplication (homing) in naturally occurring systems is

623   typically well below 100% (Chan et al., 2011; Windbichler et al., 2011). Recently,

624   however, "synthetic" gene drives have been developed, based on variations of the

625   bacterially-derived CRISPR gene editing system (Esvelt et al., 2014). CRISPR-based

626   gene drives are not only widely applicable, but can achieve homing rates in excess of

627   90% (Gantz and Bier, 2015; Gantz et al., 2015). The potential of synthetic gene

628   drives for cost-effectively engineering wild populations has received considerable

629   attention in the technical and popular media (Champer et al., 2016; Committee on

630   Gene Drive Research in Non-Human Organisms, 2016; Harmon, 2016), including its

631   potential to suppress pest populations currently deemed unmanageable (Hammond et

632   al., 2016; Szymczak, 2016; Hall, 2017; Prowse et al., 2017). However, the threat such

633   technology poses to non-target species has resulted in recommendations for rigorous

634   containment protocols for laboratory studies of the technology (Araki et al., 2014;

635   Oye et al., 2014), and a variety of suggestions to reduce the risks to non-target

636   populations. The latter include incorporating a genetic "off-switch" in the construct,

637   genetically modifying the non-target population(s) to be resistant to the suppression

638   drive (Esvelt et al., 2014), and using "daisy drives", in which the essential

639   components of a series of gene drives are released separately, combine to effect

640 population suppression, but then cease to spread as "lower" elements in the drive

641 chain are lost over time (Noble et al., 2016; Esvelt and Gemmel, 2017). CRISPR

642 gene editing techniques, though not a gene drive version as yet, have already been

643 demonstrated as viable in Sea Lamprey (Square et al., 2015; Zu et al., 2016).

644   We adapted our model to evaluate a gene drive by simulating non-Mendelian

645 inheritance of a sex ratio distorting construct. As expected based on generic modelling

646 (e.g., Beaghton et al., 2016), the simulations indicate that even very modest releases

647 of "gene-drive" larvae (i.e., 100 individuals for 1 year only) results in effective

648 eradication of GL Sea Lamprey within 200 years following their release (Fig. 2C,

649 Table 2). This initial model assumes a 100% homing rate (all offspring of a modified

650 parent carry the construct). The small numbers of stocked carriers needed for control

651 suggest a program based on mass transformation of lamprey eggs rather than the

652 logistically much more demanding need for an integrated line of genetically modified

653 carriers. Virtual eradication could be achieved much more quickly with annual

654 releases of carriers similar to the Mendelian sex ratio drive presented earlier (100,000

655 GM larvae/yr for 10 years, Fig. 2C, Table 2). Despite these positive outcomes, under

656 all scenarios the "Other" (non-target) population of Sea Lamprey is also eventually

657 driven to virtual extinction due to the escape of carriers from the Great Lakes, even at

658 very low emigration rates (Fig. 2D). The extent to which this outcome can be

659 avoided by use of more complex gene-drive-based strategies, such as "daisy drives",

660 is not yet known.

661

662 **Risk Assessment**

663

664   Overall, panellists judged the risks of the six focal options as low to extremely

665   low for all five potentially impacted categories, based on median responses (Fig. 3).

666   The group judged to be at highest risk was "other" Sea Lamprey populations, due to

667   possible spread of the gene construct beyond the Great Lakes. The category judged

668   overall to be at least risk (other than "Other") was Human Health. The latter,

669   however, was predicated on the design of a Sea Lamprey-specific construct, rigorous

670   testing for off-target effects, and dosages of the effector molecules that are very low

671   in consumed fish.

672   The magnitude of perceived risks differed widely among the six focal options

673   (Table 3). Note that risks were assessed separately for deploying an artificial source

674   of a synthetic biocide as opposed to genetically modifying an alga or bacteria to

675   produce it, due to their very different risks of spread beyond the Great Lakes. Across

676   all options, "overall" risk for each correlated highly with the score for the group

677   deemed by panellists to be at highest risk (Spearman rank rho = 0.96, p = 0.002).

678   Risk, however, did not correlate with perceived technical/logistical feasibility (rho =

679   0.11, NS). Panellists considered the release of Trojan males to be the least risky

680   option; they considered gene-driven sex-ratio distortion the most risky. All panellists

681   judged the risks of the Trojan gene approach as Extremely Low to Very Low across

682   all risk categories. In contrast, panellists differed widely in their judgements

683   regarding the gene drive, as well as Mendelian sex-ratio distortion and the release of a

684   self-propagating (algal or bacterially vectored) larval biocide. At least one panellist

685   scored each of these three options as Extremely risky (>95% probability of

686   occurrence) for at least one group potentially at risk.

687   The group perceived as at high risk for the gene-driven and Mendelian sex-

688   ratio distortion options was non-target (e.g., Atlantic) Sea Lamprey populations. In

689   theory, even one construct-carrying individual could have a long-term, adverse impact

690   of such populations. The central issue and source of uncertainty among panellists for

691   the gene-drive was the extent to which dispersal of construct carriers out of the Great

692   Lakes could be maintained at essentially zero. Our models assume an escapement

693   each year of 0.01% of the Great Lakes Sea Lamprey population. The estimate is

694   crude, but at least some movement down system by Sea Lampreys is suggested by

695   evidence that Lake Trout, at least, occasionally survive going over Niagara Falls (C.

696   Krueger, Michigan State University, Lansing, Michigan, personal communication,

697   2016). More esoteric dispersal mechanisms (e.g., Najarian, 2015), including

698   intentional and unintentional human-mediated release (Johnson et al. 2009a; Drake et

699   al. 2015), also cannot be ruled out.

700   For the Mendelian approach, the main concern and source of disagreement

701   was the uncertain sex-ratio of non-target populations. Although available data

702   suggest the sex ratios of the Western Atlantic population is roughly 50:50 (and hence

703   would be resistant to the Mendelian approach), data are sparse, Atlantic populations

704   have been heavily impacted by pollution, habitat loss and over-fishing (Hansen et al.,

705   2016), all of which could lead to population depression and a consequent shift to a

706   female-biased sex-ratio, and data indicate a female bias among some European

707   populations (Beaulaton et al., 2008). Effects of released construct carriers on

708   European populations might be mitigated by limited genetic exchange between

709   Western and Eastern Atlantic populations (Wright et al., 1985; Rodriguez-Munoz et

710   al., 2004), but probably not reduced to zero. Sex-ratios for other, possibly native

711   fresh-water populations (see discussion in Eshenroder, 2014) are not known, and they

712   could also be vulnerable to the Mendelian sex-ratio option (as well as to the gene

713   drive).

714   Experimental work demonstrates that small landlocked Sea Lamprey parasites,

715   in particular, have difficulty adjusting to seawater (Beamish et al., 1978; Beamish,

716   1980b), suggesting a partial osmoregulatory impediment to GL Sea Lampreys

717   surviving in the marine waters of the Atlantic and breeding with native Sea Lamprey.

718   In order to minimize further these risks, the effector construct in GM Sea Lampreys

719   could be designed such that it is linked to expression of an osmoregulatory gene

720   sequence (Mateus et al., 2013; Covolo-Soto et al., 2015).   Several such designs are

721   available (e.g., an IRES or 2A fusion peptide that promoted both salt intolerance and

722   sex-ratio distortion sequences). These construct designs, however, would still be

723   vulnerable to failure due to point mutations in the salinity intolerance coding region.

724   A containment mechanism based on salinity intolerance would also not eliminate

725   risks to native freshwater populations. Other containment options for the gene-drive

726   have been noted above, but are either as yet untested (e.g, a multi-component daisy

727   drive) or were judged by the panel as likely to be unacceptable (e.g., genetically

728   engineering the Atlantic Sea Lamprey population to be resistant to a population

729   suppression drive released into the Great Lakes).

730   The third option rated as potentially high risk by panellists was the

731   deployment of a self-propagating larval biocide. Again, opinions were divergent

732   among panellists, with scores for risks against both native lampreys and non-target

733   Sea Lamprey populations ranging from Extremely low (1) to Extremely high (7). The

734   central issues were the extent to which a genetically modified alga or bacteria could

735   be limited to Great Lakes drainages, and the species specificity of an siRNA effector

736   molecule, along the lines discussed above.

737   The panel also discussed risk elements that span the six options (Table 4).

738   These elements ranged from the risk of horizontal gene transfer, which has been

739　suggested between lampreys and their hosts (Kuraku et al., 2012; Zhang et al., 2014),

740　to the risk of inadequate rapid response capability by lake managers in the case of re-

741　invasion following Sea Lamprey suppression. Overall, the risks of vertical and

742　horizontal gene transfers of the constructs were judged to be extremely low in the

743　time frame (decades) being considered for the control program, the risks of off-target

744　effects of the constructs manageable given modern construct design, the risks of

745　escapement of carriers from the Great Lakes high and possibly difficult to manage,

746　and the risk of inadequate response capacity to Sea Lamprey re-invasion uncertain,

747　but possibly high if, after a successful control program, resources were to be diverted

748　to other issues.

749

750　**Stakeholder Consultation**

751

752　　　　A large majority of the stakeholders (84.7%) and the fishing community

753　(95.9%) supported research on one or more genetic options for controlling Sea

754　Lamprey in the Great Lakes; for steps towards possible implementation, the figures

755　were similar (86.3% and 95.9%, respectively). Details are provided in Thresher et al.,

756　(in press). In both surveys, support was widespread across all respondent groups, all

757　age groups and irrespective of years of professional or fishing experience. Among all

758　respondents, the three main reasons given for opposition to genetic approaches overall

759　were concerns about effects on non-target species and populations, the adequacy of

760　safeguards, and insufficient knowledge by the respondent to be comfortable with the

761　idea. Concerns about risks to human health were not widespread, nor were ethical

762　concerns about the use of the technology to control Sea Lamprey.

763   At the level of focal options, support was widespread and positive for the

764   Mendelian sex-ratio distortion, Trojan males, and development of non-parasitic Sea

765   Lamprey, divided for vaccinated prey, and negative for a synthetic larval biocide and

766   the use of a gene drive (Fig. 4). Across all options, support levels by stakeholders and

767   the fishing community correlated very highly ($R^2 > 0.96$ for both R&D and

768   implementation), with the conspicuous exception of vaccinated prey. Stakeholders

769   were split evenly with regard to researching this option (50% opposed), whereas

770   recreational fishers were broadly opposed to it (69% opposed). Overall, costs of

771   project development, ethical issues, potential availability of non-genetic options and a

772   perception that existing methods of Sea Lamprey control were adequate did not rate

773   highly as concerns for any of the focal options, whereas concerns about impacts on

774   non-target species and populations and about the adequacy of safeguards were

775   widespread. Regression tree analyses to identify the primary co-variates (attitudes)

776   that distinguished between opponents of each approach and those supporting it

777   (analyses do not include strong supporters, which as a group expressed few concerns)

778   accounted for a relatively small amount of the variance in predicted level of support

779   for each option (mean 30.1% for R&D and 25.5% for implementation). These results

780   indicated that factors beyond those assessed in the survey should account for the

781   unexplained variation in the level of support for R&D or implementation of a given

782   focal option. As we incorporated a large number of diverse explanatory variables as

783   co-variates, it is unclear what additional information would have led to greater

784   variance explained, especially given that perspectives about the overall importance of

785   Sea Lamprey control and the importance of developing alternative control tactics

786   were included as possible predictor variables. In general, identifying the unsupportive

787   fraction of respondents was usually straightforward. For example, in terms of the

788   level of support for future research and development for the heritable sex ratio drive,

789   strong opponents indicated that the approach was unethical; whereas, despite concerns

790   about safeguards, fishery biologists and managers were likely to be supportive of the

791   approach relative to other respondent types, possibly reflecting long-standing

792   frustration with conventional control tactics (Fig. 5). For the gene drive, the belief that

793   non-target risks were too high, and the belief that existing control tactics were good

794   enough, both signified low overall support for R&D.

795

796   **Discussion**

797

798   Our analyses indicate widespread support among key stakeholders and

799   recreational fishers for research on the use of one or more forms of genetic biocontrol

800   to manage Sea Lamprey in the Great Lakes (Thresher et al., in press). The breadth of

801   support is likely to reflect an appreciation of historical and on-going difficulties in

802   managing the pest, environmental consciousness among respondents regarding the

803   impacts of the species on lake ecosystems, and a hope that biotechnology might allow

804   the problem to be "solved" rather than, as is currently the case, managed. With regard

805   to preferred options, we scaled the six focal options, with the larval biocide split into

806   synthetic versus self-propagating sub-options, by risk, technical/logistical feasibility

807   and stakeholder/fishing community acceptability (Fig. 6). The ideal option is one that

808   is low risk, feasible, effective and acceptable to a broad audience of stakeholders and

809   the fishing community. Mendelian sex ratio distortion currently comes closest to

810   fulfilling all four criteria. Vaccinated prey is assessed to be feasible and low risk, but

811   is marginally acceptable as an option for stakeholders, and opposed by most of the

812   surveyed fishing community. The release of Trojan males was both acceptable and a

813   low risk approach, but panelists doubted whether male mating attractiveness could be

814   enhanced enough to have an impact on the GL Sea Lamprey population. The other

815   three options either scored very low in terms of acceptability (synthetic biocides, the

816   gene-drive) or were not considered likely to be effective (non-parasitic Sea Lamprey).

817   This landscape is dictated in part by information gaps. Human health

818   implications of a particular option, as a prime example, are determined by dosage and

819   the nature of the effecting molecules, neither of which is known at this stage. The lack

820   of detail not only makes estimating risk difficult even by informed experts, but also

821   has a large impact on community attitudes towards the different options, i.e., human

822   health concerns largely determined opposition to synthetic biocides and vaccinated

823   prey. However, unique aspects of the physiology of the lamprey reproductive and

824   feeding systems could well be exploited to design effector molecules with potentially

825   no impact on humans or any other non-lamprey target. Similarly, the risks associated

826   with, and the efficacy of Mendelian sex ratio distortion will be informed by better

827   data on the current sex ratios of the Great Lakes and Atlantic Sea Lamprey

828   populations, of a gene-drive by developments in containment technology and the

829   actual rates of downstream gene exchange with the Atlantic population, and of the

830   Trojan gene by the extent to which male attractiveness can be enhanced and its effect

831   on mating success in a non-deterministic stream environment.

832   The choice of focal options is also likely to be a function of how that option is

833   deployed. At this stage, the key considerations in assessing the future viability of a

834   biocontrol program are associated with efficacy, risks, and support by stakeholders

835   and the fishing community. Consequently, our analyses did not focus on

836   technological limitations, on the basis that given adequate resourcing, suitable gene

837   targets, construct designs and methods for insertion would be found. A key technical

838   challenge for some options, however, could be the need to create and maintain an

839   integrated brood line of genetically modified Sea Lamprey. This is not an issue with

840   synthetic biocides or vaccinated prey (for which germ line modification and

841   husbandry techniques are likely to be readily available). For Sea Lamprey, methods

842   of germ line modification are well developed (Kusakabe et al., 2003). However, in a

843   typical genetic engineering program, several generations of carriers need to be

844   screened and, often, back-crossed to select and fix optimal gene configurations and

845   phenotype. Doing so with Sea Lamprey could take 10-15 years (even in a best-case

846   scenario without complications) due to the prolonged larval stage (3-7 years) and

847   subsequent two-year parasitic and adult stages. Maintenance of a Sea Lamprey brood

848   line is further complicated by the species' semelparous life cycle. A single pair of

849   genetically modified carp can produce large numbers of carrier offspring for decades,

850   whereas Sea Lamprey adults breed once, die and need to be replaced. Consequently,

851   Sea Lamprey breeding programs would need to be carefully managed as not to lose

852   lineages of valuable brood stock.

853   An alternative strategy is suggested by the relatively small numbers of carriers

854   required to achieve Sea Lamprey suppression in the Great Lakes using the sex ratio

855   distorting options. As noted above, in theory, releasing even a single carrier of a gene-

856   driven carrier could permanently alter a target population. The models suggest larger,

857   but still logistically manageable numbers, need to be released for the Mendelian

858   approach. Typical integration rates for fish using micro-injection techniques are 3-5%

859   (e.g., Stuart et al., 1988). Several geneticists advised us that micro-injecting DNA

860   into lamprey eggs is relatively easy, and can be done at a rate of 1000s of eggs per

861   hour by experienced staff. At a 3% integration rate and 1000 eggs/hour, 10,000 single

862   copy carriers could be produced by 10 individuals working full time on the project in

863   less than a week. Sub-optimal integration and off-target effects could reduce the

864   efficiency of such a program, but the basic analysis suggests that mass transfecting

865   eggs annually could be a viable alternative to developing and managing Sea Lamprey

866   brood lines. In practice, bulk techniques, e.g., biolistics and electroporation, if

867   suitable for lamprey eggs, could transform 10s of thousands of eggs per hour, making

868   such a program highly effective. Options are also available that can substantially

869   increase rates of transgene integration (e.g., Soroldoni et al., 2009), though with a cost

870   in insertional stability. The program could also benefit from the single generation

871   contribution of episomal expression in non-integrated carriers.

872   Several additional issues need to be factored in to these evaluations of genetic

873   control. First, except under conditions of very high stocking rates, all genetic options

874   take time to affect a targeted population. In the case of the Great Lakes, at logistically

875   feasible stocking rates most options will take 20-25 years to have a discernible impact

876   on the Sea Lamprey population and strong pest suppression could take in excess of 50

877   years. Whether or not the public and managers would invest in a program with such

878   long delivery times is uncertain. Second, options that require carriers be stocked

879   could result in a short duration (ca. 5 years) but nonetheless controversial increase in

880   Sea Lamprey abundance and associated impacts. This could be compensated for,

881   however, by a short-term increase in conventional control methods, e.g., biocidal

882   treatment of nursery habitats. Third, there needs to be community consultation and

883   agreement about the level of ecological and social risk deemed acceptable. Is any

884   threat to the Atlantic Sea Lamprey population acceptable? Although several of the

885   options canvassed were judged by experts as being of low to extremely low risk

886   across multiple endpoints (native lampreys, human health), none are completely risk

887   free and a decision will need to be made on the trade-off between potentially severe

888   adverse effects and the benefits of a possible long-term suppression in the Great

889   Lakes. Fourth and perhaps most importantly, it is not clear at what level and by

890   whom decisions to implement a program should be made, and the extent to which

891   these decisions need to be supported by additional social license research (including

892   broader canvassing of the public as a whole) in concert with future bio-technology

893   R&D. Decision-making in the U.S. context alone could be complicated, due to the

894   number of jurisdictions that will or could be affected and the as yet ambiguous

895   regulatory pathways for genetically modified animals (Otts, 2014). Managing Sea

896   Lamprey in the Great Lakes is inherently a trans-national issue. The possibility that

897   genetically modified Sea Lampreys could escape to and modify permanently, even if

898   not threaten, the North Atlantic population further confounds the international scope

899   of the issue. A compelling argument can be made that European countries should be

900   involved in deciding acceptable levels of risk for genetic biocontrol options that could

901   spread if seeded into the Atlantic population, and consulted for those that should not.

902   International agreements dictate such a process (Garforth and Miranda, 2014;

903   Committee on Gene Drive Research in Non-Human Organisms, 2016). As such,

904   focal options that are inherently confined to the Great Lakes, notably deployment of a

905   synthetic biocide and vaccinated freshwater prey, might warrant greater examination

906   if effector molecules can be unambiguously and rigorously demonstrated to have no

907   impacts on native species or human health.

908

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925   **References**

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Table 1. Genetic approaches and phenotypic objectives considered as potential genetic

biocontrol options for use against Sea Lamprey in the Great Lakes.

Genetic approach/delivery method	Desired phenotypic effect/objective
Genetically modified pathogen or parasite	Increase mortality
Diet-delivered products	Reduce fertility
Chromosomal modification	Bias sex ratio
Mendelian-inherited constructs	Induce mortality
Super Mendelian (Gene driven)-inherited constructs	Cause non-parasitic maturation
	Increase mating competitiveness while reducing fertility
	Increase or renew sensitivity to existing biocides
	Replace existing biocides

Table 2. Results of 200-year simulations of the effect of 5 genetic control tactics on a Great Lake Sea Lamprey population and a downstream ("other") population that receives emigrants from the Great Lake population at a rate of 0.01% of the latter per year. We did not model the non-parasitic Sea Lamprey option (see text). Each control scenario is summarized below. See the Supplemental Material for modelling details.

Scenario	Stocking regime	Population at year 50 relative to initial population	Population at year 200 relative to initial population	Final proportion of GM individuals in GL population	Final proportion of GM individuals in other population
Heritable sex ratio, DD	100,000 larvae each year for 10 years	0.68	0.03	0.23	<0.001
Heritable sex ratio, not DD	100,000 larvae each year for 10 years	0.81	0.78	0.02	< 0.001
Trojan gene, equal mating	1,000,000 larvae each year for 50 years	0.02	0.03	0	0
Trojan gene, 3X mating	200,000 larvae each year for 50 years	0.02	0.03	0	0
GM larval biocide, +20%	NA	< 0.001	0	0	0
Vaccinated prey, 4%	200,000 hosts spanning 1 - 10 years	0.08	0.001	0	0
Gene-drive sex ratio, 1 yr only	100 larvae for 1 year	0.95	0	1	0.08
Gene-drive sex ratio, 10 years	100,000 larvae each year for 10 years	0.02	0	1	0.81

Explanation of scenarios:
1. Heritable (Mendelian) sex ratio drive, DD. Sex ratio density dependent with 65% females at low adult densities.
2. Heritable (Mendelian) sex ratio drive, Not DD. Sex ratio not density dependent with 50% females.
3. Trojan gene, equal mating. Sterile adult GM males are equally competitive with wild type males for mates.
4. Trojan gene, 3X mating. Sterile adult GM males are 3 times as successful as wild type males for mates.
5. GM larval biocide, + 20%. Simulated 20% increase in larval mortality due to biocide release
6. Vaccinated prey, 4%. Based on a host population size of 5,000,000, a 4% host vaccination rate achieved by stocking a total of 200,000 GM hosts over a several year period, 100% sterility or death resulting from a single attack of a vaccinated host, and an average of 10 hosts attacked per parasite. The model is based on the consequent 34% reduction in parasitic Sea Lamprey survival.

7. <u>Gene-driven sex ratio distortion, 1 year only</u>. Assumes 100% homing and no counter-selection.
8. <u>Gene-driven sex ratio distortion, 10 years</u>. As above, but higher and longer stocking effort

Table 3. Mean (and range among panellists) of the risk scores of adverse outcomes as assessed by a panel of experts for six "focal" genetic control options against five risk categories, and overall risks. Note that the risks for artificially sourced and self-propagating synthetic biocides were assessed separately. Risk estimates were scored by panellists as 1 through 7, where 1 = probability of occurrence during the control program < 1 in 1,000,000; 2 = < 1 in 10,000; 3 = < 1 in 100; 4 = 1-10%; 5 = 10-50%; 6= 50-95% and 7 = > 95% probability of occurrence. Technical and logistical feasibility was scored qualitatively on a scale of 1 to 7.

Option	Native lampreys	Other SL populations	Other organisms	Human health	Other risks	Overall risk	Feasibility
Mendelian sex ratio	1.95 (1-4)	3.09 (2-7)	1.59 (1-3)	1.41 (1-2)	1.33 (1-3)	3.00 (1-4)	4.96 (4-6)
Trojan males	1.45 (1-2)	1.55 (1-2)	1.09 (1-2)	1.14 (1-2)	1.13 (1-2)	1.55 (1-2)	3.45 (3-4)
Non-parasitic SL	2.82 (2-4)	2 (1-3)	1.2 (1-2)	1 (-)	1 (-)	2.36 (2-3)	2.85 (2-4)
Vaccinated Prey	2.36 (1-4)	1.14 (1-2)	1.8 (1-3)	1.55 (1-2)	1.29 (1-4)	2.45 (1-4)	4.80 (2-6)
Gene driven sex ratio	2.45 (1-5)	4.32 (2-7)	1.77 (1-4)	1.45 (1-3)	1.67 (1-5)	4.11 (2-6)	4.44 (2-6)
Synthetic larval biocide	2.23 (1-4)	1.18 (1-2)	1.59 (1-3)	1.27 (1-2)	1 (-)	1.8 (1-3)	4.41 (2-6)
Self-propagating larval biocide	2.91 (1-7)	3.55 (1-7)	2.23 (1-7)	1.32 (1-2)	1.17 (1-2)	3.8 (2-7)	3.13 (1-4)

Table 4. Key over-arching risk elements based on the risk assessment workshop and consultations with experts in the field of genetic technology and Sea Lamprey biology. *including rogue human activities (e.g., intentional movement or illegal live sale) facilitate breaking through that gap when measured over decadale scales.*

Off-target effects of constructs

> *Depends critically on effecting molecule. RNAi likely key enabling technology. Cross-reactivity of siRNA with humans or other species potentially high as although nominally it requires 21 bp overlap, it can occur with as little as 6 bp overlap in nucleotide 2-7 position. Hence probability of siRNA match is $1/(4^6)$ not $1/(4^{21})$; small, but not minute. However, impacts are likely to be dose dependent, so normal, e.g. dietary uptake, would be single or few molecules and hence exceedingly low dose. RNAi is a relatively mature science and methods for sequence design and minimization of off-target effects are well developed and could be applied to SL*

Escapement (physical containment) from Great Lakes

> *Central issues are whether there is probable movement of SL down system into the Atlantic and if they get there, will they successfully breed with Atlantic SL. Downstream movement over falls highly likely to be successful, while canals could provide alternative pathways, along with ship/boat traffic and hitching rides on parasitized migrating fish. There is a distributional gap of SL in the St Lawrence seaway, but anthropogenic vectors, including rogue human activities (e.g., intentional movement or illegal live sale) facilitate breaking through that gap when measured over decadal scales. facilitate breaking through that gap. There are no obvious genetic, osmoregulatory or developmental barriers to interbreeding, particularly if downstream transport is slow (2 generations suggested) and involves larvae.*

Vertical Gene Transfer

Native North American lampreys – No evidence of hybridization between SL and native species. Probability exceedingly low.

Atlantic SL population – No obvious behavioral or other barrier to interbreeding

Horizontal Gene Transfer (HGT)

- *HGT operates, but on the scales of 10,000 to millions of years. On the time scales we are considering (decades) likelihood of HGT exceedingly low.*

- *Transposable and other mobile genetic elements have a higher probability of HGT than less mobile elements, so construct design should avoid e.g., transposon mediated integration*

- *Despite publications, HGT among vertebrates (or animal metazoans) is still highly debated, and may prove more or less frequent than suggested*

Risk of re-infestation of the Great Lakes

- *A possible problem for any non-heritable (= "permanent") control option*

- *Re-invaders need to be treated logically as any other potential invasive species*

- *Prevention of re-infestation and development and maintenance of preventative measures could be a problem due to the diversity of vectors, human and otherwise*

- *Near or complete eradication of GL SL could lead to a decline in the capacity to mobilize a rapid response containment/eradication program should re-infestation be detected. There needs to be a recognition of the risk, a game plan on how to deal with it, and an on-going capacity for early detection of a re-inoculation event.*
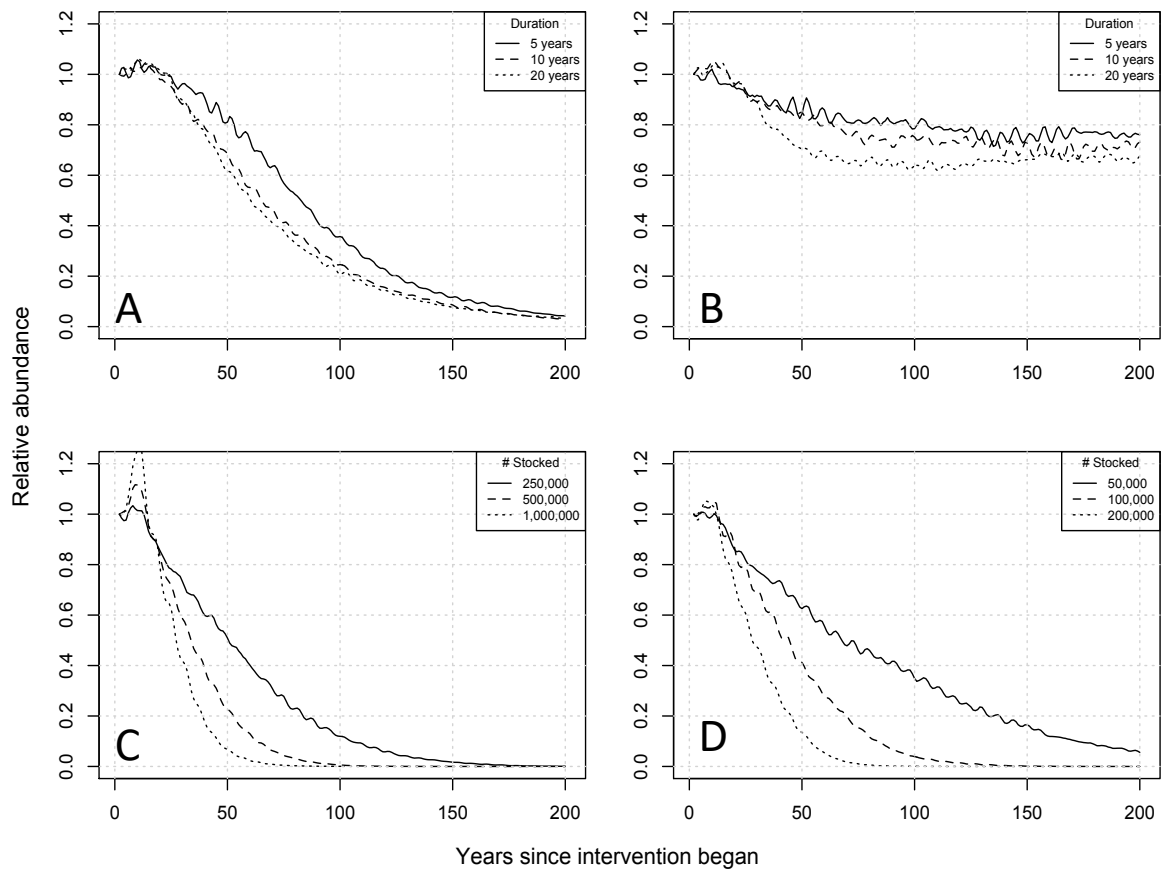
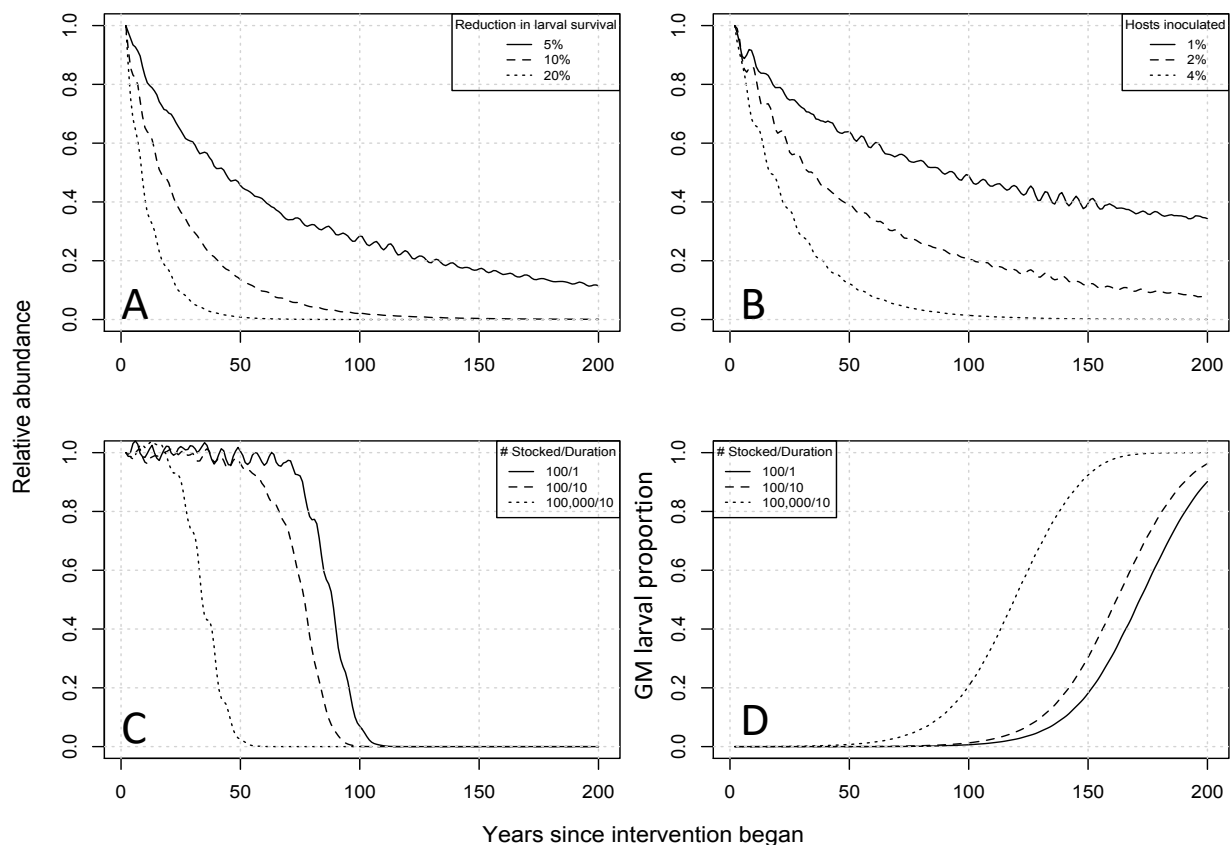Figure 1. Simulated changes in relative abundance of Sea Lamprey as a result of genetic control options: (A) heritable sex ratio drive option with intrinsic sex ratio assumed to be density dependent; (B) heritable sex ratio drive option with intrinsic sex ratio assumed to be fixed at 50% females; (C) Trojan gene option with GM males assumed to be sterile and equal in competitiveness with wild type males for matings, at stocking rates ranging from 250,000 to 1,000,000 males per year; and (D) as in Fig. C, but GM males 3X as effective as non-GM males at attracting mates, and at stocking rates of 50,000 to 200,000 males per year. Note that for the heritable sex ratio drive (A and B), we simulated three duration periods for the stocking of genetically modified larvae after which no additional stocking occurred; for C and D we simulated three different levels of stocking of larvae, which continued for the entire length of the simulation. See Supplemental Material for details of the simulation model.

Figure 2. Simulated changes in relative abundance of Sea Lamprey as a result of genetic control options: (A) addition of a genetically-based larval biocide that reduces larval survival, on average, by 5%, 10%, or 20%; (B) inoculation of 1%, 2%, or 4% of Sea Lamprey hosts with a gene-based vaccine that causes mortality of Sea Lampreys that attack the inoculated hosts: (C and D) introduction of larvae containing a non-Mendelian gene drive using three scenarios: 100 GM larvae for 1 year; 100 GM larvae for 10 years, and 100,000 GM larvae for 10 years. The left panel (C) shows the abundance of Sea Lamprey in the Great lakes as a result of the interventions; the right panel (D) shows changes in the prevalence of the genetically modified larvae in a non-target Sea Lamprey population connected to the Great Lakes population by a 0.01% per year emigration rate. See the Supplemental Material for details of the simulation model.
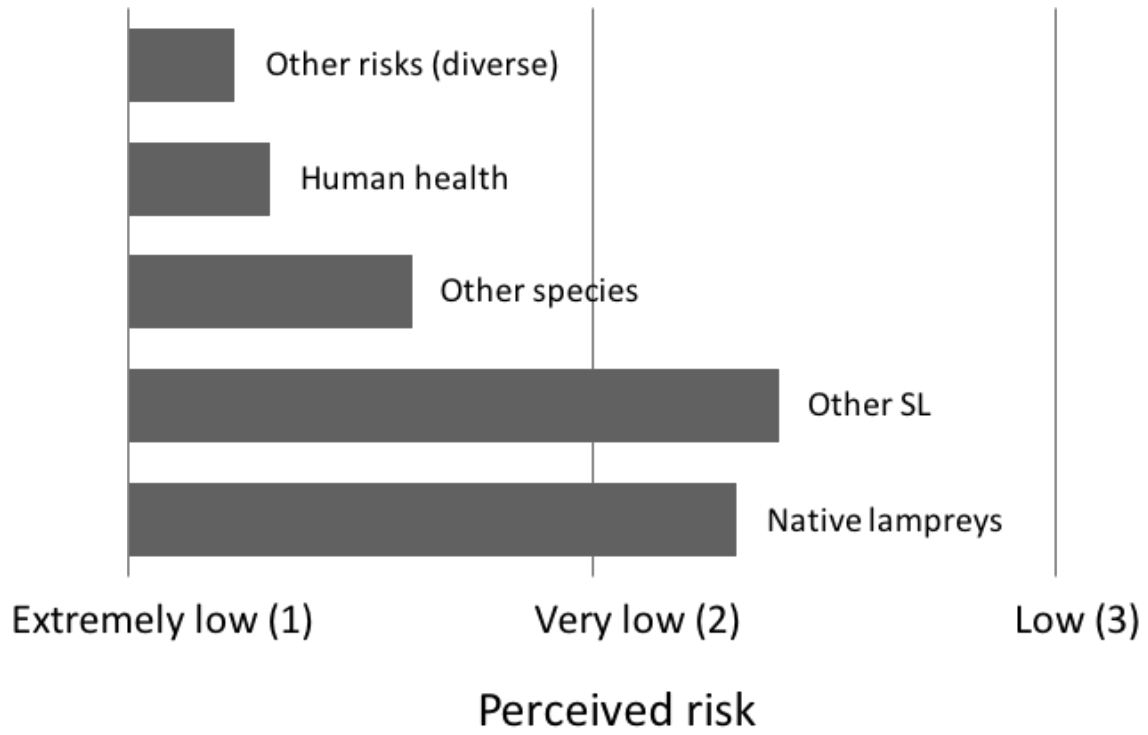
Figure 3. Level of risk as assessed by an expert panel for each identified risk category averaged (mean) across six focal options and eleven panel members.
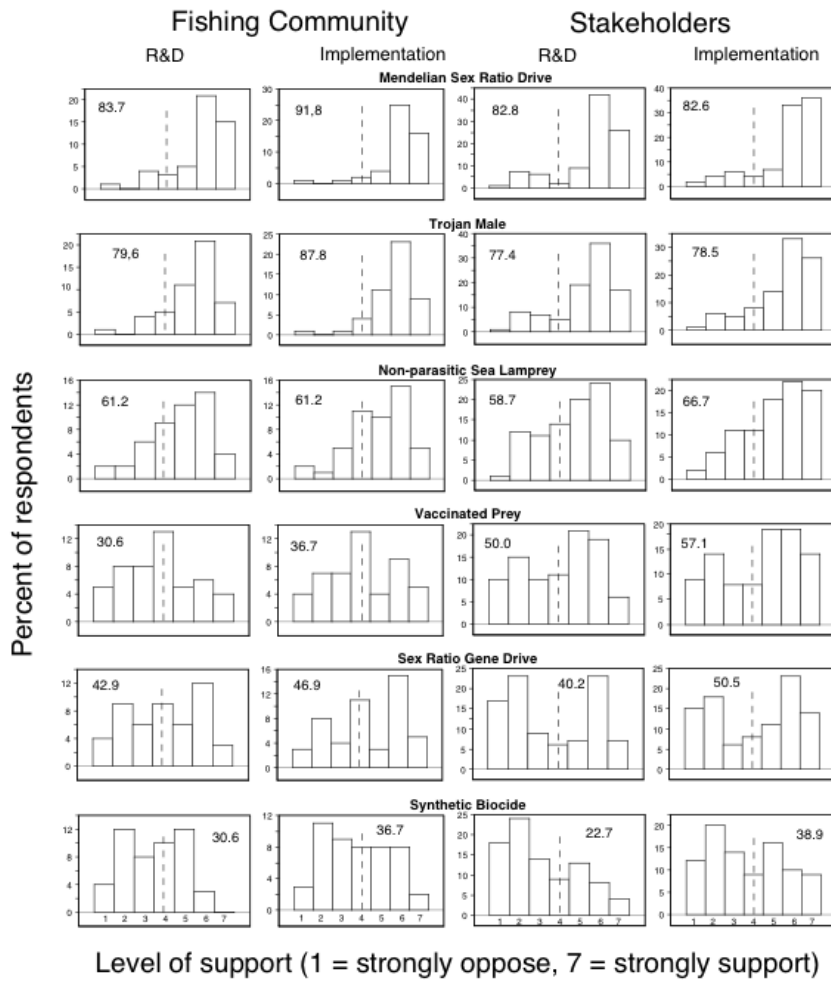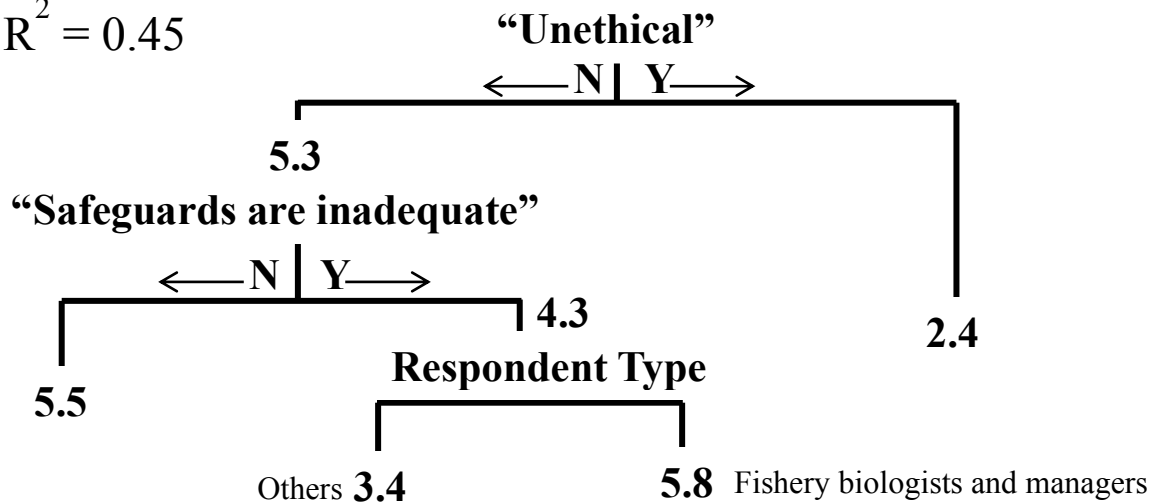
Figure 4. Distribution of level of support or opposition to focal options by the fishing community and stakeholders. Numbers are the percent of respondents that supported (score ≥ 5) R&D or Implementation for each option. Dashed lines indicate neutral response. The self-propagating and artificially dispersed nursery area synthetic biocides have been pooled for this analysis.

Heritable Sex Ratio Drive

$R^2 = 0.45$

"Unethical"

⟵N | Y⟶

5.3

"Safeguards are inadequate"

⟵N | Y⟶

5.5

4.3

2.4

Respondent Type

Others **3.4**

5.8 Fishery biologists and managers

Gene Drive

$R^2 = 0.32$

"Non-target risks too high"
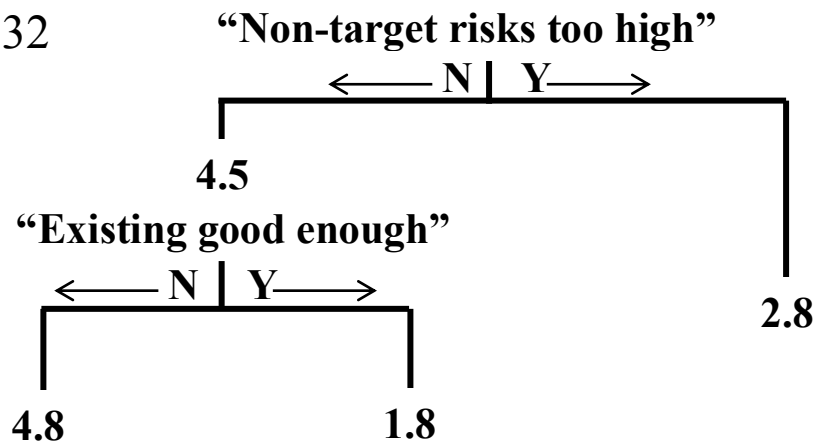
⟵ N | Y⟶

4.5

"Existing good enough"

⟵ N | Y⟶

4.8

1.8

2.8

Figure 5. Representative pruned regression trees for a widely and a weakly supported option (heritable [Mendelian] sex ratio drive and a gene drive, respectively) among stakeholders and fishers, pooled, identifying factors predictive of support/opposition for R&D within the 'unconvinced fraction' of respondents. Numeric values are mean levels of support (where Strongly opposed = 1, Opposed = 2, Moderately opposed = 3, Neutral = 4, Moderately supportive = 5, Supportive = 6) and node labels indicate survey responses. The model explained 42% and 32% of the variance in the level of support for R&D, respectively.

Regression tree analyses for Implementation of the two options are essentially identical, though capturing less variance (33% and 24%, respectively).
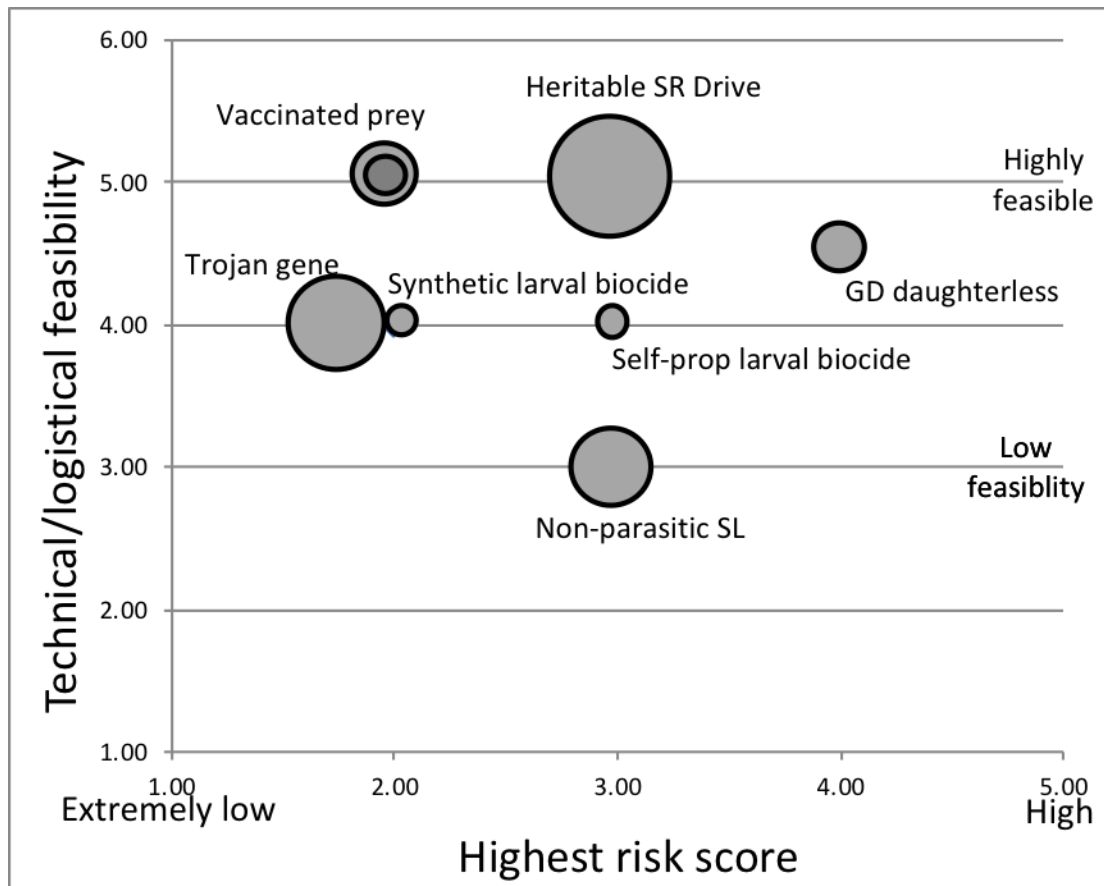
Figure 6. Scatterplot of median feasibility versus median highest risk scores for each of the seven focal options as assessed by an expert panel. Diameter of each point indicates the amount of survey respondent support for R&D for each option. Support levels were similar for both surveyed groups except for the vaccinated prey option, which was supported by 50% of engaged stakeholders, but 30.6% of the fishing community.

Evaluating active genetic options for the control of Sea Lamprey (*Petromyzon marinus*) in the Laurentian

Great Lakes

Supplementary Material

Sea Lamprey population model

Model description – Effects of five of the six genetic biocontrol options considered in this paper were examined using an age-structured model of Sea Lamprey population dynamics. The non-parasitic Sea Lamprey option was not modeled. Here we provide a detailed description of the model originally developed to examine the first option – the heritable (Mendelian) sex ratio drive. We refer to this option below as "daughterless" to reflect the idea that all offspring carrying the construct will be phenotypically male. Following this we explain how the model was adapted to simulate the other four options.

The model included two populations: a single population in the Great Lakes presumed to be under active control using lampricides and barriers, as per current practice; and, a second, uncontrolled, downstream population. The model simulated the entire life cycle of both populations, and the populations were assumed to be connected by a small amount (nominal value: 1% per year) of unidirectional movement from the Great Lakes population to the downstream population. Although bidirectional movement is plausible, our focus was on risks associated with emigration of genetically modified Sea Lamprey from the target Great Lakes population to the non-target downstream population. For simplicity and because little is known of the population dynamics of Sea Lamprey outside of the Great Lakes, we assumed that the two populations had identical demographics.

We used a stage/age structured approach to model both populations of Sea Lamprey, with larvae ages 0-6, transformers, parasites, and adults. Within each stage/age there were males and females. The males were also subdivided by the number of copies of the construct (i.e., the number of loci where the effect is expressed) they had. We allowed for up to eight copies of the construct to be present in a genetically modified male. We assumed that any Sea Lamprey containing at least one copy of the construct would be a phenotypic male; therefore, by definition all females had a copy number of zero and all offspring with a copy number > 0 were classed as males. To simulate the effect of strategic distribution

of genetically modified larvae to enhance their survival, we also needed to separately track these individuals.

The number of Sea Lamprey larvae of sex k, copy number g, age a, in year t was given by:

$$N_{t+1,a+1}^{k,g} = N_{t,a}^{k,g} \cdot s_N \cdot (1 - pm_a) \cdot (1 - m_{cl})$$

$$G_{t+1,a+1}^{k,g} = G_{t,a}^{k,g} \cdot s_G \cdot (1 - pm_a) \cdot (1 - m_{cG})$$

(S1)

All model parameters and state variables are defined and assumed value listed in Table S1. The number of transformers (recently metamorphosed larvae), parasites, and adults of sex k, copy number g, in year t were calculated from:

$$T_{t+1}^{k,g} = \sum_{a=2}^{6} N_{t,a}^{k,g} \cdot pm_a \cdot (1 - m_{cT})$$

$$GT_{t+1}^{k,g} = \sum_{a=2}^{6} G_{t,a}^{k,g} \cdot pm_a \cdot (1 - m_{cGT})$$

(S2)

$$P_{t+1}^{k,g} = (T_t^{k,g} + GT_t^{k,g}) \cdot s_T$$

(S3)

$$A_t^{k,g} = P_t^{k,g} \cdot s_P$$

(S4)

We modeled reproduction using a stochastic Ricker stock-recruitment function with log-normal process errors on recruitment:

$$N_{t,0}^* = \alpha \cdot A_t^{1,0} \cdot e^{-\beta \cdot A_t^{1,0} + \varepsilon_t}$$

(S5)

Dawson and Jones (2009) examined recruitment dynamics at the spatial scale of individual streams, whereas the spatial scale of our model was an entire lake. To re-scale the parameters of the Ricker model to an entire lake, we used forecasted lake-scale recruitment dynamics aggregated from a more detailed model of Sea Lamprey population dynamics (Jones et al 2009) for which recruitment operated at the scale of individual spawning streams and that used Ricker parameter estimates from Dawson and Jones (2009). We fit these forecasted dynamics to a Ricker model to estimate the structural parameters and the variance term (Table S1).

To allocate age zero larvae to sexes and copy numbers we needed to assign copy numbers to gametes. For the daughterless construct, all individuals with at least one copy of the construct are effectively males, so female gametes will necessarily have a copy number of 0, and the proportion of female gametes with copy number 0 ($p^{1,0}$) equals 1. To calculate the overall proportion of male gametes

with copy numbers from 0 to 8 we used the binomial probability distribution function. We compute the probability of a male gamete with h copies of the construct deriving from a male adult lamprey with g copies and then sum that proportion, weighted by the relative abundance of male adult lamprey with g copies, over all cases where $g \geq h$:

$$p^{0,h} = \sum_{g=h}^{8} \left[\frac{g!}{h!(g-h)!} \cdot 0.5^g \cdot 0.5^{(g-h)} \cdot \frac{A_t^{0,g}}{\sum_{g=0}^{8} A_t^{0,g}} \right] \tag{S6}$$

This calculation assumes that the loci containing the construct are not linked (i.e., they disassociate independently during meiosis) and that the construct is never homozygous at a locus. Finally, by assuming random assortative mating, and noting that all female gametes have copy number 0, the proportion of offspring with copy number g will be

$$O^g = p^{0,g} \cdot p^{1,0} = p^{0,g} \tag{S7}$$

The number of age 0 larvae by sex and copy number is then:

$$
\begin{aligned}
N_{t,0}^{1,g} &= N_{t,0}^* \cdot O^0 \cdot \pi_1 && g = 0 \\
N_{t,0}^{1,g} &= 0 && g > 0 \\
N_{t,0}^{0,g} &= N_{t,0}^* \cdot O^0 \cdot \pi_0 && g = 0 \\
N_{t,0}^{0,g} &= N_{t,0}^* \cdot O^g && g > 0
\end{aligned}
\tag{S8}
$$

where π_1, π_0 are the expected proportions of females and males with copy number 0 in the population ($\pi_1 + \pi_0 = 1$).

The sex ratios of adult Sea Lamprey in the Great Lakes changed substantially from the pre-control (1950s) to the post-control (1980s) period (see Jones et al. 2003), with a preponderance of males prior to control and a preponderance of females more recently. We assumed that this change in sex ratio has been a compensatory response to changes in Sea Lamprey densities, and model the effect by allowing the proportion of males to decrease linearly from 65% at an adult abundance of 1,500,000 to 35% at an adult abundance of 150,000. This approximates pre- and post-control abundances in one of the upper Great Lakes. Below 150,000 and above 1,500,000 the proportions were fixed at 35% and 65% respectively. We also simulated a scenario where the proportion of males was not assumed to be density dependent but remained at 0.5 regardless of population size.

We calibrated the model to produce forecasts of Sea Lamprey abundance, A, in the Great Lakes population consistent with levels observed recently in the upper Great Lakes, in the absence of any genetic biocontrol option being simulated. The downstream population was initialized at roughly four times the upper Great Lakes adult abundance (Table S1). Calibration was accomplished by adjusting larval survival rates.

Implementation of Options – The heritable (Mendelian) sex ratio drive option was simulated by introducing a fixed number of genetically modified larvae into the Great Lake population over a specified period of years. We assumed that when genetically modified larvae were introduced, they would be stocked in streams not subject to lampricide control (m_{cG}, $m_{cGT} = 0$). The model was designed to allow copy numbers for the construct from 1 to 8 – for this analysis we only simulated scenarios with a copy number of 1. The Trojan gene option was also simulated by introducing a fixed number of genetically modified larvae over a specified period of years, but in this case surviving individuals with the construct would develop into sterile adult males. Sterile males would compete with wild, fertile males for mates, reducing the number of effective female spawners just as would be the case for classic sterile male release tactics (e.g., Twohey et al. 2003). We also simulated a scenario where the genetically modified males were 3x as effective as wild males at successfully mating, resulting in a substantially greater effect on reducing the number of effective female spawners. The non-Mendelian gene drive sex ratio distortion option was modeled similarly to the Mendelian option except that all offspring of individuals carrying the construct were assumed to carry the construct as well. We included a parameter for "leakage" of the construct (i.e., loss of the construct in some fraction of offspring) but assumed leakage was zero for the scenarios presented in this paper.

The vaccinated prey option was modeled by reducing the survival of parasitic Sea Lamprey. We modeled three levels of inoculation for host fishes: 1%, 2%, and 4%, and assumed that any Sea Lamprey attacking an inoculated host would die. Assuming that Sea Lamprey attack, on average, ten hosts during their parasitic life stage, this would translate into 8.5%, 17%, and 34% reductions in parasitic survival, respectively. The GM larval biocide option was simulated by decreasing average larval survival rates. We examined three levels of reduced survival: 5%, 10%, 20%.

Table S1. Model parameters and their assumed values.

Parameter symbol	Definition	Value(s)
k	Sex index	0 – male, 1 - female

g	Construct copy number	0-8
t	Year of simulation	1-200
a	Age	0-6
N	Numbers of wild larvae	Dynamic state variable
G	Numbers of stocked larvae	Scenario specific
s_N, s_G	Annual larval survival (wild = N, stocked = G)	wild: 0.395; stocked: 0.395
pm_a	Age-specific proportion of larvae entering metamorphosis	age 0-6: 0,0,0,0.2,0.5,0.75, 1
m_{cl}, m_{cG}	Larval mortality due to chemical control (wild = l, stocked = G)	wild: 0.2; stocked: 0
m_{cT}, m_{cGT}	Transformer mortality due to chemical control (wild = T, stocked = GT)	wild: 0.46; stocked: 0
T	Numbers of wild transformers	Dynamic state variable
GT	Numbers of stocked transformers	Dynamic state variable
P	Number of parasitic Sea Lamprey	Dynamic state variable
A	Number of adult Sea Lamprey	Dynamic state variable: initialized at 110,000 adults for Great Lake population; 450,000 adults for downstream population
s_T	Annual transformer survival	0.75
s_P	Annual parasitic survival	0.75
α,β	Ricker structural parameters	469, 0.0000016
σ_r^2	Process error variance for recruitment	0.19
$p^{k,g}$	Proportion of gametes of sex k with construct copy number g	$p^{1,0}= 1$; $p^{1,g}$ (g>0) = 0 $p^{0,g}$ = calculated from eqn. S6
O^g	Proportion of offspring with construct copy number g	calculated from eqn. S7
π_k	Expected proportions by sex k in the wild population	Scenario specific, see text