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Field study suggests that sex determination in sea lamprey is directly influenced by larval growth rate

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19 **ABSTRACT**

20 Sex determination mechanisms in fishes lie along a genetic-environmental continuum and
21 thereby offer opportunities to understand how physiology and environment interact to determine
22 sex. Mechanisms and ecological consequences of sex determination in fishes are primarily
23 garnered from teleosts, with little investigation into basal fishes. We tagged and released larval
24 sea lamprey (*Petromyzon marinus*) into unproductive lake and productive stream environments.
25 Sex ratios produced from these environments were quantified by recapturing tagged individuals
26 as adults. Sex ratios from unproductive and productive environments were initially similar.
27 However, sex ratios soon diverged, with unproductive environments becoming increasingly
28 male-skewed and productive environments becoming less male-skewed with time. We
29 hypothesize that slower growth in unproductive environments contributed to the sex ratio
30 differences by directly influencing sex determination. To our knowledge, this is the first study
31 suggesting that growth rate in a fish species directly influences sex determination; other studies
32 have suggested that the environmental variables to which sex determination is sensitive (e.g.,
33 density, temperature) act as cues for favorable or unfavorable growth conditions. Understanding
34 mechanisms of sex determination in lampreys may provide unique insight into the underlying
35 principles of sex determination in other vertebrates and provide innovative approaches for their
36 management where valued and invasive.

37

38 **Keywords:** sex ratio, sex determination, sex reversal, growth, condition factor, lamprey

39 1. Introduction

40 Mechanisms of sex determination in fishes can range from entirely genetic to entirely
41 environmental, and their study provides opportunities for understanding how physiology and
42 environment interact to determine sex^{1,2}. Fishes can also exhibit environmental sex reversal,
43 where factors such as social structure and rearing temperature can override the primary
44 genotypic sex, and result in a reversal of phenotypic sex that is then generally fixed for life^{2,3,4}.
45 Environmentally-triggered sex determination and reversals (herein termed sex determination)
46 can lead to highly variable population sex ratios and are important when considering
47 management tactics for valued, invasive, and hatchery-reared fishes^{4,5}.

48 Mechanisms and ecological consequences of sex determination in fishes are primarily
49 garnered from teleost fishes, and are little studied in basal fishes such as Petromyzontiformes¹,
50 despite their importance for comparative studies⁶ and their increasing economic and cultural
51 value⁷. The few studies on Petromyzontiformes suggest that environmentally-triggered sex
52 determination occurs and may be influenced by density. For example, prior to the initiation of
53 large-scale efforts to control invasive sea lamprey (*Petromyzon marinus*) adult populations in the
54 upper Laurentian Great Lakes (Lakes Superior, Huron, Michigan) were predominately male-
55 biased (~65% male). After control efforts reduced sea lamprey populations by 90%, adult sea
56 lamprey populations became female-biased (~40% male), even though there was no evidence
57 that sexes differed in their vulnerability to control efforts^{8,9,10,11}. Presently, sea lamprey
58 populations are still suppressed to 10% of historic highs and adult sea lamprey sex ratio in the
59 Upper Great Lakes is estimated to be 55% male¹². Populations of least brook lamprey
60 (*Lampetra aepyptera*) also have widely varying sex ratios, with high-density populations being

61 more likely to be male-biased than low density populations¹³; sex-specific differences in
62 mortality is not believed to contribute to the varying sex ratios^{13,14}.

63 Lamprey sex differentiation appears to be complete when larvae reach approximately 90
64 mm¹⁵. However, some histological analyses of sea lamprey describe a large number of atypical
65 gonads in larvae longer than 90 mm, which could indicate a longer period of indeterminacy than
66 previously thought¹⁵ or even sex reversal^{16,17}. Furthermore, gonadal biopsy experiments on sea
67 lamprey found that in some cases sex could be labile until larvae reach 140 mm and capable of
68 changing in 8 to 16 weeks^{18,19}, although it is not yet clear whether sex change of larvae greater
69 than 90 mm occurs in natural populations. Once metamorphosis from the larval stage to the
70 hematophagic parasitic stage begins, sex appears to be fixed through adulthood¹⁹.

71 Although a hypothesis of environmentally-triggered sex determination in lampreys has
72 been supported by observational studies, the environmental triggers and physiological
73 mechanisms are still unknown. Incidental to several other experimental studies^{20,21,22,23,24}, a
74 serendipitous opportunity arose to evaluate sex ratios of adult sea lamprey produced from tagged
75 sea lamprey larvae that were stocked in Great Lake tributaries and those stocked in the Great
76 Lakes themselves near tributary mouths (herein termed lentic areas; Supplemental Table 1).
77 Prior to stocking the larvae, these areas were treated with selective pesticides (herein termed
78 lampricide) to remove wild larvae²⁵. As such, the density of larvae in both stream and lentic
79 environments was likely similar and lower than most other streams and lentic areas in the upper
80 Great Lakes. However, larval growth and metamorphosis differed between the stream and lentic
81 environments; larvae stocked in lentic areas grew slower and metamorphosed at smaller sizes,
82 presumably due to lower quality and quantity of food. Annual survival rates did not differ
83 between the environments^{22,24}(Supplemental Table 2).

84 Given observed differences in growth and size at metamorphosis of the larval
85 populations, but that larval densities were presumably similar and low, we conceptualized that
86 evaluating the sex ratio of adults recovered from these two environments could reveal possible
87 environmental triggers of sex determination in lampreys. Accordingly, our objective was to
88 determine if the sex ratio of adult sea lamprey produced from these populations differed from
89 each other and the sex ratio of at-large untagged adult sea lamprey. Given the underlying
90 hypothesis that sex determination is labile until metamorphosis into the parasitic stage¹⁹ and is
91 influenced by environmental conditions⁸, we predicted that introduction of larvae into drastically
92 different environments yielding different rates of growth and metamorphosis would result in the
93 populations expressing different sex ratios at the adult stage. This study was unable to
94 distinguish between labile sex determination and sex reversal, so we combined the processes of
95 sex determination and sex reversal together under the term sex determination. Many fishes may
96 have labile sex determination, but not sex reversal².

97

98 **2. Material and methods**

99 Detailed descriptions of the collection, tagging, release locations, and recovery of sea
100 lamprey used for this study have been published previously^{22,23,24}, so what follows is a brief
101 general description of the overall approach and analysis of adult sex ratios.

102 Larval sea lamprey between 40 and 140 mm were collected via electrofishing from
103 tributaries to Lakes Huron and Michigan several months to one year prior to regularly scheduled
104 lampricide treatments. As such, the larvae used for this study were previously located in
105 productive streams that contained substantial numbers of larvae. These larval sea lamprey were
106 then coded wire tagged and released into tributaries of Lakes Huron and Michigan ($n=5$) and into

107 areas of Lakes Huron and Michigan near stream mouths ($n=3$) between 2005 and 2007, after
 108 lampricide treatment (Supplemental Figure 1). Between 1,500 and 3,000 larvae were released
 109 per tributary or river mouth. Tagged sea lamprey were recovered in the larval stage during
 110 subsequent larval surveys and lampricide treatments. Tagged sea lamprey surviving to
 111 adulthood were captured in traps operated in tributaries to Lakes Huron and Michigan.
 112 Population parameters associated with tag recovery including survival and metamorphosis
 113 probabilities have been previously published^{22,24} (Supplemental Table 2) Here, we report the sex
 114 of tagged sea lamprey recaptured in the adult stage as determined via visual inspection of the
 115 gonad while removing the tag. We also report the overall population sex ratio as determined by
 116 visual assessment of untagged adult sea lamprey captured during the same years and in the same
 117 traps as tagged sea lamprey²⁶.

118 A Bayesian hierarchical logistic regression model was used to estimate adult sea lamprey
 119 sex data for the different stream and lentic areas in which sea lamprey were stocked. Whether a
 120 tagged sea lamprey from a stream or lentic area captured as an adult in a particular year was male
 121 was modeled as a Bernoulli random variable with the probability of being male equal to

$$122 \text{logit}(p_{i,y}^{type}) = \alpha_i^{type} + \beta_i^{type} \cdot y$$

123 where i indexes an individual stream or lentic area, y is number of years after stocking that
 124 maturation occurs ($0 \leq y \leq 5$), $type$ indexes whether a location is a river or lentic area, and α_i^{type}
 125 and β_i^{type} are the location-specific intercepts and slopes, respectively, relating the probability of
 126 being male (on a logit-scale) to y . Location-specific parameters were decomposed into type-
 127 specific population-averages and location-specific deviations from the averages

$$128 \begin{aligned} \alpha_i^{type} &= \alpha_0^{type} + \delta_i \\ \beta_i^{type} &= \beta_0^{type} + \gamma_i \end{aligned}$$

129 where α_0^{type} and β_0^{type} are the type-specific population averages for the parameters and δ_i and γ_i
130 are location-specific deviations from α_0^{type} and β_0^{type} , respectively. The following vague priors
131 were specified for the model: $\begin{matrix} \alpha_0^{type} \\ \beta_0^{type} \end{matrix} \sim MVN(\mathbf{0}, \mathbf{\Sigma}^{type})$, $\delta_i \sim N(0, \sigma_\delta)$, $\gamma_i \sim N(0, \sigma_\gamma)$,
132 $\mathbf{\Sigma}^{type} \sim Wish(2 \times 2 \text{ identity matrix}, 3)$, $\sigma_\delta \sim Unif(0, 100)$, and $\sigma_\gamma \sim Unif(0, 100)$. The model was
133 estimated using JAGS²⁷ executed from within R²⁸ using the R2JAGS package²⁹. Three parallel
134 chains, each with 2 million iterations, were run from overdispersed initialization values. The first
135 1 million iterations were discarded as a burn-in and every 100th iterations was retained, resulting
136 in a total of 30 thousand saved samples across all chains. Chain convergence for each parameter
137 was determined by examining trace plots, scale reduction factors, and posterior distribution plots.
138 Effective sample sizes of the chains were also evaluated to ensure there was sufficient
139 independent information for quantifying summary statistics of the saved chains. Medians of the
140 saved MCMC chains were used as point estimates for parameters and derived variables and 95%
141 highest posterior density intervals (HPD) were used as measures of uncertainty for the point
142 estimates.

143 **3. Results**

144 Overall, sex ratios of adult sea lamprey from the lentic, stream, and at-large populations
145 differed substantially (Table 1; Supplemental Tables 3, 4, and 5). Sex ratios of adult sea lamprey
146 stocked as larvae in lentic and stream environments were biased toward males at a ratio of 3.8
147 males to 1 female in lentic areas and 2.3 males to 1 female in streams. Sex ratio of untagged
148 adult sea lamprey captured from the same traps during the same years was 1.4 males to 1 female.

149 The population-average parameters relating probability of being male (on a logit scale) to
150 number of years after stocking that maturation occurs for lentic areas were 0.876 (95% HPD: -
151 0.142 – 2.037) and 0.122 (95% HPD: -0.325 – 0.577) for the intercept and slope, respectively
152 (Supplemental Results). Conversely, for stream environments the population-average
153 parameters were 0.930 (95% HPD: 0.048 – 1.826) and -0.236 (95% HPD: -0.726 – 0.236) for the
154 intercept and slope, respectively. Based on these point estimates, probability of being male in
155 lentic and stream environments initially were similar [lentic environments = 71% (95% HPD:
156 49-90%; stream environments = 72% (95% HPD: 53-88%)]. Whereas in lentic environments the
157 probability of being male increased slightly over time, in stream environments the probability of
158 being male decreased over time (Figure 1). For sea lamprey that metamorphosed 3 years after
159 stocking, percent male was 78% (95% HPD: 53 – 94%) for lentic environments and 56% (95%
160 HPD: 28 – 79%) for stream environments. From the saved MCMC iterations, we calculated how
161 likely that the probability male would be greater in lentic versus stream environment. Initially
162 after stocking, there was only a 48% chance that the probability of being male would be greater
163 in lentic environments. One year after stocking there was a 73% chance that the probability of
164 being male would be greater in lentic environments. For two to six years after stocking, the
165 chance that the probability of being male would be greater in lentic versus stream environments
166 ranged from 89 to 92%.

167 Most individual lentic and stream locations exhibited relationships that were similar to
168 the population-averages. The only exception was that one of the lentic areas (Carp River)
169 exhibited a decline in probability of being male over time, which was more in line with what was
170 observed for stream environments (Figure 1).

171 The saved MCMC chains for all parameters and derived variables from the estimated
172 Bayesian hierarchical logistic regression model were judged to have converged on stable
173 stationary distributions by all evaluated criteria. The lowest effective sample size for the saved
174 MCMC chains for parameters and derived variables was 11,000.

175

176 **4. Discussion**

177 Given these results, we hypothesize that growth and/or condition of sea lamprey
178 influences sex determination. Initially after stocking, percent male sea lamprey from both
179 productive and unproductive environments were similar and high relative to the at-large sea
180 lamprey populations. We attribute this to the physical process of tagging. In a study evaluating
181 effects of coded wire tagging on larval sea lamprey²³, tagged larvae were significantly shorter on
182 average than untagged larvae up to 2 years after tagging; the conclusion being that displacement
183 and handling associated with tagging decreased growth rate initially. In a productive stream
184 environment where environmental features were conducive to growth and condition, larval sea
185 lamprey quickly recovered from the tagging effect, sex ratios becoming less skewed towards
186 males. Conversely, in unproductive lentic environment, environmental features resulted in
187 further skewing of sex ratios. Previous population demographic work showed that larvae reared
188 in lentic environments grew two to four times slower and metamorphosed into the parasitic stage
189 at smaller sizes than larvae from the stream environments^{22,24}. An exception was the Carp River
190 lentic area where tagged sea lamprey larvae had condition factors 1.5 to 1.7 times greater than
191 tagged larvae in the other lentic areas²⁴, but also exhibited a decline in probability of being male
192 over time like stream environments. Although sex determination in some fishes has been
193 previously linked to rearing temperature and social context^{1,2}, if our hypothesis is substantiated

194 with future research, this would be the first time that growth rate of a fish has been linked to sex
195 determination.

196 Given the serendipitous nature of this study, some aspects of the design were not ideal,
197 but do not necessarily discount our observation of skewed sex ratios and our working hypothesis.
198 One deficiency was that larvae stocked in stream and lentic environments were collected from
199 different source streams and during different years. Therefore, the observed differences in sex
200 ratios could simply be an artifact of the streams from which the larvae were sourced. However,
201 we think this is unlikely because the sex ratios were initially very similar between stream and
202 lentic environments and diverged over time. Further, in both studies the larvae were collected
203 from multiple sea lamprey producing tributaries typical to Lakes Huron and Michigan. As such,
204 the sex ratio observed from populations reared in stream and lentic environments should have
205 been similar to the sex ratio of at-large adult sea lamprey. A second deficiency was that we do
206 not know the actual growth rate and condition for sea lamprey that were recovered as adults.
207 Instead, we are only able to broadly assume that the larvae reared in lentic areas on average grew
208 slower and metamorphosed at smaller sizes than larvae in stream environments^{22,24}. A third
209 deficiency was that we are unable to exclude the possibility that the observed sex ratios were the
210 result of differential rates of mortality or metamorphosis between the sexes. Females could
211 experience higher mortality under growth limiting conditions because of higher energetic
212 demands during gonadal development³⁰. Also, if females were more likely to delay
213 metamorphosis relative to males in slow growth conditions, differential mortality between the
214 sexes could be significant because of additional years in the larval stage. In at least two non-
215 parasitic lamprey species, females appear to metamorphose at larger sizes and older ages than
216 males^{13,31}. However, in sea lamprey, where body size and fecundity increase dramatically during

217 the parasitic stage, a similar difference in the size and age at metamorphosis has generally not
218 been reported³². In one case, the opposite trend was suggested; female sea lamprey
219 metamorphose at a younger age where males were smaller at metamorphosis³³. Taken together,
220 while differential rates of mortality and metamorphosis among the sexes have not been
221 previously reported in sea lamprey^{13,14} and overall rates of mortality did not differ between
222 stream and lentic environments^{22,24}, they remain plausible alternative hypotheses that require
223 further investigation.

224 Our working hypothesis is consistent with studies that linked male-biased sex ratios in
225 lampreys to high larval density^{10,11,13} because high density larval lamprey populations often grow
226 slowly^{34,35,36}. For example, when density of sea lamprey in the Great Lakes were reduced by
227 about 90% after initial lampricide treatments, the surviving larvae had less competition for
228 habitat and food, grew faster, and were more likely to be female^{10,11}. Our conclusion is also
229 consistent with the observation that condition factor of recently metamorphosed female sea
230 lamprey is higher than that of males and that the sex ratio of metamorphosed sea lamprey
231 became male-biased after preventing sea lamprey reproduction in a stream for five years³³;
232 perhaps because slower growing larvae require more years to achieve a size and condition factor
233 required for metamorphosis. Furthermore, in a laboratory study where gonadal biopsy was used
234 to track sex change in coded wire tagged larval sea lamprey fed Brewer's yeast, the proportion of
235 larvae with ovaries decreased as a direct result of oocyte atresia and the proportion of larvae with
236 testes increased as a result of development from atypical gonads and female sex reversal¹⁸. This
237 finding is also consistent with our conclusion because, although changes in length and weight
238 were not reported¹⁸, in previous laboratory studies when larvae were coded wire tagged and fed
239 Brewer's yeast, larvae did not grow and decreased in condition^{37,38}. The energetic investment to

240 produce oocytes is generally greater than spermatocytes³⁰, so when habitat and food is limiting
241 becoming male may provide a fitness advantage.

242 As a basal vertebrate, understanding mechanisms of sex determination in lamprey may
243 provide unique insight into sex determination in other vertebrates⁶ and may yield crippling
244 control strategies such as “Trojan” sex gene carriers and daughterless technology for sea lamprey
245 in the Great Lakes^{4,39,40}. Here, we provide the first evidence that sex determination in sea
246 lamprey may be triggered by growth rate rather than population density, and expect our hypothesis
247 will be rigorously challenged and tested in future experimental studies.

248

249 **Ethics.** Experimental protocols involving the handling of fishes were carried out in accordance
250 with United States federal guidelines for care and use of animals and were approved by the
251 American Fisheries Society through the “Use of Fishes in Research Committee, 2014”.

252 **Data accessibility.** Summarized data are available in the electronic supplementary material. R
253 code and data for conducting the Bayesian hierarchical logistic regression model described in the
254 text are available through figshare: <https://doi.org/10.6084/m9.figshare.4704724.v1>.

255 **Author’s contributions.** N.S.J. and W.D.S. conceived and conducted the study. T.O.B. and
256 N.S.J. analyzed the data. N.S.J. and T.O.B. drafted the manuscript and W.D.S. contributed to
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374 **Table 1.** Percent of the tagged adult sea lamprey that were male according to the environment
375 they were stocked in. ‘At large’ refers to adult sea lamprey without a tag that were captured in
376 the same sea lamprey assessment traps from 2007 to 2014.

Environment	N	% Males	95% CI
Lentic	171	79%	73-85%
Stream	209	66%	60-72%
At-large	59,522	59%	58-60%

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378

379 **Figure 1.** Predictions from the Bayesian hierarchical logistic model estimating percent male of
380 coded wire tagged (CWT) adult sea lamprey according to where they were stocked (Lentic
381 versus Stream) and the year after stocking in which metamorphosis occurred. The top panel
382 shows population-average predictions whereas the lower panel shows predictions for individual
383 locations. The horizontal line on each panel indicates percent model of adult sea lamprey
384 without CWT that were captured in the same traps from 2007 to 2014 as adults with tags.