Aggressive mimicry lure polymorphisms in the parasitic mussel *Lampsilis fasciola* model fish or leech host prey and differ in morphology and pigmentation, but not in display behavior

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Unionoid freshwater mussels (Bivalvia: Unionidae) are free-living apart from a brief, obligately parasitic, larval stage that infects fish hosts and gravid female mussels have evolved a spectrum of strategies to infect fish hosts with their larvae. In many North American species, this involves displaying a mantle lure: a pigmented fleshy extension that acts as an aggressive mimic of a host fish prey, thereby eliciting a feeding response that results in host infection. The mantle lure of *Lampsilis fasciola* is of particular interest because it is apparently polymorphic, with two distinct primary lure phenotypes. One, described as "darter-like", has "eyespots", a mottled body coloration, prominent marginal extensions, and a distinct "tail". The other, described as "worm-like", lacks those features and has an orange and black coloration. We investigated this phenomenon to 1) confirm that it is a true polymorphism; 2) investigate its ecological persistence; 3) identify the range of putative model species targeted by this mimicry system within a river drainage; 4) determine whether the mantle lure polymorphism includes a behavioral component. Detection of within-brood lure variation and within-population phylogenomic (ddRAD-seq) analyses of individuals bearing different lures confirmed that this phenomenon is a true polymorphism. It appears stable over ecological timeframes: the ratio of the two lure phenotypes in a River Raisin (MI) population in 2017 was consistent with that of museum samples collected at the same site 6 decades earlier. Within the River Raisin, four main "darter-like" lure motifs visually approximated four co-occurring darter species (*Etheostoma blennioides, E. exile, E. microperca,* and *Percina maculata*) and the "worm-like" lure resembled a widespread common leech, *Macrobdella decora*. Darters and leeches are typical prey of *Micropterus dolomieui* (smallmouth bass), the primary fish host of *L. fasciola*. *In situ* field recordings were made of the *L. fasciola* "darter" and "leech" lure

display behaviors, in addition to the non-polymorphic lure display of co-occurring *L. cardium*. Despite having putative models in distinct phyla, both *L. fasciola* lure morphs have similar display behaviors that differ significantly from that of sympatric *L. cardium* individuals. We conclude that the *L. fasciola* mantle lure polymorphism does not include a behavioral component. Discovery of discrete within-brood inheritance of the lure polymorphism implies potential control by a single genetic locus and identifies *L. fasciola* as a promising study system to identify regulatory genes controlling a key adaptive trait of freshwater mussels.

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ABSTRACT

Unionoid freshwater mussels (Bivalvia: Unionidae) are free-living apart from a brief, obligately parasitic, larval stage that infects fish hosts and gravid female mussels have evolved a spectrum of strategies to infect fish hosts with their larvae. In many North American species, this involves displaying a mantle lure: a pigmented fleshy extension that acts as an aggressive mimic of a host fish prey, thereby eliciting a feeding response that results in host infection. The mantle lure of *Lampsilis fasciola* is of particular interest because it is apparently polymorphic, with two distinct primary lure phenotypes. One, described as "darter-like", has "eyespots", a mottled body coloration, prominent marginal extensions, and a distinct "tail". The other, described as "worm-like", lacks those features and has an orange and black coloration. We investigated this phenomenon to 1) confirm that it is a true polymorphism; 2) investigate its ecological persistence; 3) identify the range of putative model species targeted by this mimicry system within a river drainage; 4) determine whether the mantle lure polymorphism includes a behavioral component. Detection of within-brood lure variation and within-population phylogenomic (ddRAD-seq) analyses of individuals bearing different lures confirmed that this phenomenon is a true polymorphism. It appears stable over ecological timeframes: the ratio of the two lure phenotypes in a River Raisin (MI) population in 2017 was consistent with that of museum samples collected at the same site 6 decades earlier. Within the River Raisin, four main "darter-like" lure motifs visually approximated four co-occurring darter species (*Etheostoma blennioides, E. exile, E. microperca,* and *Percina maculata*) and the "worm-like" lure resembled a widespread common leech, *Macrobdella decora*. Darters and leeches are typical prey of *Micropterus dolomieui* (smallmouth bass), the primary fish host of *L. fasciola. In situ* field recordings were made of the *L. fasciola* "darter" and "leech" lure display behaviors, in addition to the non-polymorphic lure display of co-occurring *L. cardium*. Despite having putative models in distinct phyla, both *L. fasciola* lure morphs have similar display behaviors that differ significantly from that of sympatric *L. cardium* individuals. We conclude that the *L. fasciola* mantle lure polymorphism does not include a behavioral component. Discovery of discrete within-brood inheritance of the lure polymorphism implies potential control by a single genetic locus and identifies *L. fasciola* as a promising study system to identify regulatory genes controlling a key adaptive trait of freshwater mussels.

52   **INTRODUCTION**

53   In ecology, mimicry refers to a convergent adaptive trait prevalent in many biological

54   communities: the deceptive resemblance of one organism to another (Pasteur, 1982; Schaefer &

55   Ruxton, 2009; Maran, 2015). It involves three categories of interacting ecological players: mimic

56   (organism displaying the deceptive resemblance), model (organism being mimicked), and

57   receiver (organism being deceived) (Pasteur, 1982; Maran, 2015). Mimicry occurs across a wide

58   variety of ecological contexts and sensory modalities, but conceptually (Jamie, 2017), individual

59   cases can be categorized by the traits being mimicked (signals or cues), as well as by the degree

60   of deceptiveness and the fitness consequences being communicated to the receiver (aggressive,

61   rewarding, Müllerian or Batesian mimicry).

62   Mimetic systems that are polymorphic (multiple within-species mimic morphs with

63   discrete models) have been particularly influential in uncovering the genetic basis of complex

64   adaptive traits in natural populations (Jay et al., 2018; Palmer & Kronforst, 2020). Such

65   polymorphisms are rare in nature, with the most well studied examples occurring in papilionid

66   butterflies (Hazel, 1990; Joron & Mallet, 1998; Nijhout, 2003; Jay et al., 2018; Palmer &

67   Kronforst, 2020). For instance, polymorphisms in *Heliconious* species are determined by

68   presence/absence of an introgressed chromosomal inversion 'supergene' (Jay et al., 2018), and

69   alleles of a single ancestral gene (*doublesex*) control female-specific polymorphisms in *Papilio*

70   species (Palmer & Kronforst, 2020).

71   In contrast to papilionid butterflies, the genetics of mimicry trait evolution among

72   unionoid mussels is poorly understood. Unionoida comprise ~75% of the planet's freshwater

73   bivalve species and are free-living apart from a brief, obligately parasitic, larval stage that infects

74   fish hosts (Bogan, 2007; Haag, 2012). Gravid female mussels have evolved a spectrum of

75 strategies to infect hosts with their larvae (Zanatta & Murphy, 2006; Barnhart, Haag & Roston,

76 2008; Hewitt, Wood & Ó Foighil, 2019). Females in many species use a mantle lure (Welsh,

77 1933): a pigmented fleshy extension that acts as an aggressive mimic (Jamie, 2017) of a host fish

78 prey item (e.g., small fish, aquatic invertebrate, egg mass) visual cue and elicits a feeding

79 response resulting in host infection (Haag & Warren, 1999; Barnhart, Haag & Roston, 2008;

80 Figure 1a). Mimetic mantle lures predominate in Lampsilini, a major clade of North American

81 freshwater mussels recently identified as a cryptic adaptive radiation centered on larval ecologies

82 and specialized host-infection behaviors (Hewitt, Haponski & Ó Foighil, 2021). Ortmann (1911)

83 and Kramer (1970) reported the production of rudimentary mantle lures in juveniles and male

84 lampsilines but noted that formation of fully developed lures is restricted to sexually mature

85 females, and that only gravid females engage in lure display behaviors. Surprisingly, neither

86 Ortmann (1911) nor Kramer (1970) depicted male mussel lure rudiments, nor could we find any

87 such depictions in the literature, and we therefore included examples for two species, *Lampsilis*

88 *cardium* and *L. fasciola* (Supplementary Figure 1).

89   Although mimetic mantle lures are a key adaptive trait of freshwater mussel

90 diversification, the genetic regulators underlying their formation (Kramer, 1970), variation

91 (Haag, Warren & Shillingsford, 1999; Zanatta, Fraley & Murphy, 2007; Barnhart, Haag &

92 Roston, 2008), and evolution (Zanatta & Murphy, 2006; Hewitt, Haponski & Ó Foighil, 2021)

93 remain completely unknown. This gap in our knowledge is exacerbated by the stark conservation

94 status of North American freshwater mussels, with 2/3rds of species classified as threatened or

95 near-threatened (Lopes-Lima et al., 2018). As with papilionid butterflies (Jay et al., 2018; Palmer

96 & Kronforst, 2020), targeting polymorphic lampsiline mantle lures for in-depth study may

97 represent a tractable route to closing that gap between genes and phenotypes.

98   *Lampsilis fasciola,* the Wavy-Rayed Lampmussel, is a promising candidate species in

99   that it produces a number of distinct mantle lure phenotypes (Zanatta, Fraley & Murphy, 2007)

100   across its Eastern North America distribution, extending from southern Ontario to northern

101   Alabama (Parmalee & Bogan, 1998). Two range-wide lure phenotypes predominate in northern

102   populations. The more common of the two, labeled "darter-like" by Zanatta et al. (2007), has

103   "eyespots", a mottled "main body" pigmentation composed of lateral and dorsal spots that can

104   vary substantially in color, numerous and prominent marginal extensions (AKA "appendages" or

105   "tentacles"), and a distinct "tail" region (Kramer, 1970; Zanatta, Fraley & Murphy, 2007) – see

106   Figure 1b. A rarer lure phenotype, labeled "worm-like" by McNichols (2007), lacks the above

107   features and has instead a uniform bright orange coloration underlain with a black basal stripe

108   (Zanatta, Fraley & Murphy, 2007) – see Figure 1c. The latter lure phenotype is highly distinctive

109   within the genus *Lampsilis* where fish-like mantle lures are the norm (Kramer, 1970). Based on

110   the results of laboratory larval infection experiments, and on the degree of ecological overlap,

111   *Micropterus dolomieu* (Smallmouth Bass), and to a lesser extent *Micropterus salmoides*

112   (Largemouth Bass), have been identified as *Lampsilis fasciola's* primary fish hosts (Zale &

113   Neves, 1982; McNichols, 2007; Morris et al., 2009; McNichols, Mackie & Ackerman, 2011;

114   VanTassel et al., 2021). Both host species are generalist predators of aquatic invertebrates and

115   vertebrates (Clady, 1974).

116   Our study aimed to address outstanding, inter-related questions to develop *Lampsilis*

117   *fasciola* into an integrated mantle lure polymorphism study system. First among them was

118   residual uncertainty that the mantle lure morphs represent polymorphisms rather than cryptic

119   species. Zanatta et al. (2007), using microsatellite markers, did not detect evidence of cryptic

120   species but qualified their conclusions due to small sample sizes, and their result require

121 molecular phylogentic corroboration (Fisheries and Oceans Canada, 2018). Secondly, we lack any

122 data on the relative persistence of the lure polymorphisms in natural populations through time.

123 Thirdly, one set of important ecological players – the respective models of each *L. fasciola* mantle

124 lure mimic – has not been determined with any specificity. Finally, mantle lure display behavior is an

125 important component of effective mimicry in freshwater mussels (Welsh, 1933; Jansen, Bauer &

126 Zahner-Meike, 2001; Haag & Warren, 2003; Barnhart, Haag & Roston, 2008), but it is unknown if

127 morphologically divergent *L. fasciola* mantle lures, that presumably mimic very distinct host prey

128 models, also differ in their display behaviors.

129   We leveraged a combination of field-collection, captive breeding, museum specimens,

130 and ecological surveys to collect genetic, phenotypic, and population data on this species. Using

131 novel data from two southeastern Michigan river populations [the Raisin (our primary study

132 location), and the Huron], and a phylogenomic (ddRADseq) approach, we corroborated Zanatta

133 et al.'s (2007) finding that mussels bearing the two mantle lure morphs do not represent cryptic

134 lineages. This was confirmed by our documentation of discrete, within-brood, inheritance of the

135 *L. fasciola* lure polymorphism in the captive specimens from the Alabama Aquatic Biodiversity

136 Center (AABC). Availability of mid-20th century museum specimens from a River Raisin

137 population revealed that the relative frequency of the two morphs has remained broadly stable

138 over 60 years. In addition, availability of a comprehensive Raisin River ichtyofauna survey

139 (Smith, Taylor & Grimshaw, 1981) allowed us to identify four darter species as putative models

140 for the predominant "darter-like" mantle lures in this population. The most palusible model for

141 the "worm-like" *L. fasciola* mantle lure appears to be *Macrobdella decorata* (the American

142 medical leech). Despite having putative models in distinct phyla, detailed video analyses of

143 gravid females in the two drainages revealed that both *L. fasciola* lure morphs have similar

144 display behaviors that differ significantly from that of sympatric *L. cardium* indivuals bearing

145 non-polymorphic fish-mimic lures. We conclude that the *L. fasciola* mantle lure polymorphism

146 does not include a significant behavioral component.

147

148 **MATERIALS AND METHODS**

149 **Tissue Sample Collection**

150   *Lampsilis fasciola* mantle tissue samples were collected for genotyping purposes by

151 taking non-lethal mantle clip biopsies (Berg et al., 1995) from wild population lure-displaying

152 female mussels during the summers of 2017, 2018, and 2021 from a total of three rivers (Figure

153 2). Two of the sampling locations were in southeastern Michigan: the River Raisin at Sharon

154 Mills County Park (42.176723, -84.092453; N=30; 24 "darter-like", 6 "worm-like", collectively

155 sampled in 2017, 2018 & 2020), and the Huron River at Hudson Mills Metropark, MI (42.37552,

156 -83.91650; N=13; 7 "darter-like", 6 "worm-like", collectively sampled in 2017, 2018, and 2020

157 under the MI Threatened and endangered species collection permit TE149). Both rivers flow into

158 Lake Erie and are part of the Saint Lawrence drainage. The third location was in North Carolina:

159 the Little Tennessee River (N=10; 35.32324, -83.52275; N=1-, all 10 were "darter-like" and

160 sampled in 2017); this river is a tributary of the Tennessee River and part of the Mississippi

161 drainage. Prior to each biopsy, photographs of the intact, undisturbed, lure display were taken

162 with an Olympus Tough TG-6 underwater camera (Supplementary figure 2).

163

164 **Captive Brood Tissue Samples**

165   In addition to the wild-sampled specimens, we also obtained tissue samples from 50

166 members of a captive-raised individuals of a single brood that had been ethanol-preserved a

167 decade earlier. In 2009, the Alabama Aquatic Biodiversity Center (AABC) established a culture

168   facility for endangered freshwater mussels. The Center's inaugural culture attempt, by co-authors

169   Paul Johnson and Michael Buntin, was a proof-of-concept trial involving a single gravid female

170   *Lampsilis fasciola*, a non-endangered species, sourced from a wild Paint Rock River (another

171   Tennessee River tributary) population (N 34° 47.733′,W 86° 14.396′) in Jackson County, AL

172   (Figure 2) on June 11, 2009. This female *L. fasciola* had a "worm-like" lure: the AABC data

173   sheet for the trial 2009 host infection (Supplemental Figure 3) records that it was "bright orange

174   and black", and it lacked the "eyespots", mottled body coloration, marginal extensions, and "tail"

175   of the "darter-like" lure phenotype (Butlin & Johnson, pers. observ.). On July 13th 2009, ~31K

176   glochidia larvae were extracted from the female's marsupia and used to infect *Micropterus*

177   *coosae* (Redeye Bass) hosts sourced from the Eastaboga Fish Hatchery (Calhoun County, AL)

178   using standard protocols (Barnhart, Haag & Roston, 2008). The female mussel was then returned

179   live to the Paint Rock River Population. Following completion of larval development on the fish

180   hosts, ~ 9.3K metamorphosed juvenile mussels were recovered and reared, initially for the first

181   few weeks in mucket bucket systems (Barnhart, 2006) then a suspended upwelling system

182   (SUPSYS) for two years with ~2.2K surviving. In 2011, this proof-of-concept culture

183   experiment was terminated, and the survivors were donated to several research groups, with the

184   large majority utilized for toxicology experiments (Leonard et al., 2014a,b).

185   Prior to the brood's termination, Johnson noticed that a few females had attained sexual

186   maturity and were displaying polymorphic lures (Figures 3b, 3c). We aimed to substantiate that

187   2011 observation by tracking down any remaining brood specimens and were successful in

188   recovering 50 individuals that had been preserved in 95% ethanol and shipped to Nathan Johnson

189   (USGS) in Gainsville, FL in 2011. Because *Lampsilis* spp. juveniles and males produce a

190   rudimentary mantle lure (Ortmann, 1911; Kramer, 1970), we were able to determine the primary

191   lure phenotype (darter-like" or "worm-like") of all 50 preserved brood members. Using a Leica

192   MZ16 dissecting microscope, individual photomicrographs were taken of the preserved

193   rudimentary lure structures (Figure 3d,e and Supplementary Figure 4), and their respective lure

194   phenotypes were identified independently by both T. Hewitt and by D. Ó Foighil. Additionally,

195   tissue samples were acquired from all 50 individuals and included in the downstream

196   phylogenomic analyses.

197

198   **Phylogenomic analyses**

199

200   Genomic DNA was extracted from tissue samples using E.Z.N.A. Mollusk DNA kit

201   (Omega Bio-Tek, Norcross, GA) according to manufacturer's instructions and then stored at -

202   80°C. The quality and quantity of DNA extractions were assessed using a Qubit 2.0 Fluorometer

203   (Life Technologies, Carlsbad, CA) and ddRADseq libraries were prepared following the

204   protocols of Peterson et al. (2012). We then used 200 ng of DNA for each library prep. This

205   involved digestion with Eco-RI-HF and MseI (New England Biolabs, Ipswich, MA) restriction

206   enzymes, followed by isolating 294-394 bp fragments using a Pippen Prep (Sage Science,

207   Beverly, MA) following the manufacturer's instructions. Prepared ddRADseq libraries then were

208   submitted to the University of Michigan's DNA sequencing core and run in four different lanes

209   using 150 bp paired-end sequencing on either an Illumina HiSeq 2500 or Illumina novaseq

210   shared flow cell. Two control individuals of *Lampsilis fasciola* were run in each lane and reads

211   for both individuals clustered together in every analysis with 100% bootstrap support, indicating

212   no lane effects on clustering among individuals. Raw demultiplexed data were deposited at

213   genbank under the bioproject ID PRJNA985631 with accession numbers SAMN35800743-

214   SAMN35800847. Individuals included in phylogenomic analyses can be found in Table 1, and

215   museum ID numbers can be found in Supplementary Table 1.

216   The alignment-clustering algorithm in ipyrad v.0.7.17 (Eaton, 2014; Eaton & Overcast,

217   2020) was used to identify homologous ddRADseq tags. Ipyrad is capable of detecting insertions

218   and deletions among homologous loci, which increases the number of loci recovered at deeper

219   evolutionary scales compared to alternative methods of genomic clustering (Eaton, 2014).

220   Demultiplexing was performed by sorting sequences by barcode, allowing for zero barcode

221   mismatches (parameter 15 setting 0) and a maximum of five low-quality bases (parameter 9).

222   Restriction sites, barcodes, and Illumina adapters were trimmed from the raw sequence reads

223   (parameter 16 setting 2), and bases with low-quality scores (Phred-score <20, parameter 10

224   setting 33) were replaced with an N designation. Sequences were discarded if they contained

225   more than 5 N's (parameter 19). Reads were clustered and aligned within each sample at an 85%

226   similarity threshold, and clusters with a depth < 6 were discarded (parameters 11 and 12). We

227   also varied the number of individuals required to share a locus from ~50% to ~75%.

228   We analyzed the two concatenated ddRAD-seq alignment files (50% and 75% minimum

229   samples per locus) using maximum likelihood in RAxML v8.2.8 (Stamatakis, 2014). A general

230   time-reversible model (Lanave et al., 1984) was used for these analyses that included invariable

231   sites and assumed a gamma distribution. Support was determined for each node using 100 fast

232   parametric bootstrap replications. Lure phenotype information was recorded and mapped on to

233   the phylogenetic tree. Phylogenetic signal of lure phenotype was tested using Pagel's λ (Pagel,

234   1999) in R (R Core Team, 2018) with the 'phylobase' package (R Hackathon et al., 2013).

235

236   **River Raisin Mantle Lure Phenotype Ratios Over Time**

237   Mid-20th century *Lampsilis fasciola* specimens collected at the Sharon Mills County Park

238   site (Raisin River, MI; Fig. 2a) are preserved as part of the University of Michigan's Museum of

239   Zoology wet mollusk collection. They stem from 8 different collecting events between 1954 and

240   1962 (Table 2) and their presence afforded an opportunity to assess the stability of the *L. fasciola*

241   "darter/worm" mantle lure polymorphism in that population over a six-decade time interval. All

242   of the museum specimens, males as well as females, were examined to determine whether their

243   fully-formed (female) or rudimentary(male) mantle lures were "darter-like" or "worm-like". For

244   females, this could be achieved by simple visual examination, but male lure classification

245   required a dissecting microscope. The ratio of mantle lure phenotypes observed in the Sharon

246   Mills County Park population was compared among mid-20th century (UMMZ preserved

247   females and males) and 2017 (field photographs and videos of displaying females) samples using

248   a Fisher's exact test, implemented in R.

249

250   **Putative Lure Mimicry Models**

251   Population-specific putative model species for the *Lampsilis fasciola* mantle lure mimicry

252   system were investigated at the River Raisin Sharon Mills County Park study site (Figure 2) in

253   part because of the availability of a comprehensive ecological survey of Raisin River fishes

254   (Smith, Taylor & Grimshaw, 1981). "Darters" – members of the speciose North American

255   subfamily Etheosomatidae – have been implictly identified as models for the predominant

256   "darter-like" mantle lure phenotype (Zanatta, Fraley & Murphy, 2007), and they are preyed upon

257   by *Micropterus dolomieu* (Surber, 1941; Robertson & Winemiller, 2001; Murphy et al., 2005), *L.*

258   *fasciola's* primary fish host (Zale & Neves, 1982; McNichols, 2007; Morris et al., 2009;

259   McNichols, Mackie & Ackerman, 2011; VanTassel et al., 2021). Ten species of Etheosomatidae

260   occur in the River Raisin, as does *M. dolomieu* (Smith, Taylor & Grimshaw, 1981).

261   River Raisin gravid female *Lampsilis fasciola* engage in mantle lure displays from May-

262   August. During the summer of 2017, a total of 27 different displaying females were

263   photographed along a 15um stretch downstream of the dam at Sharon Mills county park using an

264   Olympus Tough TG-6 underwater camera. The lures were first categorized into broad groupings

265   based on visual similarity (in terms of morphology and coloration; Supplementary Figure 2) and

266   these groupings were then used to identify putative host prey fish model species from those

267   present in the River Raisin drainage (Smith, Taylor & Grimshaw, 1981), in terms of convergent

268   size, shape, and coloration. Putative model species were further assessed based on their relative

269   local abundance (Smith *et al.*, 1981) and on their range overlap with both mimic and receiver.

270   Geographic ranges of *L. fasciola,* the primary host *M. dolomieu,* and each prospective model

271   species were carefully produced by hand in Arcgis software (ESRI, 2022), and the overlap

272   between *L. fasciola, M. dolomieu,* and each putative model species was assessed using Arcgis

273   software.

274

275   **Behavioral Analyses**

276   Standardized video recordings of 30 mantle lure-displaying female *L. fasciola* were

277   recorded using a Go Pro Hero 6 camera in the Summer of 2018 at the two different southeastern

278   Michigan study sites: Sharon Mills County Park (River Raisin) and Hudson Mills Metropark

279   (Huron River). An additional 4 video recordings of the lure behavior for the sympatric congener

280   *Lampsilis cardium* were collected from the Sharon Mills site to assess interspecific variability in

281   lure behavior. Recordings were captured from a top-down perspective during daylight hours

282   using a standardized frame that included a metric ruler and a Casio TX watch to record date,

283   time, and water temperature data within the video frame. For each displaying female, videos of

284   the lure movements were recorded for 10 minutes at 120 frames-per-second. Analysis of the

285   videos involved manually recording mantle lure movements for 20,000 frames, starting at 5,000

286   frames to to avoid any initial setup effects on mussel display behavior. The frame numbers when

287   an individual movement began and ended were noted, and movements of the left and the right

288   mantle lure flaps were assessed seperately.

289   To quantitatively assess behavioral differences among samples, gait analysis diagrams

290   were created in R for each displaying mussel. Averages and standard deviations for the time

291   intervals between lure undulations were calculated for each individual, as well as speed of

292   undulation and proportion of movements synchronized. A Kruskal-Wallis test was used to test

293   for overall differences among lure groups (*L. fasciola* "darter-like", *L. fasciola* "worm-like", and

294   *L. cardium*), and pairwise Wilcoxon Signed rank tests were used to compare groups directly with

295   a Bonferroni *P* value adjustment to correct for multiple tests. A Spearman correlation was used

296   to test for water temperature on time interval between lure undulations.

297   To further explore differences in lure behavior among groups, we used a General Linear

298   Mixed Model (GLMM), with sample ID as a random factor, to test for differences in lure

299   movement intervals. The GLMM appoach, unlike simple mean comparisons, allows the

300   inclusion of all lure movements for all individuals in the model. Because displaying mussels all

301   varied in the number of lure movements recorded over 20,000 frames analyzed, a dataset of 1000

302   random bootstrap values was constructed for each individual by randomly sampling values, with

303   replacement. Models were fitted using the 'lmerTest' package in R, and Satterthwaite's Method

304 (Satterthwaite, 1946; Kuznetsova, Brockhoff & Christensen, 2017) was used to test for

305 significance of fixed effects of lure phenotype on the interval between lure undulations.

306

307 **RESULTS**

308 **Captive Brood**

309   Mantle lure microphotographs (Figure 3d, e) of all 50 available AABC-cultured and 95%

310 ethanol-preserved *Lampsilis fasciola* of the same maternal brood are individually presented in

311 Supplementary Figure 4. These specimens were 2 years post-metamorphosis when preserved in

312 2011 and independent classification of their mantle lure phenotypes concurred the ratio of

313 "darter-like" to "worm-like" phenotypes was respectively 33/17 for this brood subsample.

314

315 **ddRAD-seq and Phylogenomic analyses**

316   Genomic sequencing returned raw reads ranging from 258,664 to 13,366,692 per

317 individual across the 108 unionid specimens included in the analyses comprising samples of the

318 ingroup *Lampsilis fasciola*, sourced from 4 different populations, along with outgroups *Lampsilis*

319 *cardium* and *Sagittunio nasuta*. Mean coverage depth for the 85% clustering threshold ranged

320 from 2.03 (L_fasciola_AL_mom_2) to 14.25 (L_fasciola_Raisin_16; Table 1). Between 28,725

321 and 16,161 homologous loci were identified across the two best ddrad datasets (85%-50% and

322 85%-75% respectively) and the number of loci recovered was generally consistent among all

323 samples.

324   The maximum likelihood tree produced by RAxML (Supplementary Figure 5) recovered

325 the following ingroup/outgroup topology: (*S. nasuta* (*L. cardium, L. fasciola*)) with outgroup

326 branch lengths greatly exceeding those of the ingroup. To optimize the legibility of ingroup

327   relationships, a compressed, color-coded graphic excluding *S. nasuta* was constructed (Figure 4).

328   A nested series of phylogenetic relationships was recovered for the four *L. fasciola* fluvial

329   populations with the two Michigan drainages being paraphyletic: (Little Tennessee River (Paint

330   Rock River (River Raisin (River Raisin, Huron River))). The ingroup topology also showed

331   evidence of within-population genealogical relationships with all Paint Rock River brood

332   members forming an exclusive clade (Figure 4).

333   The respective primary mantle lure phenotypes – "darter-like" or "worm-like" – of all 92

334   *Lampsilis fasciola* ingroup individuals is indicated in Figure 4. Note that 3 of the 4 population

335   samples – Little Tennessee River, River Raisin and Huron River – were exclusively composed of

336   mantle-lure displaying wild females, and the latter two samples were polymorphic in mantle lure

337   composition. Regarding the Paint Rock River sample, polymorphic lures were restricted to the

338   50 captive-raised AABC brood members sourced from a gravid, wild female in 2009 (not

339   included in the analyses). The ingroup phylogeny (Figure 4) contained two polymorphic mantle

340   lure clades, one composed of both Michigan populations (River Raisin and Huron River), the

341   other consisting only of the AABC brood, and both clades had individuals of either lure

342   phenotype interspersed across their respective topologies. Little phylogenetic signal associated

343   with either primary mantle lure phenotype ($\lambda = 0.21$; $P = 0.13$).

344   **Phenotypic Ratios Over Time**

345   Table 2 summarizes the sex and primary lure phenotypes of 57 *Lampsilis fasciola*

346   specimens collected from 1954-1962 at the River Raisin Sharon Mills County Park study site

347   (Figure 2a) and preserved in the University of Michigan Museum of Zoology's wet mollusk

348   collection (Figure 5b, c). These historical samples had a collective "darter-like" to "worm-like"

349   ratio of 48:9, with 15.8% of individuals having the less common "worm-like" mantle lure

350   phenotype. Figure 5a contrasts the mid-20th century lure phenotype ratios with a contemporary

351   (2017) estimate in that same population, based on photographic recordings of 27 displaying

352   females. The contemporary estimate was 23:4, with 14.8% of individuals having the less

353   common "worm-like" mantle lure phenotype. A Fisher's exact test found no significant

354   differences between these two temporal estimates of relative mantle lure phenotype frequencies

355   in the River Raisin Sharon Mills County Park *L. fasciola* population ($P = 1$).

356   **Putative Raisin River Lure Mimicry Models**

357   The field photographs of 27 displaying female *L. fasciola* mantle lures in the Raisin River

358   Sharon Mills County Park population in 2017 (Supplementary Figure 2) were categorized into

359   either "darter-like" (Zanatta, Fraley & Murphy, 2007) or "worm-like" (McNichols, 2007), as

360   summarized in the Materials & Methods section. In addition to the specific features that separate

361   these two primary mantle lure phenotypes (presence/absence of "eyespots" mottled

362   pigmentation, marginal extensions and a "tail"), "darter-like" lures exhibited a much higher

363   degree of variation than did "worm-like" lures, both within populations and across the species

364   range. The latter lure phenotype exhibited a relatively simple, uniform morphology combined

365   with a bright orange coloration underlain with a black basal stripe phenotype in Michigan

366   (Figure 6f-h), in Alabama (Fig. 6i, j), and in North Carolina populations (Fig. 2A in Zanatta et al,

367   2007). In contrast, Raisin River "darter-like" mantle lures exhibited individual-level variation

368   that was sometime quite marked, especially in details of their pigmentation, and to a more

369   limited degree in their marginal extensions (Figures 6a-d; Supplementary Figure 2). Among

370   individual variation was most pronounced for inter-population camparisons, e.g., see the much

371   larger "tail" in the lure displaying Paint Rock River, Alabama specimen shown in Figure 6e, and

372   also the wider range of phenotypes present in North Carolina populations (Fig. 2b-d in Zanatta et

373   al, 2007).

374   Despite the considerable individual variation among the 24 photographed Raisin River

375   "darter-like" mantle lures (Supplementary Figure 2), it was possible to identify some shared

376   phenotypic motifs, especially in pigmentation pattern, and to informally categorize 23/24 mantle

377   lures with those shared motifs into 4 general groupings. Group 1 "darter-like" mantle lures were

378   characterized by prominent, chevron-like, darker pigmented blotches, spaced regularily along the

379   flanks of the lure, over a lighter background coloration (Figure 6a). This general pattern occurred

380   in 7/24 Raisin River "darter-like" lures examined. Group 2 was rarer (3/24 individuals) and

381   consisted of a darker background coloration with large orange blotches spaced regularily along

382   the lure flanks, some divided into "dorsal" and "ventral" elements (Figure 6b). Group 3 (9/24

383   individuals) lures were characterized by prominent dark lateral maculation spatially divided into

384   a "ventral" pattern of larger, regularly spaced blotches and a "dorsal" pattern of more numerous,

385   irregular blotches of different sizes (Figure 6c). Finally, Group 4 (3/24 individuals) lures were

386   characterized by an evenly-dispersed, fine grained freckling of numerous pigmented spots over a

387   lighter background (Figure 6d).

388   To explore putative model species for the four *Lampsilis fasciola* Raisin River "darter-

389   like" mantle lure groupings (Figure 6a-d), potential matches (in terms of size, shape and

390   coloration) were sought among the 10 species of Etheosomatidae that occur in the River Raisin

391   (Smith *et al.*, 1981), many of which display pronounced sexual dimorphism in body coloration

392   (Kuehne and Barbour, 2014). The best apparent matches, depicted in Figure 7, are as follows:

393   Group 1 (Fig. 6a)-*Etheostoma blennioides* (female coloration*)*, Group 2 (Fig. 6b)-*Etheostoma*

394   *exile* (male coloration), Group 3 (Fig. 6c)-*Percina maculata* (male and female coloration) and

395   Group 4 (Fig. 6d)-*Etheostoma microperca* (female coloration).

396   The distinctive color combination of the *Lampsilis fasciola* "worm-like" lure - solid

397   orange with a black underlay (Figure 6 f-j) - did not match that of any Raisin River darter, or

398   other Raisin River fishes (Smith, Taylor & Grimshaw, 1981). It does, however, match the

399   coloration and size/shape, of the common North American leech, *Macrobdella decora*, which is

400   widespread and abundant in eastern North America watersheds and typically feeds on aquatic

401   vertebrates (Klemm, 1982; Munro et al., 1992). *M. dolomieu*, *L. fasciola*'s primary host fish, is a

402   generalist predator with a diet of aquatic invertebrates, including leeches, in addition to small

403   fishes (Clady, 1974) and recreational fishers frequently use live and/or artifical leeches as bait to

404   catch this species (Cooke et al., 2022). Based on the available data, it seems that *Macrobdella*

405   *decora* may be the best model species candidate for the "worm-like" (McNichols, 2007) *L.*

406   *fasciola* mantle lure phenotype, and will hereafter be referred to as the leech phenotype.

407   The geographic range of the mimic, *Lampsilis fasciola*, is a subset of that of its

408   receiver/host *M. dolomieu* (Figure 2), and the extent of range overlap with all 5 putative River

409   Raisin mantle lure models were calculated using Arcgis (Table. 3) and are shown in Figure 8.

410   Three of the five putative models -*Etheostoma blenniodes, Percina maculata* and *Macrobdella*

411   *decorata* have extensive overlap with *L. fasciola*'s range, but *E. exile* and *E. microperca* are

412   restricted to northern portions.

413   **Behavioral Analyses**

414   Lure movement data was extracted from video recordings of 30 *L. fasciola* and 4 *L.*

415   *cardium.* Qualitatively, *L. fasciola* and *L. cardium* have very different mantle lure display

416   behaviors. Gait diagrams show a clear distinction between both primary *L. fasciola* lure

417   phenotypes ("darter" and "leech") and *L. cardium*. A representative individual for each group is

418   shown in Figure 9, and gait diagrams for all individuals can be found in Supplementary Figure 6.

419   *L. cardium* consistently exhibited a synchronized lure undulation of both mantle lure flaps,

420   whereas *L. fasciola* samples frequently moved left and right mantle flaps independently. Gait

421   diagrams also qualitatively showed that while *L. fasciola* behavior is charcterized by a high level

422   of variability in undulation interval, *L. cardium* is comparitavely much more regular in

423   undulation interval with a steady beat frequency (Figure 9a-c).

424   Supplementary Table 2 details the time, date, location, temperature and summary

425   statistics of all 34 lure display field recordings. Movements of the left and right mantle flaps in *L.*

426   *fasciola* were recorded seperately. Boxplots for means and standard deviations of intervals

427   between left lure movements are displayed in Figure 10a,b. Boxplots of mean duration of lure

428   movements (L) are shown in Figure 10c and the proportion of synchronized lure movements are

429   displayed in Figure 10d. Only left mantle flap movements are displayed in Figure 10a-c,

430   however the distributions for the right mantle flap movements are highly similar. Kruskal-Wallis

431   tests suggest highly significant differences among all groups for average inter-undulation interval

432   and for standard deviation of inter-undulation interval ($P < 0.001$). Pairwise Wilcoxon tests

433   revealed highly significant differences in lure inter-movement interval (left) between *L. cardium*

434   and both *L. fasciola* groups ($P = 0.001$ and 0.001 for "darter" and "leech" respectively) and

435   between both primary *L. fasciola* mantle lure phenotypes as well ($P = 0.01$). Pairwise Wilcoxon

436   tests also revealed significant differences in the standard deviation of left inter-movement

437   interval between *L. cardium* and both *L. fasciola* groups ($P = 0.001$ and 0.01 for "darter" and

438   "leech" respectively) but not between the two *L. fasciola* mantle lure groups ($P = 0.09$). These

439   results suggest that, in addition to the qualitative differences between *L. cardium* and *L. fasciola*

440   lure behavior, the *L. cardium* lures are beating at a faster rate and with greater regularity. There

441   also appears to be a slight difference in the rate of lure beats between the two *L. fasciola* mantle

442   lure morphs, with the "darter" lure beating slightly less frequently, but with no differences in

443   variability. No significant relationship was observed between lure inter-movement interval (left)

444   and water temperature (Supplementary Figure 7; $P = 0.428$).

445   General Linear Mixed Models (GLMM) were used as an alternative analytical approach

446   that included a large, bootstrapped dataset of lure movements. GLMM results were similar to

447   those of the mean comparisons, with *L. cardium* individuals having faster movement frequency

448   than either *L. fasciola* lure morphs (an estimated 0.21 seconds for *L. cardium* versus 3.2 and 1.0

449   seconds respectively for *L. fasciola* "darter" and "leech" lures). However, these fixed effects are

450   not significant.

451

452   **DISCUSSION**

453   Two new lines of data, phylogenomic and genetic, corroborated Zanatta et al's. (2007)

454   preliminary finding that the primary mantle lure morphs in *Lampsilis fasciola* (Figure 1b, c)

455   represent a within-population polymorphism rather than cryptic taxa. In phylogenomic analyses,

456   all three polymorphic population samples (Huron, Raisin, and Paint Rock Rivers), collectively

457   spanning the species range (Figure 2a-c), produced tip clades that were comprehensively

458   polyphyletic regarding lure morph type (Figure 4) and the "darter vs leech" dichotomy yielded a

459   low estimate of phylogenetic signal ($\lambda = 0.21$). However, the phylogenomic data did reveal clear

460   evidence of geographic structuring (Figure 4), even among regional populations with a

461   continuous freshwater connection. For example, the Huron and Raisin drainages empty in

462   Western Lake Erie and the Little Tennessee and Paint Rock drainages empty into the Tennessee

463   River (see also VanTassel et al. (2021)). The Paint Rock River (AL) population was sister to the

464   Michigan populations (Figure 4), a result consistent with phylogeographic associations of

465   multiple other North American species, including unionid mussels and *Micropterus dolomieu*,

466   attributed to hypothesized glacial refugia in the southern Appalachian mountains (Soltis et al.,

467   2006; Borden & Krebs, 2009; Zanatta & Harris, 2013; Hewitt et al., 2018).

468   Discovery of within-brood mantle lure heterogeneity (Figure 3), apparently the first such

469   record for unionids, confirms that the *Lampsilis fasciola* "darter-like" and "leech-like" mantle

470   lures are true polymorphisms and provides initial, although limited, genetic insights into lure

471   phenotype inheritance. Of the 50 available offspring, the maternal "leech" phenotype was

472   inherited by 17, the remaining 33 had the "darter" phenotype, but none exhibited a recombinant

473   phenotype, *e.g.*, "leech" coloration with "darter" marginal extensions or "darter" coloration

474   without marginal extensions. Evidence of discrete, within-brood segregation of the mantle lure

475   polymorphism implies potential control by a single genetic locus, and expression of the maternal

476   phenotype in ~1/3rd of the offspring is inconsistent with a dominant hypothetical "leech" allele.

477   Additional pedigree insights are currently inhibited by not knowing the number of sires that

478   contributed to the brood: the dam was a wild-mated Paint Rock River individual. Freshwater

479   mussel broods frequently have multiple paternity (Ferguson et al., 2013; Wacker et al., 2018) and

480   this may well be the case also for the AABC *L. fasciola* brood (Figure 3), although additional

481   analyses of its RADseq dataset are needed to resolve that issue.

482   There are well-known cases of a single genetic locus controlling a mimic polymorphism

483   in other systems. In butterflies, polymorphic mimicry in wing pigmentation is controlled by an

484   introgressed mimicry supergene in *Heliconius* species (Jay et al., 2018) and by mimicry alleles of

485   the transcription factor *doublesex* (*dsx*) in some *Papilio* species (Palmer & Kronforst, 2020).

486   Note, however, that the *Lampsilis fasciola* mantle lure mimicry polymorphism differs in

487   important ways from these butterfly systems. It is more complex because it involves putative

488   models (darters and leeches) from disparate phyla rather than from similar morphospecies (other

489   butterflies), thereby requiring polymorphic trait differentiation in pigmentation and in

490   morphology (Figure 1b, c). It is also a case of aggressive mimicry (Jamie, 2017), different from

491   the Müllerian mimicry of *Heliconius* (Kronforst & Papa, 2015) or the Batesian mimicry of

492   *Papilio* (Kunte, 2009).

493   Persistence of *Lampsilis fasciola* mantle lure polymorphism across a broad geographic

494   scale (Michigan to Alabama, Figure 2) is notable although the mechanism responsible for

495   widespread maintenace is unclear. One hypothesized mechanism for the persistence of

496   polymorphisms in a species or population is frequency-dependent selection, where selection for

497   rare phenotypes are selected cause the ratio of phenotypes to vary over time (Ayala & Campbell,

498   1974). Frequency-dependent selection has been observed in other polymorphic mimicry systems

499   (Shine, Brown & Goiran, 2022) and it has been suggested as a possible mechanism for

500   persistence of the *L. fasciola* polymorphism (Zanatta, Fraley & Murphy, 2007; Barnhart, Haag &

501   Roston, 2008; Hewitt, Haponski & Foighil, 2021). One criterion for frequency-dependent

502   selection is that phenotype ratios oscillate over time as initially rare phenotypes become more

503   successful. However, the historical (1954-1962) and contemporary (2017) data from Sharon

504   Mills County Park (Figure 5) did not show evidence of such oscillation: the frequency of the

505   more common "darter" lure – 84.2% vs. 85.2% - and the ratio of "darter" to "leech" lures – 48:9

506   vs. 23:4 - remained essentially the same for both time windows, although we of course lack data

507   for intervening years. Theoretically, there are other mechanisms for balancing selection to

508   maintain polymorphisms over long time-scales, including heterozygote advantage or opposing

509   selection pressures favoring different alleles at polymorphic loci (Prout, 2000; Mérot et al.,

510   2020), but underlying genetics of the *L. fasciola* polymorphism is unknown at this time and more

511   data are clearly needed.

512   An integrative graphic outline of the *Lampsilis fasciola* mimetic system at the River

513   Raisin study site was assembled containing 4 "darter" and 1 "leech mantle lure phenotypes

514   together with their putative model species (Figure 7). The four putative River Raisin darter

515   model species – *Etheostoma blennioides*, *E. exile*, *E. microperca* and *Percina maculata* – are all

516   common and widespread members of the drainage's ichthyofauna with 300-900 specimens of

517   each species recovered from 30-100 sampling locations by the Smith et al. (1981) ecological

518   survey. The relative uniformity of the "leech" mantle lure phenotype in the River Raisin and

519   throughout the *L. fasciola* range (Figure 6f-j) stands in sharp contrast with much higher local and

520   range-wide variation shown by "darter" lures (Figure 6a-e). That phenotypic lure disparity

521   mirrors the collective phenotypic variability of darters vs. *Macrobdella decora*; darters are the

522   second-most diverse fish clade in North America, with ~170 species (Warren & Burr, 1994;

523   Stein & Morse, 2000). Another possibility is that at least some *L. fasciola* "darter-like" lures

524   across the mussel's range are composite mimics of visual elements from more than one member

525   of their local darter fauna, but that remains to be established.

526   While the behavior of mantle lures in *Lampsilis* mussels has been documented and

527   studied for many decades (Ortmann, 1911; Kramer, 1970; Haag & Warren, 1999), detailed

528   analysis of lure undulation behavior is currently lacking, and the relative importance of behavior

529   versus coloration and morphology is not well understood. The lure undulation for both *L.*

530   *cardium* and *L. fasciola* starts about 2/3rds of the way to the "posterior" ("tailed") side of the

531   lure, and then travels forward toward the "eyespot"-bearing "anterior". This is quite different

532   from the oscilatory "S" shaped routine swimming movements used by many fishes (Liao, 2007;

533   Smits, 2019), however, it shares some resemblence to the "C" start behavior that many fishes use

534   as an escape mechanism (Witt, Wen & Lauder, 2015). The unusual motion of the mantle lures

535   may therefore be mimicing an escape behavior to some extent to strike.

536   Although the *L. fasciola* behaviors differ significantly from those exhibited by *L.*

537   *cardium*, there appears to be no behavioral polymorphism to distinguish the darter from leech

538   lure phenotypes. Our putative model for River Raisin *L. cardium* mantle lures is a species of

539   pelagic minnow, *Pimephales notatus* (Figure 7), whose swimming behavior and ecology differs

540   markedly from that of darters (Burress et al., 2017). Darters are primarily benthic in habit, have

541   lost or greatly reduced their swim bladder (Demski, Gerald & Popper, 1973; Zeyl et al., 2016),

542   and they swim by "hopping" across the river bed in a manner that is much more erratic and

543   intermittent than the pelagic swimming behavior of most minnows (Winn, 1958). This matches a

544   general difference observed between *L. cardium* and *L. fasciola* lures: *L. cardium* moves faster

545   and more regularly in a highly synchronized way, in contrast with the erratic, often left-right-

546   unsynchronized movements of *L. fasciola* lures. The only major difference in lure behavior

547   between the "darter" and "leech" lures of *L. fasciola* is a slightly slower rate exhibited by the

548   "darter" lures (Fig. 9a). Both *L. fasciola* morphs have a similar erratic motion, despite the

549   polymorphism putatively modeling taxa from disparate phyla. Leeches swim by a dorsoventeral

550   bending wave moving from head to tail, caused by a central pattern generator (CPG) comprised

551   of interneurons in the ventral nerve cord (Jordan, 1998). This swimming behavior is very

552   different from the lure undulations observed in the leech-like *L. fasciola* lures. Despite small

553   differences in overall lure beat frequency between "darter" and "leech" mimics, it seems as

554   though the polymorphic mimicry is only skin deep. The rhythmic movements of lure undulations

555   are likely caused by a CPG, but we currently have no information about how these motor

556   patterns are activated or modulated (Marder et al., 2005).

557   The *Lampsilis fasciola* mantle lure aggressive mimicry polymorphism is complex, each

558   morph containing correlated morphological and pigmentation distinctions, however, the

559   polymorphism does not appear to be linked to any large differences in lure behavior, or in the

560   CPG that controls lure behavior. Our discovery of discrete within-brood inheritance of the *L.*

561   *fasciola* lure polymorphism is of particular interest because it implies potential control by a

562   single genetic locus. There are a number of parallel cases in the recent literature, *e.g.,* in

563   butterflies, the regulation of polymorphic mimicry in wing pigmentation also involves single

564   genetic loci (Jay et al., 2018; Palmer & Kronforst, 2020)Timmermans et al. (2020). Timmermans

565   et al. (2020) used SNP data from *Papilio dardanus* to discover a genomic inversion associated

566   with its mimetic polymorphism, and this approach is likely also tractable for *Lampsilis fasciola*

567   given the availability of polymorphic broods – we are currently raising an additional

568   polymorphic brood at the AABC. Mantle lures are a key adaptive trait in Lampsiline evolution

569   and diversification (Hewitt, Haponski & Foighil, 2021) and *Lampsilis fasciola* is a promisinga

570   highly tractable model system to uncover the genetics of lure development and variation in

571   unionoid mussel.

572

Literature cited

Ayala FJ, Campbell CA. 1974. Frequency-dependent selection. *Annual review of Ecology and systematics* 5:115–138.

Barnhart MC. 2006. Buckets of muckets: A compact system for rearing juvenile freshwater mussels. *Aquaculture* 254:227–233. DOI: 10.1016/j.aquaculture.2005.08.028.

Barnhart MC, Haag WR, Roston WN. 2008. Adaptations to host infection and larval parasitism in Unionoida. *Journal of the North American Benthological Society* 27:370–394. DOI: 10.1899/07-093.1.

Berg DJ, Haag WR, Guttman SI, Sickel JB. 1995. Mantle Biopsy: A Technique for Nondestructive Tissue-Sampling of Freshwater Mussels. *Journal of the North American Benthological Society* 14:577–581. DOI: 10.2307/1467542.

Bogan AE. 2007. Global diversity of freshwater mussels (Mollusca, Bivalvia) in freshwater. In: Balian EV, Lévêque C, Segers H, Martens K eds. *Freshwater Animal Diversity Assessment*. Dordrecht: Springer Netherlands, 139–147. DOI: 10.1007/978-1-4020-8259-7_16.

Borden WC, Krebs RA. 2009. Phylogeography and postglacial dispersal of smallmouth bass (Micropterus dolomieu) into the Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 66:2142–2156.

Burress ED, Holcomb JM, Tan M, Armbruster JW. 2017. Ecological diversification associated with the benthic-to-pelagic transition by North American minnows. *Journal of Evolutionary Biology* 30:549–560. DOI: 10.1111/jeb.13024.

Clady MD. 1974. Food habits of yellow perch, smallmouth bass and largemouth bass in two unproductive lakes in northern Michigan. *American Midland Naturalist*:453–459.

Cooke SJ, LaRochelle L, Danylchuk AJ, Danylchuk SC, Griffin LP. 2022. Influence of hook barbs on the "through-the-gill" hook removal method for deeply hooked Smallmouth Bass. *Fisheries Research* 251:106322.

Demski LS, Gerald JW, Popper AN. 1973. Central and peripheral mechanisms of teleost sound production. *American Zoologist*:1141–1167.

Eaton DAR. 2014. PyRAD: assembly of de novo RADseq loci for phylogenetic analyses. *Bioinformatics* 30:1844–1849. DOI: 10.1093/bioinformatics/btu121.

Eaton DAR, Overcast I. 2020. ipyrad: Interactive assembly and analysis of RADseq datasets. *Bioinformatics*:btz966. DOI: 10.1093/bioinformatics/btz966.

ESRI. 2022. ArcGIS Desktop.

Ferguson CD, Blum MJ, Raymer ML, Eackles MS, Krane DE. 2013. Population structure, multiple paternity, and long-distance transport of spermatozoa in the freshwater mussel Lampsilis cardium (Bivalvia: Unionidae). *Freshwater Science* 32:267–282.

Fisheries and Oceans Canada. 2018. *Management plan for the Wavyrayed Lampmussel (Lampsilis fasciola) in Canada. Species at Risk Act Management Plan Series*. Ottawa: Fisheries and Oceans Canada.

Haag WR. 2012. *North American Freshwater Mussels: Natural History, Ecology, and Conservation*. Cambridge University Press.

Haag WR, Warren ML. 1999. Mantle displays of freshwater mussels elicit attacks from fish: Mussel and fish interactions. *Freshwater Biology* 42:35–40. DOI: 10.1046/j.1365-2427.1999.00454.x.

617 Haag WR, Warren, ML. 2003. Host Fishes and Infection Strategies of Freshwater Mussels in
618   Large Mobile Basin Streams, USA. *Journal of the North American Benthological Society*
619   22:78–91. DOI: 10.2307/1467979.
620 Haag WR, Warren ML, Shillingsford M. 1999. Host Fishes and Host-attracting Behavior of
621   Lampsilis altilis and Villosa vibex (Bivalvia: Unionidae). *The American Midland*
622   *Naturalist* 141:149–157. DOI: 10.1674/0003-0031(1999)141[0149:HFAHAB]2.0.CO;2.
623 Hackathon R, Bolker B, Butler M, Cowan P, De Vienne D, Eddelbuettel D, Holder M, Jombart
624   T, Kembel S, Michonneau F. 2013. phylobase: Base package for phylogenetic structures
625   and comparative data. *R package version 0.6* 5.
626 Hazel WN. 1990. Sex-limited variability mimicry in the swallowtail butterfly Papilio polyxenes
627   Fabr. *Heredity* 65:109–114.
628 Hewitt TL, Bergner JL, Woolnough DA, Zanatta DT. 2018. Phylogeography of the freshwater
629   mussel species Lasmigona costata: testing post-glacial colonization hypotheses.
630   *Hydrobiologia* 810:191–206.
631 Hewitt TL, Haponski AE, Foighil DÓ. 2021. Evolution of diverse host infection mechanisms
632   delineates an adaptive radiation of lampsiline freshwater mussels centered on their larval
633   ecology. *PeerJ* 9:e12287.
634 Hewitt TL, Wood CL, Ó Foighil D. 2019. Ecological correlates and phylogenetic signal of host
635   use in North American unionid mussels. *International Journal for Parasitology* 49:71–
636   81. DOI: 10.1016/j.ijpara.2018.09.006.
637 Jamie GA. 2017. Signals, cues and the nature of mimicry. *Proceedings of the Royal Society B:*
638   *Biological Sciences* 284:20162080. DOI: 10.1098/rspb.2016.2080.
639 Jansen W, Bauer G, Zahner-Meike E. 2001. Glochidial Mortality in Freshwater Mussels. In:
640   Bauer G, Wächtler K eds. *Ecology and Evolution of the Freshwater Mussels Unionoida*.
641   Berlin, Heidelberg: Springer Berlin Heidelberg, 185–211. DOI: 10.1007/978-3-642-
642   56869-5_11.
643 Jay P, Whibley A, Frézal L, Rodríguez de Cara MÁ, Nowell RW, Mallet J, Dasmahapatra KK,
644   Joron M. 2018. Supergene Evolution Triggered by the Introgression of a Chromosomal
645   Inversion. *Current Biology* 28:1839-1845.e3. DOI: 10.1016/j.cub.2018.04.072.
646 Jordan CE. 1998. Scale effects in the kinematics and dynamics of swimming leeches. *Canadian*
647   *Journal of Zoology* 76:1869–1877. DOI: 10.1139/z98-131.
648 Joron M, Mallet JLB. 1998. Diversity in mimicry: paradox or paradigm? *Trends in Ecology &*
649   *Evolution* 13:461–466. DOI: 10.1016/S0169-5347(98)01483-9.
650 Klemm DJ. 1982. *Leeches (Annelida: Hirudinea) of North America*. Cincinnati, Ohio: U. S.
651   Environmental Protection Agency.
652 Kramer L. 1970. The mantle flap in three species of Lampsilis. *Malacologia* 10:225–282.
653 Kronforst MR, Papa R. 2015. The functional basis of wing patterning in Heliconius butterflies:
654   the molecules behind mimicry. *Genetics* 200:1–19.
655 Kunte K. 2009. The diversity and evolution of Batesian mimicry in Papilio swallowtail
656   butterflies. *Evolution: International Journal of Organic Evolution* 63:2707–2716.
657 Kuznetsova A, Brockhoff PB, Christensen RHB. 2017. **lmerTest** Package: Tests in Linear
658   Mixed Effects Models. *Journal of Statistical Software* 82. DOI: 10.18637/jss.v082.i13.
659 Lanave C, Preparata G, Sacone C, Serio G. 1984. A new method for calculating evolutionary
660   substitution rates. *Journal of Molecular Evolution* 20:86–93. DOI: 10.1007/BF02101990.

661 Leonard JA, Cope WG, Barnhart MC, Bringolf RB. 2014a. Metabolomic, behavioral, and
662   reproductive effects of the aromatase inhibitor fadrozole hydrochloride on the unionid
663   mussel Lampsilis fasciola. *General and Comparative Endocrinology* 206:213–226.
664 Leonard JA, Cope WG, Barnhart MC, Bringolf RB. 2014b. Metabolomic, behavioral, and
665   reproductive effects of the synthetic estrogen 17 α-ethinylestradiol on the unionid mussel
666   Lampsilis fasciola. *Aquatic Toxicology* 150:103–116. DOI:
667   10.1016/j.aquatox.2014.03.004.
668 Liao JC. 2007. A review of fish swimming mechanics and behaviour in altered flows.
669   *Philosophical Transactions of the Royal Society B: Biological Sciences* 362:1973–1993.
670   DOI: 10.1098/rstb.2007.2082.
671 Lopes-Lima M, Burlakova LE, Karatayev AY, Mehler K, Seddon M, Sousa R. 2018.
672   Conservation of freshwater bivalves at the global scale: diversity, threats and research
673   needs. *Hydrobiologia* 810:1–14. DOI: 10.1007/s10750-017-3486-7.
674 Maran T. 2015. Scaffolding and Mimicry: A Semiotic View of the Evolutionary Dynamics of
675   Mimicry Systems. *Biosemiotics* 8:211–222. DOI: 10.1007/s12304-014-9223-y.
676 Marder E, Bucher D, Schulz DJ, Taylor AL. 2005. Invertebrate Central Pattern Generation
677   Moves along. *Current Biology* 15:R685–R699. DOI: 10.1016/j.cub.2005.08.022.
678 McNichols KA. 2007. Implementing recovery strategies for mussel species at risk in Ontario.
679 McNichols KA, Mackie GL, Ackerman JD. 2011. Host fish quality may explain the status of
680   endangered Epioblasma torulosa rangiana and Lampsilis fasciola (Bivalvia: Unionidae)
681   in Canada. *Journal of the North American Benthological Society* 30:60–70.
682 Mérot C, Llaurens V, Normandeau E, Bernatchez L, Wellenreuther M. 2020. Balancing selection
683   via life-history trade-offs maintains an inversion polymorphism in a seaweed fly. *Nature*
684   *communications* 11:1–11.
685 Morris TJ, McGoldrick DJ, Metcalfe-Smith JL, Zanatta DT, Gillis P. 2009. Pre-COSEWIC
686   assessment of the federally endangered Wavyrayed Lampmussel (Lampsilis fasciola).
687   *DFO Canadian Science Advisory Secretariat*.
688 Munro R, Siddall M, Desser SS, Sawyer RT. 1992. The leech as a tool for studying comparative
689   haematology. *Comparative Haematology International* 2:75–78.
690 Murphy GW, Newcomb TJ, Orth DJ, Reeser SJ. 2005. Food habits of selected fish species in the
691   Shenandoah River Basin, Virginia. *Proceedings of the Annual Conference of the*
692   *Southeastern Association of FIsh and Wildlife Agencies* 59:325–335.
693 Nijhout HF. 2003. Polymorphic mimicry in Papilio dardanus: mosaic dominance, big effects, and
694   origins. *Evolution & development* 5:579–592.
695 Ortmann AE. 1911. *A monograp of the naiades of Pennsylvania*.
696 Pagel M. 1999. Inferring the historical patterns of biological evolution. *Nature* 401:877–884.
697 Palmer DH, Kronforst MR. 2020. A shared genetic basis of mimicry across swallowtail
698   butterflies points to ancestral co-option of doublesex. *Nature Communications* 11:6. DOI:
699   10.1038/s41467-019-13859-y.
700 Parmalee PW, Bogan AE. 1998. *Freshwater mussels of Tennessee*. University of Tennessee
701   Press.
702 Pasteur G. 1982. A classificatory review of mimicry systems. *Annual Review of Ecology and*
703   *Systematics* 13:169–199.
704 Patterson MA, Mair RA, Eckert NL, Gatenby CM, Brady T, Jones JW, Simmons BR, Devers JL.
705   2018. *Freshwater mussel propagation for restoration*. Cambridge University Press.

706 Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE. 2012. Double Digest RADseq: An
707   Inexpensive Method for De Novo SNP Discovery and Genotyping in Model and Non-
708   Model Species. *PLoS ONE* 7:e37135. DOI: 10.1371/journal.pone.0037135.
709 Prout T. 2000. How well does opposing selection maintain variation? In: *Evolutionary Genetics*
710   *from Molecules to Morphology*. Cambridge: Cambridge University Press, 157–181.
711 R Core Team. 2018. R: A Language and Environment for Statistical Computing.
712 Robertson MS, Winemiller KO. 2001. Diet and growth of smallmouth bass in the Devils River,
713   Texas. *The Southwestern Naturalist* 46:216–221.
714 Satterthwaite FE. 1946. An Approximate Distribution of Estimates of Variance Components.
715   *Biometrics Bulletin* 2:110. DOI: 10.2307/3002019.
716 Schaefer HM, Ruxton GD. 2009. Deception in plants: mimicry or perceptual exploitation?
717   *Trends in Ecology & Evolution* 24:676–685. DOI: 10.1016/j.tree.2009.06.006.
718 Shine R, Brown GP, Goiran C. 2022. Frequency-dependent Batesian mimicry maintains colour
719   polymorphism in a sea snake population. *Scientific Reports* 12:4680. DOI:
720   10.1038/s41598-022-08639-6.
721 Smith GR, Taylor JN, Grimshaw TW. 1981. Ecological survey of fishes in the Raisin River
722   drainage, Michigan. *Michigan Academician* 13:275–305.
723 Smits AJ. 2019. Undulatory and oscillatory swimming. *Journal of Fluid Mechanics* 874:P1.
724   DOI: 10.1017/jfm.2019.284.
725 Soltis DE, Morris AB, McLachlan JS, Manos PS, Soltis PS. 2006. Comparative phylogeography
726   of unglaciated eastern North America. *Molecular ecology* 15:4261–4293.
727 Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of
728   large phylogenies. *Bioinformatics (Oxford, England)* 30:1312–1313. DOI:
729   10.1093/bioinformatics/btu033.
730 Stein BA, Morse LE. 2000. A Remarkable Array: Species Diversity in the United States. In:
731   *Precious Heritage*. Oxford University Press,. DOI:
732   10.1093/oso/9780195125191.003.0009.
733 Surber EW. 1941. A quantitative study of the food of the smallmouth black bass, *Micropterus*
734   dolomieu, in three Eastern streams. *Transactions of the American Fisheries Society*
735   70:311–334.
736 Timmermans MJTN, Srivathsan A, Collins S, Meier R, Vogler AP. 2020. Mimicry
737   diversification in *Papilio dardanus* via a genomic inversion in the regulatory region of
738   *engrailed – invected*. *Proceedings of the Royal Society B: Biological Sciences*
739   287:20200443. DOI: 10.1098/rspb.2020.0443.
740 VanTassel NM, Morris TJ, Wilson CG, Zanatta DT. 2021. Genetic diversity maintained in
741   comparison of captive-propagated and wild populations of *Lampsilis fasciola* and
742   *Ptychobranchus fasciolaris* (Bivalvia: Unionidae) [1]. *Canadian Journal of Fisheries and*
743   *Aquatic Sciences* 78:1312–1320. DOI: 10.1139/cjfas-2020-0373.
744 Wacker S, Larsen BM, Jakobsen P, Karlsson S. 2018. High levels of multiple paternity in a
745   spermcast mating freshwater mussel. *Ecology and evolution* 8:8126–8134.
746 Warren ML, Burr BM. 1994. Status of Freshwater Fishes of the United States: Overview of an
747   Imperiled Fauna. *Fisheries* 19:6–18. DOI: 10.1577/1548-
748   8446(1994)019<0006:SOFFOT>2.0.CO;2.
749 Welsh JH. 1933. Photic stimulation and rhythmical contractions of the mantle flaps of a
750   lamellibranch. *Proceedings of the National Academy of Sciences* 19:755–757.

751 Winn HE. 1958. Comparative reproductive behavior and ecology of fourteen species of darters
752   (Pisces-Percidae). *Ecological Monographs* 28:155–191.
753 Witt WC, Wen L, Lauder GV. 2015. Hydrodynamics of C-Start Escape Responses of Fish as
754   Studied with Simple Physical Models. *Integrative and Comparative Biology* 55:728–739.
755   DOI: 10.1093/icb/icv016.
756 Zale AV, Neves RJ. 1982. Fish hosts of four species of lampsiline mussels (Mollusca:
757   Unionidae) in Big Moccasin Creek, Virginia. *Canadian Journal of Zoology* 60:2535–
758   2542.
759 Zanatta DT, Fraley SJ, Murphy RW. 2007. Population structure and mantle display
760   polymorphisms in the wavy-rayed lampmussel, Lampsilis fasciola (Bivalvia: Unionidae).
761   *Canadian Journal of Zoology* 85:1169–1181. DOI: 10.1139/Z07-089.
762 Zanatta DT, Harris AT. 2013. Phylogeography and genetic variability of the freshwater mussels
763   (Bivalvia: Unionidae) Ellipse, Venustaconcha ellipsiformis (Conrad 1836), and Bleeding
764   Tooth, V. pleasii (Marsh 1891). *American Malacological Bulletin* 31:267–279.
765 Zanatta DT, Murphy RW. 2006. Evolution of active host-attraction strategies in the freshwater
766   mussel tribe Lampsilini (Bivalvia: Unionidae). *Molecular Phylogenetics and Evolution*
767   41:195–208. DOI: 10.1016/j.ympev.2006.05.030.
768 Zeyl JN, Malavasi S, Holt DE, Noel P, Lugli M, Johnston CE. 2016. Convergent Aspects of
769   Acoustic Communication in Darters, Sculpins, and Gobies. In: Sisneros JA ed. *Fish
770   Hearing and Bioacoustics: An Anthology in Honor of Arthur N. Popper and Richard R.
771   Fay*. Cham: Springer International Publishing, 93–120. DOI: 10.1007/978-3-319-21059-
772   9_6.
773

Figure 1

Illustration of Lampsilis fasciola life cycle

a) Summary diagram representing the life cycle of the freshwater mussel *Lampsilis fasciola.*
The gravid female mussel displays a mantle lure, here a darter mimic, to the primary fish
host, *Micropterus dolomieu*. This elicits an attack through which the host is infected by
mussel parasitic larvae (glochidia)*.* After a short infective period (~2 weeks), the parasitic
larvae metamorphose into juvenile mussels that detach from the host and fall to the
sediment. Panels b ("darter-like") and c ("worm-like") depict the two primary phenotypes of
lure observed in *L. fasciola*. The former (b) has "eyespots", a mottled "main body"
pigmentation composed of lateral and dorsal spots that can vary substantially in color,
numerous and prominent marginal extensions, and a distinct "tail" region, whereas the latter
lacks those features and has instead a uniform bright orange coloration underlain with a
black basal stripe. Illustration by John Megahan.

a)

d) glochidia attach and
encyst on host fish

Lampsilis fasciola
Life Cycle

c) host fish attacks lure, extracting
brooded glochidia,

e) metamorphosed juvenile
mussels detach from host

a) spermatozeugmata

♀   ♂

f) benthic juvenile mussels

b) fertilization, brooding,
and mantle lure display

b)

c)
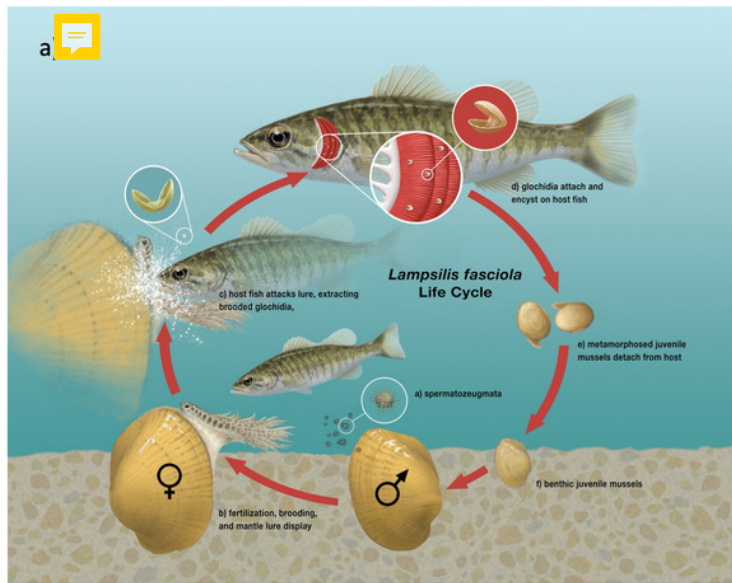
Figure 2

Map displaying geographic range of Lampsilis fasciola and its primary host, Micropterus dolomieu, as well as sampling locations

Map of Eastern North America showing the sampling sites of *Lampsilis fasciola* and the estimated ranges of this freshwater mussel species and of its primary host fish *Micropterus dolomieu*. A indicates the Raisin River sampling site at Sharon Mills County Park and B the Huron River sampling site at Hudson Mills Park, both in southeastern Michigan. Site C indicates the Paint Rock River sampling site in Alabama, and site D is the Little Tennessee River site in North Carolina.
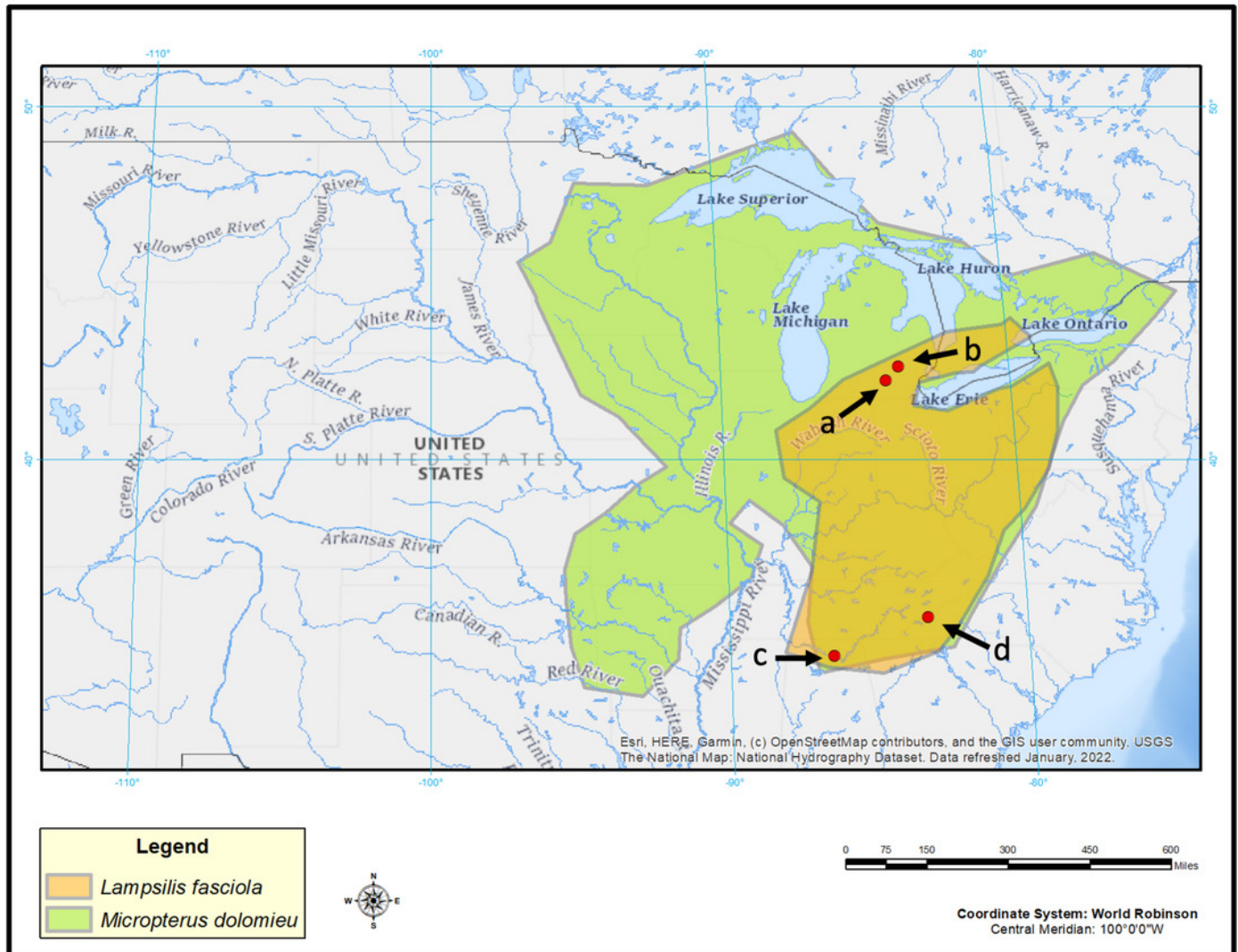
Figure 3

Photographs of Lampsilis fasciola brood raised at the Alabama Aquatic Biodiversity Center, as well as photographs of preserved lure rudiments.

The *Lampsilis fasciola* brood raised at the Alabama Aquatic Biodiversity Center from a wild, gravid female, with a "worm-like" mantle lure (Supplementary Figure 3), sampled from the Paint Rock River (Figure 2c) in June 2009. Panel a) shows juvenile members of the brood after ~16 months in culture. Panels b) and c) show single, sexually maturing, females after ~2 years of culture. The young female in b) displayed a developing "darter-like" mantle lure (with "eyespots", mottled lateral coloration, marginal extensions, and a "tail") whereas her full- or half-sibling in c) displayed a "worm-like" mantle lure (lacking the "darter" characteristics and having orange pigmentation with a black underlay). Panels d) and e) respectively show photomicrographs, taken with a dissecting microscope, of 95% ethanol-preserved rudimentary "darter-like" and "worm-like" lures from two additional brood members, part of a 50-individual subsample preserved in 2011.
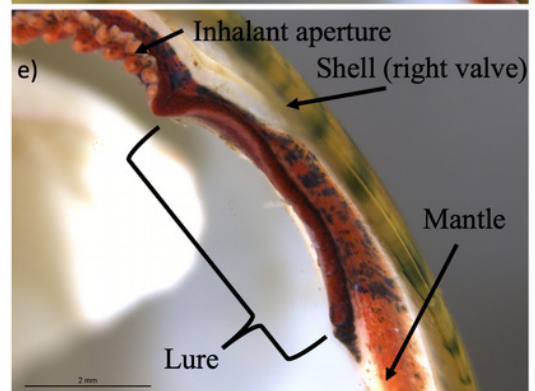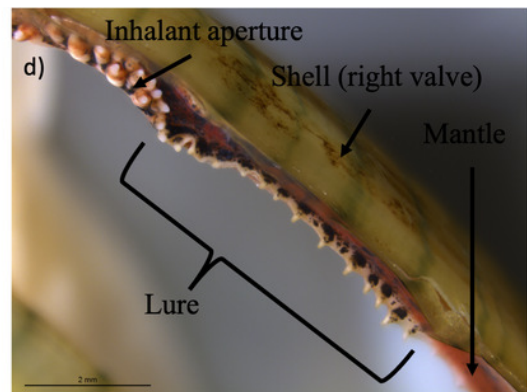
Figure 4

Phylogenomic tree displaying Lampsilis fasciola from two MI populations, one NC population, and the Paint Rock River brood raised at the Alabama Aquatic Biodiversity Center.

Phylogenomic tree of 96 *Lampsilis fasciola* individuals created in RAxML using 28,735 concatenated ddRAD-seq loci with 85% clustering threshold and 50% loci shared across samples. Gravid, lure-displaying females sampled from two Michigan drainages, River Raisin (Fig. 2a) and Huron River (Fig. 2b), are respectively highlighted in peach and pink. Specimens sampled from the Paint Rock River, Alabama (Fig. 2c) are highlighted in blue and consisted of three gravid, lure-displaying females, in addition to 50 larval brood members raised at the Alabama Aquatic Biodiversity Center in the zoomed-in tip clade. Gravid, lure-displaying females sampled from the Little Tennessee River in North Carolina (Fig. 2d) are highlighted in yellow. The respective primary mantle lure phenotypes – "darter-like" or "worm-like" of all *L. fasciola* individuals is indicated by gray and yellow boxes, respectively
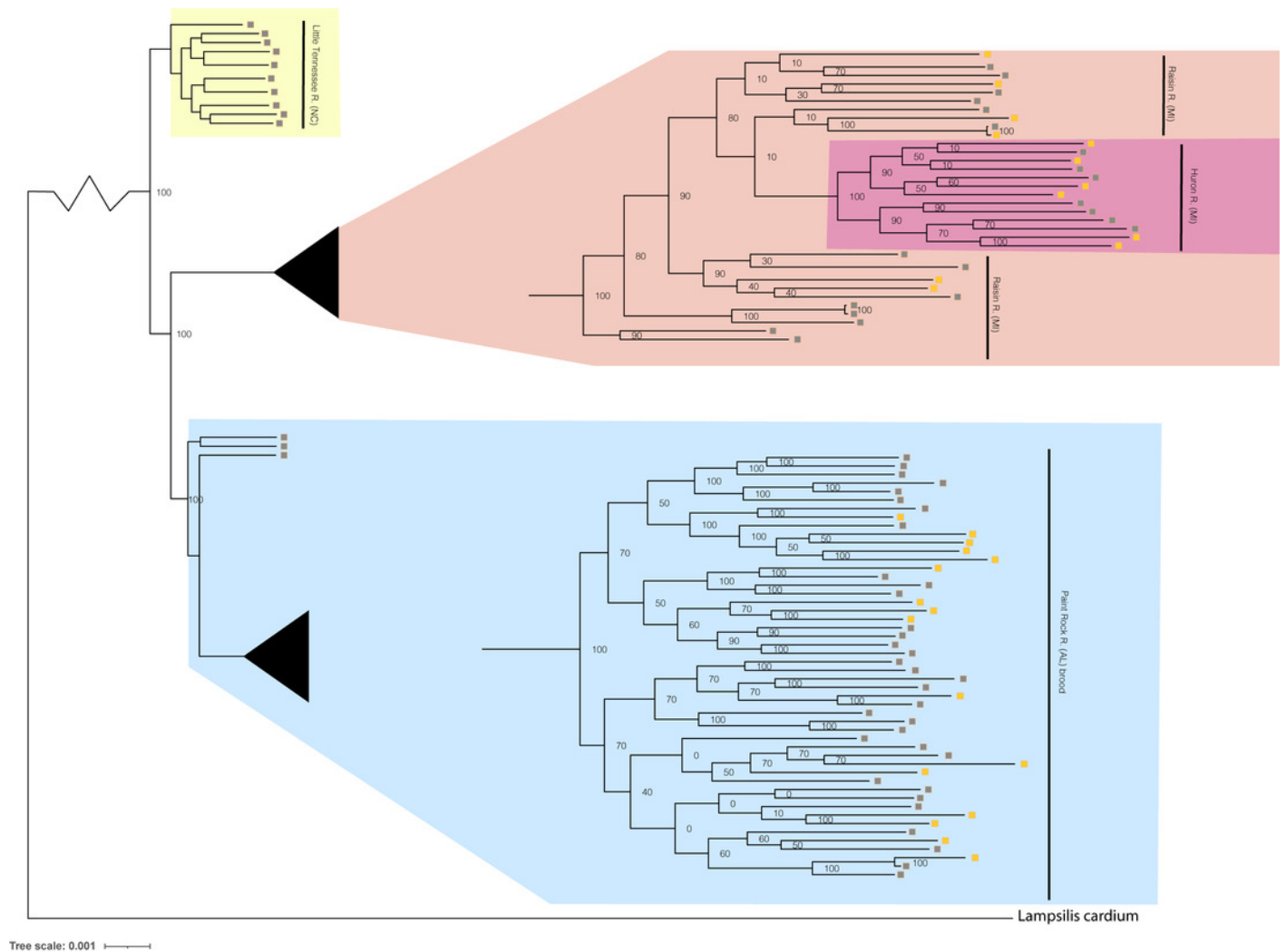
Figure 5

The ratio of "worm-like" and "darter-like" Lampsilis fasciola lures over time in the River Raisin, MI, using historical and contemporary samples.

Figure 5: a) The observed frequency of River Raisin *Lampsilis fasciola* primary mantle lure phenotypes ("darter-like" vs "worm-like") at the Sharon Mills County Park study site during two different time periods. The 1954-1962 data were obtained from the University of Michigan Museum of Zoology (UMMZ) collection specimens, both female and male. The 2017 data were based on field observations of displaying females. b) a jar of preserved UMMZ Sharon Mills specimens showing a "darter-like" and a "worm-like" mantle lure. c) a "eyespot", lateral pigmented blotches, and marginal extensions.
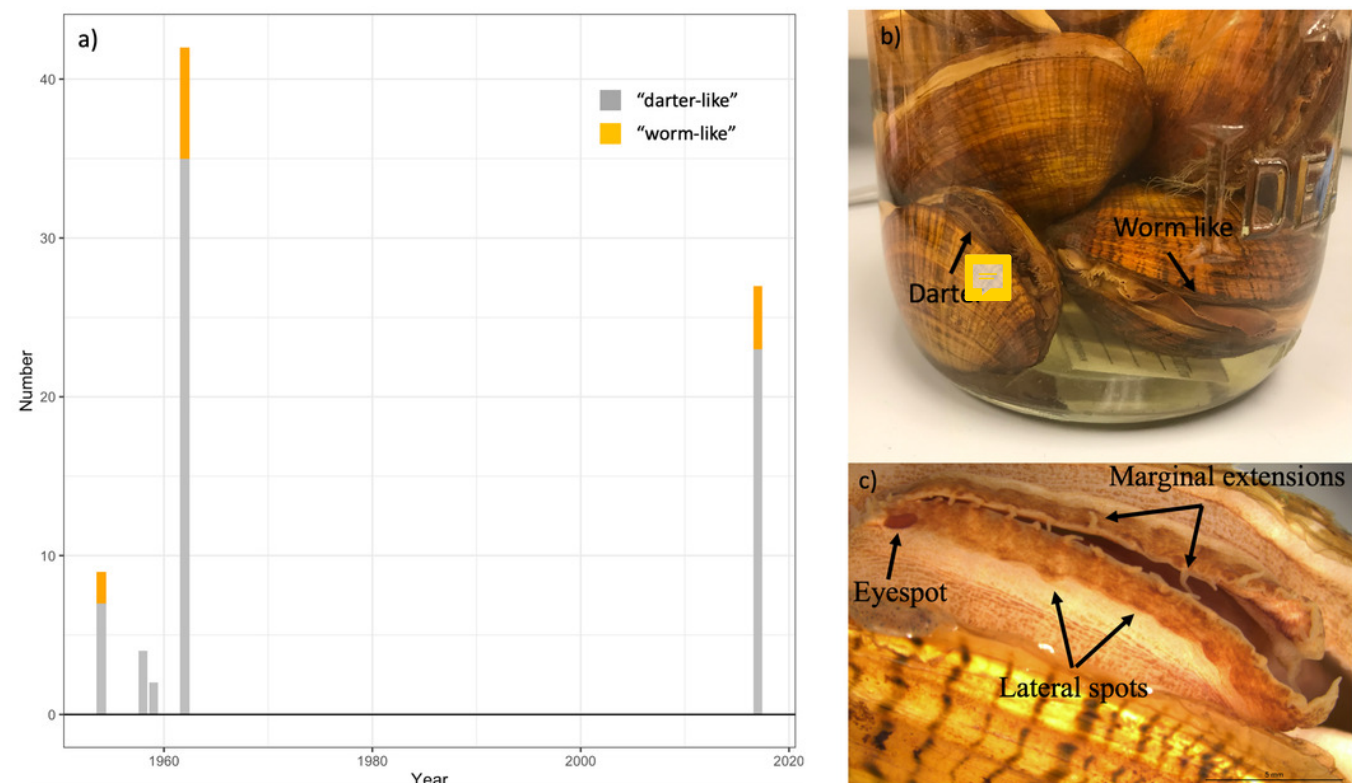
Figure 6

Panel displaying variability in the two primary lure phenotypes of Lampsilis fasciola

This panel displays some of the variability in lure phenotype, both within a population and across the range of *Lampsilis fasciola*. a-d are "darter-like" Raisin River (MI) lures photographed in the field at Sharon Mills County Park. e) depicts a "darter-like" lure displayed by a Paint Rock River (AL) female. f-h show field photographs of "worm-like" lures displayed by three Sharon Mills females, with specimen h being a younger adult. i,j are photographs of two captive AABC specimens, with "worm-like" lures, sourced from the Paint Rock River. The former photo (i), taken in 2011, shows a young (2-year old) female, a member of the captive brood, displaying her lure, and the latter photo (j) is of a female field-sampled in 2022, and showing a partially retracted mantle lure.
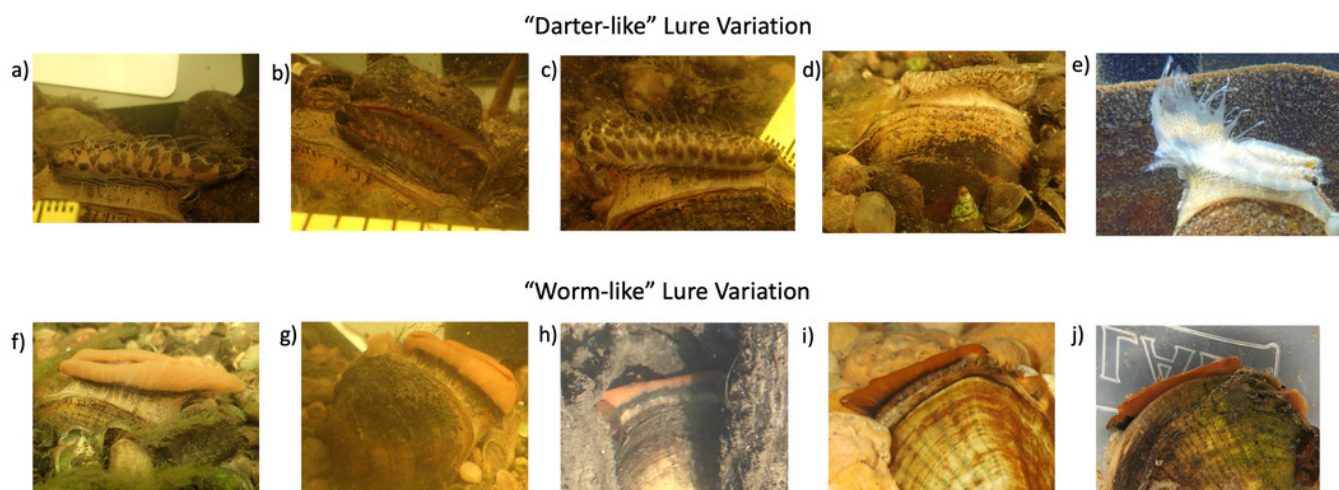
Figure 7

Illustration of hypothetical benthic assemblage of main Lampsilis fasciola lure groups, and proposed models.

A hypothetical Raisin River (Michigan) benthic assemblage showing displaying exemplars of the putative 5 main *Lampsilis fasciola* mimetic mantle lure groups (Figure 6a-d, f) present at the Sharon Mills County Park study site, together with their respective model species, and their primary receiver/fish host, *Micropterus dolomieu*. Also shown is a displaying *Lampsilis cardium* with a "small minnow" mimetic mantle lure (Patterson et al., 2018) and its putative model, *Pimephales notatus*, the most common fish species in the River Raisin (Smith, Taylor & Grimshaw, 1981). Illustration by John Megahan.

Figure 8

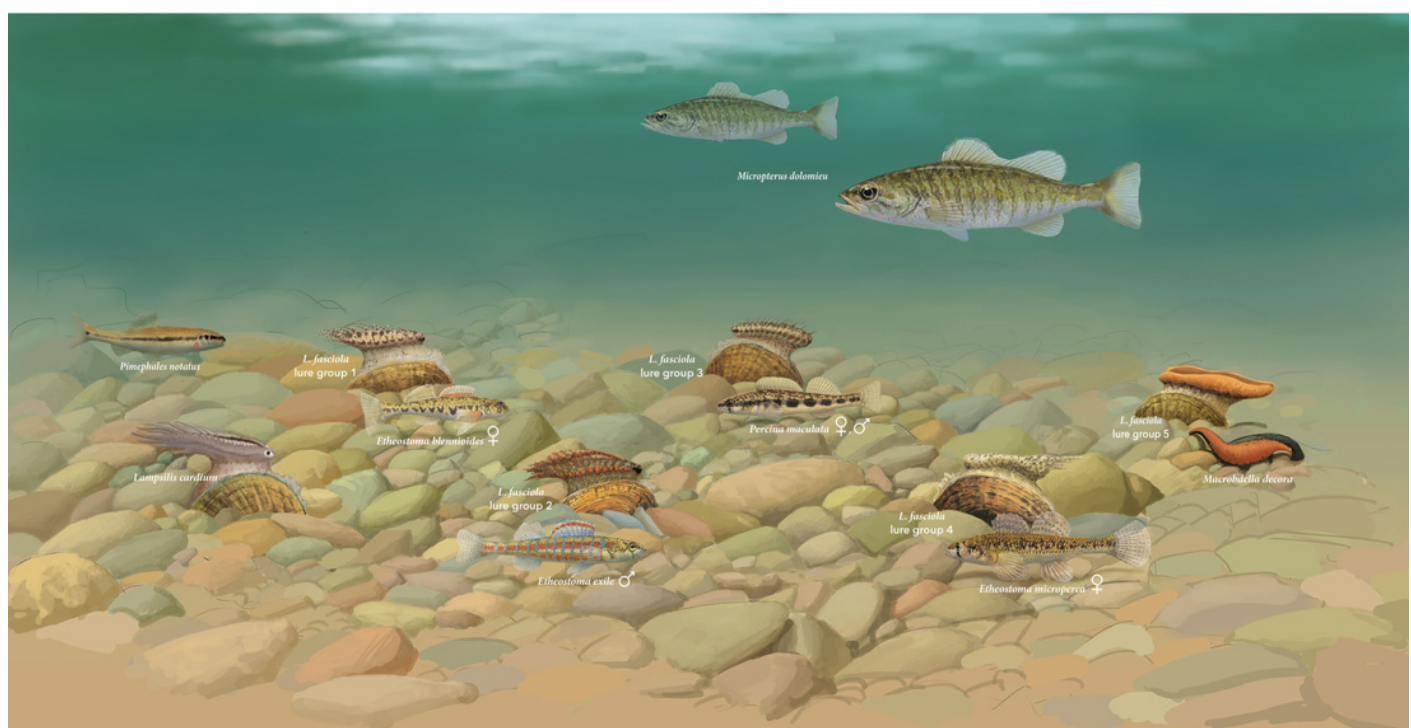Estimated range maps for proposed models of Lampsilis fasciola lures.

Estimated range maps for 5 proposed models for *Lampsilis fasciola* lures. Range maps for each proposed model is compared to the estimated geographic range of *Lampsilis fasciola*. a) *Etheostoma blennoides*, b) *Etheostoma exile*, c) *Percina maculata*, d) *Etheostoma microperca*, and e) *Macrobdella decora*. Model Illustrations by John Megahan.
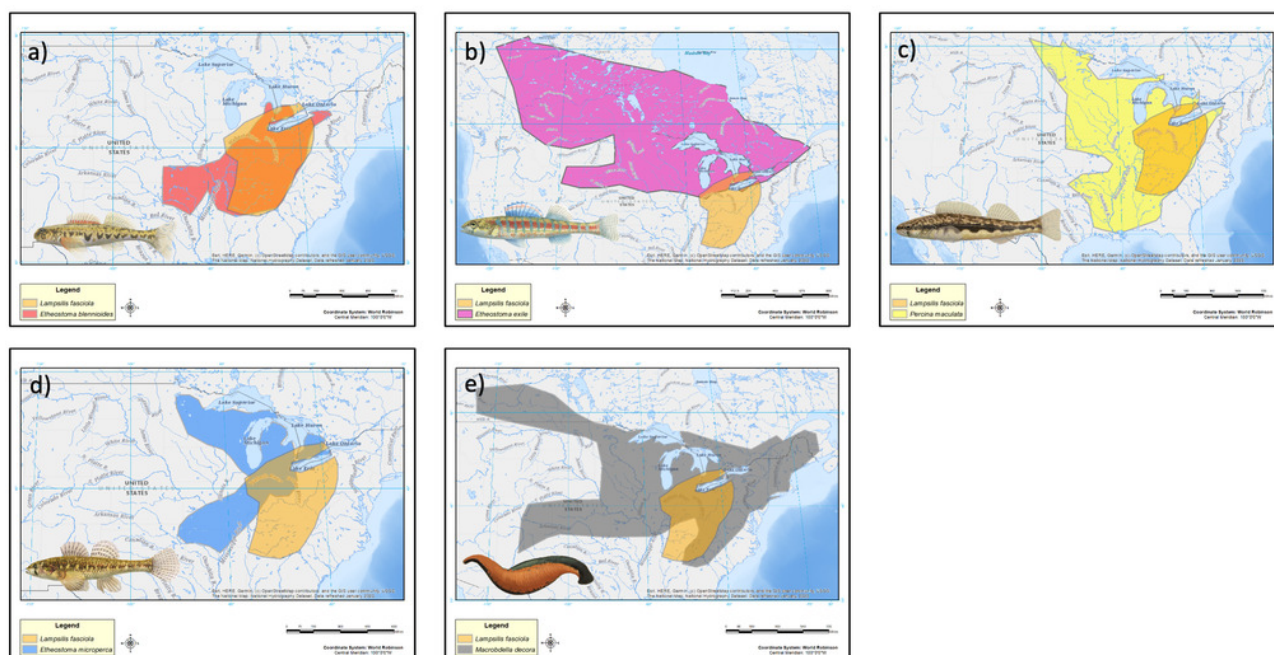
Figure 9

Gait diagrams for three exemplar mussel displays; a "darter-like" L. fasciola, a "leech-like" L. fasciola, and a Lampsilis cardium.

Mantle lure gait diagrams for three representative individuals that were field recorded using a Go Pro Hero 6 in 2018. Panel a) shows a *Lampsilis fasciola* "darter" lure sample, Panel b) displays *Lampsilis fasciola* "leech" lure sample, and Panel c) shows a *Lampsilis cardium* sample. Red center lines indicate synchronized lure movement for both left and right mantle flaps, and black lines above and below the center line indicate independent left and right movements respectively. The x-axis denotes frame number (120fps).
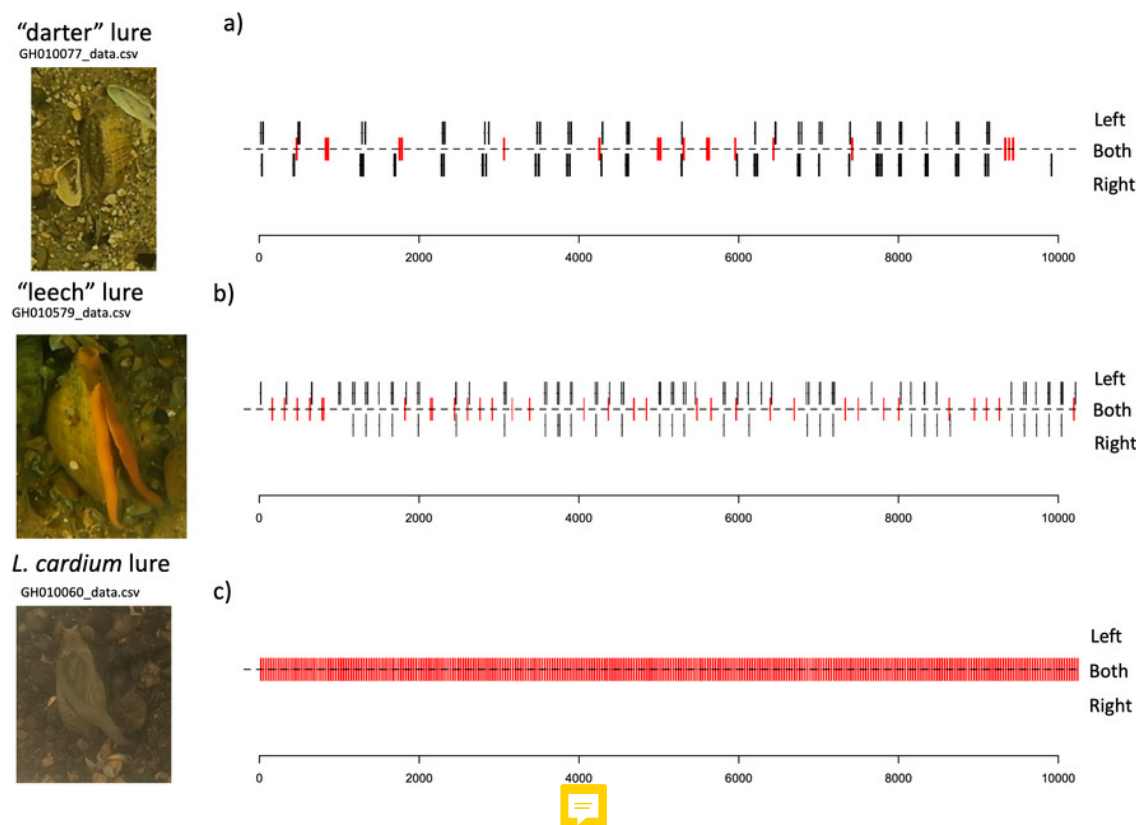
Figure 10

Summary plots for behavioral analysis of the two primary Lampsilis fasciola lure phenotypes and Lampsilis cardium.

Summary boxplots from behavioral analyses of the two primary *Lampsilis fasciola* mantle lure phenotypes ("darter" vs. "leech") and of *Lampsilis cardium*. Panel a) comparison of the mean interval between lure movements. Panel b) the mean standard deviation in lure movement interval as a proxy of variability. Panel c) the mean duration of lure movement. Panel d) the proportion of movements that are left-right synchronized. Panels a-c show means for left mantle flap movements only.

Table 1(on next page)

Table 1 displaying the name, phenotype, and sequencing metrics.

Raw reads, total clusters, and total loci in assembly from the ddRAD sequencing are displayed for each genotyped sample of *Lampsilis fasciola* and of the outgroup taxa. Individual *Lampsilis fasciola* lure phenotype designation followed Zanatta et al. (2007). Museum ID numbers can be found in Supplementary Table 1.

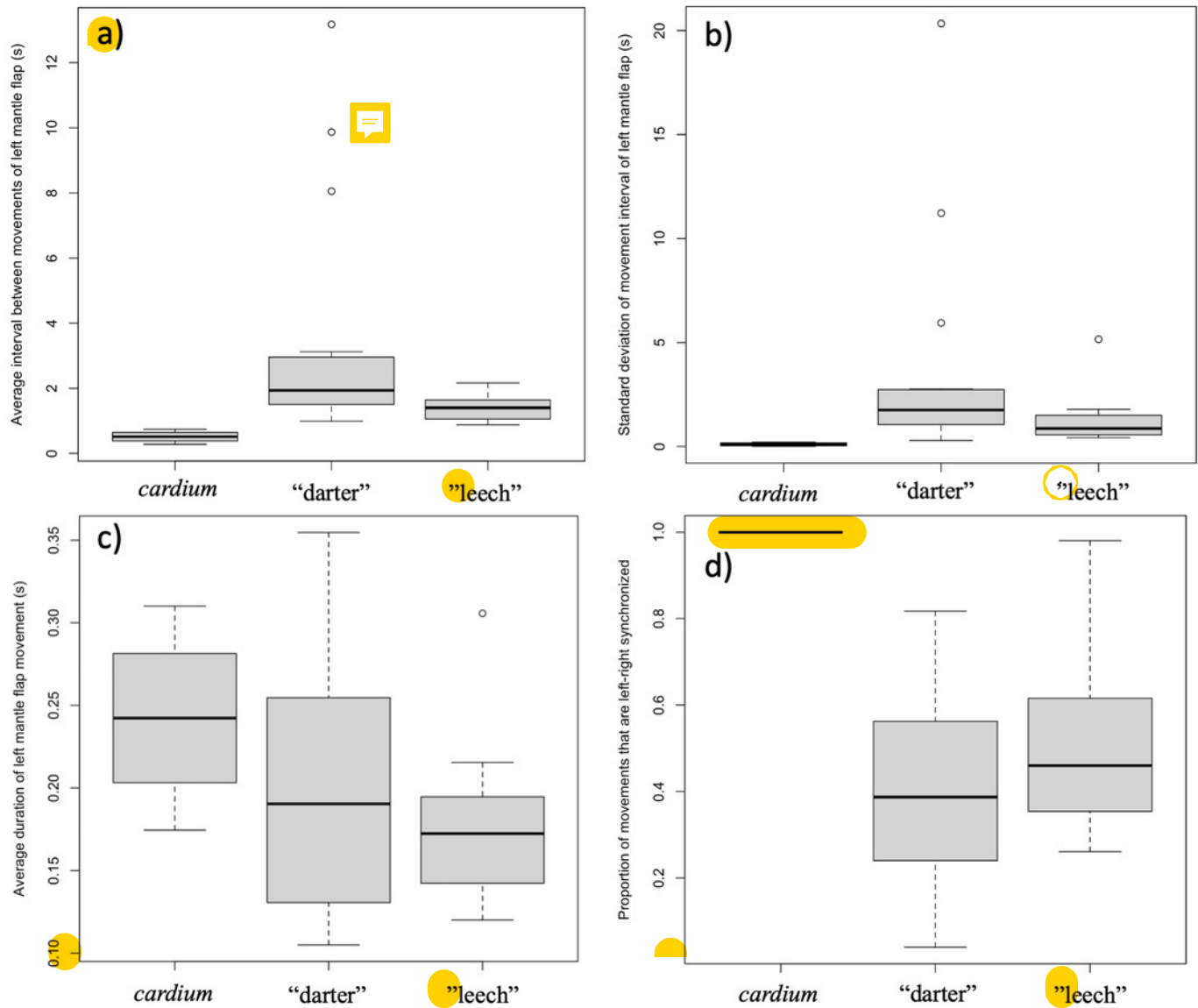| Sample Name	Lure Phenotype	Raw reads	Total clusters	Average clustering depth	Loci in assembly
L_fasciola_AL_brood_1	Worm-like	258664	97681	2.14	483
L_fasciola_AL_brood_2	Darter-like	5201836	1120710	3.28	25686
L_fasciola_AL_brood_3	Worm-like	5492519	1126749	3.4	25703
L_fasciola_AL_brood_4	Darter-like	2429494	632254	2.84	21398
L_fasciola_AL_brood_5	Worm-like	3152003	760260	3.02	23761
L_fasciola_AL_brood_6	Darter-like	3212851	810898	2.87	23434
L_fasciola_AL_brood_7	Darter-like	3649891	593765	4.22	25363
L_fasciola_AL_brood_8	Darter-like	4869307	1462723	2.29	19089
L_fasciola_AL_brood_9	Worm-like	3158818	718169	3.08	23033
L_fasciola_AL_brood_10	Darter-like	4000321	915881	3.12	24916
L_fasciola_AL_brood_11	Worm-like	5679854	1171842	3.35	25770
L_fasciola_AL_brood_12	Darter-like	4212783	979265	3.04	24693
L_fasciola_AL_brood_13	Worm-like	1300563	399134	2.51	12145
L_fasciola_AL_brood_14	Darter-like	4100372	1043360	2.79	23521
L_fasciola_AL_brood_15	Darter-like	5804293	1412102	2.91	25570
L_fasciola_AL_brood_16	Worm-like	1555906	427061	2.7	14099
L_fasciola_AL_brood_17	Darter-like	2073968	598680	2.59	13668
L_fasciola_AL_brood_18	Worm-like	6919783	1574429	3.08	25811
L_fasciola_AL_brood_19	Darter-like	3434210	829507	2.94	23708
L_fasciola_AL_brood_20	Darter-like	4778853	994416	3.35	25500
L_fasciola_AL_brood_21	Worm-like	2462560	590095	2.91	20588
L_fasciola_AL_brood_22	Worm-like	6600876	1406451	3.26	26080
L_fasciola_AL_brood_23	Darter-like	7090859	1628965	3.06	25932
L_fasciola_AL_brood_24	Worm-like	4546435	1061394	3	24174
L_fasciola_AL_brood_25	Worm-like	5379577	1135906	3.35	25703
L_fasciola_AL_brood_26	Worm-like	5592652	1501130	2.67	23965
L_fasciola_AL_brood_27	Worm-like	4893957	825855	4.09	25924
L_fasciola_AL_brood_28	Darter-like	2596873	519103	3.59	22103
L_fasciola_AL_brood_29	Darter-like	3401334	883485	2.87	21377
L_fasciola_AL_brood_30	Worm-like	3876395	1014133	2.8	22072
L_fasciola_AL_brood_31	Worm-like	5391442	1246528	3.07	25009
L_fasciola_AL_brood_32	Darter-like	4365005	1084596	2.85	23030
L_fasciola_AL_brood_33	Darter-like	5116507	1117916	3.16	24667
L_fasciola_AL_brood_34	Darter-like	7480755	1601100	3.19	26163
L_fasciola_AL_brood_35	Darter-like	8121426	1825135	3.02	25972
L_fasciola_AL_brood_36	Darter-like	5521997	1414238	2.78	24163
L_fasciola_AL_brood_37	Darter-like	6562641	1579514	2.88	25476

L_fasciola_AL_brood_38	Darter-like	6303766	1596624	2.76	24448
L_fasciola_AL_brood_39	Darter-like	6206795	1488925	2.91	24648
L_fasciola_AL_brood_40	Darter-like	8630897	1891164	3.11	26176
L_fasciola_AL_brood_41	Darter-like	7293683	1716571	2.95	25604
L_fasciola_AL_brood_42	Darter-like	4896252	1193262	2.88	22829
L_fasciola_AL_brood_43	Darter-like	6098052	1471714	2.9	25074
L_fasciola_AL_brood_44	Darter-like	7495994	1698871	3.04	25701
L_fasciola_AL_brood_45	Darter-like	3937758	670698	4.06	24947
L_fasciola_AL_brood_46	Darter-like	6370942	1343655	3.26	25855
L_fasciola_AL_brood_47	Darter-like	5542864	1318463	2.96	24550
L_fasciola_AL_brood_48	Darter-like	6313913	1469606	2.98	24983
L_fasciola_AL_brood_49	Darter-like	3163000	789239	2.9	24776
L_fasciola_AL_brood_50	Darter-like	1728370	548529	2.35	17837
L_fasciola_Huron_5	Darter-like	953302	259898	2.8	10996
L_fasciola_Huron_6	Worm-like	1682931	362706	3.31	16809
L_fasciola_Huron_7	Worm-like	746944	157212	3.29	10644
L_fasciola_Huron_8	Worm-like	1899689	402515	3.25	16584
L_fasciola_Huron_9	Darter-like	1213655	293090	2.97	11818
L_fasciola_Huron_10	Darter-like	7775910	1275602	3.87	22035
L_fasciola_Huron_11	Darter-like	1533281	295767	3.55	15386
L_fasciola_NC_1	Darter-like	1308813	254002	3.61	11873
L_fasciola_NC_2	Darter-like	4862573	852380	3.77	18321
L_fasciola_NC_3	Darter-like	663874	165869	2.95	9960
L_fasciola_NC_4	Darter-like	2610453	465228	3.76	13790
L_fasciola_NC_5	Darter-like	6927947	1459334	3.05	20804
L_fasciola_NC_6	Darter-like	1051195	202171	3.27	12415
L_fasciola_NC_7	Darter-like	1948092	382878	3.61	17101
L_fasciola_NC_8	Darter-like	3475751	669278	3.69	20683
L_fasciola_NC_9	Darter-like	5693936	1634946	2.46	22325
L_fasciola_NC_10	Darter-like	2175381	464794	3.38	17094
L_fasciola_NC_11	Darter-like	2189933	516643	3.05	17580
L_fasciola_Redo_1	Darter-like	1455864	327622	2.62	13478
L_fasciola_Redo_2	Darter-like	1839020	436418	2.43	13181
L_fasciola_Raisin_2	Darter-like	8235827	1716137	3.29	25555
L_fasciola_Raisin_3	Darter-like	6032935	1488448	2.85	25006
L_fasciola_Raisin_4	Darter-like	12947164	3587458	2.45	25245
L_fasciola_Raisin_1	Darter-like	6639384	1086218	3.97	23458
L_fasciola_Raisin_5	Darter-like	10059843	1997619	3.41	25363
L_fasciola_Raisin_6	Darter-like	8019689	1847955	3.01	25769
L_fasciola_Raisin_7	Darter-like	3816242	681697	3.95	24606

L_fasciola_Raisin_8	Darter-like	6117037	1282299	3.27	22439
L_fasciola_Raisin_9	Worm-like	5170380	775979	4.64	25798
L_fasciola_Raisin_10	Darter-like	761451	176858	3.14	11477
L_fasciola_Raisin_11	Worm-like	7140657	1670143	2.97	25519
L_fasciola_Raisin_12	Darter-like	890521	203114	2.91	10582
L_fasciola_Raisin_13	Darter-like	1071361	225030	3.47	13512
L_fasciola_Raisin_14	Darter-like	3644379	946273	2.82	21995
L_fasciola_Raisin_15	Darter-like	3578043	482446	5.04	17514
L_fasciola_Raisin_16	Darter-like	2351544	114072	14.25	516
L_fasciola_Raisin_17	Darter-like	5272816	1304726	2.87	23305
L_fasciola_Huron_1	Worm-like	13366692	4050829	2.26	17555
L_fasciola_Huron_2	Darter-like	2819896	928226	2.24	20205
L_fasciola_Huron_3	Darter-like	662275	186602	2.66	7653
L_fasciola_Huron_4	Darter-like	4792093	855457	3.88	24512
L_fasciola_AL_mom_1	Darter-like	8095030	1840917	2.95	25420
L_fasciola_AL_mom_2	Darter-like	10329331	3504027	2.03	24488
L_fasciola_AL_mom_3	Darter-like	10384477	2987559	2.34	25056
L_fasciola_Huron_12	Worm-like	6906349	1672394	2.87	25281
L_fasciola_Huron_13	Worm-like	6955496	1670627	2.88	25593
L_fasciola_Raisin_18	Worm-like	5506215	1301878	3	25373
L_fasciola_Raisin_19	Worm-like	6611596	1524682	3.03	25604
L_fasciola_Raisin_20	Worm-like	4894495	1276608	2.74	24931
L_fasciola_Raisin_21	Worm-like	8396562	1736736	3.26	25490
L_cardium_1		6864226	1710220	2.8	14625
L_cardium_2		4898330	1091622	3.11	13433
L_cardium_3		7109883	2005565	2.5	14563
L_cardium_4		4637077	997208	3.27	13860
S_nasuta_1		4544989	1169260	2.55	10441

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Table 2(on next page)

Summary table showing the sex and lure phenotype of historical Lampsilis fasciola samples from the River Raisin.

Summary of the sex and lure phenotypes of all 57 University of Michigan Museum of Zoology

*Lampsilis fasciola*individuals present in 8 separate mid-20th century collections made from

the River Raisin at Sharon Mills County Park (Fig. 2a).

Collection Date	Male darter-like	Male worm-like	Female darter-like	Female worm-like	Total darter-like	Total worm-like	Total
9/30/54	2	1	5	1	7	2	9
5/21/58	0	0	4	0	4	0	4
7/28/59	0	0	2	0	2	0	2
4/24/62	3	0	3	0	6	0	6
5/10/62	5	1	4	1	9	2	11
6/19/62	4	2	1	0	5	2	7
6/25/62	3	1	2	0	5	1	6
7/20/62	5	1	5	1	10	2	12

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Table 3(on next page)

Estimated range overlap between Lampsilis fasciola and 5 proposed models

The 5 broad categories of lure phenotypes (Groups a-e) observed at the River Raisin Sharon Mills County Park population of *Lampsilis fasciola* (Fig. 2a), as well as the estimated geographic range overlap between *Lampsilis fasciola* and its 5 Raisin River putative model species.

Type	Proposed Model	Range overlap (km^2)
Group a	*Etheostoma blennioides*	480,731
Group b	*Etheostoma exile*	87,796
Group c	*Percina maculata*	525,772
Group d	*Etheostoma microperca*	164,539
Group e	*Macrobella decora*	419,259

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