

Darter egg viability decreases over time

1 **Egg viability decreases rapidly with time since ovulation in the rainbow darter:**
2 **implications for the costs of choosiness**

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24 **Abstract**

25 Egg viability in the rainbow darter *Etheostoma caeruleum*, a fish apparently lacking female mate
26 choice, was found to decline rapidly after ovulation. We observed that the majority of a female's
27 clutch may fail to hatch if she is prevented from mating for as little as six hours. These data
28 suggest that exercising female mate preferences may be selectively disfavoured in *E. caeruleum*
29 due to the high cost of delaying mating.

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31 Keywords: egg overripening; *Etheostoma caeruleum*; rainbow darter; female mate choice; mate
32 choice cost

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33 The degree of discrimination exhibited by female animals when choosing a mate ranges from
34 none to extreme. This diversity exists because female mate choice is shaped by a number of
35 variables, including the subset of males made available to her by environmental factors and
36 male-male interactions (Beehler & Foster, 1988; Jennions & Petrie, 1997; Wong & Candolin,
37 2005), and the costs of mate choice behaviour (Janetos, 1980; Real, 1990; Kokko *et al.*, 2003). If
38 the costs of choosing outweigh the benefits, the most favoured strategy would be to mate with
39 the first male the female encounters, i.e. random mating. Several costs to female mate choice
40 (e.g. time and energy expenditure, predator exposure) have been investigated across a variety of
41 taxa (reviewed in Reynolds & Gross, 1990; Jennions & Petrie, 1997), but one has thus far
42 received little attention: decline in gametic quality over time. In fishes, the phenomenon of
43 decreasing egg viability post-ovulation, termed egg overripening, has been reported across a
44 range of species including Atlantic salmon *Salmo salar* Linnaeus 1758 (de Gaudemar & Beall,
45 1998), goldfish *Carassius auratus* (Linnaeus 1758) (Formacion *et al.*, 1993), Atlantic halibut
46 *Hippoglossus hippoglossus* (Linnaeus 1758) (Bromage *et al.*, 1994), and turbot *Scophthalmus*
47 *maximus* (Linnaeus 1758) (McEvoy, 1984). Having eggs susceptible to overripening would
48 pressure females to spawn quickly, as waiting could incur a substantial fitness cost. Such
49 selection for rapid mate acquisition may consequently lead to a decrease in female choosiness
50 and/or influence the expression of female mating preferences.

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52 The rainbow darter *Etheostoma caeruleum* Storer 1845 is a small benthic fish common in
53 freshwater streams across the eastern United States (Page, 1983). During the breeding season
54 from late March to early June, brightly coloured males attempt to guard gravid females from
55 rival males. Females signal their readiness to spawn by performing nosedigs, wherein she pushes

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56 her head into the gravel. Spawning involves the female burying herself shallowly in gravel with
57 an arched posture; once a male takes position above her, both vibrate and release eggs and
58 sperm. No parental care is practiced (Winn, 1958; Fuller, 2003).

59

60 Although there is evidence that female *E. caeruleum* favour some types of males in dichotomous
61 trials, such preferences have little apparent effect on the outcome of mating due to male-male
62 competition (Fuller, 2003). In any case, female *E. caeruleum* demonstrate no overt choice when
63 allowed to interact freely with males: having assumed the spawning position, the female always
64 spawns with the first male to arrive regardless of his characteristics (pers obs), suggesting that
65 exercising choice may be disadvantageous. Good reasons exist to suspect that mate choice in
66 female *E. caeruleum* may harbour a cost in prolonging the time between ovulation and
67 spawning: in previous experiments, females have been observed expelling and subsequently
68 eating unfertilized eggs when held in isolation (Zhou & Fuller, 2014). Furthermore, isolated
69 females subsequently allowed to spawn with males often produce entirely inviable clutches (R.
70 Moran, pers obs). This study aimed to formally test the hypothesis that delaying spawning is
71 costly for female *E. caeruleum*, by quantifying change in egg viability as a function of time since
72 ovulation.

73

74 *E. caeruleum* were collected by kick seine from Mill Pond Outlet (Kalamazoo Co., Michigan) in
75 April and May 1998 (year 1) and from an unnamed tributary of the Saline Branch Drainage
76 Ditch (Champaign Co., Illinois) in March 2017 (year 2). Fish from year 1 were maintained at the
77 Kellogg Biological Station. Fish from year 2 were maintained at the University of Illinois at
78 Urbana-Champaign. In both years, fish were housed in male-female pairs in 38 litre aquariums

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79 with gravel substrate, maintained at external ambient temperature and light:dark cycle. The fish
80 were fed frozen bloodworms (chironomid larvae) and live tubifex worms twice per day.
81
82 Fish were monitored during daylight hours over four to five days post-capture. A female was
83 assumed to have recently ovulated when she performed a nosedig. After a female performed a
84 nosedig, she was moved to an empty tank. Females were kept in isolation for various lengths of
85 time: 0 hours (n=10), 6 hours (n=6), 12 hours (n=7), 24 hours (n=5). Following the isolation
86 period, a male was introduced and the fish were allowed to freely spawn. The resulting eggs
87 were collected with a siphon and placed in small tubs filled with water; dilute methylene blue
88 was added to inhibit fungal growth. Hatching success was recorded as the number of eggs
89 yielding fry out of total number of eggs collected. To test for a relationship between hatching
90 success and female isolation time, we used a quasibinomial regression with a logit link function.
91 To determine whether there was an effect of collection year and location on hatching success,
92 year was included as a covariate in the model. The quasibinomial error distribution was used to
93 account for overdispersion in the response variable (i.e., hatching success). Statistical analysis
94 was performed in R (version 3.4.0).
95
96 All females spawned following the introduction of a male. The number of eggs collected ranged
97 from 17 to 110, and was uncorrelated with isolation time or year. Hatching success declined
98 strongly as a function of increasing female isolation time ($F_{1,25} = 5.91$, $p < 0.05$; Fig. 1). There
99 was no effect of year on hatching success ($F_{1,25} = 3.30$, $p = 0.08$). Although hatching success
100 varied at each female holding time (Fig. 1), these data suggest that on average, greater than 50%

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101 of a female's clutch is likely to become non-viable if retained for as little as six hours after
102 ovulation.

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104 Given that hatching success declines precipitously over time, gravid female *E. caeruleum* appear
105 to be under strong pressure to fully spawn a clutch of eggs in less than 24 hours. Female who fail
106 to do so risk substantial egg mortality. The time constraint for spawning may be exacerbated by
107 the fact that female *E. caeruleum* release only a small fraction of ovulated eggs per spawning
108 bout and thus must spawn multiple times to fully expel an entire clutch (Fuller, 1998). Under
109 these conditions, the cost for a female *E. caeruleum* to reject a male may be unacceptably high.

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111 Female mate choice in *E. caeruleum* may be further disfavoured by strong male-male
112 competition. Male *E. caeruleum* fight vigorously for access to gravid females, attempting to
113 monopolize spawning and prevent the participation of "sneaky" males (Winn, 1958; Fuller,
114 2003). Hence, the choice of males within a single patch is likely limited to those that are
115 competitively superior. Furthermore, there may be little additional benefit for the female to
116 choose following male-male competition if male competitive ability predicts fitness benefits to
117 females and her offspring (Wong & Candolin, 2005).

118

119 Darters are a highly speciose clade that have received increasing attention from evolutionary
120 biologists over the past decade. Spectacular and diverse male colouration in darters has been
121 suggested to act as an agent of speciation by sexual selection, with the most commonly posited
122 mechanism being divergent female mate choice (Mendelson, 2003; Williams & Mendelson,
123 2010; Williams *et al.*, 2013). However, evidence is mounting that female choice is limited in at

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124 least some darter species (Pyron, 1995; Fuller, 2003; Zhou *et al.* 2015; Moran *et al.*, 2013, 2017).
125 This study suggests that female preference in darters is likely costly due to the need to spawn
126 shortly after ovulation while egg viability remains high. Considering that egg overripening seems
127 to be common across a variety of fish species, often occurring over time frames comparable to or
128 shorter than in *E. caeruleum* (Kjørsvik *et al.*, 1990), its importance as an evolutionary force on
129 mate choice may be underappreciated.

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132 **Acknowledgements**

133 This work was supported by the Cooperative State Research, Education, and Extension Service,
134 US Department of Agriculture, under project number ILLU 875-952, the National Science
135 Foundation (DEB 0953716 and IOS 1701676), and the University of Illinois. The treatment of
136 animals was approved by the Institutional Animal Care and Use Committee under protocol
137 #17031.

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140 **References**

141 Beehler, B. M. & Foster, M. S. (1988). Hotshots, hotspots, and female preference in the
142 organization of lek mating systems. *The American Naturalist* **131**, 203–
143 219. doi:10.1086/284786
144 Bromage, N., Bruce, M., Basavaraja, N., Rana, K., Shields, R., Young, C., Dye, J., Smith, P.,
145 Gillespie, M. & Gamble, J. (1994). Egg quality determinants in finfish: the role of
146 overripening with special reference to the timing of stripping in the Atlantic halibut

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- 147 *Hippoglossus hippoglossus*. *Journal of the World Aquaculture Society* **25**, 13–
148 21.doi:10.1111/j.1749-7345.1994.tb00799.x
- 149 de Gaudemar, B. & Beall, E. (1998). Effects of overripening on spawning behaviour and
150 reproductive success of Atlantic salmon females spawning in a controlled flow channel.
151 *Journal of Fish Biology* **53**, 434–446.doi:10.1006/jfbi.1998.0716
- 152 Formacion, M. J., Hori, R. & Lam, T. J. (1993). Overripening of ovulated eggs in goldfish: I.
153 Morphological changes. *Aquaculture* **114**, 155–168.doi:10.1016/0044-8486(93)90258-z
- 154 Fuller, R. C. (1998). Fecundity estimates for rainbow darters, *Etheostoma caeruleum*, in
155 southwestern Michigan. *Ohio Journal of Science* **98**, 2–5.
- 156 Fuller, R. C. (2003). Disentangling female mate choice and male competition in the rainbow
157 darter, *Etheostoma caeruleum*. *Copeia* **2003**, 138–148.doi:10.1643/0045-
158 8511(2003)003[0138:dfmcam]2.0.co;2
- 159 Janetos, A. C. (1980). Strategies of female mate choice: A theoretical analysis. *Behavioral*
160 *Ecology and Sociobiology* **7**, 107–112.doi:10.1007/bf00299515
- 161 Jennions, M. D. & Petrie, M. (1997). Variation in mate choice and mating preferences: a review
162 of causes and consequences. *Biological Reviews* **72**, 283–
163 327.doi:10.1017/s0006323196005014
- 164 Kokko, H., Brooks, R., Jennions, M. D. & Morley, J. (2003). The evolution of mate choice and
165 mating biases. *Proceedings of the Royal Society of London B: Biological Sciences* **270**,
166 653–664.doi:10.1098/rspb.2002.2235
- 167 Kjørsvik, E., Mangor-Jensen, A. & Holmefjord, I. (1990). Egg quality in fishes. *Advances in*
168 *Marine Biology* **26**, 71–113. doi:10.1016/S0065-2881(08)60199-6

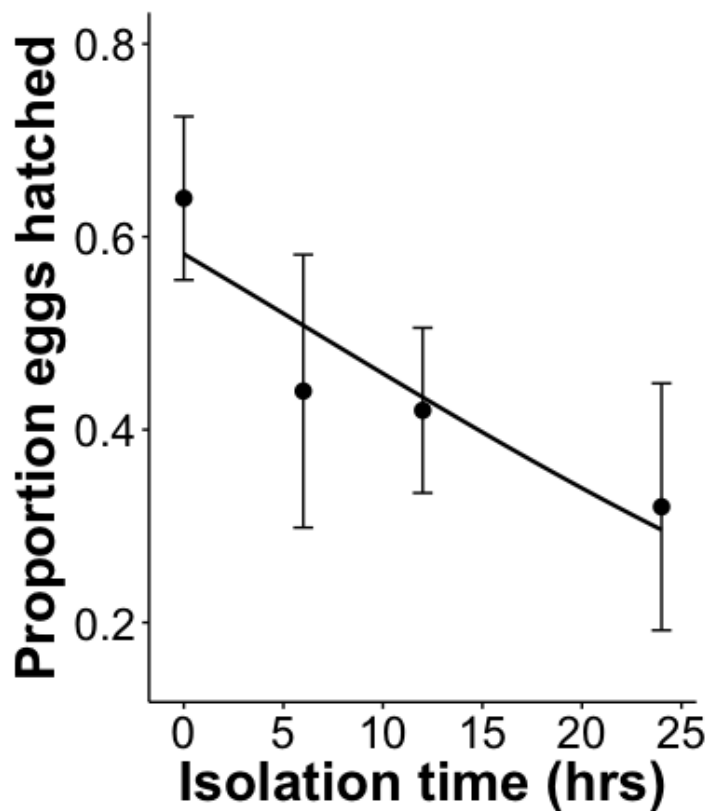
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- 169 McEvoy, L. A. (1984). Ovulatory rhythms and over-ripening of eggs in cultivated turbot,
170 *Scophthalmus maximus* L. *Journal of Fish Biology* **24**, 437–448. doi:10.1111/j.1095-
171 8649.1984.tb04814.x
- 172 Mendelson, T. C. (2003). Sexual isolation evolves faster than hybrid inviability in a diverse and
173 sexually dimorphic genus of fish (Percidae: *Etheostoma*). *Evolution* **57**, 317–327.
174 doi:10.1554/0014-3820(2003)057[0317:siefth]2.0.co;2
- 175 Moran, R. L., von Ende, C. N., & King, B. H. (2013). Mate choice copying in two species of
176 darters (Percidae: *Etheostoma*). *Behaviour* **150**, 1255-1274. doi:10.1163/1568539X-
177 00003092
- 178 Moran, R. L., Zhou, M., Catchen, J. M., & Fuller, R. C. (2017). Male and female contributions to
179 behavioral isolation in darters as a function of genetic distance and color distance.
180 *Evolution*. doi:10.1111/evo.13321
- 181 Page, L. M. (1983). *The Handbook of Darters* Neptune City, NJ: TFH Publications.
- 182 Pyron, M. (1995). Mating patterns and a test for female mate choice in *Etheostoma spectabile*
183 (Pisces, Percidae). *Behavioral Ecology and Sociobiology* **36**, 407–412.
184 doi:10.1007/bf00177336
- 185 Real, L. (1990). Search theory and mate choice. I. Models of single-sex discrimination. *The*
186 *American Naturalist* **136**, 376–405. doi:10.1086/285103
- 187 Reynolds, J. D. & Gross, M. R. (1990). Costs and benefits of female mate choice: is there a lek
188 paradox? *The American Naturalist* **136**, 230–243. doi:10.1086/285093
- 189 Williams, T. H. & Mendelson, T. C. (2010). Behavioral isolation based on visual signals in a
190 sympatric pair of darter species. *Ethology* **116**, 1038–1049. doi:10.1111/j.1439-
191 0310.2010.01816.x

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- 192 Williams, T. H., Gumm, J. M. & Mendelson, T. C. (2013). Sexual selection acting on a
193 speciation trait in darters (Percidae: *Etheostoma*). *Behavioral Ecology* **24**, 1407–1414.
194 doi:10.1093/beheco/art080
- 195 Winn, H. E. (1958). Observation on the reproductive habits of darters (Pisces–Percidae).
196 *American Midland Naturalist* **59**, 190–212. doi:10.2307/2422384
- 197 Wong, B. & Candolin, U. (2005). How is female mate choice affected by male
198 competition? *Biological Reviews*, **80**, 559–571. doi:10.1017/s1464793105006809
- 199 Zhou, M. & Fuller, R. C. (2014). Reproductive isolation between two darter species is enhanced
200 and asymmetric in sympatry. *Journal of Fish Biology* **84**, 1389–1400.
201 doi:10.1111/jfb.12364
- 202 Zhou, M., Loew, E. R. & Fuller, R. C. (2015). Sexually asymmetric colour-based species
203 discrimination in orangethroat darters. *Animal Behaviour* **106**, 171–179.
204 doi:10.1016/j.anbehav.2015.05.016
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217 **Figure 1.** The proportion of eggs that hatched from a clutch decreased with increasing time that

218 a female was held in isolation (i.e. prevented from spawning) after ovulation occurred.