Poeciliid male mate preference is influenced by female size but not by fecundity.

While female mate preference is very well studied, male preference has only recently begun to receive significant attention. Its existence is found in numerous taxa, but empirical research has mostly been limited to a descriptive level and does not fully address the factors influencing its evolution. We attempted to address this issue using preference functions by comparing the strength of male preference for females of different sizes in nine populations of four poeciliid species. Due to environmental constraints (water toxicity and surface versus cave habitat), females from these populations vary in the degree to which their size is correlated to their fecundity. Hence, they vary in how their size signals their quality as mates. Since female size is strongly correlated with fecundity in this subfamily, males were sequentially presented with conspecific females of three different size categories and the strength of their preference for each was measured. Males preferred larger females in all populations, as predicted. However, the degree to which males preferred each size category, as measured by association time, was not correlated with its fecundity. In addition, cave males discriminated against smaller females more than surface males. These results suggest that factors other than fecundity have a stronger influence on female fitness in these species.

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8 **Introduction:**

The existence and evolution of female mate choice has received substancial attention in
10 both theoretical and empirical grounds. However, male mate choice has been comparatively
neglected until recently. This is because females typically have a larger a priori investment in any
12 given mating event, and they are also inherently limited in the number of offspring they are able
to produce (Trivers 1972). The selective pressures giving rise to female mate choice are therefore
14 obvious. However, while these pressures are often stronger in females, similar pressures are also
experienced by males in many species. Males are limited in the proportion and quality of females
16 they are able to fertilize, and can therefore maximize their fitness by selectively allocating their
resources towards certain females. Theory thus predicts that male mate choice can be selected for
18 under the following circumstances: 1) There is substantial male effort in terms of searching,
courtship, mating, and mate guarding (Pomiankowski 1987); 2) Females are scarce due to a
20 biased operational sex ratio (van den Berghe & Warner 1989); 3) Female quality varies
(Johnstone et al. 1996); 4) Males invest in parental care (Sargent et al. 1986); and 5) Males'
22 mating opportunities are limited and/or insemination success varies between different females
(Nakatsuru & Kramer 1982; Verrell 1985; also see reviews of Bonduriansky 2001; and Edward &
24 Chapman 2011).

There is some evidence that these factors have indeed resulted in male preference in
26 species ranging from sexually cannibalistic arthropods, fish and birds with heavy parental
investment, and polygynous species without parental care (see reviews by Amundsen 2000;
28 Bonduriansky 2001; Edward & Chapman 2011). However, it difficult to determine the specific
factors driving the evolution of male choice in these systems since multiple factors predicted to
30 drive male mate choice evolution are present in these species. Previous empirical research has
often been limited to demonstrating the existence of male mate choice and describing its
32 manifestation in particular species. We are not aware of research examining the evolution of male
preference in response to specific selective pressures. We attempted to address this by comparing
34 poeciliid populations in which variation in female quality is likely to be the main driving force
behind male choice evolution.

36 Poeciliids are a family of internally-fertilizing, promiscuous fish that form mixed-sex
shoals and give birth to live young. Previous studies have demonstrated male preference for
38 larger females in many species (Abrahams 1993; Bisazza et al. 1989; Dosen & Montgomerie

2004a; Gumm & Gabor 2005; Herdman et al. 2004; Hoysak & Godin 2007; Jeswiet & Godin

40   2011; Plath et al. 2006; Ptacek & Travis 1997b). This is likely a result of the strong correlation

between female size and fecundity (Herdman et al. 2004; Hughes 1985; Marsh-Matthews et al.

42   2005; Milton & Arthington 1983; Reznick & Endler 1982; Riesch et al. 2009b), suggesting that

size is used as a signal of female quality and has played a role in the evolution of male mate

44   choice.

While previous studies have shown that males prefer more fecund females, how the

46   strength of this preference changes as female fecundity evolves has not been investigated. To

address this, we selected a number of populations from four poeciliid species (*Poecilia mexicana*,

48   *Limia sulphurophila, Gambusia sexradiata*, and *Gambusia eurystoma*) that exhibit different

relationships between female size and fecundity. These different relationships evolved as a

50   response to living in different habitats (Fig 1). Living in a toxic habitat or living in a cave

independently led to larger and fewer offspring; in other words, larger and fewer offspring are

52   found in toxic habitats (whether on the surface or in a cave), as well as cave habitats (whether

toxic or nontoxic); smaller and more numerous offspring are found in nontoxic surface habitats

54   (Riesch et al. 2009b; Riesch et al. 2010b; Riesch et al. 2010c). Because female size and fecundity

decoupled from each other in this system, it is possible to comparatively determine how female

56   fecundity affects the evolution of male preference.

Mate preference is most commonly studied using dichotomous choice tests, where focal

58   individuals are given a choice between two stimuli (Ritchie 1996). While this is a powerful

approach to assess mate preferences within populations, this approach makes it difficult to

60   compare between populations. Absolute preference functions are an alternative method that

allows the preferences between populations to be compared (Wagner 1998). Absolute preference

62   functions measure the preference of individual males for females varying in a continuous trait.

This is done by sequentially presenting individual females to each male, allowing the shape of a

64   male's preference for that trait to be quantified. The resulting correlation can thus be thought as

being the probability that a given male will accept a particular female trait (Ritchie 1996). Such

66   association preferences are commonly used to study male mating preferences in poeciliids and

have been shown to correlate well with actual mating preferences (Dosen & Montgomerie 2004b;

68   Plath et al. 2006; Schlupp & Ryan 1997; Wong et al. 2005).

The present study had two goals; 1) to test whether male preferences can be detected
70 using preference functions, and 2) to see if male preference tracks changes in female fecundity in
these populations. Our prediction was that male choice would be more acute in populations from
72 toxic environments, where the relative increase in female fecundity is higher as compared to
surface populations.

74 **Materials & Methods**

Species and populations:

76 Nine populations of four poeciliid species representing different habitat types were used
(summarized in Table 1). *Gambusia eurystoma* is a surface species endemic to the sulfidic Baños
78 del Azufre in Tabasco, Mexico (Tobler et al. 2008c). *Limia sulphurophila* is another surface fish
living in a sulfidic habitat, but it is endemic to a small pool in the island of Hispaniola (Rivas
80 1984). The population of *G. sexradiata* used lives in non-sulfidic surface waters. The six
populations of *P. mexicana* used in this study live in different habitats. The Oxolotan population
82 is named after the non-sulfidic, surface river it originates from. The PS0 population also lives in a
surface creek, but whose water is sulfidic. The water from this creek, named El Azufre, originates
84 from Cueva del Azufre, a sulfidic cave from which three of the other populations originated.
These populations, inhabiting a dark and toxic environment, are the PSV, PSX, and PSXIII
86 populations (For a schematic map of the region and of the cave, see Plath et al. 2010, and Tobler
et al. 2006). They are named after the chamber of the cave in which they live. The sixth
88 population is the Luna population, which originates from a non-toxic cave of the same name
(Tobler et al. 2008c).

90 Fish from all of the populations are maintained in flow-through stock tanks in the Aquatic
Research Facility at the University of Oklahoma, and have been in captivity for varying lengths
92 of time (Table 1). These tanks were the immediate source of the fish used in this study. All are
maintained in nonsulfidic, common garden conditions inside a greenhouse that receives natural
94 light.

Fish from the stock tanks were caught with a small seine and segregated by sex. Mature
96 females were then selected using minimum standard length (tip of the snout to the end of the
vertebral column) as the criterion for sexual maturity. Due to natural size differences between
98 species, the exact criterion used varied (*P. mexicana:* 29mm for the Luna population, and 30mm
for all others; *G. eurystoma:* 22mm; *G. sexradiata*: 18mm; and *L. sulphurophila*: 21mm). The

100   mature females were then sorted into roughly equally sized groups according to size (small, medium, and large), and were then placed in different 38L stock tanks. Males were randomly

102   assigned an ID number that determined the order in which they would be tested, and they were housed in individual 5L tanks that were out of sight of the females.

104

Experimental Setup:

106   Preference functions are established by measuring the amount of time a focus individual spends in association with different stimulus fish. These stimulus fish are presented sequentially

108   and differ in the variable in question. In this case, females of different sizes were sequentially presented to a male, and the time that the male spent with each female was recorded. To do this, a

110   76L aquarium, with gravel spread evenly to reduce potential bias from fish being distracted by uneven gravel, was divided lengthwise into three equal sections with two vertical lines drawn on

112   the glass (Fig 2). The outer two sections were considered the "preference zones", while the central section was considered a "neutral zone".

114   Three hollow square prisms (or "cylinder") made out of clear plexiglass were located in the center of each section of the tank. The cylinder in the center of the tank had solid walls, while

116   the two outer cylinders were perforated with seven circular holes 6mm in diameter to allow for chemical and mechanosensory signals. Chemical and mechanosensory signals have been found to

118   be important factors in poeciliid mating behavior, influencing the repeatability of individual preferences as well as the overall preference (Coleman 2011; Hoysak & Godin, 2007; Plath et al.

120   2006; Rüschenbaum & Schlupp 2013). All three cylinders were 8.5cm long by 8.5cm wide, and tall enough to stick through the water. To reduce visual distractions, three sides of the tank were

122   covered. The observer sat in a chair 2m from the tank and observed the fish through the front pane of the tank. A light with a 60W, "soft white" light bulb was placed 30cm above the center of

124   the tank for illumination.

126   Testing Procedure:

        A randomly selected female from the predetermined size category (small, medium, or

128   large) was placed in the cylinder in one of the outer preference zones. The order of the females which each male would be presented with, as well as the side in which each female would be

130   placed, were randomly determined. A male was then placed in the central cylinder for 5 minutes.

After the 5 minutes of acclimation, the cylinder around the male was gently removed. Using two
132 stopwatches, the amount of time the male spent in the preference zone containing the female was
measured by the observer. This was done for 5 minutes, after which the fish were removed from
134 the tank. The water was then stirred to homogenize any lingering chemical signals from affecting
the results of future trials. Another pair of fish was then placed in their corresponding cylinders to
136 acclimate. Every three pairs, a partial water change was also made previous to the acclimation
period of the next fish. Male weight and standard length were also measured and used as
138 covariates, but were not included in the final model because neither was significant.

140 Statistical analysis:

After checking the assumptions, a mixed between-within subjects ANOVA was performed
142 to determine the effect of two habitat variables on male preference for small, medium, and large
females. The two habitat variables used were "cave habitat", whether the population originated
144 from a cave or from a surface stream, and "toxicity", whether the population originated from a
toxic or non-toxic stream. Because the raw results were not normally distributed, the male
146 preference variables were reflected and square root transformed to meet the normality
assumption.
148 Experiments were approved by the University of Oklahoma IACUC (R09-030).
Results:
150   All statistical assumptions were met after the data transformation, with the exception of
homogeneity of variances for the time males spent with medium females ($p = 0.026$). However,
152 this violation was not deemed to be severe enough to invalidate the ANOVA. As expected, there
was a significant main effect for time spent with larger females, regardless of the habitat of origin
154 (Wilks' Lambda = 0.911, $F_{(2, 123)}$= 5.99, p= 0.003, η_p^2= 0.089). The main between-subjects effect
comparing toxicity was not significant ($F_{(1, 124)}$= 0.047, p= 0.829, η_p^2= 0.000), nor was the main
156 effect for cave habitat ($F_{(1, 124)}$= 1.043, p= 0.309, η_p^2= 0.008), or the interaction between cave
habitat and toxicity ($F_{(1, 124)}$= 0.011, p= 0.917, η_p^2= 0.000). These results suggest that, correcting
158 for the effect of female size, habitat type does not affect male preference values.

There was also a significant interaction between cave habitat and the time males spent
160 with females (Wilks' Lambda= 0.934, $F_{(2, 123)}$= 4.36, p= 0.015, η_p^2= 0.066; Fig 3). However, there

was no significant interaction between the time males spent with females and the toxicity of the

162 habitat from which they originated (Wilks' Lambda = 0.996, $F_{(2, 123)}$= 0.26, p= 0.77, h_{p}^{2}= 0.004;

Fig 4). There was also no significant interaction between time, cave habitat, and toxicity together

164 (Wilks' Lambda= 0.956, $F_{(2, 123)}$= 8.86, p= 0.06, h_{p}^{2}= 0.044). These results suggest that the

presence or absence of hydrogen sulfide in the population's habitat of origin does not influence

166 males' preference for female size, but the cave habitat does. Descriptive statistics are summarized

in Table 2.

168 **Discussion:**

As predicted, males did exhibit a general preference for larger females when all

170 populations were considered in aggregate. This result is consistent with previous

dichotomous-test studies finding preference for larger females in poeciliids (Bisazza et al. 1989;

172 Herdman et al. 2004; Hoysak & Godin 2007; Jeswiet & Godin 2011; Plath et al. 2006) and

indicates that absolute preference functions are an accurate tool to study individual preferences.

174 Because preference functions can be used to compare preferences between individuals, they can

also be used to address a more specific and broader range of questions than is possible using only

176 dichotomous choice tests.

In addition to an overall preference for larger females, the strength of male preference

178 should reflect how tightly correlated female size is with female fecundity. The populations tested

originated from habitats with different combinations of two variables- water toxicity as a result of

180 the presence or absence of H_2S ("toxicity"), and epigean or hypogean habitat ("cave habitat").

Previous studies have shown that in *P. mexicana* (Riesch et al. 2009b; Riesch et al. 2010c), as

182 well as in *G. sexradiata* and *G. eurystoma* (Riesch et al. 2010b), female fecundity is strongly

correlated with toxicity. Females from toxic habitats have much larger, but fewer, offspring.

184 Because of this, there is a larger change in fecundity from small to large females in nontoxic

habitats. The main hypothesis was therefore that the preference function of males from toxic

186 habitats would be less steep than that of males from nontoxic habitats. The results did not support

our hypothesis, as there was no significant interaction between time spent with a female and the

188 toxicity of the habitat the male originated from.

This result suggests that the change in female fecundity experienced from benign to toxic

190 habitats is only weakly correlated with the change female quality. The reason for this is unclear

and could be due to a combination of factors. It is possible that female size in nontoxic habitats is

192   correlated with increased female mortality and/or with a decrease in offspring quality. An

alternative possibility is that female size and quality are highly correlated, but that there has been

194   insufficient time for male preference to change as a response to female adaptation. It is currently

unknown how long the populations in toxic habitats have been adapting to their environments,

196   and how recently the changes in female fecundity have evolved. Since male preference is likely

under weaker selection than other traits, it is possible that males from toxic populations have

198   either not had a sufficient amount of time to adapt, or that the amount of gene flow from nontoxic

populations has been able to counteract the effects of selection. It is currently unknown how

200   much gene flow there is between toxic (*P. mexicana:* PS0, *G. sexradiata:* populations not used in

the present study) and nontoxic (*P. mexicana*: Oxolotan, *G. sexradiata)* surface populations.

202   While the amount of gene flow between toxic and non-toxic populations still needs to be

determined, it is known that there is very little gene flow between *P. mexicana* populations from

204   the Cueva del Azufre (PSV, PSX, and PSXIII) and those in the surface (Plath et al., 2010). The

genetic isolation of cave fish from surface fish might be an important reason why cave habitat did

206   have a significant effect on male preference. This difference seems to be mainly derived from

cave males' relatively low preference for small females (Fig 3), as the change in preference for

208   medium to large females is nearly identical in males from both habitats. This suggests that males

are not responding to fecundity or offspring size per se, since one would expect that the change of

210   preference from medium to large females would differ between the two environments. This same

logic also suggests that the difference is not due to cave males being choosier than surface males.

212   If males preferred larger females due to the cave habitat leading to a greater cost in male effort,

males would disproportionally prefer large females over medium females as well. Instead, these

214   results indicate that there is a relative disadvantage for cave males to mate with smaller females,

which could result if small cave females have a lower fitness than small surface females.

216   A fitness difference could occur if mortality rates for small cave female are higher than

those of medium or large females, perhaps as a result of differential predation pressures. No

218   direct evidence exists on the relative predation pressures between the two habitats, but there is

reason to believe that this possibility might be true. Cave populations of *P. mexicana* are known

220   to be preyed upon by predators which hunt by sensing tactile and/or chemical signals from fish in

close proximity (Horstkotte et al. 2010; Tobler et al. 2008a; Tobler et al. 2009), and may prey

222   disproportionally upon smaller females. At the same time, surface populations experience very

different predation pressures: surface fish are preyed upon by large visual predators (Riesch et al.

224 2009a, 2010a; Tobler et al. 2006) that target larger fish (Trexler et al. 1994). Thus, medium and

large surface females are likely to experience greater predation pressure than small females.

226 In summary, we have shown that (1) male preference for larger female size exists. This is

consistent with previous research, indicating that absolute preference functions are a valid

228 approach in this system. (2) Hydrogen sulphide does not affect the shape of male's preference

function for female size in this system. Since H_2S greatly affects female fecundity, this suggests

230 that inter-population differences of fecundity are not very highly correlated with inter-population

differences in fitness. Alternatively, there has not been enough time or enough selection pressure

232 to allow male preference to evolve as a response to changes in female fecundity, or gene flow has

been large enough to negate the effects of these pressures. (3) Cave habitat, independent of water

234 toxicity, does affect male preference. Cave males had a relative lack of preference for small

females. We suggest that this could be a result of differences in predation pressure which could

236 lead to relatively increased mortality for small females in the caves, relatively increased mortality

for medium and large females in the surface, or both. If true, this would highlight the role that

238 predators play in the evolution of male mate choice.

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Figure 1

Effect of habitat toxicity on the size/fecundity relationship

Figure 1: Schematic illustration of the previously-established relationship between female poeciliid size and her fecundity in toxic and non-toxic habitats.

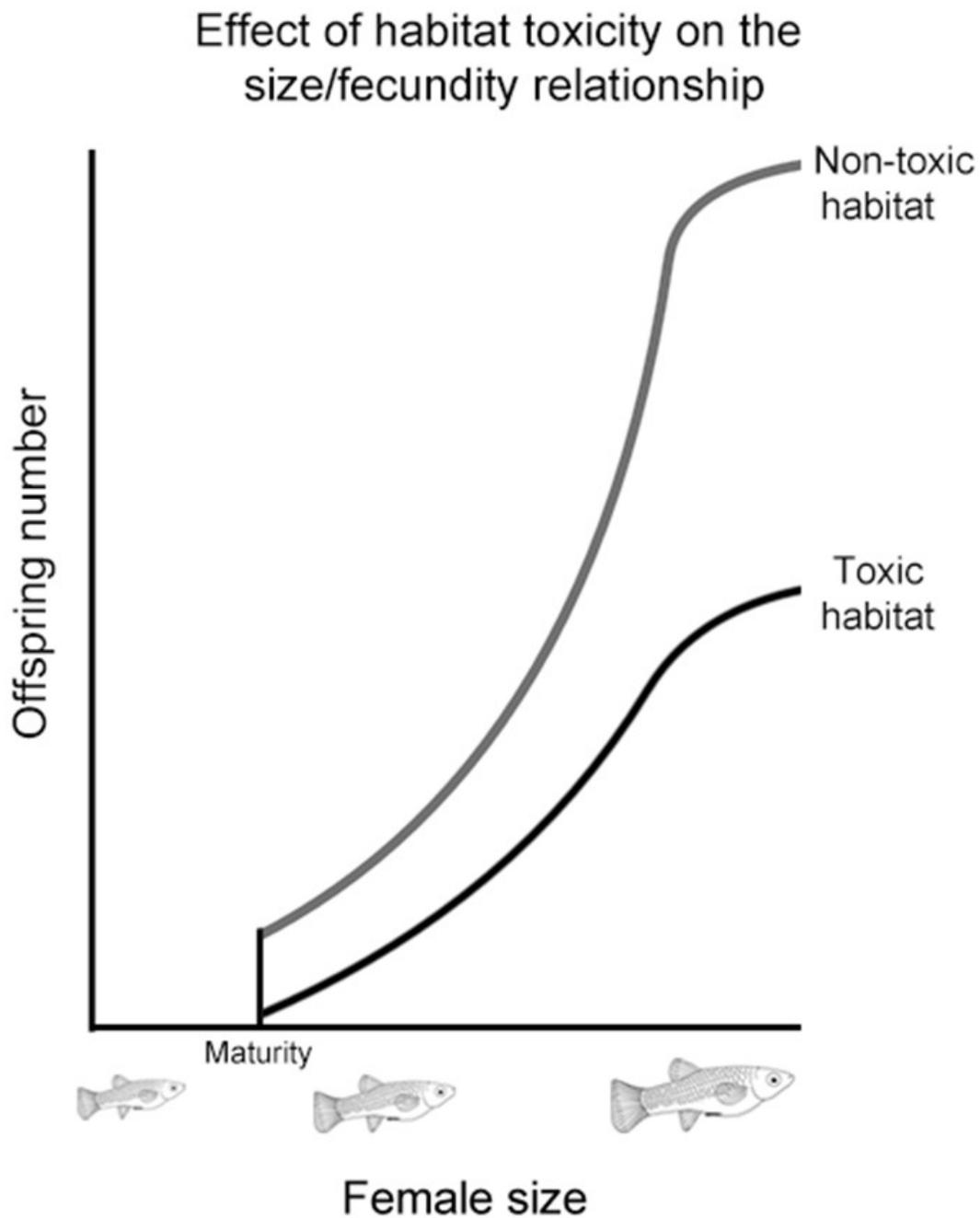



Table 1_(on next page)

Populations used

Table 1: Collection details and habitats characteristics of the populations from which the individuals used originated. All individuals were descendants of these original populations, and were raised in common garden conditions.

Population	Source location	Year	Toxic/non-toxic	Cave/Surface
*P. mexicana-*Oxolotan	17°26'55"N 92°45'55"W	2005	non-toxic	Surface
*P. mexicana-*PS0	17°26'30"N 92°46'30"W	2005	Toxic	Surface
*P. mexicana-*Luna	17°26'35"N 92°46'39"W	2006	non-toxic	Cave
*P. mexicana-*PSV	17°26'30"N 92°46'30"W	2005	Toxic	Cave
*P. mexicana-*PSX	17°26'30"N 92°46'30"W	2005	Toxic	Cave
*P. mexicana-*PSXIII	17°26'30"N 92°46'30"W	2005	Toxic	Cave
G. eurystoma	17°33'10"N 92°59'51"W	2006	Toxic	Surface
G. sexradiata	17°59'56"N 93° 8'11"W	2006	non-toxic	Surface
L. sulphurophila	18°23'52"N 71°34'12"W	2006	Toxic	Surface

Figure 2

Experimental setup.

Figure 2: Schematic representation of the experimental setup during acclimation period. Gravel and cylinder perforations were omitted for clarity.

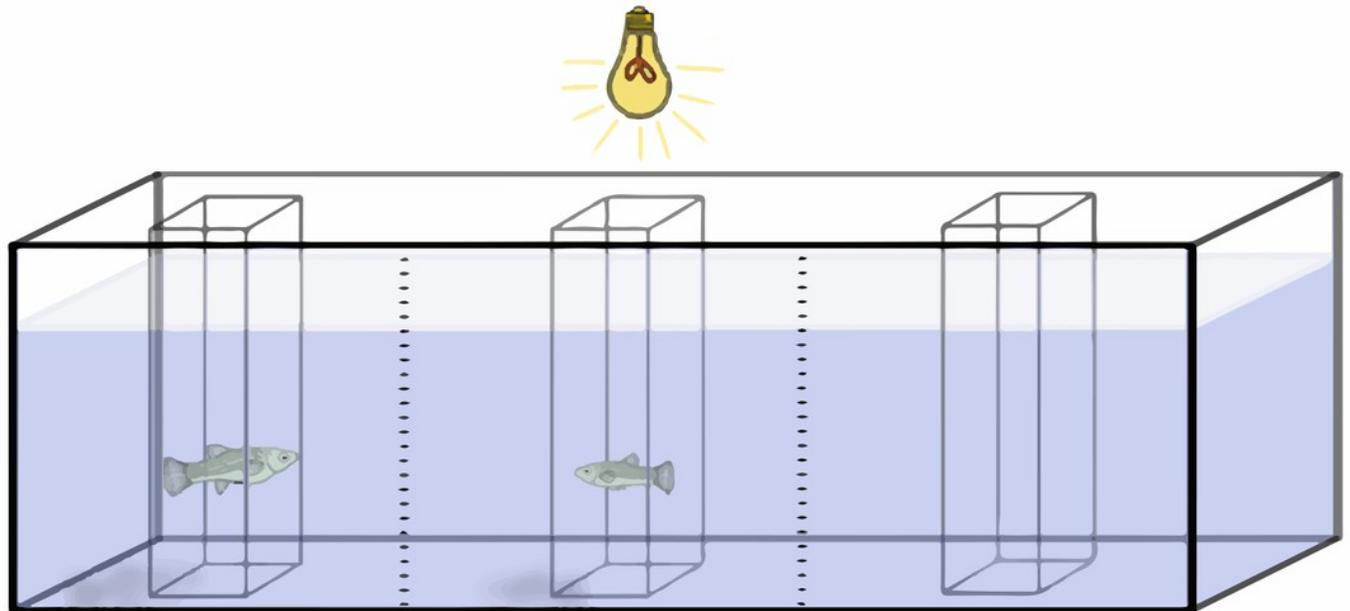



Figure 3

Male preference for female size (cave vs. surface)

Figure 3: Average transformed male preference for female size in cave vs. surface habitats. There is a significant difference between the two preference functions.

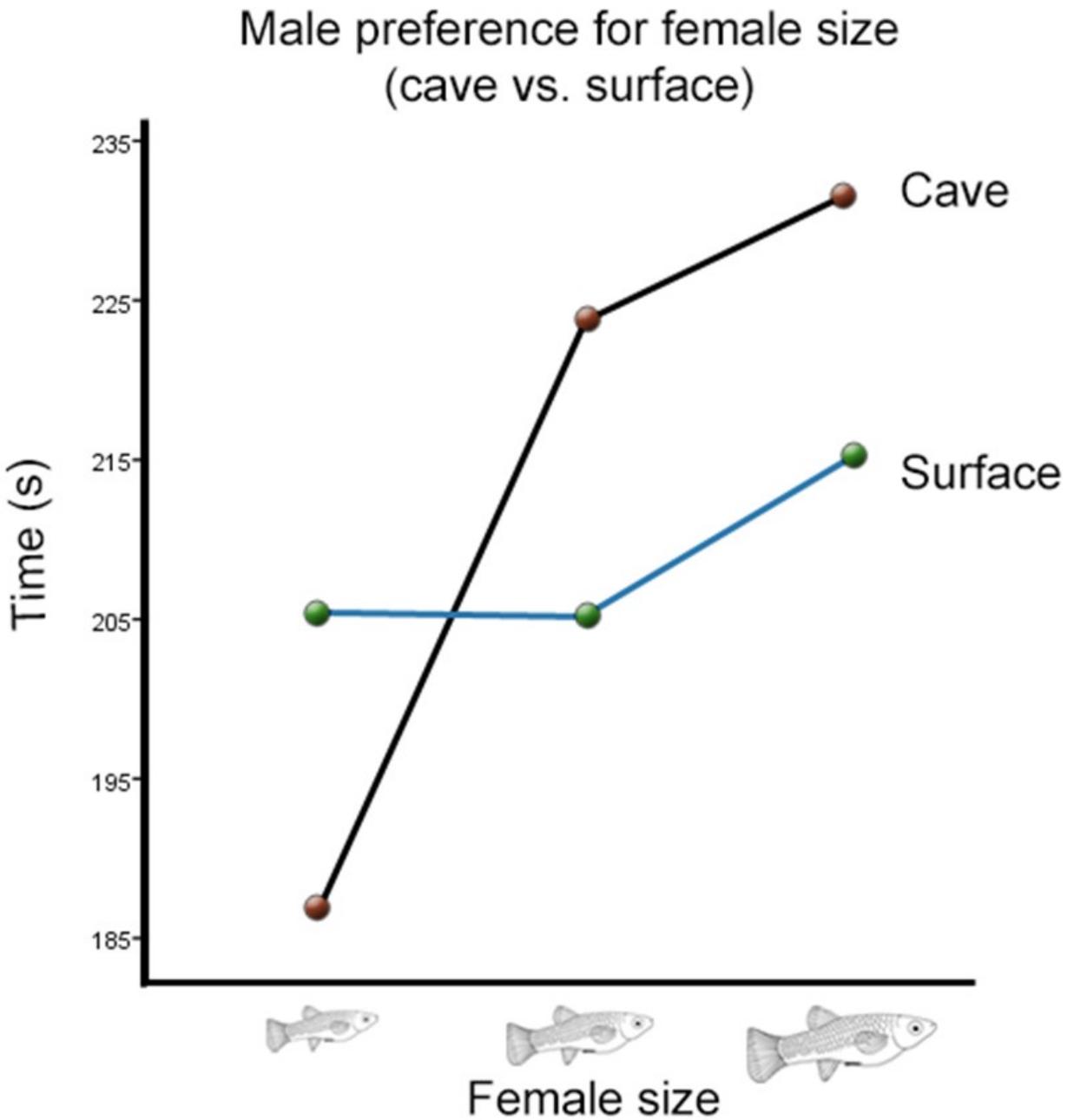



Figure 4

Male preference for female size (toxic vs. non-toxic)

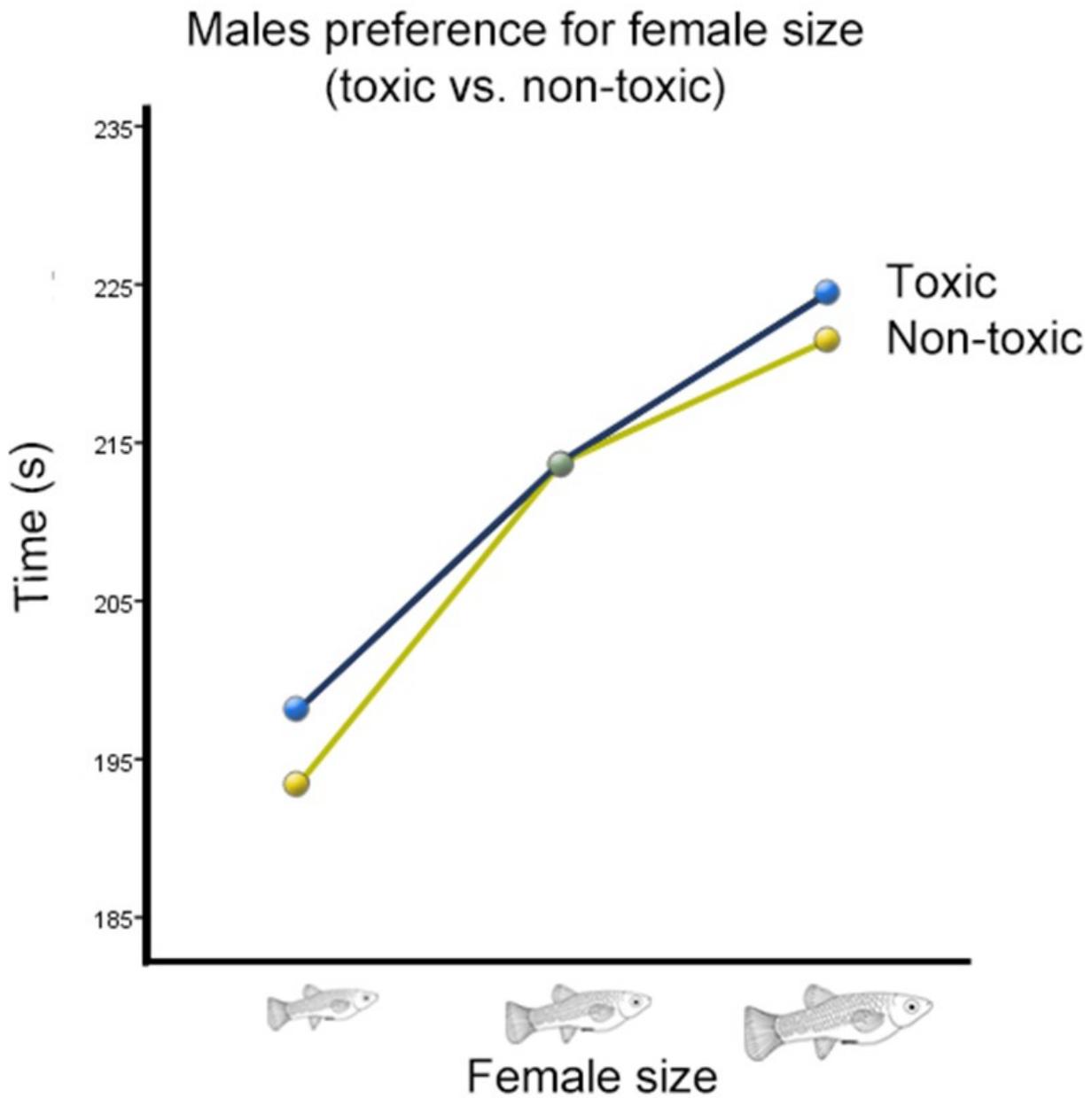
Figure 4: Average transformed male preference for female size in toxic vs. benign habitats. Preference functions are not significantly different from each other.

Descriptive statistics

Table 2: Sample size, average time, and standard deviation that males from each of the populations spent with each female size category.

		N	Mean association time (s)	Std. Deviation
Small Female	Cave	56	191.4	74.9
	Surface	72	205.1	59.1
	Nontoxic	43	199.5	75.7
	Toxic	85	198.8	61.9
Medium Female	Cave	56	218.1	59.4
	Surface	72	206.9	50.9
	Nontoxic	43	206.8	62.4
	Toxic	85	214.3	50.8
Large Female	Cave	56	233.2	62.2
	Surface	72	215.1	62.2
	Nontoxic	43	219.1	56.4
	Toxic	85	225.0	65.7