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. Assuming that male preference is correlated with female fitness, these results suggest that factors other than fecundity have a strong 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 the

strength of male preference in response to specific selective pressures. We attempted to address

34   this by comparing 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), and have been found to persist even in fish raised

in common garden conditions (Riesch et al. 2009b, Riesch et al. submitted). Living in a toxic

52   habitat or living in a cave independently led to larger and fewer offspring; in other words, larger

and fewer offspring are found in toxic habitats (whether on the surface or in a cave), as well as

54   cave habitats (whether toxic or nontoxic); smaller and more numerous offspring are found in

nontoxic surface habitats (Riesch et al. 2009b; Riesch et al. 2010b; Riesch et al. 2010c). Because

56   female size and fecundity decoupled from each other in this system, it is possible to

comparatively determine how female fecundity affects the evolution of male preference.

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

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

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

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

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

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

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

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

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

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

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

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

70   The present study had two goals; 1) to test whether male preferences can be detected

using preference functions, and 2) to see if male preference tracks changes in female fecundity in

72   these populations. Our prediction was that male preference for larger females would be stronger

in populations from nontoxic environments, where the relative increase in female fecundity is

74   higher as compared to populations from toxic environments.

Materials & Methods

76   Species and populations:

Nine populations of four poeciliid species representing different habitat types were used

78   (summarized in Table 1). *Gambusia eurystoma* is a surface species endemic to the sulfidic Baños

del Azufre in Tabasco, Mexico (Tobler et al. 2008c). *Limia sulphurophila* is another surface fish

80   living in a sulfidic habitat, but it is endemic to a small pool in the island of Hispaniola (Rivas

1984). The population of *G. sexradiata* used lives in non-sulfidic surface waters. The six

82   populations of *P. mexicana* used in this study live in different habitats. The Oxolotan population

is named after the non-sulfidic, surface river it originates from. The PS0 population also lives in a

84   surface creek, but whose water is sulfidic. The water from this creek, named El Azufre, originates

from Cueva del Azufre, a sulfidic cave from which three of the other populations originated.

86   These populations, inhabiting a dark and toxic environment, are the PSV, PSX, and PSXIII

populations (For a schematic map of the region and of the cave, see Plath et al. 2010, and Tobler

88   et al. 2006). They are named after the chamber of the cave in which they live. The sixth

population is the Luna population, which originates from a non-toxic cave of the same name

90   (Tobler et al. 2008c).

Fish from all of the populations are maintained in flow-through stock tanks in the Aquatic

92   Research Facility at the University of Oklahoma, and have been in captivity for varying lengths

of time (Table 1). These tanks were the immediate source of the fish used in this study. All are

94   maintained in nonsulfidic, common garden conditions inside a greenhouse that receives natural

light.

96   Fish from the stock tanks were caught with a small seine and segregated by sex. Mature

females were then selected using minimum standard length (tip of the snout to the end of the

98   vertebral column) as the criterion for sexual maturity. Due to natural size differences between

species, the exact criterion used varied (*P. mexicana:* 29mm for the Luna population, and 30mm

100   for all others; *G. eurystoma:* 22mm; *G. sexradiata*: 18mm; and *L. sulphurophila*: 21mm). The

mature females were then sorted into roughly equally sized groups according to size (small,

102   medium, and large), and were then placed in different 38L stock tanks. Males were randomly

assigned an ID number that determined the order in which they would be tested, and they were

104   housed in individual 5L tanks that were out of sight of the females.

106   Experimental Setup:

Preference functions are established by measuring the amount of time a focus individual

108   spends in association with different stimulus fish. These stimulus fish are presented sequentially

and differ in the variable in question. In this case, females of different sizes were sequentially

110   presented to a male, and the time that the male spent with each female was recorded. To do this, a

76L aquarium, with gravel spread evenly to reduce potential bias from fish being distracted by

112   uneven gravel, was divided lengthwise into three equal sections with two vertical lines drawn on

the glass (Fig 2). The outer two sections were considered the "preference zones", while the

114   central section was considered a "neutral zone".

Three hollow square prisms (or "cylinder") made out of clear plexiglass were located in

116   the center of each section of the tank. The cylinder in the center of the tank had solid walls, while

the two outer cylinders were perforated with seven circular holes 6mm in diameter to allow for

118   chemical and mechanosensory signals. Chemical and mechanosensory signals have been found to

be important factors in poeciliid mating behavior, influencing the repeatability of individual

120   preferences as well as the overall preference (Coleman 2011; Hoysak & Godin, 2007; Plath et al.

2006; Rüschenbaum & Schlupp 2013). All three cylinders were 8.5cm long by 8.5cm wide, and

122   tall enough to stick through the water. To reduce visual distractions, three sides of the tank were

covered. The observer sat in a chair 2m from the tank and observed the fish through the front

124   pane of the tank. A light with a 60W, "soft white" light bulb was placed 30cm above the center of

the tank for illumination.

126

Testing Procedure:

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

large) was placed in the cylinder in one of the outer preference zones. The order of the females

130   which each male would be presented with, as well as the side in which each female would be

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

132 After the 5 minutes of acclimation, the cylinder around the male was gently removed. Using two

stopwatches, the amount of time the male spent in the preference zone containing the female was

134 measured by the observer. This was done for 5 minutes, after which the fish were removed from

the tank. The water was then stirred to homogenize any lingering chemical signals from affecting

136 the results of future trials. Another pair of fish was then placed in their corresponding cylinders to

acclimate. Every three pairs, a partial water change was also made previous to the acclimation

138 period of the next fish. Male weight and standard length were also measured and used as

covariates, but were not included in the final model because neither was significant.

140

Statistical analysis:

142   After checking the assumptions, a mixed between-within subjects ANOVA was performed

to determine the effect of two habitat variables on male preference for small, medium, and large

144 females. The two habitat variables used were "cave habitat", whether the population originated

from a cave or from a surface stream, and "toxicity", whether the population originated from a

146 toxic or non-toxic stream. Because the raw results were not normally distributed, the male

preference variables were reflected and square root transformed to meet the normality

148 assumption.

Experiments were approved by the University of Oklahoma IACUC (R09-030).

150 **Results:**

    All statistical assumptions were met after the data transformation, with the exception of

152 homogeneity of variances for the time males spent with medium females ($p = 0.026$). However,

this violation was not deemed to be severe enough to invalidate the ANOVA. As expected, there

154 was a significant main effect for time spent with larger females, regardless of the habitat of origin

(Wilks' Lambda = 0.911, $F_{(2, 123)}$= 5.99, p= 0.003, h_p^2= 0.089). The main between-subjects effect

156 comparing toxicity was not significant ($F_{(1, 124)}$= 0.047, p= 0.829, h_p^2= 0.000), nor was the main

effect for cave habitat ($F_{(1, 124)}$= 1.043, p= 0.309, h_p^2= 0.008), or the interaction between cave

158 habitat and toxicity ($F_{(1, 124)}$= 0.011, p= 0.917, h_p^2= 0.000). These results suggest that, correcting

for the effect of female size, habitat type does not affect male preference values.

160   There was also a significant interaction between cave habitat and the time males spent

with females from different size categories (Wilks' Lambda= 0.934, $F_{(2, 123)}$= 4.36, p= 0.015, h_p^2=

162   0.066; Fig 3). However, there was no significant interaction between the time males spent with

females of different sizes and the toxicity of the habitat from which they originated (Wilks'

164   Lambda = 0.996, $F_{(2, 123)}$= 0.26, p= 0.77, η_p^2= 0.004; Fig 4). There was also no significant

interaction between time, cave habitat, and toxicity together (Wilks' Lambda= 0.956, $F_{(2, 123)}$=

166   8.86, p= 0.06, η_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 males' preference for female size, but the

168   cave habitat does. Descriptive statistics are summarized in Table 2.

Discussion:

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

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

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

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

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

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

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

dichotomous choice tests.

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

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

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

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

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

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

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

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

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

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

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

toxicity of the habitat the male originated from.

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

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

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

14   7

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

224 different predation pressures: surface fish are preyed upon by large visual predators (Riesch et al.
2009a, 2010a; Tobler et al. 2006) that target larger fish (Trexler et al. 1994). Thus, medium and
226 large surface females are likely to experience greater predation pressure than small females.

In summary, we have shown that (1) male preference for larger female size exists. This is
228 consistent with previous research, indicating that absolute preference functions are a valid
approach in this system. (2) Hydrogen sulphide does not affect the shape of male's preference
230 function for female size in this system. Since H_2S greatly affects female fecundity, this suggests
that inter-population differences of fecundity are not very highly correlated with inter-population
232 differences in fitness. Alternatively, there has not been enough time or enough selection pressure
to allow male preference to evolve as a response to changes in female fecundity, or gene flow has
234 been large enough to negate the effects of these pressures. (3) Cave habitat, independent of water
toxicity, does affect male preference. Cave males had a relative lack of preference for small
236 females. We suggest that this could be a result of differences in predation pressure which could
lead to relatively increased mortality for small females in the caves, relatively increased mortality
238 for medium and large females in the surface, or both. If true, this would highlight the role that
predators play in the evolution of male mate choice.

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Figure 1

Effect of habitat toxicity on the size/fecundity relationship

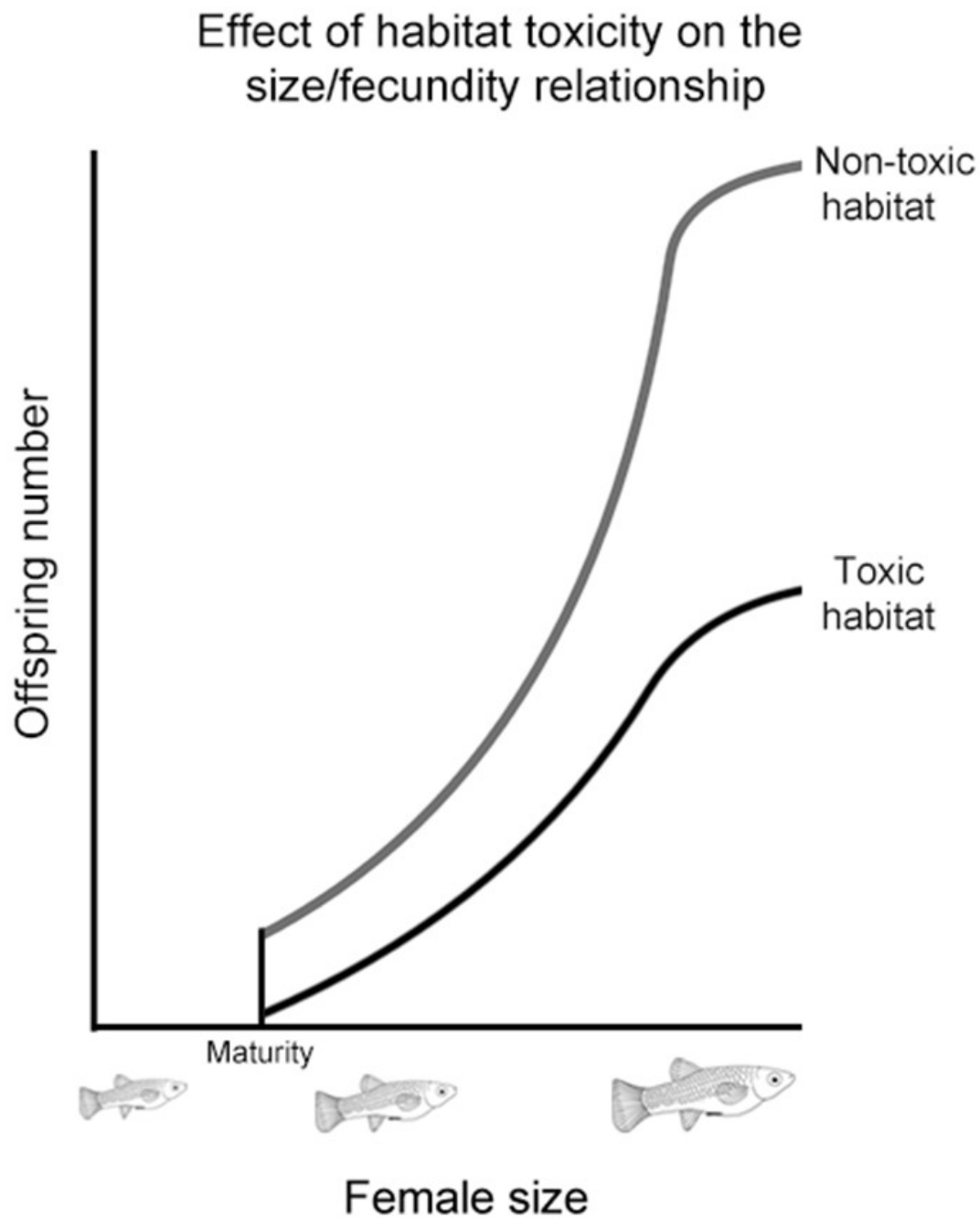
Figure 1: Schematic illustration of the previously-established relationship between female poeciliid size and her fecundity in toxic and non-toxic habitats.

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.

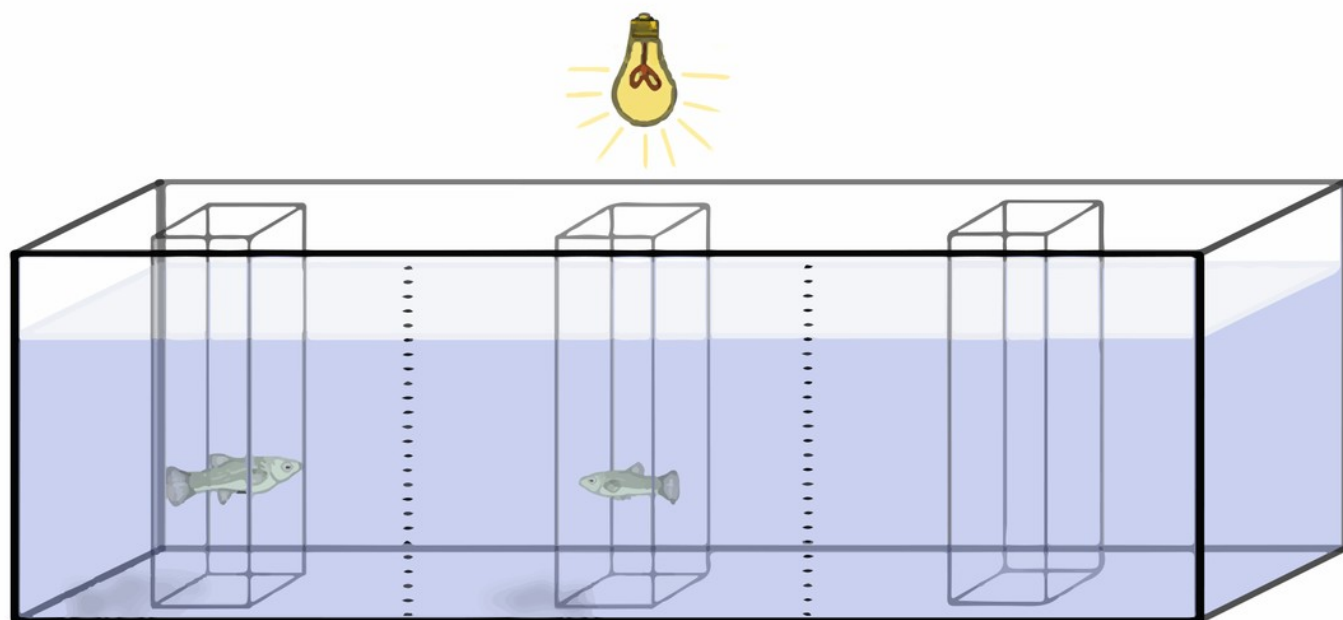



Figure 3

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.

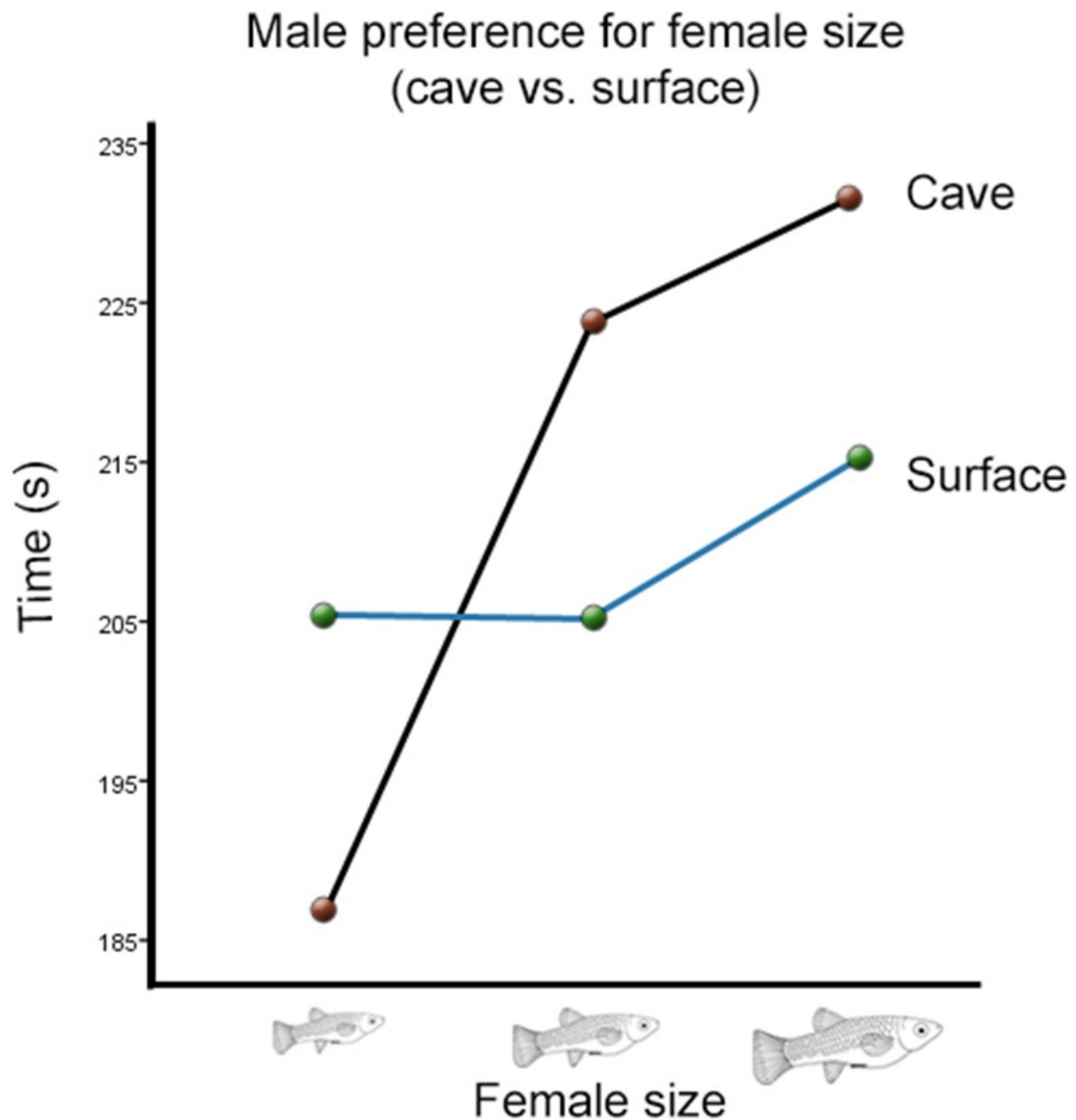



Figure 4

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

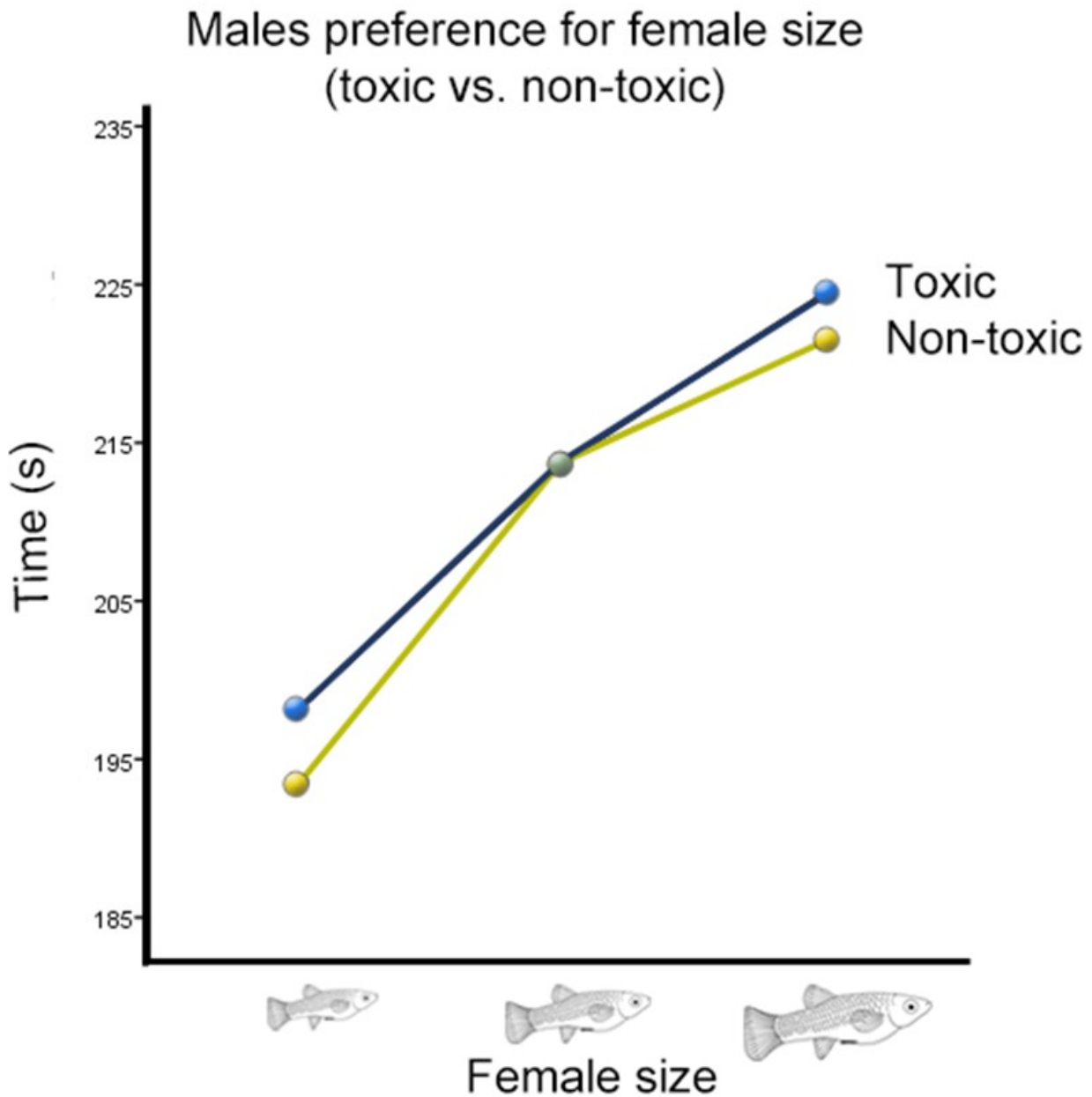
Figure 4: Average transformed male preference for female size in toxic vs. benign habitats. Preference functions are not significantly different from each other.

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