OXFORD

Article

Predator-induced changes of male and female mating preferences: innate and learned components

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Abstract

While many mating preferences have a genetic basis, the question remains as to whether and how learning/experience can modify individual mate choice decisions. We used wild-caught (predator-experienced) and F_1 laboratory-reared (predator-naïve) invasive Western mosquitofish *Gambusia affinis* from China to test whether mating preferences (assessed in a first mate choice test) would change under immediate predation threat. The same individuals were tested in a second mate choice test during which 1 of 3 types of animated predators was presented: 1) a co-occurring predator, 2) a co-evolved but not currently co-occurring predator, and 3) a non-piscivorous species as control. We compared preference scores derived from both mate choice tests to separate innate from experiential effects of predation. We also asked whether predator-induced changes in mating preferences would differ between sexes or depend on the choosing individual's personality type and/or body size. Wild-caught fish altered their mate choice decisions most when exposed to the co-occurring predator whereas laboratory-reared individuals responded most to the co-evolved predator, suggesting that both innate mechanisms and learning effects are involved. This behavior likely reduces individuals' risk of falling victim to predation by temporarily moving away from high-quality (i.e., conspicuous) mating partners. Accordingly, effects were stronger in bolder than shyer, large- compared with small-bodied, and female compared with male focal individuals, likely because those phenotypes face an increased predation risk overall. Our study adds to the growing body of literature appreciating the complexity of the mate choice process, where an array of intrinsic and extrinsic factors interacts during decision-making.

Key words: female choice, male mate choice, non-independent mate choice, predator recognition, sexual selection

Sexual selection via mate choice drives the evolution of morphological and behavioral diversity within and among species, and female preferences for male sexual ornaments and courtship displays have been documented for a vast number of species (Andersson 1982; Sigmund 1983; Basolo 1990; Wilkinson and Reillo 1994; Rosenthal 2017). While many mating preferences are innate (Bakker

and Pomiankowski 1995; Rosenthal 2017), biotic and abiotic ecological factors may still affect the strength and direction of individual mating decisions (Endler and Houde 1995; Jennions and Petrie 1997; Widemo and Sæther 1999; Cotton et al. 2006). For example, mating preferences can depend on the costs of mate finding (Milinski and Bakker 1992; Wong and Jennions 2003), on social factors like intrasexual competition (Jennions and Petrie 1997) or on the nutritional state of the choosing individual (Plath et al. 2005; Fisher and Rosenthal 2006). Another factor altering female mating preferences of prey species is brought about by the presence of potential predators (Kirkpatrick and Ryan 1991; Magnhagen 1991; Houde 1993; Sih 1994).

A central question of this study was whether and how learning (i.e., experience with certain co-occurring predators) affects the extent to which individuals alter their mate choice when confronted with different predator types (Curio et al. 1978; Kelley and Magurran 2003; Griffin 2004; Bierbach et al. 2011). We addressed this question using the invasive mosquitofish *Gambusia affinis* (Poeciliidae) as our study organism. We asked 3 additional (partly interrelated) questions: 1) do females and males alter their mating preferences to a similar degree in response to predators? We further asked whether potential predator-induced changes of mating preferences would depend on 2) consistent individual variation in behavioral tendencies (animal personality; Réale et al. 2007; Kralj-Fiser and Schuett 2014), and 3) body size of the choosing individual. Personality traits (here: boldness/exploration) and body size could interact with potential learning effects and sex differences to create variation in the extent to which individuals respond to the presence of a predator during their mate choice.

Predator-induced changes in mate choice

Predator-induced changes in mating behaviors include altered search strategies, courtship behavior, mating duration, and mating preferences (Sih et al. 1990; Magnhagen 1990; Crowley et al. 1991). Temporary alterations of mating behaviors under predation threat are thought to increase individuals' survival probability, as shown for pipefish *Syngnathus typhle* that copulated and courted less frequently, swam alone less often and waited longer before commencing courtship to avoid predators (Fuller and Berglund 1996), whereas *Gammarus duebeni* (Amphipoda) reduced pair formation (amplexus) under perceived predation risk (Dunn et al. 2008). Male guppies *Poecilia reticulata* switch from displaying courtship behavior—which is conspicuous not only to females but also predators—to coercive mating tactics (Endler 1987; Magurran and Seghers 1990; Magurran and Nowak 1991; Godin 1995). Regarding mating preferences, female green swordtails *Xiphophorus hellerii* typically prefer males sporting long swords (an ornamental caudal fin elongation) over short-sworded ones, but reverse their preference when predators are present (Johnson and Basolo 2003; Pilakouta and Alonzo 2014). Guppy females typically prefer brightly ornamented males but associate more with drab males under predation risk (Godin and Briggs 1996; Gong and Gibson 1996). Females benefit from altering their mate choice behavior, as conspicuous males are more likely to attract predators, thus increasing females' own predation risk (Zuk and Kolluru 1998; Rosenthal et al. 2001; Godin and Mcdonough 2003; Hernandez-Jimenez and Rios-Cardenas 2012). Potential predator-induced changes in male mate choice, however, have not been investigated yet.

Innate and learned (experiential) effects

In a study on female mate choice in another poeciliid fish *Poecilia mexicana*, predator-naïve (laboratory-reared) females altered their preferences for large-bodied males when exposed to a naturally co-evolved predator and instead showed a preference for small-bodied males (Bierbach et al. 2011). This response was weaker when 2 non-piscivorous fishes were presented, and the authors discuss that the pattern of altering mate choice in presence of certain predator types is likely part of the species' innate behavioral repertoire. By contrast, predator-experienced (wild-caught) females showed much weaker responses. Bierbach et al. (2011) argue that their study involved living predators that were largely inactive and showed "freezing behavior" upon transfer into the testing tanks (see also Lawrence and Smith 1989; Godin 1997; Saaristo et al. 2017).

Predator-experienced focal fish may have been better able to discern the predators' motivational state, that is, they may have not perceived an imminent predation risk by the motionless predators. Given this methodological shortcoming, we revisited the question of whether and how learning about predators and/or innate behaviors affects the extent to which individuals alter their mate choice when a predator is present. We avoided effects of predators' inactivity by using computer-animated predator images to study how male and female *G. affinis* alter their mate choice for large-bodied mating partners.

We conducted dichotomous mate choice tests (Dugatkin and Godin 1992; Kodric-Brown 1993; Walling et al. 2010) and repeated the tests while presenting either a co-evolved predator (green sunfish, *Lepomis cyanellus* [Centrarchidae]) that does not currently co-occur with mosquitofish in the part of the invasive distribution area from which we collected our test fish, or a currently co-occurring but not co-evolved predator (Northern snakehead, *Channa argus* [Channidae]). Moreover, we used both wild-caught (predator-experienced) and laboratory-reared (predator-naïve) individuals. We hypothesized that the predator-naïve cohort of test fish would change their mating preferences when facing the co-evolved predator (alluding to innate effects), whereas the predator-experienced cohort could respond more to the currently co-occurring predator if learning about predators also plays a role (Peterson et al. 2007; Kozak and Boughman 2015).

Since invasive *G. affinis* populations in China are rather young (<100 years old; Gao et al. 2017), we predicted behavioral responses to currently co-occurring predators to be based on learning. However, should rapid evolution (rather than learning) be the main driving force underlying behavioral responses to presently co-occurring predators, we would expect similar responses in wild-caught and F_1 laboratory-reared fish.

Personality effects

Consistent inter-individual variation in behavioral tendencies (animal personality; Sih et al. 2004; Réale et al. 2007) can affect mate choice decisions (Schuett et al. 2010; Bierbach et al. 2015; Chen et al. 2018). For example, Sommer-Trembo et al. (2016a) found that exploration tendencies affected females' strength of preference for conspecific versus heterospecific males in *P. mexicana*. Personality and body size may also interact to influence mating preferences. For instance, Chen et al. (2018) studied mate choice in *G. affinis* and found that within the cohort of large-bodied focal females, mating preferences for large-bodied males increased with increasing levels of boldness of the focal females. Given the complexity of these interactions and the lack of empirical studies focusing on the effects of animal personality on predator-induced changes

in individual mating decisions, we did not formulate specific predictions. However, we expected shy individuals to change their preferences in response to the presence of a predator to a larger degree than bold individuals since they are, per definition, more risk-averse (Réale et al. 2007).

Effects of body size

We also included the choosing individuals' body size as a potential explanatory variable when analyzing variation in the extent to which focal individuals alter their mate choice behavior in presence of a predator. For example, in the aforementioned study by Chen et al. (2018), personality effects became manifest only within the cohort of focal fish whose body size was larger than average. Body size is a correlate of age in female *Gambusia* spp. (which have indeterminate growth) but to a lesser extent in males, which grow less after reaching maturity (Johnson 1976; Hughes 1986; Zulian et al. 1993; Vargas and Sostoa 1996; Pyke 2005). Age usually correlates with experience, and experience can play a role in predicting individuals' anti-predator responses (Griffin et al. 2001). For example, Hawkins et al. (2007) found that salmon *Salmo salar* aged 16–20 weeks had learned to recognize predators while 3-week-old juveniles exclusively showed innate behavioral responses. Again, empirical studies on the potential relationship between body size and predator-induced changes in mating decisions are as yet lacking, but we tentatively predicted that the effects of predator-experience and personality, as outlined before, become more evident within the cohort of large-bodied focal individuals (especially in females).

Materials and Methods

Origin and maintenance of test subjects

We used both predator-experienced and -naïve adult Western mosquitofish *Gambusia affinis* from the species' invasive distribution range in China as our test subjects. Western mosquitofish were introduced to mainland China for malaria prophylaxis less than 100 years ago (Gao et al. 2017). Predator-experienced (wild-caught) fish were collected near the city of Hangzhou (Zhejiang Province, 120°15.58′ N, 30°27.70′ E) in June 2017. Predator-naïve (lab-reared) fish were first-generation descendants of wild-caught individuals collected in and around the cities of Hangzhou, Ankang (Shaanxi Province, 108°80.88′ N, 32°72.63′ E), Lishui (Zhejiang Province, 119°92.28′ N, 28°46.76′ E) and Quanzhou (Fujian Province, 118°67.57′ N, 24°87.41′ E). Chinese regulations dictate preventing the release of *Gambusia* spp. into natural water bodies (Ministry of Environmental Protection of the People's Republic of China 2016, Index No. 000014672/2016-01463), and capturing mosquitofish in the wild does not require any official permit.

All wild-caught test subjects were acclimated to laboratory conditions for 1 month before the behavioral tests. We maintained them in aerated and filtered 200-L tanks at $25 \pm 0.5°C$ under a 12 h light: 12 h dark photoperiod. Aquaria were well equipped with plants, twigs and stones and contained approximately 40 adults of both sexes per tank. We fed the fish twice a day *ad libitum* amounts of commercially available flake food and frozen blood worms (chironomid larvae), as well as *Artemia salina* nauplii and shrimps. Every week we removed feces from the bottom and replaced 30% of the water. We used aged and filtered tap water for the water changes and throughout the behavioral tests.

We identified sexually mature females based on the presence of a gravid spot (Hoese and Moore 1977). The standard length (SL) of focal females ranged from 19 to 38 mm (mean $\pm$ SD: 29.90 ± 0.50 mm, $n = 90$), which reflects body size variation of mature females in natural populations (Pyke 2005). Most focal females were likely pregnant as they displayed a distended abdomen. Poeciliid females are more receptive to male approaches for some days after giving birth (Plath et al. 2001; Magurran 2005), and so we avoided using postpartum females in our tests. Sexually mature focal males (18–29 mm; 23.04 ± 0.24 mm, $n = 90$) were identified based on the presence of a fully developed gonopodium—a modified anal fin used to inseminate females (Hoese and Moore 1977)—and based on behavioral observations (i.e., sexual interactions with females inside the stock tanks; Collier 1936).

Behavioral tests were conducted between 6 July and 17 September 2017. We isolated focal individuals, separated by sex, in 96-L tanks with an external water filter and an air stone attached to an air pump, 24 h prior to the tests. To avoid aggressive interactions and to enable tracking test subjects' ID through the course of the behavioral assessments, we kept each focal fish separately in 1.5-L transparent perforated plastic bottles before and between the different behavioral tests. The perforation allowed exchange of water and oxygen with the environment (Polverino et al. 2016; Chen et al. 2018). Longer isolation from females can alter poeciliid males' motivation to mate (Franck 1975; Plath et al. 2008), and so we kept the isolation time short to avoid such effects.

Assessment of mating preferences
Generation of computer animations
Computer-animated stimuli have been successfully applied to study animal behavior in an array of species and in various contexts (e.g., Baldauf et al. 2009; Chouinard-Thuly et al. 2017; Scherer et al. 2017a), including male and female mate choice in *G. affinis* (Chen et al. 2018). In this study we used computer-animated stimulus pairs to study mate choice decisions in a dichotomous choice situation. Stimulus pairs showed 2 images of the same individual, which we manipulated in a way that they differed in body size, but not in other morphological or behavioral traits that could affect mate choice decisions (Chouinard-Thuly et al. 2017). The computer animations ($n = 24$ per sex) were generated from high-resolution photos previously used in Chen et al. (2018). Each photo showed stimulus individuals in lateral view. We largely followed the procedures and settings described in Chen et al. (2018) to generate animations, but increased body size difference. To do so, we assessed the mean SL of an empirical sample of $n = 127$ males (mean $\pm$ 2 SD: 22.30 ± 5.72 mm) and $n = 141$ females (29.10 ± 9.34 mm) collected in the wild and subtracted (small-bodied animation) or added (large-bodied animation) 2 SD (instead of 1 SD: Chen et al. 2018). This decision was made because pre-trials revealed that focal individuals of our study species express stronger baseline preferences for large mating partners using those animation parameters.

We asked if focal individuals would alter their mate choice decisions when facing imminent predation risk (Johnson and Basolo 2003). However, predators may show freezing behavior, where individuals cease to move and stay close to the bottom for extended periods of time upon introduction into a novel environment (see discussion in Bierbach et al. 2011). To avoid such effects, we used computer-animated images of piscine predators. We obtained images of a resolution >544 pixels (width) × 364 pixels (height), that is >198,016 pixels, showing the predator in full lateral view, from fish encountered in local fish markets and during field work, as well as from publicly available internet sources (for details see Supplementary Figure S1). We successfully generated $n = 11$

animations showing Northern snakehead (*Channa argus*), which is a widespread, voracious fish predator in large parts of China, and has even expanded its natural distribution range after escaping from aquaculture (Courtenay and Williams 2004). We included $n = 11$ animations showing an originally co-evolved (North American) predator, namely green sunfish (*Lepomis cyanellus*; Peterson et al. 2007). This species was introduced to China in 1998 and became an economically important species in some regions like Guangxi, Guangdong, Hunan and Hubei Provinces (Li et al. 2014). However, *L. cyanellus* does not currently co-occur with *G. affinis* in those areas from which we collected our test fish (information obtained from local Fisheries Bureaus). To test if focal individuals would show consistent mate choice behavior when no predatory fish was presented, we also generated control animations showing a non-piscivorous species, for which we used the widespread (and invasive) Asian topmouth gudgeon (*Pseudorasbora parva* [Cyprinidae]; $n = 10$ animations). Body sizes of the animated predators (on screen; see below) were adjusted as 75.0 mm for *P. parva* and 250.0 mm for *C. argus* and *L. cyanellus*. Each computer animation showed 1 virtual predator swimming in a straight line from left to right and back in front of a uniformly light gray background (2.63 cm s^{-1}), with an invisible turn of 1 body length before changing swimming direction, that is, we let the animated fish continue to swim outside the display window for 1 body length and then change swimming direction without being seen by the focal fish (Bierbach et al. 2013; Chen et al. 2018).

Association preference tests

Our set-up for dichotomous association preference tests consisted of a transparent glass tank (60 × 30 × 35 cm) with 2 computer screens (L1510A, 38 cm diagonal length, Lenovo, Beijing, China) placed on both smaller ends to show a pair of stimulus males or females, respectively. The calibration configuration of both screens was identical with respect to brightness and hue. We placed another screen (LS27D360, 69 cm diagonal length, Samsung, Tianjin, China) behind the backside of the test tank for the presentation of a predator (Figure 1C). The test tank was visually divided into 3 zones by black marks: the central zone was designated as the neutral zone (40 cm) and the 2 lateral zones as preference zones (10 cm). To minimize disturbance from the outside, the front of the test tank was covered by black plastic foil. We filled the tank with aged and filtered tap water to a level of 25 cm, which matched the height of the screens. Water temperatures matched those in the holding tanks.

Mate choice tests were conducted between 8 a.m. and 5 p.m. To initiate a trial, we introduced a focal individual into a clear Plexiglas cylinder (10 cm diameter), placed centrally into the neutral zone, and started playback of the first pair of animations. We gave the focal individual 3 min for acclimation, during which it could see both animations. Then, we gently removed the cylinder and measured the time the focal individual spent in each preference zone during a 5 min observation period (Sato and Karino 2006; Scherer et al. 2017b). To avoid potential side-biases, we interchanged both animations (from left to right and vice versa) immediately after the first 5 min observation period and repeated the assessment of association preferences. This episode is henceforth called the 1st part of the mate choice tests.

During the entire time of the 2nd part of the tests, we presented predator animations on the screen at the backside of the test tank (Figure 1C). In Treatment 1, focal fish were presented with currently coexisting, non-piscivorous *P. parva*, which served as a control for consistency of mating preferences. In Treatment 2, we presented focal fish with the currently coexisting, piscivorous *C. argus*. Treatment 3 used *L. cyanellus*, a co-evolved predator of *G. affinis*
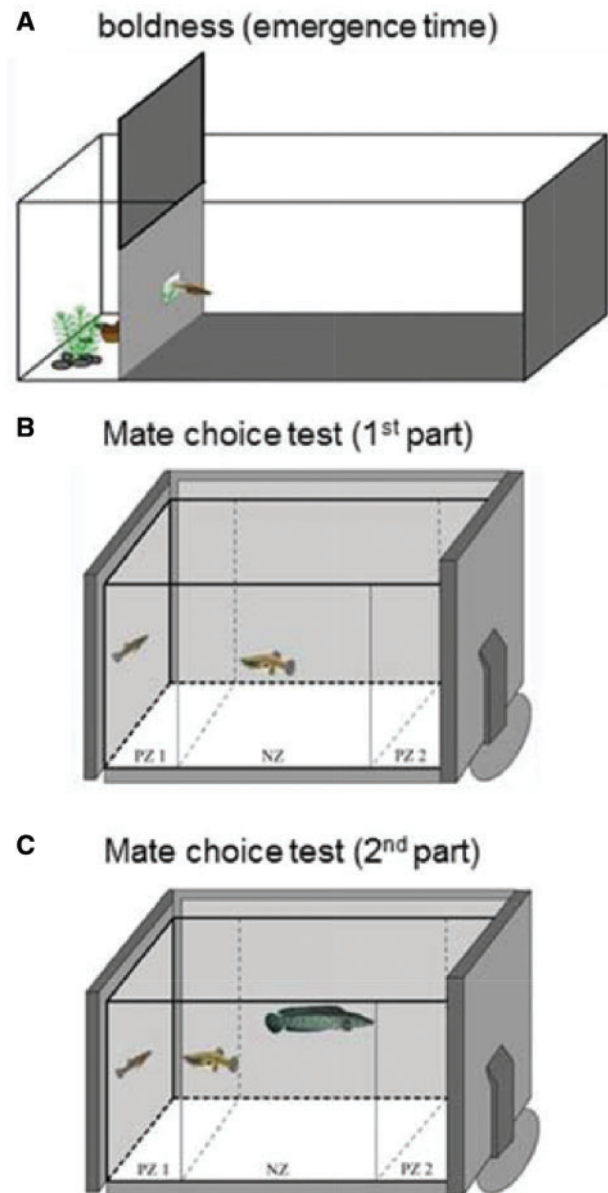

Figure 1. Schematic view of the experimental set-ups used to determine **(A)** boldness and **(B, C)** changes of mating preferences under predation risk. **(B)** We assessed the time focal individuals spent in both preference zones (*pz 1*, *pz 2*) near 2 monitors showing animated stimulus individuals of the opposite sex (large- and small-bodied) and **(C)** repeated tests whereas an animation showing a predator (here: *Channa argus*) was presented. *nz*, neutral zone. Fish are not drawn to scale.

(Peterson et al. 2007) that does not currently co-occur with *G. affinis* in those parts of China from which we collected our test subjects. We repeated measurement of individual association times (including changing side-assignments of the stimulus animations) as described for the 1st part of the tests. We thus tested $n = 15$ test subjects per "sex—rearing environment—treatment" combination, coming to a total of 180 individual trials.

Personality assessment and measurement of body size

The same individuals were also characterized twice for boldness (on 2 consecutive days), for which we used an established experimental

design: the emergence test ([Brown and Braithwaite 2004](); [Brown et al. 2005](); [Scharnweber et al. 2011]()). We used a transparent glass tank ($80 \times 30 \times 30$ cm) as our test arena, which was filled with aged and filtered tap water to a height of 18 cm ([Figure 1A]()). We covered all outer sides with gray plastic foil to minimize disturbance and placed gray cardboard under the bottom of the tank. The tank was divided by a gray plastic divider (30×30 cm) into 2 parts: a shelter area ($20 \times 30 \times 30$ cm) and an unknown area ($60 \times 30 \times 30$ cm). The divider contained a trapdoor (a 30×30 cm opaque plastic plate could be lifted by a remote pulley system so as to open a 4×4 cm hole in the center of the divider; [Figure 1A]()). Light-colored gravel, artificial plants and stones provided structural diversity in the shelter area similar to the stock tanks. Illumination was provided by a 35 W LED lamp 40 cm above the tank in addition to diffuse room illumination.

To initiate a trial, we gently transferred a focal individual into the shelter area where it was given 2 min for acclimatization before the trapdoor was remotely opened. We determined the time the focal fish needed to emerge from shelter (latency time), with bolder fish emerging faster to explore the novel environment. We terminated a trial when the focal fish had left the shelter completely or after a maximum ceiling value of 10 min (i.e., if the focal fish did not leave the shelter). This concerned $n = 33$ trials (11 males and 22 females), equaling 18.3% of our test subjects.

All focal individuals were measured for SL upon completion of the behavioral tests by briefly laying them flat on laminated millimeter paper. We made sure that no fish was tested twice by keeping tested individuals in separate tanks until completion of all experiments.

Statistical analyses

All statistical analyses were conducted in SPSS19. We tested for normality of error distributions using Kolmogorov-Smirnov-tests and arcsine (square root)-transformed all relative data prior to the statistical analyses. All descriptive statistics are presented as mean values with associated standard errors (SEM).

To evaluate preferences for large-bodied mating partners ([Chen et al. 2018]()), we compared the amount of time focal fish spent near the large and small stimulus fish during the 1st part of the preference tests as well as during the 2nd part of all 3 treatments using paired t-tests. Males and females were analyzed separately. Our study addressed questions regarding sex- and body size-specific, as well as personality-dependent changes of individual mate choice decisions in response to currently co-occurring (but not co-evolved) and co-evolved (but not currently co-occurring) predators. To answer these questions, we calculated a score expressing individual changes in mate choice decisions from the 1st to 2nd test part ([Bierbach et al. 2011]()). The preference score was calculated as the difference between focal individuals' relative association times near the initially preferred virtual stimulus fish during the 2nd part of the tests (independent of whether or not this was the larger individual) and relative association times near the same individual during the 1st part (before presentation of a predator). No change in focal individuals' preferences would lead to a score of zero, negative values would indicate that focal individuals spent less time near the initially preferred fish in the 2nd part of a trial, and positive values would indicate that focal individuals spent relatively more time near the initially preferred fish. Scores were used as the dependent variable in a univariate General Linear Model (GLM) using "sex," "animation type" (treatment) and "predator experience" as factors. We

included "SL" (standard length) and "boldness" (means from both boldness assessments) as covariates.

Initially, we included 3-way interaction terms to test, for example, whether personality differentially affects the responses of both sexes to different predator types ("animation type $\times$ sex $\times$ boldness"), or whether personality effects become apparent only in predator-naïve individuals ("animation type $\times$ predator experience $\times$ boldness"; [Sommer-Trembo et al. 2016b]()). However, we step-wise excluded all 3-way interactions as none were statistically significant ($F < 0.05$, $P > 0.95$).

We also initially included all 2-way interactions to address, for example, the question of whether both sexes differ in their responses to different predator types ("animation type $\times$ sex") or whether personality has different effects in both sexes irrespective of the animation type ("sex $\times$ boldness"). However, we excluded non-significant interaction terms from our final model (sex $\times$ SL: $F_{1,\ 165} = 0.72$, $P = 0.40$; predator experience $\times$ boldness: $F_{1,\ 165} = 1.58$, $P = 0.21$; predator experience $\times$ SL: $F_{1,\ 165} = 2.32$, $P = 0.13$; sex $\times$ predator experience: $F_{1,\ 165} = 0.59$, $P = 0.45$).

We also addressed the question of whether focal individuals spent less time associating with both stimulus individuals combined during the 2nd part (with predator) compared with the 1st part of the tests (without predator). This information was intended to augment the interpretation of our results on predator-induced changes in mating preferences and is presented in [Online Supplementary Material S2](). We ran a GLM with a similar model structure as outlined above, this time using differences in total association times (2nd part − 1st part) as the dependent variable.

We tested the repeatability (R) of boldness to quantify the degree of behavioral consistency ([Bell et al. 2009]()). We used univariate mixed models to obtain within- and among-individual variance estimates of the data from our boldness assessment, using emergence times as the dependent variable, and "sex" and "predator experience" as fixed factors. Significant deviations of R from zero were tested by likelihood ratio tests, comparing the model fit of a model including individual ID as a random factor and 1 excluding it (i.e., constraining individual variance to zero; [Nakagawa and Schielzeth 2010]()).

Results

Association preferences for large-bodied mating partners

Both predator-experienced (wild-caught) and predator-naïve (lab-reared) focal females spent significantly more time in association with larger males during the 1st part of the preference tests (i.e., before a predator was presented; [Figure 2A and B]()). By contrast, only predator-experienced focal males showed a significant directional preference for large-bodied females during the 1st part of the tests ([Figure 2C]()), whereas predator-naïve males showed no overall preference ([Figure 2D]()).

We predicted that initial preferences should remain unchanged during the 2nd part of the tests (suggesting behavioral consistency) in the control treatment using *P. parva* animations. However, a more complex pattern became apparent: in most treatment groups (including the *P. parva* treatment), focal individuals no longer expressed an overall preference for either stimulus type. Only predator-experienced males ([Figure 2C]()) and predator-naïve females ([Figure 2B]()) spent significantly more time in association with large-bodied stimulus fish in the *L. cyanellus* treatment. Notably, a "reversed" pattern (i.e., preference for small-bodied females) was
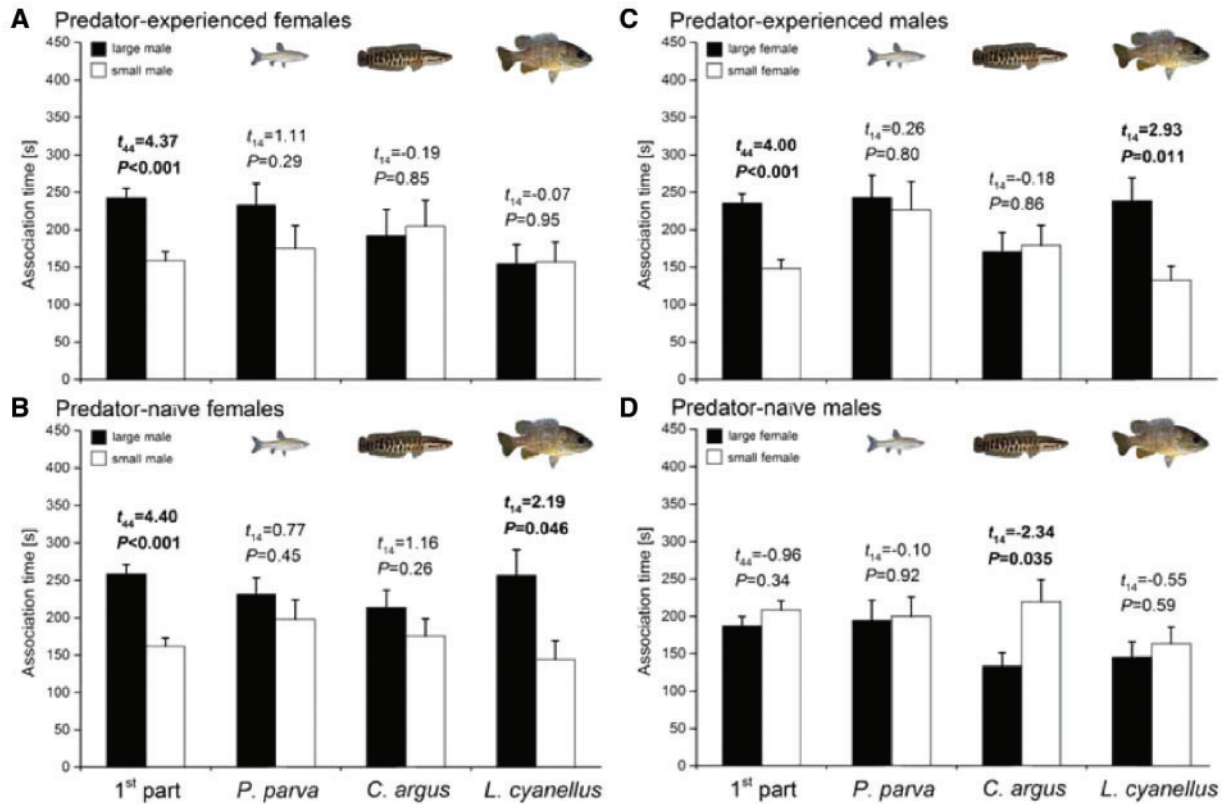
Figure 2. Mean (± SEM) time focal individuals spent in association with the large (black bars) and the small stimulus fish (open bars) during the 1st part of the choice tests (*left*) and during the 2nd part, when an animation showing a predator was presented (*from left to right: Pseudorasbora parva* [control]; *Channa argus* [co-occurring predator]; *Lepomis cyanellus* [co-evolved but not co-occurring predator]). Results are shown separately for **(A, B)** females and **(C, D)** males, and for **(A, C)** predator-experienced (wild-caught) and **(B, D)** predator-naïve (laboratory-reared) individuals. Significant results from paired *t*-tests are highlighted in bold.

observed in predator-naïve focal males in the *C. argus* treatment (Figure 2D). For all subsequent analyses, we condensed information from both test parts into a score expressing changes in individual mate choice decisions between both test parts (independent of whether or not the focal individual had preferred the large- or small-bodied stimulus during the 1st part).

Changes in individual mate choice decisions

We compared the preference score between sexes, rearing environments (experience with predators), and animation types and found a significant main effect of "animation type," whereas no other main effect was statistically significant (Table 1). However, the overall effect of "animation type" needs to be interpreted in light of 4 significant interactions terms involving this factor. Interaction terms related to learning/experiential effects ("animation type × predator experience"), sex-specific responses to the different animations ("animation type × sex"), and personality-dependent responses to the different animations ("animation type × boldness"). Finally, a significant interaction effect of "animation type × body size" (SL) was uncovered (Table 1).

Learning/experiential effects

The degree to which individuals changed their preferences from the 1st to 2nd part of the tests in response to the different types of predator animations differed significantly between wild-caught (predator-experienced) and laboratory-reared (predator-naïve) individuals (Figure 3A). Predator-experienced test subjects showed

Table 1. Results of a univariate GLM using preference scores (see main text) as the dependent variable

Source of variation	Df	F	P	Wilks' partial η_p^2
Animation type	**2**	**4.42**	**0.014**	**0.051**
Predator experience	1	1.26	0.26	0.008
Sex	1	0.52	0.47	0.003
Boldness	1	1.78	0.18	0.011
SL	1	0.08	0.78	0.001
Animation type × predator experience	**2**	**3.76**	**0.025**	**0.044**
Animation type × sex	**2**	**3.24**	**0.042**	**0.038**
Animation type × boldness	**2**	**6.27**	**0.002**	**0.071**
Animation type × SL	**2**	**3.52**	**0.032**	**0.041**
Error	165			

Preference scores express changes of individuals' mating preferences when a predator was presented. Significant effects are highlighted in bold.

pronounced changes (i.e., negative preference score-values) in mate choice behavior when presented with the presently co-occurring (but not co-evolved) Northern snakehead *C. argus*, whereas considerably weaker responses (preference score-values closer to zero) were observed in both other treatments. By contrast, predator-naïve individuals responded most to the animations showing the co-evolved (but not presently co-occurring) *L. cyanellus*, but also to the non-piscivorous (not co-evolved but presently co-occurring) *P. parva* (Figure 3A).
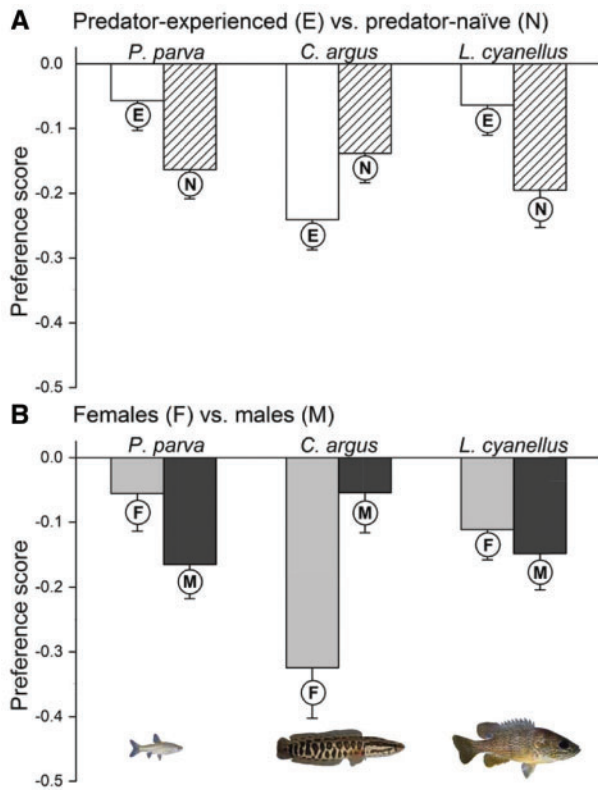
Figure 3. Changes of mating preferences in *G. affinis* when focal individuals were confronted with a piscine predator. Visualized are significant interaction effects of **(A)** "animation type × predator experience" and **(B)** "animation type × sex" (compare Table 1). We depict preference scores (residuals, corrected for other model terms), whereby negative values indicate that focal individuals spent less time in association with the initially preferred stimulus individual during the 2nd part of a trial (when a predator was presented).

Sex-specific responses to different predator types

We found that female focal individuals altered their preferences most when we presented Northern snakehead *C. argus* as a predator during the 2nd test part, whereas males showed a different response pattern and changed their mating preferences least in this treatment (Figure 3B). Although males did not show the strong alteration of mating preferences observed in females in any treatment, they did show moderate responses to the *P. parva* and *L. cyanellus* animations (Figure 3B).

Personality-dependent responses to different predators

In the cohort of wild-caught fish, we found significant estimates of repeatability (R-values) for boldness (i.e., emergence times) in females ($R = 0.24$, $P = 0.020$), but low and non-significant estimates in males ($R = 0.15$, $P = 0.24$). In the laboratory-reared cohort of test fish, we detected significant repeatability in both sexes, with females showing higher estimates ($R = 0.50$, $P < 0.001$) than males ($R = 0.27$, $P = 0.043$). Altogether, our results suggest that laboratory-reared individuals show higher consistency in emergence times than wild-caught ones, and females show higher behavioral consistency than males.

Our measure of consistent individual variation in boldness (i.e., emergence times) differentially affected predator-induced changes in mating preferences according to the type of predator presented during the 2nd part of the tests (Figure 4A). Based on our measure of
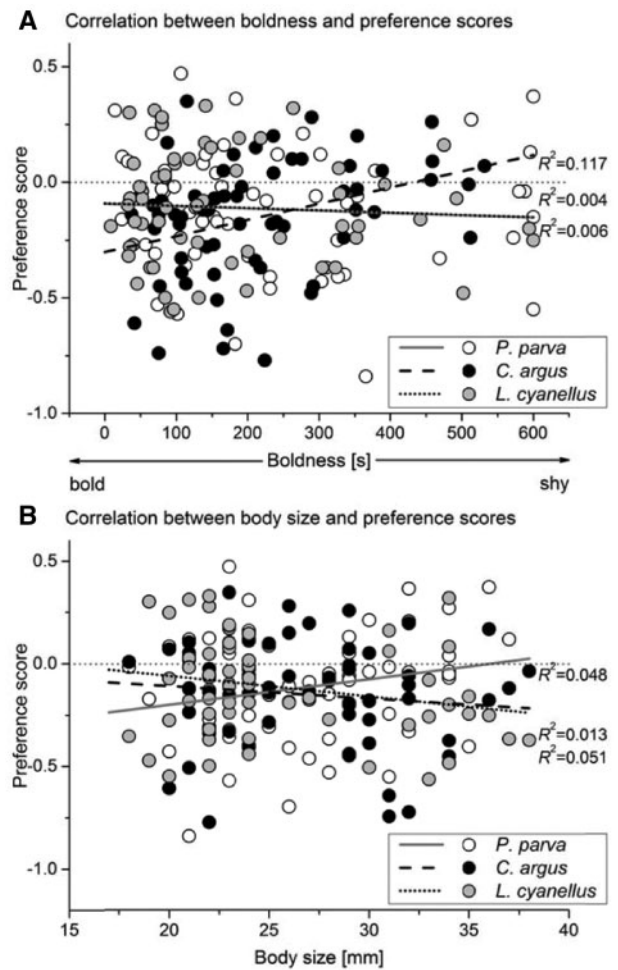

Figure 4. Changes of individual mating preferences in presence of a piscine predator. Visualization of significant interaction effects of **(A)** "animation type × boldness" (whereby longer emergence times represent shy individuals) and **(B)** "animation type × body size" (Table 1). Depicted are preference scores, whereby negative values indicate that the strength of individual mate choice decisions decreased when a predator was presented. Results of Pearson correlations are presented (R^2). Note that **(A)** bolder individuals changed their mating preferences in presence of the predatory *C. argus* whereas shy individuals were more consistent in their mate choice, whereas no such effect was seen in both other treatments. **(B)** Weak effects of focal individualss SL became evident, with larger individuals being more consistent in their mate choice decisions in the control treatment (*P. parva*), whereas the opposite pattern was observed for both predator treatments (*C. argus*, *L. cyanellus*).

effect strengths (partial η_p^2), the interaction effect between "animation type × boldness" had almost twice the effect strength of other significant interaction terms (Table 1). When *C. argus* was presented, individuals that were bold—that is, showed shorter emergence times (left portion of the scatterplot shown in Figure 4A)—changed their preferences to a much greater extent than shy individuals (right portion of the scatterplot). Accordingly, a *post hoc* Pearson correlation revealed a significant positive correlation between preference scores and our measure of boldness ($R^2 = 0.12$, $P = 0.004$, $n = 60$). In both other predator treatment groups, no significant correlations were uncovered (*P. parva*: $R^2 = 0.004$, $P = 0.32$, $n = 60$; *L. cyanellus*: $R^2 = 0.006$, $P = 0.28$, $n = 60$; Figure 4A).

Body size-dependent responses to different predators

In the GLM, the interaction of "animation type × body size" had a significant effect (Table 1), and a *post hoc* Pearson correlation using preference scores revealed a significant positive correlation of preference scores with body size when *P. parva* was presented ($R^2 = 0.048$, $P = 0.047$, $n = 60$; Figure 4B); in other words: larger individuals were more consistent in their mate choice. By contrast, we found larger individuals to be less consistent in their mate choice in the *L. cyanellus* treatment ($R^2 = 0.051$, $P = 0.041$, $n = 60$), and qualitatively (but not statistically significantly) in the *C. argus* treatment ($R^2 = 0.013$, $P = 0.20$, $n = 60$; Figure 4B).

Discussion

In this study, we used body size as a mate choice criterion. Early studies on another poeciliid fish *P. reticulata* provided first evidence that males assess females' body size during mate choice (Haskins and Haskins 1949, 1950), which was later confirmed for *Gambusia* spp. (Bisazza et al. 1989; Hoysak and Godin 2007). Likewise, female preferences for large male body size have been demonstrated for various poeciliid fishes (Reynolds et al. 1993; Rosenthal and Evans 1998; Plath et al. 2007; Tobler et al. 2008), including *Gambusia* spp. (Mcpeek 1992; Bisazza and Marin 1995). In our study, we found a female preference for large male body size in both cohorts of test fish (predator-experienced and -naïve), whereas a male preference for large females was seen only in predator-experienced but not in predator-naïve males. We argue that the specific rearing conditions of our test fish may explain this pattern: we raised offspring of a similar age class in separate tanks so as to prevent cannibalism by larger individuals (Thibault 1974; Hubbs 1991; Weeks and Gaggiotti 1993; Nilsson and Persson 2013; Pereira et al. 2017). Therefore, males were exposed to females of a relatively uniform body size, probably precluding the emergence of mating preferences for large-bodied females. Males, by contrast, developed the natural polymorphism in body size that appears to be under both genetic and social control in many poeciliid fishes (Kallman and Borkoski 1978; Kallman 1983; Snelson 1985; Ryan et al. 1992).

Several aquatic predators of poeciliids prefer large- over small-bodied prey (Trexler et al. 1994; Plath et al. 2003; Tobler et al. 2007), including predators of our study species (Ouyang et al. 2017). This should select for both females and males evolving the ability to alter their mate choice when facing predators to decrease their own predation risk. This study is the first to confirm such an effect in both sexes of the same species, even though sexes may differ in their responses to different predator types (see below).

Learning/experiential effects

We asked whether and how learning and/or innate effects play a role in determining the extent to which individuals change their mating preferences under predation risk. Wild-caught *G. affinis*—which had experienced certain predator types in their natural environment—showed pronounced changes in their mate choice behavior when presented with the currently co-occurring (but not co-evolved) Northern snakehead *C. argus*, whereas laboratory-reared (predator-naïve) test subjects responded most to the co-evolved (but not presently co-occurring) *L. cyanellus*.

Our finding that predator-experienced individuals responded most when confronted with *C. argus* confirms previous reports that predator recognition can build upon learning (e.g., Kelley and Magurran 2003 for guppies). Fish can learn about predators when visual, chemical, or both types of predator cues are presented (Tulley and Huntingford 1987; Magurran 1990; Berejikian 1995; Chivers and Smith 1998; Brown and Godin 1999; Utne-Palm 2001), and individuals can learn to respond to specific predator types after observing the responses of predator-experienced con- or heterospecifics (i.e., social learning, Box 1984; Mathis et al. 1996). For example, juvenile guppies from a low-predation environment acquired avoidance responses towards predators when associating with conspecifics from a high-predation environment (Kelley et al. 2003). Overall then, learning about co-occurring predators appears to allow individuals of our study species to mitigate the costs of mate choice for otherwise preferred phenotypes (here: large-bodied mating partners) by temporarily altering their mate choice behavior (and thus, own predation risk) when encountering certain co-occurring predators.

Meanwhile, predator-naïve individuals responded more when confronted with the co-evolved (but not currently co-occurring) *L. cyanellus*. Our results suggest that inexperienced individuals of our study species show innate predator recognition patterns that can, however, be modified by learning (see also results of additional analyses on total association times presented in Online Supplementary Material S2). Likewise, laboratory-reared Atlantic molly (*P. mexicana*) females responded differently towards 4 types of predators, with the strongest response being observed when the voracious molly predator *Cichlasoma (Trichromis) salvini* was presented (Bierbach et al. 2011). Some studies on predator recognition in aquatic vertebrate prey species, such as amphibians, reported innate predator recognition mechanisms (Kats and Dill 1998), whereas acquired predator recognition is regularly observed (Suboski 1992; Miklósi et al. 1997; Woody and Mathis 1998; Wildy and Blaustein 2001; Mandrillon and Saglio 2005). We argue that innate predator recognition—while mainly driven by direct survival selection—allows individuals of our study species to adjust their mate choice behavior to predation threat in novel environments (before learning becomes possible) or at a young age (see below).

Sex-specific responses to different predator types

Females responded most to animations showing *C. argus*, a voracious fish predator (Courtenay and Williams 2004; Landis and Lapointe 2010). Most poeciliids show a pronounced sexual size dimorphism, with females being larger than males (Bisazza 1993; Bisazza and Marin 1995; Bisazza and Pilastro 1997), and large prey are usually preferred by predators, unless gape limitations lead to a different pattern (Webb and Shine 1993; Persson et al. 1996). Moreover, pregnancy affects swimming performance and decreases the likelihood of escaping piscine predators in livebearing fishes (Plaut 2002; Ghalambor et al. 2004; see also Shine 1980 for reduced running speed in 6 species of Australian scincid lizards). Finally, Plath et al. (2011) reported that a predatory insect (*Belostoma* sp.) preferentially preys on pregnant *P. mexicana* females.

Given that the majority of our female test subjects was likely pregnant (judging by the observation of swollen abdomina), we argue that our study species shows risk-sensitive adjustment of individual mating preferences, with the more vulnerable sex (and possibly pregnant females in particular) showing stronger responses. Future studies will need to test this hypothesis by comparing, for example, the responses of females with known gestational status to predators during their mate choice. Also, future studies may wish to compare the responses of male and female guppies. Guppy males show flamboyant nuptial coloration (Endler 1978, 1980, 1991), rendering them particularly vulnerable to visually-oriented predators (Godin and Briggs 1996; Godin and Mcdonough 2003). Possibly,

male guppies alter their mate choice more in response to predators than females.

Personality-dependent responses to different predators

We found bolder individuals to change their mate choice more than shy individuals in the *C. argus* treatment (see also discussion on personality effects uncovered in additional analyses on total association times presented in Online Supplementary Material S2). This is contrary to our expectation, which we derived from the assumption that shy individuals would be generally more risk-aversive than bold individuals (Réale et al. 2007; Toms et al. 2010; White et al. 2013). However, a meta-analysis testing for fitness consequences of behavioral types found boldness to be associated with a reduced survival rate (Smith and Blumstein 2008). The authors argue that bold individuals are more prone to falling victim to predation than shy individuals. In support of this idea, a recent study by Hulthén et al. (2017) found bold roach *Rutilus rutilus* to be more susceptible to predation by cormorants than shy ones. If bold individuals of our study species also experience an increased predation pressure, our results could be explained by risk-sensitive behavior, where bold individuals show a stronger (adaptive) adjustment of their mate choice behavior under predation threat.

Body size-dependent responses to different predators

Meanwhile, we observed that large individuals were more consistent than small ones in the *P. parva* treatment. We argue that while small fish may perceive a range of other fish species as a potential threat, large individuals 1) may have partly learned to not respond to the non-piscivorous topmouth gudgeon (as large individuals may be older, at least in the female sex; Johnson 1976; Hughes 1986; Zulian et al. 1993; Vargas and Sostoa 1996; Pyke 2005), and 2) small individuals may actually be at a (mild) threat of being caught as prey by a range of otherwise non-piscivorous fishes (Edge et al. 1993; Belk and Lydeard 1994; Schaefer et al. 1994; Howe et al. 1997). The observed pattern could also be a result of large-bodied fish having larger body appendages (especially tail fins and caudal peduncles), resulting in enhanced movement performance and allowing them to escape predation quicker. However, this interpretation does not align with the observation of high behavioral consistency of large-bodied focal individuals only in the *P. parva* treatment.

In the *L. cyanellus* treatment the pattern was reversed, which may be explained by large individuals representing the preferred prey type of several aquatic predators (Plath et al. 2003; Tobler et al. 2007) as they have a higher nutritional value compared with small ones. For example, Plath et al. (2011) demonstrated that giant water bugs preferred large-bodied or pregnant *P. mexicana* as prey. The 2 predator types used in this study are certainly not gape-limited with respect to the body size range characteristic of *G. affinis* (Pyke 2005).

Acknowledgments

Funding

Authors' Contributions

Conflict of Interests

The authors declare that they have no conflict of interest.

Ethical Statement

All experiments studied here accord with current laws and regulations of the PR China and were approved regarding ethics and treatment of animals in research by the Animal Welfare commissioner at the Department of Animal Science of the College of Animal Science and Technology (Dr Lin-Sen Zan; Approval No. 137, 2016). All authors agreed on the publication of the data presented here and contributed during data collection and manuscript writing.

Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

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