



Male mate preference and size-assortative mating in convict cichlids: A role for female aggression?



A.N. Bloch^{a,*}, V.J. Estela^a, J.M. Leese^b, M. Itzkowitz^a

^a Department of Biological Sciences, Lehigh University, Bethlehem, PA, USA

^b Department of Natural Science, DeSales University, Center Valley, PA, USA

ARTICLE INFO

Article history:

Received 17 February 2016

Received in revised form 16 July 2016

Accepted 16 July 2016

Available online 18 July 2016

Keywords:

Amatitlania siquia

Pair formation

Assortative mating

Mate preference

Mate choice

ABSTRACT

Many monogamous species demonstrate size-assortative mating patterns within natural populations. To better understand the role of intersexual selection in this process, we examined the effect of male preference for female body size in the convict cichlid (*Amatitlania siquia*). We provided males with a choice between females that differed in size, relative to each other and in relation to the focal male. Based on previous work, we expected males to prefer the largest available female mates across all treatments. Surprisingly, males spent more time near the smaller of two available females, but only when the other female was larger than the male. Additionally, males spent little time with either of two potential female mates when both females were larger than the male. We hypothesized that while males might prefer the largest of available females, female behavior might limit males from acting on this preference. To test this, males were force paired with a smaller or larger female. Pair formation only occurred when the female was smaller than the male, and females that were larger than their male counterparts showed significantly more aggression when compared to smaller females. Together, these data suggest that in the absence of intrasexual competition, male mate preference for large females in convict cichlids might be limited by female aggression.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Assortative mating by size, defined as a positive correlation between male and female sizes of mate pairs within a population (Arnqvist et al., 1996; Crespi, 1989; Rowe and Arnqvist, 1996), is a common non-random mating pattern in nature (Crespi, 1989; Ridley, 1983). Because size-assortative mating affects genetic demographics (Coyne and Orr, 2004), it can profoundly impact a population's evolution (Arnqvist et al., 1996; Coyne and Orr, 2004; Crespi, 1989). Despite numerous descriptions of this pattern across a wide range of taxa (e.g. arthropods: Crespi, 1989; reptiles: Shine et al., 2001; amphibians: Lu et al., 2009; crustaceans: Bollache and Cézilly, 2004; birds: Helfenstein et al., 2004; mammals: Preston et al., 2005; fish: Rowland, 1989), it remains unclear how the mechanism of assortative mating acts to organize mate pairs (Arnqvist et al., 1996; Ridley, 1983).

To examine the process leading to size-assortative mating, we used the serially monogamous convict cichlid fish (*Amatitlania siquia*). This species forms pairs with males almost uniformly being larger than the females, in both the laboratory (Beeching and Hopp, 1999; Beeching et al., 2004; Santangelo, 2005; but see Leese et al., 2010) and the field (McKaye, 1986; Perrone Michael, 1978; Wisenden, 1994). This mating pattern seems to be influenced by both intrasexual competition and intersexual selection. Regarding the former, cichlid fish are highly aggressive, and within-sex conflicts prior to pair bond formation are common. Size has been shown to be an important factor in determining the outcome of these contests (Haley 1987), with the result being that the largest individuals of each sex generally win contests and are thus available to mate. If these contests are the main factor influencing pair formation, the observed size-assortative pairing in convict cichlids may occur simply because males have a larger maximum body size than females. As competitions occur, the largest male and largest females win contests leading to the formation of a pair bond, with the next largest of each sex doing the same, and the pattern continues throughout the population. In this scenario, intrasexual competition alone could drive size-assortative mating.

* Corresponding author at: Department of Biological Sciences, Lehigh University, 111 Research Drive, Bethlehem, PA 18015, USA.

E-mail address: anb212@lehigh.edu (A.N. Bloch).

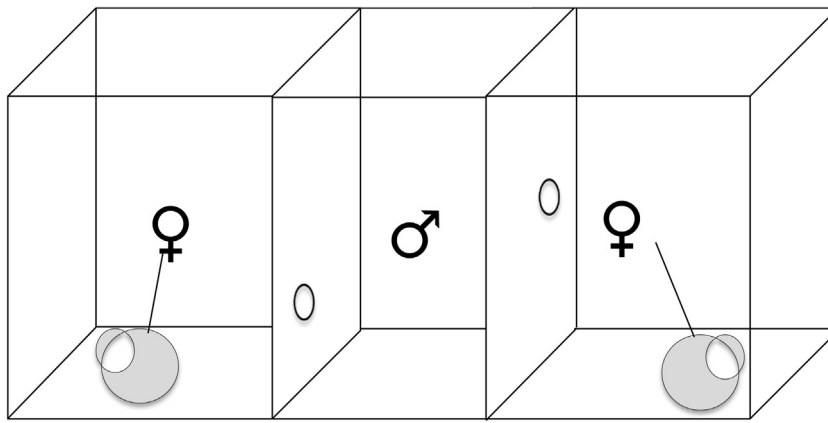


Fig. 1. Diagram of the male choice tank. On each side, a female was tethered to a small flower pot and were unable to leave their compartment. The central compartment (“neutral” compartment) initially held the male. The male had free-access into both female compartments via a small hold placed in the opaque dividers. See Methods for further details.

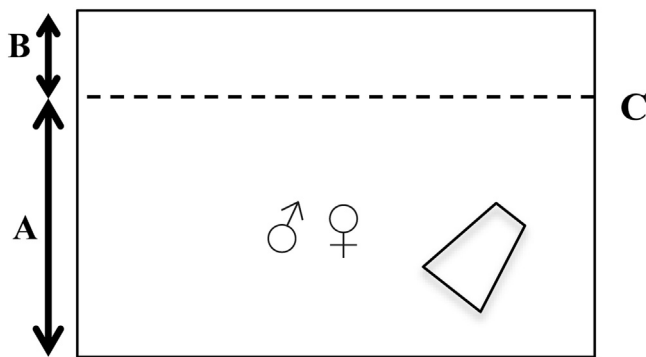


Fig. 2. Diagram of tank used in Experiment II. (A) The male/female (♂/♀) focal compartment (22.8 cm) with flowerpot. (B) intruder compartment (7.6 cm). (C) clear divider.

Intersexual selection, i.e. mate choice, has been shown to influence size-assortative mating in convict cichlids (Noonan, 1983; Beeching et al., 2004; Gagliardi-Seeley et al., 2009). Studies have primarily focused on female preference for large body size in males, but male preference for female body size has also been explored. Beeching and Hopp (1999) observed that convict cichlid males prefer to associate with the largest of available females, even if the female size exceeded their own, suggesting that male preference alone would not be responsible for male-larger size-assortative mating patterns in nature. This study, however, observed male preference when males could view females through a clear partition, which prevented individuals from physically interacting and reverse-size assorted pairs (i.e. female-larger) from actually forming. Whether the interaction and/or behavior of one or both individuals would impede or completely prevent female-larger pairs from forming is unclear. Leese et al. (2010) suggested this might be the case by demonstrating low rates of pair bond formation in convict cichlids when males were placed with females that were larger than themselves.

To more thoroughly examine these interactions in the convict cichlid, we observed male preference for potential female mates by allowing males to visit and physically interact with females in the

absence of direct intrasexual competition. We provided males with one of three treatments: (1) two females that differed in size, with the smaller female being size-matched to the male and the larger female being larger than both (2) two females size-matched to each other, but both larger than the focal male, and (3) two females size-matched to each other and the focal male. If intrasexual competition alone is responsible for pair formation (*sensu* Haley, 1987), we predicted that males would show a time-based preference for the larger of two females available, when females differed in size. When given the choice between size-matched females, we predicted that males would still show a preference, albeit a weaker one, for one female over another, and that this would occur regardless of whether females were larger than the male or not. If males did not show a clear preference for the larger of two females, when the female was larger than the male, or showed differences in preference patterns, when females were size-matched to each other but larger than the focal male, it might suggest that female behavior toward a male smaller than herself could impact or limit a male's preference.

To further explore this possibility, we also examined the behavioral interactions between males and females (*sensu* Leese et al., 2010) in a second ‘no-choice’ experiment. We placed a single male with a female that was either larger or size-matched together in a tank. We monitored aggression levels of the females in the pair to see if larger females treated males differently than size-matched females. We know from previous work that female-larger pairs are less likely to form, but this was the first attempt to determine if female behavior contributed to this result. We also observed whether male aggression levels were different when the female was smaller or larger.

2. Methods

The fish used in this study were a mixture of fish that were wild-caught and purchased through a distributor. All fish were separated by sex and maintained at $25 \pm 3^\circ\text{C}$, on a 14:10 light:dark cycle. To avoid pseudoreplication, all fish were tested only once. All experimental procedures were conducted in accordance with Lehigh Institutional Animal Care and Use Committee protocol #176.

Table 1

Mean total length of focal males and stimulus females for all treatments. Different letters within each treatment indicate significant differences ($p < 0.05$).

	Treatment 1A			Treatment 1B			Treatment 1C		
Total Length (mm)	Small Female	Male	Large Female	Large Female 1	Male	Large Female 2	Small Female 1	Male	Large Female 2
	63.76 ^a	63.76 ^a	73.88 ^b	71.72 ^b	61.74 ^a	71.78 ^b	64.2 ^a	64.2 ^a	64.2 ^a

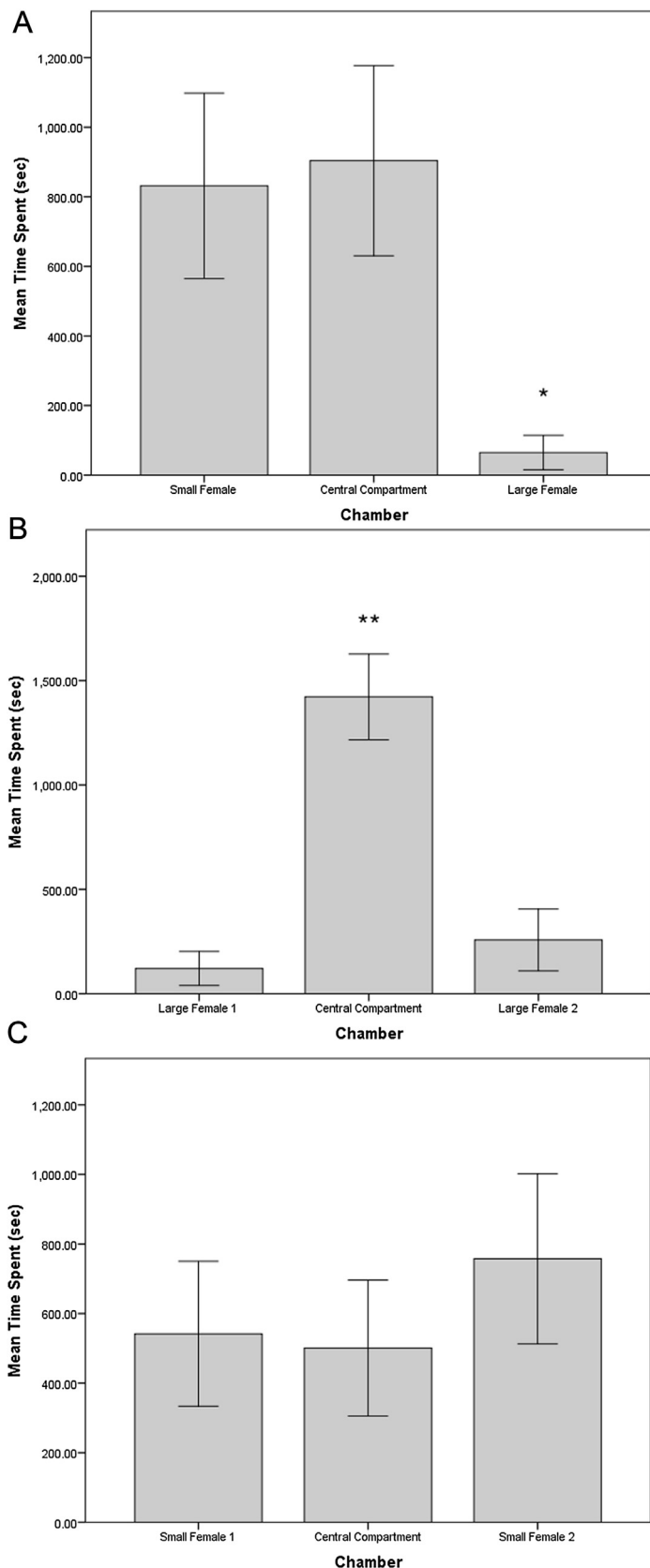


Fig. 3. Time based male preference in Experiment I. Bars show the mean time (sec), with standard error, that focal males spent in the chambers of each treatment ($n = 10$). A) Treatment IA; Small Female: 831.6 ± 267 mm, Central Compartment: 903.6 ± 273.1 mm, Large Female: 64.8 ± 49.5 mm. B) Treatment IB; Large Female 1: 120.6 ± 81.7 mm, Central Compartment: 1422 ± 205.4 mm, Large Female 2: 257.4 ± 148.3 mm. C) Treatment IC; Small Female 1: 541.8 ± 208.4 mm, Central Compartment: 500.8 ± 195.6 mm, Small Female 2: 757.4 ± 244.9 mm * $p < 0.05$; ** $p < 0.001$.

2.1. Experiment I

This experiment presented males with a choice of two different females and allowed males to physically interact with either female, while preventing the females from interacting with each other. Males were presented with one of three female size treatments (Table 1). In the first treatment (Treatment IA; $n = 10$), the focal male was offered a choice between one female that was size-matched to the focal male (± 2 mm) and the other that was 10 ± 1 mm larger than the focal male. The second treatment (Treatment IB; $n = 10$) provided a male with two females; both of which were 10 mm (± 1 mm) larger than the focal male. The third treatment (Treatment IC; $n = 10$) offered the focal male a choice between two females size-matched to each other (± 2 mm), as well as the focal male (± 2 mm).

Two opaque partitions divided a 75.7 L experimental aquaria into three compartments, two-side compartments being 30.5 cm long and a 15.2 cm central compartment (Fig. 1). Dividers had a single hole to allow focal males access to all compartments. Before the start of a replicate, females were anaesthetized with MS-222, then implanted with a thin microfilament line passed through each fish's epaxial muscle. These microfilament lines were then quickly tied into a small loop. To tether females to a particular compartment, a second microfilament line (15.2 cm) connected each female's implanted loop to a flowerpot that also acted as a nesting site. This tethering procedure caused no infection or mortality. Tethering was necessary to limit the range of fish, and caused minimal distress to the animals. Tethered fish do not appear stressed and are able to normally interact with nearby fish, are able to perform aggressive behaviors, and spawn successfully (Nilsson and Nilsson, 2000; Draud and Itzkowitz, 2004; Santangelo, 2005; Leese, 2012). After surgery, females and their flowerpots were immediately placed into each tank's respective side compartment. One hour later, the male was placed in the center compartment. To limit the stress and potential discomfort to stimulus fish, 24 h after the male's introduction, a video recording (20 min) was taken of the entire tank then later analyzed using JWatcher Behavioral Analysis software (www.jwatcher.ucla.edu).

2.2. Experiment II

To further examine how relative size might influence the pair formation process, aggression in male-larger and female-larger pairs was observed. This was performed using two non-tethered treatments titled Treatment IIA and Treatment IIB. In Treatment IIA, females were 10 ± 1 mm smaller than the male ($n = 11$). In the second treatment (Treatment IIB), the single female was 10 ± 1 mm larger than the male ($n = 11$).

Each 75.7 L experimental tank was divided lengthwise to have a large focal chamber, with a clay flowerpot, at the front of each tank (22.8 cm) and a narrower 'intruder' compartment at the back (7.6 cm; Fig. 2). Dividers were drilled with small holes to allow water flow between compartments. To encourage pair formation, three juvenile convict cichlids were placed in the intruder compartment (Itzkowitz and Draud, 1992; Leese et al., 2010). At the start of each replicate, a male and female convict cichlid were placed simultaneously into the focal chamber of each tank, and 24 h later all replicates were video recorded for 20 min then analyzed for aggression (bites and chases).

2.3. Statistics

In choice tests, the amount of time an individual spends in each stimulus compartment is a common indicator of preference and eventual mate choice (Beeching and Hopp, 1999; Nuttall and Keenleyside, 1993). Consequently, the time males spent in the com-

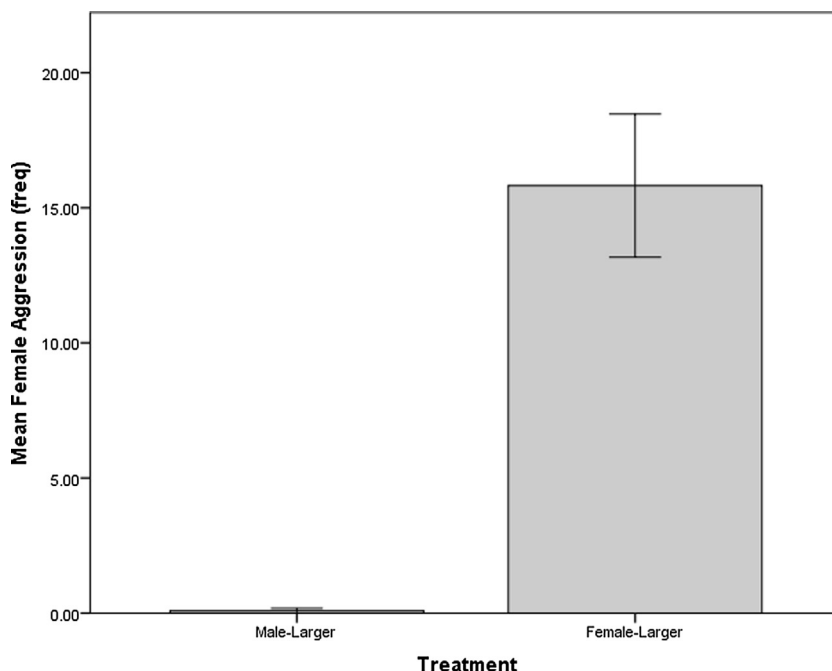


Fig. 4. Female aggression in 'no choice' paradigm Experiment II. Bars show mean frequency of female to male aggression (\pm SE) observed in each treatment of Experiment II ($n = 11$, *Male-Larger* = 0.1 ± 2.1 , *Female-Larger* = 15.7 ± 2.8 , $p < 0.001$).

partments of Experiment I was analyzed using a one-way ANOVA for each treatment. If a treatment's homogeneity of variance was violated, a Welch correction was used as this correction offers more power than a Brown-Forsythe correction (see Tomarken and Serlin, 1986). In Experiment II, male and female intersexual aggression was analyzed using independent *t*-tests.

3. Results

3.1. Experiment I

As the homogeneity of variance was violated for Treatment IA, the main effect was calculated using a Welch correction as this correction grants slightly more power than a Brown-Forsythe correction when outliers are not present. There was a main effect of compartment preference in Treatment IA ($F(2, 12.767) = 7.882$, $p = 0.006$, partial $\eta^2 = 0.245$). Planned contrasts revealed that males preferred to spend significantly more time in the smaller female compartment than in the large female compartment (Fig. 3A, $p = 0.022$). Furthermore, focal male preference for small female stimuli did not differ from male preference for the central chamber ($p = 0.821$).

When both females were larger than the male, an ANOVA of Treatment IB showed that there was a main effect of compartment preference (Welch corrected: $F(2, 15.757) = 16.662$, $p < 0.0001$, partial $\eta^2 = 0.616$). Planned contrasts revealed that males did not prefer one female over another ($p = 0.433$, Fig. 3B), instead focal females spent the majority of their time in the central chamber ($p < 0.0001$). When females were smaller than the male, an ANOVA of Treatment IC showed no significant effect of compartment preference ($F(2, 27) = 0.402$, $p = 0.673$, partial $\eta^2 = 0.029$, Fig. 3C). This lack of an effect shows that males preferred all chambers similarly.

3.2. Experiment II

Both male-initiated and female-initiated aggression was compiled for both the male-larger ($n = 11$) and female-larger ($n = 11$) treatments. An independent *t*-test of aggression revealed that

females of the female-larger treatment were significantly more aggressive than females of the male-larger treatment (*Mean difference* = 15.72, *S.E.* = 2.65, $t = -5.935$, $df = 20$, $p < 0.0001$, Fig. 4). Male aggression remained unchanged between the two treatments (*Mean Difference* = 7.91, *S.E.* = 5.6, $t = 1.4$, $df = 20$, $p = 0.176$).

4. Discussion

We set out to understand part of the process (intersexual selection) leading to size-assortative mating in convict cichlids. If intrasexual competition alone guided pair formation in convict cichlids, an individual would be predicted to mate with the winner of within sex contests, irrespective of size. Given that size generally determines these outcomes, the likelihood of size-assortative mating would be high given that males have larger maximum body sizes than females. If intrasexual competition were removed, as in this study, individuals of any size should theoretically be able to pair and mate with any size of partner. Our results indicate that this is not the case in the convict cichlid. Female convict cichlids appear highly resistant to form pairs with males smaller than themselves, suggesting that intersexual selection (mate choice) is an important component of the pair formation process. Interestingly, this seems to occur even when females are not provided with alternative male mates.

The finding of Beeching and Hopp (1999) that male convict cichlids prefer the largest of available females is almost certain to be true given the fitness benefits that come from mating with a large female, namely higher fecundity. It is likely that there would be strong selection for males to evolve this preference for female body size. However, our finding that males prefer the smaller of two available females seems to directly contradict this idea (Fig. 2A). Alone, this finding would be particularly puzzling. However, when the results of the other two treatments are considered, a pattern clearly emerges, with males appearing to choose not to spend time with females larger than themselves, even when larger females are their only option (Fig. 2B). We suggest that rather than males preferring small females, a more likely explanation is that the larger

females are preventing males from acting on the preference for large body size through some type of behavioral response.

While it might seem difficult to imagine a scenario where a female would forgo reproduction with a small male when there is no other viable option present, such a situation is extremely unlikely to occur in nature. Outside of the breeding season, when pairs are territorial, convict cichlids form mixed sex shoals and having access to a choice of potential mates is almost guaranteed (e.g., Cleveland and Itzkowitz 2008). In these conditions, the best strategy for a female might be to never 'settle' for a male smaller than herself as there would almost certainly be a better option. If this is the case, it is theoretically possible for a strategy to evolve where females always reject pairing with males smaller than themselves.

Even if this is the case, there are examples of reverse size-assorted (female-larger) pairs forming in this system. Both Itzkowitz et al. (2005) and Leese et al. (2010) were able to pair large females to small males, but this was only likely to occur when multiple small males were present. It remains unclear why female resistance to smaller males would change in a different social context, but Itzkowitz and Draud (1992) observed that pair formation in the Texas cichlid occurred much more quickly, and with less aggression, when conspecific intruders were present. Thus, perhaps by providing multiple small male mates, aggression toward each male was decreased, allowing the large female to receive more courtship that, in turn, may have changed her receptivity. Irrespective of the mechanism, the female's preference for males larger than herself can be circumvented if larger males are no longer present in the population.

In summary, previous studies have emphasized the role of intrasexual competition in maintaining the size assortative mating pattern. This implies that irrespective of an individual's actual mate preference, it will mate with the winner of an intrasexual fight. Our results indicate that large females do not readily mate with males smaller than themselves. This is almost certainly the result of female aggression toward smaller males, preventing female-larger pairs from forming. This type of pattern should be explored in other cichlids, as well as more diverse taxa to determine if this is a larger phenomena or specific to this system.

Acknowledgements

If so, I would like it to read: We would like to thank Layla Al[HYPHEN]Shaer and Dr. Bridget Dever for their advice in the design and analysis and of this experiment.

References

Arnqvist, G., Rowe, L., Krupa, J.J., Sih, A., 1996. Assortative mating by size: a meta-analysis of mating patterns in water striders. *Evol. Ecol.* 10, 256–284.

- Beeching, S.C., Hopp, A.B., 1999. Male mate preference and size-assortative pairing in the convict cichlid. *J. Fish Biol.* 55, 1001–1008.
- Beeching, S.C., Wack, C.L., Ruggner, G.L., 2004. Female convict cichlids (*Archcentrus nigrofasciatus*) prefer to consort with same sized males. *Ethol. Ecol. Evol.* 16, 209–216.
- Bollache, L., Cézilly, F., 2004. State-dependent pairing behaviour in male *Gammarus pulex* (L.) (Crustacea: amphipoda): effects of time left to moult and prior pairing status. *Behav. Processes* 66, 131–137.
- Cleveland, A., Itzkowitz, M., 2008. The role of sex ratios and resource availability on the courtship of a monogamous fish. *Behav. Processes* 80, 46–50.
- Coyne, J.A., Orr, H.A., 2004. Speciation. Sinauer Associates Incorporated.
- Crespi, B.J., 1989. Causes of assortative mating in arthropods. *Anim. Behav.* 38, 980–1000.
- Draud, M.J., Itzkowitz, M., 2004. Mate numbers or mate quality: female mate choice in the polygynandrous variegated pupfish (*Cyprinodon variegatus*). *Ethol. Ecol. Evol.* 16, 1–13.
- Gagliardi-Seeley, J., Leese, J., Santangelo, N., Itzkowitz, M., 2009. Mate choice in female convict cichlids (*Amatitlania nigrofasciata*) and the relationship between male size and dominance. *J. Ethol.* 27, 249–254.
- Haley, M., 1987. Pair formation in the Texas cichlid, *Cichlasoma cyanoguttatum* (Baird and Girard). *Biol. Behav.* 12, 177–185.
- Helfenstein, F., Danchin, E., Wagner, R.H., 2004. Assortative mating and sexual size dimorphism in black-legged kittiwakes. *Waterbirds* 27, 350–354.
- Itzkowitz, M., Draud, M.J., 1992. Conspecific intruders influence pair formation in a monogamous fish. *Behav. Processes* 28, 59–64.
- Itzkowitz, M., Santangelo, N., Cleveland, A., Bockelman, A., Richter, M., 2005. Is the selection of sex-typical parental roles based on an assessment process?: A test in the monogamous convict cichlid fish. *Anim. Behav.* 69, 95–105.
- Leese, J., Wilson, H., Ganim, A., Itzkowitz, M., 2010. Effects of reversed size-assortative mating on spawning success in the monogamous convict cichlid, *Amatitlania nigrofasciata*. *Ethol. Ecol. Evol.* 22, 95–100.
- Leese, J.M., 2012. Sex differences in the function of pair bonding in the monogamous convict cichlid. *Anim. Behav.* 83, 1187–1193.
- Lu, X., Ma, X., Li, Y., Fan, L., 2009. Breeding behavior and mating system in relation to body size in *Rana chensinensis*, a temperate frog endemic to northern China. *J. Ethol.* 27, 391–400.
- McKaye, K.R., 1986. Mate choice and size assortative pairing by the cichlid fishes of Lake Jiloa, Nicaragua. *J. Fish Biol.* 29, 135–150.
- Nilsson, S.O., Nilsson, G.E., 2000. Free choice by female sticklebacks: lack of preference for male dominance traits. *Can. J. Zool.* 78, 1251–1258.
- Noonan, K.C., 1983. Female mate choice in the cichlid fish *Cichlasoma nigrofasciatum*. *Anim. Behav.* 31, 1005–1010.
- Nuttall, D.B., Keenleyside, M.H.A., 1993. Mate choice by the male convict cichlid (*Cichlasoma nigrofasciatum*; pisces, cichlidae). *Ethology* 95, 247–256.
- Perrone Michael, J., 1978. Mate size and breeding success in a monogamous cichlid fish. *Environ. Biol. Fishes* 3, 193–201.
- Preston, B.T., Stevenson, I.R., Pemberton, J.M., Coltman, D.W., Wilson, K., 2005. Male mate choice influences female promiscuity in Soay sheep. *Proc. Biol. Sci.* 272, 365–373.
- Ridley, M., 1983. *The Explanation of Organic Diversity: the Comparative Method and Adaptations for Mating*. University Press, Oxford.
- Rowe, L., Arnqvist, G., 1996. Analysis of the causal components of assortative mating in water striders. *Behav. Ecol. Sociobiol.* 38, 279–286.
- Rowland, W.J., 1989. The ethological basis of mate choice in male threespine sticklebacks, *Gasterosteus aculeatus*. *Anim. Behav.* 38, 112–120.
- Santangelo, N., 2005. Courtship in the monogamous convict cichlid; what are individuals saying to rejected and selected mates? *Anim. Behav.* 69, 143–149.
- Shine, R., O'Connor, D., Lemaster, M.P., Mason, R.T., 2001. Pick on someone your own size: ontogenetic shifts in mate choice by male garter snakes result in size-assortative mating. *Anim. Behav.* 61, 1133–1141.
- Tomarken, A.J., Serlin, R.C., 1986. Comparison of ANOVA alternatives under variance heterogeneity and specific noncentrality structures. *Psychol. Bull.* 99, 90–99.
- Wisenden, B.D., 1994. Factors affecting mate desertion by males in free-ranging convict cichlids (*Cichlasoma nigrofasciatum*). *Behav. Ecol.* 5, 439–447.