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ARTICLE

Male mate choice in the annual fish *Austrolebias reicherti* (Cyprinodontiformes: Rivulidae): when size matters

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Abstract

While the importance of male mate choice is increasingly recognized, it continues to be an understudied aspect of sexual selection. Here, the effect of female body size on male mate choice was evaluated in the annual killifish *Austrolebias reicherti*, a species with marked sexual dimorphism in which males are more conspicuous than females. In dual-choice mate choice trials with unequal sized females we found that males spent significantly more time with larger females. Furthermore, larger females spawned more and larger eggs when spawning was allowed in non-choice trials. Therefore, male selection is probably attributable to the higher reproductive success of larger females. To our knowledge, this is the first study that provides evidence of both male mate choice in annual fishes and its possible fitness advantages. The implications of male mate choice for sexual selection in these fish highlight the need for further studies.

Keywords Annual killifish · Body size · Fecundity · Male choice · Operational sex ratio · Sexual selection

Introduction

Mate choice occurs whenever traits in one sex increase the probability of mating or reproductive investment with specific individuals of the opposite sex (Andersson 1994). Theory predicts that females maximize reproductive success by choosing mates that will contribute the best resources, genes, or both to their offspring (Trivers 1972; Andersson 1994). Traditionally, it has been proposed that males are not selective; instead, they maximize their reproductive success by increasing the number rather than the quality of partners (Bateman 1948). However, theoretical approaches also suggest that males can gain fitness advantages by being choosy under certain conditions (Bergstrom and Real 2000; Kokko and Johnstone 2002; Edward and Chapman 2011; Fitzpatrick and Servedio 2018). Indeed, choosy males have been documented across a wide range of species (revised in Edward and Chapman 2011). Nevertheless, although male mate choice is probably more common than previously thought, attention has been focused on female choice, particularly in species with sexual dimorphism in which males are more conspicuous than females (Houde 2001). Consequently, male mate choice remains understudied (Bonduriansky 2001).

A key factor in the evolution of male mate choice is the relationship between the number of mates available and male capacity to mate (Edward and Chapman 2011). The number of mates available depends largely on the operational sex ratio (OSR), which is the ratio of sexually active males to females (Emlen and Oring 1977). Numerous examples show that males can be choosy when the OSR is female-biased (e.g. Candolin and Salesto 2009; Amundsen 2018). Male capacity to mate could be limited by energetic constraints. For example, males are more likely to be choosy if the courtship behaviour is energetically expensive (Judge and Brooks 2001), or risky in terms of attracting predators (reviewed in Kotiaho 2001). Furthermore, variation in the quality of available females is a fundamental requirement for the evolution of male mate choice: the broader this variation is the larger the potential benefits of male choice (Edward and Chapman 2011). Males should have mating preferences and exhibit mate choice whenever they can adequately discriminate among females that differ noticeably in quality (Andersson 1994). In many taxa, particularly in fishes, males should prefer bigger females since female fecundity is positively correlated with body size (Charnov 1993; Helfman

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et al. 2009). Indeed, male preference for large females has been demonstrated in several fish species (e.g. Basolo 2004; Dosen and Montgomerie 2004; Werner and Lotem 2006; Tudor and Morris 2009; Callander et al. 2012; Schlupp 2018). While body size is often used as a proxy for fish female fecundity, few studies report data on both the male mating preferences for large females and the relationship between fecundity and female size (e.g. Kraak and Bakker 1998; Pélabon et al. 2003; Herdman et al. 2004).

In the present study, male mate preferences for female body size were examined in the annual killifish *Austrolebias reicherti* (Loureiro and García 2008), a species endemic to Uruguay eastern wetlands. Like other *Austrolebias* species, *A. reicherti* has marked sexual dimorphism (Loureiro and García 2008). Males are larger than females, exhibit bright coloration, perform elaborate and energetically expensive courtship displays, and compete aggressively for mates. On the other hand, females are relatively cryptic and express mating preferences for different male phenotypes (Passos et al. 2016). At the beginning of the reproductive season, females prefer to mate with larger males (Passos et al. 2014). However, female preference for larger males disappeared when male availability declined over the season (Passos et al. 2014). The OSR changes dramatically over the season, from 1:1 at the beginning to strongly female-biased at the end (Passos et al. 2014). This female-biased ratio in the late season provides an opportunity for males to be selective when mating (Jennions and Petrie 1997; Edward and Chapman 2011); however, male mating preference still remains to be evaluated in annual killifish.

The aim of this study was to evaluate sexual selection on females of *A. reicherti* via male mate choice. Specifically, classical dual-choice trials were performed in which males choose between contrasting female sizes. We hypothesize that males prefer bigger females due to their higher fecundity. To evaluate this, the relationship between female body size and fecundity (i.e., egg number and size) was also analysed.

Materials and methods

Collection and laboratory conditions

Adult individuals of *A. reicherti* were collected using hand nets from temporary ponds located in Treinta y Tres Department, Uruguay (32°58′54″S; 53°52′14″W; Fig. 1), at the end of the reproductive season (October 2016). Fish were transported to the laboratory and females were kept in communal aquariums (40 cm × 13 cm × 15 cm, length × width × height) in groups of up to five individuals while males were kept in individual aquariums (20 cm × 9 cm × 15 cm) to avoid harmful agonistic interactions. All fish were kept in the laboratory for 5–7 days for acclimation prior to behavioural trials under constant temperature (19 °C). Natural photoperiod conditions were maintained and they were fed ad libitum every day with the sludge worm *Tubifex* sp. In all cases, aquariums were visually isolated from each other by covering them with opaque plastic boards. Collection and experimental procedures were approved by the ethical committee of Universidad de la República, Uruguay (Comisión de ética en el uso de animales, Facultad de Ciencias, UdelaR, approval file no. 241000-001186-12 date 31-10-2012). Fish were returned to the same aquarium after experiments were completed and retained as breeding stock.

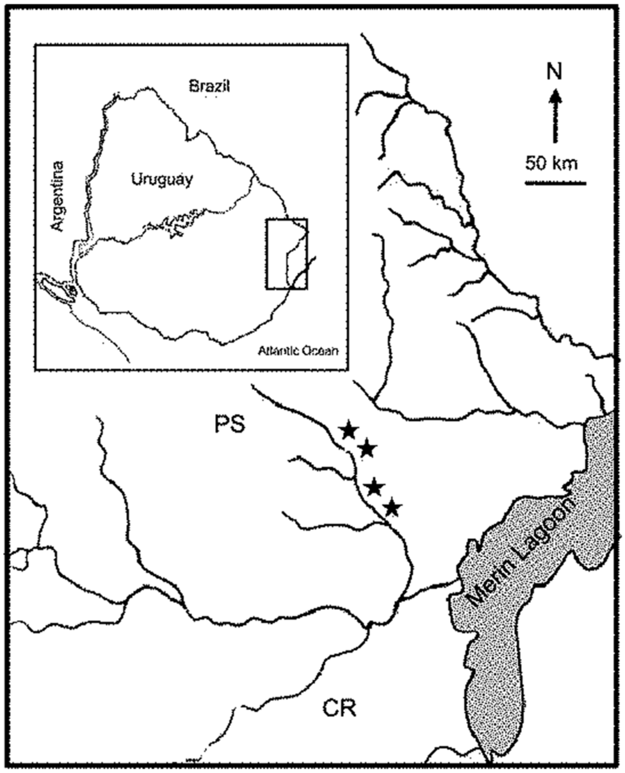

Fig. 1 Distribution of *A. reicherti* and collection sites. The ponds are indicated by *black stars*. *CR* Cebollatí River, *PS* Parao stream

Male mate choice trials

Male mating preferences were tested using a dual choice setup in which males were placed with two stimulus females of different body size. The experimental setup consisted of aquariums (60 cm × 20 cm × 20 cm) divided by two perforated transparent acrylic slides to allow water flow but preclude the individuals from moving between compartments. Consequently, the aquariums were divided into a central compartment of 40 cm and two lateral compartments of 10 cm each (Fig. 2). In the central compartment, three zones were defined by external markings: a central zone of 30 cm

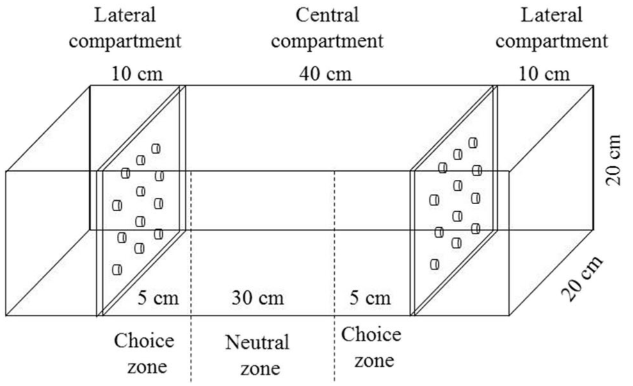

Fig. 2 Scheme of the experimental set-up design divided by two perforated transparent acrylic slides. Neutral and choice zones were defined by external markings (*dotted lines*). Illustration not drawn to scale

long (i.e. neutral zone), and two side zones of 5 cm long (i.e. choice zones) (Fig. 2). In order to reduce stress and to mimic a natural environment, the bottoms of the aquariums were completely covered with a thin layer of peat moss, and small pieces of *Myriophyllum* sp. were placed in all compartments as this macrophyte is typically abundant in temporary ponds. To reduce any external disturbance, and to provide a uniform background, black screens were placed covering the side walls and rear wall of the aquariums.

Mating preferences of 16 males were examined using 16 different pairs of females varying in their body size by 9.5 ± 1.1 mm (paired t-test: $t_{15} = 8.8$, $P < 0.001$). For this, two size categories were used: small (standard length: mean ± SE = 27.0 ± 0.6 mm, range = 23.0–31.3 mm) and large (standard length: mean ± SE = 36.5 ± 1.1 mm, range = 31.1–46.7 mm). Each individual was used in only one trial. In each trial, one male was placed alone in the central compartment to acclimate for 20 min; then, a large and a small female were simultaneously placed in the lateral compartments. Females were randomly assigned to the right or left side in each trial to avoid side bias. Behaviour of males was then video-recorded during four sessions of 15 min each and separated by 1-h intervals (Passos et al. 2014). In order ensure that males were motivated to mate, only males that displayed courtship behaviour were used in analyses (12 out of 16 males).

Female size and fecundity

Female fecundity (i.e. egg number and size) was evaluated by allowing stimulus females of different size categories to spawn with the tested males. After mate choice trials, each male was housed with two females of the same size category (six males were individually housed with two large females each, and six males were individually housed with two small females each) in aquariums (40 cm × 15 cm × 15 cm). A Petri dish with glass beads of 500 µm diameter (Thomas Scientific, Swedesboro, NJ) was supplied as spawning substrate. Groups consisting of one male and one or two females are a common practice in breeding procedures in this species (Papa et al. 2016). To avoid familiarity among individuals, the male and the two females were taken from different male choice trials. After 16 h, the Petri dish was carefully removed and the spawned eggs collected and counted. Further, eggs from eight trials (four of each size category) were photographed under a microscope with a digital camera, and the diameter of eggs was measured along their longest axis (±0.01 mm).

Additional spawning trials with fish not used in mate choice trials were performed in order to test the relationship between female size and number of eggs. We conducted 29 additional spawning trials in which each individual was used only once. A male and a female taken from a breeding stock were housed together and spawned eggs were collected and counted 48 h later.

Variables measured and statistical analysis

Prior to the assays, fish were placed carefully in a plastic container with a scale and photographed. These digital images were used to measure fish standard length (measured from the tip of the snout to the distal end of the caudal peduncle), using TpsDig version 2.26. Recorded videos were manually inspected to obtain the time that males spent in the two choice zones with different stimuli (i.e. association time). To avoid observer biases, the analysis of the videos was made without a priori knowledge of which side either female size class occupied. Differences between association times with different stimuli were evaluated using a paired t-test. The time spent in the proximity of a stimulus is a strong predictor of mating preference (Kingston et al. 2003; Basolo 2004; Walling et al. 2010), and has been used previously to evaluate female choice in *Austrolebias* (Passos et al. 2013, 2014). A particular female was considered preferred if the male spent more than 50% of the total association time interacting with her. The number of trials showing a preference for either the large or the small female was tested by the mean of a binomial test. We measured the strength of preference as the difference in the time that the males spent with the large female minus the time that the male spent with the small female. We examined the relationship between the strength of the preference and the difference in size between stimulus females (larger standard length – smaller standard length) using a Pearson correlation to test whether the difference in size between females affects male preferences. We compared the number and the average size of spawned eggs by each female size class using Mann–Whitney tests. Moreover, we evaluated the relationship between female size and the number of eggs obtained in the 29 additional spawning trials by Spearman rank correlation. Data were checked for normality and homoscedasticity using Shapiro-Wilks and Levene tests, respectively. We applied standard parametric tests (i.e. paired *t*-test, Pearson correlation) to the data unless they violated the assumptions of normality and homoscedasticity. In such cases we applied a corresponding nonparametric test (i.e. Mann–Whitney test, Spearman rank correlation). Statistical analyses were performed using SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Differences were considered significant when $P < 0.05$.

Results

Male mate-choice trials

Males spent significantly more time in the choice zone of larger females (paired *t*-test: $t_{11} = 2.867$, $P < 0.05$; Fig. 3), with 10 of 12 males preferring the larger female (binomial test, $P = 0.019$). However, there was no relationship between strength of preference and stimulus female size difference (Pearson correlation: $r_{10} = -0.35$, $P > 0.05$).

Female size and fecundity

Females spawned eggs in all 12 trials. The total number of spawned eggs was 226, and was significantly higher for the large female size class (median = 31.5, range = 2–39) than for the small female size class (median = 9, range = 2–18) (Mann–Whitney test: $U = 5.5$, N1 = N2 = 6, $P < 0.05$). Moreover, large females spawned bigger eggs (median = 1.73 mm, range = 1.52–1.87 mm) than small females (median = 1.45 mm, range = 1.31–1.48) (Mann–Whitney test: Z = − 2.3, N1 = N2 = 4, $P < 0.05$). In the additional spawning trials, female size was positively correlated with number of spawned eggs (Spearman rank correlation: $r_s = 0.85$, $N = 29$, $P < 0.001$; Fig. 4).

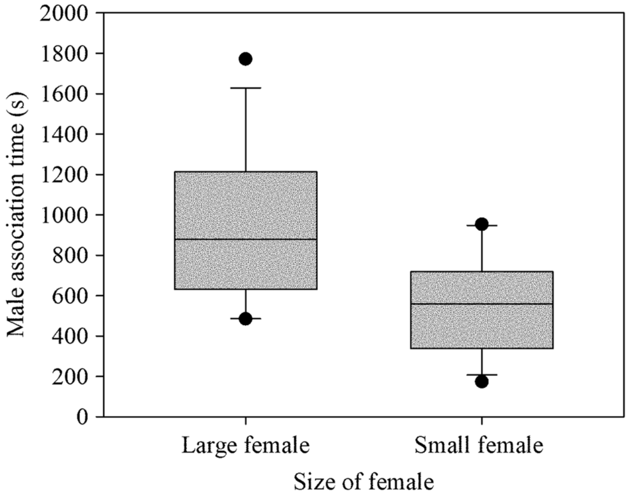

Fig. 3 Box plots of male association time (s) for both stimulus female sizes: large females (36.5 ± 1.1 mm) and small females (27.0 ± 0.6 mm) in a dual choice test ($N = 12$). *Horizontal lines* represent the median, *vertical lines* represent maximum and minimum

Discussion

In this study, evidence of male mate choice was found in *A. reicherti* and to our knowledge this is the first time that male mate choice has been demonstrated in annual fishes. Males placed with two females of unequal size spent significantly more time with larger females. Larger females had higher fecundity (i.e. produced more and larger eggs), and the number of eggs was positively correlated with female body size. These results agree with previous studies in several taxa examining both male preferences for female size and size-fecundity relationships (Andersson 1994; Bonduriansky 2001; Pélabon et al. 2003; Herdman et al. 2004).

Male choosiness is expected when OSR is biased towards females, i.e. when there are many females available to mate (Edward and Chapman 2011). Thus, male mate choice in *A. reicherti* is expected since mate choice assays were performed using males collected at the end of the reproductive season when the OSR is typically female biased (Passos et al. 2014). Another scenario in which male choosiness is expected is when males invest a significant amount of energy in reproduction, usually in the form of parental care (Kokko and Monaghan 2001). While *A. reicherti* do not exhibit parental care, males perform elaborate and costly courtship displays. These consist of fin vibrations and high-speed

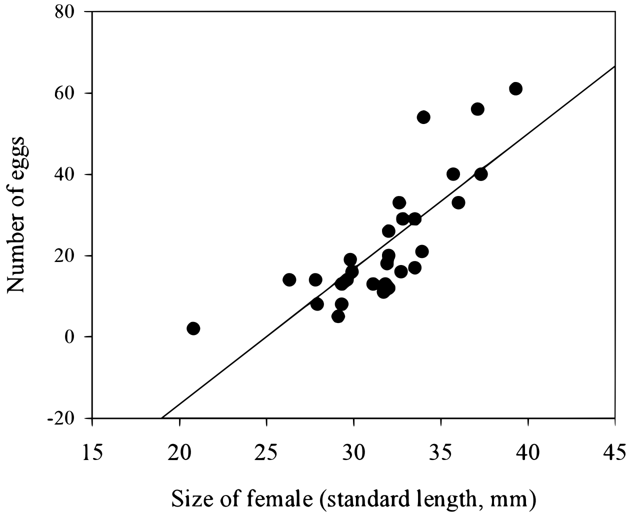

Fig. 4 Relationship between female standard length (mm) of *A. reicherti* and number of spawned eggs

undulating body movements coupled with an intensified coloration, enhancing both body and fin pigmentation design simultaneously (García et al. 2008). During these courtship displays, males vigorously ward off rivals by biting and chasing them (Passos et al. 2016). Thus, courtship is probably energetically expensive and may increase predation risk. It is reasonable then that males strategically allocate their mating effort according to female attractiveness.

Male mate preference for larger females may have several causes. First, males may obtain direct benefits from mating with larger females because female body size is correlated with fecundity (Andersson 1994) as in many other annual fish species (e.g. Schalk et al. 2014; Gonçalves et al. 2011; Vrtílek and Reichard 2016). Larger females spawned more and larger eggs. Consequently, males choosing to mate with larger and more fecund females should have higher reproductive success. A higher number of eggs will lead to more offspring and potentially allow more hatching opportunities. In addition, egg size is often under selection in fishes (Einum and Fleming 1999; Einum et al. 2002). While it is still unknown whether larger eggs have a fitness advantage in *Austrolebias*, evidence exists for several other fish species in which larger eggs produced larger fry with higher survival probabilities (e.g. Wallace and Aasjord 1984; Einum et al. 2002). Second, larger females could cause greater visual stimulation and exploit pre-existing sensory bias underlying male preferences (Rosenthal and Evans 1998).

Males of *A. reicherti* were able to evaluate the relative sizes of females and make seemingly adaptive mate choices based on that assessment. However, our results also indicate that strength of preference for larger females was not affected by their difference in size. The amount of variation in the difference of female size may not have been great enough to reveal a correlation (in most dyads the big female was between 1.3 and 1.6 times greater than the small female). Additional studies are required to assess the relationship between the strength of preference and the difference in size between females. Also, it is important to note that the sample size used was relatively small, with only 12 of 16 trials analysed. Despite these constraints in experimental design, we found strong and significant evidence for male choosiness in this species.

While female mate choice is most often reported, the importance of mate choice in males is increasingly recognized (Clutton-Brock 2007). Our results contribute to the growing number of studies demonstrating that male mate choice indeed occurs, even in species without parental care, in which males exhibit bright coloration, perform courtship behaviour, and engage in intrasexual contests. We have shown that male *A. reicherti* prefer larger and more fecund females. Male mate choice may thus play an important role in the annual killifish mating system, and the implications for sexual selection highlight the need for further research. An important direction for future surveys is to investigate the relative importance of male and female choice according to substantial changes in OSR over the course of the short breeding season.

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Compliance with ethical standards

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Conflict of interest The authors declare that they have no conflict of interest.

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