

## **Effects of reversed size-assortative mating on spawning success in the monogamous convict cichlid, *Amatitlania nigrofasciata***

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Many vertebrate taxa show size-assortative mating where large males typically pair with large females. The mechanism behind size-assortative mating has been studied primarily in the context of intrasexual and intersexual selection, where multiple potential mates differing in relative size are provided to focal individuals. Little is known about how pairs form and reproduce when larger mates are unavailable. Using the convict cichlid (*Amatitlania nigrofasciata*), we examined the likelihood of spawning behavior when the relative size of sexes are reversed from that found in nature. In one experiment, reverse size-assorted pairs never spawned, in comparison to control size-assorted pairs, where spawning occurred in 60% of replicates. In a second experiment, individuals were given a choice of two small mates and reverse size-assorted pairs, spawned almost as frequently as size-assorted pairs. This suggests that a mechanism exists that hinders spawning in reverse size-assorted pairs, but that this can be overcome by providing individuals with a choice of potential mates.

KEY WORDS: *Amatitlania nigrofasciata*, mate choice, pair formation, sexual selection, size-assortative mating.

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### INTRODUCTION

In those vertebrates where size varies greatly among adults, size assortative mating is common, e.g. large males typically pair with large females (MATHIS 1991; MAEKAWA et al. 1994; BOLL & LINSSENMAIR 1998; BEECHING & HOPP 1999; SHINE et al. 2003). Size is often positively correlated with fecundity and may be an indicator of genetic quality, making it an advantage to mate with the largest available individual. For the most part, the mechanism to achieve size assortative pairs has been studied in the context of intrasexual competition (MATHIS 1991; BEECHING & HOPP 1999; SUTHERLAND 2007) and/or mate preferences (NOONAN 1983; CRESPI 1989; ARNOVIST et al. 1996; BASOLO 2004). In both types of sexual selection, the difference between

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potential mates is relative, making it unclear how individuals respond when larger mates are unavailable. It is possible that in the absence of a choice, both sexes will mate with any sized mature individual, perhaps indicating that the benefits gained by mating with a larger partner may not compensate for the costs of continuing the search (REAL 1991).

This seems most likely for polygynous males that have the ability to mate with several females in rapid succession. Here, the cost associated with mating with small females is minimal compared to waiting for a large female to appear. If such a female does appear, polygynous males remain available for mating. For species with long term pair bonding that includes biparental care, neither sex can quickly 'recover' from mating with individuals that are too small. Furthermore, in biparental species, the size of one parent may impact on the pair's ability to raise their offspring. For example, studies on biparental cichlid fish (Cichlidae) have shown that the parental roles performed by a parent correlate to its size relative to its mate (AWATA & KOHDA 2004). In convict cichlid fish, *Amatitlania nigrofasciata*, females benefit from a large male's ability to protect the offspring against predators (GAGLIARDI-SEELEY & ITZKOWITZ 2006). ITZKOWITZ et al. (2005) reversed the size-typical relationship in the convict cichlid fish and found that when presented with a conspecific intruder, a female paired with a smaller male spent less time with offspring than females mated to larger males, perhaps making the offspring more vulnerable to predation. Thus unlike polygynous species, biparental species may be more resistant to mating with small individuals, even when alternative mates are unavailable.

Using the biparental convict cichlid fish, we examined how males and females respond when they are provided with potential mates of a single given size, as would not occur under natural conditions. In observations of over 100 pairs of convict cichlids at four different study sites, WISENDEN (1995) found only one instance in which a male's standard length was smaller than his female partner. The rest of the pairs showed size differences with a mean of 13 mm standard length, and ranging as high as 30 mm. Because reverse size-assortative pairs almost never form when size typical mates are available (personal observation), we used a no-choice and choice paradigm in which small individuals of one sex were placed with one large individual of the opposite sex. For the convict cichlid, the size-assorted (control) group consisted of the male being larger than the female. We tested the following hypothesis: that males and females actively avoid forming reverse size-assorted pairs. Previous studies on the pairing of size-assorted convict cichlids revealed a spawning success rate in laboratory conditions between 60–70% (BOCKELMAN & ITZKOWITZ 2008); thus, we assumed that fewer reverse size-assorted pairs would form and those that did would take longer to produce fertilized eggs.

## METHODS

All replicates were conducted at Lehigh University using a combination of fish purchased from local pet suppliers and laboratory-raised animals bred from fish originally obtained in Costa Rican streams. Previous studies have shown no differences in the mating and courtship behaviors of laboratory and wild caught individuals. Prior to testing, individuals were maintained in same sex stock aquaria (473.5 l) at  $20 \pm 2$  °C on a 14:10 light:dark cycle and fed commercially available trout crumbles every other day. After testing, individuals were placed into separate stock tanks to eliminate pseudoreplication.

Experimental aquaria (284 liters) were divided into two compartments by placing a clear plastic divider near one end of the tank ( $\approx$  15 cm from end). At the beginning of each replicate, a conspecific juvenile was placed behind the divider. The presence of intruders has been shown to

facilitate pair bond formation in monogamous cichlids (ITZKOWITZ & DRAUD 1992). An artificial breeding site (flower pot) was placed in the aquaria opposite the intruder compartment and an air stone was added to the tank. All fish in experimental aquaria were fed trout crumbles *ad libitum* daily.

In the first experiment, one large and one small individual were placed together in the experimental aquaria. In the reverse size-assorted group ( $n = 10$ ), the large individual was a female and the small individual a male. All large females were between 75.0 and 105.0 mm in total length and small males were between 54.0 and 85.0 mm. In the size-assorted group ( $n = 10$ ), the size of the sexes was reversed. All large males were between 70.0 and 105.0 mm and all small females were between 50.0 and 85.0 mm. In any given pair, the large individual was  $2.0 \pm 0.3$  cm larger than the small individual. Each pair was observed daily for signs of aggression or injury. The replicate was ended if aggression between subjects caused severe injury and/or death was likely to occur. This was observed when the smaller individual was confined to a corner of the aquarium and could not move to another location in the tank without being attacked and harassed by the larger fish. Otherwise, the pairs were given 21 days to successfully spawn before the replicate was ended. Thus one of three possible outcomes was recorded for each replicate: (1) spawning; (2) >21 days; or (3) termination of the replicate due to excessive aggression with the potential for injury.

In the second experiment, a large individual was placed with two small size-matched individuals of the same sex. In the reverse size-assorted group ( $n = 10$ ), the large individual was female and the two small individuals were male, and in the size-assorted group ( $n = 10$ ), the sizes of the sexes were reversed and the large individual was male and the small individuals were female. All large females were between 70.0 and 85.0 mm, all small males between 50.0 and 65.0 mm, all large males between 70.0 and 85.0 mm and all small females between 45.0 and 65.0 mm in total length. If pair formation occurred in this experiment, the unselected small individual was removed from the experimental tank to avoid injury. Replicates were ended after 21 days, upon spawning, or when both small individuals were removed due to high levels of aggression from the large individual.

The frequency of spawning was compared between the size-assorted and reverse size-assorted groups in both the choice and no choice experiments using a Wilcoxon rank-sum test. In the choice experiment, the number of days to spawning was compared between size-assorted and reverse size-assorted groups using an independent samples t-test. Results were considered significant at  $P \leq 0.05$ .

## RESULTS

In the no choice experiment, the number of pairs that spawned in less than 21 days differed significantly between the reverse size-assorted and the size-assorted groups (Fig 1.,  $N = 20$ ,  $z = -2.854$ ,  $P = 0.004$ ). Six of ten replicates in the size-assorted group successfully spawned in less than 21 days. Two replicates had to be ended because of injury to the small female and two replicates went 21 days without spawning. In the reverse size-assorted group, none of the pairs spawned in less than 21 days. Three replicates were ended due to injury and seven replicates lasted 21 days (Table 1).

In the choice experiment, there was no difference in the number of pairs that spawned between the reverse and size-assorted groups (Fig 1.,  $N = 20$ ,  $z = -0.890$ ,  $P = 0.374$ ). Seven of 10 replicates in the size-assorted group spawned successfully, while three replicates went 21 days without spawning. In the reverse size-assorted group, five replicates spawned, two went 21 days without spawning, and three were ended prior to 21 days because of injury to the small males (Table 1). There was no difference in the number of days to spawning between the reverse and size-assorted groups of the choice experiment ( $t = 0.703$ ,  $df = 10$ ,  $P = 0.498$ ).

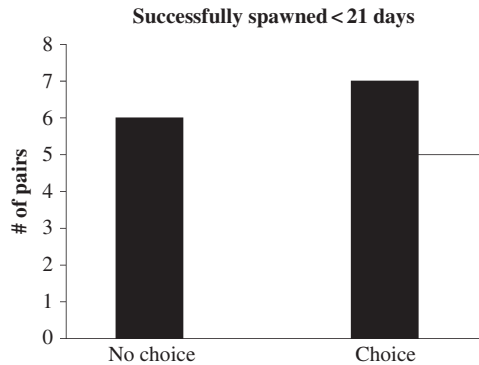


Fig. 1. — The number of spawning pairs in size-assorted (solid) and reverse size-assorted (open) pairings when given a single small individual (no choice) or a choice of two small size-matched individuals (choice).

Table 1.

The frequency of outcomes of replicates for both the choice and no choice experiments.

	Replicate outcome (N)			Avg # of days to spawning
	Spawned	>21 days	Aggression	
No choice				
Size-assorted	6	2	2	8.3
Reverse size-assorted	0	7	3	—
Choice				
Size-assorted	7	3	0	8.9
Reverse size-assorted	5	2	3	11.2

## DISCUSSION

The results from the first experiment clearly show that reverse-size assorted pairs do not spawn as readily as size-assorted pairs in the convict cichlid. This suggests that size-assortative mating may be controlled by a mechanism outside of intrasexual competition. That is, even when same-sex competition is removed, reverse size-assorted pairs are not as likely to spawn as size-assorted pairs. Given that it often benefits both sexes to mate with the larger of available mates, in reverse size-assorted pairs, it seems likely that the female is not receptive to the small male. It is possible that females have a certain threshold size criteria for potential mates and males that do not surpass this threshold are not considered suitable. Thus sexual selection, via mate choice, appears to be playing a role even when the choice is to spawn with a small individual or forgo reproduction.

In the size-assorted group, pairs formed 60% of the time; a success rate that was consistent with previous laboratory studies (BOCKELMAN & ITZKOWITZ 2008). Large males were able to form pair bonds with females much smaller than themselves, suggesting that perhaps they do not have a certain size threshold for acceptable females. When given a choice, the higher fecundity of large females may be selected for by males, but the cost of forgoing reproduction may incur a higher risk than pairing with a small female.

In the second experiment, it was somewhat surprising that reverse-size assorted pairs were able to spawn almost as frequently as size-assorted pairs when given a choice of two small individuals. The presence of conspecific convict cichlids has been shown to facilitate pair bond formation, but all pairs in both experiments were exposed to an intruder, so this does not explain the patterns observed. Intrasexual competition between the two small males may have stimulated the large female to pair with one of the small males at a rate similar to small females pairing with large males. The role of intrasexual competition on pair formation is not well studied in monogamous species but there is some evidence that increasing the numbers of alternative mates does increase the likelihood of pair formation in the convict cichlid fish (CLEVELAND-ROBERTS & ITZKOWITZ unpublished data).

It is evident that mate choice and pair bond formation in this monogamous system consists of a dynamic and complex interaction of multiple factors. While reverse size-assorted pairs are less likely to spawn when forced together, it seems that providing females a choice of small individuals compensates for this incompatibility. Further studies monitoring courtship and aggression behaviors during reverse size-assortative mating may elucidate the reason why a choice of mates has this effect on reverse size-assorted pairs.

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