Reproductive success and female preference in the amazonian cichlid angel fish, *Pterophyllum scalare* (Lichtenstein, 1823)

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The angel fish, *Pterophyllum scalare* is a cichlid native to the Amazon Basin of Brazil and is exported as an ornamental fish. In this study the importance of the experience and previous reproductive success of males in mate selection was investigated. In order to investigate reproductive experience, six pairs of males (experienced and inexperienced) and six females were used. Males were placed in an aquarium, where one female was released. Mate selection was verified by the time spent by a female near one of the males. To evaluate reproductive success, six pairs of males were tested, each pair consisting of a successful male and an unsuccessful one. Again, time spent with one of the males was considered as an indication of preference by the female. Each female was then paired with an unsuccessful male and their reproductive success was assessed. Females preferred larger, aggressive, territorial and experienced males. Correlations between male aggressiveness, aeration and egg survival were significant. During larval care, male aggressiveness was significantly related to larvae survival. Furthermore, intrabucal care and larval survival showed significant correlations with care provided by the experienced, mated and isolated fish. Survival of offspring resulted from mating with experienced and inexperienced males showed significant differences. Correlations between time spent by females with successful males during reproduction and survival rate of eggs and larvae were significant. Females assess the capacity and willingness of males in investing efforts to raise the offspring through their courtship behavior. Experienced and successful males are preferred by females and thereby achieve greater reproductive success.

O acará bandeira, *Pterophyllum scalare* é um ciclídeo nativo da Bacia Amazônica do Brasil, e é exportado como espécie ornamental. Neste estudo foi investigada a importância da experiência dos machos e do sucesso na reprodução anterior para escolha do parceiro. Para as investigações relativas à experiência reprodutiva foram utilizados seis pares de machos (experientes e inexperientes) e seis fêmeas. Os machos foram colocados em aquários, onde foram adicionadas as fêmeas (uma por aquário). A escolha do parceiro foi verificada através da permanência das fêmeas junto a um dos machos. Para as investigações referentes ao sucesso reprodutivo foram testados seis pares de machos, cada par com um macho bem sucedido e outro mal sucedido na reprodução anterior, frente a uma fêmea bem sucedida. Foi observada a permanência da fêmea com um dos machos, como indicativo de preferência. Em seguida, cada fêmea foi pareada com um macho mal sucedido e avaliado o sucesso reprodutivo dos casais. As fêmeas preferiram machos maiores, mais agressivos, territoriais e experientes. As correlações entre agressividade dos machos, cuidado através da aeração e sobrevivência dos ovos foram significativas. Durante o cuidado com larvas a agressividade dos machos experientes foi significativa em relação à sobrevivência delas. Além disso, cuidado intrabucal e sobrevivência das larvas mostraram correlações significativas em relação ao cuidado realizado pelos peixes experientes, acasalados e isolados. Comparando a sobrevivência de proles resultantes de acasalamentos com os machos experientes e inexperientes, foram observadas diferenças significativas. As correlações entre permanência das fêmeas com machos bem sucedidos na reprodução e sobrevivência de ovos e de larvas foram significativas. As fêmeas avaliam a capacidade e disposição para investimento na criação da prole através do comportamento de corte dos machos. Os machos experientes e bem sucedidos têm a preferência das fêmeas e alcançam maior sucesso reprodutivo.

Key words: reproductive behavior, courtship, mate selection.

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Introduction

In most animals, females choose mates based on contribution that they may offer towards the success of the offspring (Gould & Gould, 1989). In monogamous species, both sexes are selective and each member of the pair must contribute in the best manner possible to increase reproductive success (Wickler & Seibt, 1985). These species generally develop biparental care and during courtship, females, by means of phenotypic characteristics, assess the ability and willingness of males to invest in raising the offspring (Goodenough *et al.,* 1993). Females prefer territorial males that display parental skills, since this naturally predicts high reproductive success (Cluton Brock, 1988). In fish, acquiring a high quality territory for spawning purposes is one of the main requirements of male's reproductive success. (Huntingford, 1979; Cacho *et al.*, 1999; Chellappa *et al.,* 1999a). In this group, territories are generally occupied by large aggressive males which are usually able to defend them (Torricelli *et al.,* 1993), and then, attracting a higher number of females and achieving greater reproductive success (Cacho *et al.*, 1999; Chellappa *et al.,* 1999b). In cichlids, most species are territorial and defend mating sites during reproductive periods (Keenleyside, 1991; Chellappa, 2000).

The angel fish *(Pterophylim scalare)* is a cichlid native to the Amazon Basin of Brazil and is exported as an ornamental fish. Males of this fish are territorial and compete for females. Couples adopt serial monogamy, a rare system in which partners, after investing in their descendants for a given time, seek several new mates during the same breeding season (Gould & Gould, 1989). In nature, the angel fish reproduces in a highly competitive system, where there is intense parental involvement with the offspring, mainly during the initial breeding phase, when males are more diligent in parental care (Cacho *et al.,* 1999). Participation of male is important for reproductive success, since females alone cannot protect the eggs from predators. This study aimed at investigating the importance of prior male experience and reproductive success in mate selection and reproductive success of *P. scalare*.

Materials and Methods

Acquisition and maintenance of fish. The forty angel fishes (*P. scalare*) used in this study (twenty males and twenty females), were captured in rio Tapajós (Amazon Basin). These fish were acquired from an ornamental fish shop in Natal (Rio Grande do Norte State). The observations were carried out in the Ichthyology Laboratory of the Department of Oceanography and Limnology, Federal University of Rio Grande do Norte, Natal, Brazil, during 2004.

Males and females were maintained in two 1000 L cement tanks (provided with constant aeration) separately, fed daily *ad libitum* with commercially available fish food. Dissolved oxygen was maintained at 8.0 mgL^{-1}, pH values ranged from 6.8 to 7.0 and water temperature around 27 ^{0}C. Precautions were taken to minimize disturbance to the fish and the natural photoperiod of 06:00 to 18:00h was maintained in the laboratory.

Procedures. Fish used in this study were over one year old and considered potential competitors, with males measuring over 120 mm in total length and females between 100 and 120 mm. Male selection was based on aggressiveness displayed toward their own image in a mirror. Aggressiveness was expressed by the attacks and bites quantified during six 30 minutes sessions. Fish were considered very aggressive when they displayed a mean frequency of aggressiveness above 70% on a scale of 70 to 100%. The scale of aggression used in this study was based on Rogers (1995) who studied *Cichlasoma citrinellum* (another cichlid).

Fish breeding were observed under semi-natural conditions, but similar to those encountered in nature. In nature, these fish inhabit river margins with clear water and dense aquatic vegetation, where they are usually found among roots. In laboratory, fish were placed in a glass aquarium (1.20 x 0.58 x 0.50m), which bottom was covered by crushed river stones and sand, adorned with different species of Amazonian aquatic lager plants (*Anubia* sp. and *Echinodorous amazonensis*, Fasset) and narrow leaves (such as *Valisneria gigas* and *Elodea* sp., Graebener).

Fish were maintained in aquariums for two months and observed for 30 days (1 hour each day). Breeding of randomly-formed pairs was observed during one breeding cycle (a cycle of approximately 21 days, corresponding to the period between territory occupation and independence of the offspring). At the end of the first breeding cycle, couples were marked by clipping their dorsal and pelvic fins in order to facilitate individual identification.

To investigate the importance of reproductive experience on female preference, six experiments were performed, each with six observations, totaling 36 hours data. Six pairs of males were used (placed in aquariums measuring 70 x 40 x 40 cm), and each pair consisted of an experienced and inexperienced individual of similar size and conditions. Males that managed to protect their offspring until independence in the earlier reproductive cycle were considered experienced and inexperienced if they did not manage to go beyond courtship during the first mating attempt. A female was further released into each aquarium in order to investigate male selection by the female. Selection was verified through time spent by the female with one of the males. Experienced males were more aggressive, exhibited courtship skills and cleaned substrate for eggs deposition. Females selected only experienced males. Subsequently, six more tests were performed using only inexperienced males. Reproduction was monitored and eggs and larvae of each couple were counted.

For investigations regarding reproductive success, six tests were carried out using six pairs of angel fish (male with prior reproductive success and another with reproductive failure) and six females. In each aquarium, one pair of males was placed with one female with previous reproductive success. Time spent by the female with one of the males was considered indicative of preference. Offspring survival rates for couples with reproductively successful males were assessed during one reproductive cycle. Females were paired

with unsuccessful males during a new reproductive cycle and the offspring survival rate was assessed for each couple.

Males which did not manage to go beyond courtship during the first mating attempt were considered inexperienced. On the other hand, males capable of reproducing were considered experienced. Males that managed to protect their offsprings until independence in the earlier reproductive cycle were considered successful and males which did not manage to protect their offspring until independence were considered unsuccessful.

Data analyses. Statistical tests were performed with SAS software using analysis of variance (ANOVA). Student's t test, Fisher/Snedecor's F test were used to identify differences between treatments and Pearson's correlation was used to verify the association between aggressiveness and parental care of experienced males for the survival of their offspring. Also, correlation was used to verify the association between parental care of experienced couples and survival of larvae and to verify the association between the time spent by the female with experienced successful males and inexperienced males for the survival of their offspring.

Results

Reproductive experience. In all tests performed females preferred experienced males and remained near them (ANOVA, t - test, t = 14.02; p < 0.001). Reproductively experienced males were more aggressive while taking care of the eggs and they were more diligent in protecting and aerating them. Correlation between the aggressiveness of experienced males and egg survival (Fig. 1) was significant (Pearson, r = 0.96; p < 0.01).

Experienced males effectively contributed to egg care. Then, the correlation between parental care exhibited by these males through aeration and egg survival (Fig. 2) was significant (Pearson, r = 0.94; p < 0.01).

During larval care, experienced males were more aggressive and protected the offspring better (through intra-bucal care) than inexperienced males. Correlation between aggressiveness of experienced males and larval survival (Fig. 3) was significant (Pearson, r = 0.97; p > 0.01).

During larval care there was a significant correlation between the care provided by reproductively experienced males and females and survival of larvae (Pearson, males: r = 0.95; p < 0.01; females: r = 0.96; p < 0.01).

Angel fish females remained only with inexperienced males when there was no other option. Time spent by females with these males was equal to the previous condition (Fisher / Snedecor, F = 0.21; p = 0.65). However, when offspring survival resulting from matings was compared in reproductively experienced and inexperienced males, significant differences were observed in the survival percentage of eggs and larvae (Fisher /Snedecor, percentage of surviving eggs: F = 151.38; p < 0.001; percentage of surviving larvae: F =11.08; p < 0.01).

Reproductive success. In angel fish, correlations between time spent by females with experienced males (successful in the previous reproduction, egg and larval survival) were significant (for eggs - Pearson, r = 0.97; p < 0.01; for larvae - Pearson, r = 0.90; p = 0.04). On the other hand, correlations between time spent by females with males unsuccessful in the previous reproduction and egg and larva survival were not significant (for eggs - Pearson, r = 0.06; p = 0.92; for larvae - Pearson, r = 0.32; p = 0.59).

Discussion

Results indicate that reproductive experience influenced male selection in female angel fish. Courtship behavior of experienced males of this species may indicate their parenting skills, possibly perceived by females, leading them to select males that demonstrate capacity and willingness to invest in raising the offspring.

It is well known that characteristics of agonistic encounters between males may also appear in courtship displays. As affirmed by Huntingford (1993), in many male fish species, the exhibition of these characteristics and their use may be controlled by both aggressive and sexual motivational systems.

In angel fish *P. scalare*, during parental care, the diligence and the aggressive response of experienced males towards intruders was fundamental for protecting eggs. This characteristic was responsible for the reduced risk of predation of the offspring. During egg care, experienced males made an expressive contribution through aeration. The preference for experienced males is a part of female strategy in obtaining advantages for their offspring.

During larval care, experienced males protected their young better with significant effects on their survival. Therefore, the aggressive response to intruders and the reproductive experience of males were fundamental to the care and survival of the offspring and the pairs formed with these males were more successful.
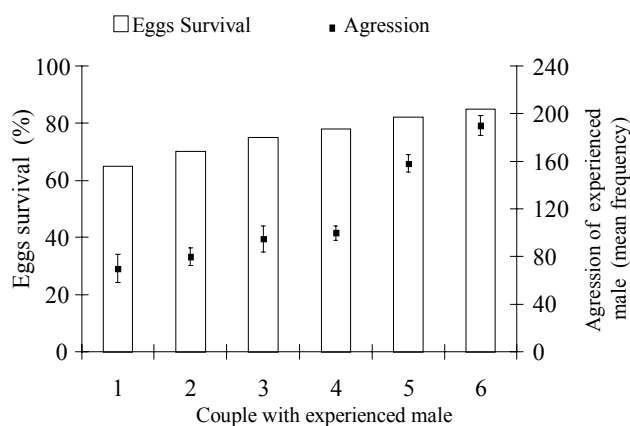

Fig. 1. Survival of the eggs in relation to the aggressiveness of experienced males (Pearson, p < 0.01).

Females of angel fish remained with inexperienced males only when there was no other option. However, this decision led to lower reproductive success. In these fish, males that were larger, more aggressive and possessed more parenting skills were preferred by females. Torricelli *et al.,* (1993) observed that the competitive ability of larger males made them more able to defend their territory and offspring against predators, what distinguished themselves as better care providers. Rogers (1995), studying the behavior of mate selection in the Midas Cichlid, *Cichlasoma citrinellum*, observed that females preferred larger, more aggressive and more reproductively experienced males. Couples formed by these males obtained advantages in defending territory and protecting offspring against predators. Our results agree with the data obtained by these authors and show that enhanced performance during angel fish reproduction was observed in couples with males that were larger, more aggressive, more experienced and more diligent in parental care.

Moreover, in *P. scalare*, the experience of the males may be accompanied by other characteristics, such as territory quality and social status. Larger and more aggressive male angel fish very often compete for access to territory preferred
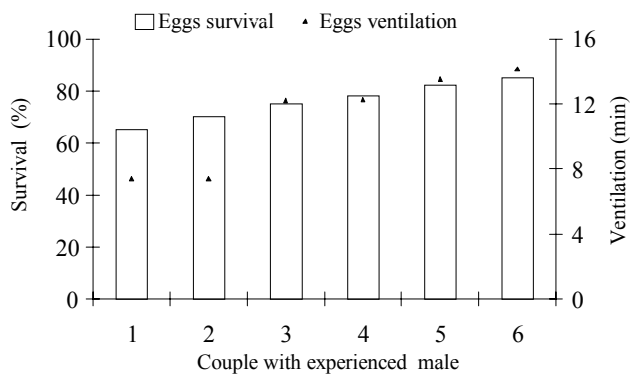
by females for reproduction and therefore, females select large and aggressive males that defend the best egg-laying areas (Cacho *et al.*, 2004; in press). Then, acquiring high-quality territory for reproduction represents security in raising offspring, as occurs with various other cichlids, such as the Java tilapia, *Oreochromis mossambicus* and the Nile tilapia, *Oreochromis niloticus* (Keenleyside, 1991). Social status may also contribute to successful competition for sexual partners, resulting, in most cases, in reproductive opportunities. In *P. scalare*, results suggest that selecting the best partner was one of the factors ensuring reproductive success in this species, as was described in other fish species, such as the Midas Cichlid, *Cichlasoma citrinellum*, and the goby, *Padogobius martensi* by Rogers (1995) and Torricelli *et al.*, (1993), respectively.

Conclusions

With regards to experience, male reproductive success and mate selection in angel fish it is clear that: Females assess the characteristics that indicate ability and willingness to invest in raising the offspring, through male courtship behavior. Females prefer larger, more aggressive and sexually experienced males for mating because couples with these males are more successful in acquiring and maintaining mating and breeding sites. The aggressive response and the reproductive experience of the males are fundamental in offspring care. Consequently, experienced males are preferred by females because they achieve greater reproductive success.

Fig. 2. Survival of the eggs in relation to care provided by experienced males through aeration (Pearson, p < 0.01).
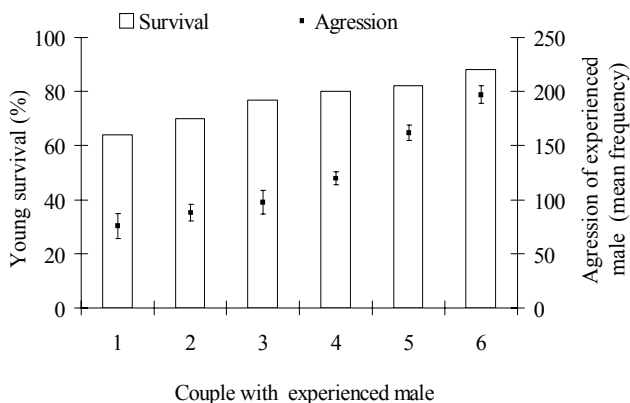
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Fig. 3. Survival of the larvae in relation to experienced male aggressiveness (Pearson, p < 0.001).

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