

Behaviorally Induced Sex Reversal of *Metriaclima* cf. *livingstoni* (Cichlidae) from Lake Malawi

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Sex reversal in the Lake Malawi cichlid, *Metriaclima* cf. *livingstoni*, was documented. A functional male in an aquarium was separated from three functional females by a divider of clear Plexiglas® sealed to tank walls. After the male was removed, one of the functional females developed male secondary sexual characteristics and fertilized eggs produced by the other females. In a second experiment, 6–7 functional females were placed in tanks without males. Again, sex change was noted with one of the former females fertilizing several broods.

NATURALLY occurring sex reversal, although common in a number of groups of marine fishes, is rare in freshwater fishes. Steroid-induced sex reversal of freshwater fishes is commonplace in aquaculture facilities. Tilapia (Tilapiae: Cichlidae) in particular is one of the most often manipulated groups of fishes. Aquaculturists have discovered that the sexual orientation of these fishes is fairly plastic in comparison with other vertebrates and easily manipulated by steroid hormones (Nakamura, 1981). Natural occurring sex reversal, though, has rarely been observed. *Monopterus albus*, the Rice Field Eel, is one of the few documented cases of naturally occurring sex reversal in freshwater fishes (Chan and Phillips, 1967).

Kaufman and Liem (1982) linked cichlids with members of the Labroidei on the basis of three pharyngeal characters (Stauffer et al., 2006). Although the monophyly of Labroidei is confused, Sparks and Smith (2004) believed that the sister group of the cichlids may include a large assemblage of diverse perciform lineages including but not limited to other labroid fishes. Sex changes in social groups of wrasses (Labroidei) have been shown (Ross et al., 1990). Because of the similarities of cichlids with marine forms, it is not surprising that cichlids show characters that suggest sex reversal. Many cichlids display several of the less reliable indicators of protogynous sex reversal, including yellow bodies or oocytes in the testes of males (Peters, 1975), bimodal size frequency distributions, with males being larger than females, and biased sex ratios, with females outnumbering males (Shapiro, 1987). Cichlids can also display more reliable features, such as changes in spawning behavior or gender specific coloration and morphology (Bass, 1988). Such changes are considered indications of transitional individuals. Preliminary investigations (Peters, 1975; Ohm, 1980, 1985) suggest that sex reversal occurs in cichlid fishes. Carruth (2000) reported histological evidence that suggested that *Crenicara punctulata* is a protogynous hermaphrodite in captivity. Naish and Ribbink (1990) showed that many cichlids, which Peters (1975) suggested were capable of sex reversal, were cases in which male secondary sexual characters were displayed by females. A similar phenomenon was reported in *Melanochromis auratus* from Lake Malawi (Hale et al., 1999). Definitive proof of sex reverses requires finding transitional individuals or direct observation of a specific individual spawning first as one sex and later as the opposite sex (Sadvoy and Shapiro, 1987;

Shapiro, 1987). The purpose of this paper was to determine experimentally if individuals of the shell-dwelling *Metriaclima* cf. *livingstoni* can change sex.

MATERIALS AND METHODS

Adult *M. cf. livingstoni* were captured while SCUBA diving off Mazinzi Reef (14°07.813'S, 34°57.744'E) in Lake Malawi, Africa and then transported to our laboratory at Penn State University. All fish used in the experiments were the F₁ generation from wild-caught fish. Water temperature was 25°C (±1°C) and photoperiod was 12L/12D. Water was filtered with a corner charcoal bubble filter. Twice daily, fish were fed live brine shrimp and a commercial flake food in an amount that they could consume in 10 min.

All fishes were held in tanks with one male and multiple females. The tanks contained no substrate, but a broken clay pot was provided for shelter and to facilitate spawning. In Lake Malawi, these fish use large empty snail shells for shelter. We did not use shells in our experiments because it was too difficult to determine if females were holding eggs when they were hiding in shells.

Metriaclima cf. *livingstoni* is a maternal mouthbrooder, as are all the haplochromine cichlids native to Lake Malawi (Konings, 2001). Occasionally, the female will deposit several eggs on the substrate where they are fertilized by the male before the female gathers them into her mouth. Alternatively, the female immediately takes the eggs into her mouth and then approaches the anal fin of the male at which time the eggs are fertilized.

We initially identified the sex of all individuals by color and length of the pelvic fins; subsequently, we examined the urogenital openings to support our initial determination. We used the following observations of behavior to corroborate our use of secondary sexual characters. Fishes that we initially identified as males exhibited strong territorial behavior and were observed courting females. In some instances, spawning was directly observed, but in most cases, sex was determined by morphology, behavior, and mouth brooding. In no instances did we observe a fish that we identified as a male brooding eggs or fry. All females used in the experiments had been observed mouth brooding at least one batch of fry or eggs. Whenever a fish that we determined to be female was found to be holding eggs or fry, she was removed and placed in a separate tank until the fry

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were released. Each fish, which we identified as being a male, had exhibited territorial behavior and was the only fish identified as a male that was held in the same aquarium as a mouth-brooding female. Therefore, we concluded that all fish used were either functional males or females.

The initial experiment consisted of eight 75.5-L fish tanks divided into two equal compartments by a piece of clear Plexiglas® sealed to the tank walls with silicon. A functional male was placed in one compartment and three functional females were placed in the other. The tanks were lined with black plastic on three sides to prevent visual interaction with fish in other tanks. After one month, the males were removed from six of the eight tanks. The two tanks in which the male remained served as controls. The fish were then observed for 2–4 months.

The second experiment was designed to increase the number of females present and determine if the initial presence of a male was necessary to induce sex change. Six functional females were placed in each of two 75.5-L tanks and seven functional females were placed in each of two other 75.5-L tanks. The fish were observed for 3–5 months for signs of sex reversal. Any individuals showing male coloration were left in the tank to see if they would breed with any of the remaining females. Once mating took place, females noted to be holding eggs were isolated in 37-L tanks to observe whether eggs would develop into fry.

RESULTS

In the first experiment, a female in one of the eight tanks developed male secondary characteristics that are usually seen only in dominant males. The fish's mouth and gular region became enlarged and it developed a protruding forehead. This individual fertilized eggs produced by one of the remaining females in the experiment, resulting in three large broods of fry. No other females in this experiment developed male behaviors or coloration.

The second experiment was designed to remove the initial male interaction. After approximately two months, one individual in each of the two tanks with six individuals began to display male coloration and behavior. During the third month, a female in one of these tanks was noted to be holding eggs. The female was removed and placed in a separate tank and observed. Two weeks later she released a brood of fry. None of the females in the other tank were observed holding eggs. No individuals in the other two tanks showed signs of male secondary sexual characters.

DISCUSSION

Many gonochoristic fishes first pass through an early ovarian stage before developing a permanent adult sex (Takahashi and Shimizu, 1983). We do not know if all *M. cf. livingstoni*, or all rock-dwelling cichlids from Lake Malawi in general, pass through an ovarian stage such that all males are derived secondarily, or if some males develop primarily. Large dominant *M. cf. livingstoni* males are easily recognized based on external morphology and coloration, but smaller submissive males will retain female coloration in the presence of a dominant male. Large dominant females will sometimes take on male coloration but do not develop other male secondary characteristics (Hale et al., 1999). Taking these factors into consideration, we thought that it was essential to use in our experiments only females that had previously produced fry, essentially excluding the possibility

that sex reversal was a delayed version of juvenile differentiation, or that the males produced in the experiments had always been males that were simply expressing female coloration.

Wikert (1993) histologically examined gonads of 40 individuals of *Metriaclicma cf. livingstoni* and found no evidence of transitional states indicating sex reversal. This may be in part because he did not collect fishes from shells, where it would be likely that a transitional individual would be establishing a territory. He determined that there was a biased sex ratio of 1.6 females per male and that males in some size classes were larger than females. Observations in our laboratory confirm Wikert's conclusions, but in the laboratory, the sex ratio tends to be somewhat higher with approximately five to ten females to every male developing in all broods. The males in the laboratory tend to grow exceptionally larger than the females. Wikert (1993) also found oocytes present in active testes, but as mentioned before, these findings are considered unreliable indicators of sex reversal (Sadvoy and Shapiro, 1987; Shapiro, 1987). Transitional individuals or individuals documented to breed as functional members of both sexes are needed to definitively prove sex reversal. Our experiments are the first known successful attempts at documenting a Malawian cichlid that first bred as a female and later as a male.

The first experiment yielded limited success, with only one functional female switching sex. We expected that sex reversal was socially controlled by the presence of a large male that inhibited the females from changing sex. In marine fishes, it has been documented that a minimum number of the initial sex must be present for sex reversal to occur (Shapiro and Boulon, 1982; Shapiro, 1990).

The second experiment was designed to increase the number of females initially present and remove male interaction from the beginning. After approximately two months, two of the tanks had an adult female, which showed signs of male behavior. One of these individuals completely switched sex and fertilized eggs from a female. The fish in the other two experimental tanks did not appear to show any signs of switching sex. Although our experiments showed sex reversal, further studies are needed to document whether sex reversal occurs in the wild. Histological examinations of transitional individuals need to be completed to identify the changes that are associated with sex reversal in these fish.

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