

SEX REVERSAL IN MUD LOACH, *MISGURNUS MIZOLEPIS*

BY IMMERSION

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Introduction

Mud loach, *Misgurnus mizolepis*, is an important both as a food fish and as a fish that is used in religious ceremonies by Buddhists in Korea (Kim et al., 1994). About 4,000 tons of loaches were produced annually both by capture and culture. Mud loach exhibits sexual dimorphism in that the female grows faster than the male. The ability to produce and culture a monosex female population would improve yields.

In aquaculture, monosex female populations are traditionally produced either by hormonal treatments of sexually undifferentiated fry or by mating sex reversed males (genetic females but phenotypic males) with normal females in species where the female is homogametic (Johnstone et al., 1979; Tave, 1993). However, the sex determination mechanism of this species has not been understood yet.

The use of hormone-treated feed is the preferred way to produce sex-reversed fish, but it has several liabilities: it cannot be used in species which do not accept artificial feed during the labile period; the timing of the treatment is critical; if fish have access to natural food, sex reversal will not be complete; if the feed is not consumed immediately, the hormone can leach from the feed. Immersion technology, in which sexually undifferentiated fry are raised in water containing hormones, can circumvent some of these problems, because the hormone does not have to be consumed to enter the fish, and because the hormone is present in the water, it is present throughout the critical period when each individual undergoes sexual differentiation.

The objective of this study was to evaluate the effectiveness of various concentrations and treatment durations of both estradiol-17 β , and 17 α -methyltestosterone on the production of sex-reversed mud loach by immersion treatments. The effect of the immersion treatments on survival, growth, gonad histology and morphology, and pectoral fin morphology were also evaluated.

Materials and methods

Gametes were stripped from five male and eight female 3-year-old broodfish. Eggs and sperm were pooled and fertilized as described by Kim et al. (1995). Fertilized eggs were incubated at 25°C. Thirty six hours after hatching, 330 fry were randomly allocated to each of 36 groups for

feminizations, also allocated to 48 groups for masculinizations. Each group was stocked in an 80 cm x 50 cm x 40 cm aquarium containing 40 l of well-aerated water. Temperature was 25°C. For feminizations, fry were raised in water containing 0, 50, 100, or 200 ug of estradiol-17β/l for either 1, 2, or 3 weeks. And for masculinizations, fry were also immersed in water containing 0, 25, 50, 100, or 200 ug of 17α-methyltestosterone for either 1, 2, or 3 weeks. There were 3 replicate aquaria per each treatment combination. During the immersion treatments, fry were fed *Artemia* nauplii and 39% protein carp feed *ad libitum*.

When each immersion treatment was finished, 33 fish were randomly taken from each of the aquaria in order to determine the individual weight, total length, standard length, and condition factor. The remaining fish in each aquarium were counted by capture to evaluate the percentage of fish surviving after treatment. After hormone treatment, the fish from each replicate were randomly assigned to a 90-l aquarium, and grown as described by Kim et al. (1994) for 9 months.

When fish were 2 months old, 50 fish were randomly sampled from each replicate, and their gonads were examined histologically as described by Goetz et al. (1979). Gonads containing both oocytes and spermatocytes were classified as intersex. Morphology of the pectoral fins and gonad morphology of sex reversed fish were compared to those of normal control fish at 9 months. Differences were assessed by ANOVA and/or Chi-square analysis at the level of $p < 0.05$.

Results and discussion

Survival and growth

Survival rates decreased as dose and duration of treatment increased; in most instances they were significantly lower than those of the controls ($p < 0.05$) (Table 1). Survival of fish treated with the highest dose (200 ug/l of estradiol-17β or 17α-methyltestosterone) were significantly lower than those of all other groups after all three time periods.

Mean weights, lengths, and condition factors are presented in Table 2. The fish in the 200 ug/l estradiol-17β treatments revealed significantly inhibited growth rates, regardless of treatment duration. However, growth of fish in all 17α-methyltestosterone treated groups were unaffected; they were similar to those controls in all three detection points.

Sex reversal and sex ratio

Mean sex ratios of the control groups were approximately 1:1 for all three time periods. In general, both hormone concentration and treatment duration had a significant effect on sex reversal. The 50 ug estradiol-17β/l treatments for 1 and 2 weeks, and 25 ug 17α-methyltestosterone did not significantly alter the sex ratios, but all other treatment combinations did. The 200 ug estradiol-17β/l for 2 and 3 weeks were the only treatment combinations which produced monosex female

Table 1. Percent survival 1, 2, and 3 weeks after estradiol-17β (ES) or 17α-methyltestosterone (MT) treatments (mean±s.d.) of mud loach fry

Treatment group	Survival (%)		
	1 week	2 weeks	3 weeks
ES			
0 ug/l	96.8±1.3 ^x	87.1±1.7 ^x	82.0±2.1 ^x
50 ug/l	96.2±2.1 ^x	75.1±3.9 ^y	68.3±4.2 ^y
100 ug/l	92.9±2.3 ^x	74.0±4.5 ^y	67.1±3.4 ^y
200 ug/l	72.0±3.7 ^y	48.8±5.5 ^z	37.0±6.0 ^z
MT			
0 ug/l	96.1±2.6 ^a	88.8±3.4 ^a	82.8±0.9 ^a
25 ug/l	95.7±2.7 ^a	87.2±0.9 ^a	78.4±1.6 ^b
50 ug/l	95.2±1.2 ^a	82.3±5.6 ^a	74.7±1.6 ^b
100 ug/l	94.8±1.4 ^a	73.9±2.2 ^b	67.1±1.2 ^c
200 ug/l	85.0±2.1 ^b	67.8±2.3 ^c	56.7±2.4 ^d

Means within a column superscripted with different letters are significantly different ($p < 0.05$).

Table 2. Weight (W), total length (TL), and condition factor (CF) (mean±s.d.) of experimental groups after 1, 2, and 3 weeks estradiol-17β (ES) or 17α-methyltestosterone (MT) immersion treatments

Treatment dose (ug/l)	1-week treatment			2-week treatment			3-week treatment		
	W (mg)	TL (mm)	CF	W (mg)	TL (mm)	CF	W (mg)	TL (mm)	CF
ES 0	2.5±0.6 ^a	15.4±0.6 ^a	0.7±0.3 ^a	25.5±0.6 ^a	31.5±0.8 ^a	0.8±0.1 ^a	432.8±25.5 ^a	39.0±0.2 ^a	7.3±0.3 ^a
50	3.4±0.7 ^a	16.3±1.0 ^a	0.8±0.1 ^a	21.3±1.3 ^a	30.5±1.4 ^a	0.8±0.2 ^a	460.0±16.9 ^a	39.4±0.7 ^a	7.6±0.5 ^a
100	3.5±0.1 ^a	16.9±0.6 ^a	0.8±0.1 ^a	25.3±1.6 ^a	31.5±0.2 ^a	0.8±0.1 ^a	498.7±12.7 ^a	40.6±1.3 ^a	7.5±0.6 ^a
200	1.0±0.1 ^b	10.8±0.2 ^b	0.8±0.1 ^a	12.6±0.6 ^b	26.3±1.0 ^b	0.7±0.1 ^a	21.2±1.2 ^b	30.1±0.4 ^b	0.8±0.1 ^b
MT 0	5.0±1.3 ^x	18.7±0.3 ^x	0.8±0.2 ^x	33.4±3.9 ^x	27.2±2.0 ^x	1.7±0.3 ^x	479.1±35.3 ^x	36.8±2.4 ^x	9.7±1.2 ^x
25	5.4±1.5 ^x	18.5±0.4 ^x	0.9±0.2 ^x	37.3±3.0 ^x	26.1±1.3 ^x	2.1±0.3 ^x	504.3±32.5 ^x	38.5±2.2 ^x	8.9±1.1 ^x
50	5.3±1.2 ^x	18.6±0.5 ^x	0.8±0.2 ^x	39.3±2.4 ^x	27.9±1.7 ^x	1.8±0.3 ^x	487.3±29.0 ^x	37.7±2.0 ^x	9.2±1.0 ^x
100	5.0±1.3 ^x	18.3±0.3 ^x	0.8±0.2 ^x	37.9±1.6 ^x	25.7±1.7 ^x	2.3±0.4 ^x	506.7±22.9 ^x	38.2±3.0 ^x	9.3±1.4 ^x
200	5.1±0.9 ^x	18.1±0.5 ^x	0.9±0.2 ^x	37.2±2.6 ^x	25.6±1.9 ^x	2.3±0.4 ^x	483.4±53.6 ^x	38.0±4.2 ^x	9.2±1.8 ^x

Means within a column superscripted with different letters (a, b in ES; x, y in MT) are significantly different (p < 0.05).

populations. The 100 ug estradiol-17β/l for 3 week treatment produced a near monosex population (98.7%); the other fish were classified as intersex. All male populations were produced by 200 ug 17α-methyltestosterone/l for 2 and 3 weeks, and 100 ug 17α-methyltestosterone/l for 3 weeks also produced higher percent males than 99% (Table 3 & 4).

Table 3. Effects of estradiol-17β immersion treatments on the sex ratios of mud loach

Dose (ug/l)	1-week treatment			2-week treatment			3-week treatment		
	Female (%)	Male (%)	Intersex (%)	Female (%)	Male (%)	Intersex (%)	Female (%)	Male (%)	Intersex (%)
0	49.3±2.5 ^{a,x}	50.7±2.5	0.0±0.0	54.0±2.8 ^{a,x}	46.0±2.8	0.0±0.0	52.0±3.3 ^{a,x}	48.0±3.3	0.0±0.0
50	56.7±2.5 ^{a,x}	40.0±1.6	3.3±0.9	55.3±3.8 ^{a,x}	39.3±2.5	5.3±2.5	64.7±2.5 ^{b,y,+}	34.0±3.3	1.3±0.9
100	66.7±2.5 ^{b,x,+}	28.7±1.9	4.7±0.9	78.7±2.5 ^{b,y,+}	20.0±1.6	1.3±0.9	98.7±1.9 ^{c,z,+}	0.0±0.0	1.3±0.9
200	73.3±3.8 ^{c,x,+}	26.0±2.8	0.7±0.9	100.0±0.0 ^{c,y,+}	0.0±0.0	0.0±0.0	100.0±0.0 ^{c,y,+}	0.0±0.0	0.0±0.0

Means within a column superscripted with different letters (a, b, c) are significantly different (p < 0.05). Means within a row superscripted with different letters (x, y, z) are significantly different (p < 0.05). +s indicate significant deviations from 1 : 1 ratio.

Table 4. Effects of 17α-methyltestosterone treatments on the sex ratios of mud loach

Dose (ug/l)	1-week treatment			2-week treatment			3-week treatment		
	Male (%)	Female (%)	Intersex (%)	Male (%)	Female (%)	Intersex (%)	Male (%)	Female (%)	Intersex (%)
0	48.0±4.3 ^{a,x}	52.0±4.3	0.0±0.0	49.3±3.4 ^{a,x}	50.7±3.4	0.0±0.0	51.3±3.4 ^{a,x}	48.7±3.4	0.0±0.0
25	51.3±3.4 ^{a,x}	46.7±5.6	2.0±1.6	66.0±1.6 ^{b,y,+}	32.7±1.9	1.3±0.9	80.0±2.8 ^{b,z,+}	16.0±1.6	4.0±1.6
50	60.7±2.5 ^{b,x,+}	38.0±3.3	1.3±0.9	72.0±3.3 ^{c,y,+}	25.3±2.5	2.7±2.5	94.0±3.3 ^{c,z,+}	4.7±2.5	1.3±0.9
100	75.3±2.5 ^{c,x,+}	23.3±1.9	1.3±0.9	83.3±4.1 ^{d,y,+}	16.0±4.9	0.7±0.9	99.3±0.9 ^{d,z,+}	0.7±0.9	0.0±0.0
200	81.3±2.5 ^{d,x,+}	18.0±1.6	0.7±0.9	100.0±0.0 ^{e,y,+}	0.0±0.0	0.0±0.0	100.0±0.0 ^{d,y,+}	0.0±0.0	0.0±0.0

Means within a column superscripted with different letters (a, b, c, d, e) are significantly different (p < 0.05). Means within a row superscripted with different letters (x, y, z) are significantly different (p < 0.05). +s indicate significant deviations from 1 : 1 ratio.

These data show that estradiol-17 β and 17 α -methyltestosterone are effective in sex reversing mud loach. The results also indicate that this species can be effectively sex reversed by immersion and that the hormone does not have to be added to feed. This is similar to that observed in coho salmon and chinook salmon (Goetz et al., 1979; Hunter et al., 1986). These results also show that the optimal immersion dosages of estradiol-17 β and 17 α -methyltestosterone are 100-200 ug/l.

The hormone was administered during the critical period of gonadal differentiation (Kim et al., 1990), and the results show that the phenotypic development of the undifferentiated gonad could be directed by an external steroids. The ability to produce a sex-reversed population depends, to a great extent, on the administration of the hormone during this short period of development (Goetz et al., 1979; Yamazaki, 1983; Hiott and Phelps, 1993).

Further research is needed to determine the optimal treatment combination that can be used to produce 100% sex-reversed mud loach with minimal adverse effects on growth and survival. Further research is also required to establish the rate at which the administered steroids are cleared from the fish. It would also be valuable to determine the concentration of hormones in normal fish and hormone-treated fish at the time of harvest. A recent study of the clearance of isotopically labeled estradiol-17 β from salmon fry has shown that clearance is very rapid (Piferrer and Donaldson, 1994).

Gonad histology

Histological examination of the gonads from 2-month-old control females showed normal oocytes at various stages of development; those of 2-month-old normal males revealed normal testicular tissue containing spermatocytes and spermatozoa. Gonadal tissue from sex-reversed hormone-treated fish was similar histologically to that from normal fish. Gonadal tissue from intersex individuals contained both oocytes and spermatocytes, in various morphological arrangements and developmental stages (Fig. 1).

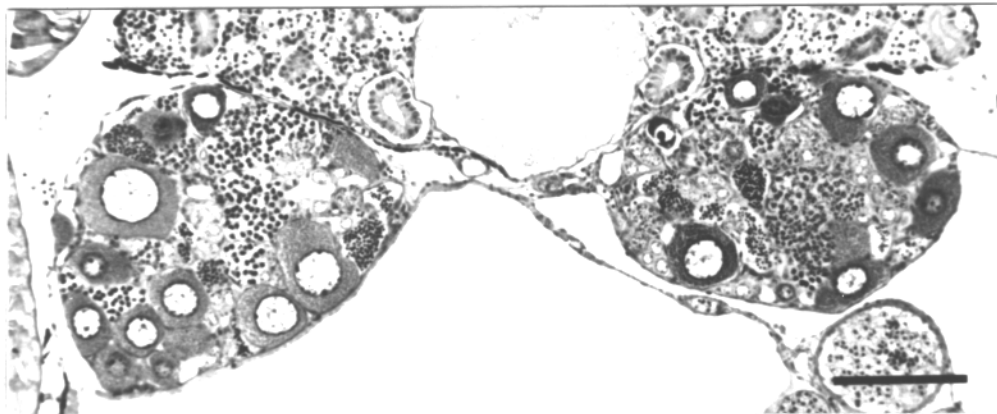


Figure 1. Transverse sections of gonads from 2-month-old intersex fish. Bar indicates 100 μ m.

Morphology of gonads and pectoral fins of sex reversed fish

External morphology of ovaries from 9-month-old control fish revealed a single sac-shaped organ, with well-developed yolk-laden oocytes. Testes from 9-month-old control males were a pair of rod-shaped structures, with milky white sperm. In sex reversed females, the development of oocytes in the ovaries did not extend to the genital pore, which suggests the possibility that sex-reversed mud loach might not be able to spawn. On the other hand, the masculinized fish have similar testes morphology compared to normal males.

The size and shape of the pectoral fins exhibit sexual dimorphism in mud loach. Those in the males are larger and the fins have a distinct lamina circularis. Sex reversed fish had pectoral fins that were intermediate in size between those of normal males and normal females. Furthermore, most, if not all, laminae circulares in sex reversed fish were vestigial, although the size varied (Fig. 2).

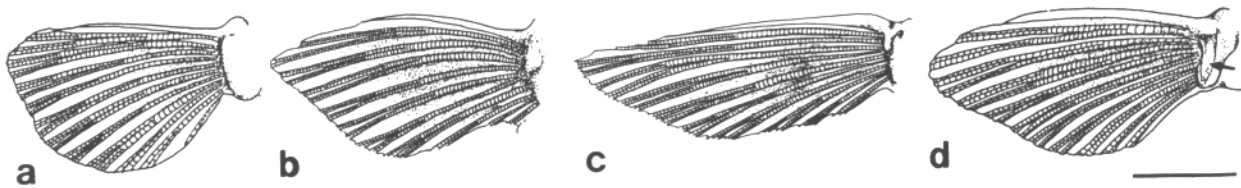


Figure 2. Shape and structure of pectoral fins: normal female (a); feminized fish (b) masculinized fish (c); normal male (d). Arrow indicates the lamina circularis. Bar indicates 5 mm.

This paper presents for the first time a protocol to ensure 100% feminization and masculinization in a cyprinid mud loach using steroid immersion technique alone.

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