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Health & Welfare

## Influencing sex ratios in Nile tilapia

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### Interactions between genotype, temperature



Setup for artificial incubation of Nile tilapia eggs.

The importance of monosex (male) culture for profitable Nile tilapia aquaculture has long been recognized. Masculinizing with hormones is in many cases done to increase the percentage of males. A growing number of consumers is interested in environmentally-friendly production and do not accept products that were produced with the use of hormones or similarly active substances. Environmental factors can influence the sex determination of reptiles and fishes, and this knowledge could be useful to develop all-male Nile tilapia stocks without the use of hormones in the near future.



Setup for temperature treatment of first-feeding Nile tilapia fry.

## Temperature and sex ratios

Initial indications on the influence of temperature on sex ratios in lizards go back to the mid-1960s. Numerous studies conducted since then have shown temperature-dependent sex determination in other reptiles like turtles and alligators, and later also in some fish species.

Different species models have been reported, where sex ratios are skewed to one sex at high or low temperatures, or where one sex results from intermediate and the other sex from lower and higher temperatures during egg incubation or fry rearing (Lemma, 2001).

## Study setup

We recently conducted a study to test the effect of different fry rearing temperatures on the sex ratios of Nile tilapia (*Oreochromis niloticus*) from two different sources: Lake Manzala in Egypt and Lake Rudolph in Kenya. Male and female breeders from both sources were individually tagged and maintained in glass aquaria. Fish were manually stripped when the females showed signs of readiness for spawning (swollen and reddened urogenital papilla and territorial behavior). After artificial fertilization, eggs and hatching larvae were incubated at 28 degrees-C up to day 9 after fertilization.

## Temperature treatments

At day 9 the individual offspring groups were separated into treatment and control groups. Controls were maintained at a constant 28 degrees-C. Temperature treatments were applied at 18 degrees-C for 20 days or at 36 degrees-C and 38 degrees-C for 10 days starting on day 10, and then survival rates in the treatment and corresponding control groups were recorded. Then all groups were separately reared at 28 degrees-C until individual fishes could be sexed by external examination. Differences in sex ratios between treatment and control groups were analyzed for significance by  $\chi^2$ -tests ( $p < 0.05$ ).

## Results

Table 1 shows data on the sex ratios observed within full sib groups of randomly selected male and female breeders from the two original sources, after different temperature treatments and in the corresponding control groups. Treatments with 18 degrees-C for 20 days typically did not result in sex ratios significantly different to controls. In contrast, in most cases the 36 degrees-C treatment for 10 days led to a significantly higher percentage of males when compared with the corresponding controls.

**Table 1. Influence of temperature treatments on offspring sex ratios in Nile tilapia from different origins.**

<b>Origin: Lake Manzala</b>											
Control (28° C)			18° C for 20 days			36° C for 10 days			38° C for 10 days		
N <sub>1</sub> <sup>(1)</sup>	N <sub>2</sub> <sup>(2)</sup>	% M. <sup>(3)</sup>	N <sub>1</sub>	N <sub>2</sub>	% M.	N <sub>1</sub>	N <sub>2</sub>	% M.	N <sub>1</sub>	N <sub>2</sub>	% M.
21	2,264	50.1	11	1,555	54.2	20	2,050	79.3	11	1,168	68.8
<b>Origin: Lake Rudolph</b>											
Control (28° C)			18° C for 20 days			36° C for 10 days			38° C for 10 days		
N <sub>1</sub>	N <sub>2</sub>	% M.	N <sub>1</sub>	N <sub>2</sub>	% M.	N <sub>1</sub>	N <sub>2</sub>	% M.	N <sub>1</sub>	N <sub>2</sub>	% M.
13	865	53.0	6	484	53.3	13	894	61.0	5	371	52.0

<sup>1</sup> Number of tested offspring groups resulting from different matings.

<sup>2</sup> Number of sexed individuals.

<sup>3</sup> Percentage of males.

Increasing the treatment temperature to 38 degrees-C did not increase male percentages, when compared to the 36 degrees-C treatment. Because survival rates in controls and treatment groups were similar (controls at 28 degrees-C, 95 percent survival; at 18 degrees-C treatments: 91 percent survival; at 36 degrees-C treatments: 95 percent survival; at 38 degrees-C: 87 percent survival), differences in survival rates between sexes are probably not responsible for the observed differences between treatment and control groups.

Considerable differences in the sensibility of the sex determining mechanism to temperature treatments were observed between animals of different origin (Table 1). In the 36 degrees-C treatment, 17 of the 20 tested offspring groups with a Lake Manzala origin showed significantly more males than the corresponding control groups. The mean percentage of males after 36 degrees-C treatment was 79.3 percent (range 44 to 100 percent). For animals of Lake Rudolph origin, only three of the 13 tested groups showed a significantly increased percentage of males. The mean value of 61.0 percent (range 44 to 79 percent) was also lower.

## Repeatability and individual influence

To examine the repeatability of the response of sex ratios to temperature treatment, spawns were repeated (Table 2). The percentages of males in the 36 degrees-C treated replicated spawns differed only by 1.7 to 3.7 percent.

**Table 2. Sex ratios within repeated spawns of Nile tilapia from Lake Manzala origin.**

Male No.	Female No.	Control (28° C)			36° C for 10 days		
		N <sub>2</sub> <sup>(1)</sup>	% S.R. <sup>(2)</sup>	% M. <sup>(3)</sup>	N <sub>2</sub>	% S.R.	% M.
1	35	368	97.4	50.8	352	91.4	89.8*
1	35	179	90.3	51.4	202	89.9	86.1*
1	40	324	94.1	50.9	321	96.7	72.3*
1	40	276	91.5	48.9	275	92.7	68.7*
7	10	216	93.0	50.0	246	98.5	94.3*
7	10	107	98.8	50.5	94	100.0	92.6*

<sup>1</sup> Number of sexed individuals

<sup>2</sup> Survival rates during treatment in percent

<sup>3</sup> Percentage of males

\* Significantly different from control ( $\chi^2$  – test at sex ratios,  $p < 0.05$ )

**Table 3. Sex ratios within paternal half sib groups in Nile tilapia from Lake Manzala origin.**

Male No.	Female No.	Control (28° C)			36° C for 10 days		
		N <sub>2</sub> <sup>(1)</sup>	% S.R. <sup>(2)</sup>	% M. <sup>(3)</sup>	N <sub>2</sub>	% S.R.	% M.
1	2	190	96.5	54.2	119	98.0	83.2*
1	4	246	100.0	50.8	329	97.1	61.4*
1	10	325	99.4	47.7	325	97.3	91.3*
5	7	150	98.1	45.3	153	99.4	92.8*
5	8	198	100.0	51.0	173	100.0	89.0*
5	11	302	98.8	46.4	201	94.4	81.6*
7	9	70	98.8	54.3	77	92.2	80.5*
7	10	313	94.9	48.3	324	93.6	89.4*
7	40	147	95.8	49.0	150	95.0	76.7*

<sup>1</sup> Number of sexed individuals

<sup>2</sup> Survival rates during treatment in percent

<sup>3</sup> Percentage of males

\* Significantly different from control ( $\chi^2$  – test at sex ratios,  $p < 0.05$ )

Observations within groups of paternal half sibs showed the individual influence of breeders on the response of the sex ratio to the temperature treatment. Table 3 shows data from three different males each mated with three randomly selected females. Compared to their control groups, all groups treated at 36 degrees-C showed significantly higher percentages of male offspring, and the level of response to the treatment depended on the individual females. Differences in sex ratios of up to approximately 20 percent were observed within half sibs of single males.

## Conclusion

Results of this study showed that simple temperature treatments can repeatedly increase the percentage of males in the offspring of Nile tilapia of different origins. The resulting sex ratios in offspring of populations of different origin and individual fish from the same origin after temperature treatments evidence the interactions between genotype and the sensitivity of the sex determination in Nile tilapia to temperature treatment.

The genetic component of the observed response of sex ratios to temperature treatments is promising for genetic improvement. Further experiments will evaluate selection programs to generate Nile tilapia strains that can efficiently and cost-effectively produce all-male stocks by temperature treatments.)

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