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Performance testing of clonal Nile tilapia lines

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Maintenance of the developed clonal groups required almost no additional costs



This experimental tank setup was used to produce clonal lines of Nile tilapia at the Institute of Animal Husbandry and Genetics in Germany.

Fish populations with good growth performance and the highest possible phenotypic uniformity are preferred by the aquaculture industry, to reduce labor and use of resources in the production and processing of products. One approach to improve these characteristics is the reduction of genetic variability by artificial production of clonal lines and use of these isogenic fish or isogenic crossbreds from two clonal lines for commercial production.

Homozygous clones

In fish, external fertilization and embryonic development allow the development of homozygous clones in only two generations through the use of induced gynogenetic or androgenetic reproduction. Whether a breeding strategy including clones or clone crosses is relevant for practical applications depends on the performance of the clone crosses, as well as the cost-effectiveness of the development and maintenance of the clonal lines.

Clonal lines of Nile tilapia

In recent years, a research group at the University of Göttingen's Institute of Animal Husbandry and Genetics in Göttingen, Germany produced clonal lines of Nile tilapia (*Oreochromis niloticus*), one of the most important species in commercial aquaculture. Although clones have also been developed in other fish species, limited data have been published on their reproductive and growth performance in comparison to normal, genetically variable controls.

Development, reproduction

During the first step, homozygous *O. niloticus* were produced through "late" heat shock treatments at 41 degrees-C for 6 minutes, applied 25-35 minutes after egg activation with sperm that was genetically inactivated by ultraviolet irradiation. Six homozygous females produced through this mitotic gynogenesis procedure were fertile and served as clone mothers.

During the second step of clone development, eggs from these homozygous females were artificially stripped and given an "early" heat shock treatment (41 degrees-C for 4 minutes), 4.5 minutes after egg activation with genetically inactivated sperm (meiotic gynogenesis).

The clonal status of the resulting individuals was verified by gene marker studies. To continue the clonal lines, clonal females were again reproduced by meiotic gynogenesis.

Reproductive performance

Table 1 provides data on the reproductive performance of females belonging to the six developed clonal lines. Compared to nonclonal control groups with a mean value of 43.9 percent for first-feeding fry, the tested *O. niloticus* clones were much more difficult to reproduce.

The treatment for gynogenetic reproduction seemed to be responsible for the reduced survival rates within clonal groups. Also, conventionally fertilized eggs from clonal females not exposed to shock had mean survival values as low as 11.2 percent for first-feeding fry, which indicated the reduced quality of eggs from clonal females.

Table 1. Reproductive traits of first-generation clonal *O. niloticus* females from six clonal lines.

		Clone I	Clone II	Clone III	Clone IV	Clone V	Clone VI
No. of shock-treated eggs	N	9,733	6,021	3,015	6,365	507	4,833
First-feeding fry	N	475	133	30	297	2	101
	%	4.88	2.21	0.99	4.67	0.39	2.09
Adult fish	N	159	75	28	158	2	72
(Second-generation clones)	%	1.63	1.25	0.93	2.48	0.39	1.49

Growth performance

Clonal and control groups were incubated and reared under identical conditions at 28 degrees-C. On day 20 after hatching, fish selected for growth performance testing were transferred into 80-liter glass aquaria. Depending on the number of available clonal fish per egg batch, each aquarium was stocked with up to 80 individuals.

Control groups were reared at corresponding stocking densities. As all gynogenetic clonal fish were females, all female control groups were used for the growth comparisons.

All groups were fed three times a day *ad libitum* with commercial pelleted feed. Fish were individually weighed on day 132 after hatching. Differences in mean body weights and variances of body weights between groups were analyzed statistically.

Table 2 presents body weight data obtained from clonal groups and corresponding controls. Differences in weight between clonal groups and controls were not as marked as they were for reproductive traits. Altogether, clonal groups showed slightly lower growth compared to controls, but these differences were insignificant.

Table 2. Mean body weights and coefficients of variation from clonal groups and corresponding controls.

Stocking Density	Group	No. of Tanks	No. of Fish	Mean Body Weight (g)	CV ¹ F-Test
Fish/tank: 21-50	Control	11	385	49.6	31.1
	Clone I	5	140	43.2	18.2
	Clone II	4	155	41.7	25.3
	Clone III	2	90	68.3	21.4
	Clone IV	1	29	62.5	21.0
	Clone V	1	39	22.2	19.7
	Clone VI	–	–		
	All clones	13	453	48.0 n. s. ²	22.5*
All stocking densities	Control	22	670	50.2	34.1
	All clones	29	825	45.5 n. s.	23.1*

¹ coefficient of variation, ² not significantly different from control

* significantly different from control ($P \leq 0.05$)

For tested groups with a stocking density of 21 to 50 individuals per tank, clones III and IV showed better growth than controls. All clonal *O. niloticus* groups had lower variabilities for body weight than the control groups, probably due to their genotypical uniformity. Maternal and environmental influences seemed to be responsible for the remaining variability observed within clonal groups.



Homozygous females were artificially stripped in the study.

Conclusion

This study demonstrated the difficulties of development and reproduction of clonal *O. niloticus* lines through induced gynogenesis. However, maintenance of the developed clonal groups required almost no additional costs and care, because their growth performance was comparable to, and in some cases better, than control groups.

It remains to be determined if continuation of clones by natural spawning of females with sex-reversed functional clonal males will be easier than through gynogenetic reproduction. From results of ongoing diallel crosses between the developed clonal lines, the Institute of Animal Husbandry and Genetics will determine if the development and continuation of clones in *O. niloticus* is worthwhile for practical applications in breeding programs.

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