Growth, Metabolic Rates and Body Composition of Individually Reared Triploid Tilapia (*Oreochromis niloticus*) in Comparison to Diploid Full-Sibs

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Introduction

The culture of tilapia, mostly Nile tilapia, *Oreochromis niloticus*, has been expanding all over the tropics for the past decades. In contrast to many other species, it is cultured in a wide range of environments from freshwater to marine and at all possible levels of intensity. However, problems common for many tilapia culture systems are the reduction of growth rates at the onset of sexual maturity and undesired reproduction, leading to high numbers of small fish (stunting). Brämick *et al*. (1995) confirmed triploidization as a tool to prevent stunting effects in extensive pond culture of Nile tilapia, *Oreochromis niloticus*. In their experiments, no differences in growth between triploids and diploid controls could be observed at age of maturation (6 weeks of grow-out), however, at the time of harvest (25 weeks of grow-out), triploids were significantly heavier ($p < 0.01$) than control fish. This was contrary to results gained from experiments under laboratory conditions (Don and Avtalion, 1986; Penman *et al*., 1987, Puckhaber and Hörstgen-Schwark, 1991). It was speculated that triploid tilapia may have gained the observed growth advantage only due to stunting effects in control ponds, although predator controlled cultivation of diploid tilapia was used. In addition, breeding activities of fish seemed to be responsible for decreasing growth in diploid females at time of maturation. In order to evaluate the (physiological) effects of triploidy in Nile tilapia we set up an experiment to compare growth, metabolic rates and body composition of individually reared triploid Nile tilapia with diploid full-sibs.

Materials and Methods

Experimental fish

The same population of *Oreochromis niloticus* (originating from Lake Mansala, Egypt) as in the earlier lab (Puckhaber and Hörstgen-Schwark, 1991) and field experiments (Brämick et al, 1995) was used. The brood stock of this population was kept at the Institut für Tierzucht und Haustiergenetik, Universität Göttingen, where the experimental groups were established. In order to produce diploid and triploid full sibs, an egg batch was randomly taken from a single pair mating and divided into two groups. While one group remained untreated (diploid control), eggs of the other group were heat-shocked to induce retention of the second polar body. Heat-shock treatment was applied at 41°C for a duration of 4.5 min, 4 min post fertilization, as described by Puckhaber and Hörstgen-Schwark (1991). Triploidization success was confirmed by chromosome preparations (Kligerman and Bloom, 1977, adapted by Puckhaber and Hörstgen-Schwark, 1991) in a random sample of ten embryos out of the

treated group. At the age of 90 days 60 diploid and 60 triploid fish with an average weight of 15 g were transfered to the aquaculture laboratory of the Department of Animal Nutrition and Aquaculture in the Tropics and Subtropics, Hohenheim University, where they were group reared untill the beginning of the experiment.

Experimental Setup

At the age of 128 days, diploid and triploid tilapia with an initial body mass of 27g (20 fish each) were stocked individually in aquaria (20*l*) connected to recirculating systems. Concurrently, 7 diploid and 8 triploid fish were stocked individually in the boxes (5*l*) of a recirculating respirometric system (Focken *et al.*, 1994) for the continuous individual recording of oxygen consumption. All systems were equipped with biological filters for nitrification. NH_3 and NO_2^- levels were below 0.2 and 2mg/*l* respectively at any time. In order to prevent high nitrate levels, 10% of water were exchanged every day. Water temperature was kept at 27°C (± 0.1°), an artificial light regime was set at 12h light:12h dark.

Feeding Regime

The fish were fed a diet with 33.8% crude protein, 13.5% lipids and 7.1% ash, gross energy was 21.7kJ/g (all dry matter base). This diet was mostly plant derived, with soybean meal as main protein source. Feed allowance was based on the metabolic body mass (power 0.8) of each individual fish and was adjusted weekly according to body mass changes. Feeding rate at a given day was identical for all fish. During the first week of experiment, fish were fed at maintenance level in order to determine their metabolic rates at this level. Feed allowance at this time was 3.5g/$kg^{0.8}$/d. During the second week, feed allowance was gradually increased to 17.5g/$kg^{0.8}$/d and kept at this level until week 8. To ensure complete ingestion of the feed by all fish, from week 9 to week 17, feed allowance was reduced to 14g/$kg^{0.8}$/d. The daily ration was given in 6 equal installments during the light time by means of automatic feeders. Fish were fed 6 days/week, there was no feeding on the day of the weekly sampling.

Sampling and Analytical Procedures

The fish were sampled every week in order to monitor their body mass development. For this, the fish were taken out from their aquaria by a scope net and weighed to 0.1g in a pre-tared bucket half filled with water. The metabolic growth rate has been calculated as body mass gain per metabolic body mass per day (Dabrowski et al., 1986). Oxygen consumption data were taken every 45 minutes for each box. From these raw data, hourly rates were calculated, averaged over 1 week and standardized for the metabolic body mass.

The experiment was terminated after 17 weeks. For chromosome examination of each fish, colchicine solution (2%) was injected into the dorsal muscle (2 mg/kg). Four hours later, the fish were killed. Gill tissue was then taken and treated like the embryonic tissue described above The correct ploidy staus was confirmed for each individual. Body mass, length and height of each fish were measured. After opening the abdominal cavity, sex and maturity status were recorded and the weight of the gonad taken. The carcass was autoclaved, homogenized, deep frozen, lyophilized and ground to a fine powder. This was analyzed for dry matter, protein, lipid and ash content according to the official German procedure as outlined by Naumann and Basler (1983), i.e. macro-Kjeldahl-N times 6.25 for protein, Soxleth extraction with petrol-ether for lipids, combustion at 480°C for ash. Gross energy

was determined by isoperibolic bomb calorimetry (IKA C 7000) using a benzoic acid standard.

Statistics
All data were analyzed for significant effects (sex or ploidy or interaction between these) by Two-Way ANOVA Option of GRAPHPAD Prism 3.0. Sigificance level was set at 5%.

Results

Body mass development and metabolic growth rates
The body mass development of the experimental fish is given in Fig. 1. At the beginning of the experiment, there were no significant differences in body mass between male and female

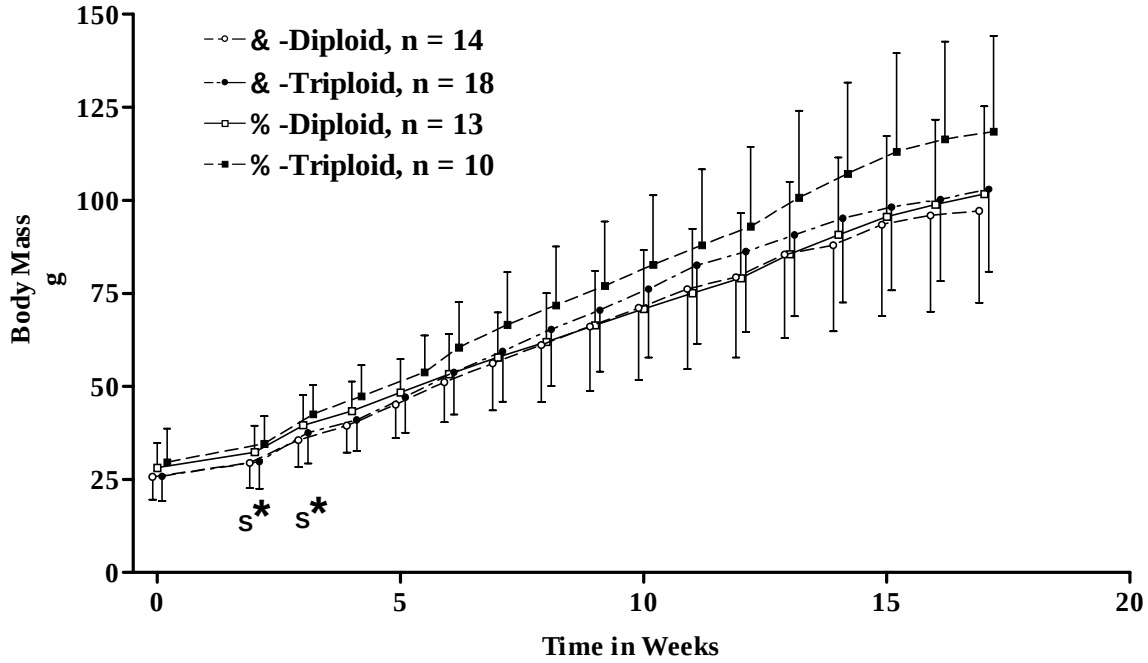

Figure 1: Body mass development of diploid and triploid tilapia *Oreochromis niloticus*.
S : effect of sex on body mass, * : significant at $p < 5\%$,

or diploid and triploid fish. Triploid males had the highest average body mass throughout the experiment. For the first 5 weeks, diploid males rank second. After 2 and 3 weeks, males are significantly heavier than females. After week six, triploid females replace diploid males in the second rank, but the difference between diploid and triploid fish is not significant at any time throughout the experiment. The metabolic growth rates (Figure 2) were around $6g/kg^{0.8}/d$ in the second week (gradual increase in feeding rate), males performed significantly better than females, the same holds true for triploid fish compared to diploid fish. In the following week, at a constant feeding level of $17.5g/kg^{0.8}/d$, growth rates almost double and than decrease gradually towards the end of the experiment. From week 3 to week 11, typically the metabolic growth rate of triploid females is highest, after that date, the triploid males rank first. Except for the first week and week 14, where the poor performance of females was highly significant, there are no statistically significant differences due to sex

or ploidy status, significant interaction (different effect of triploidy on males and females) could be observed only once in week 15, but as there is no significant effect of sex or ploidy status at that time, this result must be considered incidential. The metabolic growth rates calculated for the entire experiment are given in Table 1, there are no significant effects of either sex, ploidy status or interaction.

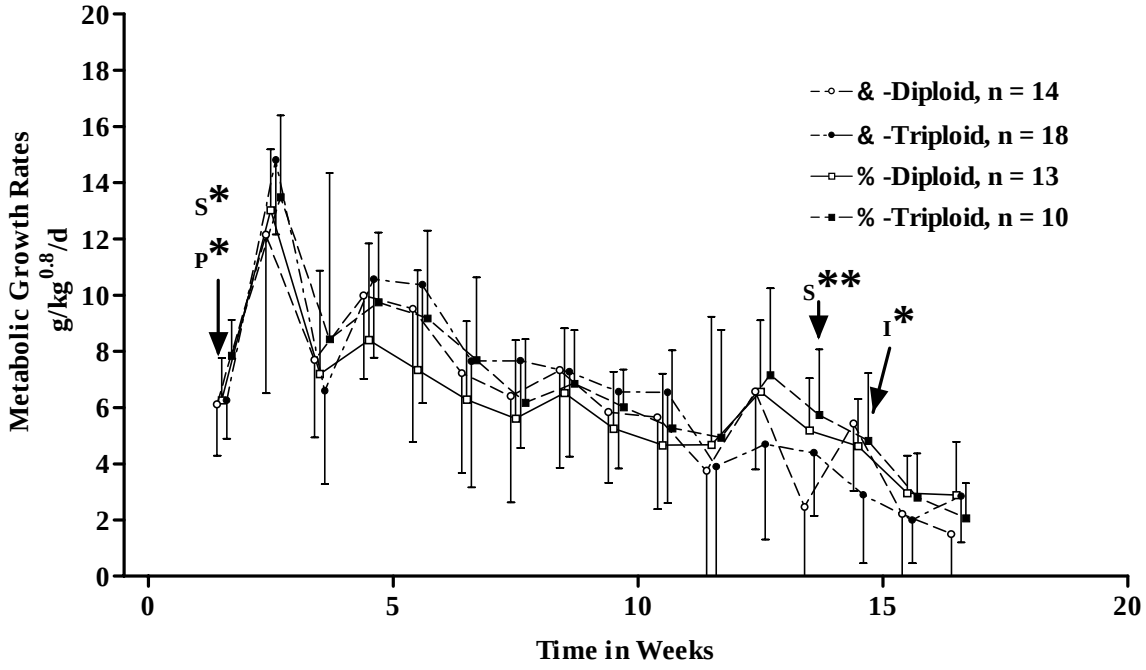

Figure 2: Metabolic growth rate of diploid and triploid tilapia *Oreochromis niloticus*.
S : effect of sex on growth rate, P : effect of ploidy status on growth rate,
I : interaction between sex and ploidy status, * : significant at $p < 5\%$, ** : significant at $p < 1\%$

Feed conversion

Feed conversion (FC, Figure 3) follows a pattern similar to metabolic growth rate. In the first week of intensive feeding, all groups have FC values above 1, however, these cannot be sustained, from week 4 to week 13, FC is around 0.5, after that date, it drops further towards the end of the experiment. Triploid females usually perform best up to week 10, after that date triploid, later diploid males rank first. Again, significant effects can be observed only in weeks 14 (highly significant sex effect and significant ploidy effect) and 15 (significant interaction). Data for FC for the entire period are given in Table 1, there are no significant effects.

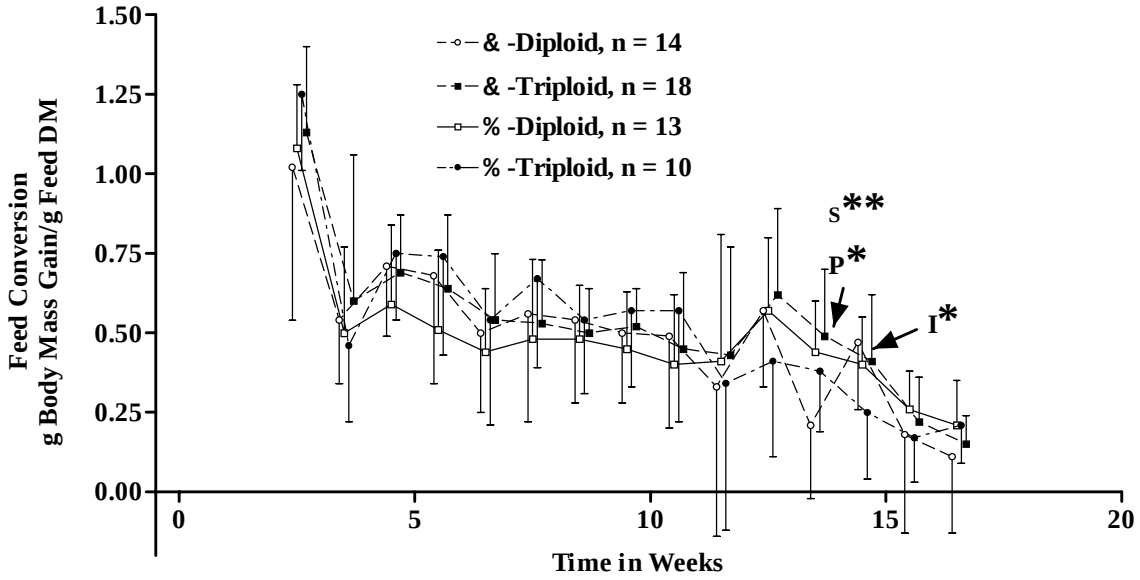

Figure 3: Feed conversion of diploid and triploid tilapia *Oreochromis niloticus*.
S : effect of sex on growth rate, P : effect of ploidy status on growth rate,
I : interaction between sex and ploidy status, * : significant at p<5%, ** : significant at p<1%

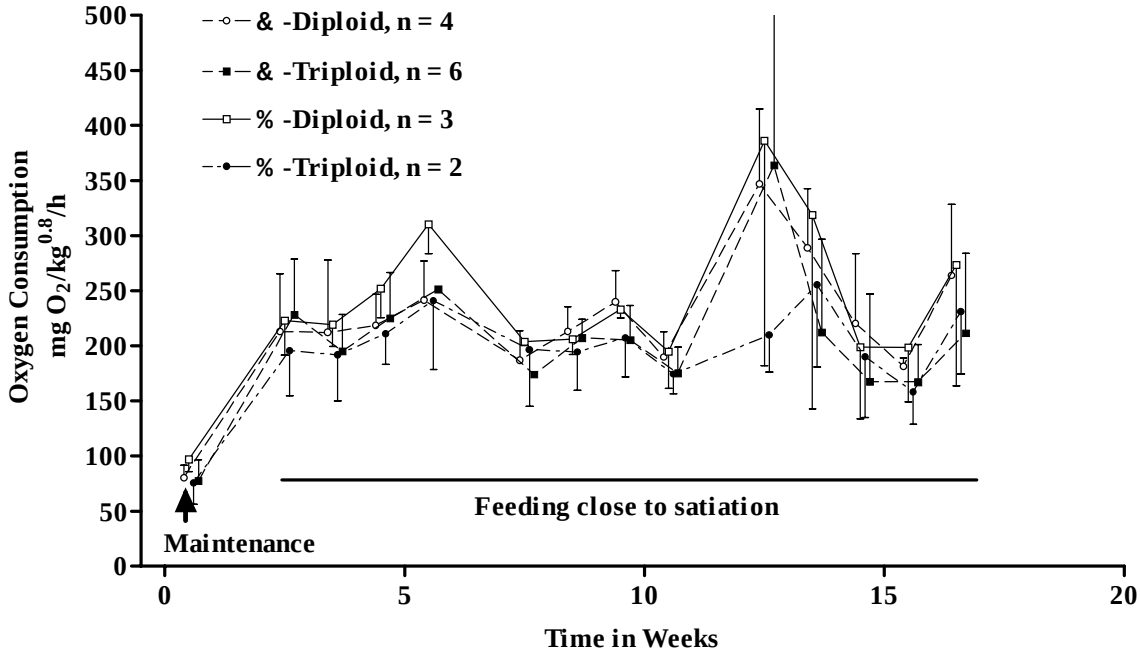

Figure 4: Oxygen consumption rates of diploid and triploid tilapia *Oreochromis niloticus*.

Oxygen consumption

Oxygen consumption data show a marked difference between the first week at a feeding rate of 3.5g/kg$^{0.8}$/d (maintenance level) and the remaining time when fish were fed close to satiation (Figure 4). Diploid fish tend to have slightly higher oxygen consumption compared to their triploid sibs, but this is not statistically significant at any time.

Morphometric data
Length, height and condition factor of the fish at the end of the experiment are given in Table 1. While the effect of sex on length is not significant, triploid fish are significantly longer than their diploid sibs. Condition factor of triploid fish is slightly lower (although not quite significant), indicating a more elongate shape compared to the diploid fish.

Gonadal state and gonadosomatic index
All fish had reached sexual maturity (at least for females stage 3 according to the scale by Kronert *et al*., 1989, >20 eggs visible, for males stage 4 according to scale by Oldorf et al., 1989, testes white, thickened) at the end of the experiment. First mature diploid females were observed in week 6 (average body mass of diploid females 45 - 50g). At the end of the experiment, most triploid females were in the phase of resorption of eggs, while diploid ones were in any stage between 3 and 6 (spent). Diploid and triploid males were mostly in stage 7 (ripe-running) at the end of the experiment.

The gonadosomatic index (Table 1) of diploid fish was almost double that of the triploid ones in both sexes, this effect is highly significant. For both diploid and triploid fish, GSI is about 50% higher in females compared to males (significant), there are no indications for interaction between sex and ploidy status.

Chemical composition of carcass and GE
Dry matter content in fresh matter as well as protein, ash, ether extract and gross energy of dry matter are given in Table 1. Females have a higher content of dry matter in fresh matter, this effect is very significant. Effects of ploidy and interaction on this parameter are not sigificant. Protein and ash content of dry matter are relatively homogenous, there are no significant effects for these. Sex has a very significant effect on the ether extract in dry matter, ploidy has a significant effect. This results in the highest content in female triploids (21.6%), followed by diploid females and triploid males (18.8% and 18.4%, resp.), diploid males had lowest content (16.9%). For gross energy content, the ranking is the same as for ether extract, however, only effect of sex is significant.

Discussion
For interpretation of the results of this study, the experimental conditions should be kept in mind, especially that all fish were reared individually, and that feeding rate (relative to metabolic body mass) was the same for all fish. This setup allows for the precise analysis of the physiological potential of the experimental fish, as factors like differences in voluntary feed intake and in competitional behavior are completely eliminated.

Body mass development, metabolic growth rate and feed conversion follow a pattern typically observed under our experimental conditions, i.e. an initial boost followed by a rather stable phase at lower levels. Towards the end of the experiment, there may have been a

growth limitation due to available space especially in the respirometric boxes. Growth rates are similar to those achieved in another experiment using the same feed formula (Schreiber *et al*. 1998), but the feed seems not to be optimal as higher growth rates could be achieved by fishmeal based diets under identical experimental conditions (Santiago *et al.*, 1998).

No obvious reason can be given for the dramatic increase in oxygen consumption and drop in growth rates and feed conversion and the respective standard deviations around week 13. As all groups are affected by this more or less the same way, it is most likely an external effect, e.g. an influence from the tap water used in partial exchange of water in the recirculating systems, however, no change has been recorded in the external parameters monitored in this experiment.

Combining the results for metabolic growth rate, oxygen consumption and body composition of diploid and triploid fish, it can be concluded that the triploid fish had slightly lower energy expenditure (oxygen consumption), but this advantage cannot be channeled into overall growth but only into lipid accretion. Puckhaber (1992) reports that triploid females had twice as much lipid accretion in the abdominal cavity as diploid ones, while there were no differences between diploid and triploid males. Lipid content in muscles was higher in triploid fish of both sexes.

The relatively homogeneous growth of diploid and triploid tilapia is in contrast to the findings of an earlier lab study by Puckhaber and Hörstgen-Schwark (1991) in which triploid fish had a significantly lower weight at 136, 178 and 220 days compared to a diploid control. In contrast to the study presented here, fish were reared in groups. There is quite good agreement between the growth observed in our experiment and in the pond experiments by Brämick *et al*. (1995) until the onset of sexual maturity. The fact that in our experiment the growth of diploid and triploid fish was comparable after the onset of sexual maturity supports the hypothesis of Brämick (1995) and Brämick *et al*. (1995) that the significant difference in body mass between diploid and triploid fish in pond culture was due to reproduction and increasing competition of the diploid fish.

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Table 1: Summary of data for growth, morphometry, body composition and energy content

		♀ Diploid		♀ Triploid		♂ Diploid		♂ Triploid		Effects		
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Sex	Ploidy	Inter-action
n		*14*		*18*		*13*		*10*				
Initial body mass	g	25.71	6.09	25.95	6.74	28.22	6.58	29.66	8.98	n.s.	n.s.	n.s.
Final body mass	g	97.17	24.73	102.99	22.15	101.76	23.48	118.52	25.71	n.s.	n.s.	n.s.
MGR (week 2-17)	$g/kg^{0.8}/d$	6.00	0.94	6.29	1.10	5.85	1.17	6.42	0.92	n.s.	n.s.	n.s.
FC (week 3-17)	g/g	0.44	0.07	0.46	0.09	0.44	0.09	0.48	0.07	n.s.	n.s.	n.s.
Final length	cm	17.24	1.59	17.68	1.18	17.58	1.55	18.83	1.18	n.s.	*	n.s.
Final height	cm	5.86	0.52	6.05	0.49	5.85	0.57	6.01	0.72	n.s.	n.s.	n.s.
Condition factor		1.87	0.14	1.84	0.11	1.84	0.09	1.75	0.09	n.s.	n.s.	n.s.
Gonadosomatic index	%	2.47	1.06	1.24	0.97	1.63	0.50	0.75	0.44	**	***	n.s.
Dry matter content in fresh matter	%	28.38	1.65	29.56	1.80	27.46	1.08	27.96	1.14	**	n.s.	n.s.
Crude protein	% DM	58.90	2.42	57.02	2.41	59.03	1.92	58.58	1.55	n.s.	n.s.	n.s.
Crude ash	% DM	16.70	1.34	15.60	2.92	17.73	1.47	16.78	1.12	n.s.	n.s.	n.s.
Ether extract	% DM	18.83	3.33	21.59	3.72	16.93	2.44	18.42	2.67	**	*	n.s.
n		*13*		*8*		*9*		*9*				
Gross energy	kJ/g DM	21.70	1.54	20.92	1.19	20.47	1.22	20.44	0.63	*	n.s.	n.s.

MGR Metabolic Growth Rate, FC Feed Conversion (body mass gain /feed dry matter)
n.s. not significant, * significant at $p < 5\%$, ** significant at $p < 1\%$, *** significant at $p < 0.1\%$