Evaluating the Benefits of Size-Sorting Tilapia Fry Before Stocking

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ABSTRACT. Size-grading is routinely practiced in fish hatcheries for reasons such as increased growth among small fish, reduction of cannibalism, decrease in size variability among harvested fish, and facilitated feeding; yet there is little information with regards to growth potential of sorted groups. Two growth trials were conducted with 30-day-old, size-sorted tilapia, to evaluate growth depensation and the influence of social interactions. In the first experiment, sibling juvenile tilapia were sorted into three size classes, small (2.6 g), medium (3.7 g), and large (4.9 g), and 15 fish were stocked into 60-L aquaria with three replicate aquaria per treatment. A fourth treatment (mixed) consisted of five fish from each size category stocked together. Diet was offered at 5% body weight per day over a 7-week growth trial. Average final weights of the fish at harvest were 13.3, 18.8, 27.0, and 24.7 g for small, medium, large and mixed treatments, respectively. Fish in the large treatment grew faster than fish in the medium treatment, which in turn grew faster than fish in the small treatment. Condition index of fish in the large treatment was significantly greater than in the small treatment ($P < 0.05$). In the second experiment sex-reversed juvenile tilapia were sorted and divided into small (0.9 g), medium (1.2 g), and large (1.5 g). Methods were as

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previously described except after eight weeks of culture, the tilapia were harvested and then transferred into larger tanks (150 L) to accommodate for their increased size. Average final weights after 13 weeks were 61.3, 78.8, 95.0, and 75.8 g for small, medium, large, and mixed treatments, respectively. Fish in the large treatment grew significantly faster ($P < 0.05$) than fish in the medium, small, and mixed treatments. Results suggest that size grading before stocking would improve commercial returns for farmers.

KEYWORDS. Tilapia, *Oreochromis niloticus*, sorting, growth depensation

INTRODUCTION

Tilapia culture is an established industry in the United States with production in the year 2001 reaching eight million kilograms (DeWandel 2002). Producers range from small growout facilities that purchase fry from specialized hatcheries to large facilities that produce their own seed. These producers cannot afford the time and labor to count the thousands of fish needed to stock large facilities. Consequently, subsamples are counted and weighed to determine the number of fry per unit weight (Jensen 1988) and the weight of fingerlings required to provide a pre-determined density is calculated. Sorting or grading fingerlings by size before stocking would allow producers to more accurately determine stocking size and number (Huner et al. 1984). A sample of ungraded fish can result in under- or over-stocking and may lead to poor feeding practices and increased production costs (Lovshin and Phelps 1993).

Size-grading is routinely practiced in fish hatcheries (Gunnes 1976) for several reasons, such as increased growth among small fish (Jobling and Reinsnes 1987), reduction in cannibalism (Hecht and Pienaar 1993), decrease in size variability among harvested fish (Purdom 1974; Jobling 1982), and facilitated feeding by using a smaller range of food particles for each graded group (Wallace and Kolbeinshavn 1988). A further benefit of size-grading in tilapia would be sex separation, the male fish generally being larger than the female (Pruginin and Shell 1962). Jobling and Reinsnes (1986) state that the rationale for sorting fish is the expectation that the growth of small fish will improve once large fish are removed.

This is, however, based on the assumption that small and large fish have the same, or similar, physiological potentials for growth and that it is social interaction which is the major cause of growth depensation (Jobling and Reinsnes 1986).

The study described below was divided into two separate experiments. The first experiment evaluated the effects of size-grading Nile tilapia, *Oreochromis niloticus*, fry on growth, feed conversion, sex separation, and growth depensation following an eight-week growout trial. The second experiment evaluated the same parameters over a 13-week period in sex-reversed fish. The goal was to determine whether all fish possessed the same genetic potential for growth and some are stunted due to social interactions, or if fish grow at different rates due to variable genetic predispositions, irrespective of dominance hierarchies within a population.

MATERIALS AND METHODS

Experiment 1

All studies were performed at the North Auburn Fisheries and Aquaculture Research Station at Auburn University, Alabama. Nile tilapia, *Oreochromis niloticus*, males and females were stocked into aquaria and the eggs from one female were removed, hatched, and maintained for 30 days in a 600-L round tank located outdoors. The larvae were offered a commercial diet continuously for 12 hours each day using automatic belt feeders. At the end of 30 days, fish were harvested and hand-sorted into three sizes; large, medium, and small (Table 1). Fish were stocked into 60-L aquaria as described below. Water in the aquaria was re-circulated through a settling tank, a biological filter, and a sand filter before returning to the aquaria. Temperature was maintained at 28±1°C using submersible heating elements placed in the settling tank and oxygen concentration was maintained above 6 mg/L using air diffusers in all the aquaria and the biological filter. Ammonia concentration in the water was determined twice weekly using the Solorzano (1969) method, and never exceeded 0.1 mg/L. The pH was maintained between 7.2 and 8.0 using soda ash (Na_2CO_3).

Fifteen fish were stocked per aquarium in four treatments and three replicates per treatment. The treatments were: large, medium, small and mixed sizes. The mixed treatment consisted of five fish from every size group stocked together in an aquarium. Fish were maintained in the

TABLE 1. Initial weight (IW), final weight (FW), final length (FL), Fulton-type condition index (CI), coefficient of variation in fish weight within each treatment at harvest (CV) and feed conversion ratio (FCR) for tilapia sorted by weight at stocking. Values in the same column sharing the same letter are not significantly different ($P = 0.05$).

Treatment	IW (g)	FW (g)	FL (mm)	CI	CV	FCR
Small	2.6a	13.3a	90.5a	1.74a	0.31a	1.57a
Medium	3.7b	18.8b	97.6a,b	1.88a,b	0.30a	1.56a
Large	4.9c	27.0c	110.5c	1.94b	0.26a	1.57a
Mixed	4.2d	24.7b,c	106.4b,c	1.86a,b	0.49b	1.42a
PSE[1]	0.06	1.68	2.90	0.038	0.041	0.039

[1]PSE = pooled standard error.

aquaria for seven weeks, and weighed weekly. They were offered a 50% protein commercial diet (Aquamax Starter Fingerling 300 by PMI Nutrition International, Brentwood, Missouri,[1] at 5% body weight. At the end of the seven weeks, all fish were harvested, group-weighed, and then individual weight and length determined. The fish were then preserved in a 10% formalin solution.

The sex of the fish in the small treatment and large treatment was identified by visual inspection of gonads of preserved fish. The gonads were removed and placed on a microscope slide, stained with bromocresol green, crushed under a second microscope slide and inspected at 10× magnification. Males and females were differentiated by identifying testicular and ovarian tissue, respectively.

Experiment 2

A second experiment was conducted to assess whether differences in growth among size-sorted treatments was related to the sex of the fish. Fertilized eggs from three female tilapia were removed, hatched and cultured for 30 days on a commercial fry diet supplemented with 60 mg methyl-testosterone/kg diet (Rangen Inc., Buhl, Idaho). Water quality parameters were tested as described above and remained within acceptable limits. Following the 30-day period, the sex-reversed fish were harvested and graded into three sizes; large, medium, and small (Table 2),

1. Use of trade or manufacturer's name does not imply endorsement.

TABLE 2. Comparisons of mean weights and Fulton-type condition index (CI) of tilapia size sorted and stocked as small, medium, large to similar size-class fish stocked in the mixed-size treatments and reared for 7 and 8 weeks in Experiments 1 and 2, respectively. *P* denotes level of significance for ANOVA.

	Experiment I		Experiment II	
	Mean weight (g)	CI	Mean weight (g)	CI
Small	13.3	1.74	16.6	1.83
Mix small	11.2	1.72	11.9	1.75
P	0.406	0.701	0.047	0.269
Medium	18.8	1.88	22.2	1.92
Mix medium	23.9	1.95	20.5	1.90
P	0.052	0.39	0.040	0.565
Large	27.0	1.94	27.3	1.94
Mix large	38.0	1.90	29.8	2.06
P	0.036	0.476	0.387	0.182

and stocked as previously described in 60-L aquaria. As in experiment 1, water in the aquaria was re-circulated through a settling tank, a biological filter, and a sand filter before returning to the aquaria. Fish were maintained in the aquaria for eight weeks, weighed weekly and offered a high protein (40%) commercial diet (Silver Cup, Murray, Utah) at 5% body weight. At the end of the eight weeks, all fish were harvested, anesthetized, group weighed, and then individual weight and length determined for each fish. The tilapia were then re-stocked into 150-L tanks to accommodate for their larger size and maintained on a commercial 35% protein growout diet (Melick Aquafeed Inc., Catawissa, Pennsylvania) at 4% body weight for an additional 5 weeks of culture.

Data Analysis and Statistics

Final weight, survival, and feed conversion ratio [FCR = $F/(W_F - W_I)$, where F is the dry weight of feed offered to the fish, W_F is the final weight of the fish, and W_I is the initial weight of the fish at stocking; all feed offered was consumed] of the fish were calculated. The Fulton-type condition index (CI) of the fish at harvest was estimated using the formula: CI =

$(W/L^3) \times X$, where W is the weight in grams, L is the length in millimeters, and X is a constant equal to 100,000 (Anderson and Gutreuter 1983). The coefficient of variation (CV = standard deviation/mean) in weight of individual replicates was compared among treatments. Furthermore, data for individual weights from the mixed treatment were divided into five large fish from each replicate (MixL), five medium (MixM) and five small (MixS). The effect of interaction among various sizes of fish on growth was evaluated in both experiments by comparing Large to MixL, Medium to MixM and Small to MixS using ANOVA. Differences in sex ratio between the Small and Large treatments were evaluated in Experiment 1 by comparing the percentage (arcsin-transformed) of males in the two treatments using one-way ANOVA.

In Experiment 1, two methods of analysis were used to determine if growth rates differed among treatments. In the first method, fish weights were compared among treatment at stocking and at harvest using a level of significance of $P = 0.006$ (0.05/8, where 8 is the number of times fish were weighed). In the second method, data were corrected for differences in initial weight of fish by selecting incremental four week periods. This allowed for an estimate of growth over a four-week period where all fish started at similar weights and were maintained under similar conditions. Weights of treatments large, medium, and small were compared at days 0, 7 and 14, respectively, and at days 28, 35, and 42, respectively, using analysis of covariance with treatment (initial weight) and weights at days 0, 7, and 14 as co-variates.

In Experiment 2, the eight-week growth data for the various size classes was analyzed as previously described. Additionally at the conclusion of the growth trial, final weights, lengths, CI, CV, and FCR were analyzed. Statistical analyses were performed using SAS (V8.2, SAS Institute Inc., Cary, North Carolina). ANOVA was used to determine significant differences ($P < 0.05$) among treatment means, and Student-Newman Keuls' t-test was utilized to determine significant differences among means.

RESULTS

Experiment 1

When tilapia are size-sorted and reared separately in aquaria, large individuals appear to maintain their size advantage after eight weeks of culture. Fish that were heavier than their congenerics at stocking, remained heavier throughout the experiment (Table 1; Figure 1). The CI of the fish in the large treatment was significantly greater than the CI of the fish in the small treatment, but similar to CI in the medium and mixed treatments. The CV in the small, medium, and large treatments were all similar (0.26-0.31) and significantly smaller than the CV in the mixed treatment (0.49). The FCR was similar among all treatments (1.42-1.57) (Table 1).

The average weight of the smallest five fish in the mixed treatment (11.21 g) appears to be lower than the average fish weight in the small treatment (13.29 g), although no statistical differences were found (Table 2). However, the average weight of the largest five fish in the mixed treatment (38.00 g) was significantly higher than the average weight of fish in the large treatment (26.97 g). The CI of fish is not significantly different from each other when small is compared to MixS, medium to MixM and large to MixL (Table 2).

When four-week growth of fish that started at similar weights were compared, we find that fish in the large treatment grew faster than fish in the other treatments (Table 3). When growth was observed for four weeks starting at day-0 for the large treatment (mean weight = 4.92 g), day-7 for the medium treatment (mean weight = 4.96 g), and day-14 for the small treatment (mean weight = 4.39 g), the final mean individual weights for the three treatments were 16.00 g, 13.11 g, and 11.06 g, respectively.

FIGURE 1. Growth of size sorted tilapia juveniles over seven weeks. Thirty-day aged fish were sorted into large (L; 4.9 g), medium (M; 3.7 g) and small (S; 2.6 g) size classes.

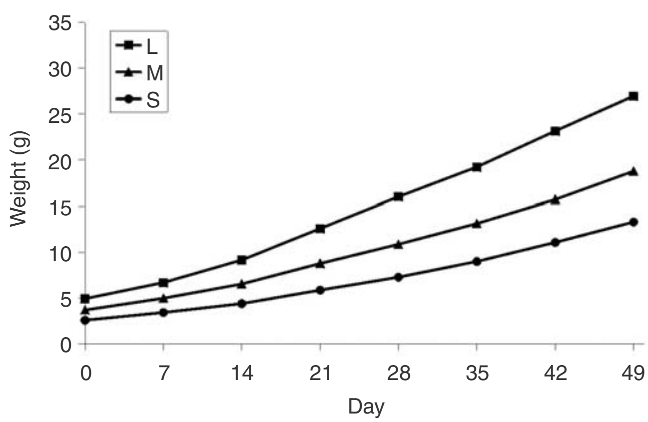


TABLE 3. Four week growth comparisons between the various treatments were made when starting date was corrected so that initial weights were similar. Values in the same column sharing same letter are not significantly different ($P = 0.05$).

	Weight after 4 weeks	
	Initial (g)	Final (g)
Small	4.4a	11.1a
Medium	5.0a	13.1a
Large	4.9a	16.0b
PSE[1]	0.18	0.64

[1]PSE = pooled standard error.

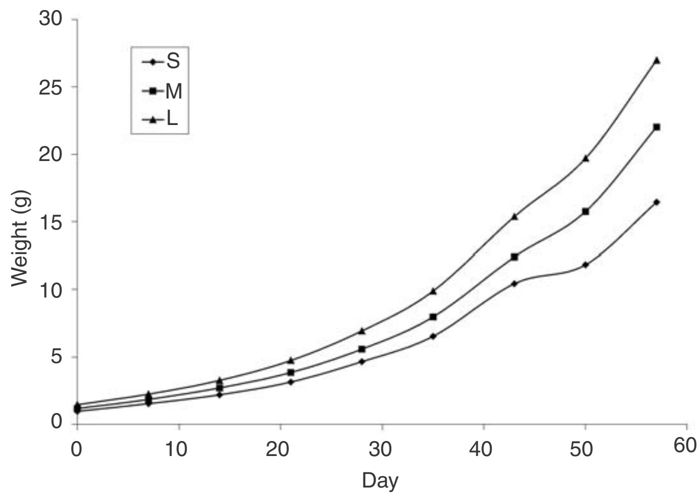
The sex ratio (males to females) in the large treatment was significantly different from the sex ratio in the small treatment ($P = 0.0085$). The average sex ratio in the large treatment was 81.4% males to 18.6% females while in the small treatment it was 37.7% males to 62.3% females.

Experiment 2

After eight weeks of culture, the average weight of fish in the large treatment (27.3 g±0.46) (mean±SE) was greater than the weight of fish in the medium treatment (22.2 g±0.29) which in turn was greater than the weight of fish in the small treatment (16.6 g±0.21) (Figure 2). Average weight of the five smallest fish in the mixed treatment (11.9 g) was significantly lower than the average fish weight in the small treatment (16.6 g) and the average weight of the five largest fish in the mixed treatment (29.8 g) appeared higher than the average weight of fish in the large treatment (27.8 g), although significance could not be demonstrated (Table 2). The average weight of medium fish in the mixed treatment (20.5 g) was significantly lower than the average weight of fish in the medium treatment (22.5 g). Comparisons of CI did not reveal significant differences among treatments when small was compared to MixS, medium to MixM, and large to MixL (Table 2).

Fish survival in Experiment 2 was 100%. The FCR, and CI were similar among all treatments (Table 4). However, significant differences in biomass, final weight, and final length were observed (Table 4). The final weight of the large treatment (95.0 g) was significantly different from the

FIGURE 2. Growth of size sorted, sex-reversed tilapia juveniles over eight weeks. Thirty-day aged fish were sorted into large (L; 1.5 g), medium (M; 1.2 g), and small (S; 0.9 g) size classes.



medium (78.8 g), small (63.1 g), and mixed (75.8 g) treatments. The mean final weight in the medium treatment was significantly different from the small treatment but not significantly different from the mixed treatment. Tilapia in the large treatment grew an average of 1.00 g/day, while fish in the small treatment grew an average of 0.65 g/day.

DISCUSSION

Size-grading of tilapia fingerlings prior to stocking appears to select for faster growing fish while reducing size variability at harvest. In the present experiment, size-sorted tilapia appeared to grow at different rates irrespective of the size hierarchies within a population. When tilapia were graded and stocked into uniform size groups, large fish grew at a faster rate relative to their initial body size than did smaller fish.

Size variation in a batch of same age fish could be due to genetic differences or physiological differences where some fish hatch earlier or feed earlier and thus acquire a size advantage and maintain it (Brown 1946). This initial size differential could allow large fish to compete with

TABLE 4. Initial weight (IW), final weight (FW), final length (FL), Fulton-type condition index (CI), coefficient of variation in fish weight within each treatment at harvest (CV), and feed conversion ratio (FCR) for tilapia sex-reversed with 60 mg methyl-testosterone sorted by weight at stocking (Experiment 2) and maintained for 13 weeks. Values in the same column sharing the same letter are not significantly different ($P = 0.05$).

Treatment	IW (g)	FW (g)	FL (mm)	CI	CV	FCR
Small	0.9a	61.3a	139.7a	2.15a	0.29a	1.04a
Medium	1.2b	78.8b	151.7b	2.22a	0.26a	1.03a
Large	1.5c	95.0c	160.3c	2.25a	0.25a	1.03a
Mixed	1.1d	75.8b	148.7b	2.22a	0.33b	1.02a
PSE[1]	0.005	0.81	0.46	0.15	0.013	0.005

[1]PSE = pooled standard error.

smaller fish of the same age, thus increasing growth depensation (variability in growth rates among conspecifics reared communally). However, our results suggest that growth differential among same age tilapia is related to their genetic component and initial size difference resulted in an increased growth depensation. The growth rate of large fish remained faster than that of small fish even when they were reared separately. Nonetheless, competition does play a role in growth of tilapia. Large fish grew larger in the mixed treatment than in the large treatment, while small fish were smaller in the mixed treatment than in the small treatment. Koebele (1985) observed similar results in the redbelly tilapia, *Tilapia zillii*. He postulated that dominant fish control food acquisition and modify the appetite of subordinates. However, similar results were not always observed in other teleost species.

Wickins (1987) and Kamstra (1993) found that growth rates of stunted eel were mainly governed by physiological responses and not necessarily social interactions. Purdom (1974) and Jobling (1982) found that size variation at harvest of communally-reared sole, *Solea solea*, and plaice, *Pleuronectes platessa*, depended on size variation at stocking. Conversely, Wickins (1985) observed an increase in size variability of elvers reared in isolation as opposed to communally. Moreover, Lambert and Dutil (2001) found that the effects of size-sorting on growth of Atlantic cod, *Gadus morhua*, were affected by stocking densities. Since both growth rate and growth depensation vary among species, ages, and sizes of fish, as well as among fish exposed to various environmental conditions (Wootton 1992), making comparisons among studies is difficult.

Genetic differences are responsible for variation in growth rate of tilapia (Basiao and Doyle 1999). However, growth depensation is also related to social rank and competition. Goldan et al. (1997) report four non-exclusive mechanisms related to social rank that affect growth. The first three, direct competition, social stress and increased motor activity may depress growth in small fish. The fourth, dominance cost, depresses the growth rate of larger fish. It is impossible to determine the existence of all four mechanisms in the present study nor the relative importance of each in regulating fish growth. However, the present results do demonstrate that large fish grew better when competing with small fish, rather than fish of the same size, while small fish grew better when competing with fish their size rather than large fish.

The higher growth rate in the large treatment in Experiment 1 may be partially due to sex of the fish. Male tilapia grow faster than females (Hanson et al. 1983; Toguyeni et al. 1997) and the proportions of males in the large and small treatments were 81.4% and 37.7%, respectively. Pruginin and Shell (1962) found that size-sorting of tilapia at an early age results in a sex ratio separation similar to that obtained in the present experiment. In order to determine if growth differences were due to the phenotypic sex of the fish or its genotypic makeup, the fish in Experiment 2 were sex-reversed. In this case, growth differences between the small and the large fish were still apparent even after 13 weeks. Irrespective of differences in genotype and phenotype, it is clear juvenile tilapia that are larger than their siblings at an age of 30 days remain larger and continue to grow faster even after 13 weeks.

Observations at our lab (unpublished data) indicate that small tilapia do not catch up with larger siblings even after an extended culture period. Consequently, size separation of tilapia fingerlings prior to stocking for intensive culture appears to have economic benefits. Fish grow faster, have a better condition index, and are more standard in size at harvest. Tilapia are prolific fish and their husbandry is relatively simple. By discarding small fish right after the nursery phase of production, the aquaculturist could save money, time and effort by only rearing fast growing fish. Furthermore, growth rate variations among fish fry affect analysis of experimental results. Results of the present work suggest that tilapia juveniles should be size-sorted before being used in growth trials.

Thus, size-sorting tilapia fry at 30 days selects for individuals with varying growth rates. Sorting reduces size variability, thus simplifying the feeding process during growout, and product processing after harvest. Moreover, size-sorting and removal of smaller fish will reduce the opportunity cost of culturing slow growing fish, allowing for a more efficient operation. Further, hatchery managers should grade 30 day-old tilapia fry and discard small fish. This will cull the slow growing fish and will shift the populations to favor faster growing males. Lastly, size sorting fry before stocking in research experiments will reduce variability and improve experimental results.

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