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[Article]

Establishment of Mostly Male Groups of Bluegills by Grading Selection and Evaluation of their Growth Performance

H. P. WANG* AND G. K. WALLAT

Ohio Center for Aquaculture Research and Development, Ohio State University South Centers, 1864 Shyville Road, Piketon, Ohio 45661, USA

R. S. HAYWARD

Department of Fisheries and Wildlife Sciences, University of Missouri–Columbia, 302 Anheuser-Busch Natural Resources Building, Columbia, Missouri 65211, USA

L. G. TIU, P. O'BRYANT, AND D. RAPP

Ohio Center for Aquaculture Research and Development, Ohio State University South Centers, 1864 Shyville Road, Piketon, Ohio 45661, USA

Abstract.—Concerns over the economic feasibility of commercial aquaculture production of bluegills *Lepomis macrochirus* have heightened the need for strategies to enhance growth. Recent studies have shown that individually reared bluegill males can grow twice as fast as females; such studies have generated increased interest in development of mostly male or all-male populations. In this study, we developed a practical procedure to establish mostly male bluegill groups through grading selection and we tested their growth against that of a normal population. A single cohort of bluegill juveniles was cultured in a pond for 1 year; when the fish reached a mean weight of 30.1 g, the cohort was graded and divided into two mostly male groups (top 25% and top 50% of fish by total length) and a mixed-sex control group. The percentage of males in each group was as follows: 50.0% in the mixed control group, 75.4% in the top 25% group, and 69.7% in the top 50% group. Weight gain per fish in the top 25% group was significantly greater ($P < 0.05$) than that in the mixed group throughout most of the experiment. There were no significant differences detected in survival among the three groups, although the top 25% group had survival of 96.0% compared with 90.6% and 90.5% for the top 50% group and the mixed control group, respectively. The top 25% group had the highest percentage (46.3%) of fish reaching 150 g at the end of the experiment, followed by the top 50% group (28.3%), and the mixed control group (12.7%). The coefficient of variation (CV) for weight decreased in all three groups over time; the mostly male groups maintained lower initial and final CV values than did the mixed control group. Results indicate that mostly male bluegill groups are able to grow faster than typical mixed-sex populations, and social interaction costs among communally reared males did not significantly decrease growth of mostly male populations in the aquaculture settings.

The bluegill *Lepomis macrochirus* and its F_1 hybrid (female green sunfish *L. cyanellus* × male bluegill) have long been commercially cultured to support recreational fishery stocking needs throughout the mid-southern and southeastern United States (Brunson and Robinette 1986; Tidwell and Webster 1993; Flickinger et al. 1999; Brunson and Morris 2000). In the north-central region of the country, effort has more recently been directed towards rearing lepomid sunfishes for food markets (Garling et al. 1992; Garling and Sheehan 1999; Wang et al. 2000; Hayward and Wang 2002, 2006). Whereas mostly small- to intermediate-sized sunfish have been reared for recreational stocking purposes, substantially larger fish (225–340 g) are desired for food markets (Brunson and Morris 2000).

Because of their desirable production traits, bluegills and hybrid bluegills are considered the most feasible among the lepomid sunfishes for rearing to food market size (Brunson and Morris 2000; Hayward and Wang 2002, 2006). These traits include palatability, willingness to accept a pelleted diet (Lewis and Heidinger 1978; Ehlinger 1989), availability of broodstock and capacity for both natural and manipulated spawning, tolerance of poor water quality, and the ability to grow over a wide range of temperatures. In a survey conducted by the North Central Regional Aquaculture Center (NCRAC), midwestern brokers, wholesalers, retailers, and restaurants listed the bluegill as one of the top three species they would like to purchase for their customers (Brunson and Morris 2000). Nile tilapia *Oreochromis niloticus* have been labeled and sold as

* Corresponding author: wang900@ag.osu.edu

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"sunfish" in some major market outlets, indicating the public demand for sunfish.

The need to grow sunfish to large sizes (225–340 g) for the food market has led to the concern that required grow-out times may be too long for commercial rearing to be economically feasible. Available data indicate that with the present technology, more than 2 years of rearing are necessary for sunfish to reach food market size in the Midwest (Ellison and Heidinger 1978; Tidwell et al. 1994). The consensus of NCRAC's Sunfish Workgroup is that for economic reasons, reduction of grow-out periods to within 2 years is essential for the development of commercial rearing of bluegills or hybrid bluegills as food fish in the north-central United States (Hayward and Wang 2002, 2006).

Recently, a number of NCRAC-funded studies have shown that bluegills possess the inherent capacity to grow to food market sizes substantially faster than hybrid bluegills (Hayward and Wang 2002). Male bluegills appear to hold the greatest potential for the food market due to their more rapid growth capacity relative to that of females (Hayward and Wang 2006). Male bluegills outweighed female bluegills by 111.9% at the end of a 300-d experiment and were also significantly larger than both male and female hybrid bluegills that were reared in parallel (Hayward and Wang 2006). These findings suggest that monosex culture of a male bluegill population could be advantageous. However, in the aforementioned studies, fish were held individually and growth was assessed from the means of eight replicates. There is a need to determine whether male bluegills that are communally cultured in aquaculture settings will still grow faster than female or mixed-sex populations because strong social interactions among males could decrease their growth in groups.

A size grading method for forming groups of mostly male bluegills from a larger group of bluegills with balanced sex ratios (hereafter, "mixed-sex" groups) was recently developed under laboratory conditions (Doerhoff 2007) using multicohort fish reared in a commercial producer's pond. Findings indicated that the size grading method can be applied to bluegills after the first growing season (fish weight = 15–25 g) to form groups in which approximately 80% of the fish are male (Doerhoff 2007).

We considered it necessary to (1) conduct additional evaluations of the size grading method's ability to form mostly male groups of bluegills from a single cohort on a commercial scale and (2) evaluate the growth potential of these groups relative to typical mixed-sex bluegill groups. Hence, the purpose of this study was to further evaluate the capacity to establish mostly male bluegill groups through the size grading method and to then compare the growth performance of mostly male groups with that of normal populations by use of tank rearing followed by rearing in commercial-scale cage systems.

Methods

Fish stocking and grading.—Approximately 20,000 bluegill fingerlings from a single cohort were obtained from the Hebron State Fish Hatchery, Ohio, in September 2004 and were cultured in a 0.1-ha pond for one growing season. When the fish reached a mean weight of 30.1 g, the pond was harvested and the fish were randomly allocated to two large tanks (2.1-m diameter) such that biomass was similar between tanks. Three sample groups were taken randomly from each tank to form a mixed-sex (ungraded) control group; an additional 100 fish were taken randomly from each tank, their total lengths were measured, and the fish were arranged by size to obtain lower size cutoff points for the top 25% group (by length) from one-half of the cultured population and the top 50% group from the other half of the cultured population. Based on these cutoff points, 10 fish were selected from each tank for use in testing and setting the bar gap widths of two graders. The top 25% fish were then passively graded from one tank, and the top 50% fish were passively graded from the other tank. Overall, this produced three groups: (1) the top 25% fish group, (2) the top 50% fish group, and (3) a mixed-sex, ungraded control group that reflected the sex ratio of the original bluegill group before grading. The three groups were stocked at an equal biomass of 3 kg/tank into nine 400-L experimental tanks (3 replicates/group) supplied with well water.

Culture conditions and samples.—The experiment was conducted in two culture phases: indoor tanks and outdoor cages. At the beginning, experimental fish were raised in the nine 400-L round tanks provided with flow-through well water at ambient temperatures for 5 months (November 2005–March 2006). When the outdoor air and water temperature increased in April 2006, all groups were transferred to nine 1-m^3 cages situated in a 2-ha reservoir. The nine cages were positioned around a rectangular dock (4×8 m) in the reservoir. An aerator was installed in the center of the dock and operated throughout the cage culture phase (April–September 2006). Fish were fed to apparent satiation twice daily (at 0900 and 1600 hours) using a commercially available diet (Silver Cup Number-4 trout crumble [45% protein, 11% fat] during the tank culture phase; 1.5-mm Silver Cup trout diet during the cage culture phase). Dissolved oxygen and water temperature were measured once daily using a YSI 51B dissolved oxygen meter (Yellow Springs Instru-

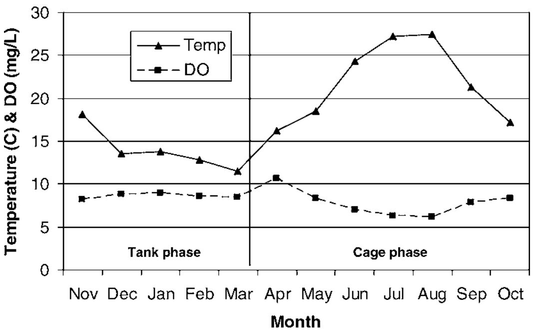

FIGURE 1.—Monthly mean temperature (Temp; °C) and dissolved oxygen (DO; mg/L) readings for bluegill experimental culture conditions in tanks and in cages.

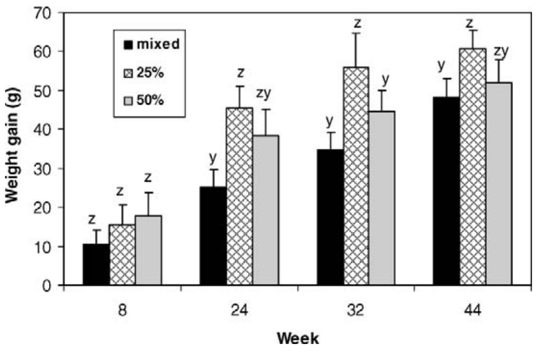

FIGURE 2.—Mean weight gain (g) of bluegills in three experimental groups (mixed = ungraded, mixed-sex control group; 25% = graded, mostly male treatment group containing the top 25% [by total length] of fish in the population; 50% = graded, mostly male treatment group containing the top 50% of fish in the population) during 44 weeks of culture. Within a sample week, means denoted by the same letter are not significantly different ($P > 0.05$).

ments, Yellow Springs, Ohio) and were used to calculate monthly mean values. The mean water temperature was 18.5°C with a range of 15.4–30.1°C over the period of the experiment. The mean level of dissolved oxygen over the 11-month experiment was 8.2 mg/L with a range of 5.7–11.3 mg/L (Figure 1). The pH of the water ranged from 7 to 8, which was a desirable range for growth of bluegills.

Fish were sampled ($N = 50$) every 8–12 weeks in both tanks and cages to determine weight and total length. For individual measurements, fish were weighed to the nearest 0.1 g and measured to the nearest millimeter. At the end of experiment, all fish were individually weighed and measured and fish were euthanized to determine sex ratios in each group.

Calculations and statistical analysis.—For the fish in each replicate, weight gain (g) was calculated as $(W_f - W_i)/n$, where W_i and W_f are the initial and final cumulative weights, respectively, of all fish in each tank or cage and n is the number of fish in each replicate. The coefficient of variation (CV; %) of weight was used to examine interindividual weight variation among the fish in each tank or cage and was calculated as 100 × (SD/mean weight of the fish in each tank or cage). Considering the range in CVs across the three treatment groups at the start of the experiment, percentage of relative change in CVs was calculated as $100 \times (CV_f - CV_i)/CV_i$, where CV_i and CV_f are the initial and final CVs, respectively, of the fish weights in each tank or cage. Differences in mean weight gain among the three treatment groups were evaluated by one-way analysis of variance (ANOVA; $\alpha = 0.05$) followed by Duncan's test for means separation when significant differences were indicated by ANOVA. Analysis of covariance ($\alpha = 0.05$) was conducted to test for differences in slopes of linear models of weight growth between treatment and control groups.

Results

Sex Ratio and Survival

At the end of the 44-week culture period, all fish were sacrificed to determine sex ratio. The mixed-sex control group had 50.0% males (49.2, 51.2, and 49.6% for the three replicates), the top 25% group had 75.4% males (79.3, 76.3, and 70.6%), and the top 50% group had 69.7% males (72.6, 71.8, and 63.6%). There were no significant differences detected in survival among the three groups; the top 25% group had a survival rate of 96.0% (93.5, 97.4, and 97.1% for the three replicates) compared with 90.6% (86.7, 89.9, and 95.1%) for the top 50% group and 90.5% (94.4, 86.7, and 90.5%) for the mixed control group.

Weight Gain and Growth

By week 24, weight gain was significantly higher ($P < 0.05$) in the top 25% group than in the mixed-sex control group, but the top 25% and top 50% groups did not differ (Figure 2). By week 32, weight gain of the top 25% group was significantly greater than that of both the mixed-sex control and top 50% groups (61.0% and 26.0% greater, respectively). At the end of the experiment, the top 25% group had gained significantly more weight (24.9%) than the mixed-sex control group; there was no significant difference detected between the top 25% and top 50% groups, although the former gained 19.0% more than the latter. However, the weight gains of the three groups were similar during the low-temperature period (winter) that occurred during the first 8 weeks of the study (Figure 2).

The increase in weight was described by the following linear models: $y = 16.27x + 76.99$ for the

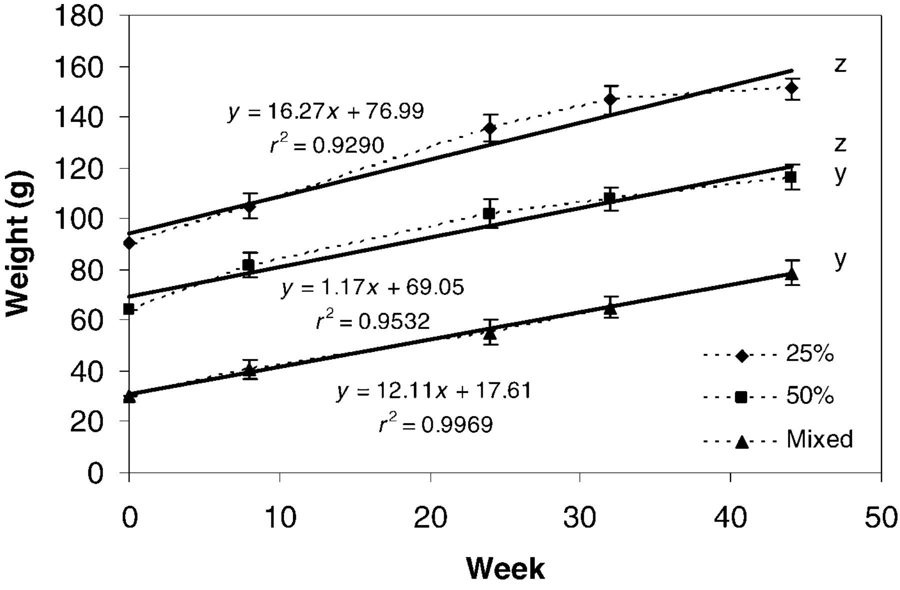

FIGURE 3.—Mean weights (g) and linear growth models of bluegills in three experimental groups (mixed = ungraded, mixed-sex control group; 25% = graded, mostly male treatment group containing the top 25% [by total length] of fish in the population; 50% = graded, mostly male treatment group containing the top 50% of fish in the population) over a 44-week period. Linear regressions denoted by the same letter had slopes that were not significantly different ($P > 0.05$).

top 25% group ($r^2 = 0.9290$), $y = 1.17x + 69.05$ for the top 50% group ($r^2 = 0.9532$), and $y = 12.11x + 17.61$ for the mixed control group ($r^2 = 0.9969$). There was a significant difference detected in slopes of the linear regressions between the top 25% treatment and mixed control groups (Figure 3), indicating that the overall growth in body weight of the 25% group was significantly higher than that of control fish.

Production Structure

Initial mean weights of the top 25% (90.5 g) and top 50% (63.7 g) groups were higher than that of the mixed-sex control group (30.1 g) as a result of the grading selection. This overall trend among group weights continued throughout the experiment; final mean weights of the top 25%, top 50%, and mixed control groups were 151.1, 116.1, and 78.6 g, respectively (Figure 3). Similarly, differences in initial mean total length existed among the three groups due to the grading selection. Mean initial lengths for the top 25%, top 50%, and mixed control groups were 15.6 cm (6.1 in), 13.6 cm (5.4 in), and 10.3 cm (4.1 in), respectively. These differences in length likewise tended to persist throughout the experiment; the top 25% group had the highest final mean total length (18.8 cm [7.4 in]), followed by the top 50% group (17.6 cm [6.9 in]) and the control group (15.5 cm [6.1 in]). However, there were no significant differences in length increases among the three groups.

The top 25% group demonstrated the highest weight increase and the least reduction in the CV for total length among the three groups, while the mixed-sex control group had the highest length increase and greatest reduction in CV for both weight and length (Table 1). Overall, the CV for weight in all three treatments decreased over the course of the experiment (Figure 4). The top 25% group had the lowest CV values for both weight and length at the beginning and throughout the experiment. The top 25% group also had the highest percentage (46.3%) of fish reaching 150 g by the end of the experiment, followed by the top 50% group (28.3%) and the mixed-sex control group (12.7%).

Discussion

Selective removal of the upper 25% and 50% of fish from mixed-sex bluegill groups belonging to a single cohort yielded subgroups in which 75.4% and 69.7% of the fish were males, on average, versus 50.0% male bluegills in the ungraded groups. These results indicate that the grading strategy allows the formation of bluegill groups consisting of 70–75% males from background populations with sex ratios near 50:50. This practical procedure for establishing mostly male bluegill groups from mixed-gender populations can be

TABLE 1.—Initial and final mean (±SE) body weight and total length, initial and final coefficient of variation (CV), difference in mean length or weight (Δ Mean), percent difference in CV (Δ CV%), and percentage of fish above 150 g for bluegills cultured over a 44-week period in three groups (control = ungraded, mixed-sex group; top 25% = graded, mostly male treatment group containing the top 25% [by total length] of fish in the population; top 50% = graded, mostly male treatment group containing the top 50% of fish in the population).

	Week 0			Week 44				
Group	Mean	CV	*N*	Mean	CV	Δ Mean	Δ CV%	>150 g (%)
				Body weight (g)				
Control	30.1 ± 3.2	130.8	433	78.6 ± 4.7	73.9	+48.5	−43.5	12.7
Top 50%	63.7 ± 4.1	78.2	157	116.1 ± 4.9	50.3	+52.4	−35.7	28.3
Top 25%	90.5 ± 3.9	43.8	105	151.1 ± 4.2	27.8	+60.6	−36.5	46.3
				Total length (cm)				
Control	10.3 ± 0.3	34.4	433	15.5 ± 0.2	18.6	+5.2	−45.9	
Top 50%	13.6 ± 0.3	23.8	157	17.6 ± 0.2	14.7	+4.0	−38.2	
Top 25%	15.6 ± 0.2	12.0	105	18.8 ± 0.2	8.5	+3.2	−29.2	

readily carried out by commercial fish producers. The capacity to form mostly male bluegill groups has the potential to increase bluegill production efficiency and the ability to rear large bluegills, which are in demand as food fish (Hayward and Wang 2006) and command high prices (R. Butz, Windridge Farm, Germantown, Maryland, personal communication). Producers can consider culturing larger fish for food markets and holding or rearing small- to intermediate-sized fish for recreational fishery stocking. However, culturing single-cohort or similar-sized bluegill fingerlings might require additional facilities; thus, an economic analysis should be done before applying this procedure.

The top 25% bluegill group, which contained the highest percentage of male fish, grew significantly faster than the mixed-sex control population throughout most of the experiment, as indicated by weight gain. There was also a significant difference detected in slopes of the linear regressions between the top 25% treatment and mixed control groups. These results indicate that communally reared male bluegills are able to grow faster than mixed-sex or female bluegill populations in regular aquaculture settings and that the social interaction costs that can impede the growth of grouped male bluegills (Hayward and Wang 2006) were not high enough to override the males' higher growth capacity. However, it appears that the top 25% and top 50% treatments exhibited superior growth during weeks 8–32 of the culture period but relatively inferior growth during the latter part of experiment. We

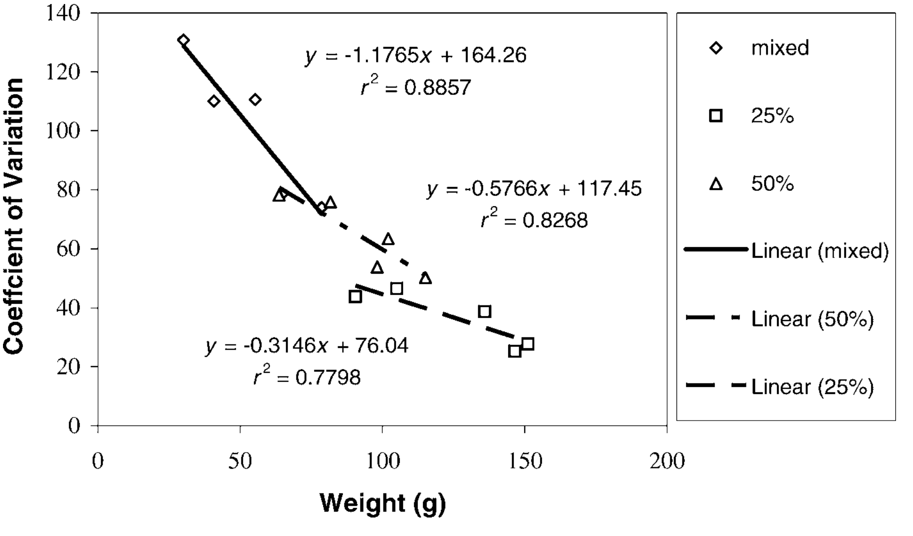

FIGURE 4.—Coefficient of variation (*y*) in bluegill weight plotted against mean weight (*x*) for three experimental groups (mixed = ungraded, mixed-sex control group; 25% = graded, mostly male treatment group containing the top 25% [by total length] of fish in the population; 50% = graded, mostly male treatment group containing the top 50% of fish in the population).

believe that the maturation of the top two size-groups was the major reason for this decreased growth response. During this experiment, the fish in the top 25% and 50% groups matured and sperm could be detected in most of the fish from June to September. The weight gain of the top 25% group was similar to that in the other two groups during the first 8 weeks. We believe this was related to low temperature because bluegills grow slowly at temperatures below 20°C (Beitinger and Magnuson 1979).

Our findings further indicate that rearing monosex male bluegill populations would be advantageous, particularly for rearing large bluegills. Monosex culture has been applied in several fish species to improve production (Donaldson and Hunter 1982; Hunter et al. 1983; Yamazaki 1983; Beardmore et al. 2001). Monosex populations have been produced by incorporating sex steroids, such as methyltestosterone or trenbolone acetate, into fish diets or by adding these to the culture water during the phenocritical period of development when fry are sexually undifferentiated (Hunter and Donaldson 1983). Hanson et al. (1983) compared growth and feed conversion of (1) manually selected populations of male Nile tilapia, (2) male hybrids of Nile tilapia × Wami tilapia *O. urolepis hornorum* or Wami tilapia × Mozambique tilapia *O. mossambicus*, (3) sex-reversed male populations of Nile tilapia, and (4) untreated Nile tilapia females. Sex-reversed male groups showed higher weight gain and food conversion than the manually selected male groups, and all-male groups grew faster than the female group. The use of monosex populations could also result in fish of more uniform size. Developing monosex male populations of bluegills through selective breeding may hold the greatest potential to improve production efficiency of bluegills and reduce the culture period for food market-sized sunfish to within 2 years.

In fact, the capacity to produce monosex male bluegill populations by means of sex reversal has existed for some time (Al-Ablani 1997). However, we are aware of no commercial bluegill producers that rear such fish in the north-central region of the USA, since commercial culture and sales of hormonally sex-reversed fish require more costly regulatory permits and these fish may be rejected by potential customers. This would appear to elevate the value of a capacity to readily produce mostly male groups through simple size grading. Although rearing mostly male bluegill groups through size grading may be less beneficial than rearing monosex male bluegills or selectively bred monosex male bluegills, the practical size grading approach would permit some commercial producers to take advantage of the substantially higher growth capacity of male bluegills relative to females.

Survival in all three of our treatment groups exceeded 90%, and the top 25% group had a survival rate of 96%. Male bluegills might have higher survival rates than do females, either inherently or in certain culture settings. However, given the low total mortality rates, we do not believe that differential mortality between the sexes significantly influenced sex ratios within our three groups but rather that the grading procedure accounted for the differences among the groups.

Increases in CV of fish weights over time can indicate the presence of dominance hierarchies wherein larger individuals outcompete smaller individuals for available food, leading to variable growth rates (Carmichael 1994; Jobling 1995). The CV for weight in all three treatments decreased over the course of the experiment. The mixed-sex control group showed the greatest reduction in CV of the three treatments throughout the experiment; however, the final CV value for weight of the control group remained the highest among the three groups. The top 25% group maintained the lowest CV values for weight throughout the experiment. The initial differences in CV values among the three treatments are easily understood based on the physical grading that was performed to establish the top 25% and top 50% groups. The continued decrease of CV in the mostly male groups suggests that any intensification of trophic competition or social interactions did not significantly affect the growth rates throughout the experiment (Jobling and Koskela 1996).

We acknowledge that there might be some differences for behavior and social interactions due to differing fish numbers per unit. We were unable to obtain similarly sized male and female juveniles for this study, and the same is true for most current bluegill culture operations. Therefore, we believed that for growth comparisons, stocking equal biomasses would be better than stocking equal numbers. If we had stocked equal numbers as in the mixed groups, there would have been a large biomass difference in each culture unit at the beginning, which would have had a significant effect on growth. Although there was greater biomass in the mixed-sex culture units, the biomass was still low in the 1-m^3 cages. Therefore, we believe this factor did not significantly limit growth of the mixed-sex treatment group. However, further studies comparing growth using equal biomasses and equal numbers (similar size) will be conducted once we have obtained all-male or mostly male populations from our selective breeding program.

In this experiment, the top 25% group showed the highest percentage (46.3%) of fish reaching 150 g at the end of the experiment, followed by the top 50% group (28.3%) and the mixed group (12.7%). The principal challenge faced during this experiment was that ambient water temperatures (in Ohio) were well below the optimum growth temperature for bluegills. Lemke (1977) found that the optimum temperature for bluegill growth was 30°C. During the 11-month experiment, there were only 4 months when mean temperature was above 20°C (only 2 months when the temperature was over 25°C). Generally, growth rates of bluegills increase with increasing temperatures up to approximately 30°C and then decrease as temperatures increase above 30°C (Carlander 1977; Lemke 1977; Beitinger and Magnuson 1979). Undoubtedly, the suboptimal rearing temperatures throughout much of the experiment impeded bluegills in all groups from reaching their growth and production potentials. Under such conditions, it is reasonable to expect that the largest fish (top 25% group) would be the most negatively affected in terms of body mass. Nonetheless, quantifying the loss of growth from this effect is difficult. We believe that the top 25% group, which had previously shown significantly faster growth than the mixed-sex group and at times the top 50% group, would have grown larger during the study if temperatures had remained more favorable and allowed this group to continue to grow at rates near the potential maximum. Therefore, we expect that the majority of fish in the mostly male groups (or at least in top 25% group) would reach market size within 1 year if they are cultured under optimum or near-optimum temperatures.

In summary, this study demonstrates that a practical grading strategy allows the formation of 70–75% male bluegill groups from source bluegill populations with approximately 50:50 sex ratios. This capacity to obtain mostly male bluegill groups holds the potential to increase bluegill production efficiency and the ability to rear large bluegills. Moreover, communally cultured male bluegills are still able to grow faster than typical mixed-sex or female populations in regular aquaculture settings. We conclude that social interaction costs among communally reared males did not significantly decrease growth of mostly male populations in the culture settings and that the rearing of monosex male bluegill populations would be advantageous for bluegill aquaculture.

Acknowledgments

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