ESTABLISHING MOSTLY-MALE BLUEGILL GROUPS AND EVALUATING THEIR GROWTH BENEFITS IN INDOOR REARING SYSTEMS

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled

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presented by Adam J. Doerhoff

a candidate for the degree of Master of Science

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I have dedicated this thesis to my parents and grandparents, for whom I treasure more each day. They never passed up the chance to ask about my bluegill, and thought I had the coolest research project in the world.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter I Page

Chapter II

Chapter III

ESTABLISHING MOSTLY-MALE BLUEGILL GROUPS AND EVALUATING THEIR GROWTH BENEFITS IN INDOOR REARING SYSTEMS

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OVERVIEW

Bluegill *Lepomis macrochirus* and hybrid bluegill (F_1: male bluegill x female green sunfish *Lepomis cyanellus*) are leading candidates for the culturing of large, food-size sunfish ($\geq$0.5 lb.; 225g) due to their acceptance of commercial feed, their fast growth, and palatability. Hybrid bluegill tend to grow faster and to larger sizes than do bluegill in ponds. However, recent evaluations in indoor tanks have shown that male bluegill grow faster than female bluegill and both sexes of the hybrid bluegill, indicating that male bluegill have the greatest potential to reach food-market size within the preferred grow-out periods of two years. A practical means to establish monosex or mostly-male bluegill groups at the juvenile stage currently does not exist, aside from treating young bluegills with androgens. However, such an ability would be useful to assist fish producers not able to carry out androgen treatment, but also to take advantage of the male bluegill's rapid growth.

The major goals of my research were to (1) develop techniques for establishing monosex or mostly-male groups of juvenile bluegill, and then to (2) compare growth rates of mostly-male and mixed-sex bluegill groups reared indoors in tanks, to determine whether the former exhibit faster growth to food-market weight, and greater numbers of large sunfish. Three experiments are aimed toward these goals, with the major objective

being to increase the present capacity to rear bluegill to food-market size within grow-out periods of two years or less.

In Experiment 1 (Chapter 1), the inherent growth capacity (IGC) of individually-held (IH), age-1 bluegill was compared to growth rates of group-held (GH) bluegill subjected to agonistic social interactions. Both the IH and GH fish were fed *ad libitum* twice daily for 240 d. On day 240, the mean weight of IH fish (90 g) significantly exceeded that of the GH fish (57 g). Social interaction among the GH fish apparently reduced their growth but resulted in greater size separation between the sexes, which proved advantageous for forming mostly-male groups.

Data from this experiment enabled construction of a model that predicted the percentages of male fish occurring in the upper 50th and 25th percentiles of weight of a mixed-sex group of juvenile bluegills. The developed model was tested on distinct, mixed-sex groups of age 1, 2, and 3 bluegills. Predicted and observed percentages of male bluegill within each of the three age groups showed good agreement, indicating that the model had some ability for directing size-grading efforts to form subgroups of mostly-male bluegills from a larger, mixed-sex population.

In Experiment 2 (Chapter 2), trenbolone acetate (TBA) immersions provided an additional means of establishing mostly-male, juvenile bluegill groups through "sex reversal." Additional benefits associated with the sex-reversing of fish sometimes include elevated consumption and growth rates, higher feed efficiency, and decreased gonadal growth.

Although the establishment of mostly-male groups via sex-reversal appears reliable and may also benefit growth, the technique required precise treatments and

substantial time commitment. Moreover, sex reversal cannot be carried out in field settings as can the size-grading approach. Consequently, the size-grading approach for forming mostly-male bluegill groups appears the most likely to be adopted by fish producers as a practical means of forming mostly-male bluegill groups.

After developing an approach to forming mostly-male bluegill groups, the remaining obstacle appeared to be amelioration of the growth-rate-reducing effects of social interactions that occur among bluegills held in confined groups. Consequently, in Experiment 3 (Chapter 3), the growth of mixed-sex (MS) and mostly-male (MM) groups in indoor tanks was compared in a 234-d study. Overall, the MM groups grew faster than the MS groups. Higher social costs within MM groups were not observed, and mean weights and growth rates were higher in the MM groups. The presence of more males in MM groups was expected to result in greater social costs and greater size variation among fish, but didn't. The additional males in the MM groups resided in the upper 50% of the size range. A method known as "topping off" involves removing the largest fish (e.g., the upper 10-20% by size) from a group being reared. By topping off, it is believed that intermediate and smaller fish are afforded a better opportunity to grow because they are somewhat released from the consumption inhibiting social influences of the most dominant fish. Minimizing such social costs is important for closing the gap between observed growth rates and realized IGCs in order to culture large numbers of bluegill to food size within two growing seasons.

BACKGROUND

The bluegill (*Lepomis macrochirus*) Rafinesque (Centrarchidae) is a popular fish that occurs throughout much of the United States. Fish producers have historically reared bluegill in earthen production ponds (Doyle and Boyd 1984; Breck 1993; Bryan et al. 1994) to be stocked later as forage and sportfish (Brunson and Morris 2000). Bluegill are prolific spawners and often occur in high densities (Pflieger 1997; Lovshin and Matthews 2003). Their willingness to accept commercial feed while also feeding on natural foods, small size at sexual maturity, and gregarious spawning behavior have allowed these fish to be reared in abundance at relatively small sizes. Bluegill are popular with anglers (Belk 1994), not only because they are vulnerable to hook-and-line capture, but also because of their firm, white flesh (Pflieger 1997; Brunson and Morris 2000) which is considered to have good flavor. Because good demand exists for small- to middle-size bluegill (50-100 mm) for pond stocking purposes in many areas of the country, there has been little emphasis placed on culturing bluegill to larger sizes.

While the market for juvenile bluegill remains strong, interest in the potential to profitably rear sunfish (*Lepomis* spp. and their hybrids) as food fish in the U.S. has developed over the past decade (Brunson and Morris 2000). Worldwide finfish and shellfish consumption continues to rise at rates that exceed sustainable yields from capture fisheries (Riley and Secombes 1993; Nielsen 1999). Consequently, increased emphasis has been placed on commercial aquaculture as a way to meet the growing demand while potentially reducing the pressure placed on many natural fish populations (Li and Moyle 1999; Devlin and Nagahama 2002; Montaigne 2003). More specifically, feedback from commercial fish producers indicates that the demand for food-market-size

Lepomis sunfish is substantial (Brunson and Morris 2000, Morris and Mischke 2000, NCRAC 2002).

Unlike the fish for pond stocking, sunfish must be reared to approximately 227 g (0.5 lbs.) for food markets. Additionally, for food-market sunfish aquaculture to be feasible, it is believed that fish must reach this size within two years of grow-out (Ellison and Heidinger 1978, Tidwell et al. 1994). Risk of loss is expected to be too high if producers must rear fish into a third year to reach food-market weight, whereas reasonable prices can be obtained more immediately for smaller fish. Rearing sunfish to food-market size will likely require techniques that are different from those currently used to rear bluegill for pond-stocking purposes, because sunfish rarely reach food-market sizes in ponds within two years.

Among the lepomid sunfish and their hybrids, the bluegill and hybrid bluegill [F1: male bluegill x female green sunfish (*L. cyanellus*)] have received the most attention for food-fish aquaculture (Lane and Morris 2002; Lovshin and Matthews 2003). This is because both fishes are well recognized, occur across much of the U.S., are considered the fastest growing lepomids, and readily accept the commercial diets which are essential to rapid growth under high rearing density conditions. Of the two fishes, the hybrid bluegill has been perceived as the better food-fish candidate and has received the most attention in this regard to date. Studies pertaining to hybrid bluegill growth, particularly in ponds, indicate that they outgrow bluegills (Krumholz 1950; Lopinot 1972; Ellison and Heidinger 1978; Brunson and Robinette 1983). The faster growth of the hybrid bluegill has been attributed to hybrid vigor and to the composition of its populations, which are typically 80-90% male (Ricker 1948; Childers 1967; Heidinger and Lewis 1972; Ellison

and Heidinger 1978). Lepomid sunfish exhibit sexually dimorphic growth differences, with males growing faster than females (Hayward and Wang 2006). Being predominantly male, hybrid bluegill tend to produce fewer competing or interfering progeny than are produced than by bluegill (Lane and Morris 2002). Moreover, there is evidence that hybrid bluegill, unlike bluegill, benefit from their progeny by readily consuming them. This cannibalism may be related to the hybrid bluegill's substantially larger mouth.

Because hybrid bluegill have received the most attention concerning their grow-out capabilities, it has been somewhat unclear how bluegill growth rates compare. No prior study had directly compared the growth rates of these two fishes for substantial time periods under well-controlled conditions. However, a recent study (Hayward and Wang 2002) found that age-1 bluegill grew much faster than hybrid bluegill grown in parallel in a laboratory setting where a favorable growth temperature (22°C) and *ad libitum* feeding with a commercial diet were provided over an 11-month period (May-March). A follow-up analysis of these data focusing on inter-sex growth differences (Hayward and Wang 2006) showed that male and female bluegill each outgrew both sexes of the hybrids. Furthermore, the data showed that male bluegill (approx. 5 g starting weight) grew significantly faster than female bluegill and reached 150 g (70% of the minimum food-market weight of 227 g) in just 11 months.

The faster growth of bluegills versus hybrid bluegills under continuously favorable laboratory growth conditions, which is in stark contrast to what has been observed in ponds, exemplifies a well-known situation with hybrid fishes wherein their growth advantage is often highly variable across rearing environments (known as a

performance x environment interaction; Avault 1996). Hybrid bluegill apparently outgrow bluegills in ponds for reasons relating to seasonal thermal regimes (Hayward and Wang 2006), and because the hybrids make better use of the natural feeds in ponds than do bluegill (Lane and Morris 2002). That the hybrids produce fewer young, and have lower social costs than do bluegill (Hayward and Wang 2002) likely contributes to their growing faster in ponds as well. Most of these impediments to bluegill growth are not present in an indoor rearing environment, where bluegill are more likely to achieve their inherently higher growth capacity. However, hybrid bluegill growth in ponds, where this fish fares the best (Tidwell et al. 1992), is much poorer than that of male bluegills in indoor recirculation tanks, where it appears that bluegill can reach food-market weight in well under two years (Hayward and Wang 2002, NCRAC 2002).

OBJECTIVES

Male bluegill are the most promising among the lepomid sunfish for food-market aquaculture, because they can grow significantly larger and faster than female bluegill and hybrid sunfish. Successful techniques to raise these fish to food-market weight (227 g) within two years are needed for their culture to be economically feasible for fish producers. To achieve this goal, techniques to establish monosex or mostly-male groups of juvenile bluegill are needed. Likewise, comparisons of growth rates, size variation development, and percentages of fish reaching food-market weight, both for mostly-male and mixed-sex bluegill groups, should be evaluated in indoor recirculation tanks. Thus, a series of experiments was conducted with the following objectives:

1) develop a technique for establishing mostly-male ($\geq$ 80%) groups of juvenile bluegill through size-grading;

2) establish monosex or mostly-male bluegill groups through a "sex reversal" approach, and determine whether growth rates of androgen-treated males differ from those of non-treated males;

3) determine whether growth rates, size variation, and thepercentage of fish reaching food-market weight (227 g) are greater for mostly-male versus mixed-sex bluegills grown in groups within indoor recirculation tanks.

Chapter I

DEVELOPMENT AND EVALUATION OF A SIZE-GRADING APPROACH FOR FORMING MOSTLY-MALE BLUEGILL POPULATIONS

ABSTRACT

Male bluegill *Lepomis macrochirus* possess a higher growth capacity than do female bluegill or either sex of hybrid bluegill (F_1: male bluegill x female green sunfish). Culturing efforts directed toward raising bluegill to food market size ($\geq$227 g) now focus on the ability to establish mostly-male bluegill groups for rearing. Additionally, a practical approach to efficiently establish all- or even mostly-male bluegill groups at the juvenile stage currently does not exist.

The inherent growth capacity (IGC) of individually-held (IH) bluegill was compared to growth rates of group-held (GH) bluegill subject to social interactions for age-1, same-cohort bluegill in this study. Bluegill were fed *ad libitum* twice daily at 22˚C for 240 d. IH and GH fish exhibited similar day-30 mean weights of about 10 g, but by day 240 the mean weight of IH fish (90 g) exceeded that of GH fish (57 g). Social costs reduced the mean weights achieved by GH fish; however, group confinement resulted in better weight-based sex separation (vs. isolation). Mostly-male groups (as high as 88%) were observed in the upper 25% of IH fish, whereas 100% males were observed in the upper 25 and 50% of fish selected from GH fish. The observed percentages of males were predictable in IH fish, but were variable and less predictable in GH fish due to differing sex ratios; however, variable sex ratios are likely to exist among bluegill populations in a commercial setting. This variability was incorporated into the formation of four multiple regression models.

One model was ultimately selected as the male-percentage prediction (MPP)

model. Models were tested on bluegill secured from a local producer, with the intent of predicting the percentage of males in the upper 25 and 50% of the group. The range in predicted percentage of males calculated by the MPP model and the observed percentage of males were similar; hence, the ability to size-grade juvenile bluegill with the model – to form mostly-male groups – appears to be both practical and reliable. Group confinement is the most common bluegill culturing method, and finding ways to reduce social costs after size-grading will help GH bluegill approach their IGC.

INTRODUCTION

Hayward and Wang (2002) provided evidence that the bluegill is a better lepomid sunfish to rear as a food fish than is the hybrid bluegill due to the former's greater inherent growth rate capacity (IGC). Their research results indicated that rearing male bluegills should give the fastest growth to food market size relative to other sunfishes, particularly if a portion of the rearing period is completed in indoor recirculating aquaculture systems (RASs) during cold periods. However, rearing predominantly male bluegills for food markets will require techniques to form monosex male, or at least mostly-male populations at the juvenile stage, which is when grow-out typically begins.

Several methods exist for separating fishes according to sex. These include methods based on inter-sex differences in size, coloration, and inner vent length or shape (McComish 1968; Ross 1984; Lebeau and Pageau 1987; Pflieger 1997). However, these methods tend to be most effective for mature fish, and are not always reliable (Casselman 1974; Ferrara and Irwin 2001). Juvenile fish of both sexes often appear similar in color,

shape, and vent characteristics, making the sexes virtually indistinguishable by external inspection (Casselman 1974; Ferrara and Irwin 2001); this also appears to be the case with bluegill. Furthermore, sex-separation techniques based on visual inspection are neither time efficient nor practical in high-fish-density settings because each fish must be individually handled and inspected. Thus, a practical approach to efficiently establishing monosex or otherwise mostly-male bluegill groups at the juvenile stage does not exist. Forming monosex male populations via "sex-reversal" is common for fish species used in aquaculture; an evaluation of the potential to do this with bluegills is treated in Chapter 2.

It is known that male bluegills grow substantially faster and larger than do females beginning in the juvenile stage (Hayward and Wang, 2006). Size-at-age in both length and weight is a sexually dimorphic trait for this species (Lane 1954; Sprugel 1954; Hayward and Wang, 2006). Sexual dimorphism, a difference in phenotypic expression according to sex, occurs in many fish species. Using sexually dimorphic traits to establish monosex male and female populations has been applied to many aquaculture species (Dunham 1990). Taking advantage of size differences that develop between male and female bluegills with age as a basis for separating males from females would seem to hold potential for forming mostly-male, and potentially even monosex male populations. However, it is not known whether a sufficient size distinction between male and female bluegills develops early enough in the ontogenetic progression for size-based separation of the sexes to be useful in aquaculture.

In addition to the sex-related influence, size variation within sunfish populations can also result from differences in fish age, the effects of agonistic social interaction

(Wang et al. 2000), and genetic differences among individuals that influence growth rate (Belk 1995). Because individual size differences within any mixed-sex bluegill population typically do not arise solely from sexually dimorphic growth, selecting males from a mixed-sex population based on size alone might not yield monosex or even mostly-male groups with good consistency.

The confounding influence on size variation within bluegill populations that arises from the simultaneous presence of multiple intra- as well as inter-annual cohorts can be substantially eliminated by working exclusively with individual, intra-annual cohorts (i.e., fish that have hatched within the same 2- to 4-week period). However, genetically based size variation within bluegill populations cannot presently be controlled, nor can that which arises from the substantial social interaction which occurs in bluegills and hybrid bluegills (Wang et al. 2000; Hayward and Wang 2002).

The conceptual basis for selecting male bluegills from a mixed-sex population involves the natural, progressive size separation of males from females within an intra-annual cohort, due to the former's tendency to grow faster. The aim with size-grading is to select an appropriate upper portion of a mixed-sex population's size spectrum at the appropriate time, so that it that will comprise all, or at least mostly male fish (Figure 1.1). Size separation between the sexes in an intra-annual cohort is expected to become more pronounced through time as the mean weight of all individuals in a cohort increases. Documenting the size separation of males and females as both groups grow, and understanding the extent to which other characteristics of the cohort (e.g., sex ratio) may influence size variation development within a bluegill group, should be useful in establishing an approach to size-selecting male bluegills.

METHODS

Approximately 750 pellet-trained, age-0 bluegills (~3.3 months post-hatch), all known to have hatched within the same two-week period in June 2001 (Steven Muich, manager, Missouri Department of Conservation's [MDC] Hunnewell Hatchery), were secured from the MDC's Hunnewell Hatchery and transported to the University of Missouri-Columbia on 4 September 2001. Fish were acclimated to laboratory conditions for 15 d by holding them as a single group at 22°C in an 1150-L recirculating aquaculture system (RAS) tank equipped with water quality, aeration, and temperature control capacities. After 1 d in residence, twice daily feeding to apparent satiation was begun with Rangen EXTR 400 pellet diet (RANGEN, Inc., Angleton, TX). A constant photoperiod of 14L:10D (light period: 0800-2000) was maintained throughout both the acclimation and subsequent experiment phases.

Following acclimation, 32 fish (mean weight = 3.55 g; SD = 1.25 g) were selected at random from the whole group and placed individually into 20-L, plastic test chambers that were submerged within two 1000-L RAS tanks, each containing 16 test chambers (Figure 1.2). Hereafter, these fish will be referred to as the individually-held (IH) fish. The test chambers had screened sides to permit water flow-through, and their open tops extended above the water surface to allow feeding and chamber cleaning. To promote water exchange, water trickled into each test chamber from a spray bar located above. In addition, eight groups of 20 bluegills each, hereafter referred to as the group-held (GH) fish, were selected at random from the remaining whole group and each placed into larger (68 L) plastic test chambers with screened sides that were submerged (with open tops above the water surface) in a third 1000-L RAS tank (Figure 1.2).

The experiment was run from September 2001 through May 2002; fish were approximately 3.3 and 11.3 months of age at the beginning and end, respectively, of the 240-d experiment period. The experiment was designed to quantify the extent that juvenile male and female bluegills became separated in size (both in length and weight) over the 240-d period, while being reared under favorable growth conditions of temperature and food supply. The data produced would ultimately be used to determine the extent to which size-based male selection from a mixed-sex bluegill group is possible, and whether predictive equations could be developed to direct size-grading efforts to form mostly-male subgroups. Parallel sub-experiments involving IH and GH fish were used to evaluate the extent to which group holding of bluegills influenced the size separation of males and females, and potentially impeded or enhanced the ability to form mostly- or monosex male bluegill populations. Although only a single group density was evaluated with the GH fish (330 fish/m^3), useful insights were expected to be gained into the extent to which fish held in groups (as is done in aquaculture) might impede the ability to select male bluegills based on size. The holding density used was close to that routinely used when rearing bluegills in our laboratory. Social effects associated with bluegills held in groups could, for example, impede size separation of the sexes. Or, they might facilitate size separation given evidence that male bluegills are more aggressive than females (Hayward and Wang 2002; Hayward and Wang, 2006) and that males may limit female access to food, thus causing them to grow more slowly than males. It was unknown whether data resulting from fish held individually or in groups might provide a better basis for constructing a predictive model for size-selecting predominantly male bluegills from a mixed-sex group.

Both the IH and GH fish were fed twice daily to apparent satiation while held at 22 °C (± 1.0°C) throughout the experiment period. All test chambers and the whole tanks that housed them were cleaned weekly by siphoning; 25% water exchanges were also made to ensure that high water quality was maintained. Monitoring of TAN, nitrite, and nitrate levels was done in all three tanks on a weekly basis. Water temperature and dissolved oxygen levels were measured daily. Dissolved oxygen was maintained at ≥8.0 ppm; TAN, nitrite, and nitrate concentrations remained below 0.025, 0.25, and 60.0 mg/L.

Total length (nearest 1.0 mm) and weight (nearest 0.1 g) of each IH fish was determined every 30 d throughout the experiment period. On the final day of experimentation, all IH fish were weighed, measured, and then euthanized and frozen so that each fish's sex could be determined at a later time. Experimentation and sampling were similar for the GH bluegills, except that one of the initial eight groups was euthanized following each of the eight successive 30-d periods, with the 20 fish in a group being measured and weighed just before euthanization and then frozen for later sex determination. This design provided data showing how weights and lengths of group-held male and female bluegills separated as the fish grew over the experiment period, and also allowed month-by-month comparisons between fish reared with and without the influence of social interaction.

For larger fish, sex was determined by direct observation of the gonads. For smaller fish, or whenever simple observation was deemed insufficient, the squash method (Guerrero and Shelton 1974) for sex determination was applied using fast-green stain. Spermatocytes or oocytes were then identified with a dissecting microscope.

Multiple regression (MR) analyses were performed separately for the IH and GH fish data in an effort to develop predictors of the percentages of male bluegills that occurred within selected, upper-weight-percentile groups, drawn either from the single group of 32 IH fish, or from the eight groups of 20 bluegills (GH setting) as the experiment progressed and the mean lengths and weights of bluegills increased. Two best MR models, one using mean fish length as a predictor variable, and the other using mean fish weight, were developed over the IH fish data, and likewise for the GH fish data.

Selected upper-weight-percentile groups of bluegills consisted of various upper portions of the ranked weight or length spectrums of fish within the whole groups of 32 fish (for IH fish) or the distinct groups of 20 fish (for GH fish). Selected groups included the upper 100% (all fish), upper 75%, upper 50%, and upper 25% of all fish in a group according to weight or length. For IH fish, MR models for predicting the percentages of males within the selected groups could involve up to three predictor variables: (1) mean weight or length of bluegills in the base population of 32 fish at each 30-d interval, (2) CV of weight or length in the base population of 32 fish at each 30-d interval, and (3) the upper portion of fish (based on ranked weights or lengths) that was selected from the base population. It is noted that the mean lengths and weights of bluegills in the single IH population of 32 fish, as well as the CV of length or weight, changed over the eight successive months. The same three predictor variables were included when developing MR models for predicting the percentages of male bluegills using the GH fish data. However, for the GH fish, it was possible to also consider sex ratio (number of females / number of males) in the base population as a fourth potential predictor variable for MR

models based on GH fish. This was possible because sex ratios varied across the eight groups of 20 fish; this was not possible for the IH fish because sex ratio among the single group of 32 fish did not change over time (except in a minor way when one fish in this group perished after the second month of the experiment).

In addition to the three (IH fish data) or four (GH fish data) individual predictor variables, all combinations of two predictor variables were also initially included in the MR analyses as interaction terms; this resulted in 6 and 10 potential predictor variables for the IH and GH fish data sets, respectively. To determine the best MR model based on fish weight and also fish length, separately for the IH and GH data sets (four MR models), a stepwise variable selection procedure was applied with the best models being decided on the basis of AIC scores and R^2 values.

Fully independent data sets were also established to evaluate the extent to which the four MR models constructed from the laboratory data could accurately predict the percentages of male fish within the selected upper size groups of bluegills that had been collected from ponds. Three groups of 50 bluegills each were collected by seining three ponds managed by Osage Catfisheries Inc., Osage Beach, Missouri, and were transported to the laboratory in April 2002. The fish were reported to be age 1, age 2, and age 3 fish, in ponds 1, 2 and 3, respectively; however, the ages were not verified. It is unlikely that the fish in each pond were from a single intra-annual cohort. Upon arrival at the laboratory, all fish were euthanatized and their lengths and weights immediately determined; the sex of each was determined subsequently using the same methods as for the laboratory-reared fish. The four MR models developed from the IH and GH

laboratory groups of bluegills were applied to each of the three age-groups of fish from the commercial producer to determine how accurately the percentages of males within various upper size groups of these fish (upper 100, 75, 50, and 25% of ranked weights) could be predicted. Finding reasonable predictive accuracy would indicate that an MR model could be used effectively to direct efforts to select high percentages of male bluegills from mixed-sex pond populations. For example, based on rough estimates of mean weight, CV of weight, and sex ratio for a pond population of bluegills, one of the MR models might indicate the percentages of male bluegills that would occur in various upper size percentiles of the pond population. Fish in the desired upper size percentile could then be selected from the pond by mechanical size grading.

RESULTS & DISCUSSION

Growth-in-weight trajectories of the IH bluegills showed a tendency for male fish to outgrow females over the 240-d experiment in the absence of social influence (Figure 1.3). The progressive weight separation of males from females over time, demonstrates an increasing potential to size-select male bluegills from a mixed-sex population, as mean weight (or length) of the fish in the whole population increases. However, as the weights of male and female bluegills became more separated with time, the growth trajectories of both sexes also exhibited depensation (fanning out) (Figure 1.3), a characteristic that would tend to impede the ability to establish purely male bluegill populations by size selection. The ability to size-select high percentages of male bluegills from a mixed-sex group well before fish in the mixed-sex group reached the highest mean weights observed for IH and GH fish in Experiment 1 would be desirable.

Of the 31 IH bluegills that survived to the end of the 240-d experiment, 16 (52%) were ultimately determined to be males (Table 1.1). Size selection of the largest 24 fish out of the total 31 IH bluegills (approximately the upper 75% of fish) was simulated by ranking individual fish weights and selecting the top 24. The same was done for the largest 16 of the 31 fish (approximately the upper 50%), and for the largest eight fish (approximately the upper 25%). Over the nine, 30-d-apart sampling dates (including the initial day), the percentages of male bluegills in the selected groups (upper 75, 50, and 25%) generally increased, as more restricted upper portions of the total 31 fish were considered and as the mean weights of fish increased (Table 1.1). Once the mean weight of all fish reached 43 g (126 mm) on day 120, increases in the percentage of male bluegills in selected groups occurred consistently as more restricted upper portions of the whole group were considered (Table 1.1). Beginning on day 120, it was possible to consistently select groups containing 88% male bluegill (seven males of eight total fish). Although the mean weights (and lengths) of bluegill increased substantially beyond day 120, higher proportions of male fish in upper 25% groups (>88% male fish) could not be achieved after this. However, some increase in the percentage of males in the upper 50% groups was evident after day 120.

Unlike the IH fish, the 240-d data set for the GH fish involved eight distinct groups of 20 bluegills. At each successive 30-d interval (excluding day 0), all bluegills in one of the initial eight groups (group selected at random) were measured, weighed, and then sacrificed for sex determination. Results for the GH fish were inherently more variable than for the IH fish due to the involvement of multiple bluegill groups versus only the single set of 32 bluegills that represented the IH fish. The eight groups of GH

fish exhibited more varied sex ratios (range = 33 to 65% male fish, Table 1.2) as well as higher weight variation (CV) relative to the IH fish (Figure 1.4), which tended to increase with time, likely from social interaction. Consequently, the GH data set was thought to have provided a richer, more realistic data set for developing a predictor of the percentage of male bluegills within selected groups. Alternatively, the greater variability within the GH data set could represent undesirable background "noise" such that better prediction models for selecting male bluegills would emerge from the IH fish data set.

The mean weight of GH fish in the test chamber sampled on day 30 was approximately 10 g (Table 1.2), similar to that of the IH fish on day 30 (Table 1.1). However, the mean weight of the GH bluegill group sampled on the final day of experimentation (day 240) was only 57 g, whereas the IH fish had reached about 90 g by day 240. The substantially lower weight gain observed for the GH versus the IH fish over the course of the experiment was likely related to the occurrences of agonistic social interaction among the fish held in groups (Wang et al. 2000). Despite the more rapid growth rate of the IH fish, the size separation of males and females, a feature that is key to the ability to size-select male bluegills from a mixed-sex group, tended to become more pronounced with time for the GH fish than for IH fish; however, this pattern was not consistent across all sampling dates (Figure 1.7).

As for the IH fish, the percentages of male bluegills in groups selected from the test chambers containing approximately 20 fish (GH fish) generally increased, as more restricted upper portions of the weight-ranked fish were considered (upper 75, 50 and 25%) and as the mean weights of bluegills increased over time (Table 1.2). We could successfully select groups containing 100% male bluegills in the upper 25% selection

groups, and occasionally in the upper 50% selection groups as well, whereas 100% male bluegill groups were never selected from the IH fish. However, an ability to consistently select 100% male bluegills in the highest selection group (upper 25%), once the fish in a group reached a certain mean weight, was not demonstrated (Table 1.2). Hence, while the potential to size-select bluegill groups with higher percentages of males (including 100% males) was achieved for the GH fish, the stability of outcomes was lower than for the IH fish, as indicated by the inconsistent increases in the percentages of male bluegills selected as mean weights of GH bluegills increased (Figure 1.8). Some of the predictive inconsistency observed for the GH fish was likely related to differences in the sex ratios among the eight distinct bluegill groups (Table 1.2). However, when developing MR models for predicting the percentages of males within selected groups, the opportunity exists to incorporate influences of differing sex ratios among the GH fish chambers as a predictor variable (in addition to the predictor variables: mean fish weight, upper percentile selected, and CV of fish length or weight); this was not reasonable for the IH fish because sex ratio was static over the course of the experiment.

From Table 1.3, the best MR model for predicting the percentage of male bluegills within a size-selected group from the IH fish data set based on fish weights included two predictor variables: 1) mean weight of all fish in the IH group preceding size-selection (hereafter, "mean weight"), and 2) mean weight interacting with the upper percentage of fish that were selected from the IH group. When mean lengths were used instead of mean weights, the best MR model for the IH fish data again included two predictor variables: 1) mean length interacting with the upper percentage of fish selected, and 2) mean length interacting with the CV of length in the whole IH group. Both MR

models yielded R^2 values of 0.89 (Table 1.3). The best MR model from the GH fish data using fish weights included three predictor variables: 1) mean fish weight, 2) mean fish weight interacting with the upper percentage of fish selected, and 3) the upper percentage selected interacting with the sex ratio within any given chamber containing GH fish. The best MR model from the GH fish data using fish lengths also included three predictor variables: 1) mean length, 2) mean length interacting with the upper percentage of fish selected, and 3) sex ratio interacting with the CV of length. The two MR models based on the GH fish had R^2 values 0.76 and 0.78, respectively.

Differences were found among the mean prediction errors associated with the four MR models (Table 1.4), when each model was used to predict observed percentages of male bluegills that occurred in the upper 100%, 75%, 50% and 25% of the ages 1, 2 and 3 fish that had been secured from the ponds of the commercial producer (ANOVA, with blocking by fish age group; $P<0.05$). The estimated mean errors (absolute value of predicted minus observed percentages of male bluegills) were 18.3, 14.3, 10.3, and 6.0% for the IH-length, IH-weight, GH-length, and GH-weight MR models, respectively. Duncan's test indicated that the GH-weight model yielded the lowest mean error when applied to the independent bluegill data set. Performance of the GH-length model was no different from that of the IH-weight model, with performance of the former being no different from that of the IH-length model. The generally better predictive performances of the two GH models versus the IH models relative to the observed percentage of males are portrayed in Figure 1.9.

The better overall predictive performance of the MR models based on the GH data can likely be attributed to their ability to account for the "background" sex ratios in

bluegill populations from which attempts to size-select male fish were made. Among the three age groups of bluegills secured from the commercial producer's ponds, sex ratios ranged from 34% to 52% males. Sex ratios in bluegill populations often vary widely (Tulin and Phelps 2004); the ability to accommodate these background ratios in a MR model for predicting male percentages in selected size groups clearly would be advantageous. Moreover, the GH data set which involved fish held in groups likely provided a more realistic representation of the male versus female bluegill size separation that would occur in a pond setting where most bluegills are produced.

Size-selective models that would direct grading efforts to select subgroups of mostly-male bluegills from a larger mixed-sex population could be of substantial practical use to commercial producers of bluegills. This approach can be readily applied by fish producers in culture settings, unlike alternative approaches such as sex-reversal. Although potentially more efficient and capable of producing fully monosex male groups, sex reversed fish require treatment with male androgens such as methyltestosterone or trenbolone acetate and require federal approval (typically requiring many years to acquire) before treated fish could be sold for human consumption. Moreover, most fish producers would have to purchase sex-reversed fish because they would be unfamiliar with the technical procedures associated with sex reversal; licenses would also be required before male androgens could be held legally at a producer's facilities.

A downside of the size-selection approach is that it would be most appropriately applied to mixed-sex bluegill populations comprising only a singe intra-annual cohort, in order to reduce background fish size variation in the mixed-sex population that would result from fish being of different ages. Using single, intra-annual cohorts of bluegills

(fish that all hatched within the same 2- to 4-week period) would better ensure that size differences are primarily related to inter-sex differences in growth rate, and should improve the ability to select male fish. Establishing mixed-sex bluegill populations comprising single intra-annul cohorts would require more attention by producers when producing seed stock in ponds than is typically given. In particular, it would be necessary to remove brood fish from ponds promptly after their first spawning effort is completed.

Another consideration is that an inter-annual cohort of bluegills should be grown to a mean size of at least 90 mm (3.5 inches) before size grading is applied to remove mostly-male fish; in a recent experiment in a producer's seed-stock pond in northern Missouri, it was observed that bluegills that hatched in Fall 2004 did not reach mean lengths of 90 mm until Fall 2005 despite being provided a commercial feed. Also, because selecting bluegill groups with high percentages of male fish (i.e., $\geq$ 80%) will typically require selecting fish in the upper 25th percentile of size (or higher) from a mixed-sex population, high numbers of bluegills will be required in the mixed-sex population to yield substantial numbers of fish in the high-percentage-male selection group. For example, if six grow-out ponds are each to be stocked with 10,000 bluegills from an upper 25% selection group, the mixed-sex population from which these fish are selected would have to number approximately 150,000 fish. However, this is not an unreasonable number of young bluegills to be produced in a nursery pond of substantial size.

To date, there has been only one application of a size-selection model outside of what was described in this chapter involving bluegills from Osage Catfisheries in

Missouri. In Chapter 3, the MR model developed from the GH data set and based on fish lengths was applied to establish mostly-male bluegill groups in RASs. Size grading procedures directed by this model and applied to an original mixed-sex group were predicted to produce groups of 300 bluegills in each of two tanks with male percentages of 69%. Sex determination of all fish in both of these tanks at the end of the study showed them to contain 70% and 66% male bluegills, respectively. Fish selected randomly from the original mixed-sex group to form two additional tanks of 300 bluegills showed sex ratios of 51% and 57%. Further evaluations of the capacity of MPP models to accurately direct size-grading operations to form bluegill groups containing targeted percentages of male fish are warranted, particular in settings where mechanical grading will be applied.

Finally, it is noted that the MPP models have been used only to predict the percentages of male bluegills that will result in size-selected groups. The extent to which male bluegill groups formed by the MPP models are composed of the various types of male bluegills, including cuckolder and parental males (Belk 1995; Neff et al 2004) has not been evaluated. Although it appears reasonable to expect that parental male bluegills are most represented in the size-selected groups, given their more delayed maturation and expected faster growth, the extent to which cuckolder males are selected against could be of interest given the aim of selecting fish with the greatest capacity to reach large sizes most expeditiously.

Table 1.1 – The mean weight (g), mean length (mm), proportion and percentage of males represented in the upper 100% (all fish), 75%, 50%, and 25% of fish selected on successive sampling dates for IH fish.

Day	N	Mean Weight (SD)	Mean Length (SD)	All Fish	Upper 75%	Upper 50%	Upper 25%
0	32	3.55 (1.25)	61.03 (5.43)	16/32 (50%)	11/24 (46%)	9/16 (56%)	5/8 (63%)
30	32	10.35 (3.26)	79.81 (6.77)	16/32 (50%)	11/24 (46%)	10/16 (63%)	5/8 (63%)
60	32	19.72 (5.46)	95.84 (7.40)	16/32 (50%)	12/24 (50%)	10/16 (63%)	5/8 (63%)
90	31	30.49 (9.67)	110.10 (8.87)	16/31 (52%)	13/24 (54%)	11/16 (69%)	5/8 (63%)
120	31	42.96 (15.25)	122.26 (10.84)	16/31 (52%)	14/24 (58%)	11/16 (69%)	7/8 (88%)
150	31	60.56 (21.86)	132.90 (12.31)	16/31 (52%)	15/24 (63%)	11/16 (69%)	7/8 (88%)
180	31	67.00 (25.22)	141.55 (13.29)	16/31 (52%)	15/24 (63%)	11/16 (69%)	7/8 (88%)
210	31	81.06 (29.71)	149.00 (13.99)	16/31 (52%)	15/24 (63%)	13/16 (81%)	7/8 (88%)
240	31	89.58 (33.59)	156.21 (15.27)	16/31 (52%)	14/24 (58%)	13/16 (81%)	7/8 (88%)

Table 1.2 – The mean weight (g), mean length (mm), and proportion and percentage of males represented in the upper 100% (all fish), 75%, 50%, and 25% of fish selected on successive sampling dates for GH fish.

Day	N	Mean Weight (SD)	Mean Length (SD)	All Fish	Upper 75%	Upper 50%	Upper 25%
0							
30	20	10.22 (4.93)	78.35 (10.63)	12/20 (60%)	9/15 (60%)	6/10 (60%)	3/5 (60%)
60	19	16.57 (7.94)	88.11 (12.48)	12/19 (63%)	11/14 (79%)	8/10 (80%)	5/5 (100%)
90	18	28.00 (16.69)	103.22 (17.07)	8/18 (44%)	8/14 (57%)	8/9 (89%)	5/5 (100%)
120	18	28.03 (18.63)	104.22 (20.18)	6/18 (33%)	6/14 (43%)	5/9 (56%)	4/5 (80%)
150	18	49.03 (25.00)	124.33 (19.29)	9/18 (50%)	9/14 (64%)	9/10 (90%)	5/5 (100%)
180	20	36.23 (22.19)	116.75 (21.20)	13/20 (65%)	12/15 (80%)	10/10 (100%)	5/5 (100%)
210	18	43.21 (32.64)	120.94 (25.05)	11/18 (61%)	9/14 (64%)	9/9 (100%)	4/5 (80%)
240	19	57.44 (41.88)	126.32 (31.56)	11/19 (58%)	10/14 (71%)	9/10 (90%)	4/5 (80%)

Table 1.3 – Best multiple regression models for predicting percentage of male bluegills in a size-selected group. Models were constructed on the basis of either fish length (mm) or fish weight (g) for IH and GH fish.

Definition of Response (Y) and Predictor Variables (X)

Y = % males observed in selected upper size group
X1 = mean weight or mean length of bluegills in whole group
X2 = selection group by weight or length (upper 25, 50, 75, or 100%)
X3 = sex ratio in whole group (# female / # male)
X4 = CV of weight (nearest 0.1 of gram) or length (nearest whole mm) in whole group
X5 = X1*X2
X6 = X1*X3
X7 = X1*X4
X8 = X2*X3
X9 = X2*X4
X10 = X3*X4

Individual-Weight Model; Y = X1 + X5
% males = 53.6 + (0.63)(mean weight) + (-0.007)(mean weight * selection group)
Y = 53.6 + 0.63(X1) – 0.007(X5)
R^2 = 0.89; AIC = 107.76; BIC = 109.16; F = 131.28; $p \leq 0.0001$

Individual-Length Model; Y = X5 +X7
% males = 47.6 + (-0.003)(mean length * selection group) + (0.036)(mean length * CV)
Y = 47.6 – 0.003(X5) + 0.036(X7)
R^2 = 0.89; AIC = 106.90; BIC = 108.23; F = 134.85; $p \leq 0.0001$

Group-Weight Model; Y = X1 + X5 + X8
% males = 79.1 + (0.8)(mean weight) + (-0.008)(mean weight * selection group)
+ (-0.25)(selection group * sex ratio)
Y = 79.1 + 0.8(X1) – 0.008(X5) – 0.25(X8)
R^2 = 0.76; AIC = 153.62; BIC = 156.66; F = 28.84; $p \leq 0.0001$;

Group-Length Model; Y = X1 + X5 + X10
% males = 45.7 + (0.7)(mean length) + (-0.005)(mean length * selection group)
+ (-0.856)(sex ratio * CV)
Y = 45.7 + 0.7(X1) – 0.005(X5) – 0.856(X10)
R^2 = 0.78; AIC =149.97; BIC = 150.18; F = 33.45; $p \leq 0.0001$

Table 1.4 – The age, sample size, mean weight (g), mean length (mm), sex ratio, CV of weight, CV of length, proportion and percentage of males observed in the upper 100%, 75%, 50%, and 25%, and the predicted percentage of males within those ranges according to the multiple regression models, for age 1, 2, and 3 bluegill selected at random from Osage Catfisheries, Osage Beach, Missouri.

Age	N	Mean Wt (SD)	Mean Ln (SD)	Sex Ratio (#F/#M)	CV Wt	CV Ln	% Males in Upper 100%	75%	50%	25%
1	58	3.85 (0.67)	64.60 (3.79)	1.07	17.40	5.87	28/58 48%	24/44 59%	20/29 69%	12/15 80%
Predicted %						Absolute Mean Error				
Individual-Weight						12.3	53	54	55	55
Individual-Length						14.8	42	47	52	56
Group-Weight						3.0	52	59	67	74
Group-Length						2.5	53	61	69	77
2	50	8.54 (3.54)	79.50 (9.80)	1.94	41.53	12.33	17/50 34%	14/38 37%	11/25 44%	8/13 62%
Predicted %						Absolute Mean Error				
Individual-Weight						13.3	53	54	56	57
Individual-Length						23.8	59	65	71	77
Group-Weight						8.0	32	44	58	71
Group-Length						20.8	50	60	70	80
3	48	32.10 (9.05)	120.90 (9.99)	0.92	28.17	8.27	25/48 52%	23/36 88%	22/24 92%	12/12 100%
Predicted %						Absolute Mean Error				
Individual-Weight						23.3	51	57	63	68
Individual-Length						22.5	47	56	65	74
Group-Weight						11.0	56	68	80	92
Group-Length						7.0	62	77	92	107

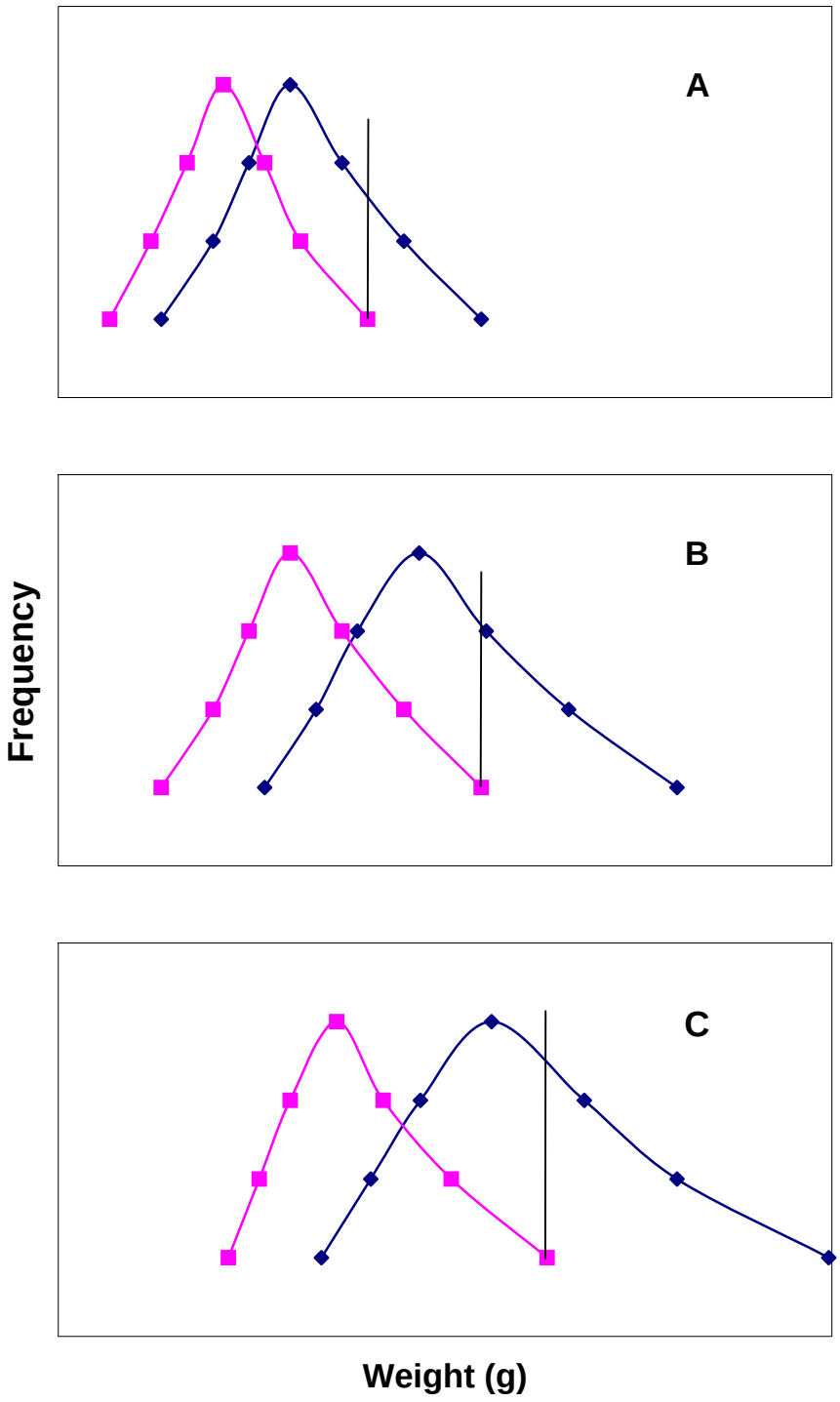
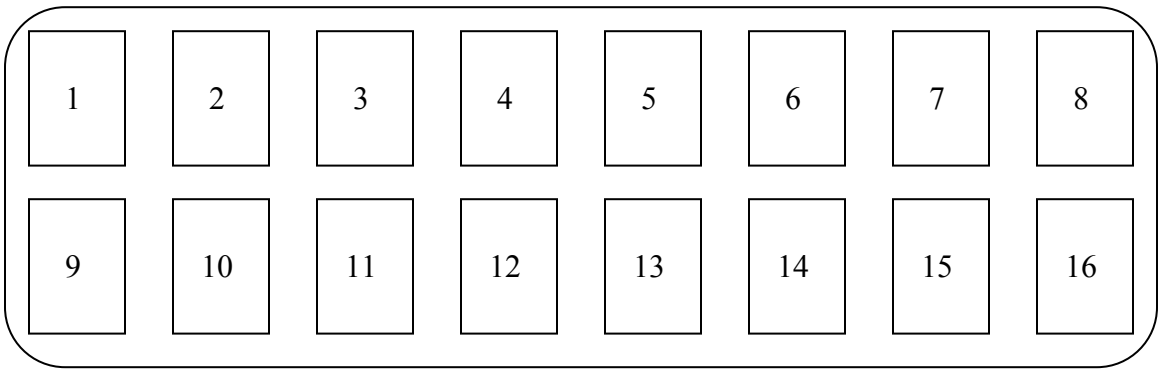


Figure 1.1 – The conceptual basis for size-selecting male bluegills from a mixed-sex bluegill population takes advantage of the natural, progressive size separation of males from females within an inter-annual cohort as the fish grow. Early on, males and females are largely indistinguishable based on their weight or size in a mixed-sex cohort (A). As time progresses, males begin to outgrow females (B). This separation in weight continues as the mean weight of all bluegills increases, making it increasingly more possible to select substantial numbers of males by selecting fish that are above the weight or size indicated by the vertical line (C).

TANK 1



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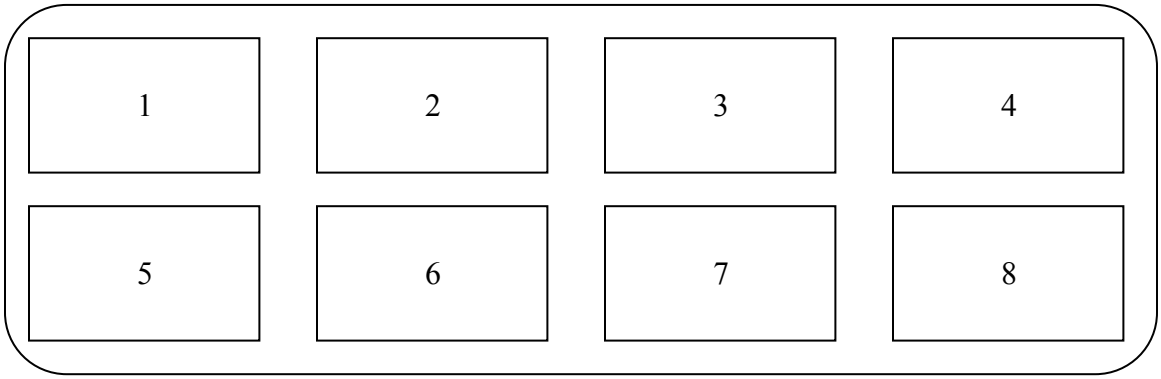

TANK 3

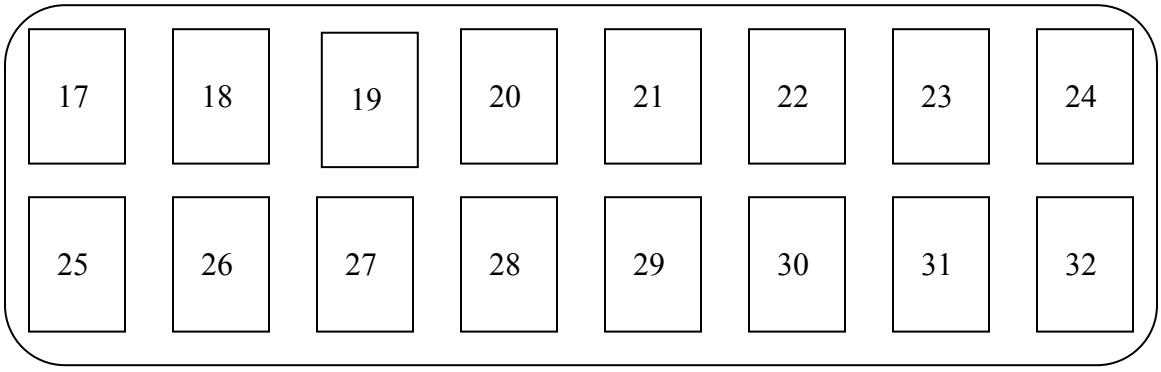

Figure 1.2 – The experimental design for Experiment 1. IH fish were reared in Tank 1 (small chambers 1-16) and Tank 3 (small chambers 17-32). GH fish were reared in Tank 2 (large chambers 1-8).

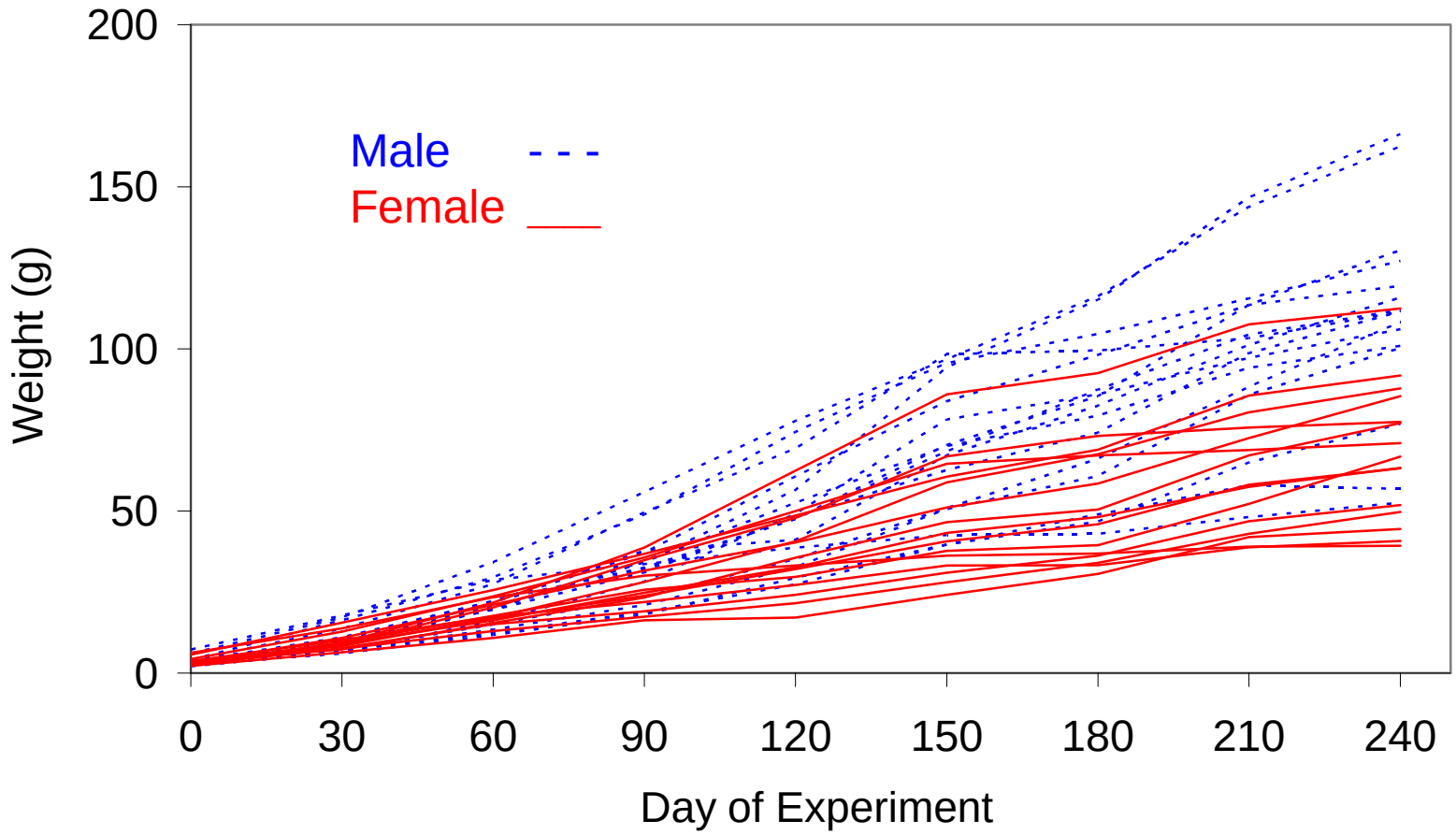

Figure 1.3 – Observed growth in weight of the IH fish over the successive, 30-d-apart sampling dates, illustrating the separation of males (N = 16) and females (N = 15) on the basis of weight over time.

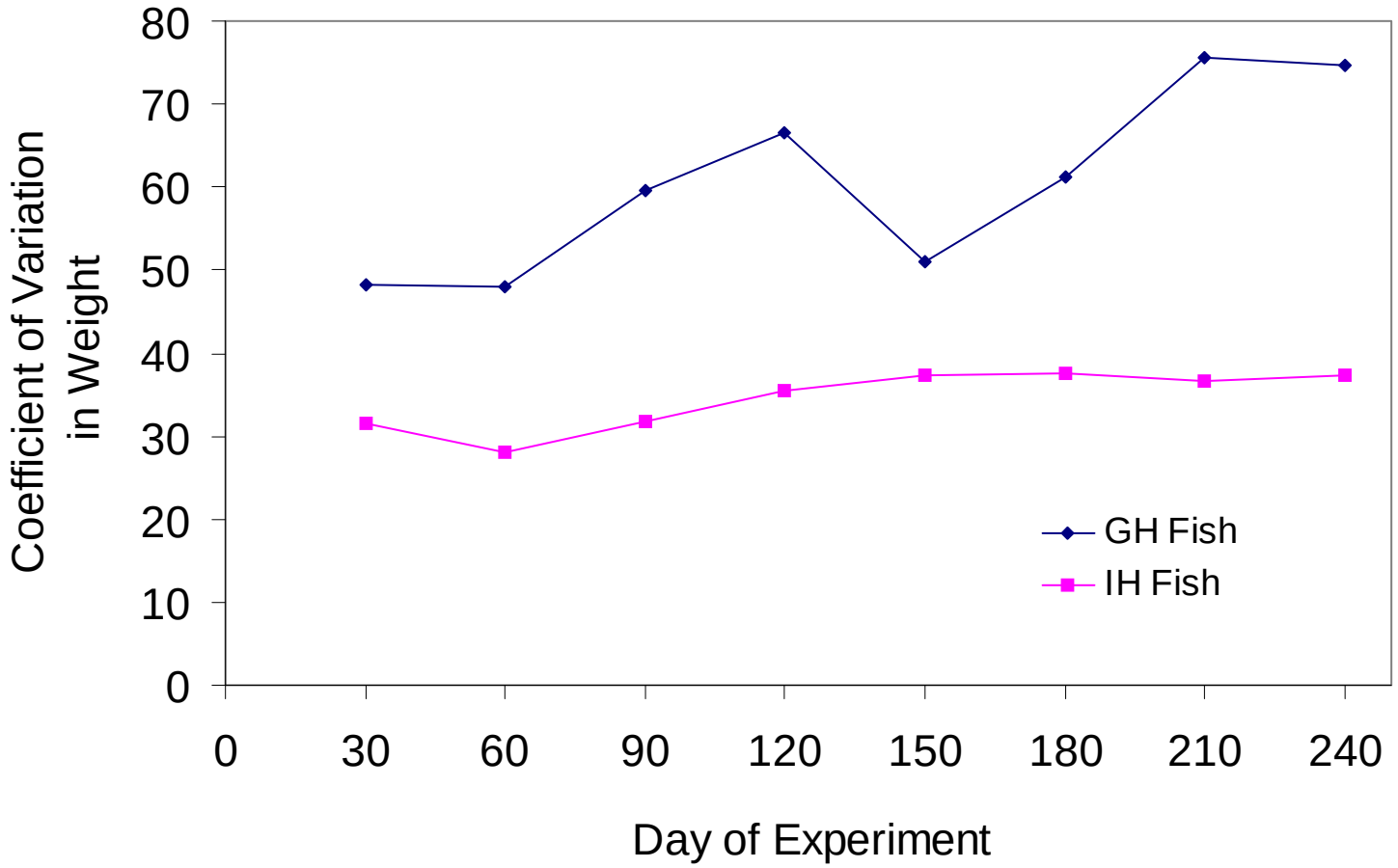

Figure 1.4 – Coefficients of variation (CV) in weight for male and female bluegills on successive sampling dates. Changes in CV among the 31 IH fish are shown over the experimental period; for the GH fish, CVs for each of the eight distinct groups of bluegills that were euthanatized on the indicated experiment days.

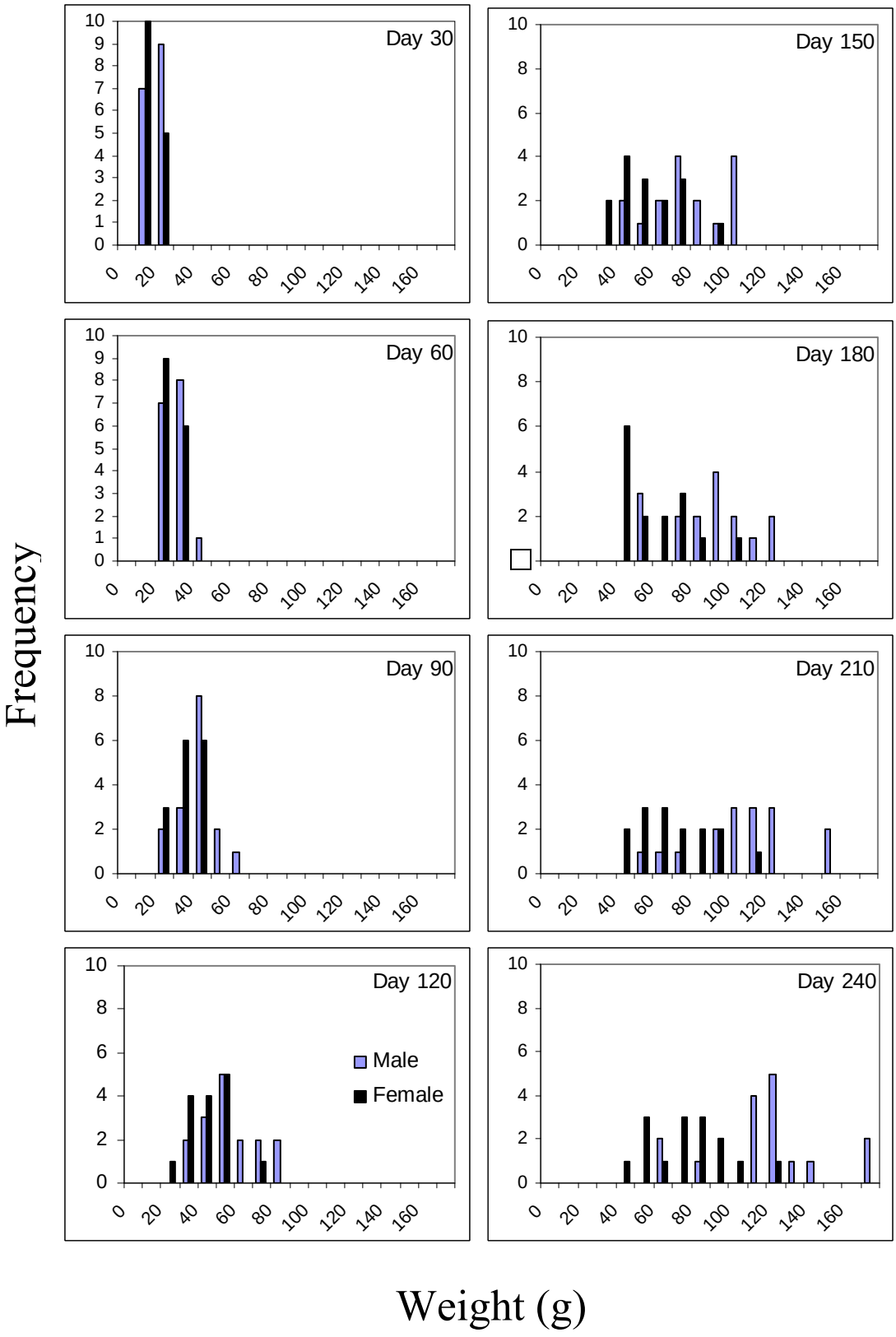

Figure 1.5 – Weight frequency histograms of IH fish on successive sampling dates.

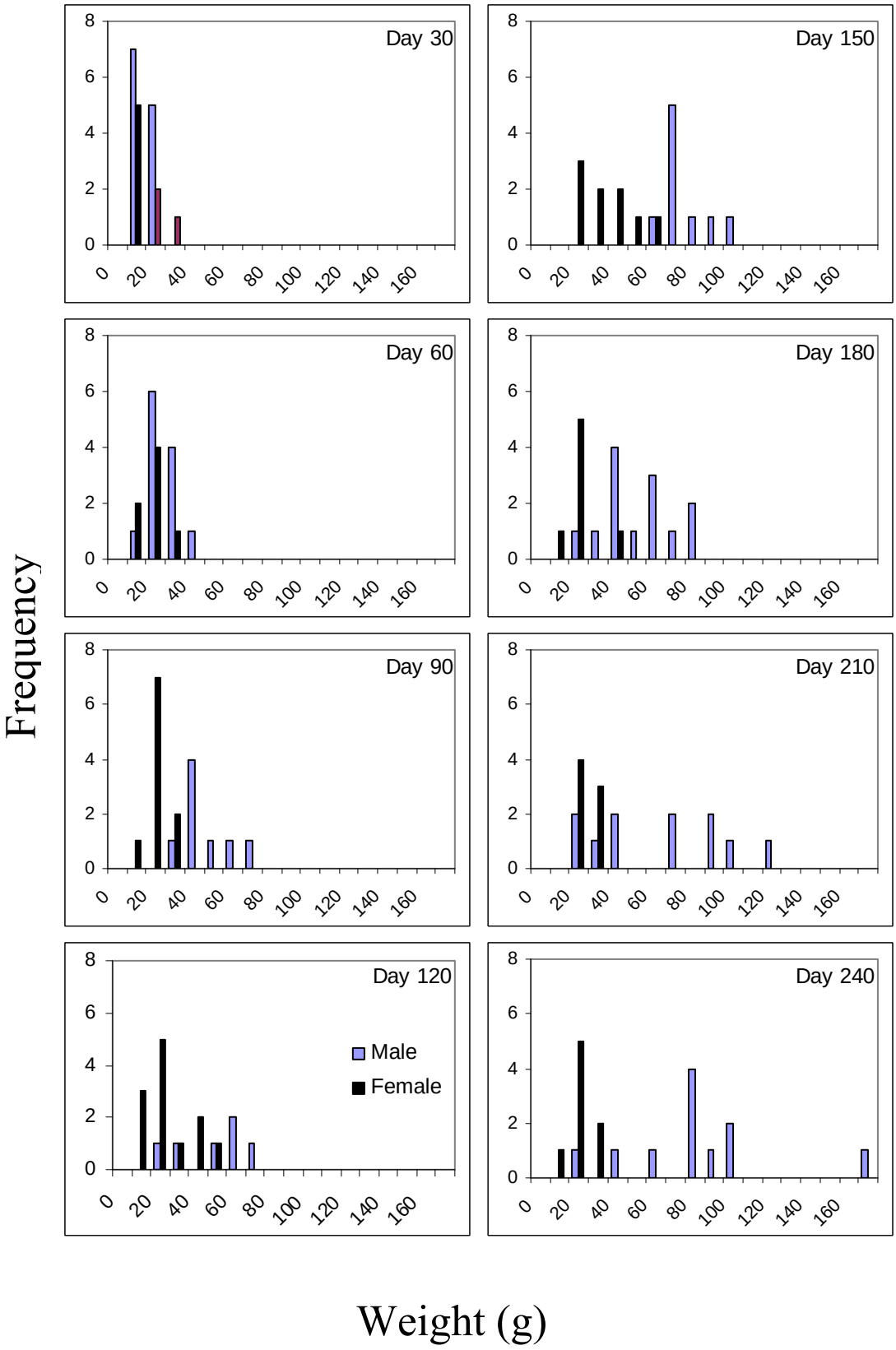

Figure 1.6 - Weight frequency histograms of GH fish on successive sampling dates.

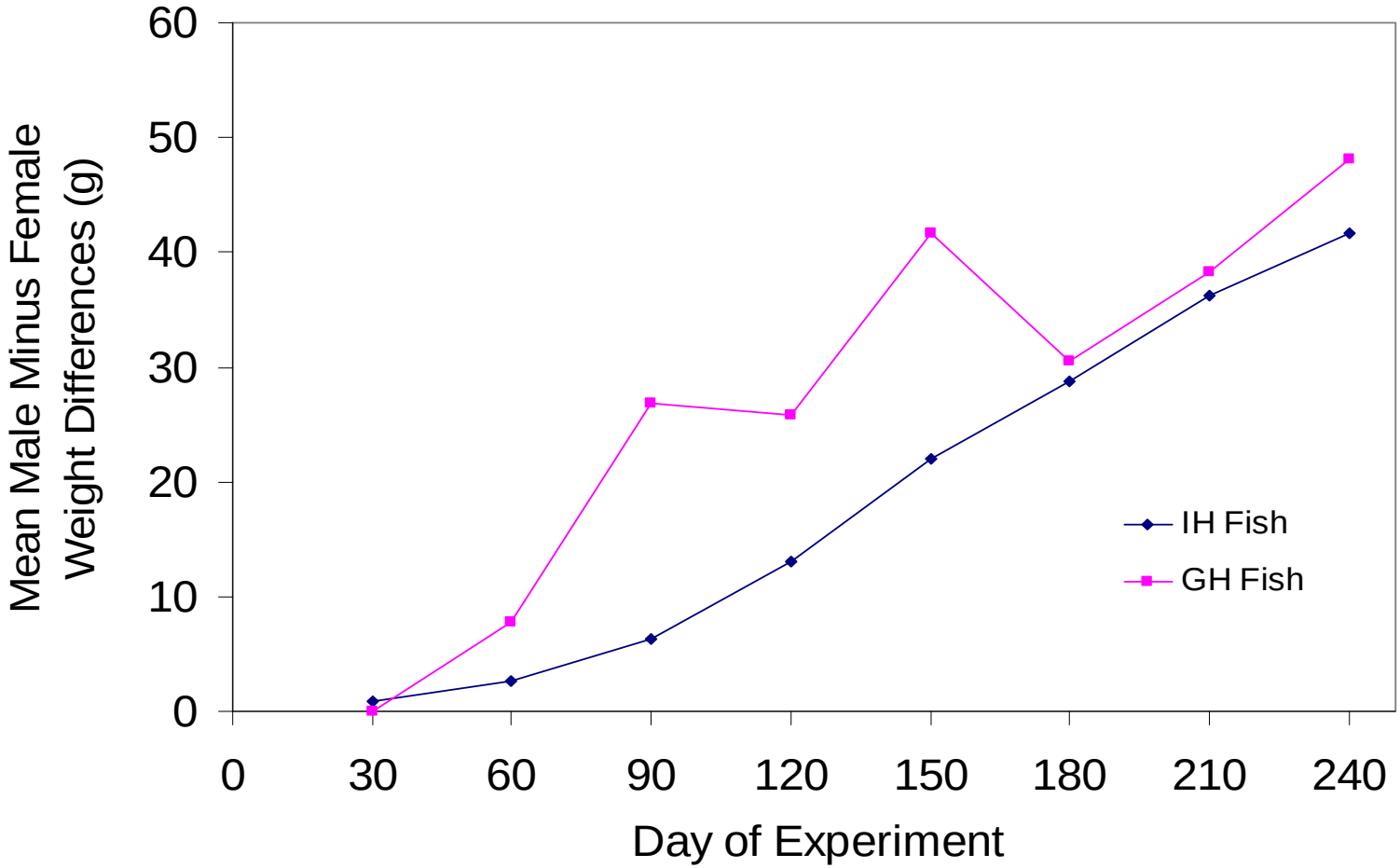

Figure 1.7 – Differences between male and female mean weights (male mean weight minus female mean weight) for the IH and GH fish on successive sampling dates. Changes in mean weight differences are tracked among 31 IH fish throughout the experiment, while results for GH fish refer to the serially sacrificed distinct fish groups.

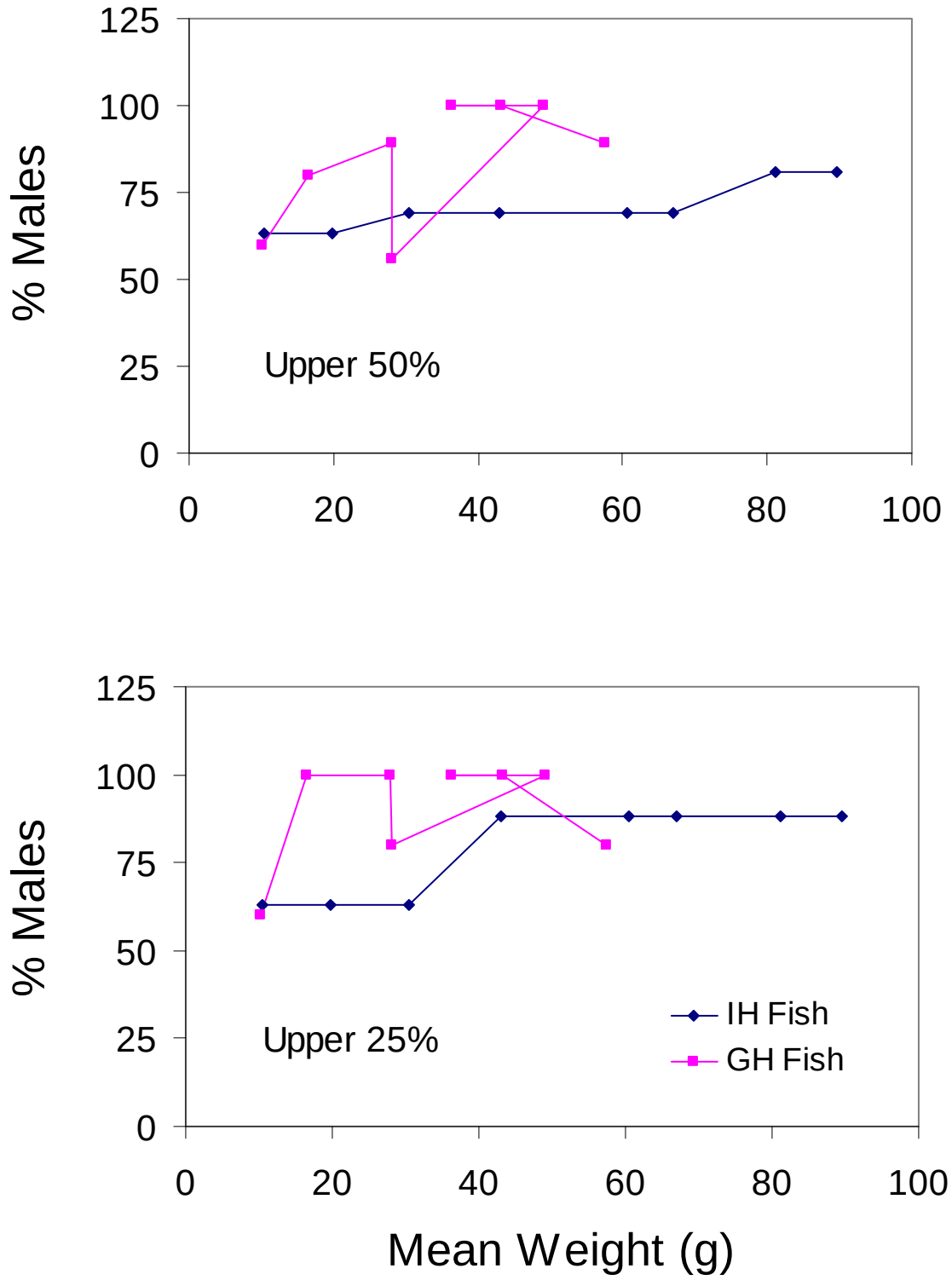

Figure 1.8 – Demonstration of the less stable, increasing trend of percentage of male bluegills selected (in upper 50% and upper 25% selection groups) for the GH fish versus the IH fish.

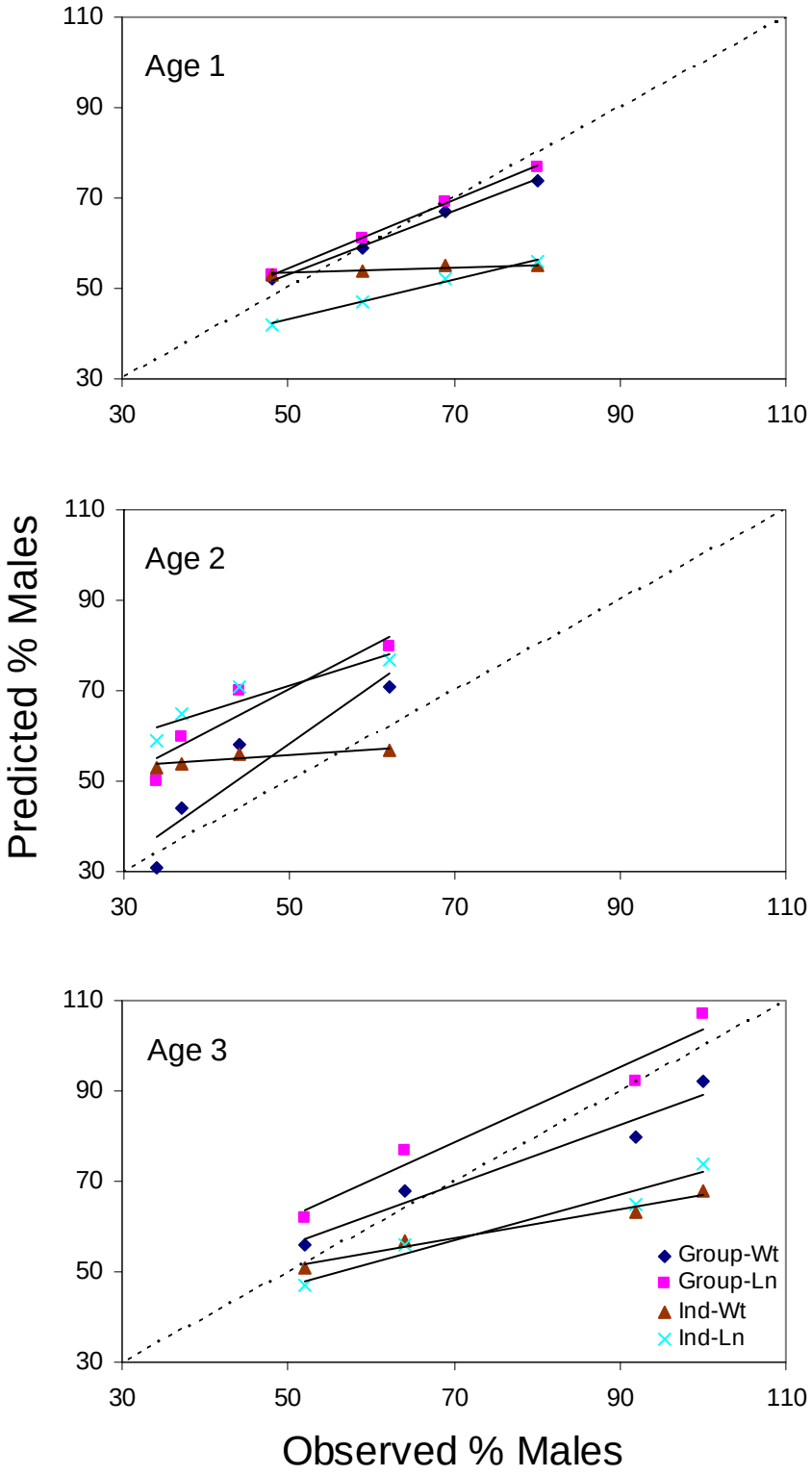

Figure 1.9 – Comparisons of the four MR model predictions of the percentages of male bluegills in upper 50-, 75-, and 100-percent selection groups, versus observed percentages of males in these groups, for ages 1, 2, and 3 bluegills secured from a commercial producer.

Chapter II

AN EFFORT TO ESTABLISH PREDOMINANTLY MALE BLUEGILL GROUPS THROUGH TRENBOLONE ACETATE IMMERSION

ABSTRACT

The ability to successfully sex reverse bluegill females to males following sex reversal by trenbolone acetate immersion has been established. This provides a means other than size-grading to establish mostly-male groups. Additionally, some sex-reversed fish have been reported to grow larger than untreated fish due to 1) higher consumption/appetites, 2) higher gross growth efficiency, 3) and decreased energy allocated to gonadal growth. The inherent growth capacities of bluegill treated with trenbolone acetate relative to controls had not previously been evaluated. Our interpretation of the period of gonadal differentiation in bluegill – when sex-reversal techniques must be executed – conflicts with that reported in the literature. Thus, this study replicated a documented approach to sex-reversing bluegill with trenbolone acetate and compared the results to a published approach where treatment with TBA was conducted 10-d later and to the control. Replication of the documented approach (treatment beginning on 35 days post-hatch – Set 1) resulted in unexpected paradoxical feminization. That same approach at a later treatment time (treatment beginning on 45 days post-hatch – Set 2) resulted in significantly more males than in the control and significantly more males than Set 1. Female mean weights in Set 1, Set 2, and their respective controls were similar; however, male mean weight differed in all three treatments, with those in Set 2 being significantly higher. Absolute growth rate was highest and lowest for Set 2 males and females, respectively, suggesting that the most intense social interactions of the three treatments occurred here. Male growth exceeded

female growth by more than two, three, and four times in the control, Set 1, and Set 2, respectively. Overall, results indicated that (1) female to male sex reversal methods for Set 1 fish were not reliable, (2) female to male sex reversal methods were significantly more reliable for Set 2 than Set 1, (3) the period of gonadal differentiation in bluegill must fall closer to 45 dph than 30 dph, and (4) treatment positively impacted male weight gain and growth rates, which were more pronounced in Set 2 than in Set 1.

INTRODUCTION

Culturing mixed-sex populations of fish often results in early and frequent reproduction, which tends to depress growth rates (Mires 1995). This is common in bluegill populations and leads to high densities and consequent stunting (Ricker 1948; Loveshin and Matthews 2003). All- or even mostly-male bluegill groups are expected to show faster growth rates than mixed-sex groups because delayed maturation in males, relative to females, allows greater investment in somatic versus gonadal growth (Hayward and Wang 2002; Hayward and Wang 2006). Also, mostly-male groups may show less prolific reproduction than mixed-sex bluegill groups.

Sex reversal is a commonly practiced means of producing monosex fish groups. Hormone-induced sex control of fish is reportedly possible in at least 47 species (15 families) of fish with 31 different steroids (16 androgens, 15 estrogens) (Pandian and Sheela 1995). Sex reversal in fishes is possible, given that an individual's genotypic sex is determined at fertilization, whereas the phenotypic sex develops later under hormonal control and is often plastic (Dunham 1990). Phenotypic sex can be manipulated if steroid treatment occurs during a critical time in the species' development. This brief period is

the time of gonad or sexual differentiation, and is unique to each species in timing and duration (Yamamoto 1958; Yamamoto 1962; Malison et al 1986).

No information is available on the growth rate potential of masculinized representatives of the three species of centrarchids in which sex-reversal has reportedly been successful. Many studies report initial growth rates, but do not explore long-term growth capacity (Pandian and Sheela 1995). However, growth rate improvements up to twofold have been reported for sex-reversed fish relative to untreated fish. Sex control often leads to infertility, such that substantially more energy is allocated to somatic rather than gonadal growth relative to untreated fish (Matty and Cheema 1978, Donaldson and Hunter 1982, Riley and Secombes 1993, Pandian and Sheela 1995). Also, masculinized fish reportedly show both increased appetite and food conversion efficiency (Cowey et al. 1973; Malison et al. 1988). Establishing mostly-male bluegill groups through sex control may therefore promote bluegill somatic growth so that market size may be attained in less time relative to untreated bluegill.

Four conditions must be met for fish to be successfully sex-reversed. During (i) the correctly determined period of sexual differentiation, (ii) an appropriate steroid must be (iii) delivered at the correct dosage (iv) for an appropriate duration. Each of these four constraints tends to differ among fish species. Skewed sex ratios, development of ovotestes, or death can result when not all the criteria are met (Hackmann and Reinboth 1974). Trial-and-error experimentation is commonly employed to determine the appropriate approach for a given species.

The most common means of administering sex steroids to fish is by application to the feed (Donaldson and Hunter 1982); however, treatment may be incomplete unless the

feed is well-consumed (Johnstone et al. 1983), and some fish species may not switch to exogenous feeding until after the time of gonad differentiation. Steroid administration via immersion of fish eggs is another common technique. Appropriate hormone doses are quite small (usually measured in ug/L) and are commonly dissolved in 70-90% ETOH to ensure accurate measurement and application.

The most commonly used hormone for producing monosex male populations in fishes is 17α-methyltestosterone (MT) (Shelton and Jensen 1979; Gale et al. 1999; Al-Ablani and Phelps 2002; Gomelsky et al. 2002). This hormone has been previously applied to centrarchids to form mostly-male groups (Garrett 1989; Al-Ablani and Phelps 1997). Two studies attempted to sex-reverse bluegill with MT to form mostly-male populations. Chew and Stanley (1973) were unsuccessful in their effort to establish mostly-male bluegill groups with MT. More recently, Al-Ablani and Phelps (2002) produced many inter-sex fish and more females than males when applying MT. Thus, the few studies completed to date with bluegill reveal difficulty in forming mostly-male groups with MT, despite successful efforts with other centrarchids.

Attempts to form all-male groups with MT at high doses or extended exposures can cause aromatization of an androgen to an estrogen that may ultimately induce feminization (Goudie 1983; Piferrer et al. 1993). Aromatization occurs in the gonad (Zanuy et al. 1999) when aromatase enzymes convert androgens to estrogens (Callard et al. 1981). The excessive release of estrogen, instead of androgen, can produce a female rather than a male.

Trenbolone acetate is a synthetic anabolic androgenic steroid hormone, similar to testosterone, but which cannot be aromatized (Neumann 1975; Rogozkin 1991; Galvez et

al. 1996). It is approved by the U.S. Food and Drug Administration for the growth acceleration of livestock (Roche and Quirke 1986) and is marketed worldwide under the brand name Finaplix-H® (Hoechst Roussel Vet Ltd., Dublin, Ireland). Trenbolone acetate is 50 times more anabolic than testosterone (Vida 1969; Neumann 1976) and is about 15-30 times stronger than testosterone (Neumann 1975). TBA exhibits both anti-gonadotropic and anti-estrogenic activity (Neumann 1976). Because trenbolone acetate is non-aromatizable, feminization should not directly result from its application. Successful masculinization of channel catfish *Ictalurus punctatus* (Galvez et al. 1995) and blue tilapia *Oreochromis aureus* (Galvez et al. 1996) has been achieved with trenbolone acetate.

Trenbolone acetate has also reportedly been used successfully to produce mostly-male groups of bluegill (Al-Ablani and Phelps 2002; Tulin and Phelps 2004). Commercial feed treated with TBA yielded fewer males than in the controls, and many intersex fish; however, TBA application via immersion yielded up to 94% males with no intersex fish (Al-Ablani and Phelps 2002). Thus, sex reversal via TBA immersion appears to be a promising technique for establishing mostly-male bluegill groups through sex control.

Here, data were collected to determine whether (1) the published approach for masculinization of female bluegill using TBA immersion is repeatable, (2) sex-reversal of bluegill through TBA exposure is more effective when treatment begins on day 35 versus day 45, and (3) TBA exposure time affects growth rates of whole fish or gonads.

METHODS

Bluegill were spawned indoors at the United States Geological Survey – Columbia Environmental Research Center (USGS-CERC), Columbia, Missouri. Brood fish were caught by hook-and-line from several private farm ponds in Cole County, Missouri on 27 April 2002 and were immediately transported to USGS-CERC, where they were placed at the same temperature (18°C) in four indoor, flow-through tanks (60 L/hr). Photoperiod, temperature, and tank dynamics (number and size of males, females, and nests) were controlled to induce indoor spawning following Mischke and Morris (1997).

Spawning occurred during the first two weeks of June 2002. Each cohort of fry spawned indoors was separately reared in re-circulating tanks at the University of Missouri-Columbia on a progressive diet of spirolina algae, rotifers, plankton, brine shrimp, and mashed commercial feed (Zeigler Bros® age-0 mash, Zeigler Bros, Inc., Gardners, Pennsylvania; Bryan et al. 1994). Plankton and rotifers were netted with a 35-micron plankton net from outdoor earthen ponds at USGS-CERC and size-graded daily with appropriately sized mesh filters. New brine shrimp were reared every 24 h and any extra were discarded at that time. Only the largest and seemingly healthiest bluegill cohort (N=1250) was selected for the sex-reversal experiment.

Twenty-five days post-hatch, the selected cohort was transferred back to USGS-CERC and held in two, 38-L glass aquaria until the sex-reversal experiments began on days 35 and 45 post-hatch. During this acclimation period, fry were fed brine shrimp and mashed commercial feed three times daily (09:30, 14:00 and 18:30 hours) without restriction. Bluegill were exposed to one concentration of TBA for 3 d during each of

two time intervals [hereafter called Set 1 (35 d post-hatch) and Set 2 (45 d post-hatch)]. The exposure interval for Set 1 was selected so as to repeat the experiments of Al-Ablani (1997) who exposed bluegill between 34 and 40 d post-hatch (dph). Replication of Al-Ablani's (1997) methods began one day later (day 35) due to equipment availability. The exposure interval for Set 2 was selected based on qualitative histological examination of bluegill gonads from 30-90 dph at the University of Missouri-Columbia, which suggested that sexual differentiation may not begin in bluegills until approximately 45 dph.

For each experiment, three replicates (A, B, and C) of 80 fry held in a 10 x 9 x 6-cm, fine screen-mesh basket, were immersed in a 38 L glass aquarium containing TBA or solvent. Glass aquaria were placed in a temperature-controlled, flow-through raceway. Finaplix-H® (Hoechst Roussel Vet Ltd., Dublin, Ireland) TBA pellets (20 mg each) were dissolved using ultrasound in 40 ml of 90% ethanol per pellet. Each TBA treatment tank received a 20-ml solution for a final tank concentration of 500 ug/L. Control tanks received 20 ml of 90% ethanol. The TBA or ethanol solutions were slowly pipetted and thoroughly stirred into the water, and each tank was equipped with two air stones to ensure constant mixing of the hormone/solvent as well as adequate dissolved oxygen levels for the bluegill. TBA exposure occurred on 35, 38, and 41 dph for Set 1, and on 45, 48, and 51 dph for Set 2. Hormone exposure time was 5 h on the treatment days (12:00-17:00 hours). Outside of that time, the mesh baskets were held in raceways with clean well water (1 TBA replicate and 1 control replicate for each Set per raceway; Figure 2.1). On the three treatment days the fry were fed only twice, once before and after treatment; the second of three feedings was omitted to prevent the possibility that this food might absorb TBA or solvent. Water temperature was maintained at $21 \pm 1\ ^{0}C$

and the photoperiod was regulated at 12L:12D beginning at 07:00 hours. Temperature, dissolved oxygen, and flow were monitored three times daily during the scheduled feeding and treatment times.

After the final immersion, one control replicate and one treatment replicate from each Set were separately reared in mesh baskets (68 L) in the three flow-through tanks at CERC, where they were spawned, until they were large enough (92 dph) to tolerate transportation to recirculation tanks at the university. Rearing of fish was continued then at 23 $\pm$ 1 ^{0}C on a commercial diet of crushed Rangen® EXTR 400 (Rangen Inc., Buhl, Idaho) and Silvercup® Steelhead 2.5 (Nelson and Sons, Inc., Murray, Utah) fed twice daily *ad libitum*. Each replicate was maintained in mesh-walled baskets (68-L) so as to continue rearing in the original exposure groupings. After the fish reached a mean length of 6.0 mm, each replicate group was thinned to 12 fish through selective sampling (fish were selected from predetermined intervals that represented the group's size range) on 11 January 2003. The removed fish were euthanatized and preserved in 10% buffered formalin. Gonads from the sacrificed fish were excised for sex determination. Gonads not readily identified as either testes or ovaries were processed and sectioned with a Cryostat® (Jencons Scientific Inc., Bridgeville, Pennsylvania). Gonad tissue was examined using the squash method (Shelton and Guerrero 1974), which involved staining with an aceto-carmine solution and viewing gonadal sections with a compound microscope.

Sex ratios based on gonad histology indicated that not all treated females were sex- reversed. Therefore, the fish were moved to tanks where they were individually held to assess growth rates beginning on 12 March 2003 (280 dph). Each fish was weighed

(g), measured (mm), and then placed in a plastic test chamber (20 L) pierced with small holes to permit constant water exchange. Sixteen test chambers were placed in each of three paired, 2000-L recirculation tanks. Water trickled freely into each test chamber from above to promote water exchange and dissolved oxygen. Excess feed and feces were removed weekly with a siphon prior to a 25% water exchange. Fish were fed an *ad libitum* diet of Silvercup® Steelhead 2.5 and Silvercup® Brood beginning 31 March 2003, and were then weaned onto AquaMax® Grower 400 (PMI Nutrition International, LLC., Brentwood, Missouri) on 23 April 2003. Feed was unable to exit the chambers, ensuring the individual rations did not mix. Lengths and weights were recorded every 30 d for 90 d (280, 310, 340, and 370 dph). They were reared at 23 $\pm$ 1 ^{0}C until being euthanatized on 10 June 2003 (370 dph) for sex determination. Each fish was individually euthanized with an overdose of MS222. Sex was determined by visually inspecting the gonads and the gonadosomatic index (gonad wt (g)/somatic wt (g) x 100%) was calculated from gonad weights.

Results of survival, sex reversal, GSI, weight gain, and growth rates were compared between the control and TBA-treated fish. Chi-square analysis was used to determine if the observed sex ratios differed from the expected 1:1 male to female sex ratio, and also to determine if the observed sex ratios differed between the control fish and the TBA-treated fish. The gonadosomatic index data did not meet assumptions of normality and homogeneity of variance, and were therefore analyzed using a non-parametric Kruskal-Wallis test to detect differences between treated and non-treated fish. Differences in sex-specific GSI means between the control sets were tested using a *t*-test to determine if the control data could be combined. Weight data were log-transformed to

meet requirements of normality and homogeneity of variances. Differences in sex-specific mean weights between the control sets were tested using a *t*-test to determine if the control data could be combined. Differences in weight gain and growth rates between male and female bluegill exposed to TBA on different days were tested by three-way ANOVA with treatment, sex, and day as class variables. Differences in growth rate were also tested with the ANCOVA heterogeneity-of-slopes/intercept test. Tests resulting in *P* values ≤ 0.05 indicate significant differences. All tests were performed using SAS® software.

RESULTS

Survival

Survival by Set and treatment was recorded for the duration of the experiment (Table 2.1). Fish were sub-sampled and removed from the study at 35-51 dph, 220 dph, and 370 dph. Natural mortality occurred throughout the experiment; approximately 10% of the fish from each replicate were lost between 35-220 dph. In addition to natural mortality, one replicate was lost from each set in each treatment due to temperature-control system failure. This accounted for an additional 10% mortality in the other replicates and treatments as well.

Sex Reversal

No intersex fish were observed. Sex ratios of fish from Sets 1 and 2 sampled at 220 dph (Group 1) and exposed to ethanol only were skewed toward being female, but did not differ significantly ($P>0.05$) from an expected 1*m*:1*f* ratio (Table 2.2).

Trenbolone-acetate-treated fish were expected to be predominantly male; however, the ratio for Set 1 (exposed at 35 dph) was female-biased while that of Set 2 (exposed at 45 dph) was male-biased; however, there was no statistical difference from a 1*m*:1*f* ratio (Table 2.2).

The sex ratios of fish from Set 1 and Set 2 that were sampled 370 dph (Group 2) and exposed only to ethanol were slightly female-biased (Table 2.3). Trenbolone-acetate-treated Set 1 and Set 2 fish were female-biased unlike the results observed for Set 2 at 220 dph. Again, Chi-square analysis indicated that none of the observed sex ratios differed ($P>0.05$) from the expected 1*m*:1*f* ratio.

The combined results of the two samples taken on 220 dph (Table 2.2) and 370 dph (Table 2.3) are presented in Table 2.4. The data were not normally distributed and were thus arcsine-transformed. *T*-tests on the arcsine-transformed control data indicated the control groups did not differ from each other ($P>0.05$); consequently, these were pooled. Overall, females predominated in the ethanol treatments and among fish treated with TBA beginning at 35 dph. Although not differing significantly ($P>0.05$) from a 1*m*:1*f* ratio, the Set 2 sex ratio was male-biased. Moreover, nearly twice as many males were observed in Set 2 as Set 1 ($P=0.001$), and Set 2 resulted in more males than in either ethanol-treated Set ($P=0.02$) (Table 2.4). Chi-square analysis revealed that only TBA-treated Set 1 resulted in a sex ratio that differed significantly from the expected 1*m*:1*f* ratio (20*m*:47*f*, $P=0.01$).

Gonadosomatic Index

The GSI was calculated for each fish sacrificed at 370 dph, and differences between ethanol-treated and TBA-treated replicates were tested for using the non-parametric Kruskal-Wallis test (Table 2.5). No significant sex differences ($P>0.05$) were detected in either the ethanol- or TBA-treated fish. Similarly, no significant differences ($P>0.05$) were detected between Set 1 and Set 2 by sex. The control GSIs were similar ($P>0.05$) and consequently pooled; this allowed the treatment data to be tested against a single control.

Weight Gain and Growth Rates

Mean weights (g) were compared between control and fish within each sex, over the period of 280-370 dph (Figure 2.2). Mean male weights tended to be highest for Set 2 males, intermediate for Set 1 males, and lowest for control males, whereas female weights were markedly lower and did not differ significantly ($P>0.05$) between the control and treatment fish (Table 2.6). Male weights were significantly higher than female weights at 310, 340, and 370 dph ($P\leq0.05$), and the Set 2 mean male weight was significantly higher than for the male controls only at 310 dph ($P\leq0.05$).

No differences ($P>0.05$) were detected in either slopes or intercepts among the control fish; all controls were thus pooled. Slopes and intercepts were both different ($P\leq0.05$) between Set 2 males and the combined controls while no differences ($P>0.05$) were detected between Set 1 males and the controls, between Set 1 and Set 2 males, or between Set 1, Set 2, and control females. Slopes and intercepts were both different ($P\leq0.05$) between males and females in Set 1, Set 2, and the control.

Absolute growth rates (AGR, g/d) were determined for individual fish and analyzed for treatment and control fish by sex (Table 2.7; Figure 2.3). Differences in growth rate were examined for four time intervals: 1 = 280-310 dph; 2 = 310-340 dph; 3 = 340-370 dph; 4 = 280-370 dph. Male growth (interval 4) exceeded female growth by more than two, three, and four times in the control, Set 1, and Set 2, respectively. Female AGR (interval 4) increased in all treatment and control groups at all intervals. Among males, AGR was highest in Set 2, intermediate in Set 1, and lowest in the control. Differences by sex, day, and interval were tested using the non-parametric Kruskal-Wallis test. A significant difference ($P \leq 0.05$) was detected at all intervals among females between Set 2 and the controls, within males between Set 1 and the control, Set 2 and the control, and finally between males and females in Set 1.

DISCUSSION

Survival

Mortality in this study (~20%) exceeded that reported by Al-Ablani and Phelps (2002) (<5%), but was due, in part (~10%), to equipment failure.

Sex Reversal

As expected, a sex ratio that was no different from $1m:1f$ was observed in the ethanol-treated fish; however, these data were slightly female-biased. It is common for sex ratios in bluegill populations to substantially deviate from a $1m:1f$ sex ratio. Across more than 20 bluegill control groups from several experiments, Al-Ablani (1997) observed percentages of males to range from 33-70%. Other centrarchids, including

largemouth bass *Micropterus salmoides* and black crappie Pomoxis *nigromaculatus* showed less variation. In those studies, groups ranged from 48-54% male (Al-Ablani 1997), although largemouth bass have been observed to be as high as 89% male (Garrett 1989).

Based on results obtained by Al-Ablani (1997), fish treated with TBA were expected to be predominantly male. The combined results of samples taken at 220 dph and 370 dph revealed that, relative to the control, males predominated in Set 2 whereas females predominated in Set 1. Observing significantly more females in Set 1 was unexpected since TBA is a non-aromatizable androgen (Neumann 1975); however, it has been reported that non-aromatizable androgens retain a feminizing effect (Goudie et al 1983; Davis et al 1990). Androgen delivery occurred via ETOH, creating suspicions that ETOH may have estrogenic properties; however, no documentation was located supporting the possibility that ETOH may have contributed to unintentional feminization.

The unintentional feminization observed in our study may have been dose-related, but this is unlikely because of prior successes using these methods (Al-Ablani 1997). Three-day exposures of 500 and 1000 ug/L TBA yielded 94% and 93% males, respectively (Al-Ablani 1997); thus, our replication of Al-Ablani's smallest, most effective dose (500 ug/L) should not have been responsible for unintentional feminization. Al-Ablani (1997) found that lower doses resulted in lower, but comparable, results since two-day exposures of 250, 500, and 750 ug/L TBA produced 85, 90, and 89% males, respectively. It appears that a dose much greater than 500 ug/L would be necessary to significantly impact sex ratio. Sub- or super-optimal hormone treatments often yield intersex fish (Pandian and Sheela 1995), whereas other failures to

successfully sex-reverse fish can paradoxically determine (or not affect) sex (Nakamura 1975).

Paradoxical feminization is common (Stanley and Chew 1973; Al-Ablani 1997). Al-Ablani (1997) reported that androgens, not estrogens, can interfere with and disrupt the natural sex determination mechanism in bluegill. Other attempts by Al-Ablani (1997) to masculinize bluegill resulted in many intersex fish, whereas the attempt to feminize bluegill with estradiol-17β resulted in 100% females with no intersex fish. Together, these findings from several methods suggest that disruption of the sex determination pathway in bluegill results in the default sex (female) being expressed.

Unlike Al-Ablani's (1997) results, we were unable to revert females to males at 35 dph; however, we were successful at 45 dph, relative to the control. Nearly twice as many males were observed in Set 2 as in Set 1, which suggests that the timing of treatment is important in bluegill, given that the only distinction between Set 1 and Set 2 was the 10-d time difference.

Al-Ablani's (1997) controls were significantly skewed ($P<0.0001$) toward males (68%) relative to our control. Al-Ablani's TBA-treated fish resulted in 26% more males than the respective control and 44% more males than the expected 1*m*:1*f* ratio. Our findings resulted in 17% more males than the respective control. Despite differences in the sex ratio of control fish between the two studies, both resulted in the production of significantly more males than in the controls.

Gonadosomatic Index

Significantly smaller gonads are commonly observed in sex-reversed fish. It is

advantageous to rear sterile fish to market size because gonads can account for as much as 30% of body weight in fish (Riley and Secombes 1993). Androgen exposure has previously been shown to cause sterility (Donaldson and Hunter 1982), with one measurable benefit being increased feed conversion efficiency (Dunham 1990). In our study fish, their gonad weights were not reduced as a consequence of TBA exposure. Thus, TBA seems unlikely to increase somatic growth at the expense of gonadal growth.

Weight Gain and Growth Rates

Relative to the controls, the mean weight gain data showed that TBA treatment did not affect female weights. In contrast, there was some indication that treatment positively affected male weight gain. Exposure of bluegill to TBA beginning at 45 dph resulted in increased weight gain. This gain was not significantly higher than the control at the end of the 90-d growth assessment, but significant differences in slopes and intercepts between Set 2 males and the control indicated that Set 2 male weights were gradually separating from those of control males.

Sex reversal has been shown to increase feed conversion efficiency in tilapia (El-Gamal 1987); however, TBA did not affect the somatic growth of channel catfish (Galvez et al 1995) or the somatic growth or feed conversion ratio of blue tilapia (Galvez et al 1996). We found that the inherent growth capacity (IGC) of bluegill, according to sex, was altered by hormone exposure and treatment time. Because same-cohort fish were held under identical conditions, the between-group differences in male AGR could not have been environmentally (Allendorf et al. 1986) or genetically based (Carter et al 1996).

Our results showed that the IGC of bluegill was not achieved due to social interactions associated with group holding. Social costs differed in severity for each group, as was evidenced by the male and female AGRs during interval 1. However, all-female groups rebounded and grew similarly through intervals 2 and 3 after any "carryover" effects from group holding had apparently been overcome. These results suggest that social interactions were more costly for the treatment than for the control fish, and more costly in Set 2 than in Set 1. They also suggest that the carryover effects of social interaction exist temporarily for both males and females when held individually. Brief continuation of subordinate behavior in fish upon being moved to isolation following group holding is common (Purdom 1974; Milinski 1982; Wang et al 1998).

The control and treatment group AGRs were not the same through intervals 1-3. As was observed for all females, the control male AGR increased through intervals 1-3. However, the Set 1 and Set 2 male AGRs were lowest in interval 2. Thus, group holding appears to have affected treated males differently than control males. The disparity between male and female AGR was the least in the control and the most in Set 2 at interval 1. In Set 1 and Set 2, the disparity between male and female AGR relative to the control at interval 1 suggests that (1) carryover effects of social interaction (and possibly the social interaction itself) promoted male growth, and (2) female growth was hampered to a greater degree than in the control, and to a greater degree in Set 2 than in Set 1.

Increased activity (expressed as chasing, dodging, and decreased consumption by smaller, subordinated fish) is consistent with other findings (Koebele 1985; Hayward and Wang 2002). In the absence of other fish, the competitiveness, metabolism, and appetite of males likely decreased, causing the eventual decline in AGR observed in interval 2. A

rebound in AGR in interval 3 likely represented a stabilization of these fluctuations. Because these fluctuations in AGR were not observed in the control males, I believe that the treated males were more competitive than the control males under group holding, which ultimately resulted in higher AGRs. The significant differences in AGR between Set 2 males and the control males in interval 4 suggest that bluegill treated at this time possessed a higher growth potential than did untreated fish.

Social costs can be severe under restricted feeding (Jobling 1994). Cumulative consumption (CC) was not measured and consequently, feed conversion ratios (FCR) could not be calculated. However, that these fish were fed to apparent satiation suggests that Set 2 males either ate more and/or better assimilated their rations than did Set 1 or control males. It is unclear whether greater appetite, a greater feed assimilation rate, or both, was responsible for the accelerated growth of Set 2 males.

It appears that the ability to establish all- or mostly-male bluegill groups via size-grading with the MPP model was both more reliable and more time- and cost-effective than was sex reversal. However, the significantly higher AGRs and mean weights of Set 2 males compared to untreated fish should not be overlooked. The establishment of mostly-male bluegill groups via TBA immersion is practical, can apparently benefit growth, and holds promise for rearing bluegill to food market size within two-year grow-out periods.

Table 2.1 - Survival and sampling chronology for the production of predominantly male bluegill and increased growth of bluegill following trenbolone acetate immersion. Each treatment set began with 240 fish. Numbers in parentheses indicate the number of fish sampled, split evenly among the three replicates. Non-parenthetic numbers indicate the remaining number of experimental fish within the set.

	Treatment				
	Solvent Control (Ethanol)		TBA Exposure (Ethanol + TBA)		
Days Post-Hatch	Set 1 (exp.d35)	Set 2 (exp.d45)	Set 1 (exp.d35)	Set 2 (exp.d45)	No Exposure
25					1250
35	240	240	240	240	290
35	(30)		(30)		(30)
41	(30)		(30)		
41	180		180		(30)
45		(30)		(30)	(30)
45					
51		(30)		(30)	
51		180		180	(30)
79[1]	120	120	120	120	
220	77	78	67	67	(170)
220	(53)	(54)	(43)	(43)	
221	24	24	24	24	
370	23	23	24	24	

[1] One replicate was lost from each set in each treatment.

Table 2.2 – The effects of trenbolone acetate and treatment time on the sex ratios of bluegill fry sampled 220 dph. Set 1 fish were exposed on 35, 38, and 41 dph; Set 2 fish were exposed on 45, 48, and 51 dph. Sex ratios are expressed both as a ratio and percentage of males by replicate and for both replicates pooled. Chi-square analysis indicated none of the observed sex ratios was significantly different ($P>0.05$) from the expected 1:1 male to female ratio.

	Solvent Control (Ethanol)		TBA Exposure (Ethanol + TBA)	
Replicate	Set 1 (exp.d35)	Set 2 (exp.d45)	Set 1 (exp.d35)	Set 2 (exp.d45)
A	12*m*:22*f*	10*m*:19*f*	5*m*:15*f*	12*m*:7*f*
	35%	34%	25%	63%
C	6*m*:13*f*	11*m*:14*f*	9*m*:14*f*	16*m*:8*f*
	32%	44%	39%	67%
Total	18*m*:35*f*	21*m*:33*f*	14*m*:29*f*	28*m*:15*f*
Mean	34%	39%	33%	65%
N	53	54	43	43

Table 2.3 - The effects of trenbolone acetate and treatment time on the sex ratios of bluegill fry sampled 370 dph. Set 1 fish were exposed on 35, 38, and 41 dph; Set 2 fish were exposed on 45, 48, and 51 dph. Sex ratios are expressed both as a ratio and percentage of males by replicate and for both replicates pooled. Chi-square analysis indicated none of the observed sex ratios was significantly different ($P>0.05$) from the expected 1:1 male to female ratio.

	Solvent Control (Ethanol)		TBA Exposure (Ethanol + TBA)	
Replicate	Set 1 (exp.d35)	Set 2 (exp.d45)	Set 1 (exp.d35)	Set 2 (exp.d45)
A	5*m*:7*f*	6*m*:6*f*	1*m*:11*f*	4*m*:8*f*
	42%	50%	8%	33%
C	5*m*:6*f*	6*m*:5*f*	5*m*:7*f*	6*m*:6*f*
	45%	50%	36%	50%
Total	10*m*:13*f*	12*m*:11*f*	6*m*:18*f*	10*m*:14*f*
Mean	43%	52%	25%	42%
N	23	23	24	24

Table 2.4 – The effects of trenbolone acetate and treatment time on the sex ratios of bluegill fry sampled on 220 dph and 370 dph. Total numbers of phenotypic males and females are presented along with the percent of males present. An asterisk indicates a sex ratio significantly different from the expected 1:1 male to female ratio using Chi-square analysis. Similar lowercase letters indicate no significant difference in percentage of males (P>0.05).

	Solvent Control (Ethanol)		TBA Exposure (Ethanol + TBA)	
Phenotypic Sex	Set 1 (exp.d35)	Set 2 (exp.d45)	Set 1 (exp.d35)	Set 2 (exp.d45)
Male	28	33	20	38
Female	48	44	47	29
% Male	37%	43%	30%* a	57%b
Pooled Control	40%a			

Table 2.5 – The mean gonadosomatic index (GSI) ± standard deviation (SD) for fish sacrificed at 370 dph. GSI ranges are in parentheses. Non-parametric Kruskal-Wallis tests indicated no significant difference (P>0.05) among or between Sets by sex.

	Solvent Control (Ethanol)		TBA Exposure (Ethanol + TBA)			
Phenotypic Sex	Sets 1 & 2 (exp.d35 & d45)	N	Set 1 (exp.d35)	N	Set 2 (exp.d45)	N
Male	0.33 ± 0.25		0.47 ± 0.32		0.45 ± 0.40	
	(0.09 - 1.08)	20	(0.15 - 0.85)	5	(0.14 – 1.44)	10
Female	3.26 ± 2.15		3.77 ± 2.65		2.79 ± 1.73	
	(0.57 - 7.03)	23	(0.12 – 8.81)	18	(0.72 – 5.39)	14

Table 2.6 – The mean weight (g) ± standard deviation (SD) for fish sacrificed at 370 dph. The number of fish sampled is in parentheses. Similar lowercase letters indicate no significant difference in mean weight by sex (P>0.05).

	Solvent Control (Ethanol)		TBA Exposure (Ethanol + TBA)	
Phenotypic Sex	Set 1 (exp.d35)	Set 2 (exp.d45)	Set 1 (exp.d35)	Set 2 (exp.d45)
Male	84.48 ± 53.22a (10)	80.61 ± 23.29ac (5)	101.22 ± 25.58ac (10)	115.49 ± 46.74abc (10)
Female	38.78 ± 25.11a (12)	49.36 ± 33.69a (18)	43.39 ± 23.92a (11)	45.11 ± 12.72a (14)

Table 2.7 – Absolute growth rate (g/d) of males and females by interval for treatment and control fish. AGR was analyzed for four intervals: 1 = 280-310 dph; 2 = 310-340 dph; 3 = 340-370 dph; 4 = 280-370 dph.

		Solvent Control (Ethanol)		TBA Exposure (Ethanol + TBA)	
Sex	Interval	Set 1 (exp.d35)	Set 2 (exp.d45)	Set 1 (exp.d35)	Set 2 (exp.d45)
Male					
	1	0.50	0.60	1.00	1.34
	2	0.63	0.71	0.85	0.83
	3	0.87	0.88	1.09	1.15
	4	0.66	0.72	0.97	1.08
Female					
	1	0.23	0.08	0.06	-0.11
	2	0.32	0.39	0.39	0.40
	3	0.41	0.49	0.41	0.48
	4	0.32	0.33	0.30	0.27

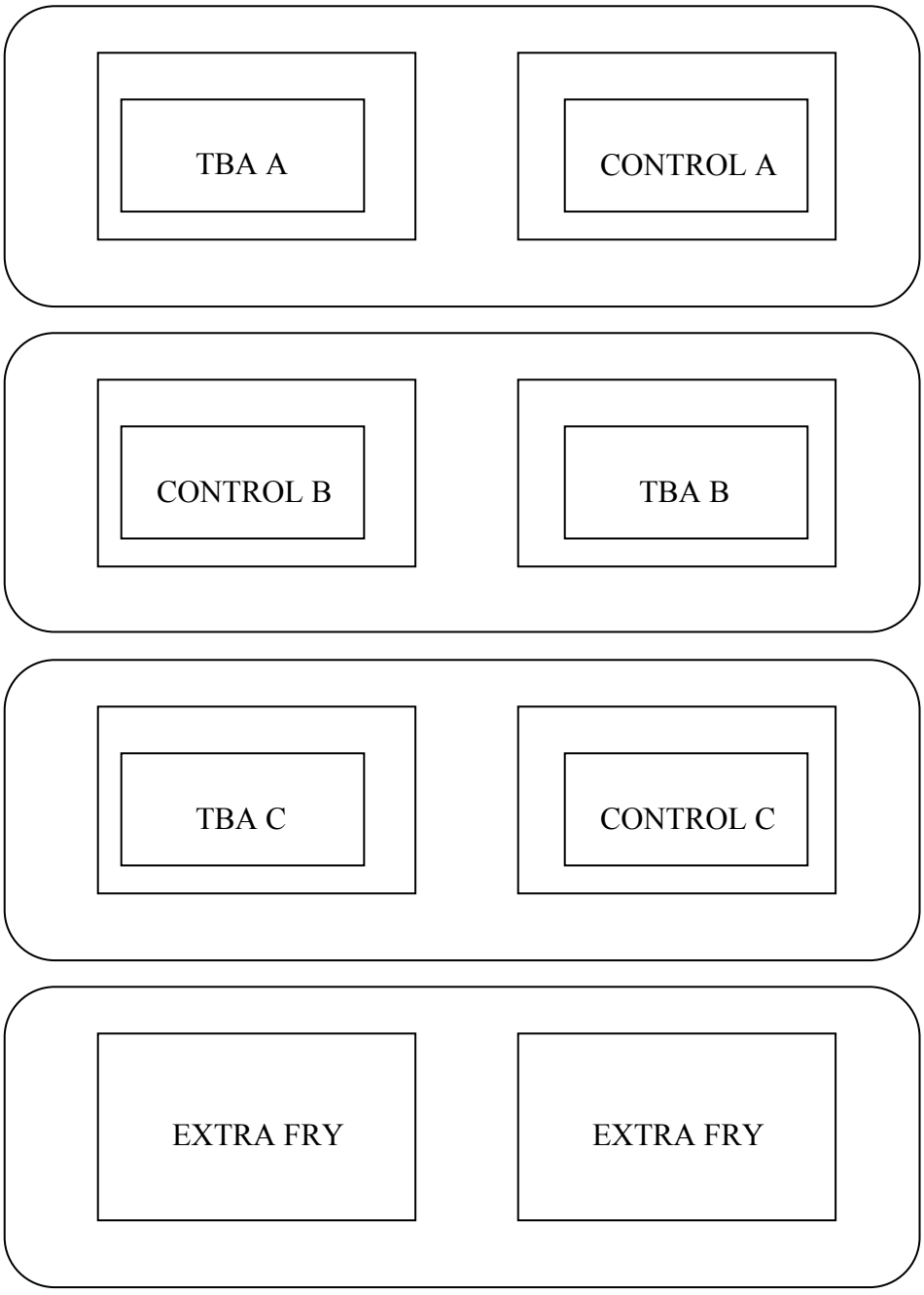

Figure 2.1 – Experimental design of Experiment 2. One mesh basket holding fry was placed in each 38-L tank holding either the treatment solution or solvent (control), two tanks per raceway. During non-treatment periods, the mesh baskets remained in their respective raceways without the tanks. Extra fry (including Set 2 fry) were placed in one of two 38-L tanks that resided in a fourth raceway. When treatment for Set 1 was complete, experimental fry were transferred and raceways were cleaned in preparation for Set 2. Three separate replicates of treatment and control groups are represented by letters A, B, and C.

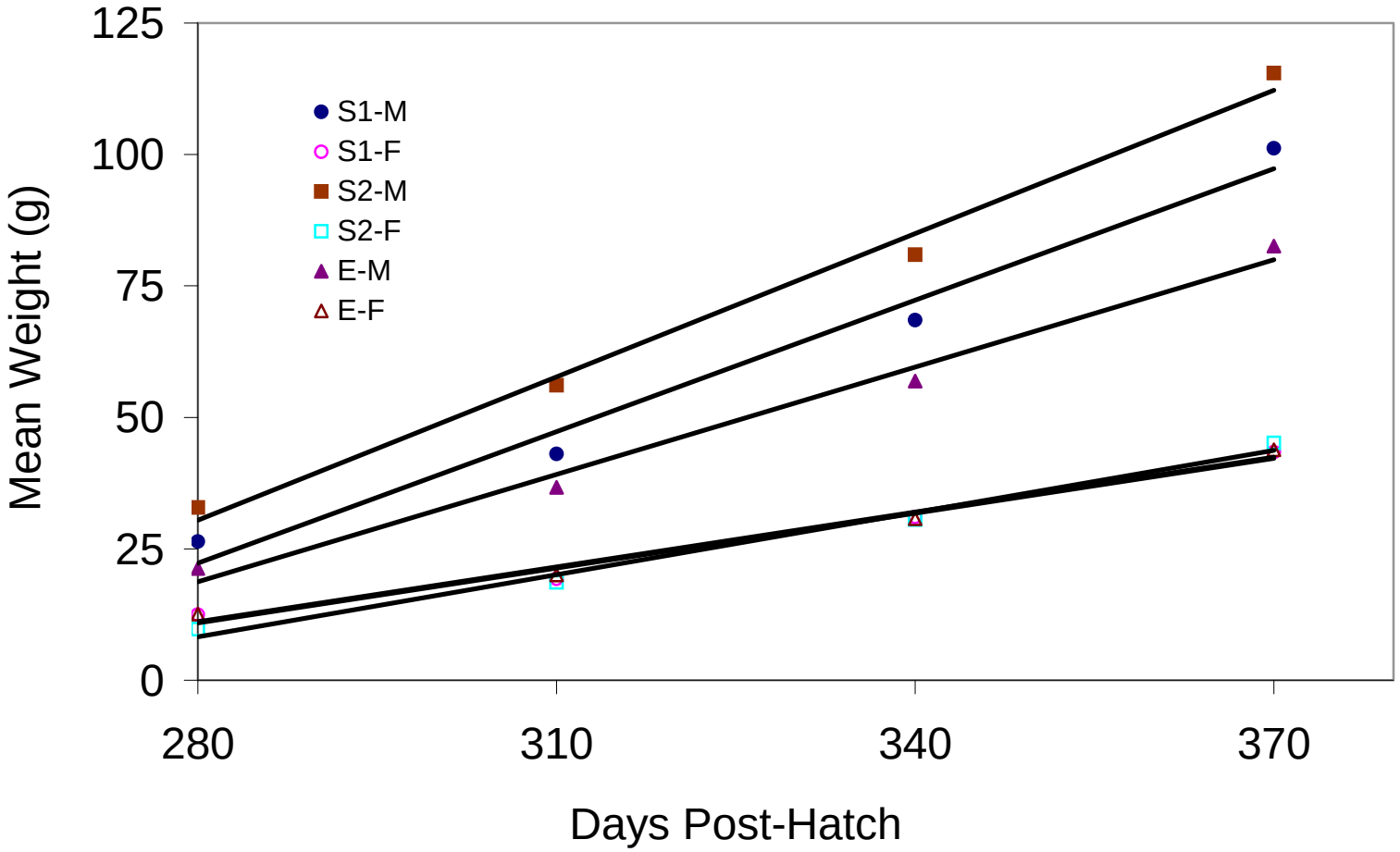

Figure 2.2 – The mean weights of Set 1 (S1), Set 2 (S2), and ethanol-treated (E) fish by sex (M or F) during the 90-day growth assessment.

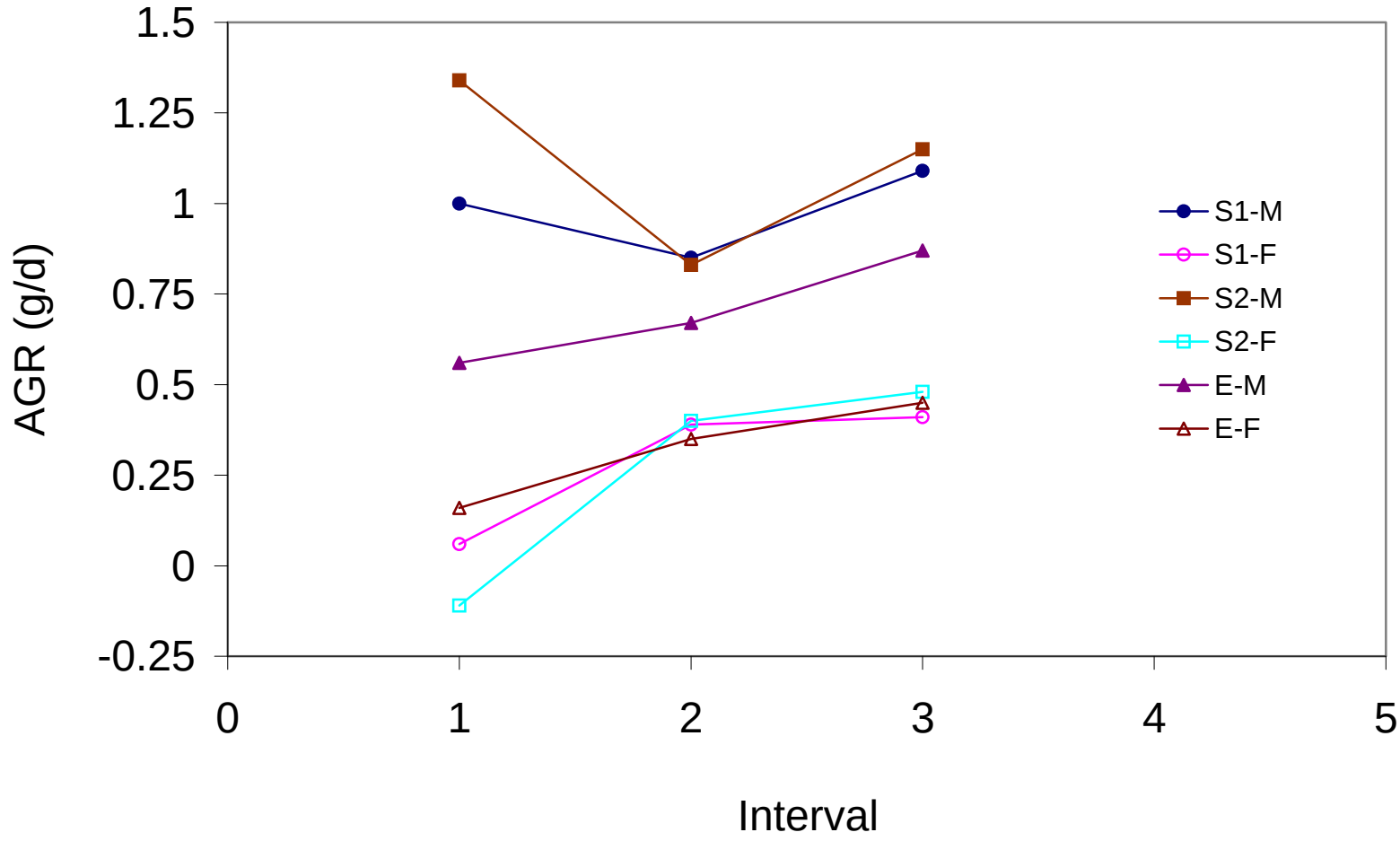

Figure 2.3 – The absolute growth rate (g/d) by interval of Set 1 (S1), Set 2 (S2), and ethanol-treated (E) fish by sex (M or F) during the 90-day growth assessment (Intervals: 1 = 280-310 dph; 2 = 310-340 dph; 3 = 340-370 dph).

Chapter III

EFFECTS OF REARING HIGH- PERCENTAGE-MALE GROUPS ON BLUEGILL GROWTH IN RECIRCULATING AQUACULTURE SYSTEMS

ABSTRACT

Research indicates that the relatively high inherent growth capacity (IGC) of male bluegill *Lepomis macrochirus* should permit them to reach food-market size substantially sooner than female bluegill or hybrid bluegill (F_1: male bluegill x female green sunfish *L. cyanellus*), if bluegill are reared indoors in tanks where the disadvantages associated with pond rearing are largely negated. However, given the evidence for high social costs among male bluegills, it was unclear how their growth would progress when reared in mostly-male (MM) groups in indoor tanks. Although male bluegills possess a comparatively high IGC, the social costs in MM bluegill groups may exceed those in mixed-sex (MS) bluegill groups where sex ratios are more balanced, because male bluegill tend to be highly aggressive which can dampen growth rates. Mostly-male bluegill groups were established using a size-grading approach (Chapter 1) that selects males from mixed-sex bluegill groups. The MM groups were grown in parallel with MS groups for 234 d; all groups were fed without restriction at a favorable growth temperature. Use of the size-grading approach yielded groups containing significantly more male bluegills (68%) than in MS groups (54%), where fish had been selected at random. The size-grading approach accurately predicted the percentages of male bluegills in the MM groups. Overall, MM bluegill groups grew faster than the MS bluegill groups, but not substantially so. Weight-frequency histograms for the final day showed that the distributions by sex were similar across treatments. The additional males in the MM tanks tended to reside in the upper 50% of the weight range by study's end.

The mean weights and SGRs for all fish were significantly higher in the MM tanks. Size variation development (SVD) was not greater among MM versus MS groups, contrary to the expectation that the greater number of males in the MM groups would yield greater social costs, and thus greater size variation among fish. Patterns of cumulative feeding efficiency (FE) and final FE were similar across the MS and MM groups and declined over the study period. Positive regression relationships between fish relative weights (Wr) and their lengths indicated the presence of strong social hierarchies wherein large fish secured greater portions of the available feed. Reducing social costs among bluegill groups in tanks will be necessary to permit these fish to grow at rates close to their IGCs. It is believed that applying a "topping off" (cull-harvesting) harvest strategy (progressively removing the largest fish in the tanks over successive harvest efforts), would result in the next-largest size groups growing rapidly to larger sizes due to their being released from social constraints. Overall, the results suggest that rearing mostly-male bluegill groups in indoor tanks, where topping-off is periodically applied to remove the large social dominants, will result in rapid growth of bluegills to food market sizes.

INTRODUCTION

A recent study found that bluegill *Lepomis macrochirus* substantially outgrew hybrid bluegills (F_1: male bluegill x female green sunfish *L. cyanellus*) when both were reared in parallel for 10 months in indoor tanks (Hayward and Wang 2002). In that study, fish were held individually in test chambers set within recirculating aquaculture systems (RASs) under continually favorable temperatures and feeding regimes for growth. These results were unexpected because hybrid bluegills have long been

considered to possess higher growth capacity than bluegills, based on comparative growth studies of the two fishes in ponds (Ellison and Heidinger 1978; Brunson and Robinette 1985, 1986). Because hybrid bluegills have been thought to grow faster than bluegills, they have received more attention as a sunfish candidate for the food market. In this setting, rapid growth to larger sizes (≥0.5 lb; 227 g) is critical, such that food-fish weights can be reached within a two-year grow-out period.

A follow-up analysis of the data from Hayward and Wang (2002) that focused on gender-related differences (Hayward and Wang 2006) revealed that the male bluegills substantially outgrew the female bluegills, and that the female bluegills outgrew both sexes of the hybrid bluegill over the 10-month trial. From a starting size of about 7 g, male bluegills reached 150 g (~70% of food-market weight), whereas female bluegills reached only 70 g; male hybrid bluegills (representing 80-95% of hybrid bluegill groups), surprisingly reached only 50 g.

Several factors may account for why hybrid bluegills outgrow bluegills in ponds when the latter apparently possess substantially higher growth capacity as age-1 and early age-2 fish. Hybrid bluegills have substantially larger mouths (inherited from the female green sunfish) than do bluegills. This may account, in part, for their having been found to feed more effectively on natural food items, including their own progeny, in ponds (Lane & Morris 2002). Natural food items can add substantially to a fish's energy intake in ponds, and can also provide necessary nutrients that may be lacking in commercial diets (Avault 1996). Also, hybrid bluegills are less prolific spawners in pond settings than are bluegills due to their male-skewed sex ratios (Kurzawski and Heidinger 1982). The progeny of hybrid bluegills apparently serve as a food source for adults in ponds and may

also enhance their growth (Lane and Morris 2002). In contrast, the more numerous progeny of bluegills are thought to compete with adults for natural food as well as for commercial feed (Loveshin and Matthews 2003). Hayward and Wang (2006) also observed that male bluegills indoors exhibited rapid growth phase August through March as age 1-2 fish, relative to male hybrid bluegill, growing substantially larger than did hybrid bluegills during this period. The male bluegill's substantial growth advantage during this period was related to a lesser energy investment in gonad development relative to the male hybrid bluegills. Because pond water temperatures during a large portion of the rapid-growth period for bluegills (November-March) are typically suboptimal for growth, much of the growth potential of bluegills may go unrealized in outdoor settings.

Overall, the findings of Hayward and Wang (2002) and subsequent analyses of these data (Hayward and Wang, 2006) indicate that rearing mostly male bluegills in indoor RASs may be an effective means for growing large, food-size sunfish. Such an approach would take fuller advantage of the male bluegill's higher growth capacity by providing continually favorable growth temperatures, while negating factors (such as reproduction) that tend to impede bluegill growth in ponds.

To date, few studies have evaluated bluegill growth in RASs, and none has evaluated the potential benefits of growing monosex or high-percentage-male bluegill groups in RASs to produce large, food-market sunfish. In part, this is because awareness of the superior growth capacity of male bluegills versus hybrid bluegills has largely gone unrecognized. Also, the capacity to form predominantly male bluegill groups has, until recently, been lacking (Chew and Stanley 1973; Arslan and Phelps 2003).

Although rearing monosex or mostly-male bluegill groups in RASs would appear to hold promise for rearing food-size sunfish, the high social costs to growth within MM tanks could negate expected growth advantages. Wang et al. (2000) found that social interaction among grouped hybrid bluegill juveniles substantially reduced mean consumption, growth, and growth efficiency by up to 24, 34 and 15%, respectively, and also contributed to the development of substantial within-group size variation. Hayward and Wang (2002) found that costs of social interaction were even greater for bluegills than for hybrid bluegills, and that social interaction costs increased for both fishes in concert with inherent growth capacity that varied over the course of the experiment. These results suggest that faster-growing fish such as male bluegills, versus female bluegills, may be highly aggressive when reared in groups. The effects of strong social interaction may be manifested not only by slower average growth rates within groups with more male bluegills, but also by poor feeding efficiency (FE) and the development of substantial size variation (Wang et al. 2000).

Accordingly, this experiment aimed to determine the extent to which rearing bluegill groups having high percentages of males in indoor RASs results in (1) higher growth rates and the production of greater numbers of food-size sunfish, (2) reduced feeding efficiency (FE), and (3) increased fish size variation, relative to when bluegill groups with more balanced sex ratios are similarly reared.

METHODS

A total of 1,780 pellet-trained, juvenile bluegill, all known to have hatched within the same two-week period (Steven Muich, Hunnewell Hatchery, Missouri Department of Conservation, personal communication) were secured from the Missouri Department of

Conservation's Hunnewell Hatchery and transported to the University of Missouri-Columbia on 26 September 2002 at ages 94-108 d post-hatch. Fish were acclimated to laboratory conditions in two 1100-L RASs (water temperature 19 $\pm$ 1^0C; photoperiod 14L:10D) and fed to apparent satiation three times daily with Aquamax Grower Diet (PMI Nutrition International, LLC., Brentwood, Missouri). Thirty fish were sampled at random from each tank on a weekly basis to track mean weight and length development.

When the two fish groups reached mean lengths of approximately 85 mm, a size at which male and female bluegill can be size-separated according to Chapter 1 results, all fish were combined into a single, 1100-L RAS (hereafter, referred to as the "original tank"). Six hundred fish were selected at random from the original tank, with random halves of these 600 fish being allocated to two other 1100-L RASs (300 fish per tank). This was done to form mixed-sex (MS) bluegill groups that reflected the sex ratio of bluegills in the original tank. Of the remaining fish in the original tank, the upper 50% by length (fish >85 mm) were selected by size grading. Because this upper 50% group contained fewer than the required 600 fish (300 fish needed for each of the two tanks), an additional 95 fish from the upper-end of the lower 50% group (size range: 82-85 mm) were added to achieve 600 fish. Random halves of these 600 fish were then stocked into each of two additional 1100-L RASs, in an effort to form mostly-male (MM) bluegill groups. Overall, the upper 51% of the size range of bluegills remaining in the original tank after formation of the MS groups was selected to establish the two MM groups.

Bluegills were 200-215 d post-hatch at the start of the experiment (day 0, 10 January 2003). Fish were fed by automatic feeders at a rate of 1.5% of body weight daily through seven daily feedings, with a combination of high-protein ($\geq$45%) commercial

feeds (Rangen® EXTR 400, Rangen Inc., Buhl, Idaho, and Silvercup® Steelhead 2.5, Nelson and Sons, Inc., Murray, Utah). On 30 March 2003 (day 80) the Rangen diet was replaced with Silvercup® Brood. Fish were given progressively larger feed pellets (multiple pellet sizes at any given time) to accommodate size increases and size disparity among fish within tanks. The feed amounts provided were adjusted over time following fish sampling to maintain a feeding rate of 1.5% body weight per day. From days 96 to the end of the experiment (day 234), feeding on weekends was stopped to increase appetites, reduce amounts of waste feed, and maintain high water quality.

A photoperiod of 14L:10D was maintained throughout the 234-d experiment, which ended on 29 August 2003. Open tops of the recirculation tanks were routinely covered with black plastic (~90%) to reduce stress on fish and to encourage feeding. Fish were reared at 25 $\pm$ 1°C throughout the experiment, which is within the reported temperature range for maximum growth of bluegill (Beitinger and Magnuson 1979). Dissolved oxygen levels remained at 7.0 $\pm$ 1.0 ppm according to twice-daily monitoring. Ammonia, nitrite, and nitrate levels were monitored biweekly with a water quality test kit (Wardley, Master Water Test Laboratory, Secaucus, New Jersey) and remained below 0.025, 0.25, and 60.0 mg/L, respectively, throughout the experiment. Siphoning of feces and waste feed was done daily in each tank, and 150 L of water was replaced up to five times weekly to maintain high water quality. The RAS bio-filters were back-washed at least once weekly throughout the experiment.

Each month, and on the first and final days of experimentation, fish in each of the four tanks were sampled by gently mixing and then capturing them with dip nets for measuring (nearest 1.0 mm) and weighing (nearest 0.1 g). Mixing of fish was found to

be critical for achieving unbiased representations of fish sizes in tanks. Thirty fish were sampled per tank on days 0, 30, 60, and 90; sample size was then increased to 50 fish per tank on days 120 and 150; all fish in the tanks were measured and weighed on days 180 and 234. Dead fish were removed daily and the losses recorded for each tank. Tank water volumes were increased from about 600 to 750 L on day 45 to accommodate the increasing fish biomass.

Upon completion of the experiment, all fish were measured, weighed, and euthanized prior to sex determination by gonad inspection. For larger bluegills, sex could be determined by simply observing the gonads by eye. For smaller fish, or whenever observation by eye was deemed insufficient, the squash method using fast-green stain (Guerrero and Shelton, 1974) was used for sex determination. Spermatocytes or oocytes were then identified with a dissecting microscope. The final-day sex ratios of bluegills remaining in each of the two MS tanks and the two MM tanks were also determined.

All outcomes of statistical analyses were decided at $P<0.05$. MS tanks were considered as controls against which responses of fish in the MM tanks (treatments) were compared. Chi-square analysis was applied to determine whether directed size grading of bluegills in accordance with the predictive model from Chapter 1 would produce fish groups (in the two MM tanks) with higher percentages of male bluegills than when bluegills were randomly selected from the original tanks to form the two MS groups. Furthermore, Chi-square analysis was used to determine whether the resulting percentages of male bluegills in each of the MM tanks differed from percentages predicted by the model from Chapter 1.

Because the distributions of bluegill weights within the tanks were consistently non-normal (Shapiro-Wilk test, else Kolmogorov statistic when $N>50$), medians were used to represent the central tendencies of fish weights within the tanks on sampling dates. Absolute growth rates (AGRs, g/d) of bluegills in each of the four tanks were determined as the slopes of linear regressions of median fish weight versus sampling day; specific growth rates (SGRs) were similarly determined, but with log_e of median fish weight serving as the response variable. Heterogeneity-of-slopes tests were applied to determine whether AGRs differed between the two control tanks (MS) and between the two treatment tanks (MM), and likewise for SGRs. Weight gain (g) by bluegills in each tank was calculated as the difference between median fish weight on the final and initial day of experimentation. Standard two-tailed *t*-tests were applied to determine whether the means of the median weight gains differed between the MS and MM groups; a similar approach was used to determine if the initial and final weights of bluegill differed between MS and MM groups. A paired *t*-test was used to assess whether the whole-experiment weight gain by male bluegills exceeded that of female bluegills over all four tanks; pairing involved differences between male and female weight gain within each tank. A standard *t*-test was applied to decide whether differences in male versus female weight gain existed between the MS and MM groups. For assessments of male versus female weight gain, median final-day weights of male and female bluegills in each tank were determined separately. However, it was necessary to use median weight of all fish in a tank (males and females combined) as initial-day weights for both sexes because the sex of individual fish was unknown at the start.

Due to non-normal bluegill weight distributions, size variation of bluegills at the beginning (SV_i) and end (SV_f) of the experiment was calculated for each tank as the difference between the 95th and 5th percentiles of fish weight, divided by the median weight of fish in the sample. Initial SVs were based on the random sub-samples of 30 fish, whereas final day SVs where based on all fish in a tank. Size variation development (SVD, %) over the experimental period was calculated for each tank as $(SV_f - SV_i) / SV_i \times 100\%$.

Overall feeding efficiency (FE) was calculated for each tank as the difference between final- and initial-day combined fish weight (tank production [g]), divided by the total feed provided (g) over the 234-d experiment period. The amount of feed provided to each tank throughout the experiment was calculated as the sum of the daily rations that were estimated at each 30-d interval**.** Feed conversion ratio (FCR) was also reported. Additionally, FE values for each tank were calculated over each of the successive sampling intervals throughout the 234-d experiment to elucidate any trends in FE over the experiment's course.

Relative weight (Wr) was calculated for sampled fish in each tank on days 0, 30 and on the final day of the experiment (all fish were measured and weighed on day 234) as W/W_s, where W is an individual fish's weight (g) and W_s is that fish's total-length-dependent (mm) standard weight as determined from the fish-length-dependent, standard-weight equation of Hillman (1982). Relative weights of sampled fish in each tank were regressed on their total lengths, and for male and female fish separately. These regressions were used to gain insights into the presence of social interaction costs within

tanks, and to assess differences that might exist between the MS and MM tanks and between male and female fish within tanks.

RESULTS

Sex Ratio

Survival of bluegill over the 234-d experiment ranged from 92-95% across tanks. On the final day of the experiment, the percentages of male bluegill in the MS tanks were 57% (156*m*/119*f*) in tank 1 and 51% (153*m*/139*f*) in tank 3; percentages of male bluegill in the MM tanks were 70% (202*m*/87*f*) in tank 2 and 66% (189*m*/96*f*) in tank 4. Chi-square analysis indicated that size-selecting bluegill from the original tank of fish according to the male-percentage prediction model (MPP) from Chapter 1, produced groups in the MM tanks (tanks 2 and 4) with higher percentages of male fish than in the MS groups (tanks 1 and 3) which were formed by randomly selecting bluegills from the original tank of fish ($P< 0.05$). Using input variables values of (1) upper % of fish length range selected = 51, (2) mean length of fish in the original tank before fish removal = 84.70 mm, (3) CV of the fish before removal = 17.77, and (4) estimated % males in the original tank before removal = 50, the MPP model predicted that the MM tanks would each contain 69% male bluegill. This predicted value was close to the observed percentages of male fish in each of the MM tanks (70% and 66%, respectively); chi-square analysis indicated that observed percentages of male bluegill were no different from the MPP model prediction ($P<0.05$). It is noted that an insufficient starting number of fish (in the original tank) precluded selection of higher, upper percentages of the total bluegill length range which, had this been possible, would likely have resulted in higher percentages of male bluegill (>70%) in the MM tanks.

Growth Rates

Sampling fish within the four tanks in a manner that accurately represented bluegill size structures proved to be challenging, likely because larger bluegills could better avoid capture, and also because the spatial distributions of the larger and smaller fish in the tanks were nonrandom. The bluegill median weight estimates on day 150 (N=50 fish sampled per tank) were substantially higher than for day 180 for all tanks, and greater than or equal to the median weights in all tanks on day 234 (final day of the experiment) (Appendix Table 1). The day 180 and 234 medians represented true medians in that all fish in each of the four tanks were measured and weighed at both times. The bluegill median weights estimated on day 150 were also markedly higher than those for day 120 (Appendix Table 1). A similar occurrence involving aberrantly high median weight values was evident for the day 90 median weight values, but this occurred in tanks 1 and 2 only (Appendix Table 1). Consequently, median weight values for day 150 were omitted for all four tanks, and those for day 90 were omitted for tanks 1 and 2 only.

Absolute growth rates (AGR, g/d) of bluegills based on regression slopes did not differ between the two MS groups or the between two MM groups (heterogeneity-of-slopes tests; N=13 and 13**;** $P \geq 0.05$). Consequently, data from the two MS tanks were pooled, and likewise for the two MM tanks. Heterogeneity-of-slopes testing based on the pooled data within the MM and MS groups indicated that AGR was significantly greater for the MM group (0.16 g/d) than for the MS group (0.12 g/d) (N= 26; P<0.05) (Table 3.1). Mean weight gain over the full experiment was 9.8 g (31%) higher in the MM versus the MS group (t-test, N=2; P<0.05) (Table 1). However, specific growth rate

(SGR), also determined from pooling of data within the MM and MS groups, was not different between the MS and MM groups (heterogeneity-of-slopes test; N=26**;** $P \geq 0.05$).

Across the four tanks, male bluegills gained significantly more weight than did female bluegills over the 234-d experiment (paired t-test; N=4; $P<0.05$); on average males gained 48.6 g (SE = 2.9) more than females. There was no indication that male weight gain relative to female weight gain either increased or decreased in the MS tanks versus the MM tanks. On average, males gained 51.9 g (SE = 5.2) more than females in the MM tank, and 45.4 g (SE = 1.7) more in the MS tanks, but these means were not found to be significantly different (t-test; N=2; $P \geq 0.05$).

On the final day of experimentation, the mean weights of bluegills at the 25th, 50th, 75th, and 100th percentiles were significantly higher in the MM tanks than in the MS tanks (ANOVA, blocking on quartile; $P<0.05$) (Table 3.2). Means of median weights of upper-quartile (Q4) bluegills were 112.8 g (50 % of food-market weight) and 124.3 g (55% of food market weight) in the MS and MM groups, respectively. Comparable values for upper 10th percentile fish were 141.8 g (62%) and 147.2 g (65%) for MS and MM groups, respectively, following 7.8 months of rearing. Absolute growth rates of bluegills in the upper weight quartiles at the end of experimentation (means of medians) averaged 0.44 and 0.46 g/d in the MS and MM tanks, respectively; comparable values for upper 10th percentile fish were 0.56 and 0.55 g/d for bluegills in MS and MM tanks respectively.

At the end of the experiment, bluegills in the lowest weight quartile (Q1) were predominantly female (67% female fish on average) in both the MS and MM tanks (Table 3). The percentages of male bluegills increased through weight quartiles 2 and 3

in both the MM and MS tanks, the increase being greater in the former (86% males occurred in quartile 3 in the MM tanks versus only 55% males in the MS tanks). The final percentages of male bluegills in the upper weight quartile were more similar, reaching averages of 98% and 94% in the MM and MS tanks, respectively. Stockpiling of male bluegills was evident in both the MS and MM tanks in the upper 3rd and lower 4th weight quartiles (Figure 3.1). This stockpiling of males was substantially more pronounced in the MM tanks where total numbers of males were greater. Despite the greater numbers of faster-growing male bluegills in the MM tanks, and indications that more of these fish were moving up in size, no more bluegill came close to food market weight in the MM tanks than in the MS tanks (Figure 3.1).

Size Variation

Size variation development (SVD, %) over the course of the experiment averaged 15.37% for the two MS tanks and 18.11% for the two MM tanks (Table 4). There was no statistical evidence that SVD was greater in the MM tanks than in the MS tanks (*t*-test, N=2, $P \geq 0.05$), even though the presence of more male bluegills in the MM tanks was predicted to promote more intense social interaction and, in turn, greater size variation among fish within these tanks.

Feeding Efficiency

Mean values of FE over the full 234-d experiment were low, averaging 0.398 (FCR= 2.529) and 0.345 (FCR=2.906) across the two MS tanks and two MM tanks, respectively (Table 3.5). No difference in mean FE was detected between the MS and MM groups (*t*-test; N =2, $P \geq 0.05$). Trends in FE over the successive experiment sub-

periods showed similar declines in all tanks (Figure 3.2). Values of FE were quite high during the first 30 d of the experiment, averaging 1.36, but declined to only 0.33 by the end of the study. FE declined rapidly during the first half of the experiment, with the rate of decline diminishing in all tanks thereafter.

Relationships between Wr and Fish Length

Significant positive relationships ($P<0.05$) were observed between fish Wr values and their lengths in each of the four tanks on the final day of the experiment. Significant regression relationships were also revealed for male and female bluegills separately within each tank (Figure 3.3). In all tanks, bluegills of the greatest lengths (predominantly males) were also in excellent condition (Wr>100), whereas fish of progressively lesser lengths were in progressively lower condition. In all tanks, the regression slopes for males were significantly higher than for females (Table 3.6), evidence that male bluegills were in higher condition than females at the upper lengths. Analysis of Covariance indicated that the relationships between Wr and fish length for all fish, males only, and for females only, did not differ between tanks 1 and 3 (MS groups), and likewise between tanks 2 and 4 (MM groups) (Table 3.6). Consequently, these regression-related data were pooled across the tanks within the MM, and likewise within the MS group. Using the pooled data, regression slopes of Wr versus fish length were higher for the MM group than for the MS group, whether the regressions were based on the sexes combined or separately by sex (Table 6).

The presence of similar relationships (sexes combined only) between fish Wr values and their lengths was also tested using data collected early in the experiment (days 0 and 30) to determine when such relationships became established. No such

relationships were observed in three of the four tanks on day 1, although a marginally significant (P=0.07) relationship was observed in one tank. However, by day 30, significant, positive relationships were present in all the tanks.

DISCUSSION

Basis for Study

Recent findings suggest that it may be advantageous to rear groups of predominantly male bluegills in indoor systems (RASs) when seeking to grow large, food-size sunfish (Hayward and Wang 2002; Hayward and Wang, 2006). Bluegills may perform better in indoor RASs than in ponds, in part because the negative influences of reproduction, which can include trophic competition between adults and offspring, are negated in the former type of culture system (Kurzawski and Heidinger 1982; Flickinger et al. 1999). Also, favorable rearing temperatures will be continually present in indoor RASs (but not in ponds) over the period when age 1-2 bluegills, particularly males, possess very rapid growth capacity (Hayward and Wang 2002). Rearing predominantly male bluegills should take fuller advantage of this gender's substantially more rapid growth capacity versus female counterparts. However, the male bluegill's high growth capacity may be accompanied by highly aggressive behavior (Hayward and Wang 2002), which has been shown to markedly reduce food consumption, feeding efficiency and, ultimately, growth rates of grouped lepomid sunfish (Wang et al. 2000). Because no studies have evaluated bluegill growth performance in commercial-scale RASs, particularly when mostly-male bluegill groups are reared, it is not known how the potential advantages and disadvantages of rearing monosex or mostly-male bluegill groups in RASs will balance out.

Performance of MPP Model

Final-day percentages of male bluegills in the two MM tanks (66% and 70%) versus those in the two MS tanks (51% and 57%) provided further evidence that the MPP model (Chapter 1) may be a practical and effective tool for establishing mostly-male bluegill groups through size grading. Results indicated that the MPP model accurately predicted percentages of male bluegills that occurred in groups formed through size grading (groups containing 69% male bluegills were predicted for each MM tank). Forming MM groups with higher percentages of male bluegills than were achieved would have been desirable for the purposes of the present experiment, but was not possible due to the limited number of total fish (~1800) that were available. Hence, a potential shortcoming of the MPP model approach may be that forming groups with very high percentages of male bluegills (e.g., $\geq$90%) will require starting with numbers of fish much greater than those in the groups that will be ultimately formed.

Growth Responses

Male bluegills grew faster than females across the four tanks, gaining 48.6 g more body weight, on average, during the experiment. Accordingly, fish in the MM tanks, which contained an average of 26% more male bluegills than did the MS tanks, showed significantly higher median growth rates (as AGR, g/d) and greater total weight gains over the 234-d experiment, versus fish in the MS groups with more balanced bluegill sex ratios. However, overall growth rate differences between fish in the MS and MM groups were relatively slight (AGRs of 0.12 versus 0.16 g/d) and were found not to be significantly different when growth rates were represented as SGR. SGR is an

appropriate growth rate metric for this comparison, given that the starting median weights of fish in the MS and MM groups differed.

Means of median final weights of bluegills in the MM tanks were consistently higher across the four weight quartiles versus those in the MM tanks. However, differences between median fish weights in the upper weight quartiles of the MS and MM groups were modest (112.8 g versus 124.3 g), and even more so in the upper 10^{th} percentiles of weight for the two (141.8g versus 147.2 g). Substantially higher weights of upper quartile bluegills were expected in the MM group versus the MS group, given that the later contained higher percentages of faster-growing male fish.

The final-day weight distributions of male and female bluegills showed evidence of stockpiling of male fish in the upper 3^{rd} and lower 4^{th} quartiles of weight, particularly in the MM tanks; stockpiling of male bluegills was also evident in the MS tanks but was less pronounced than in the MM tanks. The significant positive relationships between bluegill's size (length) and body condition (Wr) that developed in all tanks indicated that strong social hierarchies were present and caused progressively smaller fish to consume less food. Previous work has shown that the reduced food consumption and elevated metabolic costs associated with social subordination stress cause the more subordinated fish to be smaller than more dominant fish in groups (Koebele 1985l; Jobling 1994; Wang et al. 2000).

Further indicating the presence of strong social hierarchies, and their roles in influencing bluegill growth rates, was the observation that larger fish in tanks (predominantly male bluegills) exhibited AGRs that were similar to those of individually-held (no social effects) male bluegill in the study of Hayward and Wang

(2006). Male bluegills experiencing no social inhibition in the aforementioned study grew at rates in the range of 0.41-0.58 g/d, whereas upper quartile and upper 10th percentile bluegills (≥94% were male fish) grew at average rates of 0.45 and 0.56 g/d, respectively, across all tanks in the present study. This comparison suggests that the largest bluegills in both the MM and MS groups were growing at near-maximum capacity without social inhibition, and the Wr versus length relationships (Figure 3.3) show that fish of progressively lesser weights were likely experiencing increasing levels of social inhibition.

Dealing with Social Interaction

Finding evidence that social forces played important roles in impeding growth rates of bluegills in the present study indicates that strategies to reduce the effects of these forces will be required to take advantage of any benefits associated with rearing mostly-male groups in indoor RASs. Approaches for reducing agonistic social interaction among fishes and its substantial negative effects on growth performance include forced swimming (Christiansen et al. 1991; 1992), duoculture, the rearing of two species together (Nortvedt and Holm 1991), use of high rearing densities (Fleming and Johansen 1984), grading to promote size uniformity, and "topping off" (also referred to as "cull harvesting") (Avault 1996). Some of these approaches may be impractical for reducing social effects among bluegills. For example, high rearing densities have been shown to have a negative influence on growth rates of male bluegill (Hayward and Wang, 2006). Continuous swimming has been applied mainly to salmonid fishes, but would appear unnatural for bluegills which are generally more sedentary.

The stockpiling of male bluegills that was most evident in the upper 3rd and lower 4th weight quartiles in the MM tanks (Figure 1) was likely caused by growth-impeding social influences exerted by the largest, predominantly male bluegills in the upper 4th quartiles. These larger bluegills apparently enjoyed growing conditions that were unimpeded by social forces, given that they grew at rates equivalent to individually-held bluegills in an earlier study. As such, these fish likely reached the maximum growth that could be achieved by the fish grown in the tanks over the 234-d study period. It is further believed that removal of the largest fish in the upper 4th quartile from the rearing tanks would have resulted in the rapid growth of the next largest male bluegill, due to their being released from the growth-impeding influences of the larger fish. In turn, it is expected that fish of progressively smaller sizes would have also moved up in weight and size, accordingly. Importantly, it is thought that under such conditions, bluegills in the upper 3rd and lower 4th quartiles may increase in weight (and length) at super-normal rates upon removal of the larger fish through a compensatory growth response (Hayward et al. 1997). Rapid, compensatory growth of these fish is expected because these individuals have been restricted in their food consumption by the larger, more dominant fish, as indicated by their lower Wr values. When the food supply is restored following a period of its being available in "socially" restricted amounts, the under-fed fish will become hyperphagic and grow rapidly to weights (and ultimately lengths) close to those that they would otherwise have achieved had the feed restriction not been present. Often, this growth is achieved with above average efficiency (Jobling 1994; Skalski et al. 2005). The extent to which the progressively smaller fish would also increase rapidly in length and weight is unknown and warrants investigation as well.

From the observations made, it is believed that application of a "topping off" approach (Avault 1996) may be most advantageous for dealing with the high social costs that appear to be present when rearing bluegills, particularly male bluegills, at relatively high densities in indoor RASs. "Topping off," although practiced in various ways, generally involves a continuous process of stocking, rearing, and harvesting of a species in a culture setting, where only the largest fish are harvested at any given time, these being immediately replaced by an equivalent number of small "starter" fish. "Topping off" could take advantage of the strong, growth-impeding influence of hierarchical, agonistic social interaction that exists in RASs containing mostly-male bluegills, to periodically enhance growth rates via compensatory growth.

Assuming linear growth trajectories, growth rate data from the present study indicate that 14 months would be required for Q4 bluegills in the MM tanks (containing 68% male fish) to reach, on average, a food-market size of 0.5 lbs (227 g); upper 10^{th} percentile fish would reach this weight in only 12 months, well below the 24-month industry time limit. Using higher-percentage-male bluegill groups could potentially reduce the time required for upper quartile fish to reach market size. Once a sufficient number of bluegills reach food-market size, these could be harvested and replaced with starter-size fish. In turn, the harvesting should release the new largest fish from the growth-inhibiting circumstances and allow them to grow rapidly. The findings of the present study suggest that further investigation of the growth performance of mostly-male or monosex male bluegill groups reared in indoor RASs where "topping off" harvesting approaches are applied could facilitate efficiently growing large, food-market sunfish.

Feeding Efficiency

Although not known, it is considered likely that the strong social interaction forces within the four RAS tanks contributed substantially to the continuously declining and ultimately low overall FE values that were determined for this experiment. Regression relationships between Wr and fish size, believed to reflect the presence of hierarchical social interaction, were largely absent early in the study, but had become established by day 30. A common effect of hierarchical social interaction is reduced access to feed for many fish within a group, despite feed being present in quantities sufficient to satiate all fish (Koebele 1985; Jobling 1994; Wang et al. 2000). Unconsumed feed was noticed on the tank bottoms early in the study, and this was observed to increase with time (even though amounts of food provided were below estimated maximum consumption levels in accordance with tank water temperature and fish sizes and numbers). Ration levels were further reduced after a time (fish fed only 5 d weekly versus 7 d) in an effort to reduce the quantities of unconsumed feed, primarily to keep water quality favorable. Waste feed continued to be present, however in retrospect, it appears that having reduced the feeding levels may have only exacerbated the problem of food availability, as it has been shown that social forces intensify as the food amounts provided are diminished (Jobling 1994). Even when applying a "topping off" approach to bluegills reared in tanks, social hierarchies would undoubtedly continue to exist in tanks to an extent, and the majority of fish would have some level of restricted access to feed, promoting low FEs. Alternative approaches (some previously mentioned) for reducing the intensity of social interaction among bluegills within tanks could be effective in increasing fish access to food and improving FE. For example, treatment of

fish with tryptophan has been shown to reduce aggression in fish in tanks, by increasing serotonin levels (Papoutsoglou et al. 2005). Complete feeds also do not exist for bluegills. The incomplete diets used in the present study could have become progressively less adequate as the fish grew, contributing to the declining FEs observed. Hence, in addition to reducing social costs, efforts to develop more complete diets for bluegills in different life stages will be important for indoor tank rearing because, unlike in pond culture, natural food items are not available to supplement the commercial diet.

Wr versus Fish Size Relationships

Finally, it was observed that the Wr versus fish length regression relationships determined in the present study appeared to be more sensitive indicators of social interaction intensity within fish rearing groups than were the more commonly used percentage increases in size variation based on CV or SD of weight or length (Jobling 1994; Geffen 1996). The Wr versus length relationships indicated more intense social forces (via steeper slopes) in the MM versus the MS tanks, consistent with expectation, whereas %SVD did not. Consistently lower slopes of the Wr versus fish length regressions also suggest that less intense social forces may have been operating on female bluegills than male bluegills within each tank. Higher aggression in male versus female bluegills is consistent with evidence that group rearing of bluegills with higher growth capacity (e.g., male bluegills) involves higher social costs than rearing conspecifics with lower growth capacity (e.g., female bluegills) (Hayward and Wang 2002). The consistent presence of strong positive relationships between fish Wr values and size may also

indicate that social forces, more so than genetic differences in growth capacity, account for the pronounced differences in sizes of bluegills at the end of the study.

Table 3.1 – The absolute growth rate (AGR) (g/d), specific growth rate (SGR), mean of median weight gain (g), and mean of median final weight (g) for MS and MM fish. Similar lowercase letters indicate no significant difference (P>0.05).

Group	N	Mean % Male	Mean of Median Initial Weight (g) (SE)	AGR (g/d) (SE)	SGR (SE)	Mean of Median Gain (g) (SE)	Mean of Median Final Weight (g) (SE)
MS	2	54	10.95 b (0.80)	0.12 b (0.02)	0.005 a (0.0008)	31.58 b (0.13)	42.53 b (0.68)
MM	2	68	17.75 a (0.80)	0.16 a (0.02)	0.005 a (0.0006)	41.38 a (1.08)	59.13 a (1.88)

Table 3.2 – The weight (g) of bluegills and percent of food market weight at the 25th, 50th, 75th, and 100th percentiles for MS and MM groups at the end of the experiment.

Group	Tank	Percentile			
		25th	50th	75th	100th
MS	1	19.9	41.9	82.0	188.4
	3	19.9	43.2	86.9	218.0
	Mean	19.9 (9%)	42.6 (19%)	84.5 (37%)	203.2 (90%)
MM	2	30.8	57.3	98.0	186.9
	4	29.0	61.0	101.8	189.6
	Mean	29.9 (13%)	59.2 (26%)	99.9 (44%)	188.3 (83%)

Table 3.3 – The percentage of male bluegill by weight quartile in MS and MM groups at the end of the experiment.

Group	Tank	% Male	Q1	Q2	Q3	Q4
MS	1	57	35	37	62	94
	3	51	27	34	48	94
	Mean	54	31	36	55	94
MM	2	70	37	65	82	97
	4	66	32	46	90	99
	Mean	68	35	56	86	98

Table 3.4 – The initial and final size variation, and size variation development (%) for MS and MM groups. Similar lowercase letters indicate no significant difference (P>0.05).

Group	Tank	SV_i	SV_f	SVD(%)
MS	1	2.81	3.29	17.08
	3	1.83	2.08	13.66
	Mean			15.37 (1.71) a
MM	2	1.84	2.22	20.65
	4	1.80	2.08	15.56
	Mean			18.11 (2.55) a

Table 3.5 – The overall feeding efficiency (FE) and feed conversion ratio (FCR) for MS and MM groups. Similar lowercase letters indicate no significant difference (P>0.05).

Group	Tank	Combined Weight Gain (g)	Total Feed Provided (g)	FE	FCR
MS	1	11,315	31,105	0.364	2.749
	3	12,032	27,781	0.433	2.309
	Mean			0.398 a (0.35)	2.529 a (0.220)
MM	2	13,744	42,070	0.327	3.061
	4	13,613	37,458	0.363	2.752
	Mean			0.345 a (0.018)	2.906 a (0.155)

Table 3.6 – The regression slope (N) for MS and MM groups. Similar lowercase letters indicate no significant difference (P>0.05).

Group	Tank	% Male	Sexes Combined	Males Only	Females Only
MS	1	57	0.115 (274)	0.154 a (155)	0.043 b (119)
	3	51	0.110 (281)	0.139 a (142)	0.067 b (138)
	Pooled 1 & 3	54	0.112 (555)	0.148 a (297)	0.058 b (257)
MM	2	70	0.160 (287)	0.197 z (201)	0.106 x (86)
	4	66	0.143 (283)	0.167 z (189)	0.097 x (94)
	Pooled 2 & 4	68	0.152 (570)	0.183 z (390)	0.101 x (180)

Appendix Table 3.1 – The median fish weight per sample by tank. Asterisks indicate values withheld from some analysis due to apparent sampling error.

Day	Sample Size	Tank 1	Tank 2	Tank 3	Tank 4
0	30	10.2	17.0	11.8	18.6
30	30	18.8	25.7	18.4	26.4
60	30	21.5	35.5	24.7	40.2
90	30	44.2*	56.7*	35.3	43.6
120	60	33.2	46.3	24.4	37.5
150	50	46.2*	70.8*	52.6*	60.2*
180	All Fish	32.9	50.0	31.5	50.1
234	All Fish	41.9	57.3	43.2	61.0

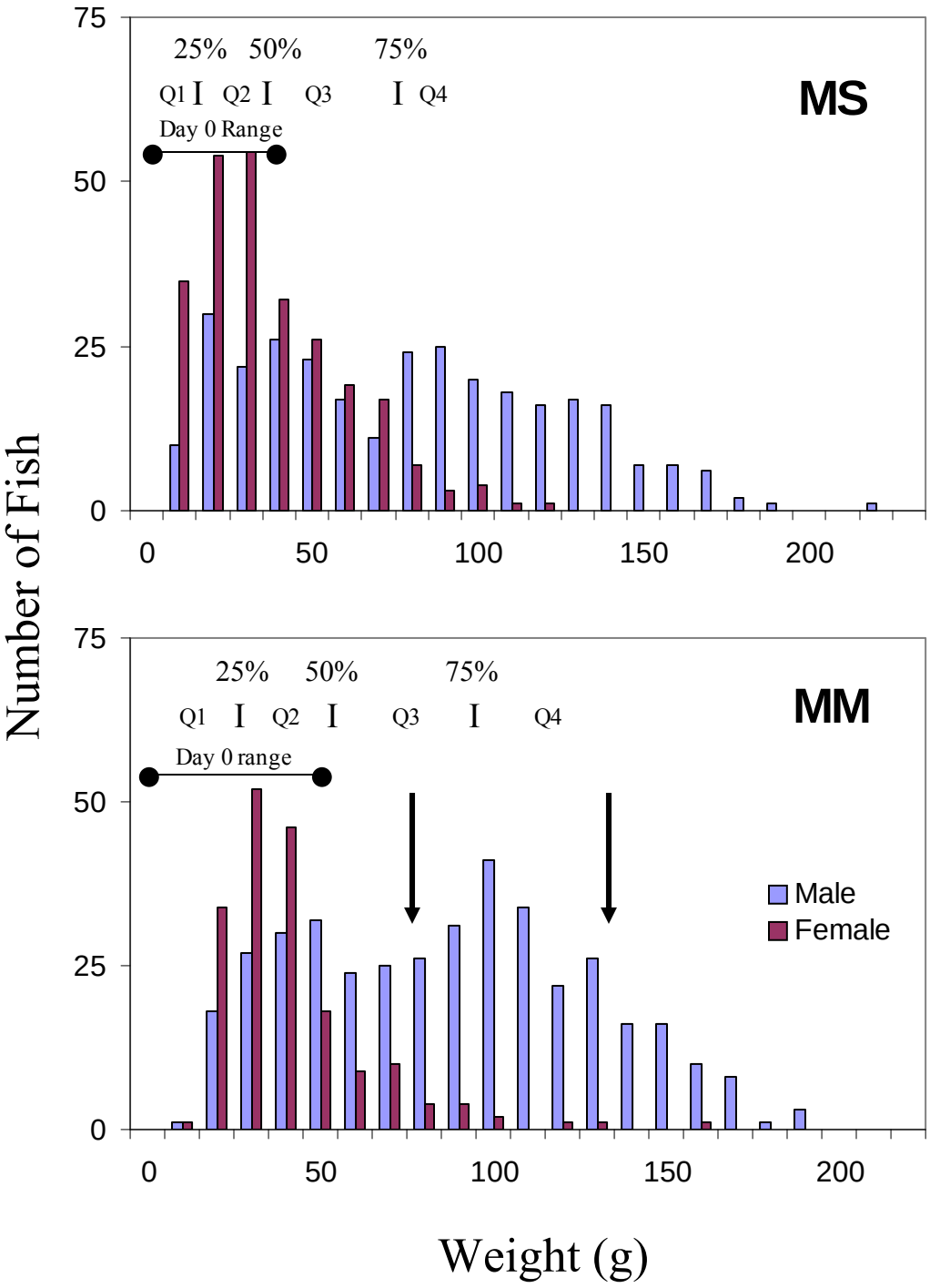

Figure 3.1 – Final day weight distributions of male and female bluegills in mixed-sex (MS – tanks 1 & 3) and mostly-male (MM – tanks 2 & 4) tanks. Shown are weight quartiles, the 25th, 50th, and 75th percentiles of weight, and stockpiling of males in the MM panel.

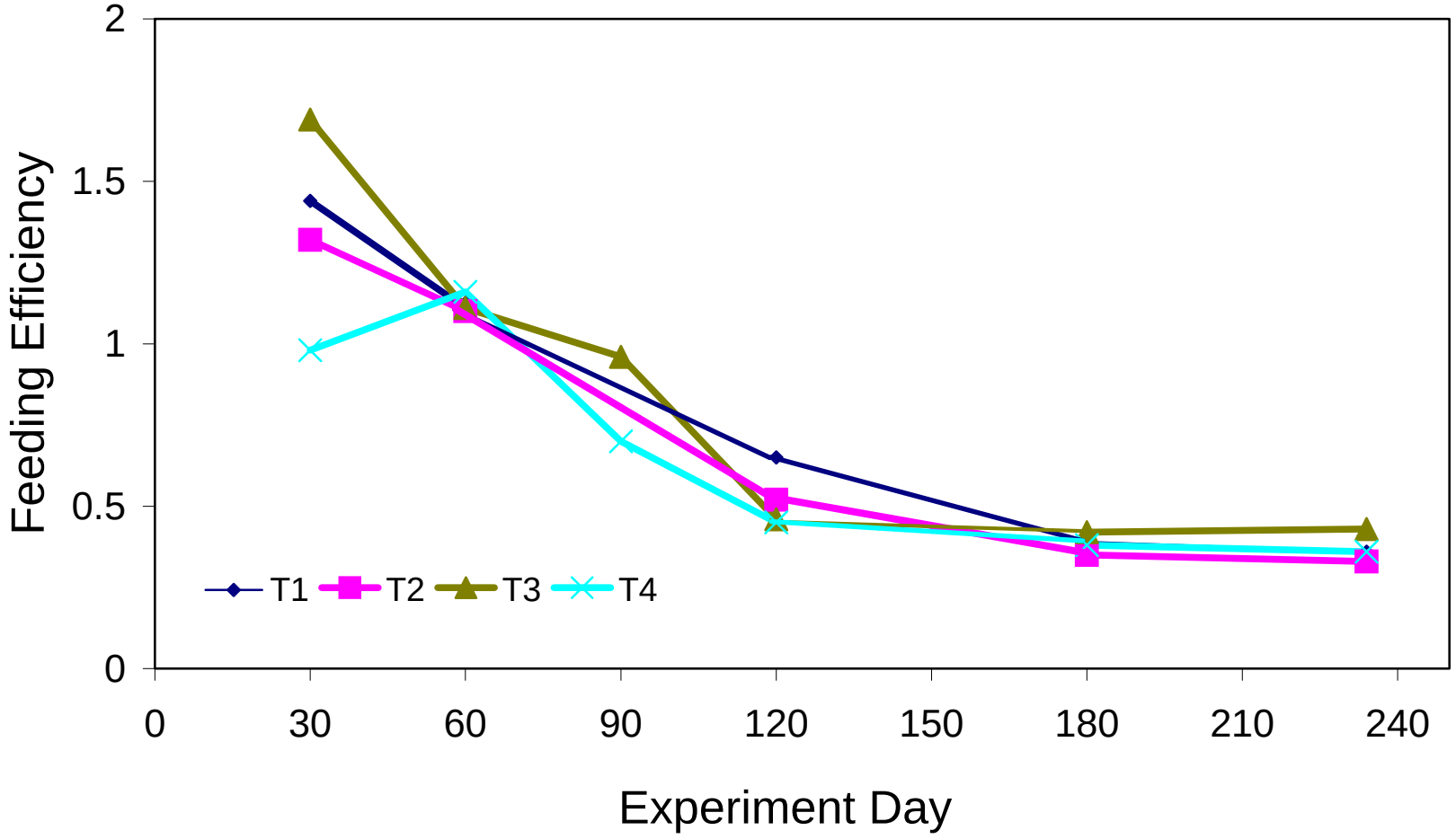

Figure 3.2 – The progression of feeding efficiency in the MS tanks (1 & 3) and MM tanks (2 & 4) throughout the 234-day experiment.

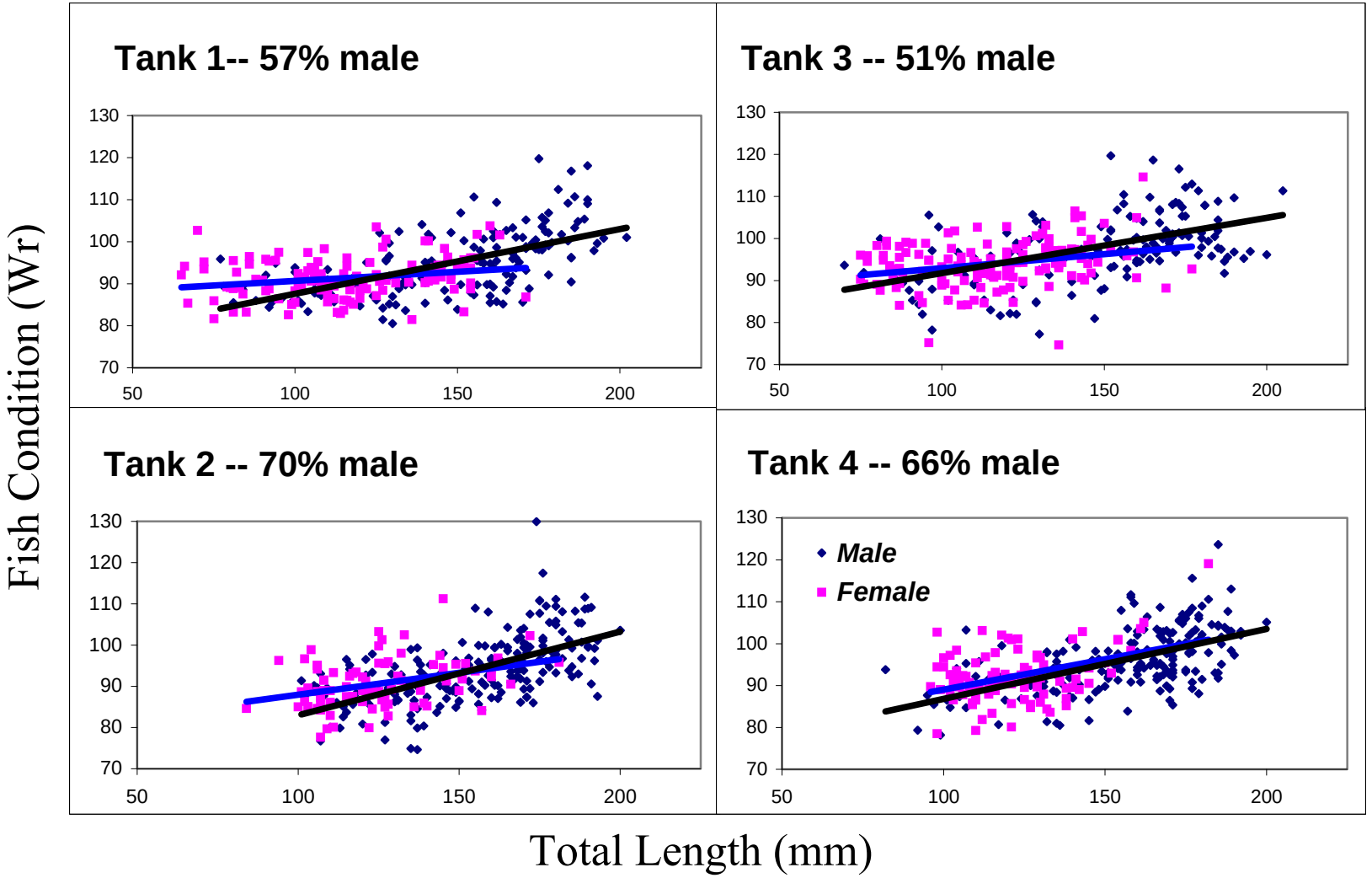

Figure 3.3 – Significant relationships between fish condition as relative weight (Wr) and fish length in each of the four tanks on the final day of the experiment. Regression lines for male and female bluegill are shown separately in each panel.

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