

# NORTH CENTRAL REGIONAL AQUACULTURE CENTER

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**Project Title:** Developing Genetically Fast-Growing Monosex Populations in Bluegill [Termination Report]

**Key Word(s):** Sunfish

**Total Funds Committed:** \$160,000

**Initial Project Schedule:** September 1, 2013 to August 31, 2015

**Current Project Year:** September 1, 2016 to August 31, 2017

**Participants:** Charles E. Hicks, Lincoln University, Missouri; Han-Ping Wang, The Ohio State University, Ohio; James Wetzel II, Lincoln University, Missouri

**Extension Liaison:** Charles E. Hicks, Lincoln University

**Industry Liaison:** Curtis Harrison, Harrison Fisheries, Inc., Missouri

## Project Objectives

1. Identify additional super - males and performance - selected females from existing populations.
2. Create all - male bluegill populations by crossing super - males with females of selected and non - selected stocks.
3. Rear populations at two or more locations in the NCR.
4. Compare sex ratios and production characteristics of sub - populations as based on maternal stocks.

## Deliverables

1. Characterize the performance characteristics and sex ratios of super - male/performance -selected cross.
2. Characterize the economic cost benefits of culturing the super - male/performance - selected cross.
3. Publication of results in journal article, and extension publications (i.e., factsheets, research tours).

## Project Summary

Improving the growth rate and broodstock of bluegill (*Lepomis macrochirus*) and its hybrids has been ranked as one of the top priorities in USDA-NCRAC. This research specifically addresses the needs identified by NCRAC. The results of this research will advance our understanding of sex-determining mechanisms in fish. Further, using this information, we expect to be able to obtain super male broodfish using data garnered from this project. The result will be genetically fast-growing all-male populations by crossing super males with genetically improved females. Not only will a monosex culture be expected to produce the greatest biomass in a given period of time, but also all male bluegill culture may promote growth by reducing the metabolic cost of sexual growth and reproduction. This will benefit fish farmers by increasing the efficiency and profitability of sunfish aquaculture production in the U.S.

In this project, we found that water temperatures and genetics affect the number of male Bluegills. Although we are not able to produce all-male offspring due to the nature of the complicated sex determination by both genetics and water temperature in bluegill, the progeny nonetheless were primarily males and the near-all-male population grew substantially greater and larger than unselected Northern Bluegill and Coppernose Bluegill stocks.

## Technical Summary and Analysis:

*Objective 1.* — Progeny test for all-male populations using improved fish from Lincoln University of Missouri was conducted. Temperature effects on sex ratio have been found in some geographic populations, i.e., local conditions with higher water temperatures yielded larger numbers of males and more females in low temperatures. These findings were published in *Aquaculture*. Follow-up investigation using four different geographic populations strongly suggests that both temperature-dependent sex determination (TSD) and genetic sex determination (GSD) exist in bluegill. This paper was published by *The Biological Bulletin*. In addition, an article on the effects of *Astragalus membranaceus* (AM) on growth performance and stress profiles in bluegill sunfish was published in *Fish Physiology and Biochemistry*.

Objective 2 - Three batches of Bluegill that were a product of Lincoln University's selective breeding program for food-fish production was transported to OSU's South Center Wet Lab. Twenty-four selected females were single-mated to 24 of OSU's males with each pair in one of 24 round flow - through tanks with each tank equipped with an artificial spawning nest. Water temperature and photoperiod were set at 25°C and 16 h light: 8 h dark. Fish were checked twice daily and when nests were found with eggs, the nests were singly placed in the bottom of aerated 400-L tanks similar to those used for mating pairs. Fifteen pairs spawned yielding 12 useable batches of expected all-male Bluegill stock.

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The number of offspring was limited due to the challenging nature of this research in a limited time span. In Spring 2017, OSU researchers produced ~5,000 this type of fish, and next year they should have enough broodfish to produce large number of all-male or mostly-male fingerling for large scale pond test.

Objective 3 - The all – male Bluegill were sent to LU for comparison of growth, sex ratios and production characteristics with unselected Northern Bluegill and Coppernose Bluegill stocks. Initial intent was to rear the all – male and reference stocks in separate tanks (three tanks per stock) of the same recirculating aquaculture system (RAS) with similar stocking densities. However, the number of fish provided by OSU was too low to allow for stocking densities needed for the tank study. Adjustment was made to rear the three stocks commingled in multiple tanks (common gardens). Each common garden contained similar numbers of fish of all stocks at time of stocking. The common gardens started in April 2016 and fish monitored for growth performance.

Growth was less for all three stocks than realized for similar sized (initial TL 5 to 10 cm) Northern Bluegill used in other trials of the same RAS at the same time. In an effort to promote faster growth, the three common gardens were split into six in August 2016 using tanks of the same size roughly halving stocking density. Estimated growth at that time caused LU to request an additional no-cost extension from October 31, 2016 through April 30, 2017. Additionally, the increased number of tanks is allowing the comparison of manual-feeding (hand-feeding) and automatic feeding. The former is most prevalent with commercial production while the latter has been shown to produce better results for Bluegill up to the average size fish used at the beginning of this trial. The extended common garden trial terminated in February 2017, at which time fish processed for determination of sex based on gonads and dress out.

Two additional trials were completed using excess Bluegill generated at LU for intended use as objective 3 reference fish. The first trial compared current commercially available diets hand-fed to Bluegill in tanks. Higher protein and energy diets generally produced better growth and feed conversion efficiency although production cost as a function of feed cost supported use of intermediate diets in terms of crude protein and energy level. Where feed conversion is more important, such as with fish reared under more intensive conditions or other management issues are more costly, then the higher protein and energy diets are likely to be preferable.

A second additional trial was looking at sex within a brood as a function of size well before fish could be sexed externally or based on appearance of gonads. Three broods where each sorted into three groups based on size. Seventy-five fish for largest and smallest group of each brood were reared to the point where they could be sexed with certainty based on appearance of gonads. Sex ratio was not a correlated with size of fry at the time of sorting before sex could be determined by gross observation. Additionally sex ratio can vary markedly between broods that differ only in the parents used to conceive them. At Piketon facility, an experiment on comparison of growth, sex ratios and production characteristics of mostly-male (~95%) and regular mixed population was completed under Objective 4.

Objective 4 - At the end of the above experiments of growth evaluation, sex ratios and production data including survival, growth rate, FCR (for separate rearing experiment) were compared between the selected mostly-male bluegill and the control groups at both locations. At LU facility, the large mostly-male and small mostly-male stocks in terms of weight started smaller and grew significantly more than control group in terms of weight increase. Percent weight increase of the mostly-male stocks was 3 to 5 times ( $P < 0.0001$ ) than observed for the reference Northern Bluegill and Coppernose Bluegill stocks; the large (mean 29.4g) and small (15.1g) mostly-male stocks increased in weight by 571% and 900% (Table 1), respectively. Specific growth rate was highest for the smaller mostly-male stock (1.43%) followed by the large mostly-male stock (1.20%) where the values for the Northern Bluegill and Coppernose Bluegill where lowest and not significantly different from each other ( $P < 0.0001$ ; Table 1).

The sex ratio (male: female) for Northern Bluegill, Coppernose Bluegill, large mostly-male and small mostly-male groups are: 50%:50%, 60%:40%, 89%:11%, and 82%:18%, respectively. At OSU Piketon facility, the experimental results showed: 1) Size was much uniform for near-all-male group and coefficient of variation (CV) for body weight ( $100 * \text{standard deviation} / \text{mean}$ ) was lower in the male groups comparing control groups (53.1 vs.76.0, and 51.8 vs. 76.9 at the beginning and end of experiment, respectively); 2) Survival of near-all-male groups was significant higher than that of mixed sex groups (25.0% v.s. 3.4% on average); CV for body weight is the most important determinant for survival because we found a few number of large size fish chased and bite small size fish and resulted in mass mortality; and 3) mostly-males grew 16.7% faster than mixed population in body weight during juvenile phase. The experiment is continuing beyond NCRAC-funded period

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**Table 1.** Results of 249-day combined common garden growth trials comparing stocks of commercially sourced Northern Bluegill (NBG) and Coppernose Bluegill (CBG) to large all-male and small all-male with feeding treatments by hand for 93 days followed by 156 days of hand-feeding 3x daily and 12-h belt feeders.

| Stock                        | Mean Weight <sub>initial</sub> (0.1 g) | Mean Weight <sub>final</sub> (0.1 g) | Weight Increase (0.1g) | Weight Increase (%) | SGR               | Standard Error for Stock*Tank |
|------------------------------|--|--------------------------------------|------------------------|---------------------|-------------------|-------------------------------|
| NBG                          | 43.4 <sup>a</sup>                      | 121.2 <sup>b</sup>                   | 77.8 <sup>b</sup>      | 180 <sup>c</sup>    | 0.67 <sup>c</sup> | 0.26 <sup>a</sup>             |
| CBG                          | 42.0 <sup>a</sup>                      | 109.7 <sup>b</sup>                   | 67.6 <sup>b</sup>      | 161 <sup>c</sup>    | 0.59 <sup>c</sup> | 0.33 <sup>a</sup>             |
| Mostly-male <sub>large</sub> | 29.4 <sup>b</sup>                      | 188.6 <sup>a</sup>                   | 159.2 <sup>a</sup>     | 571 <sup>b</sup>    | 1.20 <sup>b</sup> | 0.24 <sup>a</sup>             |
| Mostly-male <sub>small</sub> | 15.1 <sup>c</sup>                      | 139.3 <sup>b</sup>                   | 124.3 <sup>a</sup>     | 900 <sup>a</sup>    | 1.43 <sup>a</sup> | 0.28 <sup>a</sup>             |

## Principal Accomplishments

Genetically improved fast-growing mostly-male populations were created by cross of LU and OSU improved fish. Characteristics, performance and sex ratios of fast-growing male population were characterized and compared with mixed control population. At LU facility, the large mostly-male and small mostly-male stocks in terms of weight started smaller and grew significantly more than control group in terms of weight increase. Percent weight increase of the mostly-male stocks was 3 to 5 times than observed for the reference Northern Bluegill and Coppernose Bluegill stocks; large and small mostly-male stocks increased in weight by 571% and 900%, respectively. Specific growth rate was highest for the smaller mostly-male stock (1.43%) followed by the large mostly-male stock (1.20%); values for the Northern Bluegill and Coppernose Bluegill were lowest and not significantly different from each other. The sex ratio (male:female) for Northern Bluegill, Coppernose Bluegill, large mostly-male and small mostly-male groups are: 50%:50%, 60%:40%, 89%:11%, and 82%:18%, respectively.

At OSU Piketon facility, the experimental results showed: 1) Size was much uniform for near-all-male group and coefficient of variation (CV) for body weight was significantly lower in the male groups comparing control groups (53.1 vs.76.0, and 51.8 vs. 76.9 at the beginning and end of experiment, respectively); 2) Survival of near-all-male groups was significant higher than that of mixed sex groups (25.0% v.s. 3.4% on average); CV for body weight is the most important determinant for survival because we found a few number of large size fish chased and bite small size fish and resulted in mass mortality; 3) mostly-males grew 16.7% faster than mixed population in body weight during juvenile phase.

This rearing experiment is continuing beyond funded period using funds from state grants. Three journal articles on related results have been published in journals *Aquaculture*, *The Biological Bulletin*, and *Fish Physiology and Biochemistry*. One journal article is under review by journal *Aquaculture Research* (see publication list). Two papers have been accepted by a Wiley book "Sex Control in Aquaculture", which will be published by Wiley & Sons at the end of 2017 (see publication list). Four papers/abstracts were presented and published in Conference Proceedings of World Aquaculture Society after oral presentations. Two additional oral presentations were made in other conferences (see publication list). Extension publications (i.e., factsheets) are in preparation. The next step is to do commercial-scale on-farm/on-station test of all or mostly-male populations vs regular mixed populations. Once this have been done and the results confirmed, OSU can deliver all or mostly-male seeds to fish farmers. Distribution of stocks will be based on the standard (OSU) procedure for commercialize genetically improved animals.

## Impacts

The impact of this project will be primarily via the delivery of fast-growing mostly-male bluegill populations to fish farmers in Ohio, the Midwest, and other states. The greatest return on investment for this project is the ultimate reduction in production costs due to increased growth rate and reduced feed costs. A successful creation of genetically male bluegill strains can have a tremendous impact on the sunfish aquaculture industry by increasing growth rate of 30- 35% and saving energy expenditure of 20-30% for sex growth

## **Recommended Follow-Up Activities:**

Results of experiments at two locations confirmed that genetically improved fast-growing mostly-male populations grew significantly faster than mixed-sex regular populations. Percent weight increase of the mostly-male stocks was 3 to 5 times than observed for the reference Northern Bluegill and Coppernose Bluegill stocks at LU aquaculture facility. To best examine genotype by environmental interactions and obtain commercial-scale results applicable across the North Central Region, we recommend conducting both commercial-scale on-farm and on-station tests of fast-growing mostly-male or all-male (once developed) strains with local mixed-sex regular populations at four locations using both separate rearing and communal rearing methods. Ponds at four geographic locations at different latitudes, including two demonstration/research stations and two commercial farms. We can develop and adhere to consistent rearing protocols at all of the selected sites. We can evaluate all of the key production parameters of fingerling production (e.g., survival, growth, feed conversion) in Year 1, and evaluate all of the key production parameters for raising the two groups of fish to market size (e.g., survival, growth, feed conversion for separate rearing, and fillet yield) in Year 2. It is at this time that economic cost benefits of these animals can best be estimated.

## **Publications, Manuscripts, Workshops, and Conferences**

See the Appendix for a cumulative output for all NCRAC-Funded Sunfish activities.