

**DEVELOPING GENETICALLY FAST-GROWING MONOSEX MALE POPULATIONS IN  
BLUEGILL**

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**Industry Advisory Council Liaison:** Curtis, Harrison, Harrison Fisheries, Inc., MO

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

**Funding Request:** \$160,000

**Duration:** 2 Years (September 1, 2013 - August 31, 2015)

**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)

**Proposed Budgets:**

<b>Institution</b>	<b>Principal Investigators</b>	<b>Objectives</b>	<b>Year 1</b>	<b>Year 2</b>	<b>Total</b>
The Ohio State University	Han-Ping Wang	1-4	\$59,738	\$64,262	\$124,000
Lincoln University of Missouri	Charles E. Hicks James Wetzell II	3 & 4	\$4,000	\$32,000	\$36,000
<b>Totals</b>			<b>\$63,738</b>	<b>\$96,262</b>	<b>\$160,000</b>

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## JUSTIFICATION

Bluegill sunfish *Lepomis macrochirus* have become an economically important and high-value species, both from the perspective of their use in aquaculture, as well as their recreational value. In some Midwest states like Ohio and Michigan, bluegill have been listed as one of the top three culture species of fish because of their desirable characteristics (Lewis and Heidinger 1978; McLarney 1987; Ehlinger 1989) for production, and the demand for them and high value in the marketplace. Due to the excellent palatability of the sunfish family, tilapia have been labeled and sold as “sunfish” in some major market outlets, indicating the public demand for sunfish.

Because the aquaculture business in some regions in the U.S. does not appear to be able to economically sustain large market products (e.g., channel catfish *Ictalurus punctatus*), regional niche marketing of high value products will be an important business model in the future. The demand for these high value species has remained very high in the regional niche markets and there are no imported fish products comparable with these unique high value species. Bluegill have unique and niche markets in the Midwest, middle south, and southeastern United States. For example, retail prices for bluegill reached to \$29-35/kg (\$13-16/lb) as compared to \$10/kg (\$4.54/lb) retail for catfish (Jungle Jim’s market, Cincinnati, Ohio, 2007) and \$8-12/kg (\$3.64-5.45/lb) for fresh tilapia fillet (Lutz et al. 2003). Despite this opportunity, rapid expansion of the bluegill aquaculture industry has not occurred in this country. One reason in particular hindering expansion has been the relatively slow growth of currently cultured populations of this species.

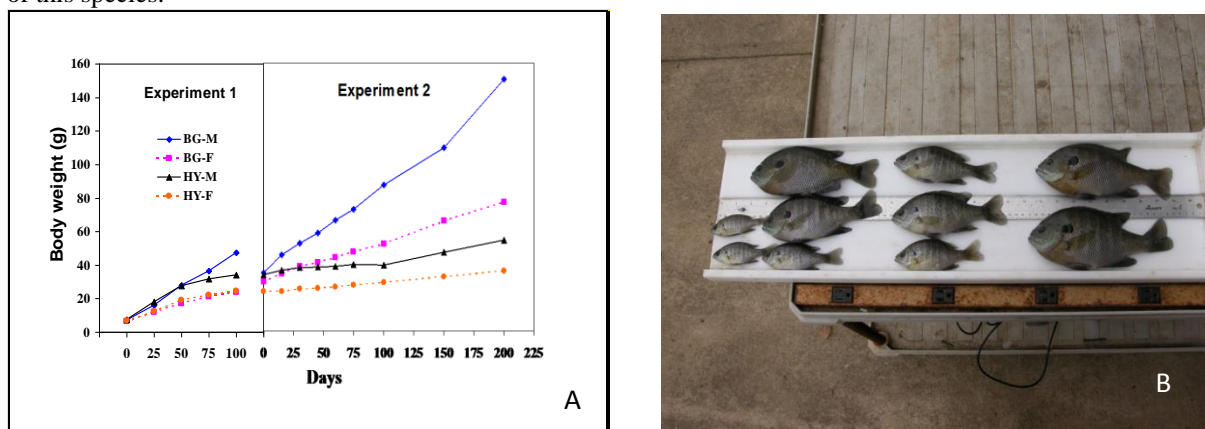


Fig.1 (A) Inherent growth of male vs female of bluegill and its hybrid. BG-M: bluegill male; BG-F: bluegill female; HY-M: hybrid (bluegill × green sunfish) male; HY-F: hybrid female (Hayward and Wang, 2006); (B) Size variation between males (large) and females (small) in the same cohort.

For the past 10 years, North Central Region Aquaculture Center (NCRAC) has funded research that focused on increasing the growth rate or creating fast-growing bluegill with the purpose of increasing sunfish aquaculture production (Wang and Hayward 2000; Hayward and Wang 2002, 2006; Hicks and Borgwordt 2009). One of the most important findings from those studies is that the inherent growth rate of bluegill males is twice that of females (Fig. 1), and males could reach commercial size (250g; 0.55 lb) in 8 months from the juvenile stage (Hayward and Wang, 2006). In this study, fish had been held individually, but growth was assessed by the mean of eight replicates.

In the follow-up research on “evaluation of growth performance of mostly-male group versus mixed-sex groups” recent results indicated that male bluegill communally reared in groups were still able to grow significantly faster than mixed or mostly-female populations in commercial aquaculture settings, and the social interaction costs among males in the group did not significantly decrease growth performance of the male population (Wang et al, 2009). Similar results are reported by Doerhoff (2007), in which, top 25% mostly-male group (80%-100% male) grew 42.3% - 62.3% faster than regular mixed-sex group (48%-52% male), and males gained 50g more than females on average by the end of the 240-day experiment. The research data and commercial practice suggested that a mostly- or all-male populations could reach market size within a year in a cage and recirculating aquaculture system (Doerhoff, 2007; Wang et al, 2009; Hicks and Borgwordt 2009 (Table 1); Windridge Farm, MD, and Woodside Farms Inc, OH, unpublished data). These results support the conclusion that a monosex culture will hold considerable potential as a method to increase the efficiency and profitability of bluegill food and recreational

aquaculture by improving growth rate, and eliminating the problem of prolific reproduction, precocious maturity and their consequences.

Bluegill sunfish and its hybrids have been identified as valuable aquaculture taxa by NCRAC. The demand for bluegill has remained very high in the Midwest U.S. and related regions because of their excellent palatability and high value. Although there are several mature aquaculture industries, such as catfish, trout and salmon in the other regions of this country, bluegill has its unique and niche market in the Midwest, middle south, and southeastern United States. For example, in Ohio and Michigan, bluegill has been listed as one of the top three culture species (Malison 2000). In attempts to profit by the popularity of this species, Tilapia is currently being marketed in parts of the Midwest as “bluegill”. However, stunting has been the most challenging management problem in farm ponds. Their prolific nature and precocious maturation have long been recognized as the cause of these problems (Ricker 1948; Emig, 1966; Bennett 1971). Recent concern for commercial production of bluegill has heightened the need for enhancing growth and reducing grow-out time to increase the efficiency and profitability of bluegill aquaculture. Importantly, as recently documented, bluegill males grow significantly faster than females (Wang and Hayward 2006; Doerhoff 2007), and now much interest has been generated concerning the development of monosex male populations to solve the recognized problems and achieve the above goals identified by NCRAC by both enhancing growth rate and eliminating the problem of prolific reproduction.

The Bluegill sunfish are currently recognized as one of the most valuable North American recreational fish. Bluegill and its F<sub>1</sub> hybrids (female green sunfish *L. cyanellus* x male bluegill) have long been commercially cultured to support recreational fishery stocking needs throughout the middle south, and southeastern United States (Brunson and Robinette 1986; Tidwell and Webster 1993; Brunson and Morris 2000). The recreation market offers a great opportunity for aquaculture of bluegill and its hybrid. The prices for catch-and-keep and catch-and-release fishing are about \$30 per 10 fish or \$29 per 1 hour (Fields et al. 2004). Therefore, monosex male populations can also greatly enhance sunfish recreational aquaculture by eliminating the problem of prolific reproduction, precocious maturity and their consequences and increasing growth.

Monosex culture has been applied in several species to improve fish production (Schreck 1974; Donaldson and Hunter 1982; Hunter and Donaldson 1983; Yamazaki 1983). The potential advantages sought from these approaches include one or more of the following features: achievement of higher growth rate, elimination of reproduction, reduced variation in harvest size, and reduction of risk of environmental impact resulting from the escape of exotic species. Sexual growth can commonly account for 20-30% of a fish's energy expenditure, and culture of males and females together results in early and frequent reproduction (Mire 1995). This is especially true for bluegill (Emig 1966; Bennett 1971).

Considering the commercial value of bluegill, recent technological advances in their culture, and the dramatic differences in growth rates between sexes, it has become increasingly important to evaluate the development of methods to control sex in bluegill with the ultimate aim of producing monosex populations of only faster-growing males. The research proposed is significant, because the outcomes of research will enable us to understand sex-determining mechanisms in bluegill; Furthermore, this new knowledge will allow us to develop technology for producing all male populations or genetically male bluegill strains that would have potential to grow more than 50% faster than mixed bluegill population by increasing growth rate of 30-35% (males growing twice faster over females, Wang and Hayward 2006) and saving energy expenditure of 20-30% for sex growth (Mire 1995). Crossing super male populations developed by OSU with the 4th generation of fast-growing line of bluegill developed by Lincoln University (LU) will further enhance growth of all-male populations. Such improvement of growth rate would significantly increase the efficiency and profitability of sunfish operations, therefore, the aquaculture production in the inland U.S.

The Ohio State University (OSU) has conducted sex-control research in bluegill for 5 years, and successfully sex-reversed regular populations to all females (Wang et al. 2008) and induced them to produce progeny. The project initially was funded by a USDA special grant. Unfortunately, all USDA special grants were cut in 2011. In this project we are proposing to continue the established project to achieve our final goal by combining the sex-control selection at OSU with genetic selection for growth trait at LU to enhance selection result and response.

Improving the growth rate and broodstock of bluegill and its hybrids has been ranked as one of the top priorities in USDA-NCRAC. *The proposed research will specifically address the needs identified by that agency.* The results of

this research can be expected to advance our understanding of sex-determining mechanisms in fish. Further, using this information, we expect to be able to obtain super male broodfish. By the completion of the proposed research, we expect to generate 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.

## **RELATED CURRENT AND PREVIOUS WORK**

### Growth Performance of Male Bluegill Communally Reared in Groups Evaluated and Confirmed

A study on “establishment of mostly-male groups of bluegill by size-grading and evaluation of their growth performance versus mixed-sex groups” was completed. In this study, a cohort of one-year-old bluegill (30.1g) were graded and divided into two mostly-male groups (top 25% and top 50% by length) and a mixed-sex control (no grading). The growth performance of the three groups of fish was tested in tanks (first 5 months) and then cages (final 6 months) at ambient water temperatures. At the end of the 11-month experiment, all fish were killed to determine sex ratios in each group. Weight gain in the mostly-male bluegill groups was significantly greater ( $P<0.05$ ) than the mixed control group throughout most of the experiment. The top 25% treatment with 75.4% males gained 61% more weight by the week 32. The top 25% treatment also had a higher survival (96.0%) than the mixed control (90.6%). The coefficient of variation (CV) for weight decreased in all three groups over time. These results indicated that male bluegill communally reared in groups were still able to grow faster than mixed or mostly-female population in commercial aquaculture settings, and the social interaction costs among males in the group did not significantly decrease growth performance of the male population. This was published in *North American Journal of Aquaculture* (Wang et al. 2008).

### Gonadal Sex Differentiation Time in the Bluegill and its Relation to Fish Size and Age clarified

Using a slow-growing batch (SGB) and a fast-growing batch (FGB) of fish, OSU staff compared the effects of fish size and age on the gonadal differentiation. The results indicated that the gonadal differentiation in bluegill sunfish was more related to body size than to age. In presumptive ovaries, germinal and somatic differentiation began between 13.2 and 16.0 mm (0.52 and 0.63 in) (60 dph in SGB and 30 dph in FGB) in total length (TL). In presumptive testes, the number of germ cells began to multiply in fish between 19.0 and 22.5 mm (0.75 and 0.89 in) (at 70 dph in SGB and 50 dph in FGB) TL. These findings indicate that the bluegill sunfish is a differentiated gonochorist. Based on these results, OSU staff suggest that the critical period of sex differentiation in the bluegill sunfish occur between 13.2 and 16.0 mm TL (0.52 and 0.63 in) and sex differentiation is distinguishable in most fish at 21.0-28.0 mm (0.83-1.10 in) TL. This has been published by *Aquaculture* (Gao et al. 2009)

### Three Strains of Bluegill Obtained and Approximately 1,500 Sex-reversed Female Brooders Developed

Currently, OSU staff have obtained three strains of bluegill from different geographic locations with a total of more than 2,000 brooders. Using some of these fish and based on above results, we successfully produced about 1,500 sex-reversed female brooders, and a paper on this was published in *Aquaculture* (Wang et al. 2008). The results indicated the estradiol-17 $\beta$  ( $E_2$ ) treated groups of 100, 150, 200 mg/kg feed all produced 100% monosex female populations, but the higher dosage groups (150, 200 mg/kg) resulted in differences in growth and survival during treatment. The histology study of gonads confirmed that fish in  $E_2$  treated groups of 100, 150, 200 mg/kg feed all had only oocytes, while the gonads from the group of 50 mg/kg feed had intersex cells. Through this study, approximately 750 pseudo-females have been generated for the proposed project. These Pseudo-females will be used in this proposed project.

### The 4<sup>th</sup> Generation of Improved Lines Developed

Lincoln University has developed improved bluegill sunfish lines utilizing mass selection techniques. Fourth generations were produced with pond spawning, indoor culture in a recycle system, and pond culture for finishing (Hicks et al. 2009). A comparison of ending size, standard growth rates, and feed conversion ratios were made

between six families. Families 1, 2 and 3 were progeny from three generations of selected families and families 4, 5 and 6 were progeny from the original parents P1 matings. The comparison was made by testing growth in an indoor recirculating system for 120 days, then in 4-0.1 ha (0.25-acre) ponds for 150 days with PIT tags in each individual fish. Results of the indoor grow-out are shown in Table 1.

Table 1. Means of Weight (g), SGR (Standard Growth Rate), and FCR (feed conversion rate) by Family.

Family	SEX	Weight(g)	MSE	SGR	FCR
1		169.51	5.9697	0.9305	1.1519
	♂	194.70			
	♀	144.32			
2		168.65	5.9914	0.8029	1.2625
	♂	194.54			
	♀	142.75			
3		172.03	5.8290	0.8634	1.2632
	♂	198.83			
	♀	145.23			
4		107.79	6.7430	1.0148	1.1980
	♂	127.08			
	♀	88.50			
5		119.72	6.6789	0.7229	1.4988
	♂	141.88			
	♀	97.56			
6		127.30	6.6157	0.8118	1.3257
	♂	132.70			
	♀	121.37			

#### ANTICIPATED BENEFITS

Improving the growth rate and broodstock of bluegill and its hybrids has been ranked as one of the top priorities in USDA-NCRAC. The proposed research will specifically address the needs identified by that agency. By the completion of the proposed research, we expect to generate genetically fast-growing all-male populations by crossing super-males with genetically improved females. These outcomes will enable us to develop GMB-producing broodstock and mass production of monosex populations. Not only will a monosex culture be expected to produce the greatest biomass in a given period of time, but also an all-male bluegill culture may promote growth by reducing the metabolic cost of sexual growth and reproduction. Therefore, this will benefit fish farmers by increasing the efficiency and profitability of sunfish aquaculture production in the U.S.

The impact of this project will be primarily via the delivery of fast-growing all-male bluegill population 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 will have a tremendous impact on the sunfish aquaculture industry by increasing growth rate of 30-35% (Wang and Hayward 2006) and saving energy expenditure of 20-30% for sex growth (Mire 1995).

#### OBJECTIVES

We hypothesize that the sex determination system of bluegill is male heterogametic (XY). Our long-term goal is to develop an all-male production technology and superior broodstock via selective breeding in bluegill sunfish for the aquaculture industry. To achieve this goal, we will pursue the following specific 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.

## APPROACH

The Ohio State University has conducted sex-control research in bluegill for 6 years, and successfully sex-reversed regular populations to all females (Wang et al 2008) and induced them to produce progeny. By crossing females with male genotype, some super-males have been produced. The project initially was funded by a USDA special grant. Unfortunately, all USDA special grants were cut in 2011. Lincoln University in Missouri has selected the fourth generation of genetically improved bluegill through selective breeding, and an on-station test (in an applied aquaculture setting) showed that the improved fish grew 16 % faster than unimproved fish (unpublished data). We are proposing to continue the established project to achieve our final goal by combining the sex-control selection at OSU with genetic selection for growth trait at LU to create fast-growing all-male bluegill populations by crossing super-males with selected females

### **Objective 1 - Identify additional super-males and performance-selected females from existing populations (Year 1).**

In Year 1, we will be rearing more progeny from crosses of the existing pseudo-females with normal males to maturity in large recirculating tanks for selection of YY-male. OSU then will conduct progeny testing of males to identify additional YY males. Thirty males will be taken from each progeny produced from previous projects at OSU and will be individually mated with 30 normal females. The fertilized eggs will be incubated in round tanks (40 x 40cm; 4 x 4 in) up to the completion of yolk sac absorption. Subsequently, they will be stocked as sib groups in small flow-through tanks. After rearing for 2 months, a minimum of 100 fish from each sib group will be sexed using gonad squash technique. The fish producing all-male population will be YY-male.

### **Objective 2 - Create all-male bluegill populations by crossing super-males with females of selected and non-selected stocks.**

#### Broodfish selection from Objective 2 and genotyping

In the year 1 of this project, LU will select 150 best females from 4<sup>th</sup> generation of improved populations and deliver them to OSU to create fast-growing all-male bluegill populations. They will be tagged and genotyped at the Aquaculture Genetics and Breeding Laboratory (AGBL) at Piketon. Molecular genetic pedigrees will be determined and a genetic relatedness chart will be constructed at AGBL. At least 72 least related, with highest breeding value will be selected from the 150 best fish for production of fast-growing monosex male progeny.

#### Cross of super males and genetically improved females

*Nested mating.*— In year 2, 24 selected and improved female and 24 selected super-male bluegill sunfish will be stocked into four 400 L round tanks with flow-through well water at a ratio of 6:6 per tank at OSU South Center Wet LAB. Out of the season spawning procedure will be used to produce the spawn in indoor tank system. Briefly, water temperature and photoperiod will be manipulated to match its natural spawning-season. First, the temperature in each tank will be gradually decreased from 23°C (73 °F) to 17-18°C (63-64 °F) at the rate of 1°C every 2 days, and photoperiod will be decreased to 8 h light/day (d) from 16 h light/d within 2 weeks. A water temperature of 17-18°C and photoperiod of 8 h light/d will be kept for 4 weeks. Then temperature will be increased to 25 °C and photoperiod to 16 h light/d over 2 weeks. When water temperature and photoperiod reach 25°C (77 °F) and 16 h light: 8 h dark, four artificial spawning nests will be placed in each tank, and will be checked twice daily. Fish in each tank will be fed 1.5% body weight at first using a high protein feed (Silver cup, 45% crude protein, 16% crude fat) daily using an automated belt feeder, then 3% body weight when temperature reaches 25°C (77 °F). Nests with eggs will be placed in the bottom of aerated 400-L tanks with flow-through well water for incubation. Eggs will be hatched in 24-36 h at 24-26°C (75-79 °F).

*Control lines.*— Two control lines will be produced: 1) regular males x regular females; 2) super males x non-selected females. For both lines, 24 female and 24 male bluegill will be randomly taken from regular related populations and nest-mate the same way as the nested mating above to make control line.

### **Objective 3 & 4 - Rear populations at two or more locations in the NCR, and compare sex ratios and production characteristics of sub-populations as based on maternal stocks.**

*Fry rearing.*— Newly hatched larvae will be transferred into 400-L (106-gal) round tanks with flow-through water of 23 °C for both mating designs. Beginning at 4 dph, the fry will be fed six times daily with the brine shrimp (Bio-Marine, INC., USA). At 10 dph, the commercial larval AP-100 micro-feed (Zeigler Bros., INC., USA) will be gradually added to the diet and then AP-200 micro-feed will be gradually added at 20 dph. From 30 dph on, the fry were fed AP-200 micro-feed exclusively and were fed three times daily. During the fry nursery stage, the rearing conditions and temperature will be kept identical among all tanks.

*Evaluation of growth rate and production of all or mostly-male populations vs. two control lines.*— Experiments will be conducted at both OSU and LU when fish reach 40 dph. At both locations, five hundred all or mostly-male bluegill will be stocked in each of three 1.5-m (4.9-ft) diameter flow-through tanks (replicates). One thousand five hundred fish from each control line will in six additional tanks. All fish will be acclimated for about two weeks. Fish will be fed to satiation, and mortality will be monitored during the period of acclimation. At Day 0 (stocking day), every two months, and the end of the experiment, 50 fish will be sampled for individual weight and total length. In addition, every month, 50 fish from each tank will be individually weighed. Feeding rates will be about 6-7% of body weight at the beginning of the experiment, and then be reduced by 1% body weight every two months until reaching 3% BW. Feeding amount and mortality will be recorded on data sheets in order to calculate feed conversion ratio (FCR) and survival rate for each tank. Feeding ration will be adjusted monthly based on new weights and survival. Daily temperature, dissolved oxygen (DO) and mortality will also be recorded for each tank. Temperatures, DO levels, and water exchange rate in all tanks will be kept the same. All tanks will be siphoned regularly. This experiment will be conducted for 7 months.

*Comparison of sex ratios and production characteristics.*— At the beginning and the end of the above experiments, sex ratios of the two groups at two locations will be determined and compared. At the end of the above experiments of growth evaluation, production data including survival, growth rate, and total production, FCR will be compared between the selected all or mostly-male bluegill and the two control lines.

### **Data analyses**

Sex-ratios among/between treatments or against 1:1 will be compared using a chi-squared ( $\chi^2$ ) test. Performance response (growth rate, food conversion rate, survival, and coefficient of variation) will be calculated over periods for the experiments on growth performance. Differences in mean responses among the three groups for the experiments at OSU and LU will be analyzed using ANOVA.

Family assignment will be carried out using the program CERVUS Version 2 (Marshall et al. 1998). The genotyping error rate for CERVUS was set at 1%. For any fish that are assigned with less than 95% confidence, the genotypes will be manually compared with their putative parent and any mismatches evaluated. The progeny not being confidently assigned will be excluded from all further analyses. Best linear unbiased prediction (BLUP) analyses in ASReml version 3.0 (VSN International Ltd (VSNi), UK) will be used to estimate breeding values (EBVs) for broodfish and progeny.

### **Deliverables**

The economic cost benefits of culturing the super-male/performance-selected cross will be characterized at the end of the experiment. A model will be used for the economic analyses. The model will describe the inputs (feed/chemicals etc.), outputs (fish products), the process itself (input-output relations), and the associated capital cost, energy consumption, labor requirement, and materials expressed in both quantities and costs. Information on the economic cost benefits, performance characteristics, and sex ratios of super-male/performance-selected cross will be delivered to industry and the public through extension publications (e.g., factsheets, websites and research tours) and journal article. The impact of this project will be primarily via the delivery of fast-growing all-male bluegill populations to fish farmers in Ohio, the North Central Region, 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 would have a tremendous impact on the sunfish



aquaculture industry by increasing growth rate of 30-35% (Wang and Hayward 2006) and saving energy expenditure of 20-30% for sex growth (Mire 1995).

Genetically male bluegill strains will be used by fish farmers. At the completion of this project, we expect that we will be able to deliver fast-growing all-male bluegill to fish farms to conduct commercial-scale on-farm demonstrations of all-male populations vs. regular mixed-sex populations in the Midwest at different environments and altitudes. Selected stations and farmers will raise the all-male populations side by side in replicated ponds with the same-sized fingerlings of the species from their farm. In this way, farmers will clearly experience the performance differences among all-male populations and their current lines of domesticated fish. By the time we have GMB producing brooders, we will deliver either broodfish or cryopreserved sperm from them, or both, to fish farmers.

This research is a part of a long-term bluegill breeding program, which needs to accomplish eight steps to achieve all YY-male brood stock. This 2-year project is part of the overall program and will enable us to complete steps 4 and 5 of the eight steps. By the end of this project, we will have some YY-males, however this is not the product meant to be distributed to the industry. We will need to use those experimental YY-males to develop YY-females through feminization. Then we will cross YY-males with YY-females to massively produce all YY-male broodstock. It is this YY-male broodstock that can be distributed to farmers to produce all male populations for culture. We will need several years to achieve the final goal. Distribution of the genetically improved fish to the industry is a complex issue. We have been working with University Specialists to try and determine what will be the fair, best and most efficient way to transfer the technology to the industry once the final product has been developed.

## **FACILITIES**

The OSU-OCARD has twelve 0.10-ha (0.25-acre) ponds, some of which will be used for this project. Up to 200 spawning, hatching and rearing tanks in the wet laboratory will be available to produce all-male progeny and grow-out experiments. A new facility with sixty 1.82-m (6-ft) tanks for housing brood stocks is completed. This new tank system will be used for this project also. The OSU aquaculture genetic laboratory at Piketon is equipped with a variety of equipment including a high-throughput ABI Genetic Analyzer for genetic analysis. Genotyping and parentage analyses will be conducted in this lab.

Lincoln University has twelve 0.10-ha (0.25-acre) ponds that can be used for on-station tests. In addition LU has a new wet laboratory that contains a number of inside rearing systems which can be devoted to this project. Specifically LU has a 24 795-L (210-gal) recirculating aquaculture system ( RAS ) that can be devoted to this project.

## REFERENCES

- Abucay, J. S., G. C. Mair, D. O. F. Skibinski and J. A. Beardmore. 1999. Environmental sex determination: the effect of temperature and salinity on sex ratio in *Oreochromis niloticus* L. *Aquaculture* 173:219-234.
- Al-ablani, S.A. and R.P. Phelps. 1997. Sex reversal in black crappie *Pomoxis nigromaculatus*: effect of oral administration of 17 $\alpha$ -methyltestosterone. *Aquaculture* 158: 155-165.
- Arslan, T. 2002. Sex determination in selected Centrarchids. Ph.D. dissertation. Auburn University, Auburn, Alabama. P 13 –161.
- Arslan, T. and R.P. Phelps 2003. Masculinization of bluegill *Lepomis macrochirus* by Multiple androgen immersion enhancers. *Journal of the World Aquaculture Society* 34: 403-411.
- Baroiller JF, D. Chourrout, A. Fostier, and B. Jalabert. 1995. Temperature and sex chromosomes govern sex-ratios of the mouthbrooding cichlid fish *Oreochromis niloticus*. *Journal of Experimental Zoology* 273:216–223.
- Baroiller JF, Y. Guiguen, and A. Fostier. 1999. Endocrine and environmental aspects of sex differentiation in fish. *Cell Molecular Life Science* 55:910–931.
- Bennett, G. W. 1971. Management of lakes and ponds. Van Nostrand Reinhold Co., New York, New York, USA.
- Bishop Y.M.M, S.E. Fienberg, and P.W. Holland. 1975. Discrete multivariate analysis: theory and practice. MIT Press, Cambridge, Massachusetts.
- Brunson, M. W. and H. R. Robinette. 1987. Reproductive isolation between a hybrid sunfish and its parental species. *The Progressive Fish-Culturist* 49:296-298.
- Chen, F. Y. 1969. Preliminary studies on the sex determining mechanism of *Tilapia mossambica* Peters and *T. hornorum* Trewavas. *Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie* 17: 719- 724.
- Childers, W.F. 1967. Hybridization of four species of sunfishes (Centrarchidae). *Illinois Natural History Survey Bulletin* 29:159-214, Urbana, Illinois.
- Childers, W. F. and G. W. Bennett. 1961. Hybridization between three species of sunfishes (*Lepomis*). III *Natural History Survey Biology Notes* No.46, 15 pages.
- Clemens, H.P. and T. Inslee. 1968. The production of unisex broods by *Tilapia mossambica* sex-reversed with methyltestosterone. *Transactions of the American Fisheries Society* 97: 18– 21.
- Conover, D. O. and B. E. Kynard. 1981. Environmental sex determination: interaction of temperature and genotype in a fish. *Science* 213:577-579.
- Conover, D. O. and M. H. Fleisher. 1986. Temperature-sensitive period of sex determination in Atlantic silverside, *Menidia menidia*. *Canadian Journal of Fisheries and Aquatic Sciences* 43:514-520.
- Conover, D. O. and S. W. Heins. 1987a. The environmental and genetic components of sex ratio in *Menidia menidia* (Pisces: Atherinidae). *Copeia* 1987(3):732-743.
- Conover, D.O. and S. W. Heins. 1987b. Adaptive variation in environmental and genetic sex determination in a fish. *Nature* 326: 496– 498.
- Crandall, P. S. and P. P. Durocher. 1980. Comparison of growth rates, sex ratios, reproductive success and catchability of three sunfish hybrids. Pages 88-104 in *Proceedings of the annual meeting Texas Chapter of the American Fisheries Society*, volume 2. Texas A&M University, College Station, Texas, USA.

- Dagnélie P. 1975. *Théorie et méthodes statistiques, Applications agronomiques*. Gembloux: Presses Agronomiques de Gembloux.
- Devlin, R. H. and Y. Nagahama. 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. *Aquaculture* 208: 191-364.
- Doerhoff, A. J. 2007. Establishing mostly-male bluegill groups and evaluating their growth benefits in indoor rearing systems. Master thesis, University of Missouri-Columbia, Missouri. Available: <https://mospace.umsystem.edu/xmlui/bitstream/handle/10355/4915/research.pdf?sequence=3> (June 2013).
- Donaldson, E.M., and G.A. Hunter. 1982. Sex control in fish with particular reference to salmonids. *Canadian Journal of Fisheries and Aquatic Science* 39: 99-110.
- Ehlinger, T. 1989. Learning and individual variation in bluegill foraging: habitat-specific techniques. *Animal Behavior* 38:643-658.
- Ellison, D. G. and R. C. Heidinger. 1976. Dynamics of hybrid sunfish in southern Illinois farm ponds. *Proceedings of the Annual Conference of Southeastern Associations of Fisheries and Wildlife Agencies* 30:82-87.
- Emig, J. W. 1966. Bluegill sunfish. Pages 375-392 in A. Calhoun, editor. *Inland fisheries management*. California Department of Fish and Game, Sacramento, California, USA.
- Gao, Z., H.P. Wang, D. Rapp, P. O'Bryant, H. Yao, G.K. Wallat, W. Wang, R. MacDonald and L.G. Tiu. 2009. Gonadal sex differentiation in the bluegill sunfish *Lepomis macrochirus* and its relation to fish size and age. *Aquaculture* 294:138-146.
- Gao, Z., H.P. Wang, D. Rapp, P. O'Bryant, H. Yao, G.K. Wallat, W. Wang, L.G. Tiu, and R. MacDonald 2010. Effects of a nonsteroidal aromatase inhibitor on sex reversal, gonadal differentiation and growth performance of bluegill sunfish *Lepomis macrochirus*. *Aquaculture Research* 41: 1282-1289.
- Guerrero, R.D., W.L. Shelton. 1974. An aceto-carmin squash method of sexing juvenile fishes. *Progressive Fish Culturist* 36: 56.
- Hayward, R. S., and H. P. Wang. 2002. Inherent growth capacity and social costs of bluegill and hybrids of bluegill and green sunfish: which fish really grows faster? *North American Journal of Aquaculture* 64:34-46.
- Hayward, R. S., and H. P. Wang. 2006. Rearing Male Bluegills Indoors May Be Advantageous for Producing Food-size Sunfish. *Journal of the World Aquaculture Society* 37: 496-508.
- Heidinger R.C. and W. M. Lewis. 1972. Potential of redear sunfish  $\times$  green sunfish hybrid in pond management. *The Progressive Fish Culturist* 34: 107.
- Hicks and Borgwordt 2009. Production Methods for Food-Sized Bluegills. *North American Journal of Aquaculture* 71:52-58
- Hubbs, C. L. and L. C. Hubbs. 1933. The increased growth, predominant maleness, and apparent infertility of hybrid sunfishes. *Papers Michigan Academy of Science* 17:613-641.
- Hulata, G., G. Wohlfarth, and S. Rothbard. 1983. Progeny-testing selection of tilapia broodstocks producing all-male hybrid progenies-preliminary results. *Aquaculture* 33:263-268.
- Hunter, G.A., E. M. Donaldson, J. Stoss, and I. Baker. 1983. Production of monosex female groups of chinook salmon (*Oncorhynchus tshawytscha*) by the fertilization of normal ova with sperm from sex-reversed females. *Aquaculture* 33: 355-364.
- Jalabert, B., Moreau, J., Planquette, and P., Billard, R., 1974. Déterminisme du sexe chez *Tilapia macrochir* et *Tilapia nilotica*: action de la méthyltestostérone dans l'alimentation des alevins sur la différenciation

sexuelle; proportion des sexes dans la descendance des males “inverses”. Ann. Biol. Anim. Biochim. Biophys. 14 (4-B), 729–739 (in French with English abstract).

- Laarman, P. W. 1979. Reproduction of F1 hybrid sunfish in small ponds. *The Progressive Fish-Culturist* 41:145-147.
- Lewis, W.M., and R. C. Heidinger. 1978. Use of hybrids in the management of small impoundments. Pages 104-108 in G.D. Novinger and J.C. Dillard, editors. *New approaches for the management of small impoundments* North Central Division, American Fisheries Society, Special Publication 5, Bethesda, Maryland.
- Li, L., Wang, H.P., C. B. Givens, S. Czesny, and B. L. Brown. 2007a. Isolation and characterization of microsatellites in yellow perch (*Perca flavescens*). *Mol Ecol Notes* 7: 600–603.
- Mair, G.C., J.S. Abucay, D.O.F. Skibinski, T.A. Abella and J.A., Beardmore. 1997. Genetic manipulation of sex ratio for the large scale production of all-male tilapia *Oreochromis niloticus* L. *Can. J. Fish. Aquat. Sci.* 54: 396-404.
- Mair, G.C. and D.C. Little. 1991. Population control in farmed tilapia. *NAGA* 1991, 8–13.
- Marshall, T.C., Slate, J., Kruuk, L., Pemberton, J.M., 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* 7: 639–655.
- McLarney, W. 1987. Characteristics of important cultured animals summarized. Pages 485-508 in *The freshwater aquaculture book*. Hartley & Marks, Point Roberts, Washington.
- Mires, D. 1995. The tilapias. Pages 133-152 in C. E. Nash and A. J. Novotony, editors. *Production of Aquatic Animals*. Elsevier, New York, New York, USA.
- NCRAC. 2002. Annual Report of the North Central Regional Aquaculture Center. Michigan State University, E. Lansing, Michigan.
- Myers, J.M., P.O. Heggelund, G. Hudson and R.N. Iwamoto, 2001. Genetics and broodstock management of coho salmon, *Aquaculture* 118: 53-62.
- Neff, B.D., P. Fu, and M.R. Gross. 1999. Microsatellite evolution in sunfish (Centrarchidae). *Canadian Journal of Fisheries and Aquatic Sciences* 56, 1198–1205.
- Pruginin, Y. S., G. Rohtbard, R. Wohlfarth, R. Halevy, R. Moav and G. Hulata. 1975. All male broods of *Tilapia nilotica* x *T. aurea* hybrids. *Aquaculture* 6:11-21.
- Ricker, W. E. 1948. Hybrid sunfish for stocking small ponds. *Transactions of the American Fisheries Society* 75:84-96.
- Wang, H.P. Z. Gao, B. Beres, J. Ottobre, G.K. Wallat, L.G. Tiu, D. Rapp, P. O’Bryant and H. Yao. 2008. Effects of estradiol-17 $\beta$  on survival, growth performance, sex reversal and gonadal structure of bluegill sunfish *Lepomis macrochirus*. *Aquaculture* 285: 216–223.
- Wang, H.P. G.K. Wallat, R. Hayward, P. L.G. Tiu, O’Bryant and D. Rapp. 2009. Establishment of mostly-male groups of bluegill by size-selection and evaluation of their growth performance. *North American Journal of Aquaculture* 71:216–223.
- Wang, H.P., L. Li, G.K. Wallat, B. Brown, H. Yao, Z. Gao L.G. Tiu, P. O’Bryant, D. Rapp and R. MacDonald. 2009. Evaluation of relative growth performance and genotype by environment effects for cross-bred yellow perch families reared in communal ponds using DNA parentage analyses. *Aquaculture Research* 40: 1363 - 1373.

- Wang, H.P., Z. Gao, P. O'Bryant, J. D. Rapp, R. MacDonald, H. Yao, X. Cao and G. K. Wallat. 2010. Sex control and sex determination mechanism in bluegill. Abstract in the Proceedings of the World Aquaculture Society Conference, page 1057. March 1-5, 2010. San Diego, California USA.
- Wang, N., R. S. Hayward and D.B. Noltie. 2000. Effects of social interaction on growth of juvenile hybrid sunfish held at two densities. *North American Journal of Aquaculture* 62: 161-167.
- Yamamoto, T. and T. Kajishima. 1969. Sex-hormone induction of reversal of sex differentiation in the goldfish and evidence for its male heterogamety. *Journal of Experimental Zoology* 168, 215– 222.
- Yamazaki, F., 1983. Sex control and manipulation in fish. *Aquaculture* 33: 329– 354.

## PROJECT LEADERS

<u>State</u>	<u>Name/Institution</u>	<u>Area of Specialization</u>
<b>Ohio</b>	Han-Ping Wang The Ohio State University	Fish Culture/Fish Breeding
<b>Missouri</b>	Charles E. Hicks Lincoln University of Missouri	Fish Culture
	James Wetzel Lincoln University of Missouri	Fish Culture

**PARTICIPATING INSTITUTIONS AND CO-PRINCIPAL INVESTIGATORS**

**The Ohio State University**

Han-Ping Wang

**Lincoln University of Missouri**

Charles E. Hicks  
James Wetzel

UNITED STATES DEPARTMENT OF AGRICULTURE  
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0039

**BUDGET**

ORGANIZATION AND ADDRESS Ohio State University South Centers 1864 Shyville Rd Piketon, OH 45661			USDA AWARD NO. _____ Year 1: Objectives 1 - 4			
PROJECT DIRECTOR(S) Han-Ping Wang			DURATION PROPOSED MONTHS: <u>12</u>  <b>Funds Requested by Proposer</b>	DURATION PROPOSED MONTHS: _____  <b>Funds Approved by CSREES (If different)</b>	Non-Federal Proposed Cost-Sharing/Matching Funds (If required)	Non-federal Cost-Sharing/Matching Funds Approved by CSREES (If Different)
<b>A. Salaries and Wages</b>			<b>CSREES-FUNDED WORK MONTHS</b>			
			Calendar	Academic	Summer	
1. No. Of Senior Personnel						
a. <u>0.05</u> (Co)-PD(s).....			0.6			4520
b. _____ Senior Associates.....						
2. No. of Other Personnel (Non-Faculty)						
a. <u>0.5</u> Research Associates/Postdoctorates.....			6			20500
b. _____ Other Professionals.....						
c. _____ Paraprofessionals.....						
d. _____ Graduate Students.....						
e. <u>1</u> Prebaccalaureate Students.....						
f. _____ Secretarial-Clerical.....						
g. _____ Technical, Shop and Other.....						3600
<b>Total Salaries and Wages</b> ..... <input type="checkbox"/>						28620
B. Fringe Benefits (If charged as Direct Costs)						10618
<b>C. Total Salaries, Wages, and Fringe Benefits (A plus B)</b> <input type="checkbox"/>						39238
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)						
E. Materials and Supplies						18,100
F. Travel						2400
G. Publication Costs/Page Charges						
H. Computer (ADPE) Costs						
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)						
J. All Other Direct Costs (In budget narrative, list items and dollar amounts, and provide supporting data for each item.)						
<b>K. Total Direct Costs (C through J)</b> ..... <input type="checkbox"/>						59738
L. F&A/Indirect Costs (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs included in on/off campus bases.)						0
<b>M. Total Direct and F&amp;A/Indirect Costs (K plus L)</b> <input type="checkbox"/>						59738
N. Other..... <input type="checkbox"/>						
<b>O. Total Amount of This Request</b> ..... <input type="checkbox"/>						59738
P. Carryover -- (If Applicable) Federal Funds: \$ _____ Non-Federal funds: \$ _____ Total \$ _____						
Q. Cost-Sharing/Matching (Breakdown of total amounts shown on line O)						
Cash (both Applicant and Third Party)..... <input type="checkbox"/>						
- Non Cash Contributions (both Applicant and Third Party)						
NAME AND TITLE (Type or print)			SIGNATURE (required for revised budget only)			DATE
Project Director						
Authorized Organizational Representative						
Signature (for optional use)						



UNITED STATES DEPARTMENT OF AGRICULTURE  
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

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**BUDGET**

ORGANIZATION AND ADDRESS Ohio State University South Centers 1864 Shyville Rd Piketon, OH 45661				USDA AWARD NO. _____ Year 2: Objectives 1 - 4			
PROJECT DIRECTOR(S) Han-Ping Wang				DURATION PROPOSED MONTHS: <u>12</u>	DURATION PROPOSED MONTHS: _____	Non-Federal Proposed Cost-Sharing/Matching Funds (If required)	Non-federal Cost-Sharing/Matching Funds Approved by CSREES (If Different)
<b>A. Salaries and Wages</b>				<b>CSREES-FUNDED WORK MONTHS</b>			
				Calendar	Academic	Summer	
1. No. Of Senior Personnel							
a. <u>0.05</u> (Co)-PD(s).....				0.6			<b>4648</b>
b. _____ Senior Associates.....							
2. No. of Other Personnel (Non-Faculty)							
a. <u>0.5</u> Research Associates/Postdoctorates.....				6			<b>21115</b>
b. _____ Other Professionals.....							
c. _____ Paraprofessionals.....							
d. _____ Graduate Students.....							
e. <u>1</u> Prebaccalaureate Students.....							
f. _____ Secretarial-Clerical.....							
g. _____ Technical, Shop and Other.....							<b>3667</b>
<b>Total Salaries and Wages</b> ..... <input type="checkbox"/>							<b>29430</b>
B. Fringe Benefits (If charged as Direct Costs)							<b>11213</b>
<b>C. Total Salaries, Wages, and Fringe Benefits (A plus B)</b> <input type="checkbox"/>							<b>40643</b>
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies							<b>19219</b>
F. Travel							<b>4400</b>
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts, and provide supporting data for each item.)							
<b>K. Total Direct Costs (C through J)</b> ..... <input type="checkbox"/>							<b>64262</b>
L. F&A/Indirect Costs (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs included in on/off campus bases.)							
<b>M. Total Direct and F&amp;A/Indirect Costs (K plus L)</b> <input type="checkbox"/>							<b>64262</b>
N. Other..... <input type="checkbox"/>							
<b>O. Total Amount of This Request</b> ..... <input type="checkbox"/>							<b>64262</b>
P. Carryover -- (If Applicable) Federal Funds: \$ _____ Non-Federal funds: \$ _____ Total \$ _____							
Q. Cost-Sharing/Matching (Breakdown of total amounts shown on line O)							
Cash (both Applicant and Third Party)..... <input type="checkbox"/>							
- Non Cash Contributions (both Applicant and Third Party)							
NAME AND TITLE (Type or print)				SIGNATURE (required for revised budget only)			DATE
Project Director							
Authorized Organizational Representative							
Signature (for optional use)							

Form CSREES-2004 (12/2000)

## BUDGET EXPLANATION FOR OHIO STATE UNIVERSITY

(Wang)

### Objectives 1-4

**A. Salaries and Wages.** PI's salary is requested for year 1: \$4,520 and year 2: \$4,648. Six month's salary for RA for year 1: \$20,500 and year 2: \$21,115. The RA will be involved in managing all experiments, experimental fish and data collection analyses. Two months' salary for a technician for year 1: \$3,600 and year 2: \$3,667.

**B. Fringe Benefits.** Year 1: the fringe benefit is 37.1% (\$10,618); Year 2: the fringe benefit is 38.1% (\$11,213).

**E. Materials and Supplies.** Year 1: Feed for feed training, and growth test and broodstock (\$4,100); PI tags for broodfish (\$2,000); lab chemicals and supplies for genotyping (\$3,500); water quality kit (\$300); ten heaters for heating water for nursery and growth test (\$1,800); eight belt feeders for feed training and growth test (\$2,400); pond and tank suppliers (nets, buckers, etc.) (\$1,000); power and water costs (\$3,000). Year 2: Feed for feed training, and growth test and broodstock (\$7,724); lab chemicals and supplies for genotyping (\$4,000); water quality kit (\$300); ten heaters for heating water for nursery and growth test (\$1,800); pond and tank suppliers (nets, buckers, etc.) (\$1,895); power and water costs (\$3,500).

**E. Travel.** Year 1: One professional meeting (American or World Aquaculture Society) for one person to present findings from project: \$2,400 - Air ticket: \$400-500; registration-\$450; Hotel: 5 x \$170=\$850; Food per Diem: 6 x 60=\$360; Travel to Airport and hotel: \$150 (round trip from Piketon to Columbus: 170 mile x 0.565 = \$96); Other-\$50. Year 2: One professional meeting (American or World Aquaculture Society) for one person to present findings from the project: \$2,400 - Air ticket: \$400-500; registration-\$450; Hotel: 5 x \$170=\$850; Food per Diem: 6 x 60=\$360; Travel to Airport and hotel: \$150 (round trip from Piketon to Columbus: 170 mile x 0.565 = \$96); Other-\$50. Two round trips for two people from OSU to LU to pick-up broodfish and deliver fry: 2,000 – each trip: 1,000 miles x 0.565 = \$565; Hotel: 2 x 140=\$280; Food per Diem: 2 x 60=\$120.

UNITED STATES DEPARTMENT OF AGRICULTURE  
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**BUDGET**

ORGANIZATION AND ADDRESS Lincoln University -Missouri Foster Hall, 904 Chestnut Jefferson City, MO 65101			USDA AWARD NO. _____ Year 1: Objectives 1 - 4			
PROJECT DIRECTOR(S) Charles E. Hicks James E. Wetzel			DURATION PROPOSED MONTHS: <u>12</u>	DURATION PROPOSED MONTHS: _____	Non-Federal Proposed Cost-Sharing/Matching Funds (If required)	Non-federal Cost-Sharing/Matching Funds Approved by CSREES (If Different)
<b>A. Salaries and Wages</b>			<b>CSREES-FUNDED WORK MONTHS</b>			
			Calendar	Academic	Summer	
1. No. Of Senior Personnel						
a. ___ (Co)-PD(s).....					1	
b. ___ Senior Associates.....						
2. No. of Other Personnel (Non-Faculty)						
a. ___ Research Associates/Postdoctorates .....						
b. ___ Other Professionals.....						
c. ___ Paraprofessionals.....						
d. ___ Graduate Students.....						
e. ___ Prebaccalaureate Students .....						
f. ___ Secretarial-Clerical .....						
g. <u>1</u> Technical, Shop and Other .....						
Total Salaries and Wages..... <input type="checkbox"/>						
B. Fringe Benefits (If charged as Direct Costs)						
C. Total Salaries, Wages, and Fringe Benefits (A plus B) <input type="checkbox"/>						
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)						
E. Materials and Supplies					3000	
F. Travel					1000	
G. Publication Costs/Page Charges						
H. Computer (ADPE) Costs						
I. Student Assistance/Support (Scholarships/ fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)						
J. All Other Direct Costs (In budget narrative, list items and dollar amounts, and provide supporting data for each item.)						
K. Total Direct Costs (C through J)..... <input type="checkbox"/>					4000	
L. F&A/Indirect Costs (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs included in on/off campus bases.)						
M. Total Direct and F&A/Indirect Costs (K plus L) <input type="checkbox"/>					4000	
N. Other..... <input type="checkbox"/>						
O. Total Amount of This Request..... <input type="checkbox"/>					4000	
P. Carryover -- (If Applicable) Federal Funds: \$ _____ Non-Federal funds: \$ _____ Total \$ _____						
Q. Cost-Sharing/Matching (Breakdown of total amounts shown on line O)						
Cash (both Applicant and Third Party)..... <input type="checkbox"/>						
- Non Cash Contributions (both Applicant and Third Party)						
NAME AND TITLE (Type or print)			SIGNATURE (required for revised budget only)			DATE
Project Director						
Authorized Organizational Representative						
Signature (for optional use)						



## BUDGET EXPLANATION FOR UNIVERSITY OF WISCONSIN-STEVENSON POINT

(Hicks and Wetzel)

### Objective 3 & 4

**A. Salaries and Wages.** Six months' salary for a RA for year 2: \$15,000. A portion of the salary for a research assistant (RA) is requested. The RA will be involved in managing all experiments, experimental fish and data collection analyses.

**B. Fringe Benefits.** Year2: RA: \$4,425 with fringe rate of 29.5% .

**E. Materials and Supplies.** Year 1: Feed for feed training, and growth test and broodstock (\$1,000); power and water costs (\$3,000). Year 2: Feed for feed training, growth test and broodstock (\$5,000); water quality kit (\$300); Belt feeders for growth test (\$2,275); power and water costs (\$4,000).

**E. Travel.** Year 1: One trip to OSU for collaborative research: \$1,000

**BUDGET SUMMARY FOR EACH YEAR FOR EACH PARTICIPATING INSTITUTIONS**

Year 1

	<b>OSU</b>	<b>LU</b>
Salaries, Wages, and Fringe Benefits	\$39,238	\$0
Materials and Supplies	\$18,100	\$3,000
Travel	\$2,400	\$1,000
All Other Direct Costs	\$0	\$0
Total Project Costs	\$59,738	\$4,000

Year 2

	<b>OSU</b>	<b>LU</b>
Salaries, Wages, and Fringe Benefits	\$40,643	\$19,425
Materials and Supplies	\$19,219	\$12,575
Travel	\$4,400	\$0
All Other Direct Costs	\$0	\$0
Total Project Costs	\$64,262	\$32,000

### SCHEDULE FOR COMPLETION OF OBJECTIVES

Start date: September 1, 2013

Completion date: August 31, 2015

Task	Calendar																							
	Year 1												Year 2											
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept
<i>Rearing more progeny with YY-males to maturity - <b>Objective 1</b></i>	■	■	■	■	■	■																		
<i>Progeny test to identify more YY-males - <b>Obj. 1</b></i>							■	■	■	■	■													
<i>Broodstock selection and genotyping; pedigree construction - <b>Obj. 2</b></i>							■	■	■	■	■													
<i>Crossing YY ♂ with improved ♀ producing all ♂ and fry rearing - <b>Obj. 2</b></i>											■	■	■	■	■									
<i>Evaluation of growth and sex ratio of all or mostly-male populations- <b>Obj. 3&amp;4</b></i>																■	■	■	■	■	■	■	■	
<i>Manuscript and project report submission</i>																						■	■	

## VITA

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## EDUCATION

B.S. Central China Agricultural University, 1982, Fisheries Science.  
M.S. equivalent Yangtze River Fisheries Institute (YFI), 1987, Aquaculture Science.  
Ph.D. University of Missouri-Columbia and Huazhong (Central China) Agricultural University  
(a joint training program), 2001, Aquaculture Science.

## POSITIONS

Adjunct Professor (2005–present), Department of Animal Science; Principal Scientist & Director (2005–present), OARDIP, OSU South Centers  
Adjunct Professor (2005–present), Key Laboratory of Fish Genetic Resources and Biotechnology, Chinese Ministry of Agriculture  
Research Associate Professor (2003) and Senior Research Associate (2000–2003). Department of Fisheries & Wildlife, University of Missouri-Columbia  
Associate Professor (1993–1999), Assistant Professor (1987–1993), Research Associate (1984–1986), Department of Aquaculture & Environment, YFI, Chinese Academy of Fisheries Sciences.

## SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

World Aquaculture Society  
American Society of Animal Sciences  
USDA-North Central Regional Aquaculture Center, Member of Technical Committee-(R)

## SELECTED PUBLICATIONS

- Wang, H.P., Z. Gao, P. O'Bryant, D. Rapp, H. Yao, G. R. MacDonald, and W. Wang. In Press. Temperature effects and genotype-temperature interactions on sex determination in bluegill sunfish. *Aquaculture*.
- Eissa, N., and H.P. Wang. In Press. Physiological stress response of yellow perch subjected to repeated handlings and salt treatments at different temperatures. *North American Journal of Aquaculture*.
- Xu, Y.J., H.P. Wang, H. Yao, P. O'Bryant, and Q.Y. Wang. In Press. GH, IGF-I and IGF-II mRNA expression in fast and slow growing strains and families of yellow perch *Perca flavescens*. *Aquaculture*.
- Cao, X, H.P. Wang, H. Yao, P. O'Bryant, J. D. Rapp and G. K. Wallat. 2012. Evaluation of one-stage and two-stage selection in yellow perch I: genetic and phenotypic parameters for growth traits of F<sub>1</sub> fish reared in ponds using microsatellite parentage. *Journal of Animal Sciences* 90:27–36.
- Xu, Y.J., X.Z. Liu, M.J. Liao, H.P. Wang, and Q.Y. Wang. 2012. Molecular Cloning and differential expression of three GnRH genes during ovary maturation of *Verasper variegates*. *Journal of Experimental Zoology* 317:434–46.
- Wang, H.P., H. Yao, P. O'Bryant, D. Rapp, G.K. Wallat, L.G. Tiu and R. MacDonald. 2011. Family-tank interactions on early growth performance of yellow perch reared in single family tanks versus mixed-family tanks as inferred using molecular pedigrees. *Aquaculture research* 42:1694–1702.



## VITA

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Lincoln University  
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E-mail: hicksc@lincolnu.edu

## EDUCATION

B.S. Utah State University, Logan, Utah, Biology  
M.S. Utah State University, Logan, Utah, Fishery Biology

## POSITIONS

Lincoln University (06/02 to present) - Cooperative Research, Assistant professor, aquaculture, research in aquaculture and sunfish culture.  
University of Missouri (11/20 to present) - Fisheries and Wildlife, Assist Fisheries and Wildlife Extension Specialist with workshops and publications. Assist with walleye and sunfish grow out projects at two commercial fish farms.  
Lincoln University (Fall 1997) - Adjunct Staff Teach upper level course in aquaculture.  
Missouri Department of Agriculture (11/93 to 11/98) - Aquaculture Specialist.  
Genesis Aquaculture, Inc. - Director Technical Services.  
South Florida Aquaculture Center, Inc. (1/87 to 3/89) - Florida City, Florida, General Manager.  
Missouri Department of Conservation (6/68 to 3/87) - Jefferson City, MO., Superintendent of Fish Hatcheries.  
Utah Division of Wildlife Resources (10/65 to 6/68)] - Salt Lake City, Utah. Supervisor of Hatcheries.  
Logan Experimental Fish Cultural Station (4/64 to 10/65) - Logan, Utah, Biologist  
Fisheries Research Institute, University of Washington - Seattle, WA, Biologist

## SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society  
Fish Culture Section of the American Fisheries Society  
Missouri Aquaculture Association

## SELECTED PUBLICATIONS

Hicks, C.E., Ellersieck, M.R., and C.J. Borgwordt. 2009. Production methods of food sized bluegill sunfish (*Lepomis macrochirus*). North American Journal of Aquaculture 71:52-58.  
Hicks, C.E., and R.A. Pierce II, Bluegill Sunfish Production in Missouri. 2012. University of Missouri Extension Aquaculture Guide g9473. University of Missouri, Columbia, Missouri.  
Pierce, R.A., Hayward, R.S., Parcell, J. and C.E. Hicks. 2007. Paddlefish Production: opportunities for Missouri Pond and Lake Owners. University of Missouri Extension Guide. University of Missouri, Columbia, Missouri.  
Graham, L. K., E. J. Hamilton, T. R. Russell, and C. E. Hicks. 1986. The culture of paddlefish—a review of methods. Pages 78-94 in J. G. Dillard, L. K. Graham, and T. R. Russell, editors. The paddlefish: status, management and propagation. American Fisheries Society, North Central Division, Special Publication 7, Bethesda, Maryland.

## VITA

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## EDUCATION

BS Purdue University, 1989, Fisheries and Aquatic Sciences  
MS Purdue University, 1993, Aquaculture  
Ph. D. Southern Illinois University, 2004, Zoology

## POSITIONS

Associate Professor of Aquaculture (2012-present), Lincoln University of Missouri  
Assistant Professor of Aquaculture (2007-2011), Lincoln University of Missouri  
Research Investigator (2005-2006), Lincoln University of Missouri  
Technical Help/Para-Professional (2003-2005), Southern Illinois University  
Researcher II (Aquaculture Technology Transfer) (2000-2002), Southern Illinois University  
Research Assistant of Aquaculture (1999-2000), Southern Illinois University  
Dissertation Fellowship (1998-1999), Southern Illinois University  
Research Assistant of Aquaculture (1994-1998), Southern Illinois University  
Research Assistant of Fisheries and Aquatic Sciences (1989-1992), Purdue University  
Assistant Forester of Martel and Darlington Forest (1989), Purdue University  
Assistant Biologist of Gypsy Moth Survey (1988), Indiana Department of Natural Resources

## SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

World Aquaculture Society  
North American Native Fishes Association  
Missouri Academy of Sciences

## SELECTED PUBLICATIONS

- Wetzel J. E., C.S. Kasper, C. C. Kohler. 2006. Comparison of pond production of phase-III sunshine bass fed 32-, 36-, and 40%-crude-protein diets with fixed energy: protein ratios. *North American Journal of Aquaculture* 68(3): 264-270.
- Wetzel, J.E. II. 2006. Spawning and raising the bantam sunfish. *American Currents* 33(1):11-15.
- Wetzel, J. E. and C. C. Kohler. 2005. Distinction between gastric digestion and evacuation in black bass fed piscine prey. *Transactions of the American Fisheries Society*. *Transactions of the American Fisheries Society* 134:533-536.
- Roberts, M.E., J.E. Wetzel, R.C. Brooks, and J.E. Garvey. 2004. Daily increment formation in otoliths of red spotted sunfish. *North American Journal of Fisheries Management* 24:270-274.
- Wetzel, J.E. 2002. Form alternation of adult female crayfishes of the genus *Orconectes* (Decapoda: Cambaridae). *American Midland Naturalist* 147:326-337.
- Brown, P.B., J.E. Wetzel, J. Mays, K.A. Wilson, and C.S. Kasper. 2002. Growth differences between stocks of yellow perch (*Perca flavescens*) are temperature dependent. *Journal of Applied Aquaculture* 12:43-56.