Ohio Aquaculture Research and Development Integration Program (OARDIP) Newsletter
The Ohio State University

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The Ohio Aquaculture Research and Development Integration Program Newsletter is published biannually to share the Program highlights

For more information on OARDIP, contact Dr. Han-Ping Wang at wang900@ag.osu.edu, or visit www.ag.ohio-state.edu/~prec/aqua/

OARDIP Researchers Meeting

The Ohio Aquaculture Research and Development Initiative Program (OARDIP) held its biannual meeting on May 20th at the OSU South Centers in Piketon OH. Investigators from the OSU South Centers, Department of Animal Sciences, School of Environmental and Natural Resources and Bowling Green Aquaculture Center (BGAC) attended the meeting. At this meeting, investigators reported on the project progress and discussed priorities and plans for aquaculture research and outreach for 2009. Updates and accomplishments from this group are published quarterly through the OARDIP newsletter. To read the latest issues, go to http://www.ag.ohio-state.edu/~prec/aqua/.

OARDIP meeting (left to right): Han-Ping Wang (OARDIP Director), Laura Tiu (Co-Director), Karolina Kwasek (Graduate student), John Mark Reddish (Research Associate), Joe Ottobre (Co-Director), Julie Strawser (Information Associate), and Macdonald Wick (Co-Director). Shawn McWhorter (manager of the BGAC) attended the meeting by conference call.

The 2nd Generation of Improved Lines and Mapping Fish of Yellow Perch has been Produced by OARDIP

OARDIP has created the 2nd generation of improved lines of yellow perch using marker-assisted cohort selection this spring from the 2007 year-class selected lines at the OSU South Centers. For this generation, 105 survival families were produced by mating 150 pairs of 1st generation improved lines produced in 2007.

Nursery and feed training of the 2nd generation fish was completed during May and June. On-station testing of the 2nd generation of improved lines is being conducted in 5 ponds, six large tanks (10' in diameter) and 10 medium tanks (3' in diameter). The first 2nd generation of improved lines were produced from 2006 year-class selected lines last year.

In addition, the 2nd generation (F_2) of mapping fish was produced this spring from the F_1 mapping families, which were obtained by crossing two females from the fastest growing strain with two males from the slowest growing strain.

Growth and maturation status of the F_2 fish are being monitored for constructing linkage maps and conducting QTL analysis for maturation timing and growth.

2009 Aquaculture Field Day to Disseminate OARDIP Research Results and Aquaculture techniques to Farmers

By Anna Copeland, Intern

Forty farmers, businesses and fish enthusiasts from all over Ohio came together June 13 for OSU South Centers Aquaculture Field Day. The event provided self-guided walking tours of the Ohio Center for Aquaculture Research and Development facility. Visitors were given the opportunity to tour the fish genetics lab, the hatchery (wet lab), the greenhouse, and the ponds to learn more about research results and cultural techniques related to breeding and genetics, indoor aquaculture production, pond management and construction and culture demonstrations.

Laura Tiu talks pond culture with field day visitors

Aquaculture specialists and researchers were available at each station to explain research, exhibit various fish species and to answer any questions the participants had. Visitors had access to books, videos and other resources on aquaculture. A curious silence fell into the room when one of the employees played a video of all things shrimp farming.

Visitors collect information and watch videos in the resource room

Dr. Wang talks about our breeding and genetics work

The visitors came from all walks of life. One couple simply came because they are fascinated with aquaculture and wanted to learn more. One couple was on the quest to become more self-sufficient by growing their own fish for meals. Matt Hein, another participant, came to learn about tilapia culture. A friend of his owns a restaurant in Marysville that provides whole foods for the less fortunate. He wanted to learn about growing tilapia to possibly contribute to the business.

Geoff Wallat educates visitors about indoor aquaculture

Acknowledgements

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- USDA Cooperative State Research, Education & Extension Service
- North Central Region Aquaculture Center (NCRAC)
- Ohio Agriculture Research and Development Center (OARDC)
- The Ohio State University Extension
- Ohio Sea Grant
- Ohio Soybean Council

Baitfish and Aquaponics Projects underway at the Bowling Green Aquaculture Center

The Bowling Green Aquaculture Center (BGAC) has been very active this Spring in preparation for several research studies this year. Several hundred brood size spot fin shiners have been brought indoors for spawning conditioning. Eggs will be collected in early summer for use in three first feeding experiments to determine the optimal starter diets for indoor nursery culture of spot fin shiners.

Adult spot fin shiners spawning at the BGAC site

The first study will compare commercially available micro-encapsulated starter diets to determine survival to 14 days post-hatch, when spot fins are large enough to accept a starter sized fish diet. The second study will repeat the use of artificial starter diets, in combination with additions of live *Nannochloropsis sp.* algae. The third study will again repeat the use of artificial starter diets with additions of live marine rotifers (*Brachionus plicatilis*), a zooplankton species that is a critical first feed for many fish.

Shawn McWhorter (manager of the BGAC) has also been developing protocols for batch culture of *Nannochloropis sp.* algae and rotifers, with the aim of repeatedly producing high numbers of organisms per milliliter of culture water. The objective is to develop a system capable of supporting 500,000 spot fin shiner fry (or more) at a time, during the

Spot fin shiner fry 5 days post hatch.

first few weeks of culture until they reach a large enough size to consume commercially available prepared diets. A long range goal of BGAC is to develop reliable indoor baitfish culture techniques to improve survivals of juvenile and adult baitfish compared to pond production, and extend the growing season of market-sized baitfish.

The aquaponics research greenhouse is also being readied for a study to compare plants and herbs grown strictly in a hydroponic system (inorganic fertilizer additions to plant grow beds) versus an aquaponics system (use of fish waste as an organic fertilizer to plant grow beds). Tilapia will be raised in two 1,000 gallon tanks to provide the organic fertilizer source to the plants. This system has potential applications for small acreage areas as well as in urban areas where there is interest in producing locally grown, fresh produce.

Yellow perch nutrition project updates

We have been investigating the relationship between reproduction and quality of progeny and the level of the essential amino acid, lysine, in plant based yellow perch diets. The study is currently in its 3rd year. Spring of 2009 was the second spawning season for fish maintained on lysine-supplemented or lysine-deficient diets. We are particularly interested in the performance of the offspring. These are the first progeny of the broodstock that had been fed experimental diets with the major source of protein from plants.

In spring 2009 we conducted a set of experiments with yellow perch larvae and juveniles to evaluate culture method for successful growth, survival and swim bladder inflation under controlled indoor rearing conditions. We proposed, for the first time, a system with conical tanks and designed new culture conditions that included saline water, the addition of algae, feeding with live and preserved live food. We are convinced that this system has a potential to be used for rising yellow perch at early life stages under commercial conditions.

Our preliminary results are very promising with approximately 90 % swim bladder inflation, a figure that has never been achieved in indoor rearing of this fish.

Our final experiment is feeding yellow perch juveniles with a commercial diet and 3 experimental wheat gluten-based diets to evaluate the response of the first generation of fish from mothers fed low lysine-containing diets to a free lysine-supplemented diet, a lysine-glycine diet (supplemented with lysine-glycine dipeptide) and control diet (deficient in lysine). The objective of the present study is to evaluate the effect of wheat gluten-based diets supplemented with different lysine forms (free lysine and lysine-glycine dipeptide) on yellow perch offspring which are the first progeny of lysine-deficient broodstock, on the growth, survival and muscle proteome.

Piketon Prawns

Freshwater prawns are back at the OSU South Centers in Piketon, Ohio. After a one year hiatus, a freshwater prawn culture demonstration is again available for viewing at the Ohio Center for Aquaculture Research and Development. On June 10, 2009, ten thousand juvenile prawn were hand counted into a one-acre culture pond. The prawn will remain in the pond until the middle of September, when they will be harvested at the conclusion of the demonstration.

There are four goals of the 2009 freshwater prawn demonstration project including: investigate the use of nitrogen and phosphorus reducing plants as additional habitat, supplemental feed and for aquatic weed suppression, develop a low-tech way to estimate stocking mortality, evaluate the use of range cubes as a less-cost alternative to formulated shrimp feed and demonstrate recently improved quality assurance protocols during harvest and marketing. The results of these projects will be shared with the freshwater prawn industry in Ohio and other states.

There are approximately 20 freshwater prawn producers in Ohio this year, down from a high of 24 in 2008. Information on prawn harvests and local sales will be available from our Center early September 2009. Information on growing freshwater prawns in Ohio is available on our website (http://www.ag.ohio-state.edu/~prec/aqua/shrimp.htm) or by calling one of our aquaculture specialists.

Anna Copeland, OSU South Centers Aquaculture Intern, assists with stocking the freshwater prawns.

OSU Researcher Uses a Combination of Hormones and Selective Breeding to Create Bigger Bluegill

By Doug Caruso, THE COLUMBUS DISPATCH

PIKETON, Ohio -- All of the fingerling bluegill swimming in the gray tank with a scarlet 36 on it look about the same. But a quarter of them are "super males."

They're the key to growing ponds full of all-male bluegill. Since male bluegill grow twice as large as females, fish farmers can provide larger fillets for people's dinner plates. That would mean larger profits.

Han-Ping Wang, principal research scientist at Ohio State University's aquaculture lab in Piketon, is close to achieving that goal using a process that starts, oddly enough, with turning all the fish into females. He introduced estrogen to tanks of bluegill that had a natural population of about half male and half female. In the right amounts, the hormone turns all the fish into egg-producing females.

Then Wang let genetics take over. First, a quick primer on genes and gender: Genetic males have an X chromosome and a Y chromosome. Genetic females have two X chromosomes. If the male's Y chromosome links up with the female's X chromosome, the offspring is male. If the male's X chromosome links up with a female X chromosome, the offspring is female. In nature, it's a coin toss -- about half the babies turn up male; about half turn up female.

Male bluegill vs. female in the same cohort

And it's all up to dad's genes. So what happens when dad also lays the eggs? Though they now produce eggs, the half that started as males keep their male X and Y chromosomes after the hormone treatment. Those fish laid their eggs this past winter, and untreated males fertilized the eggs.

The result: Half the brood should be male with an X chromosome and a Y chromosome. A quarter should be female with two X chromosomes. Another quarter should be "super males" with two Y chromosomes. "That's what we need," Wang said.

Right now, Wang knows only that 75 percent of the fish in tank 36 are male. You can't tell bluegill fingerlings apart by looking at them, so he's developed genetic tests that reveal the gender. But he doesn't know which males carry the double-Y chromosome combination he's looking for.

When you put YY males in a tank with XX females, it's like making a coin toss with a two-headed quarter: Bet on boys every time because each offspring will get a Y chromosome from super dad and an X chromosome from mom.

In the next step, Wang will put individual males in breeding tanks with females. Whenever he sees an all-male brood, he'll know that the male has two Y chromosomes. Wang knows that there's concern about hormone-treated food and hormones escaping into the environment where they can affect the natural breeding of species. But he says that his method removes the hormone treatment from the farm.

He's also looking for hormone-free ways to get more male bluegill. Simply by controlling the temperature of the water in the first weeks of a brood's life, he's gotten as many as 90 percent of the fish to turn out male.

There is a market for bluegill, but prices are too high, said Doug Denny, owner of the Fish Guys in the North Market. He sells whole bluegill, which he buys for $4 per pound. He estimates he'd have to charge about $9 per pound to offset the work put in to scale and fillet the fish. That puts it at about the same price as lake perch, which comes to him already processed. "If I could get fillets at $6 a pound, I could sell it," Denny said.

At the U.S. Department of Agriculture's last count in 2007, there were 144 bluegill farms in Ohio, said Laura Tiu, an OSU aquaculture extension expert in Piketon. She's the one who helps transfer research to the farmers who can use the information to produce bigger fish faster and potentially bring down the price.

Once he's identified the super males, Wang plans to look for genetic markers and develop a test to quickly identify them by clipping a bit of fin and analyze it in the lab. They're looking for distinct genetic markers to help them identify the fish. They already have found seven such markers that help determine whether a young bluegill is male or female.

If he can grow enough super males, he can send them to farmers who can breed them with females to produce all-male broods. Wang wants to treat some super males with estrogen to turn them into egg-producing females with two Y chromosomes. Call them "super mamas." Breeding super males with super mamas would produce nothing but super males. Then those super males can be sent out to breed with untreated females to produce ponds full of all-male fish. Another option is harvesting the super male sperm, freezing it, and sending that out to the fish farmers. "We would create the first bluegill sperm bank," Wang said.

New Publications

Gonadal sex differentiation in the bluegill sunfish *Lepomis macrochirus* and its relation to fish size and age

Zexia Gao, Han-Ping Wang, Dean Rapp, Paul O'Bryant, Geoff Wallat, Weimin Wang, Hong Yao, Laura Tiu, Russ MacDonald

Abstract: Bluegill (*Lepomis macrochirus*) monosex culture holds considerable potential as a method to improve bluegill aquaculture production as males grow much faster than females. A detailed understanding of the time of gonadal development and differentiation is critical to control sex and optimize culture.

In the present study, we systematically studied gonadal sex differentiation of the bluegill sunfish and its relation to fish size and age from hatching to 90 days post hatching (dph) using a slow-growing batch (SGB) and a fast-growing batch (FGB) of fish. The results indicated that the gonadal differentiation in bluegill was more related to body size than to age.

In presumptive ovaries, germinal and somatic differentiation began between 13.2 and 16.0 mm (60 dph in SGB and 30 dph in FGB) in total length (TL). The outgrowths from the proximal and distal portions of the gonads and the fusion of the outgrowths to form the ovarian cavity occurred between 16.0 and 21.0 mm TL (80 dph in SGB and 50 dph in FGB) with germ cells undergoing meiosis. The gonads in the females larger than 25.5 mm TL always had peri-nucleolus oocytes.

In presumptive testes, the efferent duct formed in the fish ranging from 25.4 to 28.0 mm TL (90 dph in SGB and 70 dph in FGB) with the onset of meiosis and testes contained spermatocytes exhibiting active meiosis in males larger than 33.0 mm TL.

These findings indicate that bluegill is a differentiated gonochorist and sex differentiation occurs earlier in females than males. Based on our results, we suggest that the critical period of sex differentiation in bluegill occur between 13.2 and 16.0 mm TL and histological sex differentiation is distinguishable in most fish larger than 21.0 mm TL.

Aquaculture (2009), 294: 138–146.

doi:10.1016/j.aquaculture.2009.05.024

Evaluation of relative growth performance and genotype by environment effects for cross-bred yellow perch families reared in communal ponds using DNA parentage analyses

Han-PingWang, Li Li, Geoff Wallat, Bonnie Brown, HongYao, Zexia Gao, Laura Tiu, Paul O'Bryant, Dean Rapp &Russ MacDonald

Abstract From 24 mating sets, 6300 fingerlings of yellow perch (*Perca flavescens*) were stocked into one pond and equal numbers of progeny from six representative sets out of the 24 were stocked into each of two other ponds.

After communal rearing for 21 months, total length and body weight were assessed for n=300 fish in each of the three ponds and molecular pedigrees were performed for each sampled individual to assign the progeny back to the original parents. The overall average number of alleles per locus was A = 16.4 and observed and expected heterozygosities were H_o=0.88 and H_e=0.77, respectively. The mean weight of random samples and the top 10% fast-growing fish from the pond with all sets was significantly greater than those from either of the two replicate ponds with six crosses.

For the two replicate ponds, no significant differences were found in family rankings and assignment of the top 10% heaviest fish, indicating that families with superior growth performance in one pond also exhibited the same superior growth performance in the replicate pond. However, there were no significant correlations detected in family mean weights of the top 10% fish between any two of the three ponds.

Aquaculture Research (2009), doi:10.1111/j.1365-2109.2009.02232.x

New Publications (continued from page 6)

Establishment of mostly-male groups of bluegill by grading selection and evaluation of their growth performance

H. P. Wang, G. Wallat, R. Hayward, L. G. Tiu, P. O'Bryant and D. Rapp

Abstract: Concerns over economic feasibility of commercial bluegill *Lepomis macrochirus* aquaculture production have heightened the need for strategies to enhance growth. Recent studies have shown that individually-reared bluegill males can grow twice as fast as females, resulting in increased interest in development of mostly or all male populations.

In this study, we developed a practical procedure to establish mostly male bluegill groups through grading selection, and tested their growth against a normal population. A single cohort of bluegill juveniles was cultured in a pond for a year, and then graded and divided into two mostly male groups (top 25% and top 50% of fish) and a mixed-sex control group when the fish reached a mean weight of 30.1 g.

The mixed control group contained 50.0% males; the top 25% group had 75.4% males and the top 50% group had 69.7% males. Weight gain per fish in the top 25% group was significantly greater ($P < 0.05$) than the mixed group throughout most of the experiment.

There were no significant differences detected in survival among the three groups, although the top 25% group had survival of 96.0%, compared to 90.6% and 90.5% for the top 50% group and the mixed control, respectively. The top 25% group had the highest percentage (46.3%) of fish reaching 150 g at the end of the experiment, followed by the top 50% group (28.3%), and the mixed control group (12.7%).

The coefficient of variation (CV) for weight decreased in all three groups over time, with the mostly male groups maintaining lower initial and final CV values than the control group. Results indicate that mostly male bluegill groups are able to grow faster than typical mixed-sex populations, and social interaction costs among communally reared males did not significantly decrease growth of mostly male populations in the aquaculture settings.

North American Journal of Aquaculture (2009), 71:216–223.

Temperature effects and genotype-temperature interactions on sex determination in bluegill sunfish *Lepomis macrochirus*

Zexia Gao, Han-Ping Wang, Weimin Wang, Dean Rapp , Paul O'Bryant, Hong Yao, Geoff Wallat and Russ MacDonald

Abstract: Much interest has been generated concerning the development of monosex male populations of bluegill *Lepomis macrochirus* due to their more rapid growth capacity relative to females. The methods involved to develop monosex population require a comprehensive understanding of the underlying basis of sex determination and gonadal function with development of monosex male populations.

In this study, through histological observation, the bluegill is classified as a differentiated gonochorist and its critical period of sex differentiation occurs between 13.2 and 16.0 mm total length. Effects of genotype by temperatures (23, 29, 34°C) on sex ratio were tested on two batches of fry with different parents, each having two replicates.

The fry were reared at each of three temperatures from an initial mean size of 0.7 cm (3 days post hatch) to a final mean size of 6.3 cm. The sex of fish was then determined by macroscopic and histological examination of the gonads. The potential parents and fry were genotyped at 6 polymorphic microsatellite loci to perform parentage assignment.

A chi-squared (χ^2) analysis was used to compare sex ratios among replicates and treatments, as well as between treatments and a theoretical 1:1 sex ratio. In the first batch, sex ratios were not significantly different from 1:1 in any group. In the second batch, sex ratios also did not deviate significantly from 1:1 in 23 °C group; however, sex ratios significantly deviated from 1:1 in 29 °C and 34 °C groups, in which a significantly higher proportion of males (66.7–70.6%) were yielded.

These results indicated that high rearing temperature could alter the phenotypic sex ratios and increase the proportion of males in some families of bluegill sunfish, while not having the same effect in other families. It is suggested that there are some genotype–environment interactions on bluegill sex determination.

10th International Symposium on Genetics in Aquaculture *(ISGA 2009), 22-26 June 2009 Bangkok, Bangkok, Abstract P081*