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

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OARDIP Achievements and Impacts 2010

For the past year, in collaborations with the OSU Department of Animal science, the OSU Department of Food Science, the OSU South Centers Business Development Network, the Ohio Soybean Council and several international Institutions, OARDIP accomplished 8 studies and projects, published 5 journal articles and 5 proceedings papers, received 3 research grants in a significant amount of funds, trained 5 graduate students, post doctoral fellow and scholars, generated 6 new grant proposals, conducted three intensive workshops and made 25 presentations to audiences around the state.

O'GIFT (Ohio Genetic Improvement of Farmed-fish Traits) Program

Multiple improved lines of yellow perch have been developed. The testing data show that the improved lines grew 28% - 52% faster than unimproved fish. The 3rd generation of improved fish has been created in 2010 from 2006 year-class through crossing and mating more than 100 families. Two F_2 mapping families have been grown up and monitored growth for genetic maps identifying genes important to increasing production. Three projects related to growth gene express and sex maturation mechanism under different temperature and photoperiod regimes in yellow perch were completed and three manuscripts on these have been finished. An experiment in evaluating family growth response of genetically improved fish to soybean meal diet (SMD) vs. fishmeal-based diet (FMD) has been accomplished and the results are being used to genetically improve SMD utilization rate of yellow perch. For bluegill, twenty families of all - male populations, which would grow 40-50% faster than a mixed-gender population, have been successfully created through selective breeding. In addition, a project on evaluation of hatchery stocks and wild populations of largemouth bass across North America and Asia, and a manuscript on this have been completed. The results of this study provide valuable base for developing future selective breeding program for largemouth bass. *The O'GIFT program is expected to increase aquaculture production of perch, bluegill and largemouth bass by 35-50% by developing genetically improved broodstocks.*

Bowling Green Aquaculture Program/Center (BGAC)

The BGAC has created the first hatchery of its kind in Ohio that will focus on the breeding and rearing of juvenile spot fin shiners, a new baitfish species for Ohio. BGAC has established indoor spawning protocols for spotfin shiner culture, and is making progress with live first feed types and transitions to prepared diets for the fully indoor culture of spotfin shiner juveniles and market size adults. BGAC has been collaborating with Reed Mariculture of San Jose CA to design and construct and pass on to the producers the means of producing rotifers which are a needed live first feed for the shiners. The BGAC hosted a meeting of the baitfish producers in 2010 to evaluate the past years performance and future needs of the producers. Enthusiasm in this new enterprise is high with big expectations for 2011.In December the BGAC held a "Live Feeds Workshop" which was well attended with much interest shown by producers of many species of interest in the state. The site received approximately 120 visitors and received approximately 200 phone calls from those interested in the activities of the Bowling Green Site. Visitors of note were Congress Woman Marci Kaptur and the new Director of ODA Jim Zehringer.

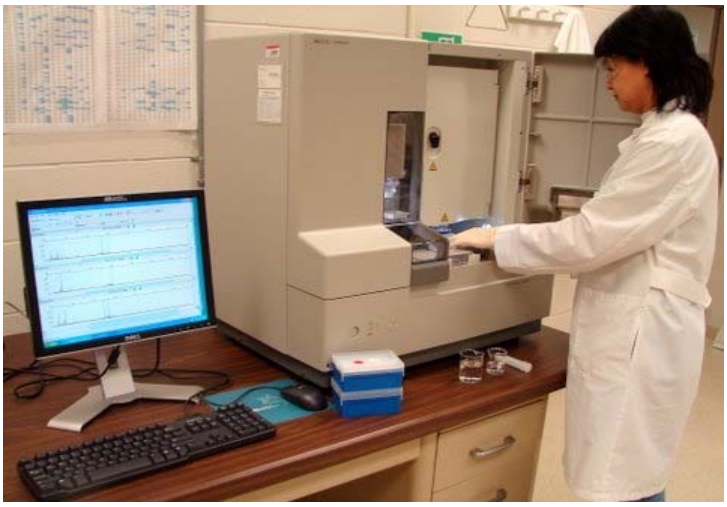
OARDIP Achievements and Impacts (continued from page 1)

Aquaculture Genetics and Breeding Lab (AGBL)

This is the first lab of its type in the Midwest and crucial to the success of the GIFT program. In this lab, genetic relatedness charts and genetic pedigree of selected broodfish have been constructed for breeding programs for the past years. Genotyping of 1500 broodfish for genetic pedigree was completed for breeding program and additional 80 microsatellite markers have been developed in 2010 for constructing genetic maps for traits of growth and VHS resistance. Family identification technology using DNA for selective breeding in yellow perch and bluegill has been established. A first genetic linkage mapping of bluegill has been completed for selective breeding and published in *Aquaculture International*. Genotyping for 1250 largemouth bass from 27 sites and 11 states was finished for stock evaluation.

Fish Muscle Growth and Nutrition Program

Proteomic analysis of muscle proteins in yellow perch have shown that 1) proteins associated with fast type muscle are associated with muscle growth of yellow perch; 2) there are at least 18 unique proteins associated with families of Yellow Perch segregated into fast and slow growing catagories; 3) Lys-Gly dipeptide supplements increase growth intestinal PepT1 gene expression in juvenile yellow perch. We have completed breakthrough research demonstrating wheat gluten based diets supplemented with lysine could replace animal protein based diets for Yellow Perch. *These diets could result in a cost savings of 10-15% in feed costs and they would reduce the impact on the natural resources.*



Aquaculture Technology Transfer (AT^2) and Dissemination

In 2010, OARDIP personnel provided 15 private business consultations and toured over 100 people through their research and demonstration facilities in Piketon and Bowling Green, Ohio. Staff made over 25 presentations to audiences around the state. Three intensive workshops including Perch School, the Ohio Soy-Aqua Bus Tour of Farms and the Live Feeds Workshop, were conducted in the past year. About 20 clients received individual business planning assistance designed to enhance the success of their business ventures

Economic and Scientific Impacts

Aquaculture sales in Ohio have tripled from $1.8 million to $6.6 million in recent years. Nationally, Ohio ranks first in sales of yellow perch for food and is the number one bluegill producing state. Ohio also ranks fourth in sales of baitfish and largemouth bass sold for sport and fifth in number of baitfish farms. *Fifty-six journal articles and proceedings papers have been published, including 5 journal publications and 5 proceedings papers in 2010.*

OARDIP co-chaired an international workshop in Aquaculture Genetics and Breeding

In June 20 – 21th, 2010, OARDIP Director Hanping Wang co-chaired an international workshop in Aquaculture Genetics and Breeding in the Fishery College of Huazhong Agriculture University in Wuhan, China. There were about 50 participants from institutions from China, Europe and America, presenting research results from population genetics to marker-assisted selection.

OARDIP Outreach/Extension 2010 : A Year in Review

The mission of the OARDIP at the OSU South Centers is to support the development of a sustainable aquaculture industry in Ohio. This is accomplished through a variety of research and Extension projects. Research and Extension efforts are designed after input from the industry and are focused on increasing the economic impact of aquaculture in Ohio.

In 2010, OARDIP personnel provided 15 private business consultations and toured over 100 people through their research and demonstration facilities in Piketon and Bowling Green Ohio. Participants included prospective fish farmers, local school groups and visitors from Poland, Tajikistan, West Virginia, the Ohio Bee Keepers Association and a local retirement community. Staff made over 25 presentations to audiences around the state at venues such as the Ohio Aquaculture Association Annual Meeting, Small Farm Conference, Marietta College, Shawnee State University and the Farm Science Review. Three intensive workshops: Perch School, the Ohio Soy-Aqua Bus Tour of Farms and the Live Feeds Workshop, were conducted in the past year. The OARDIP program was featured in multiple media pieces including television, radio and newspaper.

In addition to our primary research initiatives on Selective Breeding and Baitfish Culture, several outreach and demonstration projects were initiated as well. These include: partnering with the Ohio Soybean Association and the OSU South Centers Business Development Network in writing two proposals; working with a bluegill producer on a large scale on -farm production project, collaborating with other aquaculture Extension Specialists to develop an eXtension Community of Practice; continuing the Freshwater Prawn Quality Assurance demonstration work; and working with Ohio companies exploring Algae as a biofuel or possible aquaculture feed ingredient. Six businesses were targeted for additional support and assistance in achieving their expansion plans in the areas of locally produced fish feeds, alternative protein sources for aquaculture diets, and increased tilapia and marine shrimp production. Two Ohio businesses were assisted with submitting a Small Business Innovative Research (SBIR) grant and three other producers were assisted with submitting a Sustainable Agriculture Research and Education (SARE) grant.

A significant amount of effort this year was put toward implementing the new Ohio State Plan for Aquaculture. OARDIP worked closely with the Ohio Department of Agriculture (ODA) to develop, and now implement, this plan. Some results of this partnership include successful

implementation of a USDA feed reimbursement program, where producers received partial rebates for feed purchased in 2008 and 2009, the hiring of an Aquaculture Coordinator at the ODA to help with the development of the industry, the hiring of an Aquaculture Marketing Specialist at ODA, the support for a summer intern focusing on fish health and the completion of an in-depth feasibility and market analysis of the aquaculture industry in Ohio to help determine the best steps for industry growth.

Aquaculture continues to grow in Ohio. The industry currently consists of over 250 mostly small farms catering to niche markets like pond stocking and food fish markets. However, there seems to be a growing interest in taking aquaculture to the next level. The challenge is to figure out what is that next level and how do we go about getting there. Many feel that the next level is the development of indoor recirculating aquaculture facilities that can capture larger markets by providing consistent, safe, locally raised product year-round. Activities in 2010 put us on the path to achieving the goal of continued growth in 2011.

Ohio Aqua-Soy Initiative Bus Tour

In collaboration with Ohio Soybean Council (OSC), OARDIP organized an Ohio Aqua-Soy Initiative Bus Tour to promote the growth of aquaculture in Ohio and activities of the Soy-Aqua Initiative.

One of the best ways to learn about something new is to talk to others already doing it. This is true for learning more about the aquaculture industry as well. Talking with fish farmers and seeing existing operations are great ways to learn more about aquaculture opportunities in Ohio. In order to offer an opportunity like this, the Ohio State University's Ohio Center for Aquaculture Research and Development partnered with the Ohio Soybean Council and the Ohio Department of Agriculture to organize the 2010 SoyAqua Bus Tour of Northeast Ohio Fish Farms.

On July 16, 2010 the SoyAqua bus tour left Wooster Ohio and visited five fish farms located in northeast Ohio. The first stop was Scales to Tails Seafood Shoppe in Wooster, Ohio. Participants learned about the processing and marketing end of the business. Next, the bus visited Laurel Creek Fin Farm, where owner Chuck Jolley raises bluegill and largemouth bass in outdoor ponds. Raber's, a fish farm that raises bluegill in multiple ponds for the food fish and pond stocking markets, provided a tour and also catered a traditional Amish lunch for participants. Fenders' Fish Hatchery, in business over 50 years, was the next stop. This farm utilizes ponds in three counties to supply their large pond management company. Finally, Blue Ribbon Fish Farm, showed off their impressive processing and live fish holding facilities in New Philadelphia Ohio.

Participants strongly agreed that the SoyAqua bus tour was a worthy investment of their finances and time and of great value to their current or future enterprises. Seeing working aquaculture facilities and getting to talk to the owners was cited as a valuable part of the tour. Participants networked, made important contacts, and were encouraged by the amount of information available. Several participants suggested holding the tour annually to benefit the industry.

Education on the banks of Laurel Creek Fin Farm during the SoyAqua Northeast Ohio Bus Tour

Steve Fender talks about raising fish for stocking ponds (photo credit: Chris Kick)

What is a Rotifer Anyway?

Many of the species of fish raised for commercial production in Ohio are so small when they hatch that they require a period of time where they must feed on natural or live food before they can transition to a commercially prepared diet. This natural diet often consists of rotifers and plankton, microscopic animals that have been traditionally cultured in ponds using natural sunlight and Mother Nature. New technology, modeled after successes in the marine aquaculture industry, allows for the culture of these first feeds using indoor systems. This is important as it helps close the life cycle of many of the aquaculture species grown in Ohio, enabling them to be raised entirely indoors, and therefore, year round. The OARDIP hosted a Live Feeds and Hatchery Techniques Workshop at the Bowling Green Aquaculture Center on December 4th, 2010 to share this information and train aquaculture producers in the culture methods of live feeds. Twenty-five attendees came from Ohio, Michigan and Indiana to gain information and training on the methods and systems used to culture live aquaculture feeds in indoor systems.

OARDIP's Shawn McWhorter organized the event and invited Dr. Eric Henry, of Reed Mariculture, a leader in the larviculture feed industry, to join him in the training.

Participants reported a 20% increase in their knowledge of live feeds production after attending the workshop. One hundred percent of attendees surveyed have plans to use what they learned in the future and have already increased their experience in culturing live feeds since the workshop. One participant commented, "I tremendously enjoyed Shawn's and Dr. Henry's presentations and was able to get a lot of questions answered. The information gleaned has the potential to make my production more successful and that was worth it." Another enjoyed "just getting together with people with similar interests, and usually come away with additional usual information just chatting with them."

OARDIP plans to continue this line of research into first feeding of fish and hopes to have additional workshops in the future to share what we learn. For additional information or questions about live feeds for aquaculture, contact Shawn McWhorter, 248-231-2318 or mcwhorter.31@osu.edu.

ODA Doubles Aquaculture Coverage

With the return of Gary Stansberry as aquaculture coordinator, Lindsey Mandau will continue to work with the Ohio Department of Agriculture (ODA) in aquaculture marketing. Both positions will work in tandem and with the Ohio aquaculture industry to help the industry achieve great success. These positions will continue to work both in the field and office to keep moving Ohio aquaculture forward. The Ohio aquaculture Plan and Strategic Plan will serve a guide for Gary and Lindsey to follow. These documents identify the current industry position as well as barriers and recommendations to assist the growth of the Ohio aquaculture industry. Gary and Lindsey are looking forward to the upcoming year and the positive changes that are sure to come for Ohio!

New Publications

A first genetic linkage map of bluegill sunfish (*Lepomis macrochirus*) using AFLP markers

Wei-Ji Wang , Han-Ping Wang , Hong Yao , Geoff Wallat , Laura G. Tiu and Qing-Yin Wang

Abstract Genetic linkage maps were constructed for bluegill sunfish, *Lepomis macrochirus,* using AFLP in a F_1 inter-population hybrid family based on a double-pseudo testcross strategy. Sixty-four primer combinations produced 4,010 loci, of which 222 maternal loci and 216 paternal loci segregated at a 1:1 Mendelian ratio, respectively. The female and male framework maps consisted of 176 and 177 markers ordered into 31 and 33 genetic linkage groups, spanning 1628.2 and 1525.3 cM, with an average marker spacing of 10.71 and 10.59 cM, respectively. Genome coverage was estimated to be 69.5 and 69.3% for the female and male framework maps, respectively.

On the maternal genetic linkage map, the maximum length and marker number of the linkage groups were 122.9 cM and 14, respectively. For the paternal map, the maximum length and marker number of the linkage groups were 345.3 cM and 19, respectively, which were much greater than those on the maternal genetic linkage map.

The other genetic linkage map parameters of the paternal genetic linkage map were similar to those in the maternal genetic linkage map. For both the female and male maps, the number of linkage groups was greater than the haploid chromosome number of bluegill (2n = 48), indicating some linkage groups may distribute on the same chromosome. This genetic linkage mapping is the first step toward to the QTL mapping of traits important to cultured breeding in bluegill.

Aquaculture International **(2010)**

18:825–835

No sex-specific markers detected in bluegill sunfish *Lepomis macrochirus* by AFLP

Ze-Xia Gao, Han-Ping Wang, Hong Yao, Laura Tiu and Wei-Min Wang

Abstract The amplified fragment-length polymorphism (AFLP) technique was used to identify sex-specific markers in bluegill sunfish. A total of 12 835 loci were produced using 256 AFLP primer combinations, including 531 (4.14%) polymorphic loci among different pools. Among the 256 primer combinations, only nine (3.52%) primer combinations yielded sex-associated amplifications across the pooled DNA samples. Four AFLP loci (0.03%) were initially considered as possibly being female specific because they were only amplified in two female DNA pools, and another five AFLP loci (0.04%) were only amplified in two male DNA pools (Table II). When these loci were re-analyzed in all samples, including all individual samples composed of DNA pools, however, the sex-specific markers were only observed in a limited number of individuals of putative sex. These results revealed that for each putative sex-specific marker, the putative sex-specific bands in the pooled DNA samples were virtually caused by the individual polymorphism. This study was the first attempt to find sex-specific markers in *L. macrochirus*. Despite the failure to find such markers, these data offer useful information for further studies targeting similar goals. For future investigations, we recommend the use of an alternative approach, possibly focusing on the gene expression patterns during the course of sex determination and differentiation.

Journal of Fish Biology **(2010)**

76: 408–414

New Publications (continued from page 6)

Estimates of heritability and genotype by environment interactions for body weight in yellow perch reared in communal tanks

Yao, H., H. P. Wang, X. Cao, P. O'Bryant, J. D. Rapp, G. K. Wallat and L. Tiu.

Abstract An understanding of effects of genotype by environment interaction (G×E) is essential to ensure maximum genetic gains are achieved for a molecular marker assisted breeding program. In this study, fifty families of yellow perch (*Perca flavescens*) from different crosses were mixed and reared in the same ponds in the nursery phase. Experiment was performed under two temperature and two density regimes with the 50 families of feed-trained fingerling. All the fish were genotyped and pedigreed using 8 microsatellite markers, which allowed them to be assigned to the individual breeders used in the mating design. The overall average number of alleles per locus was A = 17.0 and expected heterozygosity was H_e=0.80. The progeny from the 50 families could be confidently assigned to their family of origin at the rate of 98.0%. Heritability and estimated breeding values for body weight was estimated using ASREML 2.0 (animal model). Genetic correlations between ages were calculated and breeding values at various ages were estimated in different treatments. This study demonstrated that molecular pedigrees established from microsatellite markers are useful and essential for tracking individual family performance of communally-reared fish and examining G×E interactions on phenotypic trait expression in yellow perch.

Proceedings of the World Aquaculture Society Conference

page 1121. March 1-5, 2010

San Diego, USA.

Evaluation of one-stage and two-stage selection in improving fish growth and breeding efficiency in yellow perch using DNA pedigree.

Cao, X, H. P. Wang, H. Yao, P. O'Bryant, J. D. Rapp and G. K. Wallat.

Abstract Twenty-five full-sib families were produced in a pair-mating design. After hatching, each family with similar fish number was stocked into ponds for the nursery phase. Feed-trained fingerlings then were reared into four 0.1 ha ponds using the same family composition and fish number. At the end of year one, 300 fish in each pond were weighed, measured and fin-clipped. Two ponds were graded and the top 50% fish were restocked to two same sized ponds for year-2 grow-out (two-stage selection), while the other two ungraded ponds were harvested and restocked to other two ponds (one-stage selection). At the end of year two, the same procedures of sampling were conducted as for the end of year one. Thirty-four parents and 1165 offspring (600 fish at the end of year one and 565 fish at the end of year two) were genotyped with 8 microsatellite markers. Parentage assignment was performed using Cervus 3.0.

Proceedings of the World Aquaculture Society Conference

page 169. March 1-5, 2010.

San Diego, USA.

New Publications (continued from page 7)

Sex control and sex determination mechanism in bluegill

Wang, H.P., Z. Gao, P. O'Bryant, J. D. Rapp, R. MacDonald, H. Yao, X. Cao and G. K. Wallat.

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 order to control sex by steroid treatment and to study the genetic sex-determining mechanism in bluegill, the first step is to optimize sex reversal using steroids. 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. Based on this result, 30-day-old fry were fed E_2 at 50, 100, 150 and 200 mg kg^{-1} diet for 60 days with positive control treatment, and 100% monosex female populations without intersex gonad were produced in 150 and 200 mg kg^{-1} groups. By crossing the largest sex-reversed females (most of them should be XY-females) with regular males, 70% - 100% males were produced, and 25% of them should be YY-males. Trails to produce all-male population by crossing the YY-bluegill with regular females are underway. Effects of genotype by temperatures (17, 23, 29, 34°C) on sex ratio were tested also on two batches of fry with different parents. In the first batch, sex ratios did not deviate significantly from 1:1 in 23 °C group ($P > 0.05$); however, sex ratios significantly deviated from 1:1 in 29 °C and 34 °C groups, in which a significantly higher proportion of males (66.67–70.64%) were yielded. The pooled sex ratios were compared to each other also and found that temperature had significant effects on sex ratios in the Batch 1 ($P < 0.001$). The percents of males in 34 °C and 29 °C groups were significantly higher than that in 23 °C and 17 °C groups ($P < 0.05$). In general, the proportion of males increased with an increase in treatment temperature from low (17 °C) to high (34 °C and 29 °C). In the Batch 2, sex ratios were significantly different from 1:1 in groups of 34 °C and 17-to-29 °C, in which a significantly higher proportion of males (58.79%) and females (63.9) were produced, respectively, while no significant deviations from 1:1 were detected in groups of 23°C and 29°C ($P > 0.05$). There was no significant difference ($P>0{\cdot}05$) in sex ratio among thermal groups of 34 °C, 29 °C and 23 °C, while significant difference was detected between 17-to-29 °C group and other three groups in sex ratio. In addition, no significant differences were detected between 17 °C and 17-to-29 °C treatments, 29 °C and 29-to-17 °C treatments ($P > 0.05$). There were significantly higher ($P<0{\cdot}05$) proportions of males in high thermal groups of 29 °C and 34 °C in the 1st batch than in the 2nd batch. In both batches, the proportion of females decreased in low temperature treatment of 17 °C or 17-to-29 °C. We also tested 12835 AFLP loci and screened a considerable proportion of the bluegill sunfish genome, not a single sex-specific marker was found. These results suggest that sex chromosomes may be absent in the bluegill and a sex determining system based on polygenic effects will need to be considered.

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San Diego, USA.

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- **Ohio Agriculture Research and Development Center (OARDC)**
- **The Ohio State University Extension**
- **Ohio Soybean Council**
- **Ohio Sea Grant**

New Publications (continued from page 8)

Estimates of genetic parameters for growth-related traits of yellow perch with one-stage and two-stage selection

Cao, X, H. P. Wang, H. Yao, P. O'Bryant, J. D. Rapp and G. K. Wallat.

Abstract Yellow perch *Perca flavescens* is an important aquacultural and ecological species in the Great Lakes Region and the Midwest USA. As a part of the effort to enhance aquaculture production of yellow perch, we are undertaking a selective breeding program for improving their growth rate. Estimation of genetic parameters for growth-related traits is an essential step in the development of a breeding program.

Twenty-five full-sib families were produced in a pair-mating design. After hatching, each family was stocked into ponds with similar fish number for the nursery phase. Feed-trained fingerlings then were reared into four 0.1 ha ponds using the same family composition and fish number. At the end of year one, 300 fish in each pond were weighed, measured and fin-clipped. Two ponds were graded and the top 50% fish were restocked to two same sized ponds for year-2 grow-out, while the other two ungraded ponds were harvested and restocked to other two ponds. At the end of year two, the same procedures were conducted as for the end of year one. Thirty-four parents and 1165 offspring (600 fish at the end of year one and 565 fish at the end of year two) were genotyped with 8 microsatellite markers. Parentage assignment was performed using Cervus 3.0. Genetic parameters were analyzed using ASREML 2.0 (animal model). Out of the 1165 offspring, 94.08% could be assigned to a single parental pair. Genetic and phenotypic correlations between weight and total length were 0.97 ± 0.031 and 0.96 ± 0.004 for one-year-old fish, 0.93 ± 0.130 and 0.92 ± 0.010 for ungraded two-year-old fish, and 0.98 ± 0.00 and 0.81 ± 0.021 for graded two-year-old fish, respectively. Heritabilities for weight and total length of graded two-year-old fish were much higher than those of ungraded two-year-old fish. In comparison, corresponding genetic parameters of the same groups of fish reared in tanks were also estimated. The results indicated that the two-stage selection might be an effective breeding method. The results of this study would be very useful for the selective breeding program of yellow perch.

Proceedings of the World Aquaculture Society Conference page 170. March 1-5, 2010.

San Diego, USA.

Effects of a nonsteroidal aromatize inhibitor on gonadal differentiation of bluegill sunfish *Lepomis macrochirus*

Ze-Xia Gao, Han-Ping Wang, Geoff Wallat, Hong Yao, Dean Rapp, Paul O'Bryant, Russ MacDonald and Wei-MinWang

Abstract In the present study, the efficacy of Letrozole, a potent nonsteroidal aromatize inhibitor (AI), on gonadal sex differentiation and sex reversal was examined on bluegill sunfish (*Lepomis macrochirus*).

In Experiment 1 with AI diet treatments (50, 150, 250 and 500 mg kg^{-1}) from 30 dph to 90 dph, AI interrupted ovarian cavity formation at a dose of 500 mg kg^{-1} diet and one intersex fish was identified in this group. The proportions of males in all treated groups were significantly higher than in the control group.

In Experiment 2 with AI immersion treatments (250, 500, 1000 µg L^{-1}) during 30 dph to 50 dph, the treated groups of 500 and 1000 µg L^{-1} produced significantly more males than control and 250 µg L^{-1} groups. Histological examination revealed no differences in ovary or testis tissues between control and AI treated fish. There were no significant differences detected in body weight and length among the AI treated and control groups ($P > 0.05$) for both experiments. The results from these two experiments suggest that inhibition of aromatize activity by AI could influence sex differentiation in bluegill sunfish.

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