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

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Inside this issue:

OSU President Visits Aquaculture Facilities	1
O'GIFT Update	1-2
BGAC Highlights	3
Aquaculture Outreach Impacts on the Industry	3
Indoor Spawning of Baitfish	4
Better Genetics Boost Perch Production	5
New Journal Publications	5-6

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.

OSU President E. Gordon Gee visits Aquaculture Programs in Bowling Green and Piketon

On Wednesday, June 25th, The Bowling Green Aquaculture Center (BGAC) conducted its official Grand Opening with a V.I.P. Ribbon Cutting Ceremony. Dr. E. Gordon Gee, President of The Ohio State University, was on hand to cut the ceremonial ribbon and tour the facilities with Shawn McWhorter, BGCA Research Associate and Manager. Special attendees also included State Representative Randy Gardner; State Representative James J. Zehringer; Dr. Bobby Moser, Vice President of Agricultural Administration and University Outreach, and Executive Dean of the College of Food, Agricultural and Environmental Sciences; Dr. Steven Slack, Director of OARDC; Dr. Ted Batterson, Director of the North Central Regional Aquaculture Center; Ohio Department of Agriculture's Director Robert J. Boggs and State Veterinarian Dr. Tony Forshey; Wood County Commissioner Alvin Perkins; and Tom Yingling, Dennis Eisenhour and Bob Calala, representing the Ohio Aquaculture Association.

On July 2nd, Dr. Gee visited the Aquaculture Program at the OSU South Centers in Piketon. Dr. Han-Ping Wang, OARDIP Director, reported to him the achievements and impacts of the Ohio Aquaculture Research and Development Integration Program (OARDIP). Aquaculture Specialist Geoff Wallat and Laura Tiu discussed the development of aquaculture industry in Ohio. Dr. Gee was very interested in the program.

OSU President E. Gordon Gee and Shawn McWhorter perform the ribbon cutting for the Bowling Green Aquaculture Center

OSU President Dr. E. Gordon Gee with Aquaculture Team in Piketon

O'GIFT Program makes significant progress

Ohio Genetic Improvement of Farmed-fish Traits (O'GIFT) Program is a long-term broodstock improvement program being funded by USDA and based at the OSU South Centers. The overall goal of the program is to create, through a long-term selective breeding approach, superior fish broodstocks with traits amenable to commercial-scale aquaculture in Ohio and the Midwest. The O'GIFT Program currently includes two major projects - Integrated genetic improvement of yellow perch broodstock through marker assisted selection and Developing mass production technology of genetically male bluegill populations and broodstock. The O'GIFT program is expected to increase aquaculture production and efficiency of perch and bluegill by 35-50% by developing genetically improved broodstocks.

The O'GIFT Program started in 2004, and is experiencing significant progress. The following are the highlights.

Yellow perch:

- Eight strains of yellow perch were obtained from eight states and stock evaluations in genetic variation and growth have been completed. The results have been published in the journal *Aquaculture*.

continued on page 2

Supported by USDA, the OARDIP with the Ohio State University has established five long-term programs and one new aqua-genetics laboratory aimed at providing outstanding scientific and outreach capabilities to support the continued development of aquaculture in Ohio and the Midwest.

O'GIFT Program *(continued from page 1)*

- Approximately 2,000 genetically superior broodfish were selected as the base breeding population for the long-term selective breeding program.
- Seven improved lines of yellow perch have been established. The data shows that the two improved lines of 2006 grew 28% and 54% faster than controls, respectively. The preliminary data for 2007 progeny shows genetic lines grew 25%-42% faster than controls.
- Approximately 59,000 improved perch fry and fingerlings were distributed to fish farmers.
- The 2nd generation of improved fish from improved lines of 2006 was created in spring of 2008.
- Approximately sixty microsatellite markers have been developed for the marker-assisted breeding program.
- A parentage analysis technique using six molecular markers in yellow perch has been developed for family identification and genetic pedigree.
- Two mapping families have been developed for next year's linkage and quantity trait loci mapping.

Bluegill:

- Three batches of sex reversal experiments have been completed and ~600 sex-reversed females have been generated for developing all-male populations.
- Sex reversed females were successfully induced to spawn. By crossing sex reversed females with regular males, a most-male (25% YY-males and 50% XY males) population has been produced.
- A study on "the effect of estradiol-17β on survival, growth performance, sex reversal and gonadal structure of bluegill" has been completed and results have been published in the journal *Aquaculture*.
- Using AFLP technique, seven sex-specific markers were identified with 64 primer combinations amplified across the female and male genomic DNA pools, respectively. A linkage map for bluegill was constructed and a manuscript on this is in review.
- A study on "gonadal sex differentiation in the bluegill and its relation to fish size and age" has been completed and a manuscript has been submitted to the journal *Aquaculture*.
- An experiment on genotype - environment effects on sex determination is underway.

An Aquaculture Genetics and Breeding Lab established in Piketon

The new Aquaculture Genetics and Breeding Lab (AGBL) is the first lab of its type in the Midwest and crucial to the success of the O'GIFT program.

In this lab, many microsatellite markers have been developed for the marker-assisted breeding program. Genetic relatedness analysis of selected broodfish is performed and large color-coded charts of relatedness are constructed every year. Parentage analysis technique, using eight molecular markers, has been developed and family identification of the selected broodfish is conducted for the breeding program.

Seven sex-specific markers were identified and a linkage map for bluegill was constructed for selective breeding. A histological method for identification of sex reversed fish has been established.

Bowling Green Aquaculture Center Highlights

The Bowling Green Aquaculture Center (BGAC) is housed at the Agricultural Incubator Foundation, located 6 miles north of the city of Bowling Green, Ohio. The Center's main focus has been to conduct research projects on the performance and economic efficiency of commercially-available recirculating aquaculture systems (RAS) and to evaluate the culture suitability of baitfish species, such as the spotfin shiner *Cyprinella spiloptera* and the golden shiner *Notemigonus crysoleucas,* for Ohio aquaculture farms.

Thus far, three research projects have been completed, and results have been presented at several regional meetings. This year, five research projects are planned, including first-feeding trials and comparison of commercially prepared diets for juvenile and adult spotfin shiners.

Additionally, an aquaponics (rearing fish and plants in tandem) research system has been constructed to test the suitability of fish effluent water on the production of a variety of high-value vegetable plants and herbs.

The following are some highlights:

- Three commercial recirculating aquaculture systems (RAS) were run for one year and tested for performance and economic efficiency. All three systems were used to raise largemouth bass and yellow perch in duoculture. All systems produced market size (>140 g) yellow perch in nine months, with one system (AquaCube) producing significantly larger perch and largemouth bass than the other two systems.

- A wild caught broodstock population has been used to produce over 75,000 eggs in 2007. Out of season spawning has also been achieved with this group enabling an additional 3 spawns from the breeders outside their normal breeding season. For 2008, we have conditioned a new group of broodstock, and are producing multiple batches of fry for feed trials and growout studies.

- Specialists worked with a local grower conducting an on-farm research project raising golden shiners and freshwater shrimp in duoculture. The grower was able to harvest over 800 lbs of golden shiners and market at $4 per pound.

The BGAC hosted over 150 visitors in 2007. Over 30 have become regular clients that routinely work with the Center either in starting up their aquaculture businesses or helping to improve their existing production.

For more information on these projects, contact: Shawn McWhorter at 419-823-1807/mcwhorter31@ag.osu.edu Or Geoff Wallat at 740-289-2071 ext.146/ wallat1@ag.osu.edu

Aquaponics research is being conducted at the Bowling Green Aquaculture Center

Aquaculture Outreach Impacts on the Industry

The success of the OARDIP at OSU is in a large part due to its strong foundation in both research and outreach. A pertinent research program must be accompanied by an effective Extension program if the results are effectively transferred to and adopted by the industry.

A website has been developed that enables 24 hour access to information, email list serves for quick dissemination of information, face to face and phone counseling, and workshops designed to educate clients on how to incorporate our research-based information into their businesses.

In 2008, we have completed two workshops and will hold a baitfish culture workshop in Bowling Green on October 25.

In mid-March, we held our biennial Perch School. This workshop is always very popular as it features a variety of hands-on activities. Thirty attendees learned techniques for spawning yellow perch and actually got to practice them on site at our research and demonstration facility.

The evaluations showed that there was a good mix of experienced (30%) and entry-level (70%) fish farmers. All the attendees reported that they gained both knowledge and skill, with 80% planning to apply what they learned. Our favorite quote came from one attendee, who when asked if they could put a dollar figure on what the workshop was worth to their business, replied "Priceless."

In response to requests from stakeholders, we organized a workshop covering the basics of what it takes to produce largemouth bass in Ohio from reproduction to marketing. The workshop was held at the Fisher Auditorium in Wooster, Ohio on April 19th, and attended by 8 largemouth bass producers. Researchers from Kentucky State University, the leading largemouth bass research center in the country, provided the presentations, sharing their culture procedures and recent research findings.

Successful Indoor Spawning May Lead to Ohio Baitfish Industry

(By Candace Pollock, OSU Reporter)

Ohio fishing enthusiasts, who bait their lines with imported shiners, may soon be catching that Lake Erie smallmouth bass, walleye or crappie with a native baitfish, spawned and raised for the first time in an indoor environment.

Ohio State University aquaculturists with OSU South Centers at Piketon have successfully induced the first known indoor spawning of the spotfin shiner and produced juvenile spotfins for the market. The research may mark the beginnings of a modest baitfish industry in Ohio.

According to the latest U.S. Department of Agriculture Census of Aquaculture, Ohio ranks fourth in the nation in baitfish sales, skyrocketing 153 percent from 1998 to 2005. Ohio also ranks fifth nationally in the number of baitfish farms, behind Arkansas, Minnesota, New York and Wisconsin. Ohio imports most of its baitfish from those states.

Geoff Wallat, an OSU South Centers at Piketon aquaculturist, hopes that an Ohio baitfish industry could help reduce some of the dependence on imported baitfish from other states, especially when faced with occasional supply shortages.

"The principal bait used on Lake Erie is the emerald shiner, wild caught from the Great Lakes, including Lake Erie. But there are times of the year (late summer) when the shiner is not available in Ohio, and must be imported. With the VHS (viral hemorrhagic septicemia) quarantine, the fish can't be held long enough for testing before being shipped to Ohio," said Wallat, who holds a research appointment with the Ohio Agricultural Research and Development Center. "So, sometimes we are facing a huge shortage of bait."

Wallat said that researchers began looking at the spotfin shiner because it looks similar to the emerald shiner, and previous studies have indicated that the native fish -- which spawns in the wild in low numbers-- performs well in ponds.

The research, part of the Bowling Green Aquaculture Program and led by research associate Shawn McWhorter, began two years ago. Within a year, researchers had successfully spawned the spotfin shiner beyond its normal summer spawning season, using thermal and light cycles to mimic spawning conditions. By inducing spawning conditions multiple times indoors, researchers have been able to build a robust broodstock. Female spotfins can lay as many as 700-900 eggs in one spawning.

Researchers have disseminated the spawning and development techniques to Ohio fish farmers. They could be raising their own spotfin shiners by 2009.

Ohio State University aquaculturists are also looking at spawning and developing golden shiners for land and inland reservoir fishing. Golden shiners, farm-raised in Arkansas, are also imported in large supply.

"The golden shiner is not well-suited for harvest at warmer temperatures, so Ohio faces shortages when it comes time to harvest the species in June," said Wallat.

Like the spotfin shiner, researchers are spawning the golden shiner indoors, hoping to extend the growing season, thereby creating a niche baitfish market in Ohio.

"By raising golden shiners in Ohio, we can reduce transportation costs and produce high-quality baitfish that last longer in holding tanks and bait stores," said Wallat. "With Ohio being a huge recreational fishing state, there's value in pursuing a baitfish industry in the state."

The research, funded by USDA Cooperative State Research, Education, and Extension Service, will continue for another five years.

Ohio baitfish sales are an integral part of Ohio's aquaculture industry, which has nearly doubled from $1.8 million in 1997 to $3.3 million in 2006, according to the USDA.

Impact

Aquaculture sales in Ohio have nearly doubled from $1,788,000 in 1997 to $3,338,000 in 2006. Ohio Aquaculture is naturally diverse. Nationally, Ohio ranks first in sales of yellow perch for food and is the number one bluegill producing state. Ohio also ranks forth in sales of baitfish and largemouth bass sold for sport, fifth in number of baitfish farms and sixth in sales of hybrid striped bass sold as a food fish. Thirty-nine journal articles and abstracts have been published.

Better Genetics Boost Perch Production

(By Candace Pollock, OSU Reporter)

With the help of genetics, Ohio fish farmers will be able to raise the crème de la crème of yellow perch - the state's No. 1 food fish - with the potential to increase production efficiency up to 50% over current growth standards.

Ohio State University aquaculturists with OSU South Centers at Piketon are analyzing the genetic traits of yellow perch lines then cross-breeding those exhibiting high-growth rates and little genetic similarities. After two years of research, data of first generation crosses have shown the fish grew 28 to 54% faster than the unimproved fish. The goal of the research is to genetically improve broodstocks of yellow perch – a species that lags in growth. "Yellow perch is an important fish species for Ohio's aquaculture industry, but a major problem for the yellow perch industry is the relatively slow growth of currently cultured populations of this species," says Han-Ping Wang, director of the Ohio Aquaculture Research and Development Integration Program. "Our goal is to help farmers raise bigger fish. Bigger fish means more dollars."

Wang and his colleagues are accomplishing this task using a state-of-the-art aquaculture facility and new genetic marker technology that improve upon traditional breeding methods. The Aquaculture Genetics and Breeding Lab at Piketon, the first of its type in the Midwest, enables researchers to track the pedigree of the parents to the offspring so when genetically unrelated fish exhibiting the best growth characteristics are bred, the genetics showcasing those traits can be traced back to the source.

"For traditional selection, to raise 150 fish families, you have to have 150 individual tanks, but that's not possible. With marker technology, all families can be communally raised in the same pond or tank. When fish are raised to harvest size, the best fish or candidates from different families can be identified with parentage analysis by using genetic tags," says Wang. "Inbreeding can be a big problem if fish are being crossed without knowing their genetics. This reduces the growth rate instead of improving the growth rate."

Now, researchers are able to develop genetic charts and compare the genetic fingerprint of the offspring with its parents, so that traits for improved growth remain intact for breeding the next generation. So far, researchers have distributed nearly 60,000 improved perch fry and fingerlings to Ohio farmers using this method.

According to the 2005 USDA Census of Aquaculture, yellow perch accounted for the largest portion of food fish sales in Ohio with 25 farms selling $222,000 worth of fish for an average price of $3.12 per pound. Ohio ranks No. 1 in the nation in sales of yellow perch, and is an integral part of Ohio's robust aquaculture industry.

New Journal Publications

Effects of estradiol-17β on survival, growth performance, sex reversal and gonadal structure of bluegill sunfish *Lepomis macrochirus*

Han-Ping Wang, Zexia Gao, Beatrix Beres, Joseph Ottobre, Geoff Wallat, Laura Tiu, Dean Rapp , Paul O'Bryant and Hong Yao

We systematically investigated the feminization of bluegill *Lepomis macrochirus* by oral administration of various doses of estradiol-17b (E_2) and evaluated their effects on the growth performance, production and gonadal structure of sex-reversed female bluegill at both sex-ratio and histological levels.

With positive control treatment, 30-day-old fry were fed E_2 at 50, 100, 150 and 200 mg kg^{-1} diet for 60 days. The survival of fish in the E_2 treated and control groups were not significantly different ($P > 0.05$). The growth of the treated fish was significantly retarded during the period of treatment, while there was no side effect detected post-treatment and the retarded fish caught up during 120 days of culture after E_2 treatment.

All the treated groups produced 100% monosex female populations based on the macroscopic shape of gonads, and there were no significant differences detected between any E_2 treatment and control group in the mean GSI of females during the spawning season from June to October ($P > 0.05$).

Histologically, 13.3% and 5.0% of the intersex fish were determined to come from the 50 and 100 mg kg^{-1} E_2 treatment groups, respectively, with 6.9% and 4.1% of the gonadal area containing spermatocytes. Most of genotypical male fish treated with exogenous E_2 developed gonadal structures histologically indistinguishable from the gonads of females.

This study suggests that 150 mg kg^{-1} E_2 is the optimal dosage for feminization in bluegill, with 50 and 100 mg kg^{-1} E_2 being sub-optimal and 200 mg kg^{-1} E_2 being over-optimal. ***Aquaculture, 2008, doi:10.1016/j.aquaculture.2008.08.041***.

New Journal Publications *(continued from page 5)*

Yellow perch strain evaluation I: Genetic variance of six broodstock populations

Bonnie Brown, Han-Ping Wang, Li Li, Chandler Givens, Geoff Wallat

As a prelude to strain selection for domestication and future marker assisted selection, genetic variation revealed by microsatellite DNA was evaluated in yellow perch, Perca flavescens, from four wild North American populations collected in 2003–2004 (Maine, New York, North Carolina, and Pennsylvania,), and two captive populations (Michigan and Ohio).

For the loci examined, levels of heterozygosity ranged from He=0.04 to 0.88, genetic differentiation was highly significant among all population pairs, and effective migration ranged from low (Nem=0.3) to high (Nem=4.5). Deviation from Hardy–Weinberg equilibrium was regularly observed indicating significant departures from random mating. Instantaneous measures of inbreeding within these populations ranged from near zero to moderate (median F=0.16) and overall inbreeding levels averaged FIS=0.18.

Estimates of genetic diversity, ΦST, and genetic distance were highest between Michigan and all other broodstock groups and lowest between New York and Ohio. Genetic differentiation among groups did not correlate with geographic distance. Overall, the patterns of variation exhibited by the captive (Michigan and Ohio) populations were similar to patterns exhibited by the other wild populations, indicating that spawning and management practices to date have not significantly reduced levels of genetic variation. ***Aquaculture, 271 (2007) 142–151.***

Isolation and characterization of microsatellites in yellow perch *(Perca flavescens)*

L. Li, H . P. Wang, C. Givens, S. Czesny and B. Brown

A total of 45 microsatellite loci from yellow perch, *Perca flavescens,* were isolated and characterized. Among the 45 microsatellite loci, 32 had more than two alleles.

A wild population of *P. flavescens* (*n*=48) was used to examine the allele range of the microsatellite loci. Mendelian inheritance of alleles was confirmed by examining the amplified products in pair-mated families.

The number of alleles for the 32 polymorphic loci varied from two to 16, and observed heterozygosity ranged between 0.024 (YP79) and 0.979 (YP60). Crossspecies polymorphic amplification in four other Percidae species was successful for 22 loci. ***Molecular Ecology Notes (2007): 600–603.***

Proteomic analysis of proteins associated with body mass and length in yellow perch, *Perca flavescens*

John Mark Reddish, Normand St-Pierre, Andy Nichols, Kari Green-Church and Macdonald Wick

The goal of commercial yellow perch aquaculture is to increase muscle mass which leads to increased profitability. The accumulation and degradation of muscle-specific gene products underlies the variability in body mass (BM) and length observed in pond-cultured yellow perch.

Our objective was to apply a combination of statistical and proteomic technologies to identify intact and/or proteolytic fragments of muscle specific gene products involved in muscle growth in yellow perch.

Seventy yellow perch randomly selected at 10, 12, 16, 20, and 26 wk of age were euthanized; BM and length were measured and a muscle sample taken. Muscle proteins were resolved using 5–20% gradient SDS-PAGE, stained with SYPRO® Ruby and analyzed using TotalLab™ software. Data were analyzed using stepwise multiple regression with the dependent variables, BM and length and proportional OD of each band in a sample as a potential regressor. Eight bands associated with BM ($R2 = 0.84$) and nine bands with length ($R2 = 0.85$) were detected. Protein sequencing by nano-LC/MS/MS identified 20 proteins/peptides associated with BM and length.

These results contribute the identification of gene products and/or proteolytic fragments associated with muscle growth in yellow perch. ***Proteomics 2008, 8, 2333–2343.***

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