

Nearly 70% of the \$1 billion dollar domestic aquaculture industry is located in the southeastern United States. One of the most important programs supporting this critical sector of southern agriculture is the USDA-CSREES Southern Regional Aquaculture Center (SRAC). The Center is the only research-funding program with the flexibility to stay abreast of industry development, identify problems on a region-wide scale, and implement cooperative, interstate projects to solve those problems.

SOUTHERN REGIONAL AQUACULTURE CENTER

ARKANSAS KENTUCKY TEXAS NORTH CAROLINA FLORIDA
LOUISIANA VIRGINIA ALABAMA SOUTH CAROLINA TENNESSEE
MISSISSIPPI OKLAHOMA GEORGIA VIRGIN ISLANDS PUERTO RICO

Nineteenth Annual SUMMARY OF PROJECTS

WHAT IS SRAC? . . . SRAC is one of five Regional Centers established by Congress and administered through the USDA Cooperative State Research, Education, and Extension Service. The thirteen states and two territories included in the Southern Region are listed in the masthead, above. Mississippi State University serves as the Host Institution for SRAC, and the Administrative Center is located at the Thad Cochran National Warmwater Aquaculture Center, Stoneville, Mississippi.

SRAC provides a mechanism for identifying and solving problems. Priority research and education needs for the Southern Region are identified by the Industry Advisory Council, which consists of aquaculture industry representatives from throughout the region, and the Technical Committee, which consists of research and extension scientists. These two groups recommend project areas to the SRAC Board of Directors, which selects projects with the highest priority for development and funding. The best scientific talent in the region is then brought together to address the problem.

IMPACT . . . In the past year, four research projects funded at nearly \$2 million were in progress. The Center's "Publications" project is in its eleventh year of funding and has produced 179 fact sheets with contributions from 164 authors from throughout the region. All publications are available at the SRAC web site (see box below).

SRAC research has led to many technologies that benefit the aquaculture industry. For example, many people believe that hybrid catfish produced by crossing the female channel catfish with the male blue catfish is a superior fish for catfish aquaculture. However, hybrid eggs and fry are difficult to produce and new breeding technologies are needed to allow the promise of this fish to be realized. Scientists are cooperating in a SRAC project to improve the efficiency of hybrid production and allow economical delivery of the hybrid technology to the catfish industry. At the beginning of this project, only about 4 to 5 million hybrid catfish fry were being hatched per year. Research results from this project have been important in increasing hybrid catfish production to more than 25 million fry hatched in 2006.

This report summarizes these projects and others currently funded by SRAC.

For further information on the Southern Regional Aquaculture Center, fact sheets and detailed reports of the results of SRAC projects, visit the SRAC web site at <<http://www.msstate.edu/dept/srac>>.

Publications, Videos and Computer Software

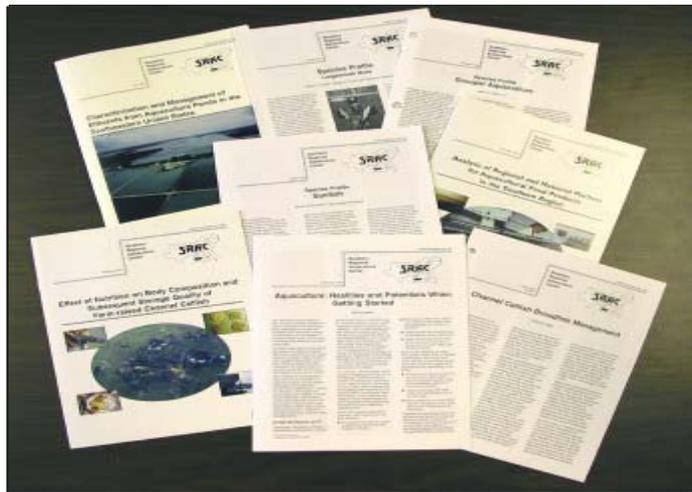
THE CHALLENGE . . .

Aquaculture currently produces almost half the world's seafood supply and is one of the most rapidly expanding agricultural industries in the world. Domestic aquaculture production is centered in the southeast region, where more than 100 species of fish, shellfish, aquatic reptiles, and plants are cultured for food or ornamental purposes. The total farm value of southeastern aquaculture is over one billion dollars, with a total economic impact exceeding five billion dollars. Aquaculture is a young, unique, and rapidly expanding industry, and the need for information to sustain growth and development has increased dramatically over the past 25 years.

OUR RESPONSE . . .

Extension and research scientists in the southeastern United States developed this project to produce research-based fact sheets, videos, and other educational materials to support regional aquaculture education, production, and marketing. The SRAC publication project uses a region-wide pool of experts to develop materials for distribution through the nationwide network of educators, Extension Specialists, County Agents and the World Wide Web. This

process makes efficient use of personnel and funds at the State level, and results in timely, high-quality educational materials. Each publication contains understandable, factual information that provides guidance for producers,



processors, consumers, students, or investors. Subject matter includes biology and life history of specific culture species, culture techniques and systems, nutrition, water quality and waste management, disease treatment, consumer education, marketing, and much more.

PRINCIPAL ACCOMPLISHMENTS . . .

The Southern Regional Aquaculture Center has now published 179 fact sheets, 4 project reports, 19 research reports, and 20 videos. These publications provide essential information for aquaculture producers, lending agencies, and consumers of aquaculture products. Educators in high schools and colleges use SRAC

publications in classrooms throughout the United States and the world. In the months from January through September 1, 2006, a total of 85,204 fact sheets were downloaded from the SRAC web site. One of the most popular series of SRAC publications is the "Species Profiles," which provides detailed technical information on the biology and culture of marine and freshwater fish with commercial potential. Among the species included in the series are yellow perch, grouper, cobia, large-mouth bass, southern flounder, queen conch, and sturgeon.

Eight publications were completed this year, with one project summary, three fact sheets and two DVD's in progress. These publications were developed by 16 scientists associated with the following institutions and agencies:

- Harbor Branch Oceanographic Institute
- Kentucky State University
- Louisiana State University
- Mississippi State University
- North Carolina State University
- Texas A&M University
- University of Florida
- University of Georgia

To download fact sheets, go to <<http://www.msstate.edu/dept/srac.edu>> or <<http://srac.tamu.edu>>. ❖

Innovative Technologies and Methodologies for Commercial-scale Pond Aquaculture

THE CHALLENGE...

Aquaculture operations in the southern region of the United States are finding it difficult to maintain profitability. Production costs are increasing, but the prices that producers receive for fish, shrimp, and other cultured aquatic animals are not keeping pace. The problems are especially troublesome for channel catfish farming, the major aquaculture activity in the region. Increasing profitability of channel catfish farming is a long-term, complex, multifaceted problem. Nevertheless, methods for reducing production costs would provide an immediate improvement in profitability.

OUR RESPONSE...

Eleven research scientists from seven institutions have joined in a four-year project to investigate new technologies and methodologies to improve the efficiency and enhance the profitability of aquaculture in the Southern Region. The scientists represent the following institutions:

- Auburn University
- Clemson University
- Louisiana State University
- Mississippi State University
- University of Arkansas at Pine Bluff
- USDA-ARS (Pine Bluff, Arkansas)
- USDA (Stoneville, Mississippi)

Several possible methods for improving efficiency and profitability of aquaculture are under investigation:

- Evaluation of new production systems and improvements in existing production systems for channel catfish;
- Improvement in equipment used for mechanical aeration and for fish harvesting in channel catfish culture;
- Evaluation of energy, material, and economic efficiency of production systems.

PRINCIPAL ACCOMPLISHMENTS...

Several variations of the partitioned aquaculture system (PAS) concept are being evaluated and all show some promise for commercialization. The PAS concept is based on physically dividing the pond into sections for holding fish and

treating waste produced during culture. The two sections are hydraulically connected by water flowing between the two systems. Research at Clemson University showed that the PAS has promise for accelerating the growth of channel catfish fingerlings and appears to have potential for commercial use. A less intensive approach, called the semi-confinement system, also appears to have potential for use in channel catfish fingerling production.

Maintenance of water quality in most pond aquaculture systems depends on plant growth (autotrophy). However, under super-intensive culture conditions, autotrophy cannot provide adequate waste treatment and is increasingly supplemented by microbial-based (heterotrophic) processes. A heterotrophic, biofloc system was tested by USDA scientists at Pine Bluff, Arkansas, and successfully produced channel

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Improving Reproductive Efficiency to Produce Channel × Blue Hybrid Catfish Fry

THE CHALLENGE...

Catfish farming needs to be more competitive and profitable in today's economy. Inefficiencies occurring at all phases of production need to be eliminated. Problems include high mortalities during the fry and fingerling production phase, as well as diseases and stress from poor water quality throughout the production cycle. Additional inefficiencies result when market-size fish evade harvest and continue to grow.

Application of the hybrid channel catfish female × blue catfish male could alleviate these problems, making catfish farming more competitive, sustainable and profitable. However, hybrid eggs and fry are difficult to produce. Technologies applicable to small-scale production have been developed, but they need to be improved to allow large-scale adoption of the hybrid.

OUR RESPONSE...

Nine scientists at five institutions are conducting research to improve the hatching rate of channel × blue hybrid catfish embryos and to improve the number of hybrid fry produced per weight of brood stock to allow economical delivery of the hybrid technology to the catfish industry.

The project focuses on four goals to address the problem of

poor hybrid egg hatching:

- Develop brood stock selection and management protocols to optimize channel × blue hybrid embryo production.



- Develop induced spawning techniques and management strategies to optimize gamete collection and storage.
- Develop techniques to identify, assess and improve gamete quality.
- Develop economically viable, standardized hatchery procedures and fertilization protocols to optimize hatching rate of hybrid embryos.

Attaining these objectives will result in techniques to induce spawning of brood stock to produce high quality hybrid embryos with improved hatch rate. The impact of wide-scale adoption of the hybrid should increase efficiency, productivity, sustainability and profitability in the catfish industry.

The project began on April 1, 2004. The following research institutions are involved:

- Auburn University
- Louisiana State University

- Mississippi State University
- University of Memphis
- USDA/ARS

Harvest Select Farms, Inverness, Mississippi has also provided resources and research facilities for this project.

PRINCIPAL ACCOMPLISHMENTS...

Temperature. A temperature × time model was developed to help predict the optimum time to initiate artificial spawning to produce hybrid fry. Channel catfish begin to spawn at approximately 100 degree-days after the pond water temperature reaches 21°C. This may be the appropriate time to initiate artificial spawning to produce

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Improving Reproductive Efficiency to Produce Channel × Blue Hybrid Catfish Fry (continued from page 4)

hybrid catfish eggs. The degree-hour response time is not linear over temperatures ranging between 24 and 28°C when using the hormone LHRHa to induce ovulation in channel catfish females. The degree-hour response time is longer at cooler temperatures and females ovulate faster at higher temperatures. This is important because reasonable work schedules can now be formulated for commercial-scale production. Early spawning can be accomplished by heating water prior to the natural spawning season without any difference in success compared to the natural spawning season. If warm water is available, channel catfish can be spawned as early as the first of January.

Ovulation rate and number of eggs released increases with increasing temperature. Hatch rate of hybrid embryos is improved if LHRHa-injected channel catfish females are stripped within 2 hours of first observed egg release. Waiting longer will increase the number of eggs stripped, but this is more than offset by much lower hatch rate.

Brood stock nutrition. Feeding



standard 32% protein floating catfish feed 6 days per week for 2 months prior to spawning gives equal or better fry production than feeding high-protein diets. Supplemental feeding with liver was detrimental to fry production. Supplementation of brood fish diets with menhaden fish oil and the long-chain, polyunsaturated fatty acids docosahexaenoic acid and arachidonic acid for 2 months prior to spawning can increase hybrid fry output 33% to 100% depending upon the initial condition of the females. Availability of forage fish, even at low levels, improves hybrid fry production.

Brood stock genetics. Strain of male blue catfish and/or strain of channel catfish female affected sperm production, hatching rate of hybrid embryos and total fry production. Genotype-environment interactions were also observed for sperm production. Utilization of genetic variation has the potential to double efficiency and productivity of hybrid embryo production. Use of crossbred channel catfish females did not improve hybrid fry production.

Selection for body weight or inbreeding in channel catfish reduced hybrid fry production in some, but not all lines of channel catfish.

Induced spawning. At one location, no significant differences were observed between LHRHa, carp pituitary extract, and catfish pituitary extract to induce spawning for production of hybrid catfish fry. At a second location LHRHa was superior to carp pituitary extract, other forms of GnRH, and



ovaprim for producing hybrid catfish fry.

Implants of LHRHa at a rate of 100 µg/kg generates the most consistent and the greatest number of fry per weight of female. At the end of the season this dose needs to be reduced to 75 µg/kg. Early in the season latency of time from initial introduction of the LHRHa until the time of ovulation for implants is longer than that for injections, but later in the season, latency is the same for both injections and implants. Ovulation of individual females in aquaria or bags resulted in greater fry production

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Improving Reproductive Efficiency to Produce Channel × Blue Hybrid Catfish Fry (continued from page 5)

than females mass-ovulated in tanks. Indirect exposure of channel catfish females to the scent of channel catfish males increases number of hybrid fry produced per kg of female. LHRHa implants had minimal, but positive effects on blue catfish male reproduction.

In general, plasma estradiol, plasma testosterone, cathepsins D and L and mean egg sizes of channel catfish females increased from May/June of one year and



then plateaued at various time periods until spawning in May of the second year. Activity of cathepsin B was variable from month to month, and mean protein content of eggs was highest in October when eggs appeared and decreased for the remainder of the year (November through April) when eggs were present. These measurements may allow screening of females most likely to produce high-quality eggs. No large differences in these variables were observed among four strains during each month.

Gamete quality. Spectrophotometric assays were used to determine sperm concentrations from

crushed testis of catfish. Using this tool should result in more efficient use of sperm, and more consistent fertilization rates. The anterior testis of channel catfish produced more sperm and more concentrated sperm with better motility than the posterior testis. This relationship should hold true for blue catfish testis and will be tested. Increased sperm concentrations gave increased fertility, and fresh sperm had almost double the fertilization rate of frozen sperm. Sperm concentrations can be reduced in currently used fertilization protocols by 100-fold, with little reduction in subsequent hatch rate.

Automated transparency scanners imaged catfish oocytes and embryos during oocyte maturation and embryogenesis, respectively. Animations of time-lapse image stacks revealed a surprising amount of cell movement in cleavage stage embryos. Other details of embryonic development included gastrulation/epiboly, neurulation, initiation of motility, and hatching. Arrested development and subsequent cytolysis of abnormal embryos could also be clearly documented, including the developmental events prior to arrest and death. Cleavage-arrested embryos continued to show movements in spite of failed development. Developmental arrest

is not necessarily followed immediately by cytolysis and death. The cause of this developmental arrest needs to be ascertained and corrected. Hopefully, this can be adapted for practical application of predicting egg and embryo quality. Water activated, but unfertilized, eggs showed the characteristic movements seen previously in normally fertilized embryos. Blastodisc enlargement and protrusion also took place mimicking normal development, however, none of the activated, unfertilized eggs underwent gastrulation or cleavage.

Ultrasound was able to identify ovarian development differences between females that ovulated and those that did not following injections of LHRHa. However, no predictive differences were observed prior to injection. After injection, use of ultrasound enabled identification of females that were at the correct stage of ovulation to allow stripping of eggs.

Hatchery practices. Various chemotherapeutants were tested

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Feed Formulation and Feeding Strategies for Bait and Ornamental Fish

THE CHALLENGE...

Commercial production of baitfish and tropical ornamental fish represents economically valuable components of the U.S. aquaculture industry. Market sizes of bait and ornamental fishes are relatively small and specific sizes are needed for specific purposes, so that repeated grading and handling during production are often required. After harvest, fish must withstand the additional demands of distribution and sales, and must survive for extended periods. Significant losses occur when fish are transported on trucks from the production facilities to distribution sites. A combination of handling stress and suboptimal environmental conditions can result in high mortality when fish are transferred between facilities. Therefore, effective management practices that enhance stress resistance and prolong survival of bait and ornamental fishes are critically needed.

OUR RESPONSE...

Six scientists from four institutions will collaborate to develop diets and feeding practices that enhance stress resistance and prolong survival of bait and ornamental fishes. Scientists from the following institutions are collaborating in this project:

- Texas A&M University
- University of Arkansas at Pine Bluff
- University of Florida
- University of Georgia

The project addresses the following objectives: 1) manipulation of diet composition and feeding strategy for economical production of “jumbo” golden shiners; 2) manipulation of diet composition and feeding strategy to increase immuno-competence and resistance to stress in bait and ornamental fish during production, transport and live display; and 3) determination of the relative contribution of natural foods and prepared diets to growth, response to low dissolved oxygen, and other health indices for bait and ornamental fish in different production systems.

PRINCIPAL ACCOMPLISHMENTS...

The overall goal of this project is to assess changes in diet composition and feeding strategies on the growth, health, and body composition of freshwater baitfish and ornamental fish.

Feeds and feeding for “jumbo” golden shiners. There is an unmet demand for large baitfish (“jumbos”) and there is currently no good way to produce these fish in one year. Our first task was to determine the best stocking density for juvenile golden shiners to maximize the production of jumbos (larger than 12g) within a single growing season. In this trial, golden shiner



juveniles were stocked at four densities and grown for 105 days while being fed once daily with a commercial 42% protein feed. Average fish weight decreased with increasing fish density, but gross yield (total pounds of fish produced) increased with density. Survival was not different among treatments. Fish stocked at 30,000/acre resulted in about 54% jumbos by weight. Stocking juvenile golden shiners in late July resulted in lower single-season yields of jumbos compared to direct stocking of hatchery fry at low densities. Direct stocking of fry in early May resulted in higher

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yield of jumbos in a single season. However, the extra production of jumbos must be balanced against other uses for the ponds.

In a second trial, we are evaluating the effects of diet composition and feeding frequency on the growth and production of golden shiners stocked at 30,000 fish/acre. Fish are being fed either once or twice daily with one of two diets (42% protein diets with or without fish meal). The most recent data shows that feeding frequency is affecting fish weight (fish fed twice daily are larger), but there is no effect of the different diets on growth.

Feeds and feeding to enhance health of bait and ornamental fish. Multiple feeding trials with dietary additives that may stimulate fish health have been attempted at Texas A&M University and the University of Arkansas at Pine Bluff. Methodological problems have hindered progress, but overall there have been few pronounced effects of these feed additives on general performance (growth, survival, feed conversion) of golden shiners in tanks. Better methods of measuring immune and stress responses of small fishes must be developed to assess these diet additives fully, and concentrated efforts are being made in this area. One promising technique is the measurement of cortisol (a stress hormone) in the whole body of the fish instead of the blood. Also, some of the feed additives may be more effective in

protecting the fish against specific pathogens (such as the bacterium that causes columnaris disease), rather than enhancing the general immune response. The physical form of diets used may also need adjustment. We are using cold-pelleted diets, but in commercial production extruded (floating) pellets are produced using steam. Diet form as well as composition can affect experimental results, so this is another area for additional research.

We have also examined the effects of diets with 4 or 10% lipid (fat) and diets with or without fish meal on performance of golden shiners in tanks. Previous studies showed some beneficial effects of high lipid levels in diets for golden shiner and goldfish. However, in this trial there were no differences in growth, survival, or whole-body lipid of fish fed diets with 4 or 10% lipid. In general, feed conversion of fish fed 10% lipid diets was higher than that of fish fed 4% lipid diets, except for fish fed diets with a prebiotic (Grobiotic®-A). It is likely that fish did not respond to higher dietary lipid in this study because they were larger at the beginning of the trial (larger fish grow more slowly), and the diets were lower in protein than previous studies. Therefore, more protein may have been used for energy instead of tissue growth, and the benefits of extra lipid were not realized. There were no major differences in performance of fish fed diets with or without fish meal, consistent with previous studies. Removal of fish meal from

production diets for baitfish would reduce cost.

We also performed a bacterial challenge on groups of golden shiners fed the standard diet (4% poultry fat), a 10% poultry fat diet, or a diet with both 10% poultry fat and 2% Grobiotic®-A. The Grobiotic®-A is a prebiotic, a non-living product containing indigestible carbohydrates that stimulate “good bacteria” in the gut. Prebiotics can enhance fish performance under stress, such as exposure to pathogens. Golden shiners fed the diet with Grobiotic®-A had higher survival than fish fed the other diets when exposed to the bacteria that cause columnaris disease. This is a significant pathogen of golden shiners and other bait and ornamental fish.

At the University of Georgia, whole-cooked soybeans are being compared to soybean meal in diets for golden shiners, feeder goldfish and fathead minnows. During initial trials in aquaria, it was necessary to increase the salinity of the water to 3 parts per thousand using artificial sea salts to get good survival of golden shiners. All remaining trials in aquaria will be conducted at this higher salinity. Goldfish and fathead minnow trials are in progress, and pond trials will begin after the aquarium trials have been completed.

Contribution of natural foods to growth and health of bait and ornamental fish. It is difficult to separate the effects of

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Feed Formulation and Feeding Strategies for Bait and Ornamental Fish (continued from page 8)

prepared diets and natural foods in outdoor systems (pools, ponds) on performance of fish, but most commercial production of bait and ornamental fishes is in outdoor systems. Studies are underway to determine the best diets to use in ponds where bait and ornamental fish will have access to both food sources.

In three feeding trials conducted in recirculating systems at Texas A&M with golden shiners, we were unable to maintain enough natural productivity to assess the relative contributions of natural and prepared foods to fish performance. A modified culture system has been developed and is currently being tested for this purpose.

A feeding trial in outdoor pools is in progress at the University of Arkansas at Pine Bluff using the same diets described in Objective 2 (diets with or without Grobionic®-A, with or without fish meal, or with 4 or 10% lipid). We are measuring chlorophyll *a* in the pools to assess algal abundance (indicator of natural foods). At 4 weeks, there were no differences in weight gain among treatments and survival has been good. The

feeding trial will last a minimum of 8 weeks, or as long as water temperature will support active daily feeding. Growth, survival, feed conversion, body composition and health indices will be determined to assess performance of the fish.

Feeding trials at the University of Florida were conducted with ornamental fish species (swordtails and zebra danios). There was a significant difference in the growth and perhaps survival of zebra danios produced in ponds receiving treatments of liquid fertilizer, cottonseed meal, an unprocessed meal diet, and a processed (pelleted and reground) diet. A significant difference in primary productivity (algae, based on chlorophyll *a*) was also observed. Zebra danios fed an unprocessed and a processed diet in tanks also demonstrated a difference in growth. The low dissolved oxygen stress test was inconclusive, and other protocols are needed to assess stress in these species also.

Other preliminary results include an apparent difference in growth, survival, and production (i.e. number of offspring produced) of swordtails based on pond trials

with the 4 treatments. The data also have suggested the need for another study which would classify and quantify secondary productivity in these ponds (i.e. crawfish, tadpoles, insects, etc.) based on the different treatments. Studies for unprocessed and processed diets in tanks for swordtails are in progress. Based on this data, several producers have altered their stocking densities and feeding regimes for zebrafish and swordtails. The producers were enticed by the rapid growth and high survival rates demonstrated by feeding a processed diet twice daily.

Additional studies are in progress to address project objectives, but preliminary results indicate that there is good potential to improve production efficiency, product quality, and marketability of bait and ornamental fishes through changes in diet composition and feeding strategy. ❖



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catfish under experimental conditions. Further testing is needed to ascertain the commercial possibilities of this system. At Stoneville, Mississippi, USDA scientists tested a motor-powered U-tube aerator and confirmed that the device is highly efficient in moving water, but the oxygen transfer rate must be improved through design modifications. Research at Auburn University

has focused on developing indicators of economic and resource use efficiency. These indicators will be further refined in the next project year. ❖



Improving Reproductive Efficiency to Produce Channel × Blue Hybrid Catfish Fry (continued from page 6)

to improve egg hatching success. Hatching success was high in the untreated controls (82.8%) and highly variable within treatments. A tendency toward increased hatching success was observed among eggs treated with 100

ppm formalin (87.7%), 100 ppm iodine (88.1%), and 2.5 ppm copper sulfate (87.0%). The frequency of formalin treatments should be three times per day to maximize hatch rate of hybrid embryos and four treatments per

day is excessive. At 28°C, hybrid embryos are chemically sensitive to formalin between 42 to 46 hours post-fertilization, and formalin treatments should be avoided during this period to maximize hatch rate. ❖

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