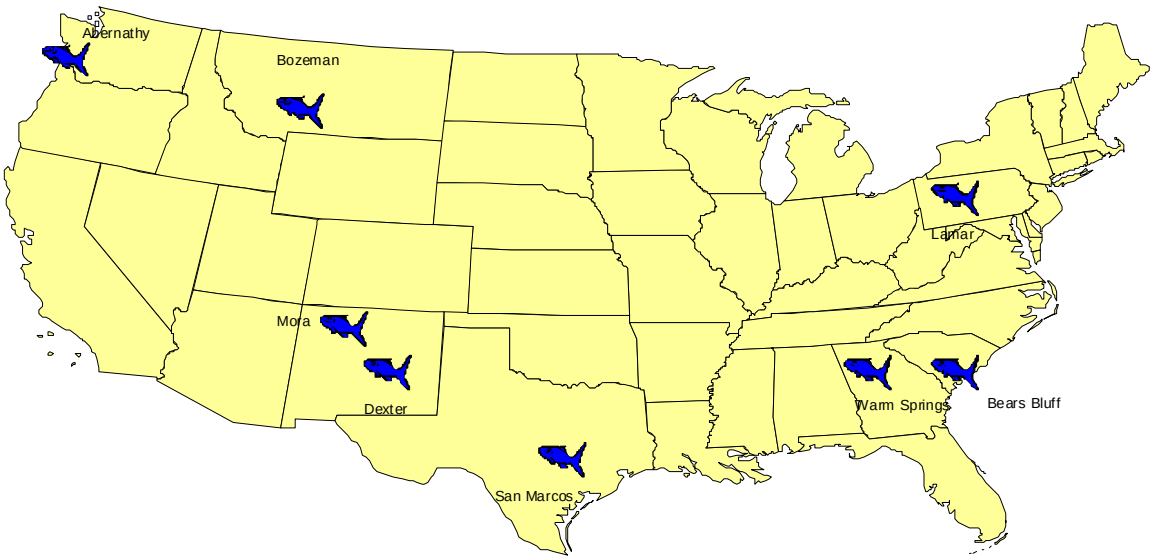
Warm Springs Fish Technology Center

Background and Overview:

Fish Technology Centers were established in 1965 to provide leadership and guidance to the fish culture community. Over the years fish culture studies focused on reducing costs, enhancing fish quality, and improving overall fish culture operations. The importance of Fish Technology Centers became clear as fisheries program managers became increasingly aware of the need to produce fish that were healthy, genetically diverse, and well-adapted to fisheries management objectives. As the Service expanded its vision under the umbrella of ecosystem management, Fish Technology Center roles have correspondingly grown. Areas of specialty now include technical support for fisheries resource programs such as interjurisdictional fishes, estuarine and riverine fishes, non-indigenous aquatic nuisance species, threatened and endangered species, and other emerging high priority aquatic resource needs. To accomplish their work, each Fish Technology Center maintains at least two areas of technical expertise. Functioning as a cohesive system, each Fish Technology Center strengthens the others, taking full advantage of various geographic differences to ensure that study results will successfully support a broad array of users. Through their partnership role with other Service arms and federal agencies, states, tribes, and the private sector, Fish Technology Centers provide a vital link in the Service's commitment to conservation of our nation's aquatic resources.

Fish Technology Centers



The Warm Springs Fish Technology Center (FTC) was established in 1993, to strengthen technology development within the Southeast Region and support other program areas through outreach from the Warm Springs Regional Fisheries Center. Since its inception, the FTC has continued to support the wide array of aquatic resource needs in the Region and the nation. The scope of the operations expanded in 1995 and the Center was charged with strengthening Service capabilities in coastal and riverine fisheries programs. Along with this charge came the responsibility and resources to develop the Bears Bluff NFH, South Carolina, as a unit of the Fish Technology Center. Accordingly, the role of the Fish Technology Center expanded and now includes program development targeted at interjurisdictional riverine and estuarine fisheries.

FTC Vision & Prospectus:

The technical focus areas of the Technology Center continued to expand, continuing to advance new technologies, especially cryopreservation and genetics. The repertoire of capabilities continues to advance, and Fish Technology Center staff envisions the following four major focus areas to be the strength of their program:

1) Fish /Aquatic Species Culture Development
   - interjurisdictional coastal/riverine fishes
   - native fishes
   - imperiled species
   - amphibians
   - other Regional high-priority species

2) Cryopreservation
   - development of new protocols
   - archiving endangered species diversity
   - enhancing hatchery spawning capabilities

3) Conservation Genetics
   - assessing population viability and stock structure
   - molecular tag development
   - assessing risks associated with hatchery propagation/supplementation

4) National Fish Strain Registry
   - assembles performance information on wild populations
   - assembles characteristics (e.g., phenotype and genotype) of aquatic domestic broodstock
   - develop a functionally integrated web-based program for customer value

Cryopreservation Laboratory Highlights

MOU with USDA's NAGP

In FY 2008, the U.S. Fish and Wildlife Service entered into a Memorandum of Understanding with the U.S. Department of Agriculture's National Animal Germplasm Program (NAGP) for the storage of cryopreserved sperm samples. The agreement allows the Service to ship cryopreserved samples to the NAGP as an insurance policy against a catastrophic event and the loss of samples at Warm Springs. The NAGP is a state-of-the-art cryopreservation facility that houses cryopreserved samples of the countries most important agricultural species. The Service retains the rights to all of the samples shipped to the NAGP, and requests for usage of samples from the NAGP must go through an authorization procedure prior to removal.

In May and June of 2008, the first samples from the Service were shipped to the NAGP. In May, 277 straws of robust redhorse (*Moxostoma robustum*) sperm were shipped to the NAGP. These samples were collected from fish from the Savannah River in 2004. In June, an additional 788 straws of robust redhorse sperm were shipped from fish collected from the Oconee River in 2004-2006. In future months, the Warm Springs Fish Technology Center plans to send more samples to the NAGP to ensure the safety of cryopreserved sperm from endangered species.

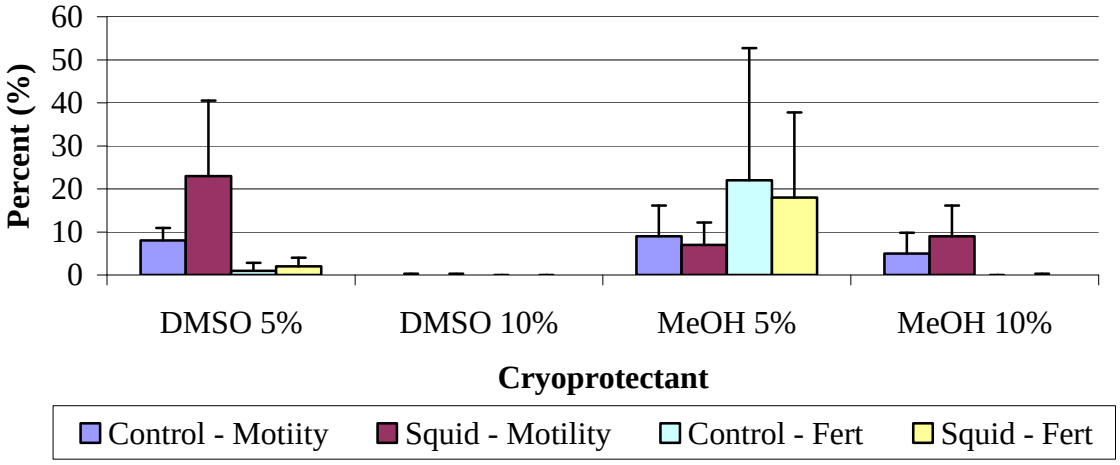
Warm Springs Fish Technology Center's Cryopreserved Sperm Repository. The repository currently holds over 14,000 straws from 16 different species.

Shortnose sturgeon cryopreservation

A study investigating the effects of diet on shortnose sturgeon sperm cryopreservation was continued in 2008. Results from 2007, showed no differences between the diets tested, but it was believed that the fish were not on the squid meal diet for long enough to have an effect. The fish were fed the diet for an additional year to see if differences could be found this year.

Sperm were collected from 3 males from each of the diet treatments. In an effort to minimize differences between groups, not attributable to diet, sperm concentrations for each sample were adjusted to 7.46 x 10^8 cells/ml. Sperm were cryopreserved using methanol (MeOH) and dimethyl sulfoxide (DMSO) at concentrations of 5% or 10% in 0.5-ml straws. The straws were frozen on a 1" styrofoam platform floating on liquid nitrogen. After 24 hours of storage, the straws were thawed for 9 sec in a 40 ^{o}C waterbath, and used in a fertilization trial.

Overall, there were no significant differences ($P > 0.05$) for sperm cryopreserved from fish fed the control diet or the squid diet. Sperm cryopreserved with 10% DMSO for either diet had significantly lower ($P = 0.0477$) post-thaw motility than did sperm cryopreserved with 5% DMSO on the squid diet. There were no significant differences ($P = 0.2535$) for fertilization rates among the treatments.

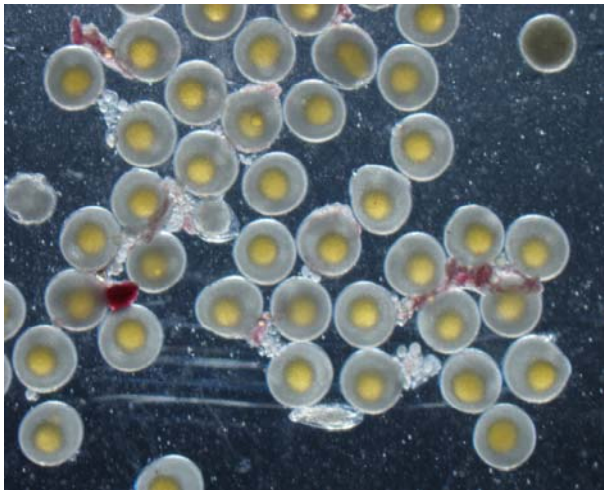


Striped bass spawning

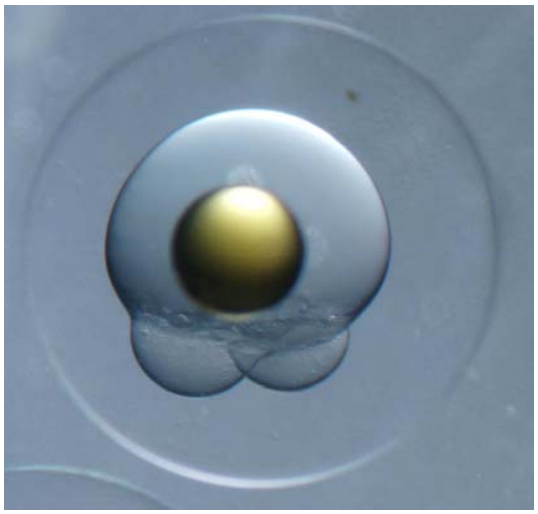
The Warm Springs Fish Technology Center began efforts to tank spawn domesticated Gulf strain striped bass broodstock in 2002. If successful, the project would enable a wider diversity of Gulf strain striped bass to be used and would help to supplement production during years when insufficient wild broodstock are able to be captured.

During November of 2007, female broodstock were received from North Carolina State University. Fish were fed a commercially available striped bass broodstock diet from Zeigler Bros. However, the fish went off feed for approximately 6 weeks after arrival at Warm Springs. The water temperature was gradually reduced to 5 ^{o}C to simulate water temperatures encountered in the wild. In March and April, the temperature in the tanks was gradually raised to 16 ^{o}C to stimulate the fish to spawn.

A total of 41 fish (15 females and 26 males) were selected for spawning. Oocyte sizes were determined by inserting a canula into the urogenital opening and collecting a small quantity of eggs from the ovary. The oocytes were transferred to a 32-well plate containing 0.9% NaCl solution. The oocyte diameter was then measured using a microscope and eyepiece reticle. A fish that was eligible for spawning typically had oocytes >1000 µm. The selected females had a mean oocyte diameter of 1044 µm. All fish were implanted with 40-60 ug/kg bodyweight Ovaplant implants. All of the males were spermiating at the time of implantation and continued to spermiate throughout the 3-week spawning period.


Striped bass oocytes

Of the 15 females that were selected for spawning, 13 females spawned and produced fry. The remaining two females died prior to spawning. Only two of the females that spawned required an injection of HCG to initiate spawning. Three of the females spawned during the night, and most of their eggs were missed due to the egg collection devices overflowing. A total of 8,515,594 eggs were collected, and produced 2,244,868 fry. A 90% increase in fry production over last year.


Striped bass egg shortly after fertilization (2-cell stage).

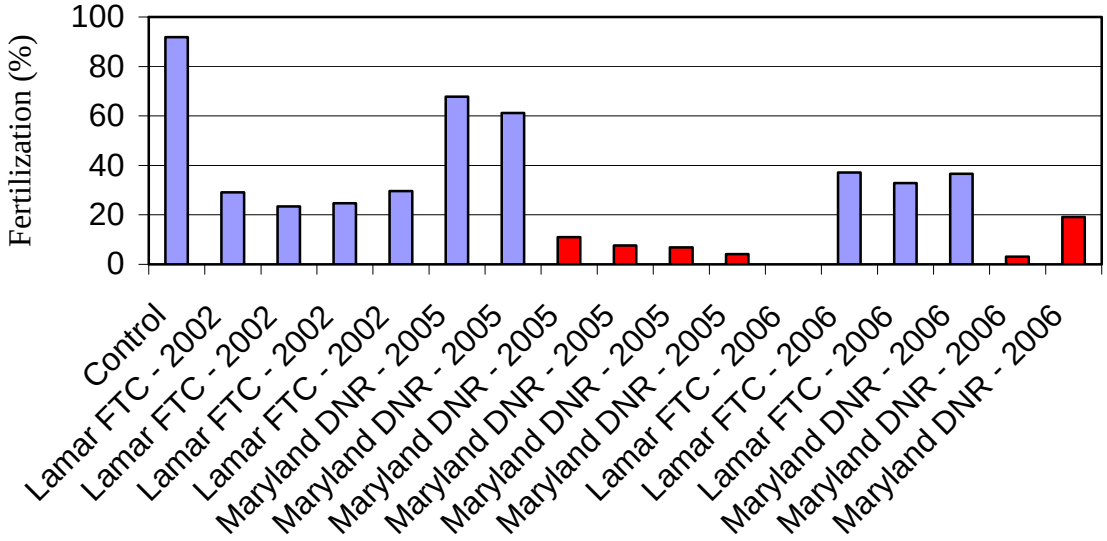
Unfortunately, nearly all of the fry died within 3-4 days after hatching. The fry were sluggish and did not swim well. Consultations with North Carolina State University led to the theory that the period of time that the fish were off feed may have contributed greatly to the loss of fry. This period is essential for the deposition of yolk into the eggs, and being off feed probably resulted in poorly nourished fry during incubation. The project will be continued next year using the same females.

Striped bass fry.

Atlantic sturgeon cryopreserved sperm evaluation

Atlantic sturgeon sperm cryopreservation research has been conducted at the FTC over the last several years as opportunities have arisen. Sperm has been cryopreserved using various extenders, cryoprotectants, and freezing rates. Unfortunately eggs have never been available to conduct fertilization trials to determine the effectiveness of the cryopreservation procedures.

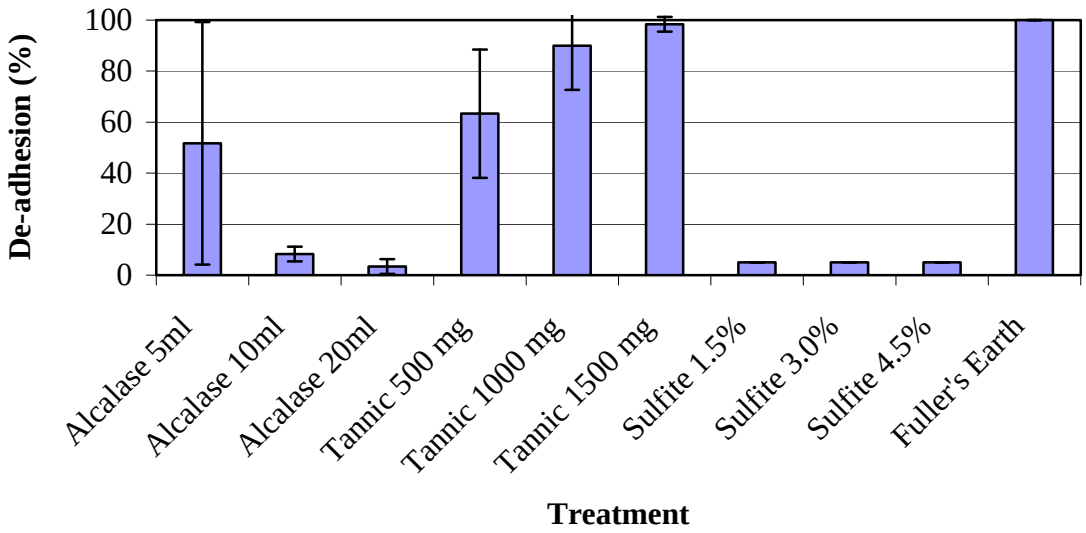
Shortnose sturgeon from the Bears Bluff National Fish Hatchery were being spawned for the final time in 2008. This presented an opportunity to get fertilization data for the cryopreserved Atlantic sturgeon sperm that had not been available using Atlantic sturgeon eggs. Shortnose sturgeon eggs were aliquoted (~700/bowl) into bowls. Straws were thawed in a 40 ^{o}C waterbath for 9 (0.5-ml) or 30 sec (5-ml). Thawed sperm (500 ul) was applied to the eggs, and water was added to fertilize. After 5-min, the water was decanted and Fuller's earth was added to de-adhese the eggs. After 30-min, the Fuller's earth was rinsed off, and the eggs were transferred to hatching jars for incubation at 16 ^{o}C. After 6-hrs, the eggs were preserved in 10% buffered formalin for the determination of fertilization rates. Blues bars are 0.5-ml straws, and red bars are 5-ml straws.



Alligator gar egg de-adhesion

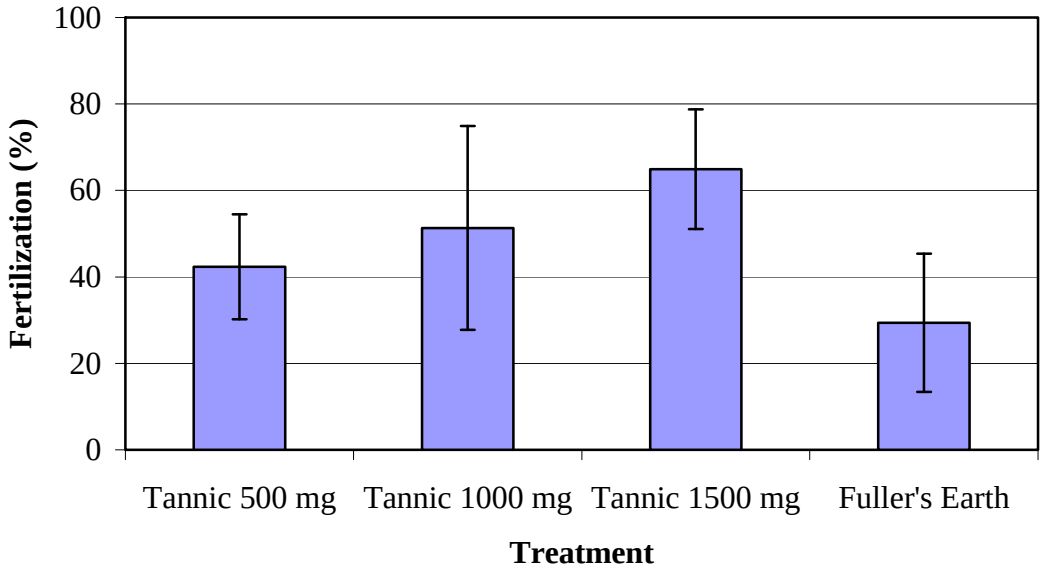
Alligator gar eggs are extremely adhesive, and begin sticking to anything they touch as soon as they are stripped from the female. In an effort to reduce handling and mechanical damage to the eggs from the current de-adhesion protocol, Fuller's earth, a study was developed to investigate the effectiveness of three chemical treatments on alligator gar egg de-adhesion. Alcalase is an enzyme that has been used successfully in Europe with tench. Tannic acid is commonly used in the de-adhesion of white bass eggs, and sodium sulfite is commonly used to de-adhese the egg masses of channel catfish.

Aliquots of eggs (~400) were distributed to three bowls for each treatment. Each bowl within a treatment was fertilized with sperm from a different male. After three minutes, excess sperm and water were decanted, and the chemical treatments were applied. After 5 minutes, chemical treatments were decanted off, and the eggs were rinsed twice with water. The control eggs were treated with Fuller's earth for 30 minutes as typically done with alligator gar eggs.

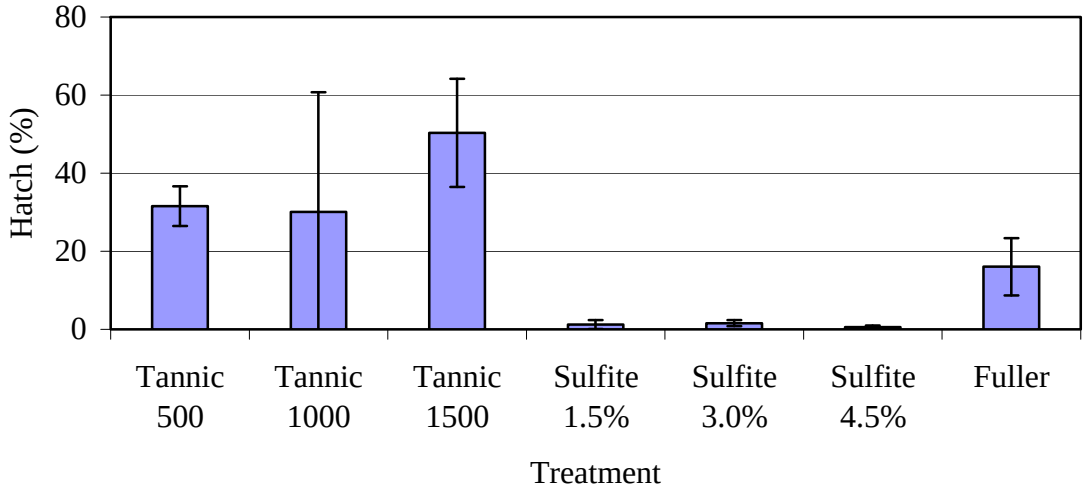


Eggs treated with all levels of tannic acid and 16 AU/L Alcalase had the same percentage of de-adhesion as did eggs treated with Fuller's earth. All levels of sodium sulfite and 32 and 48 AU/L Alcalase produced very little de-adhesion.

The eggs were transferred to jars and incubated at 21 ^{o}C. After 24 hours, all eggs treated with Alcalase had died, and they were discarded. After 48 hours, developing embryos were clearly evident in the eggs, and fertilization rates were determined. Fertilized embryos were evident in the sodium sulfite treated eggs, however the eggs were stuck to the jars and could not be counted. Eggs treated with tannic acid had fertilization rates equal to that of eggs treated with Fuller's earth.



After 120 hours of incubation, all the eggs had hatched and hatch rates were determined. Again eggs treated with tannic acid had hatch rates equal to that of eggs treated with Fuller's earth. The treatment of alligator gar eggs with tannic acid takes only five minutes, as compared to 30 minutes with Fuller's earth, and provides equal fertilization and hatch rates. Based on these results the use of tannic acid for de-adhesion of alligator gar eggs is recommended. An additional study will be done in 2009 to investigate the use of tannic acid on a production scale.



Pallid Sturgeon Sperm Cryopreservation

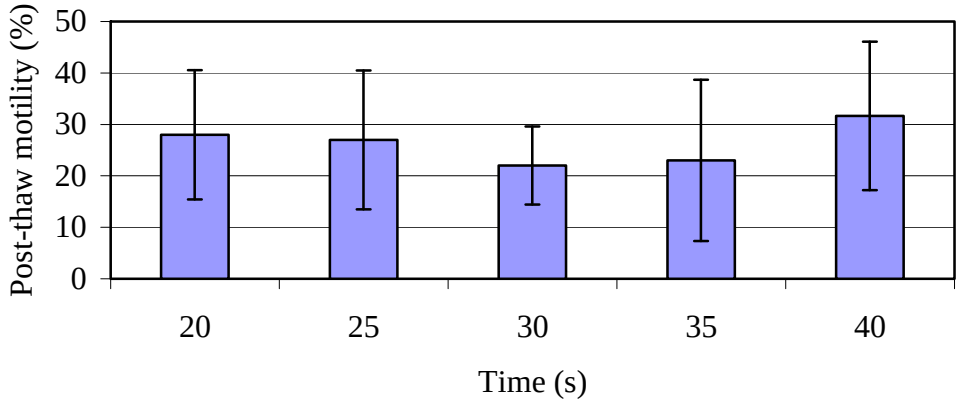
The pallid sturgeon *Scaphirhynchus albus* is a Federally listed endangered species that occurs within large rivers of the Missouri River system and Mississippi River southward from the mouth of the Missouri River. Recovery efforts have been implemented, with characterizing the remaining population, conserving the remaining diversity, and restocking being priority issues. In an effort to help conserve as much of the remaining genetic diversity as possible, cryopreservation techniques were developed in 1999. Since the development of these techniques, sperm have been cryopreserved from males collected for the restocking effort in the upper Missouri River, and are presently stored at three facilities (Warm Springs Fish Technology Center, Garrison Dam National Fish Hatchery, and Gavins Point National Fish Hatchery).

Garrison Dam National Fish Hatchery

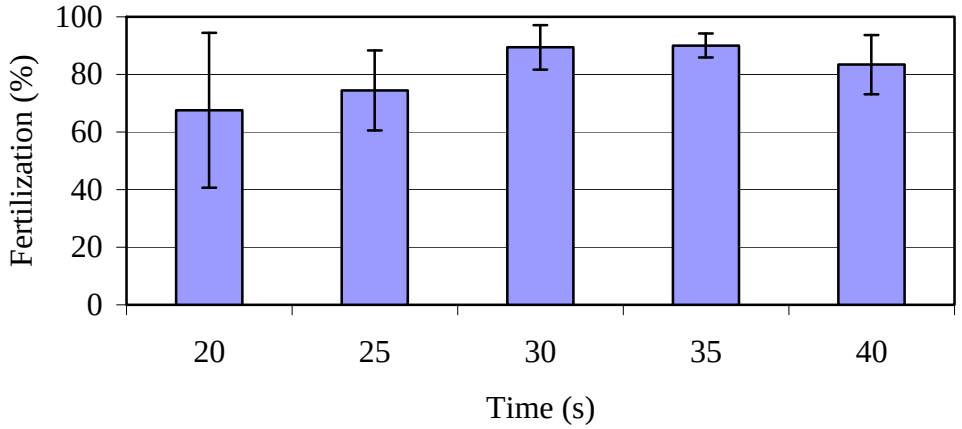
In 2008, sperm were collected and cryopreserved for archival purposes (100 straws per fish) from 20 males during the spawning season. The current total number of males held in the repository is 118. In addition studies were conducted to optimize the cryopreservation procedures that have been developed.

Thaw time study

In an effort to determine an optimal thawing time for 5-ml straws, straws were thawed for varied times in a 40 ^{o}C waterbath. After thawing, motility was estimated for each treatment. There were no significant differences (P = 0.8265) among the treatments.

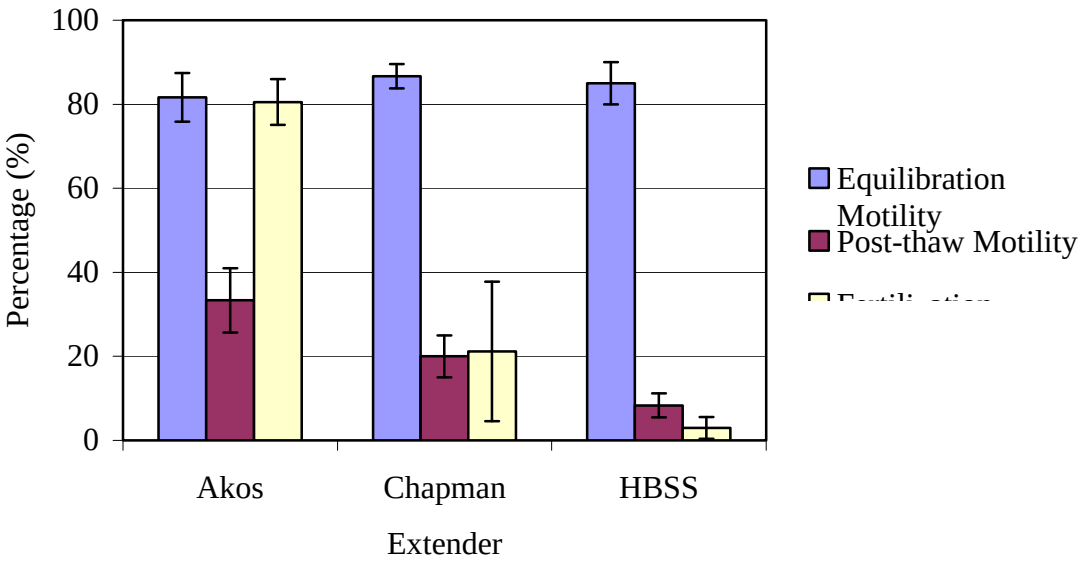


Thawed sperm (150 ul) were used to fertilize ~400 eggs for each treatment. The eggs were incubated for 6 hours, and approximately 1/3 of the eggs were preserved for fertilization determinations. There were no significant differences (*P* = 0.1313) among the treatments.



Extender fertilization study

A study was conducted to test extenders used for the cryopreservation of pallid sturgeon sperm. Three extenders were tested. Sperm were extended 1:1 (v:v) with each extender. Methanol (5%) was used as the cryoprotectant and 5-ml straws were frozen on a 2.5-cm platform floating on liquid nitrogen. Sperm were equilibrated for 9 min prior to freezing, and there were no significant differences (*P* = 0.4640) in equilibration motility among the treatments. Approximately 24-hr after freezing, the straws were thawed for 30 s in a 40 ^{o}C waterbath. There was a significant difference (*P* = 0.0044) in post-thaw motility among the treatments. Thawed sperm (150 ul) were used to fertilize ~400 eggs per treatment. Eggs were incubated for 6 hours at 23 ^{o}C, and preserved for fertilization determinations. Sperm cryopreserved using AKOS extender (81%) produced a significantly higher (P = 0.0002) fertilization rate than did Chapman (21%) or HBSS (3%).



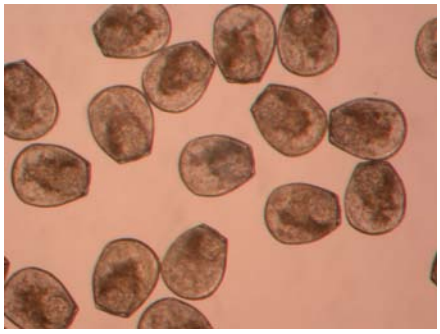
Blind Pony State Fish Hatchery

Pallid sturgeon were collected from the middle basin and brought to Blind Pony State fish Hatchery in Missouri. Sperm were collected from 5 males, diluted 1:1 (v:v) with 100 mOsmol/kg HBSS and shipped to the Fish Technology Center. Upon arrival, sperm motility was estimated, and sperm quality in 4 out of the 5 samples was high enough for freezing. One hundred straws from each male were frozen and placed in the repository. These are the first samples from pallid sturgeon of the middle basin to be placed in the repository.

Freshwater mussels

Center staff worked on new techniques to help in the recovery efforts of freshwater mussels. One potential method for the preservation and recovery of such unionid species is the collection, cryopreservation, and storage of sperm and glochidia. This would allow preservation of genetic stocks, transfer of genes from wild populations to hatchery stocks, and transfer of genetic material between wild populations.

Staff evaluated the toxicity of several cryoprotectants at three concentrations on glochidia to determine survival percentages for cryopreservation experiments. With the success of these trials, the staff is looking forward to expanding their studies in the near future



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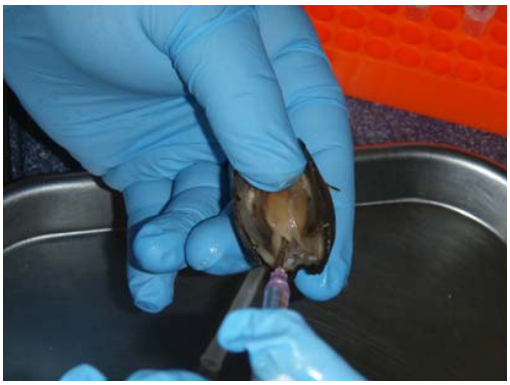
Another study conducted evaluated two methods in extracting sperm from mussels; a lethal and non-lethal technique. Results showed the lethal method provided a greater volume of sperm and with less contamination over the non-lethal technique.



Staff extracting sperm from a mussel.

Fish Passage

Staff from the WSFTC completed an extensive survey of stream crossings in the Sawhatchee Creek and Kirkland Creek watersheds of southwest Georgia to identify and prioritize barriers to fish passage. Both creeks have been designated as critical habitat for several listed mussels; shinyrayed pocketbook, oval pigtoe and Gulf moccasinshell. One of the primary constituent elements for the critical habitat designation is host fish. Host fish support the larval life stages of these three mussels. Fish barriers can disrupt connectivity between the upstream and downstream limits, impeding fish host movement, gamete transport, dispersal into newly suitable habitats, and food items transport. Approximately 30 sites were assessed in the Sawhatchee (13 sites) and Kirkland Creek (17 sites) watersheds over a total of 75 river miles. Based on criteria from the National Inventory and Assessment Procedure none of the crossings were determined to be barriers to small stream fish. Several sites were identified as good habitat for three protected mussels.

A culvert backwatered its entire length

Drought conditions affect crossing

Freshwater Mussels

Center staff are continuing to assist personnel from several FWS ES Offices (Ft. Benning and Panama City), the Fisheries Resource Office at Panama City, and Georgia Department of Natural Resources (Non-Game Section) with a mark and recapture study by collecting and tagging mussels in Sawhatchee Creek, a tributary of the Chattahoochee River. This is a five to seven year study that is in its fourth year. To date over 750 federally protected mussel have been collected and tagged.

Photograph of some of the mussels collected.

Two staff members assisted personnel from the Georgia Department of Natural Resources and the University of Georgia in conducting mussel surveys in several tributaries of the lower Flint River. The surveys assessed macro habitat selection among several species of freshwater mussels. Several non-listed, native mussels were collected for cryopreservation and genetic studies to be conducted by the center. At stream side, several mussels were swabbed to collect cells from the viscera and foot to evaluate DNA extraction techniques and mussel mortality rates. Results from this study will help to determine which sampling technique is the most efficient in providing high quality DNA for application in conservation genetics of threatened and endangered freshwater mussels.

Elephant ear (*Elliptio crassidens*) mussels collected to conduct genetic studies.

Swab kit for extracting genetic material.

One additional task Center staff worked on during the fiscal year was to assist Regional Office staff and personnel from the Ecological Services offices of Panama City and Ft. Benning in obtaining information on drought effects on aquatic species in the Southeast United States. Center staff contacted federal and state hatcheries in the Southeast to explore refugia possibilities should the need arise to relocate mussels to different hatcheries if drought conditions worsened. This was done by conducting literature searches for background material and cost proposals from different facilities to upgrade their systems to hold mussels and other aquatic species. The results of these searches were used to prioritize mussel species and holding facilities and to write the USFWS Discussion Paper for Drought Contingency Planning for Freshwater Mussels in the Southeast U.S.

Southeast drought continues.

National Fish Strain Registry Highlights

The National Fish Strain Registry (NFSR) is an internet-based program that assembles information on the life history, genetics, reproduction, and behavior of wild populations and domestic broodstock strains. This management tool can assist resource managers with decisions on which populations will be most effective for each production or conservation management application. As of September 25, 2008, the NFSR database is available for use by federal and state governments, private producers, and the general public. In order to access the NFSR website, please submit a request via email to NFSR managers, Chester Figiel (chester_figiel@fws.gov) and Nicole Rankin (nicole_rankin@fws.gov). Once registered, users can access the page at http://sds.fws.gov/nfsr. Over the next several months, NFSR managers will be contacting federal and state hatcheries to input information about fish strains they are currently managing. This database is responsive to the needs of our partners and to the mission of the USFWS (i.e., to conserve and protect aquatic resources) and is available to fisheries managers, fish producers, and researchers throughout the United States.

An Investment Performance Profile (IPP) was completed for the National Fish Strain Registry (NFSR) as part of the Fish and Wildlife Service's efforts to refine the IT Portfolio Management process. This IPP assists the Information Resources and Technology Management program in assessing the status and performance of current FWS investments in the government’s Electronic Capital Planning and Investment Control system. Additionally, this process ensures that the NFSR is aligned with the Department of the Interior’s Strategic Plan and Enterprise Architecture and that customers receive full value for the investment.

Nicole Morris Rankin joined the staff of the FTC on March 16th, 2008. Nicole's role as a fish biologist involves data management and expanding the horizons of the National Fish Strain Registry (Registry). Nicole will be challenged with two tasks: (1) updating Registry data for all fish broodstocks (hatchery and wild); and (2) expanding the Registry to include new parameters --- other aquatic species (e.g., mussels, amphibians) and ESA-listed species. Nicole brings with her valuable federal experience through working with the National Oceanic and Atmospheric Administration’s Office of Ocean Exploration and Research as a Knauss Sea Grant Fellow. Nicole graduated from the University of West Florida with a Master of Science in Biology. She received a Bachelor of Science in Biology from Jacksonville State University in Alabama.

Chytrid Fungus

An inter-regional partnership, between the Warm Springs FTC (Southeast Region) and the Dexter National Fish Hatchery and Technology Center (Southwest Region), is investigating the invasive chytrid fungus, *Batrachochytrium dendrobatidis*. Chester R. Figiel, Jr. visited Southwest region biologists to develop and standardize protocols for analyzing this fungus that has been implicated in the decline of amphibian populations worldwide. Quantitative polymerase chain reaction (qPCR) protocols were developed by Andy Pessier and Oliver Ryder (San Diego Zoo) to detect, amplify and quantify the DNA of the fungus. These protocols were standardized by Andy and taught to personnel at the Dexter National Fish Hatchery and Technology Center. These standardized protocols were also taught to Chester, who will teach them to Warm Springs Fish Health Center personnel. Additionally, recovery and management scenarios for amphibians were discussed in an effort to meet the U.S. Fish and Wildlife Service Fishery Program's broad responsibility to address national, regional, and ecosystem level aquatic resource needs and priorities.

Shortnose Sturgeon Endangered Species Permit

The Fish Technology Center completed and sent an annual report to the National Oceanic and Atmospheric Administration describing activities completed by USFWS Southeast Region personnel working on the endangered shortnose sturgeon, *Acipenser brevirostrum*. This included activities to aid in recovery efforts for this species conducted at the Bear's Bluff National Fish Hatchery, the Orangeburg National Fish Hatchery and the Fish Technology Center.

CONSERVATION GENETICS LAB HIGHLIGHTS

CGL hosts noninvasive tissue sampling and genetic workshops.

The Conservation Genetics Lab of the Warm Springs Fish Technology Center recently conducted two genetic work shops for Service biologists. The first was held at the Ecological Services/ Fisheries Resource Office in Panama City, Florida, and was a one and half day conservation genetics primer. This workshop included such topics as genetic terminology, genetic variation, microsatellites, population genetics, drift, effective population size, demography/ extinction, hybridization, and captive breeding. Seventeen staff biologists from several Ecological Services and Fisheries offices attended. The second workshop was held at the Warm Springs Regional Fisheries Center in Warm Springs, Georgia.

The second workshop showed participants a non-invasive technique to sample tissue from threatened and endangered freshwater mussels for genetic analysis. Eleven Service people, a visiting professor from Appalachian State University in Boone, North Carolina, and a biologist from the Georgia Department of Natural Resources attended. Biologists swabbed mussels to collect cells from the viscera and foot of the mussels. This technique and several others are being tested by the Fish Technology Center to evaluate DNA extraction techniques and mussel mortality rates. Results from this study will help determine which sampling technique is the most efficient in providing high quality DNA for applications in conservation genetics of threatened and endangered freshwater mussels.

Regional Geneticist invited to speak at international conference.

The Freshwater Mollusk Conservation Society, in collaboration with the Society for Conservation Biology sponsored a symposium as part of the SCB 22nd Annual Meeting held at the Chattanooga Convention Center, Chattanooga, Tennessee, July 13-18, 2008. The symposium was an opportunity for individuals to share the latest in research, monitoring approaches, and natural resource management information to support the conservation of freshwater mussel species, habitats and ecosystems. Invited speaker, Dr. Gregory Moyer, had the opportunity to present a 20 minute talk pertaining to risks associated with hatchery propagation and reintroduction. He also participated in round table discussions regarding the role of conservation genetics in freshwater mussels.

CGL in conjunction with University of Southern Mississippi completes final report on the development of molecular markers for alligator gar.

ABSTRACT: We report on the isolation of 17 polymorphic microsatellite loci from alligator gar (*Atractosteus spatula*), a large-bodied species that has experienced population declines across much of its range. These loci possessed 2 to 19 alleles and observed heterozygosities of 0.000-0.974. All loci conformed to Hardy-Weinberg

equilibrium expectations, and none exhibited linkage disequilibrium. Nine and eight of these loci were found to be polymorphic in the related species *Lepisosteus oculatus* and *L. osseus*, respectively. These microsatellite loci should prove useful in conservation efforts of *A. spatula* through the study of population structure and hatchery brood stock management.

Regional Geneticist attends USFWS Conservation Genetics Community of Practice Continuing Education Meeting.

Greg Moyer participated in the FWS Conservation Genetics Community of Practice Continuing Education Workshop recently held in Ashland, Oregon. The Conservation Genetics Community of Practice is an interactive forum that facilitates exchange of information and technologies to strengthen the use and understanding of conservation genetics within the FWS. Greg led several presentations regarding use of genetic data in conservation biology and participated in discussions of conservation genetics and ESA policy with Ecological Service staff. These interactions strengthened his understanding of Ecological Service issues and application of conservation genetics data to recovery planning for ESA listed species. His participation in this meeting has not only strengthened relationships at an individual level but also at a divisional level between Fisheries and Ecological Services.

CGL lab finishes final report on genetic evaluation of a conservation hatchery implementation program for reintroduction of sicklefin redhorse (Moxostoma sp.) in the Tuckasegee River, NC.

ABSTRACT: Due to the limited geographic distribution and perceived threats associated with physical alteration of the habitat, restoration and reintroduction efforts for the of sicklefin redhorse (*Moxostoma sp*.) have been initiated by state and federal agencies. Unfortunately, little is know about the genetic diversity of wild sicklefin redhorse and those used as brood stock for hatchery propagation. We use data from ten microsatellite loci to estimate and compare indices of genetic diversity between the Little Tennessee River population of wild adults and those used for hatchery brood stock for an initial reintroduction effort in 2007 and 2008. We also compared relatedness values for brood stock used for restoration efforts and provide guidance for future sicklefin redhorse management and reintroduction programs. The average observed heterozygosity value for sicklefin redhorse sampled in this study (0.85) was greater than that of other freshwater fishes (0.54). While no significant (all $P > 0.67$) differences existed between hatchery brood stock and wild for average gene diversity, comparisons of the average number of alleles was significantly different (all $P < 0.04$). Finally, all hatchery crosses in 2007 and 2008 appeared to be between unrelated individuals. Our data indicate that the genetic diversity of the wild population remains relatively high particularly for an organism in such a confined system with a lack of migration between populations. This observation appears to be the result of the long-lived and iteroparous nature of sicklefin redhorse. In contrast, the genetic diversity (number of alleles) of the introduced population is significantly less than that of the wild. The decrease can be attributed to sampling few numbers of individuals for brood. This situation can be remedied by either sampling more individuals (but <50% of the wild population) or by following recommended hatchery propagation guidelines.

Three funding opportunities awarded to CGL.

The CGL was awarded three separate grants in August pertaining to conservation genetics of freshwater mussels and fish. These grants include

> *Alabama Shad (Alosa alabamae) restoration for the Apalachicola-Chattahoochee-Flint River Basin* awarded by NOAA (partners: GaDNR and Clemson University; award amount $457,679),
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> *Molecular marker development for estimating genetic diversity within and among populations of eastern Gulf Slope threatened and endangered freshwater mussels awarded by FWS Quick Response program* (partners: USGS and FWS Panama City Field Office; award amount $24,420), and

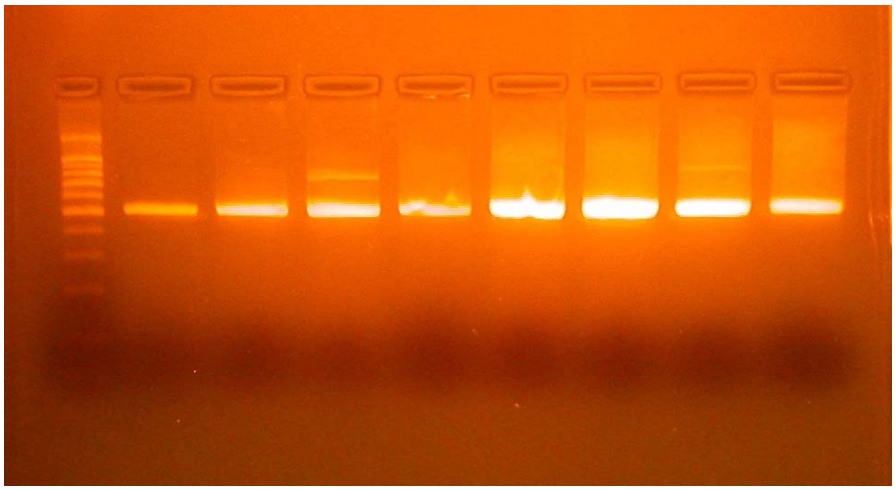
Delineating and prioritizing conservation units for freshwater mussel conservation hatchery implementation programs in the ACF awarded by FWS deferred allocation funds (partners: FWS Panama City and Columbus Field Offices; award amount $75,000).

CGL assists GaDNR and UGA with freshwater mussel sampling.

Greg Moyer assisted personnel from Georgia DNR and the University of Georgia in conducting mussel surveys in several tributaries of the lower Flint River. The surveys were conducted to assess macro habitat selection among several species of freshwater mussels. Besides conducting presence and absence surveys, several native non-listed mussels were collected and brought back for genetic studies being conducted at the WSFTC. At stream side, several mussels were swabbed to collect cells from the viscera and foot. This technique and several others are being tested by the Fish Technology Center to evaluate DNA extraction techniques and mussel mortality rates. Results from this study will help to determine which sampling technique is the most efficient in providing high quality DNA for applications in conservation genetics of threatened and endangered freshwater mussels.

CGL develops protocol for noninvasive tissues sampling of freshwater mussels.

Just like out a crime scene, a noninvasive tissue sampling technique, developed by CGL, is being employed by FWS biologists to study population structure and estimate genetic diversity for threatened and endangered mussels of the Apalachicola-Chattahoochee-Flint river system. Over the month, CGL has participated in sample collection, tissue swabbing and lab work to refine existing protocols for the extraction of DNA from T&E freshwater mussels. CGL confirmed that DNA from the tissue swabs was indeed mussels DNA by sequencing a small portion of the genome and comparing the sequence to know exemplars.



Public Outreach and Education Highlights

The FTC participated in a conservation day camp in June for approximately 1,500 children at the home field of the Columbus Catfish, a single A baseball team affiliated with the Tampa Bay Rays. Staff set up a display table with brochures about recreational fishing and endangered species. Staff displayed fish and mussel species of the Chattahoochee River which flows through Columbus, Georgia. Children enjoyed identifying relic mussel shells of the Apalachicola, Chattahoochee, and Flint Basin and examining several preserved darter species from the Coosa River Basin. Many of the children also got fish tattoos for answering questions about mussels and fish.

Staff educating children about fish and freshwater mussels.

Four FTC employees and one SCA intern helped staff the Warm Springs National Fish Hatchery Kids' Fish for Fun Event on June 7, 2008. Over 200 children participated and approximately 740 catfish were caught.

On July 21, 2008, FTC staff gave an in-depth tour of the Conservation Genetics lab, NFSR office, and Cryopreservation lab spaces to Dr. Bill Daniels and students from an undergraduate class from Auburn University.

On July 7th, Regional Geneticist Gregory Moyer provided educational outreach for a youth camp near Lagrange, GA. Camp Viola is a summer camps for youth who come from high-risk neighborhoods and economically challenged homes that do not have an opportunity to experience a summer camp. It was career-oriented outreach aimed at informing the youth about careers in biology and FWS. Greg talked about how he became interested in biology, his love for the outdoors, and why and how he chose a career in the FWS. Kids also had a chance to view live fish, salamanders, and crayfish and discuss how we as humans can impact the environment. The event was a huge success!

On August 11th, FTC staff gave a tour of the Conservation Genetics lab, NFSR office, and Cryopreservation lab spaces to Mike Harris and Jason Wisniewski, Georgia DNR, and Robert Bringolf, University of Georgia.

Bill Bouthillier participated in a Georgia Ducks Unlimited Greenwing event on August 8th. He lead a nature walk in a restored wetland in Walton County, Georgia and told children about what animals use wetlands and the importance of wetlands conservation. Over 365 people were in attendance, of which 263 were children.

Sandra Dietz, along with Regional Fisheries Center staff and Friends of the Warm Springs National Fish Hatchery, staffed an event at Warm Springs for a Chattahoochee Council of Boy Scouts retreat on September 13, 2008. Over 300 scouts and scout leaders participated and learned about different facets of the Warm Springs Regional Fisheries Center. Stations and activities were provided to display information related to the USFWS including: pollinators, invasive species, confiscated law enforcement items (showcasing illegal importation of threatened and endangered species), knot tying, cleaning/filleting fish, backyard habitats, recycling programs including monofilament, and opportunities for employment and volunteering. The most popular station was Pollinators, which featured a live working bee hive maintained and demonstrated by Sandra.

Sandra demonstrating at the live working bee hive.

On September 20, 2008, the FTC participated in the Warm Springs National Fish Hatchery Open House. This event focused on promoting the USFWS mission of working with others to conserve, protect, and enhance fish, wildlife, plants, and their habitats for the continuing benefit of the American people. FTC staff set up displays, computers, and a video screen to provide information to more than 90 children and adults about cryopreservation, conservation genetics, freshwater mussels, fish passage, amphibian conservation, and fish spawning techniques. A 50-gallon touch tank with crayfish, bull frogs, salamanders, and musk turtles was a highlight for families with children. Another popular station was the 500-gallon striped bass display tank; attendees were able to learn about striped bass spawning and stocking programs. The FTC egg trailer was the largest display and included a live freshwater mussel tank, live freshwater fish (sunfish, largemouth bass, bullhead catfish) tank, and an egg incubation demonstration. All FTC staff were present and contributed to the success of this event. Other participants in the Open House included staff from the Warm Springs Regional Fisheries Center, Fish Health Center, and National Fish Hatchery, members of the Friends of the Warm Springs National Fish Hatchery, and several other volunteers.