



*American Fisheries Society*  
*Louisiana Chapter*



ABSTRACTS OF  
THE 31<sup>ST</sup> ANNUAL MEETING OF THE LOUISIANA  
CHAPTER OF THE AMERICAN FISHERIES  
SOCIETY  
“BEYOND THE HORIZON, A BRIGHT FUTURE FOR  
LOUISIANA FISHERIES”



LAFAYETTE, LOUISIANA

JANUARY 27 – JANUARY 28, 2011

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# Louisiana Chapter of the American Fisheries Society

## Thursday, 27 January 2011 (Day 1)

Presenting author is denoted by an asterisk (\*). Student presenters are underlined. Abstract page is listed in parentheses

- 8:00AM Registration
- 8:30AM **Welcome and Opening Remarks**
- 8:40AM **Keynote Presentation**, Robert Barham, Louisiana Department of Wildlife and Fisheries
- 9:15AM **Can hemolymph lactate, protein, and glucose concentrations serve as physiological biomarkers of hypoxic stress in red swamp crayfish (*Procambarus clarkii*)?** Christopher P. Bonvillain\*, D. Allen Rutherford, Christopher C. Green, and William E. Kelso (Page 10)
- 9:30AM **Utilization of soybean-based diets supplemented with taurine and phytase by Florida pompano (*Trachinotus carolinus*).** Gregory P. Lech\* and Robert C. Reigh (Page 26)
- 9:45AM **Metabolic and embryonic responses to terrestrial incubation of Gulf killifish (*Fundulus grandis*) eggs across a temperature gradient.** Charles Brown\*, Fernando Galvez, and Christopher Green (Page 12)
- 10:00AM **Break 1:** Fifteen minutes
- 10:15AM **Effects of female size on reproductive output of the Gulf killifish (*Fundulus grandis*).** Taylor G. Allgood\*, Craig T. Gothreaux, and Christopher C. Green (Page 8)
- 10:30AM **Growth and osmoregulatory physiology of juvenile Gulf killifish (*Fundulus grandis*) across a salinity gradient.** Joshua T. Patterson\* and Christopher C. Green (Page 32)
- 10:45AM **Copper acclimation in the least killifish (*Heterandria formosa*): time-to-death and biomarker approaches.** Joseph A. Adeyemi\* and Paul L. Klerks (Page 6)
- 11:00AM **Behavior as an endpoint for copper acclimation and toxicity studies of the least killifish (*Heterandria formosa*).** Sarah K. Vogt\*, Arlene Billock, and Paul L. Klerks (Page 38)

**Day 1 Continued.**

- 11:15AM            **Zooplankton density and composition in river/floodplain habitats of the Ouachita River.** William L. Sheftall IV\*, Michael D. Kaller, and William E. Kelso (Page 35)
- 11:30AM            **Lunch on your own:** One hour and thirty minutes
- 1:00PM            **Habitat-specific fecundity of red snapper (*Lutjanus campechanus*) in the northern Gulf of Mexico.** Dannielle H. Kulaw\* and James H. Cowan, Jr. (Page 25)
- 1:15PM            **Habitat use by young lemon sharks (*Negaprion brevirostris*) at the Chandeleur Islands, Louisiana.** Jonathan McKenzie\*, Christopher Schieble, and Martin T. O'Connell (Page 28)
- 1:30PM            **Seasonal abundance of larval fishes in the upper Barataria Estuary may be limited by local hypoxic conditions.** Sean M. Jackson, Allyse M. Ferrara, and Quenton C. Fontenot\* (Page 22)
- 1:45PM            **Diet and growth rates of cultured alligator gar (*Atractosteus spatula*) stocked into a Louisiana drainage canal.** Rachel Ianni\*, Allyse Ferrara, and Quenton Fontenot (Page 21)
- 2:00PM            **Bactericidal activity of spotted gar (*Lepisosteus oculatus*) serum mediated by complement activity.** J.J. Merrifield \*, P. Bandyopadhyay, and R. Nathaniel (Page 29)
- 2:15PM            **Potential juvenile tarpon (*Megalops atlanticus*) nursery habitats in southeastern Louisiana.** William Stein, III\*, Jon McKenzie, and O. Thomas Lorenz (Page 37)
- 2:30PM            **Break 2:** Fifteen minutes
- 2:45PM            **Genetic variation of spotted seatrout (*Cynoscion nebulosus*) in Louisiana: Panmixia and genetic divergence.** Kyle R. Piller\* and Lisa Landry (Page 33)
- 3:00PM            **A review of ultrasound practices in studies of fish reproduction.** Noel D. Novelo\*, and Terrence R. Tiersch (Page 30)
- 3:15PM            **Scaling of high-throughput cryopreservation for fishery and aquaculture applications.** E Hu\*, and Terrence R. Tiersch (Page 20)

**Day 1 Continued.**

- 3:30PM **High-throughput Sperm Cryopreservation for Eastern Oysters (*Crassostrea virginica*).** Huiping Yang\*, E Hu, and Terrence R. Tiersch (Page 39)
- 3:45PM **Characterization of isolates of Group B Streptococci from diseased hybrid striped bass (*Morone saxatilis* x *M. chrysops*) and Gulf killifish (*Fundulus grandis*).** John P. Hawke\*, Esteban Soto, Rui Wang, Judy Wiles, and Wes Baumgartner (Page 18)
- 4:00PM **Ulcerative mycoses caused by *Aphanomyces invadans* and *Ochroconis* sp. in wild and cultured marine fish species.** John P Hawke\*, Wes Baumgartner, and Esteban Soto (Page 19)
- 4:15PM **The Gulf killifish (*Fundulus grandis*) project: integrating research and extension for aquacultural development.** Julie A. Anderson\*, Sunny J. Brogan, Craig T. Gothreaux, R. Glenn Thomas, and Christopher C. Green (Page 9)
- 4:30PM **Gulf killifish (*Fundulus grandis*) egg production – ratios, cycles, and mats, oh my.** Craig T. Gothreaux\* and Christopher C. Green (Page 17)
- 4:45-5:30PM **Poster Session**
- Acute effects on Atchafalaya River Basin physicochemistry associated with the passage of Hurricane Gustav.** Christopher P. Bonvillain\*, B. Thorpe Halloran, Kevin M. Boswell, William E. Kelso, A. Raynie Harlan, and D. Allen Rutherford (Page 11)
- Assessing larval blue crab (*Callinectes sapidus*) densities in the natural and artificial tidal passes of Lake Pontchartrain, Louisiana.** Rebecca Weatherall Cope\* (Page 14)
- Relation of prey availability to habitat selection for red drum (*Sciaenops ocellatus*) in Bayou St. John, an urban waterway in New Orleans, Louisiana.** Patrick W. Smith\* (Page 36)
- 6:00-9:00PM Social, student raffle and auction

# Louisiana Chapter of the American Fisheries Society

## Friday, 28 January 2011 (Day 2)

Presenting author is denoted by an asterisk (\*). Student presenters are underlined. Abstract page is listed in parentheses

- 8:00AM Registration
- 8:15AM **Flood duration and flow magnitude in the Atchafalaya River Basin and their implications for fisheries management at a basin-wide scale.** J. Brian Alford\* and Mike Walker (Page 7)
- 8:30AM **Catch and size-structure of wild red swamp crawfish (*Procambarus clarkii*) sampled from pillow traps constructed with two different mesh sizes.** Martin Plonsky\* (Page 34)
- 9:00AM **Developing and disseminating preferred catch and release methods for Louisiana saltwater recreational anglers.** Edward Chesney\*, R. Glenn Thomas, John Hawke and Cara Hoar (Page 13)
- 9:15AM **Assessment of angler exploitation of white crappie (*Pomoxis annularis*) in Poverty Point Reservoir, Louisiana.** Ryan Daniel\* and Jesse Bahm (Page 15)
- 9:30AM **Results of post-rotenone treatment monitoring for introduced tilapia (*Oreochromis* spp.) in the Port Sulphur region.** O. Tom Lorenz\* (Page 27)
- 9:45AM **A geographic information system tool for aquatic resource conservation: Red and Sabine River watersheds.** Jill A. Jenkins\*, Stephen B. Hartley, Jacoby Carter, Darren J. Johnson<sup>2</sup>, and J. Brian Alford (Page 23)
- 10:00AM **Evaluating growth and survival of three smooth cordgrass (*Spartina alterniflora*) ecotypes in coastal Louisiana.** Quenton C. Fontenot\*, Gary Fine, and Allyse M. Ferrara (Page 16)
- 10:15AM **Gonad histology of male bronze frogs (*Rana clamitans*) exposed to atrazine in the Atchafalaya River Basin, Louisiana.** Constance A. Kersten and Tyler F. Thigpen\* (Page 24)
- 10:30AM **Stabilizing blood samples in the field for laboratory analyses: Gulf sturgeon (*Acipenser oxyrinchus*) and the Mississippi Canyon 252 Oil Spill.** Heather M. Olivier\* and Jill A. Jenkins (Page 31)
- 10:45AM Break 1: Fifteen minutes
- 11:00AM Business meeting and awards presentations: Thirty minutes

## PRESENTATION ABSTRACTS

### **Copper acclimation in the least killifish (*Heterandria formosa*): time-to-death and biomarker approaches.**

Joseph A. Adeyemi\* and Paul L. Klerks

*Department of Biology, University of Louisiana, Lafayette, LA. Jaa7316@louisiana.edu*

Organisms that are exposed to low levels of a contaminant may develop increased tolerance to subsequent exposure through acclimation. In this study, we investigated copper acclimation in the least killifish (*Heterandria formosa*) using both the time-to-death and biomarker approaches. Fish were exposed to either background (control) or 15µg/L Cu for seven days. Thereafter they were exposed to 150µg/L Cu and acclimation was quantified with survival time and other biological endpoints (lipid peroxidation, catalase activity, oxygen consumption and ammonia excretion). Acclimated fish showed a significantly longer time-to-death compared to the control. Catalase activity and ammonia excretion rate were considerably higher in the acclimated group. We also observed a significant reduction in lipid peroxidation in the acclimated fish compared to the control. Similarly, oxygen consumption rate was slightly lower in the acclimated fish. Ongoing studies are looking for the underlying mechanisms of copper acclimation in this fish species as well as the associated fitness costs.

## **Flood duration and flow magnitude in the Atchafalaya River Basin and their implications for fisheries management at a basin-wide scale**

J. Brian Alford\*<sup>1</sup> and Mike Walker<sup>2</sup>

<sup>1</sup>*Fisheries Management Section, Division of Fisheries, Louisiana Department of Wildlife and Fisheries, Baton Rouge, LA. balford@wlf.la.gov;* <sup>2</sup>*Inland Fisheries Section, Division of Fisheries, Louisiana Department of Wildlife and Fisheries, New Iberia, LA. mwalker@wlf.la.gov*

The purpose of our study was to assess the impact of flooding in the Atchafalaya River Basin on fisheries production at a basin-wide scale. We modeled flood duration (number of days/year Butte La Rose gage height > 3.6 m) and flow magnitude (Mean daily gage height at Butte La Rose) of the Atchafalaya River against fish relative abundance metrics (mean catch/weight per unit effort) using long term (1987-2009) LDWF fishery-independent data collected on largemouth bass (*Micropterus salmoides*), crappie (*Pomoxis* spp.), blue catfish (*Ictalurus furcatus*), buffalo (*Ictiobus* spp.), and gizzard shad (*Dorosoma cepedianum*). We also used percentage of age-1 and age-2 largemouth bass as indicators of largemouth bass recruitment, and we modeled annual crawfish (*Procambarus* spp.) landings from fishery-dependent data obtained from trip ticket information, in addition to harvest (sacks/boat-month) from a creel survey (1994-1996) conducted on commercial fishers in the Basin. We found that when the Atchafalaya River at Butte La Rose is at flood stage ( $\geq 3.6$  m) for approximately 124-157 days/year, annual relative abundances of recreationally and commercially important fishes are optimized (nonlinear logistic functions,  $R^2 > 0.27$ ,  $P < 0.09$ ). However, gizzard shad was negatively impacted by flood duration, as its relative abundance was optimized during dry years ( $< 10$  days/year). Annual recruitment of age-1 and age-2 largemouth bass were associated positively with annual flow magnitude (linear regression,  $R^2 = 0.31-0.38$ ,  $F > 5.10$ ,  $P < 0.04$ ). Annual wild-caught crawfish and gizzard shad landings (i.e., crawfish bait), as well as crawfish sacks/boat-month from the creel survey, were associated positively with flow magnitude ((linear regression,  $R^2 = 0.44-0.68$ ,  $F > 8.00$ ,  $P < 0.02$ ). The results of this study can be used to assist the U.S. Army Corps. of Engineers in developing a flow management scheme for the Atchafalaya River that will benefit recreational and commercial fishers in the Basin, as well as the fisheries resources upon which they rely.

## Effects of female size on reproductive output of the Gulf killifish (*Fundulus grandis*)

Taylor G. Allgood\*, Craig T. Gothreaux, and Christopher C. Green

*Aquaculture Physiology Laboratory, Aquaculture Research Station, Louisiana State University Agricultural Center, LSU School of Renewable Natural Resources, Baton Rouge, LA.*  
*cgreen@agcenter.lsu.edu*

The Gulf killifish (*Fundulus grandis*) is an estuarine topminnow that occurs in coastal regions throughout the Gulf of Mexico. The hardy nature of the Gulf killifish makes it a popular baitfish, but availability to fishermen varies seasonally. Aquaculture may provide an avenue to satisfy the year round demand for Gulf killifish. The relatively low fecundity of this fractionally spawning species necessitates investigations into strategies that maximize production potential. The purpose of this study is to evaluate brood stock selection criteria by characterizing egg production of three size classes of female Gulf killifish.

Previous studies have indicated that females over 5 g are sexually mature, and our study will use four replicates of six females representing small (5.8-7.8 g), medium (13.0-15.0 g) and large (18.5-22.2) size classes held with three males of uniform size (13.0-17.0 g). The replicates will be contained in twelve 80-L aquaria within two interconnected recirculation systems at a salinity of 7.0 ppt. Fish are currently being conditioned, with tanks receiving commercially available feed (35% protein, 8% fat) at a rate of 2.5% body weight per day. Once acclimated, the photoperiod regime will be 14-hr light and 10-hr dark, and water temperature will be maintained at 26°C. To assess egg production, Spawntex<sup>®</sup> spawning mats will be placed in each tank and collected every two days for determination of total eggs spawned and viable embryos produced. Gonadosomatic indices will be measured to provide empirical data on spawning times and ovarian cycles. We will present preliminary data from these experiments designed to identify optimum brood stock sizes for efficient production of this popular marine baitfish.



Figure 1. Relative size classes of female (above) and male (below) Gulf killifish used in the study.

## The Gulf killifish (*Fundulus grandis*) project: integrating research and extension for aquacultural development

Julie A. Anderson\*, Sunny J. Brogan, Craig T. Gothreaux, R. Glenn Thomas, and Christopher C. Green

*Aquaculture Research Station, Louisiana State University Agricultural Center, LSU School of Renewable Natural Resources, Louisiana Sea Grant, Baton Rouge, LA.*

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Beginning in 2006, the LSU Agricultural Center initiated meetings to determine a prioritized agenda of future research initiatives for aquaculture in Louisiana. After multiple summits, marine baitfish culture was identified as a priority area for research. Initial funding in 2007 came from LSU AgCenter internal funds, which were followed up in 2008 with USDA Aquaculture Special Grants funding, with additional subsequent funding from USDA Special Grants, Louisiana Sea Grant, and the Louisiana Department of Wildlife and Fisheries. Research is ongoing and early results are being delivered to stakeholders and potential producers of marine baitfish.

The Gulf killifish (*Fundulus grandis*, known colloquially in Louisiana as cocahoe minnows) research project began with studies designed to determine methods to increase egg production, as previous research has indicated that low fecundity was the major bottleneck to a successful culture operation. The focus was on outdoor production in low-tech mesocosms maintained at low salinities. In 2008, an initial study was conducted comparing traditional spawning substrate (cured Spanish moss) to a commercially available substrate (Spawntex<sup>®</sup>), and results indicated that incorporation of modern spawning mats can result in the production of significantly more eggs. We have subsequently examined the effects of stocking density, sex ratios, collection cycles, and shading during the summer months on egg production.

As we continue to research production strategies for Gulf killifish, we have begun dissemination of information to stakeholders and potential producers of marine baitfish. Besides presentations at professional meetings and peer-reviewed journals, we have sent out a survey (Figure 1) to 71 marina and bait shop owners in Louisiana. We have had a good response from the surveys with a 50% return rate, with 57% of owners indicating they would attend future workshops on Gulf killifish production. Additionally we have completed multiple micro-workshops on Gulf killifish production across the state. The micro-workshops featured presentations on various aspects of biology, culture, transportation, hauling, and culture systems. Additionally, we presented a hands-on demonstration workshop on October 5, 2010 at the LSU AgCenter Aquaculture Research Station. This presentation will summarize the integration of current and future research and extension work pertaining to the marine baitfish culture initiative in Louisiana.

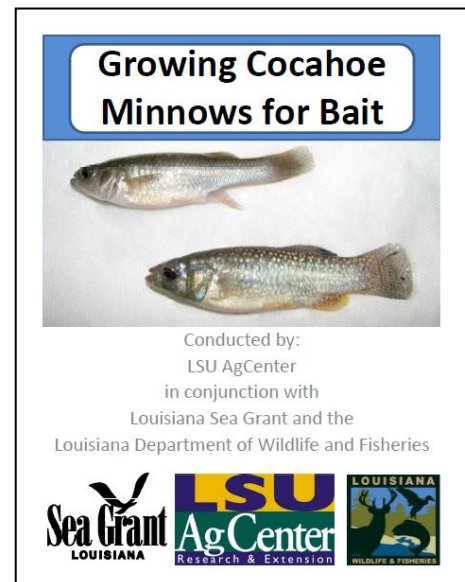


Figure 1. Cover sheet of the *F. grandis* culture survey mailed to marina and bait shop owners.

## **Can hemolymph lactate, protein, and glucose concentrations serve as physiological biomarkers of hypoxic stress in red swamp crayfish (*Procambarus clarkii*)?**

Christopher P. Bonvillain\*, D. Allen Rutherford, Christopher C. Green, and William E. Kelso  
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Crayfish harvested from the Atchafalaya River Basin (ARB) represent the majority of Louisiana wild crayfish landings. However, ARB sedimentation and anthropogenic activities have altered the historic river-floodplain connection, causing reduced water circulation and flow patterns that prompt the formation of hypoxic conditions (dissolved oxygen  $\leq 2$  mg/L) during the annual flood pulse. Physiological stress caused by chronic hypoxia exposure may lead to detrimental population effects such as reduced survival, growth, and fecundity. The purpose of our study was to determine if ARB hypoxia causes physiological stress in red swamp crayfish (*Procambarus clarkii*) by examining hemolymph lactate, protein, and glucose concentrations in individuals captured from hypoxic and normoxic sites throughout the annual ARB flood pulse. Adult *P. clarkii* hemolymph was collected by pericardial cavity puncture, with serum protein, lactate, and glucose concentrations determined spectrophotometrically. Mean crayfish hemolymph lactate and glucose concentrations were not significantly different in individuals captured from hypoxic and normoxic waters. However, crayfish from hypoxic sites displayed a significantly lower ( $P = 0.0038$ ) mean hemolymph protein concentration when compared to individuals from normoxic sites, a disparity that appeared to intensify during the latter stages of the flood pulse. Hemolymph protein concentration appears to be a good physiological biomarker of chronic hypoxic stress in *P. clarkii*. Although not seen in the field, results of laboratory experiments indicate rapid changes in lactate and glucose concentrations under hypoxic conditions. These changes suggest an additional acute response to hypoxia, possibly dampened by various biotic and abiotic factors.

## Acute effects on Atchafalaya River Basin physicochemistry associated with the passage of Hurricane Gustav

Christopher P. Bonvillain<sup>\*1</sup>, B. Thorpe Halloran<sup>1</sup>, Kevin M. Boswell<sup>2</sup>, William E. Kelso<sup>1</sup>, A. Raynie Harlan<sup>1</sup>, and D. Allen Rutherford<sup>1</sup>

<sup>1</sup>*School of Renewable Natural Resources, Louisiana State University AgCenter, Baton Rouge, LA.* <sup>2</sup>*Department of Oceanography and Coastal Sciences, Louisiana State University, Baton Rouge, LA.* *cbonvi3@tigers.lsu.edu*

On 1 September 2008, Hurricane Gustav passed over the Atchafalaya River Basin (ARB) in south-central Louisiana. Anticipating physicochemical shifts attributable to the combination of concentrated precipitation and wind stress generated by this strong category 2 storm, we deployed a continuous recording multiparameter water quality sonde in a southern ARB bayou three days prior to storm arrival to document conditions before, during, and after hurricane landfall. Quarter-hourly physicochemical measurements taken over a two-week period indicated that dissolved oxygen (DO), pH, and specific conductance all reached annual lows immediately following storm passage. The most pronounced post-storm physicochemical fluctuation involved DO. Daily mean DO concentrations dropped to hypoxic levels ( $\text{DO} \leq 2 \text{ mg/L}$ ) within 3 days of the storm passage, with near-anoxic conditions developing within five days that resulted in extensive system wide fish kills. Within six weeks, however, physicochemical parameters returned to pre-storm levels. To further assess the impact of Hurricane Gustav on ARB physicochemistry, we contrasted data on temperature, DO, pH, and specific conductance collected from 16 fixed sampling stations over a 54-day interval prior to landfall with data collected during a 45-day period after the storm. Comparisons revealed that water quality was highly dissimilar (Wilks' Lambda = 0.10,  $F_{4,91} = 209.29$ ,  $P < 0.0001$ ) between the two periods. Our observations suggest that re-establishment of typical floodplain physicochemistry and hydrology after a major perturbation like Hurricane Gustav may take many weeks, and will likely entail considerable stress to resident aquatic biota during the recovery period.

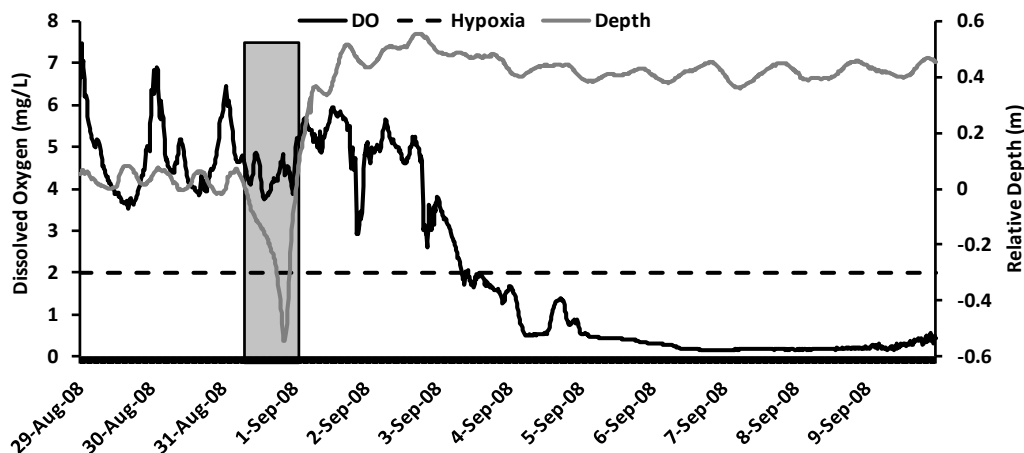


Figure 1. Little Bayou Jessie quarter-hourly dissolved oxygen and relative depth from 29 August – 10 September 2008. Hurricane Gustav made landfall on 1 September 2008. The dashed horizontal reference line indicates hypoxic level (dissolved oxygen  $\leq 2 \text{ mg/L}$ ). The grey box represents the 20 hour period of hurricane passage over the sample area.

## Metabolic and embryonic responses to terrestrial incubation of Gulf killifish (*Fundulus grandis*) eggs across a temperature gradient

Charles Brown<sup>1\*</sup>, Fernando Galvez<sup>2</sup>, and Christopher Green<sup>1</sup>

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The Gulf killifish (*Fundulus grandis*) is a euryhaline baitfish native to tidal salt marshes in the Gulf of Mexico region of the United States. Eggs are deposited on blades of marsh grass and may be exposed to air during low tide. Hatching is delayed until the eggs develop and are immersed during high tide. Because of the rapid rate of embryogenesis, embryos accumulate a large amount of potentially harmful metabolic waste that must be processed or stored during the period of terrestrial incubation. This study measures the rates of embryogenesis, nitrogen elimination, heartbeat, genetic expression of metabolic waste related enzymes, lactic acid production, maximum length of time of delayed hatching, and developmental responses of terrestrially incubated larvae.

Eggs were obtained from spawning mats that were allowed to soak for 16 hours in pools containing adult Gulf killifish. Eggs were then sorted based on stage of development and selected for formation of the gastrula. Terrestrial incubation was simulated by sandwiching approximately 1,300 eggs between 2 pieces of foam moistened with brackish water (7.6 g/L). Triplicate groups of these mats were placed in incubation chambers outfitted with external thermostats at temperatures of 20, 23, 26 and 30°C. Eggs were monitored for the rate at which they reached five critical stages of development (Fig 1): formation of the optical vesicles and embryonic keel (stage 19), onset of circulation (stage 25), functioning of the pronephrose (stage 28), formation of the lower jaw (stage 34), and extension of the head (stage 35). At each stage eggs were placed in 1.5 mL of sample water for 4 hours. The sample water was then assayed for ammonia and urea. If embryos hatched during incubation, they were preserved for morphometric analysis. At each stage, embryos were sampled for lactic acid levels, heart rate, and genetic expression of metabolite processing enzymes. After stage 35, terrestrially incubated eggs were sampled every 48 hours.

Temperature had a significant effect on rate of embryogenesis ( $P < 0.01$ ). Delayed hatching for the 20°C treatment was able to be extended 14 days past stage 35 before considerable mortalities occurred, but for only eight days for the 30°C treatment. This adaptation may be useful in aquaculture operations to manipulate hatch dates and minimize asynchronous hatching, cannibalism, and cohort size heterogeneity.

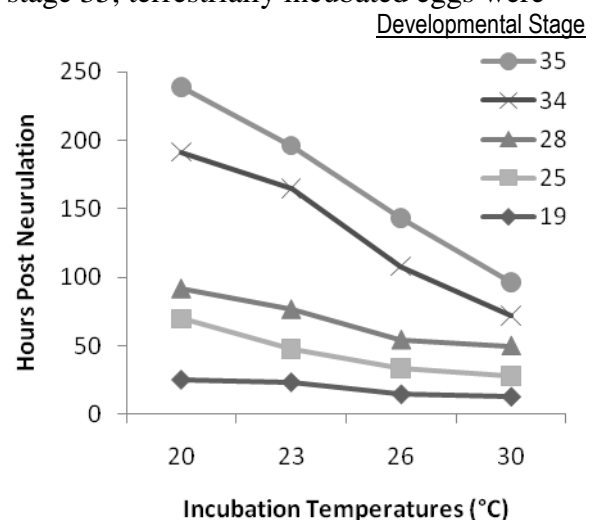


Figure 1. – Timing of developmental stages for incubation treatments of 20, 23, 26 and 30°C.

## **Developing and disseminating preferred catch and release methods for Louisiana saltwater recreational anglers**

Edward Chesney\*<sup>1</sup>, R. Glenn Thomas<sup>2</sup>, John Hawke<sup>3</sup> and Cara Hoar<sup>4</sup>

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It is well known that commercial and recreational fishing are often the most significant sources of mortality for fish populations. The Louisiana Department of Wildlife and Fisheries and the Louisiana recreational fishing task force are committed to developing and promoting better catch and release practices for Louisiana anglers in order to conserve valuable fishery resources. One of the goals of this effort is to educate the public in a way that will reduce the impacts of bycatch from Louisiana saltwater recreational fishing activities. Although saltwater catch and release fishing is uncommon in Louisiana, regulations such as species and seasonal closures and capture of non-target species results in many thousands of fishes being released every year. An often overlooked element of catch and release fishing is how to properly handle and release fish in a way that will give a released fish the best possible chances for survival. Best handling practices and release techniques vary from species to species and in different fishing situations because of water depth, hook type, hook placement, the type of tackle (rod, line weight), environmental conditions, fish type and fish size. All these factors significantly affect the level of stress a fish experiences during capture and subsequently the condition of the fish when they are released. Fish handling and release recommendations often come from fishing guides with little training in fish physiology, stress responses, disease vulnerability or after-effects of fish capture, there is also some variability in fish handling recommendations among experts. A committee of experts reviewed a variety of catch and release outreach materials from around the world, evaluated their relevance to Louisiana recreational fishing practices, and developed a number of recommendations concerning: 1) proper techniques for handling Louisiana's principal saltwater fish species, including selection and use of landing nets; 2) preferred tools and methods for de-hooking fish to be released (how to handle a gut hooked fish); 3) proper use and benefits of circle hooks; 4) handling fish suffering from moderate to severe barotraumas; and 5) other fishing techniques and strategies that will reduce fish stress and the impacts of recreational fishing bycatch.

## Assessing larval blue crab (*Callinectes sapidus*) densities in the natural and artificial tidal passes of Lake Pontchartrain, Louisiana

Rebecca Weatherall Cope\*

Nekton Research Laboratory, Pontchartrain Institute for Environmental Sciences, University of New Orleans, New Orleans, LA. [rwcope@uno.edu](mailto:rwcope@uno.edu)

Lake Pontchartrain is connected to the Gulf of Mexico via two natural passes (Chef Menteur Pass and the Rigolets) and a single artificial pass (the Mississippi River Gulf Outlet - MRGO). I assessed densities of larval blue crabs (*Callinectes sapidus*) entering Lake Pontchartrain through these three tidal passes from February 2009 to November 2010. Triplicate samples of larvae were collected in each of the three passes during the strongest flowing tide of each month with SeaGear 500 micron “Bongo” nets towed simultaneously for ten minutes at the water surface across the width of the pass, perpendicular to the incoming tide. During the collection period, the MRGO was de-authorized and closed, effectively eliminating any tidal flow and crab recruitment into Lake Pontchartrain via this corridor. As a consequence, these data will permit evaluation of the relative contributions of the three passes to the Lake Pontchartrain *C. sapidus* population, as well as changes in the crab population in response to the MRGO closure. The sampling period also serves as baseline data for determining the effects of the Deepwater Horizon oil spill on *C. sapidus* in this system. Preliminary analyses indicate that there are pulses of larval *C. sapidus* in both fall and summer, with the highest densities entering Lake Pontchartrain through Chef Menteur Pass (Figure 1).

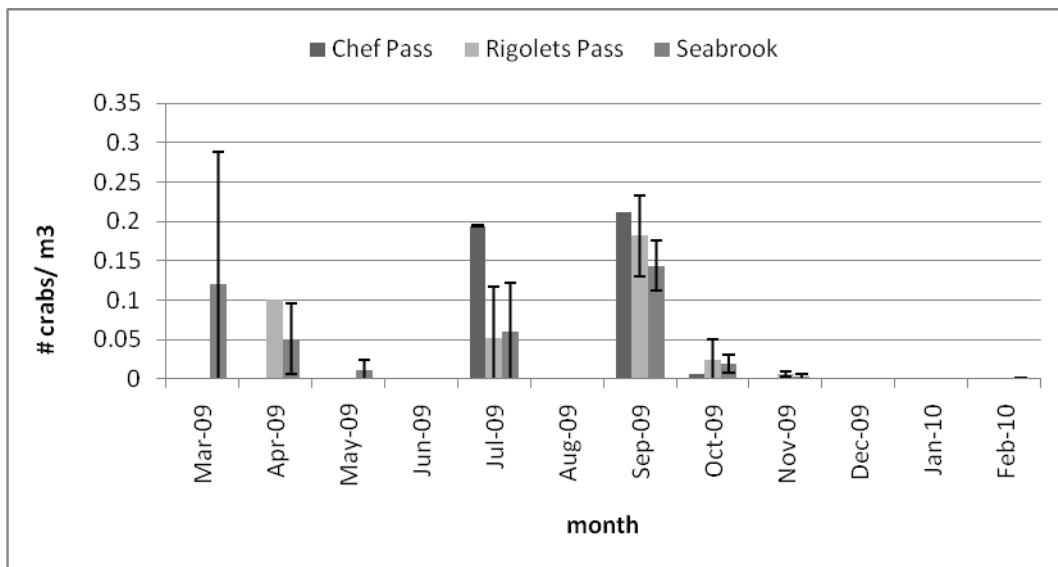


Figure 1. Densities (#crabs/m<sup>3</sup>) of larval *C. sapidus* in the Lake Pontchartrain tidal passes from March 2009 to February 2010.

## **Assessment of angler exploitation of white crappie (*Pomoxis annularis*) in Poverty Point Reservoir, Louisiana**

Ryan Daniel\* and Jesse Bahm

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Because of growing concern of detrimental angler impact to the crappie population in Poverty Point Reservoir, we estimated angler exploitation of white crappie (*Pomoxis annularis*) in this relatively new reservoir in northeast Louisiana during 2009. A tag-reward study was utilized to assess exploitation, while angling characteristics were determined through a non-uniform random access point creel survey. White crappie were tagged from January – March 2009 with Floy anchor tags labeled with REWARD and a tag number. Returns were accepted through December 31, 2009. A total of 144 of 243 tagged crappie were harvested and reported by anglers. The exploitation was estimated at 72% based on the assumption of a 20% non-reporting rate, but appeared to be nearly 80% when other variables were considered. Creel survey results (346 interviews) revealed that crappie anglers harvested 6.3 crappie per trip, with an average fish length of 290 mm. Fishing mortality and harvest data obtained from this study will be supplemented with age and growth data obtained during Fall 2010 to obtain a more accurate assessment of total annual mortality, which will be used in yield-per-recruit models to predict effects of various regulations on the population.

## Evaluating growth and survival of three smooth cordgrass (*Spartina alterniflora*) ecotypes in coastal Louisiana

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Most coastal restoration efforts include a revegetation component to secure newly created land. Louisiana coastal marshes are dominated by smooth cordgrass (*Spartina alterniflora*), which provides critical habitat for numerous ecologically and economically important fisheries species. The Louisiana Department of Natural Resources (LDNR) currently requires use of the Vermilion cultivar for all LDNR re-vegetation projects that require smooth cordgrass. Although the Vermilion cultivar grows well in a variety of habitats, the sole use of only one ecotype may reduce local genetic diversity. The purpose of this project was to compare survival and growth of the Vermilion cultivar to two wild ecotypes of smooth cordgrass collected near Fourchon, LA, and Cocodrie, LA. We used two independent study sites (Fifi Island planted 4 November 2006 and Leeville planted 18 November 2006) to evaluate growth and survival of the three smooth cordgrass ecotypes. Each planting site was divided into nine blocks and each ecotype was randomly assigned to three of the nine blocks. The mean of each ecotype-specific block (N=3) was used to calculate overall mean ( $\pm$ SD) survival, height, and number of stems at each planting site monthly from 17 December 2006 to 6 December 2007. Repeated measures analysis of variance and least-squares means ( $\alpha = 0.05$ ) were used to evaluate growth and survival among ecotypes for each planting site. Mean survival ( $74 \pm 8.0$  %) at the Fifi Island site was similar for all three ecotypes and ranged from 65 to 80%. Survival for the Vermilion cultivar ( $67 \pm 18.9$  %) was lower than survival for the Fourchon ( $80 \pm 2.4$  %) and Cocodrie ( $80.2 \pm 3.9$  %) ecotypes. Vertical growth was also similar among ecotypes throughout the study at both sites, with mean maximum plant heights (ecotypes combined) of  $177 \pm 7.6$  cm at Fifi Island and  $163 \pm 8.1$  cm at Leeville recorded on 3 October 2007. There was no difference in number of stems among ecotypes at the Leeville site, but the Fourchon ecotype ( $130 \pm 27.0$ ) produced more stems than either the Vermilion cultivar ( $121 \pm 21.4$ ) or the Cocodrie ecotype ( $120 \pm 25.5$ ) at the Fifi Island site. For all ecotypes combined, the maximum mean number of stems per  $m^2$  measured was  $124 \pm 5.3$  for the Fifi Island site and  $68 \pm 6.2$  for the Leeville site. For all metrics used, the Vermilion cultivar did not perform better than the wild ecotypes and in some cases, the wild ecotypes performed better than the Vermilion cultivar. Based on these results, we suggest that future restoration projects that use the Vermilion cultivar incorporate some local ecotypes to ensure genetic variability.

## Gulf killifish (*Fundulus grandis*) egg production – ratios, cycles, and mats, oh my

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The Gulf killifish (*Fundulus grandis*) is a popular marine baitfish for many sportfish species along the coastal waters of the Gulf of Mexico. Currently the supply of wild-caught individuals does not satisfy the demand from anglers. Although historic attempts to culture Gulf killifish identified low fecundity as the predominant bottleneck for industry success, recent research has indicated that modern culture techniques can increase production efficiency. This research builds on a series of experiments over the last few years to produce Gulf killifish eggs in above-ground tanks. During the 2010 spawning season, we investigated several practical production issues by: 1) altering sex ratios, 2) manipulating egg collection cycles, and 3) increasing spawning substrate availability.

We conducted these experiments in 9600-L above-ground mesocosms that contained river silt for filtration purposes. Tanks were filled with well water, constantly aerated via an airstone, and maintained at a salinity of 5-7 g/L with rock salt. The first experiment evaluated the effect of 2:1 and 4:1 ♀:♂ sex ratios on egg production, and incorporated parallel comparison of weekly and bi-weekly egg collections at the 4:1 ratio (Figure 1). The purpose of the egg collection cycle variation was to determine if semi-lunar peaks could be predicted and exploited (focusing labor during peak periods), and to determine whether egg output was compensatory based on substrate presence. The second experiment compared egg output from tanks with either one or two spawning mats to determine if spawning substrate availability was a limiting factor.

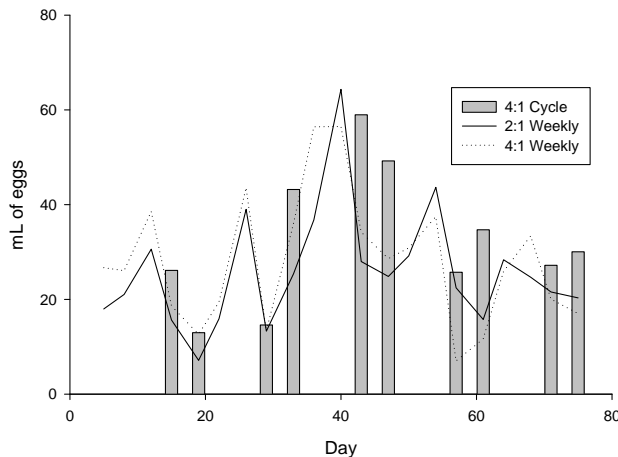


Figure 1. Average egg production (approximately 120 eggs/mL) of Gulf killifish from April through July of 2010. Mesocosms were stocked with 260 fish at either a 2:1 or 4:1 ♀:♂ sex-ratios. Egg collections were made every 3-4 days (lines) except for a separate treatment group, at a 4:1 sex-ratio, which was collected during bi-weekly cycles (bars).

Gulf killifish egg output follows semi-lunar cycles with peaks around every 14 days (Figure 1). The predicted peaks (based on the first and third quarter moon phase) did not match with the observed peaks of egg production; however, some dates towards the end of the study saw significantly more eggs collected from the cycle treatment (Figure 1). The total number of eggs collected from the cycle treatment was significantly lower than those collected from the weekly treatments. There was no significant difference between the number of eggs collected from the 2:1 and 4:1 sex-ratio treatments. The spawning substrate availability experiment is ongoing, but preliminary results indicate that mat availability does limit egg production.

## Characterization of isolates of Group B Streptococci from diseased hybrid striped bass (*Morone saxatilis* x *M. chrysops*) and Gulf killifish (*Fundulus grandis*)

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Group B streptococci have been described as disease causing agents from various species of marine fish over a wide geographic and host range. Group B *Streptococcus* sp. have been described as causative agents of fish kills of wild marine and euryhaline species in coastal estuaries of the northern U.S. Gulf Coast since the early 1970's. Isolates of group B streptococci have been obtained from cultured hybrid striped bass (*Morone saxatilis* x *M. chrysops*) and Gulf killifish (*Fundulus grandis*) from coastal Louisiana fish farms since the late 1980's. In this study we identify these U.S. Gulf Coast isolates as *Streptococcus agalactiae* group B type Ib and describe their biochemical phenotype, antibiotic susceptibility, and phylogenetic relationship to other Group B streptococci from fish cultured in Thailand, Ecuador, and Honduras. Genetic analysis was performed with partial sequence comparison of the 16S rRNA gene, *sipA*, which encodes a surface immunogenic protein, *cspA*, which encodes a cell surface-associated protein, and *secY*, which encodes components of a general protein-secretion pathway. Phylogenies inferred from *secY* and *cspA* gene sequence comparisons were more discriminative than those inferred from 16S rRNA and *sipA* gene sequences. In agreement with the molecular findings, biochemical and antimicrobial resistance analysis demonstrated similar profiles for the isolates recovered from the Gulf Coast when compared to those from other locations. Laboratory challenge methods for inducing streptococcosis in Gulf killifish were performed with strain LADL-97-151. The lethal dose 50 value, 14 days post-challenge, was  $10^{-9.43}$  (2 CFU/fish) by intraperitoneal injection.

## Ulcerative mycoses caused by *Aphanomyces invadans* and *Ochroconis* sp. in wild and cultured marine fish species

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In this paper we describe ulcerative mycotic diseases from wild populations of red drum (*Sciaenops ocellatus*) and black drum (*Pogonius cromis*) and cultured cobia (*Rachycentron canadum*). This is the first report of an invasive fungal infection in cobia. The diseases were diagnosed from specimens voluntarily submitted to the Louisiana Aquatic Diagnostic Laboratory at the School of Veterinary Medicine, Louisiana State University. Adult red drum and black drum were collected from the inshore marsh habitat in Louisiana and the juvenile cobia (10-15 cm) were from a recirculating culture system. Specimens presented with sharply demarcated, circular to irregular, ulcerative lesions in the skin penetrating into the underlying muscle tissue. Oomycete and dematiaceous hyphomycete fungi were isolated by aseptic necropsy on peptone yeast glucose (PYG) agar from infected muscle tissue. The *Aphanomyces* colonies grew slowly on the agar medium at 25°C and produced white velvety colonies that grew above and below the agar surface. The hyphomycete fungus *Ochroconis* sp. also grew slowly at 25°C and produced brown pigmented colonies on the surface of PYG agar. Histopathology of *Aphanomyces* infected fish revealed non-septate hyphae penetrating deep into the muscle surrounded by granulomatous inflammation and multinucleated giant cells. Histopathology of *Ochroconis* infections revealed melanized, septate, fungal hyphae surrounded by epithelioid granulomatous inflammation in skin, muscle, intestine, and kidney tissues. Identification of the pathogens was accomplished with a combination of morphological characters and molecular analysis by amplification and sequencing of the internal transcribed spacer (ITS) region located between the 18S and 28S rRNA genes.

## Scaling of high-throughput cryopreservation for fishery and aquaculture applications

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Cryopreservation is an effective tool used worldwide by researchers for conservation of genetic information. Broadening the application of this technology through high-throughput approaches has become important, especially for biomedical model fish such as zebrafish. This study addressed whether sperm cryopreservation in aquatic species would be sufficient for tasks related to fisheries and aquaculture on a practical scale. We chose a large-bodied species, the blue catfish *Ictalurus furcatus* for our research. Our objectives were to: 1) establish a basic pathway for high-throughput cryopreservation; 2) evaluate commercial-scale cryopreservation in hatchery production, and 3) evaluate the role of cryopreserved sperm in practical applications.

The high-throughput process combined manual sperm extraction and automated straw processing. Testes were dissected and crushed, suspensions were adjusted to  $2 \times 10^9$  sperm/ml, and an automated system (MAPI, Cryo-Bio-System, Inc., France) was used for loading, sealing, and labeling high-security 0.5-ml plastic (CBS) straws. Samples were cooled at  $5^\circ\text{C}/\text{min}$  from  $4^\circ\text{C}$  to  $-80^\circ\text{C}$ , and after storage (2 to 6 wk), 1,406 straws were transported to the hatchery, thawed at  $40^\circ\text{C}$  for 20 s, and pooled and held at the same conditions as fresh sperm collected on site. Fertilization trials with 242 females were performed at a commercial hatchery (Table 1). Using a set sperm-to-egg ratio ( $5 \times 10^5$  sperm:egg), thawed sperm had the same fertilization capability as fresh sperm. Application of cryopreserved sperm yielded production of 200,000 fry per day. From this we conclude that the use of cryopreserved sperm need not be limited to the laboratory, and offers wider utility in fisheries and aquaculture applications. The supply cost was about US\$1.50 per straw of blue catfish sperm (without labor or equipment investment), and each straw was used to produce more than 1,000 hybrid fry. Production was not different on a per-male basis for fresh and cryopreserved sperm. Thus there was no loss in production from valuable broodstock, and a significant gain in efficiency and genetic control was obtained with cryopreservation. Production at this scale would be relevant to a wide range of fisheries and aquaculture applications such as conservation programs, stock enhancement, and production of seedstock for culture.

Table 1. Commercial-scale production parameters for use of high-throughput cryopreservation of blue catfish sperm. Cryopreseved sperm from 17 males and fresh sperm from 16 males were cross-tested during 4 days with eggs from 242 females. (P-values obtained from T-test).

Parameter	Thawed sperm	Fresh sperm	P-value
Sperm use per day (cells)	$17 \pm 6 \times 10^{10}$	$24 \pm 12 \times 10^{10}$	0.173
Sperm motility (at use)	$38 \pm 7\%$	$62 \pm 3\%$	0.000
Motile sperm number	$6 \pm 2 \times 10^{10}$	$15 \pm 8 \times 10^{10}$	0.040
Hatching rate	$43 \pm 15\%$	$52 \pm 4\%$	0.158
Sac fry produced (per day)	$17 \pm 7 \times 10^4$	$28 \pm 7 \times 10^4$	0.034
Sperm use/sac fry	$12 \pm 8 \times 10^5$	$9 \pm 4 \times 10^5$	0.252
Motile sperm/sac fry	$4 \pm 2 \times 10^5$	$6 \pm 3 \times 10^5$	0.225

## **Diet and growth rates of cultured alligator gar (*Atractosteus spatula*) stocked into a Louisiana drainage canal**

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Alligator gar (*Atractosteus spatula*) are cultured in the United States to replenish historic populations and may be used as a biological control for invasive fishes. In an attempt to suppress invasive tilapia *Oreochromis* spp. that may have survived rotenone treatments, alligator gar produced in 2008 (N=159) and 2009 (N=183) were stocked in January and February 2010 into a drainage canal in Port Sulphur, Louisiana. Each alligator gar was PIT tagged, measured (mm), and weighed (kg) prior to stocking. Alligator gar recaptured with monofilament gill nets (2.5, 4.4, and 5.1 cm bar mesh) were measured and weighed, and stomach contents were retrieved via gastric lavage. Of the 60 recaptured fish examined to date for stomach contents, 41 contained identifiable food items, which included 197 fish, 34 crustaceans, 23 insects, and 2 reptiles. Based on the lengths of recaptured alligator gar, growth rates were nearly 3 mm per day, and were similar among fish recaptured and lavaged once (N=47), twice (N=9), and three times (N=3). Alligator gar produced in May 2008 had approached 1 m in total length by September 2010. Results of this study indicate that cultured alligator gar can successfully feed and grow when stocked in the wild, which may allow re-establishment of extirpated populations while simultaneously providing a potential biocontrol for invasive fishes.

## Seasonal abundance of larval fishes in the upper Barataria Estuary may be limited by local hypoxic conditions.

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The Barataria Estuary (west of Lac Des Allemands) is the southernmost western floodplain of the Mississippi River, although hydrological modifications have disconnected the floodplain and river. Because water levels in the upper Barataria Estuary are a function of local precipitation, large rain events may result in extended periods of hypoxic conditions. To describe the abundance and distribution of larval fish in the upper Barataria Estuary, larval fish were collected weekly from seven fixed sites with light traps from February through September 2007. Three sites were located in a dredged canal characterized by steep dredge spoil banks and limited floodplain access, and four sites were located in a natural bayou characterized by low banks and floodplain access during high water periods. Dissolved oxygen (mg/L), temperature ( $^{\circ}\text{C}$ ), and specific conductance ( $\mu\text{S}/\text{cm}$ ) were measured with a handheld oxygen-conductivity-salinity-temperature meter at each sample site. A total of 4,110 larval and juvenile fish representing 11 families and 15 genera were collected between 9 February 2007 and 25 September 2007. Larval *Dorosoma* spp., *Ictiobus* spp., and *Lepomis* spp. were the most abundant groups collected and were more abundant in the dredged canal than in the bayou. Bayou sites were hypoxic ( $\text{DO} < 2.0 \text{ mg/L}$ ) during 62% of sample trips and hypoxic conditions were observed in the bayou during every sample trip from 30

June 2007 to 22 August 2007 (54 d). Although *Lepomis* sp. larvae were collected throughout the summer in the canal, only two *Lepomis* sp. larvae were collected in the bayou after June 30 (Figure 1). Altered hydrology, specifically the lack of river water flushing, may contribute to chronic hypoxia in the bayou habitats of the upper Barataria Estuary, which may limit local fish recruitment.

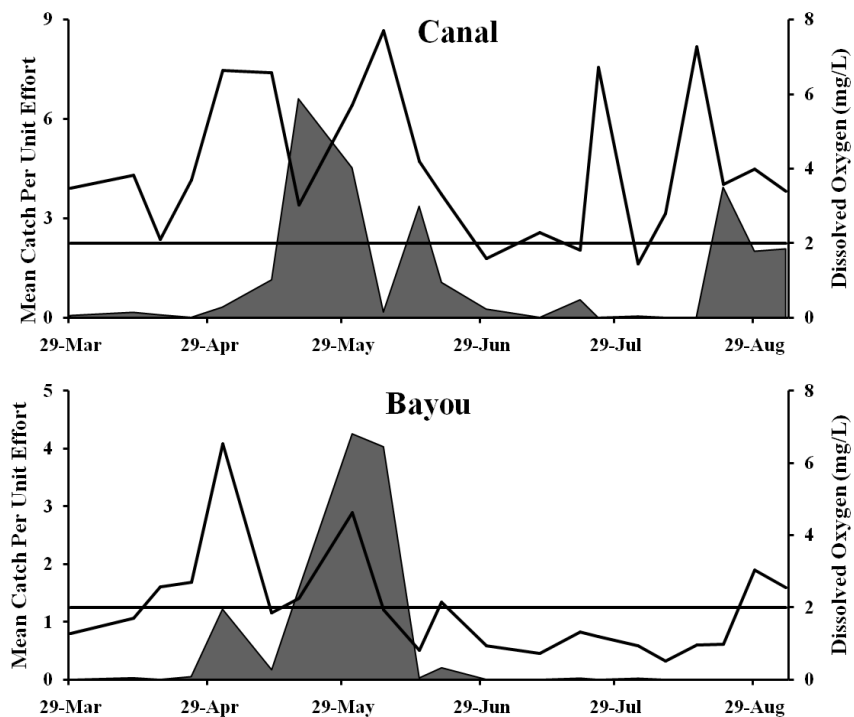


Figure 1. Mean catch per unit effort (#/trap hr) of larval *Lepomis* spp. (shaded area) and dissolved oxygen (DO; black line) for the canal and bayou habitats. Horizontal line marks  $\text{DO} = 2.0 \text{ mg/L}$ .

## A geographic information system tool for aquatic resource conservation: Red and Sabine River watersheds

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Geographic information systems (GIS) may help resource managers determine the degree to which aquatic ecosystems are healthy, degraded, or in transition. The goal of this project was to build a GIS to enhance modeling and hypothesis-testing relevant to the watersheds and associated fish fauna of the conjoined Red and Sabine Rivers (Figure 1). Fish species of concern were identified from state wildlife action plans and websites. The spatial distribution of common fish species and mercury concentrations in fillets were delineated with data from state agencies. Publicly available, georeferenced environmental data were obtained on land cover, soil type, forest canopy, impervious surfaces, wastewater facilities, 303(d) impaired waters, and superfund sites. Overlay maps of single or combined variables highlighted patterns across 8-digit hydrologic unit codes (HUCs), pointing to potential targets of managerial interest. Bowfin (*Amia calva*) and black bass (*Micropterus* spp.) were suitable indicators of bioavailable mercury, where concentrations > 0.5 ppm correlated significantly with environmental variables. Fish species occurrence and environmental relationships were analyzed with detrended correspondence analysis, and variability of fish occurrence was explained primarily by impervious surface and land cover parameters. Two-way indicator species analysis (TWINSPAN) was used to group HUCs. HUCs were grouped in three clusters based on similarity of species composition and may be potential targets of managerial interest. This GIS tool can be used to support trend analyses, reporting on water quality standards, and assessing critical habitats.

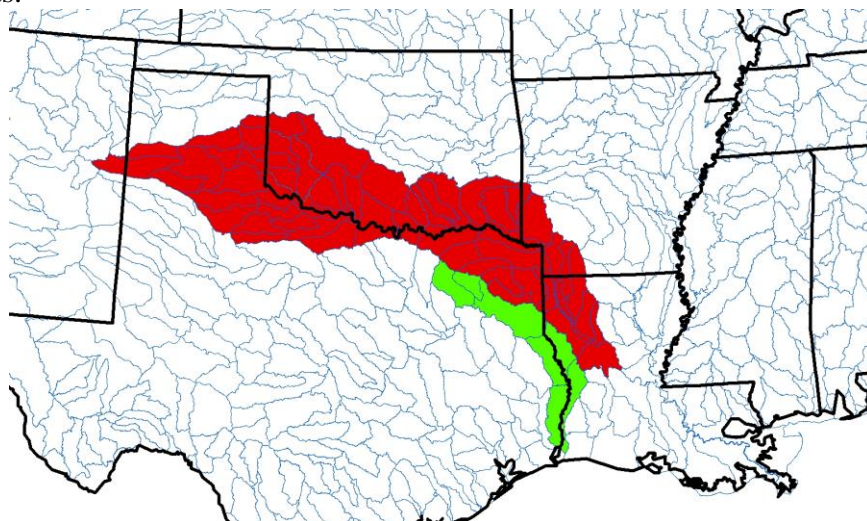


Figure 1. Red (red) and Sabine (green) River Basins.

## **Gonad histology of male bronze frogs (*Rana clamitans*) exposed to atrazine in the Atchafalaya River Basin, Louisiana**

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The Atchafalaya River Basin (ARB) is the largest contiguous bottomland hardwood forest in North America, providing water for drinking, agriculture, and recreation. Farming activities occur in the upper portions of the ARB, and water in the Atchafalaya River comes from the agriculturally-productive Mississippi and Red river basins. As a consequence, aquatic habitats in the ARB receive contamination from widespread agricultural activities. Atrazine, a triazine herbicide known to cause health problems in vertebrates, is the most commonly detected herbicide in the ARB and Mississippi River. However, no studies exist on the effects of atrazine on ARB vertebrates. In spring 2009, we measured concentrations of 0.022–2.0 µg/L of atrazine in water samples taken from 20 sites in the ARB. Subsequently, we selected four sites representing low to high atrazine presence (0.03, 0.27, 0.68, and 2.0 µg/L) and resampled these sites in spring 2010 for atrazine (0.06, 0.60, 0.10, and 1.0 µg/L.) To determine the effects of atrazine on the reproductive anatomy of lower vertebrates in the ARB, 15 male and two female bronze frogs (*Rana clamitans*) were collected from the four sites in spring 2010 (n=62). Gonads were collected and preserved in formalin, and tissues were then processed, embedded in paraffin, sectioned at 7 µm, stained with a standard hematoxylin and eosin protocol, and viewed under a light microscope. Testes from frogs inhabiting sites with increasing amounts of atrazine were comparable to those of the reference site. All testes contained sperm cells at all stages of development, and no intersex (oocytes intermingled with sperm cells) gonads were observed. This contradicts laboratory reports of intersex gonads in frogs exposed to lower levels of atrazine than those measured in the ARB.

## **Habitat-specific fecundity of red snapper (*Lutjanus campechanus*) in the northern Gulf of Mexico**

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Little information exists on the reproductive ecology of red snapper, *Lutjanus campechanus*, in the northern Gulf of Mexico (GOM). Current understanding of the stock is based upon individuals collected from artificial reef sites, or from unspecified habitats reported by the directed fishery. However, artificial reef structures constitute less than 5% of suitable habitat area on the Gulf shelf edge banks. Knowledge of natural habitat function in red snapper ecology is needed, but extremely limited. During two spawning seasons (the summers of 2009 and 2010), a total of 283 female fish were collected approximately 100 miles offshore along the continental shelf margin off the coast of Louisiana. We collected 172 fish from the shelf edge banks and 111 from two types of artificial reef sites (high-relief standing, and lower-relief toppled oil rigs) to assess differences in batch fecundity, spawning frequency and size- and age-at-maturity among the three habitat types. Preliminary results based on data collected during 2009 indicate that 81% of fish sampled from natural habitat were sexually mature, compared to only 41.7% of fish collectively sampled from artificial reef structures. Demographics and reproductive parameters from this study will contribute significantly to our understanding of habitat impacts on red snapper ecology in the northern Gulf, enhancing our ability to effectively manage the GOM red snapper stock.

## Utilization of soybean-based diets supplemented with taurine and phytase by Florida pompano (*Trachinotus carolinus*)

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Soybean meal is used in many compounded fish diets because of its balanced amino acid profile and relatively low price. However, soybean meal contains compounds that decrease palatability and digestibility in some fish species. This feeding trial was conducted to determine the optimal level of soybean meal in Florida pompano (*Trachinotus carolinus*) diets. Five fish meal-free diets were formulated to contain 0, 20, 25, 30, and 35% soybean meal (SBM), with the remaining protein provided by soybean protein concentrate (SPC) at levels of 59, 46, 43, 39 and 36%, respectively. These diets were tested with a control diet that contained 30% SBM and 30% SPC in combination with 10% fish meal. All diets were supplemented with 0.5% taurine and 1,000 FTU of fungal phytase (Natuphos™, BASF Corp.) per kg diet. Twenty pompano, with initial weight of 7.0 g per fish, were stocked in 18 circular, 227-L tanks with each diet assigned to three tanks. Fish were fed to satiation daily for 10 weeks and weighed at two-week intervals. Significant differences ( $P < 0.05$ ) were observed among pompano weight gains and feed conversion ratios (FCR; Table 1). Fish fed the SBM 0 diet had the lowest weight gain (410%) and highest FCR (2.18), the latter of which did not differ among the other treatments. Weight gain was highest in control fish (1075%), and was higher than fish fed SBM 0, SBM 20 or SBM 35 diets. Results indicate that compared to a control diet containing soybean products and 10% fish meal, Florida pompano utilize fish meal-free diets containing 25-30% SBM and 39-43% SPC without adverse effects on growth.

Table 1. Florida pompano growth parameters.

Diet	Weight gain (%)	FCR
Control	1075 a	1.56 a
SBM 0	410 c	2.18 b
SBM 20	758 b	1.76 a
SBM 25	875 ab	1.61 a
SBM 30	878 ab	1.69 a
SBM 35	793 b	1.64 a

Mean values in columns with different letters are significantly different ( $P < 0.05$ ).

## Results of post-rotenone treatment monitoring for introduced tilapia (*Oreochromis* spp.) in the Port Sulphur region.

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In December 2008, specimens of a tilapia hybrid (*Oreochromis aureus x niloticus*) were collected near Port Sulphur, LA approximately 75 km southeast of New Orleans. Tilapia have caused damage to native fish populations worldwide, and the Louisiana Department of Wildlife and Fisheries (LDWF) responded rapidly to this discovery in an attempt to control the spread of this population. In the summer of 2009, multiple rotenone applications were made, and subsequent observations suggested that tilapia dominated both biomass and numbers of fishes in freshwater habitats around Port Sulphur. Following rotenone treatments and re-introduction of native fishes, areas within and around the original range of introduced tilapia were monitored from January to September 2010. Although no tilapia were collected, I observed multiple occurrences of post-treatment reproduction by reintroduced bullhead catfish (*Ameiurus spp.*), bluegill (*Lepomis macrochirus*), green sunfish (*Lepomis cyanellus*), and warmouth (*Lepomis gulosus*). There was also evidence that small native fishes had returned to the treated canal system without intentional introductions. Western mosquitofish (*Gambusia affinis*), sheepshead minnows (*Cyprinodon variegatus*), and sailfin mollies (*Poecilia latipinna*) were all prevalent, with some interesting geographical and temporal patterns. Sites outside the treatment zone had a variety of fishes including marsh species such as ladyfish (*Elops saurus*), gizzard shad (*Dorosoma cepedianum*), and white mullet (*Mugil curema*), and freshwater species such as bluegill (*Lepomis macrochirus*), rainwater killifish (*Lucania parva*), invasive silver carp (*Hypophthalmichthys molitrix*), and juvenile tarpon (*Megalops atlanticus*). Unfortunately, tilapia were collected from the treatment zone on three separate occasions after 19 September 2010 (Figure 1). I hope to continue these monitoring efforts to determine the extent of possible tilapia resurgence in the area.



Figure 1. Map and descriptions of fall 2010 collections of tilapia in the Port Sulphur area.

## **Habitat use by young lemon sharks (*Negaprion brevirostris*) at the Chandeleur Islands, Louisiana.**

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We examined sub-adult lemon shark (*Negaprion brevirostris*) habitat use at the Chandeleur Islands, Louisiana between May 2009 and August 2010. A total of 100 sharks were collected, tagged, and measured (49 males; 51 females, fork length range = 512 to 1770 mm). For each shark, habitat and environmental variables were also recorded at the collection site. Sharks were grouped into three size/age classes (neonates < 640 mm, YOY < 900 mm, and juveniles < 900 mm). Environmental variables associated with capture sites for males and females sharks were similar (ANOVA,  $p > 0.05$ ), as were substrate preferences among shark size classes ( $p > 0.05$ ). However, there was a significant difference ( $p < 0.001$ ) among size classes regarding depth at capture, with the smaller two size classes preferring shallower habitats than the largest size class. We attached SPOT 5 satellite tags to the dorsal fin of six juveniles (4 males; 2 females; fork length range = 1100 – 1770 mm) in an effort to determine habitat preference on a larger scale. Data recovered from the tags indicated that these sharks stayed in the area throughout the summer, remaining near the islands and rarely moving to deeper offshore habitats. In the future, we will be calculating growth rate from recaptured lemon sharks marked with PIT tags and pursuing genetic analyses to determine the extent of polyandry and site fidelity in this nursery habitat. We will also be investigating effects of the oil spill and sand berm creation on the Chandeleur Islands lemon shark population.

## Bactericidal activity of spotted gar (*Lepisosteus oculatus*) serum mediated by complement activity

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Serum complement has been well characterized in teleost fish; however, little work has been done looking at holosteids. This study aims to evaluate serum complement potency of spotted gar (*Lepisosteus oculatus*) from southern Louisiana. Concentration, temperature and kinetic dependence of membrane-lytic activity were evaluated with rabbit red blood cell hemolysis. Serum hemolytic properties were tested against rabbit red blood cells at varying concentrations, temperatures and incubation times. Hemolysis was measured spectrophotometrically at 540nm. Antibacterial properties were tested by incubating  $10^6$  CFU of multiple bacteria species in serum. Bacteria were plated after two hours of incubation and enumerated by colony counts. Immunoblots for complement C3 in gar serum was performed with anti-human C3 polyclonal sera. An inhibitor against human C3 was tested with gar serum at varying concentrations to test for inhibition. The study illustrates a concentration and time dependence, with complement activity peaking near 15% concentration, with most kinetic activity in the first 2 minutes of incubation. Complement was not shown to have temperature dependence between 5°C and 35°C. Bacterial inhibition varied among species. Gar C3 was found to migrate at ~185 kDa with the C3a at ~115 kDa and C3b chains at ~70 kDa. The C3 antibody was highly inhibitory at 2 µg/mL but exhibited no significant inhibition at 0.02 µg/mL (Figure 1). This is the first report of complement activity in a gar species.

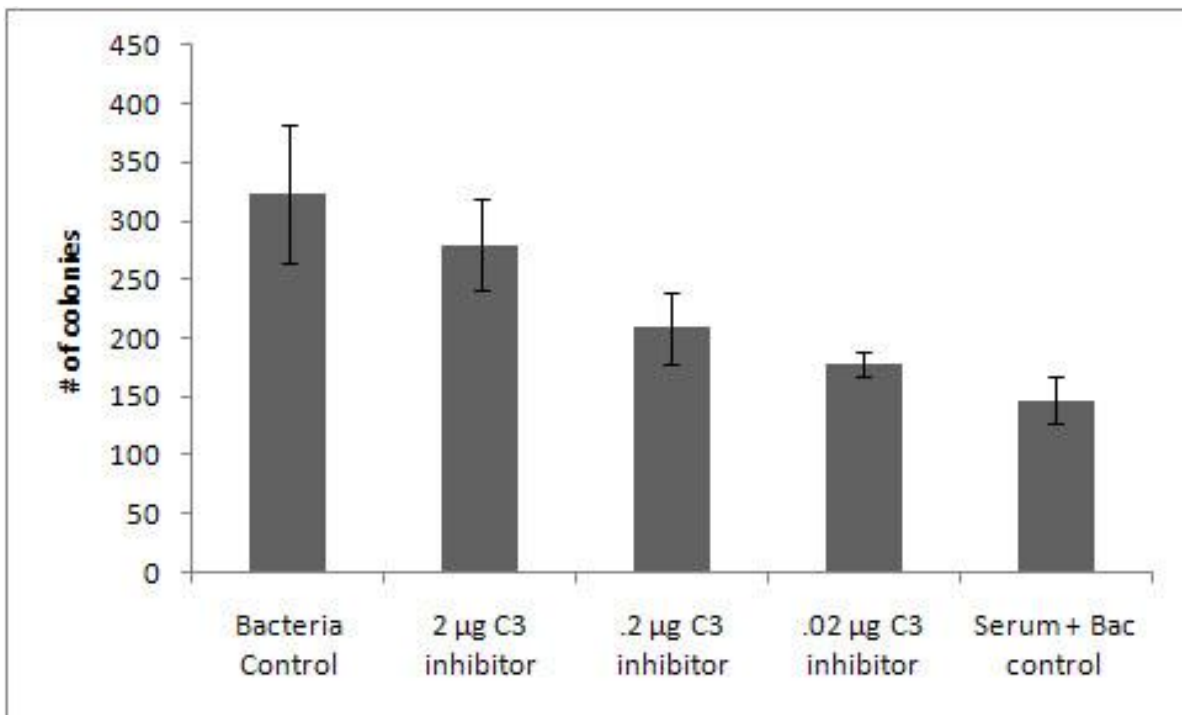


Figure 1. Mean bacterial colony number ( $\pm$  SEM) after incubation with spotted gar serum pre-incubated with C3 inhibitor. N=6

## A review of ultrasound practices in studies of fish reproduction

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Gray-scale ultrasound imaging is commonly used for viewing internal anatomy because it is a non-invasive means for real-time diagnostics. This review focuses on application of this imaging technology in studies of fish reproduction. The goal was to assemble a comprehensive data set to serve as a decision enabling tool for potential users. The objectives were to: 1) compile a literature database on assessment of aquatic species reproduction with ultrasonography; 2) compare handling and ultrasound techniques for different fishes 3) discuss these techniques in relation to principles of diagnostic ultrasound, fish biology, and the future role of ultrasonography in fish reproductive studies. Four databases (afsjournals.org, googlescholar.com, isiknowledge.com, onlinelibrary.wiley.com) were searched. The key words used were: ultrasound, fish, reproduction, and imaging. An EndNote Library (N = 36 references), and an Excel spreadsheet detailing the goal, common and scientific names, and corresponding biological, fish handling, and ultrasound technique of each study were compiled. The studies were predominantly for sex identification and measurement of reproductive indices of 29 aquatic species. The ability to view internal anatomy was dependent on whether or not the epidermis contained heavily calcified structures that prevented penetration of ultrasound into the body cavity. The second major biological determinant of ultrasonography effectiveness was body size. The difficulty in viewing gonads was highest in juvenile fish, and lowest in reproductively mature fishes, with ease of examination increasing from out-of-spawning season to actively spawning fish. There were variable combinations of handling and ultrasound procedures used within and among fish species, which ranged in size from 10 mm to 264 cm. Linear and curved array probes were used, with frequencies ranging from 3.5 to 5 MHz for larger fish and 8 – 15 MHz for smaller fish. Illustrations, or text descriptions in publications should provide a frame of reference (Figure 1) for readers and potential users of this imaging technology. Understanding the basic principles of ultrasound, biological considerations, standardized methodologies and reporting in publications will enable current and future users to compare and optimize studies within and among species.

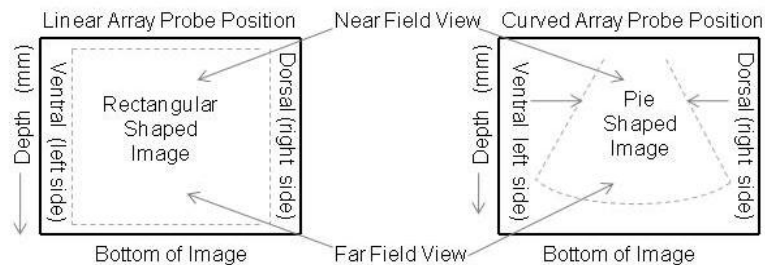


Figure 1. Schematic organization of the ultrasound image displayed by linear (rectangular shape) and curved (pie shaped) array probes. The top of the image corresponds to the position of the probe on the external anatomy (nearest to the skin) of the fish during scanning. The bottom of the image corresponds to anatomic distances furthest from the probe. The left and right side of the image can be switched.

## Stabilizing blood samples in the field for laboratory analyses: Gulf sturgeon (*Acipenser oxyrinchus*) and the Mississippi Canyon 252 Oil Spill

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Although a great deal of cell and molecular data can be derived with miniscule volumes of blood, the samples themselves must not be degraded by time in storage or suboptimal handling. Blood samples from the field are often collected far from the laboratory and typically cannot be analyzed live outside a 24-h window, while the immediate inclusion of fixatives decreases the wealth of data that could be obtained, particularly from previously unstudied species. Hence, the goal of this study was to optimize integrity of blood samples obtained in the field for lab analyses later. Prior to the US Fish and Wildlife Service's handling of Gulf sturgeon (*Acipenser oxyrinchus*) migrating into the Gulf of Mexico following the Mississippi Canyon 252 Oil Spill, blood was obtained from surrogate animal species and tested with or without a commercial cell stabilizing solution (Streck). It was formulated for human blood and immunological studies, where cellular integrity and lymphocyte antigenic sites can be maintained up to seven days at room temperature. To optimize gulf sturgeon blood sample handling, we hypothesized that the condition of blood stored in various treatments and temperatures and for different times will be similar. Using blood from koi carp (*Cyprinus carpio*), quail (*Coturnix coturnix*), and channel catfish (*Ictalurus punctatus*), several experiments were performed with acid citrate dextrose, stabilizer solution, and 4% paraformaldehyde. Biological endpoints included cell viability and nuclear DNA integrity. Over the course of 14 days, blood maintained 80% viability in the stabilizer and the optimal dilution was 3 parts blood to one part solution (Figure 1). Nuclear DNA integrity was lower at 24°C than at 4 °C, and placing blood immediately into Streck dramatically maintained cell quality as compared with samples placed into the solution even 5 minutes later. Overall, results pointed to the use of the cell stabilizing solution for optimal blood sampling of gulf sturgeon for long-term monitoring and restoration effort.

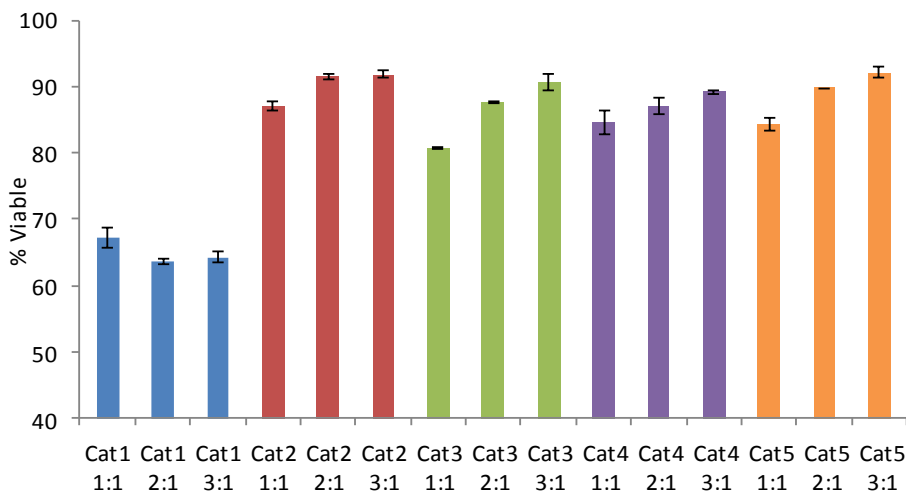


Figure 1. Average percent cellular viability of catfish blood samples in three different dilutions of cell stabilizer stored for 14 days. Blood samples were taken from five different fish.

## Growth and osmoregulatory physiology of juvenile Gulf killifish (*Fundulus grandis*) across a salinity gradient

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Gulf killifish (*Fundulus grandis*) is a species of interest for the emerging field of marine baitfish culture. Saltwater anglers support a vital industry along coastal Louisiana, but half of coastal anglers report that availability of live bait is a consistent problem. Marine baitfish aquaculture has the potential to facilitate a consistent year-round supply of high value live bait for market, which would increase angler satisfaction while generating profits for local small businesses. Gulf killifish inhabit intertidal and subtidal areas of brackish water marsh where shifts in salinity are constant and considerable. Although this life history is a valuable attribute for aquaculture, deleterious physiological effects could still exist at salinities outside the optimal range for this species. The present study was designed to examine effects of salinity on growth performance and ion regulation of juvenile Gulf killifish.

A 12-week trial was conducted in four separate but identical recirculating systems with juvenile Gulf killifish ( $0.49 \pm 0.01$ g SE). Salinity in the four systems was 0.5, 5, 8, and 12 g/L. Three 75-L tanks per treatment were stocked at one juvenile/L. Ambient temperature in the lab maintained all four systems at similar temperatures ( $\pm 0.5^\circ\text{C}$ ). Fish were fed to satiation twice daily with a commercial diet formulated to contain 32% crude protein and 4% lipid. Every two weeks juveniles were sampled for growth. Gills, intestine, and liver were sampled from three individuals per replicate after weeks 1, 3, 5, and 7. Transcriptional activity of osmoregulatory genes will be determined through quantitative PCR of gill and intestine tissue.

Regarding growth, the 0.5 g/L salinity treatment had significantly ( $P < 0.05$ ) lower weight gain and specific growth rate than brackish water treatments (Table 1). Final weight was significantly lower in 0.5 g/L salinity compared with the 12.0 g/L salinity treatment; however 5.0 g/L and 8.0 g/L we not significantly different from the other two salinity

TABLE 1. Final weight, percentage weight gain, specific growth rate (SGR), and survival. Letters denote statistical significance across rows.

	Salinity (g/L)			
	0.5	5.0	8.0	12.0
Final Weight (g/fish)	0.617 a	0.791 ab	0.839 ab	0.913 b
Weight Gain (%/fish)	16.80 a	53.31 b	70.64 b	98.50 b
SGR (%/day)	0.18 a	0.51 b	0.64 b	0.82 b
Survival (%)	59.3	86.5	96.3	89.7

treatments. These parameters indicate that Gulf killifish are able to subsist in near-fresh water environments, although growth and survival are severely compromised. Additional data from ongoing studies of gill mRNA activity and a salinity study on Gulf killifish of similar size in static outdoor pools will also be presented.

## **Genetic variation of spotted seatrout (*Cynoscion nebulosus*) in Louisiana: Panmixia and genetic divergence**

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The spotted seatrout (*Cynoscion nebulosus*) is a wide-ranging species that occurs in estuaries and shallow coastal marine habitats of the Gulf of Mexico and western Atlantic basins. Spanning the Louisiana coastline, spotted seatrout is one of the most important recreational fisheries in the state. As a result, it is critical to understand genetic variation, effective population size, and levels of gene flow among populations, which we defined *a priori* according to LDWF coastal management zones. We developed 12 new microsatellite loci and used these markers to assess genetic variation within and among Louisiana's populations of spotted seatrout. Analyses indicate that all populations are genetically variable and possess, on average, more than 13.9 alleles per population (13.90 – 15.70). The fixation index ( $F_{ST}$ ), a measure of population differentiation, indicated that the greatest degree of genetic divergence is between the Lake Pontchartrain basin (CSA 1) and all other populations. These results suggest that the Mississippi River may be acting as a partial barrier to movement and gene flow of spotted seatrout between eastern and western populations. These markers are variable and provide a tool to monitor spotted seatrout populations in the future, which may be particularly important in light of recent environmental changes in the northern Gulf of Mexico.

**Catch and size-structure of wild red swamp crawfish (*Procambarus clarkii*) sampled from pillow traps constructed with two different mesh sizes**

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This study compared the relative abundance and size structure of red swamp crawfish (*Procambarus clarkii*) sampled with two trap types of different mesh size. A total of 40 hexagonal-mesh wire pillow style crawfish traps were used to capture wild red swamp crawfish from Henderson Lake in St. Martin parish. Twenty traps consisted of 19 x 25 mm mesh wire, whereas the other 20 traps were constructed of 19 x 17.5 mm mesh wire. Each trap was baited with cut gizzard shad (*Dorsoma cepedianum*) and milled crawfish bait nuggets. All traps were fished for one day, and a total of 6 sampling trips were made between during spring 2009 and 2010. Only red swamp crawfish were captured, with samples yielding a total of 197 crawfish in the larger mesh traps and 202 crawfish in the smaller mesh traps. The mean total length (TL) of crawfish captured in the larger-mesh traps (102.4 mm) was significantly greater (ANOVA,  $F=10.30$ ,  $P = 0.001$ ) than the mean length of crawfish captured in the smaller-mesh trap (99.1 mm). Interestingly, even the minimal differences in mesh size appeared to result in a greater range of crawfish lengths captured by the smaller mesh (Figure 1).

**2009 and 2010 Henderson Captured Red Swamp Crawfish**

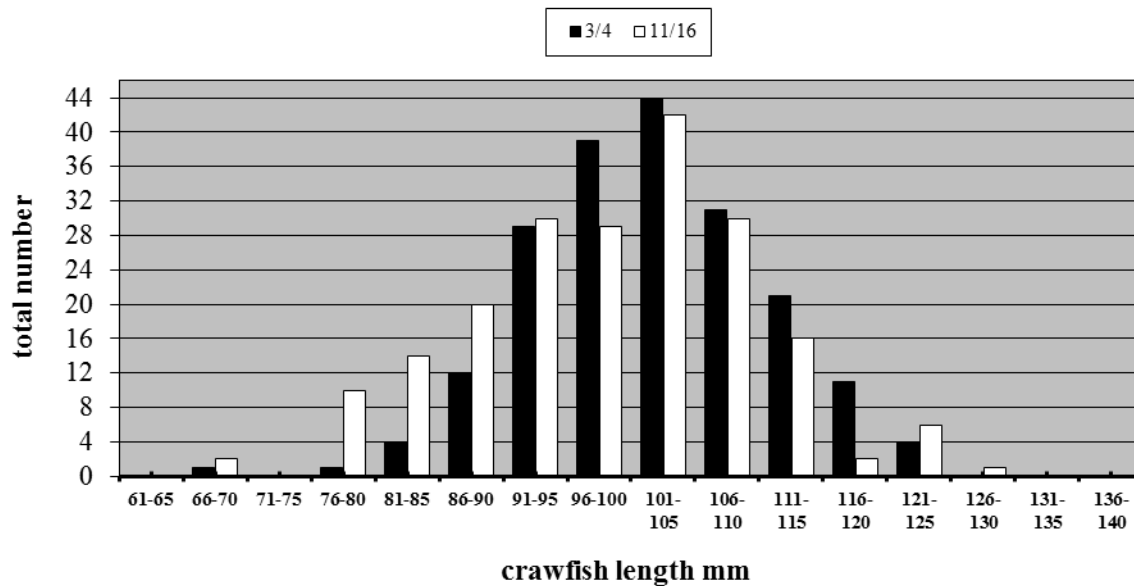


Figure 1. Total number of crawfish captured in Henderson Lake in 19 x 19 mm and 19 x 17.5 mm hexagonal wire mesh pillow type crawfish traps.

## **Zooplankton density and composition in river/floodplain habitats of the Ouachita River.**

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Zooplankton is an ecologically important component of aquatic systems, facilitating the transport of nutrients and energy from phytoplankton to higher trophic levels. In addition to predator density and food levels, water velocity and physicochemistry can significantly influence zooplankton density and community composition. We investigated the influence of habitat type (river channel, tributary creek, floodplain lake), physicochemistry, distance from the main channel, and time of year on zooplankton abundance and community composition in an Ouachita River / floodplain system downstream of Felsenthal Dam, Arkansas. This section of river is extremely flashy, with tributaries exhibiting 8-m depth fluctuations over a 1-month period. We used push nets and funnel traps to collect monthly zooplankton samples for one year from seven spatially distinct habitats in this river-floodplain system. Analyses of the factors influencing zooplankton community dynamics incorporated habitat and water quality variables, including nutrients, chlorophyll *a* and water depth. Preliminary results indicate temporal changes in both abundance and community composition among months, as well as differences among sites within months.

## Relation of prey availability to habitat selection for red drum (*Sciaenops ocellatus*) in Bayou St. John, an urban waterway in New Orleans, Louisiana

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Red drum (*Sciaenops ocellatus*) has historically been an important game fish in Bayou St. John (BSJ), an urban waterway that has been affected by many anthropogenic factors. In an effort to help restore the fishery in Bayou St. John (BSJ), I am studying red drum habitat use as a basis for future management efforts in this system. A previous study determined that red drum preferred northern portions of BSJ, although the only significant differences in habitat found were depth and width, with the northern section being deeper and wider. The current study compares red drum movements with estimates of epifaunal abundance. To quantify the epifauna, I collected triplicate samples monthly from May 2010 until October 2010 at eight stations with unbaited minnow traps throughout BSJ. During this same time period, I tagged 11 red drum with internal acoustic telemetry transmitters (VEMCO V13-1L-69 KHz transmitters; 52-96 mm in length, 13 mm in diameter, weight = 9-16 g), and monitored their movements with three moored receivers (VEMCO VR2W-coded). Results for the two most abundant epifaunal prey items indicate no differences in abundance among any of the sites (Fig. 1). The number of detections per day is being used to estimate red drum habitat preference, and data support the preferred use of the Robert E. Lee (197.80 contacts per day) site relative to the central Island (61.53), I-610 (22.39), and Cabrini Bridge (0.00) sites (sites arranged north to south). Red drum habitat preferences in BSJ do not appear to be based on differences among sites in the densities of potential epifaunal prey.

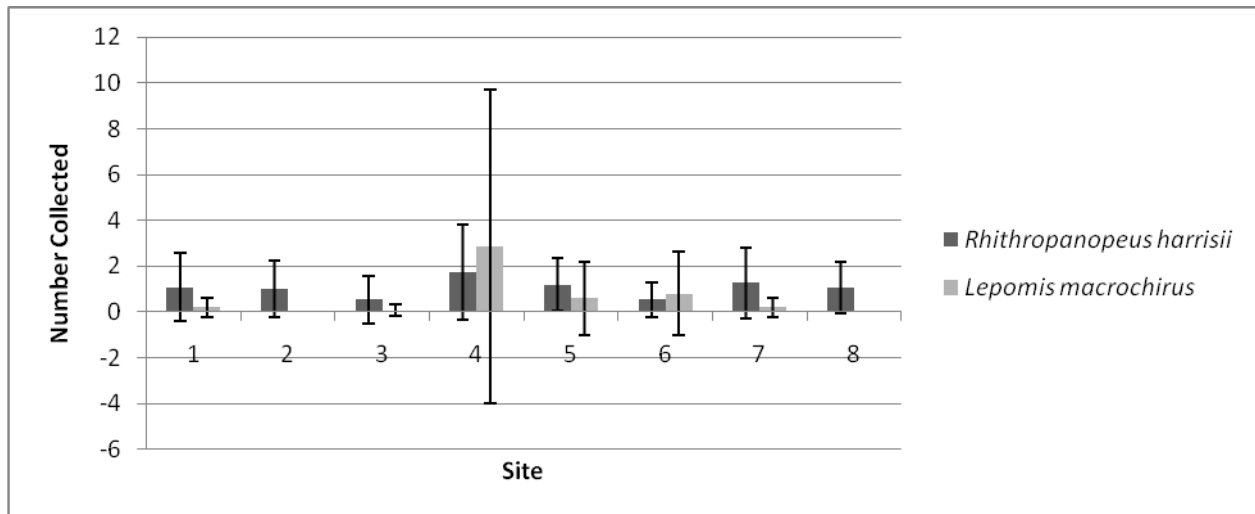


Figure 1: Chart depicting the number of *Rhithropanopeus harrisii* and *Lepomis macrochirus* (potential prey) per site from May to October 2010. The sites are ordered from north to south, with site one being the most northern.

## **Potential juvenile tarpon (*Megalops atlanticus*) nursery habitats in southeastern Louisiana**

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Reports suggest that tarpon (*Megalops atlanticus*) stocks in the northern Gulf of Mexico began to decrease precipitously in the 1950s and 1960s as a result of nursery habitat loss in the Yucatan, Mexico and South Florida. However, anecdotal evidence indicates that juvenile tarpon may use other nursery areas in the Gulf of Mexico. For example, juvenile tarpon have been collected in two sloughs near Ocean Springs, Mississippi, during several successive years since 2000, and juvenile tarpon were reported near Hopedale, Cocodrie, Grand Isle, Louisiana, during the fall of 2010. In the fall of 2010 we collected juvenile tarpon on three occasions from a roadside ditch near Port Sulphur, Louisiana, 23 km from the Gulf of Mexico. The ditch was less than 1 m deep and had a direct connection less than 1 km from marsh wetlands to the southwest. Tarpon were collected with castnets (1-2 hours sampling during midday), and early October sampling produced 14, 21, and 22 tarpon in successive weeks. Water temperatures ranged from 16.0° C to 26.0° C, air temperatures ranged from 13.8° C to 27.0° C, and salinity ranged from 8 psu to 11 psu. The juveniles ranged from 85 to 245 mm SL and had been foraging mostly on sailfin mollies (*Poecilia latipinna*) and sheepshead minnows (*Cyprinodon variegatus*), both of which were common in seine samples. Ratios of fish weight and presence of stored fat were examined, to investigate potential relationships between low temperature and cessation of feeding. The mean ratio of gut item weight to fish weight in warm water (27° C) was 3.54% (range 0.81%-7.35%), but was only 0.63% (range 0%-1.95%) when the water temperature was 16.0° C. Juvenile tarpon have been reported to be intolerant of temperatures below 10° C, but stomach samples suggest that juvenile tarpon may reduce feeding activities at temperatures well above 10° C. It is unclear how these results may affect survival, but these preliminary data suggest juvenile tarpon may not be able to survive the cold months or reach sufficient maturity to survive in the warmer waters of the Gulf of Mexico except in unusually warm winters.

**Behavior as an endpoint for copper acclimation and toxicity studies of the least killifish (*Heterandria formosa*)**

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Copper in large quantities can cause significant neurological impairments to aquatic life, although organisms may be able to acclimate to elevated levels of heavy metals. Many studies use mortality as an endpoint to determine a response to copper, but we were interested in whether a behavioral response could be used as a more sensitive indicator of toxicity and acclimation. To answer this question, we treated least killifish (*Heterandria formosa*) to a pre-exposure of 0µg/L or 15µg/L copper for 7 days, and then exposed them to 0µg/L or 100µg/L for 1 hour. Fish were put in an observation tank that contained a divider that allowed the test fish to view but not directly interact with a shoaling group of 15 least killifish, and behavior was monitored with a video recorder for a total of 3 minutes. Videos were then analyzed for the behavioral responses of shoaling encounters, time to first shoaling, duration of shoaling, and water column usage while shoaling. Fish exposed to 100µg/L copper displayed a reduction in shoaling encounters (an increase in time to first shoaling) compared to fish maintained at lower concentrations, and comparisons of shoaling behavior between fish pre-exposed 0µg/L or 15µg/L provided no evidence of acclimation.

## High-throughput Sperm Cryopreservation for Eastern Oysters (*Crassostrea virginica*)

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The Eastern oyster (*Crassostrea virginica*) is an important species in the coastal ecosystem of the Gulf of Mexico, and a major component of Louisiana's economically important annual fisheries harvest. The state's oyster industry is based on collection of natural spatfall obtained from communal seed grounds. However, this recruitment mode is especially vulnerable to the effects of environmental contaminants, such as the oil and dispersants that were released in the Gulf in 2010. Therefore it is necessary to preserve the genetic resources of oysters within a germplasm repository. Also, sperm cryopreservation can be applied to production and support of triploid oyster technology, and establishment of highly inbred families produced by self-fertilization for genome research. The goal of this study was to develop a reliable protocol for sperm cryopreservation of eastern oyster with the potential for high-throughput processing with automated equipment. Two types of 0.5-ml straws (French straws and CBS straws) were evaluated in this research to meet the processing requirements. The objectives were to: 1) evaluate the effect of 10% methanol, 1,2-propanediol (PG), and dimethyl sulfoxide (DMSO) as cryoprotectants at cooling rates of 5, 20 and 40°C/min from 5 to -80°C; 2) evaluate the effect of equilibration time (10-60 min) before freezing and thawing temperatures of 30, 40 and 50°C; 3) re-evaluate cooling rates of 10, 15, 20, 25 and 30°C/min with 10% DMSO as cryoprotectant, and 4) evaluate the final protocol by freezing sperm from a large number of males (n = 20). The optimized protocol was: collection of oyster sperm by dissection and stripping of the gonad into Ca<sup>2+</sup>-free Hanks' balanced salt solution at an osmolality of 650 mOsm/kg (HBSS650), mixing with an equal volume of 20% DMSO in HBSS650 to yield a final concentration of 1×10<sup>8</sup> sperm/ml, packaging into 0.5-ml straws, cooling at 25°C/min (French straws) or 15-25°C/min (CBS straws) from 5 to -80°C/min, transfer to liquid nitrogen for storage, thawing at 40°C for 7-10 sec (French straws) or 18-20 sec (CBS straws), and use for fertilization with freshly collected eggs (Figure 1).

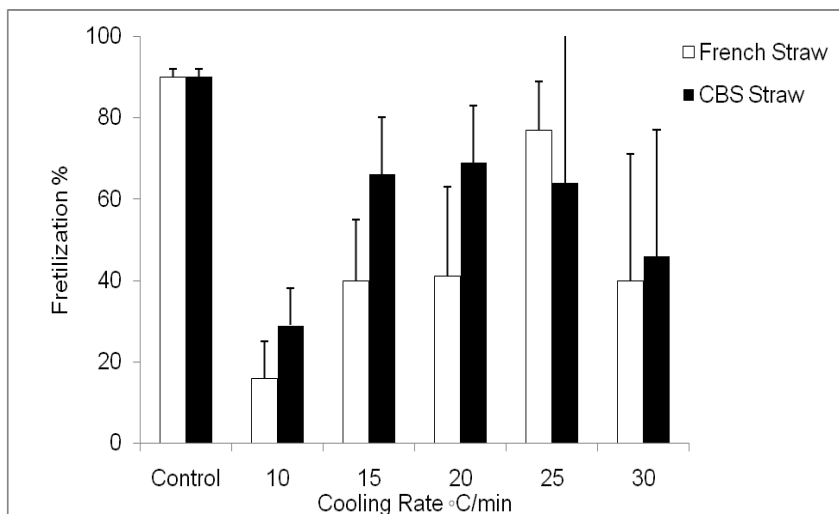


Figure 1. Percent fertilization (blastomere stage) of thawed eastern oyster sperm frozen in 0.5-ml French straws (white) or 0.5-ml CBS straws (black) with 10% DMSO as cryoprotectant at cooling rates of 10, 15, 20, 25 and 30°C/min from 5 to -80°C. Three replicates were produced by using different males.