

ABSTRACTS OF THE 41ST ANNUAL MEETING OF THE LOUISIANA CHAPTER OF THE AMERICAN FISHERIES SOCIETY



**VIRTUAL MEETING
MAY 27-28, 2021**

ACKNOWLEDGEMENTS

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Southern Division of the American Fisheries Society

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Louisiana Chapter of the American Fisheries Society

Thursday, 27 May 2021 (Day 1)

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

- 8:00 AM Meeting virtual platform opens
- 8:50 AM **Welcome and Keynote Speaker Introduction**
- 9:00 AM **Conservation, coordination, and communication: building partnerships 1 “C” at a time.** Angeline Rodgers, Project Leader, U.S. Fish and Wildlife Service, Lower Mississippi River Fish and Wildlife Conservation Office
- 9:40 AM **Break 1:** Twenty minutes
- 10:00 AM **Identifying ontogenetic shifts in primary energy pathways of invasive red lionfish (*Pterois volitans*) in the Florida Keys via stable isotope and otolith analyses.** Emily E. Shallow*, Cassandra N. Glapsie, and Michael J. Polito (29)
- 10:20 AM **Crabtivating behavioral analysis: the impacts of fipronil pesticides on Blue Crab (*Callinectes sapidus*) behavior.** Sadie A. Rawls* and Jennifer M. Hill (24)
- 10:40 AM **Status of a recently established population of Cuchia (*Amphipnous cuchia*) in Bayou St. John, New Orleans.** Victoria Rodrigues*, Susan Thomassie, Olivia Guerra, and Frank Jordan (26)
- 11:00 AM **Distribution, population status, and habitat assessment of crayfish in the Tickfaw, Tangipahoa, and Tchefuncte River basins, Louisiana.** Frederick C. Davis IV* and Christopher P. Bonvillain (14)
- 11:20 AM **River continuums and dis-continuums: trophic relationships in Louisiana coastal watersheds.** Erin E. Thayer*, William E. Kelso, and Michael D. Kaller (31)
- 11:40 AM **Temporal shifts in the diet of an oceanic predator in a river-dominated marine ecosystem.** Mitchell Lovell*, Michael J. Polito, and Michael A. Dance (20)
- 12:00 PM **Lunch on your own** (one hour twenty minutes)
- 1:20 PM **Development of a low-cost 3-D printed ‘CryoArk’ for sperm cryopreservation.** Rocky Vong*, Yue Liu, and Terrence Tiersch (33)

- 1:40 PM **Intracellular calcium signals as molecular fingerprints for assessing sperm quality in live-bearing fishes: an effort towards repository development for conservation.** Amit Sharma*, Terrence R. Tiersch, and Henrique Cheng (30)
- 2:00 PM **Using germplasm repositories to support Eastern oyster aquaculture: the biological information component.** Sarah Bodenstein*, Terrence R. Tiersch, Brian R. Callam, William C. Walton, and Jerome F. LaPeyre (10)
- 2:20 PM **Patterns and drivers of introgression in Louisiana's Largemouth Bass (*Micropterus salmoides*) stocks.** Colleen E. Walsh*, Michael D. Kaller, Sabrina S. Taylor, and William E. Kelso (34)
- 2:40 PM **Development of a low-cost automated sperm cryopreservation device for aquatic species.** Victoria J. Byrd*, Hamed Shamkhalichenar, Yue Liu, and Terrance R. Tiersch (12)
- 3:00 PM **Break 2:** Twenty minutes
- 3:20 PM **Applying open technology concepts to aquatic research.** Nikolas C. Zuchowicz* and Terrence R. Tiersch (36)
- 3:40 PM **Development of a low-cost sperm counting chamber.** Ignatius Semmes*, Yue Liu, and W. Todd Monroe (28)
- 4:00 PM **Open fabrication in 3-D printing.** Allyssa M. Oune*, Jack C. Koch, Nik Zuchowicz, and Terrence R. Tiersch (23)
- 4:20 PM **Decentralizing technology development with digital media and open-source platforms.** Jonathan W. Lai* and Terrence R. Tiersch (19)
- 4:40 PM **Break 3:** Twenty minutes
- 5:00-6:04 PM Poster Session
- 5:00-5:08 PM **Go with the flow: species-habitat associations of Lepisosteidae fishes in two restored Mississippi River floodplains.** Audrey Baetz*, KristieRae Ellis, and Solomon R. David (8)
- 5:08-5:16 PM **Cryopreservation to preserve microalgal genetic resources in repositories.** Mason L. Bailey* Allyssa Oune, Jack C. Koch, Teresa Gutierrez-Wing, and Terrence R. Tiersch (9)
- 5:16-5:24 PM **Movement and estuarine-coastal connectivity of southern flounder (*Paralichthys lethostigma*) in Rockefeller Wildlife Refuge.** Gabrielle Fignar*, Elsa Gutierrez, Philip L. Trosclair III, and Michael A. Dance (15)
- 5:24-5:32 PM **Pass the salt!: environmental predictors of Brown Shrimp abundance in Louisiana.** Caitlyn A. Fontenot*, Abigail Bockus, and Terri J. Maness (16)

- 5:32-5:40 PM **Feeding patterns of sheepshead (*Archosargus probatocephalus*) during spawning aggregations in the northwest Gulf of Mexico.** Elsa M. Gutierrez*, Jeffrey D. Plumlee, Derek G. Bolser, Brad E. Erismann, and R. J. David Wells (17)
- 5:40-5:48 PM **Effects of environmental hypoxia on red swamp crayfish *Procambarus clarkii* life history and fecundity characteristics in the Atchafalaya River Basin.** Austin Ortman* and Christopher P. Bonvillain (22)
- 5:48-5:56 PM **Fish community composition of two Mississippi River floodplain re-connectivity sites with a focus on the population demographics of gars (*Lepisosteidae*).** Derek C. Sallmann*, KristieRae Ellis, Bryan P. Piazza, and Solomon R. David (27)
- 5:56-6:04 PM ***Callinectes sapidus* reovirus 1 (CsRV1) waterborne transmission in soft shell blue crab shedding systems.** Elizabeth M. Robinson* and Julie A. Lively (25)

Louisiana Chapter of the American Fisheries Society

Friday, 28 May 2021 (Day 2)

Presenting author is denoted by an asterisk (). Abstract page is listed in parenthesis.*

8:30 AM	Meeting virtual platform opens
9:00 AM	Developing a generalizable cryopreservation pathway for protecting the genetic resources of marine invertebrates. Jack C. Koch*, Allyssa M. Oune, and Terrence R. Tiersch (18)
9:20 AM	From Australia to Louisiana: a snapshot of germplasm repositories for amphibian conservation in Australia and how I came to be in Baton Rouge. Rose Upton* and John Clulow (32)
9:40 AM	American Eel (<i>Anguilla rostrata</i>) in Louisiana: ages, growth rates, diet, and emerging patterns. Robby Maxwell*, Sean Kinney, and Kym Walsh (21)
10:00 AM	Genome-wide SNPs provide insight into migratory phenotypes of a partially migrant lake sturgeon population. Justine M. Whitaker*, Darryl W. Hondorp, James C. Boase, Charles Krueger, Louis Bernatchez, and Amy B. Welsh (35)
10:20 AM	Effects of dietary acidification on growth and postprandial physiology in fishes. Angela N. Casillo*, T. Gibson Gaylord, Christopher Green, Wendy M. Sealey, Madison Powell, and Abigail B. Bockus (13)
10:40 AM	Louisiana's Asian Carp partnership projects. Robert P. Bourgeois* and Robby Maxwell (11)
11:00 AM	Break 1. Fifteen minutes
11:15 AM	Business Meeting, Awards Presentations, and Officer Elections

ABSTRACTS

Presenting author is denoted by an asterisk (). Student presenters are underlined.*

Go with the flow: species-habitat associations of Lepisosteidae fishes in two restored Mississippi River floodplains

Audrey Baetz*, KristieRae Ellis, and Solomon R. David

GarLab, Department of Biological Sciences, Nicholls State University, Thibodaux, LA.

abaetz@nicholls.edu

Floodplain ecosystems are important habitats that offer a multitude of resources to riverine fish species. However, anthropogenic factors such as increased river channelization and construction of dams and levees have drastically reduced river-floodplain connectivity. Many fish species, including gars (Lepisosteidae), use floodplains for spawning, feeding, and nursery habitats. Due to their dependence on floodplain inundation for spawning, the largest gar species, Alligator Gar *Atractosteus spatula*, is at increased risk as vital habitats continue to suffer from reduced connectivity and altered flood pulses. To mitigate these connectivity issues, The Nature Conservancy, Louisiana Department of Fish and Wildlife (LDWF), and partners are constructing flood control structures, culverts, and weirs in two Mississippi River floodplains with the goals of improving access for fish and enhancing natural flow regimes. Loch Leven, the 5,819 acre floodplain managed by The Nature Conservancy, and the Black Hawk Scar Lakes, 147.2 acres of Richard K. Yancy Wildlife Management Area (LDWF), have been identified as ideal Alligator Gar spawning habitat. Additionally, gar species have been shown to have different habitat associations, however the extent of these preferences within the floodplain are not yet well understood. This project will aim to identify potential differences in the habitat associations and spatio-temporal distribution relationships of Lepisosteidae fishes (*Atractosteus spatula*, *Lepisosteus osseus*, *L. oculatus*, and *L. platostomus*) in two geographically similar floodplain restoration sites. Determination of potential differences in species-habitat associations within Lepisosteidae will help identify critical areas for future restoration initiatives.



Figure: Panel featuring habitat sampling types A) low-level lake B) LDWF culvert C) inundated floodplain D) TNC culvert.

Cryopreservation to preserve microalgal genetic resources in repositories

Mason L. Bailey*, Allyssa Oune, Jack C. Koch, Teresa Gutierrez-Wing, and Terrence R. Tiersch
Aquatic Germplasm and Genetic Resources Center, Louisiana State University Agricultural Center, Baton Rouge, LA. Mbail45@lsu.edu Aoune@agcenter.lsu.edu JKoch@agcenter.lsu.edu
MWing@agcenter.lsu.edu Ttiersch@agcenter.lsu.edu

Microalgae, the base of aquatic food chains, are crucial in supporting and maintaining fisheries and are routinely maintained as live cultures in laboratory settings. Furthermore, microalgae are increasingly being used in applications such as biofuels and wastewater remediation. Despite their importance, microalgal strains especially those of marine origin, lack studies to improve the development of collections for continuous availability. This project seeks to build knowledge on microalgal cryopreservation for maintaining strains for repository storage at low cost and effort. This study will fill gaps in research which will lead to outcomes fostering a more sustainable future for curated algae collection, allowing hatcheries, fisheries, and other facilities to support their core activities while preserving cultures during off-seasons or times of facility closure. One hurdle during cryopreservation of microalgae is sub-optimal freezing rates which result in ice crystal formation which can rupture and kill cells. Traditionally, chemicals called cryoprotectants are used to help minimize this. A novel method to control this risk is the use of hydrogels to suspend microalgal cultures. Hydrogels allow for carbon and oxygen exchange and do not disturb exponential growth (Figure 1). Furthermore, they absorb and retain water, potentially reducing ice crystal formation. The studies underway at AGGRC will identify efficient methods of cryopreserving microalgal species. To identify enhanced cost and time-efficient methods, three widely used marine strains (*Tetraselmis chuii* (Figure 2), *Chaetoceros gracilis*, and *Tisochrysis lutea*) will be evaluated. Our objectives are to determine the effects on viability of: 1) cryoprotectant type and exposure (equilibration) time; 2) different cooling and thawing rates; 3) hydrogels to replace cryoprotectants, and 4) using portable, dry ice and alcohol freezing in field situations.



Figure 1. Two forms of hydrogels: potassium polyacrylate (left) and sodium methacrylate (right) used to immobilize microalgal cells and prepare them for cryoprotectant-free cryopreservation.



Figure 2. A healthy culture of *Tetraselmis chuii*. The cells are plump and hold pigment which are characteristics that can be used to evaluate experimental conditions (400-x magnification).

Using germplasm repositories to support Eastern oyster aquaculture: the biological information component

Sarah Bodenstein^{*,1}, Terrence R. Tiersch¹, Brian R. Callam¹, William C. Walton², and Jerome F. LaPeyre³

¹Louisiana State University Agricultural Center, Aquatic Germplasm and Genetic Resources Center, 2288 Gourrier Avenue, Baton Rouge, LA 70803; ²Auburn University, Auburn University College of Agriculture, School of Fisheries, Aquaculture, and Aquatic Sciences, 107 Comer Hall Auburn, AL 36849; ³Louisiana State University Agricultural Center, School of Animal Sciences, 201D Animal & Food Sciences Lab Bldg., Baton Rouge, LA 70803. sboden2@lsu.edu

Louisiana has a long history of oyster farming, however, many farmers in the region are now experiencing high oyster mortalities from prolonged low salinity events. To address the challenges of these events, scientific tools such as physiology studies, genetics research, breeding programs, and sperm cryopreservation can be used. Unfortunately, information from across these disciplines has not yet been used collectively, decreasing the potential contributions of each tool. Repositories offer a solution. Germplasm repositories are collections of genetic material that benefit agriculture by storing physical genetic samples, such as sperm. A repository also stores relevant information associated with the samples, such as biological or genetic information. While a comprehensive repository system does not yet exist for aquatic species, development could support oyster aquaculture in Louisiana and help to address problems such as low-salinity events. This study focused on the biological information components of a repository, without which the repository is greatly limited in use and value. The responses (growth and mortality rate) of native oyster stocks to salinity stress in the field were measured in conjunction with gamete cryopreservation of those stocks. During the one-year field study, sperm from each stock were cryopreserved and processing information was entered into the USDA National Animal Germplasm Program repository database (NAGP Animal GRIN). The biological and genetic results from the study will also be entered into Animal GRIN to aid in developing an oyster repository (Figure 1). This can serve as a model for other aquatic species.

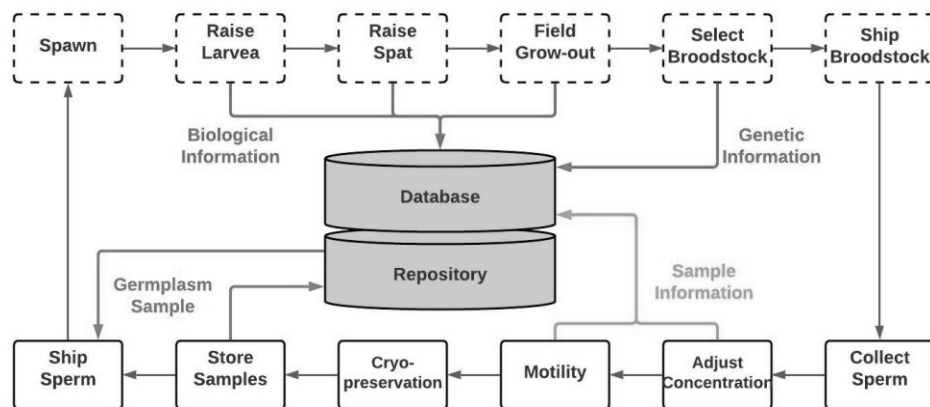


Figure 1. A process flow map outlining how information from different disciplines (such as biology & genetics) can be collected, integrated into a cryopreservation pathway, and stored alongside germplasm samples in a repository. Results from the field study (top row) can now be directly linked with other activities. Solid boxes indicate actions taken by the cryopreservation facility and dashed boxes indicate actions taken by farmers or hatchery owners.

Louisiana's Asian Carp partnership projects

Robert P. Bourgeois^{*1} and Robby Maxwell²

¹*Research and Assessment Section, Office of Fisheries, Louisiana Department of Wildlife and Fisheries, Baton Rouge, LA;* ²*Inland Fisheries Section, Office of Fisheries, Louisiana Department of Wildlife and Fisheries, Lake Charles, LA.* rbourgeois@wlf.la.gov
rmaxwell@wlf.la.gov

The first Asian carp were reported in Louisiana (LA) with the discovery of a Grass Carp in 1976. In 1985 Silver Carp, a second type of Asian carp, was documented in the state. Since that time, four species of Asian carp have spread to 56 parishes with the potential of further spread throughout the entire state due to the interconnectedness of the LA river basins. Prior to 2020, the Louisiana Department of Wildlife and Fisheries (LDWF) and Nicholls State University conducted three years of ichthyoplankton sampling to identify and quantify Asian carp spawning activities in the major river basins of the state. In 2020, LDWF expanded upon the ichthyoplankton project and developed new projects with funding provided through USFWS with the goals to expand research, prevention, and control of carp invasions. Projects funded in 2020 included an additional two years for ichthyoplankton sampling with Nichols State University and a two-year telemetry project with Louisiana State University. Proposed projects for 2021 will study impacts to native species as well as explore developing markets for Asian carp. Future projects will focus on increasing harvest, placement of barriers, and other research projects to help guide control of Asian carp in LA. LDWF would like to develop a working group of interested research partners in the state to develop ideas that may be used to combat these invasive species.

Development of a low-cost automated sperm cryopreservation device for aquatic species

Victoria J. Byrd*, Hamed Shamkhalichenar, Yue Liu, and Terrence R. Tiersch

Aquatic Germplasm and Genetic Resources Center, Department of Biological and Agricultural Engineering, Louisiana State University Agricultural Center, Baton Rouge, LA.

Vbyrd2@lsu.edu, yliu@agcenter.lsu.edu, Hshamk1@lsu.edu, ttiersch@agcenter.lsu.edu

Many aquatic species and populations in Louisiana and elsewhere are vulnerable to environmental changes. This has created a demand for cryopreservation to preserve genetic diversity and offset declining availability of wild stocks for use in breeding. Unfortunately, cryopreservation is not widely available, especially for groups that work outside of laboratories. Existing equipment for cryopreservation can be expensive (> US\$ 20,000), the literature is conflicting and confusing, and small changes in a protocol can yield very different results. In addition, work in the field requires significant investment in time and effort to bring laboratory-based equipment to the coast, or for work on a riverbank or ship. The goal of this research was to develop a 3-D printed Auto-Position Cooling Device (APCD) (Fig.1) along with electronic components to automate freezing hardware for use with portable cryogenic shipping dewars. This approach would address many of the existing problems in making cryopreservation available and would create tremendous new opportunities for much-needed standardization of procedures. The APCD design integrates a microcontroller (Arduino Uno), eight 3-D printed components, liquid a crystal display (LCD), a linear actuator, and a thermocouple. This device can control freezing rates through a user-interface by controlling sample height within a standard shipping dewar. Because of usage of consumer-level components, this device had a low material cost of about US\$ 200 total and can be constructed by users from sharing of printer files and instructions for assembly and use. This device was tested with freezing of live microalgae samples and was able to reliably produce specific programmed cooling rate profiles within the dewar. Microalgae (*Tetraselmis chuii*) samples were frozen for comparison purposes with the APCD and an IceCube (commercially available \$50,000 computer-controlled freezer) from 4°C to -80°C. The samples from both freezer methods were thawed and grew in standard culture conditions. In the future we will test the device with 0.25-ml straws and further investigate temperature ranges within the dewar to make this device available for use with other species.

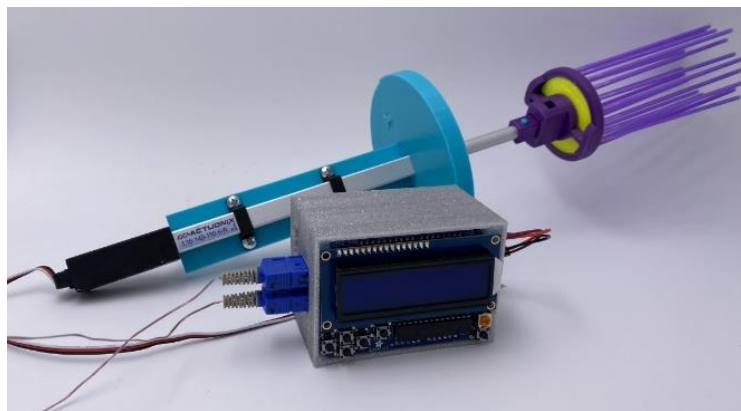


Figure 1. A prototype of the Auto-Position Cooling Device (APCD). The straws (right side) are held in a split-ring design that allows ejection of the samples in the shipping dewar after freezing. The user interface, controller, and electronics are contained in the 3-D printed box (front). A linear actuator controls the sample height based on temperatures measured through thermocouples.

Effects of dietary acidification on growth and postprandial physiology in fishes

Angela N. Casillo^{*1}, T. Gibson Gaylord², Christopher Green³, Wendy M. Sealey², Madison Powell⁴, and Abigail B. Bockus¹

¹Louisiana Universities Marine Consortium, Chauvin, LA; ²US Fish and Wildlife Service, Bozeman Fish Technology Center, Bozeman, MT; ³School of Renewable Natural Resources, Louisiana State University, Baton Rouge, LA; ⁴Aquaculture Research Institute, University of Idaho, Moscow, ID. acasillo@lumcon.edu gibson_gaylord@fws.gov cgreen@agcenter.lsu.edu wendy.sealey@usda.gov mpowell@uidaho.edu abockus@lumcon.edu

From 1990-2018, aquaculture production has risen 527%, and this industry provides over half of all fish for human consumption around the globe. Developing diets that enhance growth and optimize feed efficiency is essential to ensure maximized growth and production, contributing to increased industry sustainability. In many fish species, including important aquaculture species, a decrease in stomach pH occurs after feeding to aid in digestion. This drop is facilitated by parietal cells in the stomach epithelium that secrete protons (H^+) into the stomach lumen and bicarbonate ions (HCO_3^-) into the bloodstream. This process, as well as the return to acid-base homeostasis, is called the alkaline tide and may be energy expensive. The overall objective of this project was to analyze the effects feed pH has on the alkaline tide and the corresponding energy costs. To accomplish this, we examined the influence of diet pH on the growth and digestive physiology of two finfish species.

Feed was manufactured at pH levels of 4.5, 5.75, and 6.5 and fed to satiation to replicate experimental tanks for a duration of 6-12 weeks. At the end of the grow-out period, pH was measured in three places along the digestive tract. Whole body, blood, and tissue samples were also taken to determine blood chemistry and enzymatic activity to be analyzed moving forward. Growth and feed efficiency were also measured by recording tank weight and feed intake over time.

The results of this study aim to inform commercial feed producers of potential benefits of dietary acidification. This project also aims to determine the digestive strategy and optimal feed pH of the target species, red drum (*Sciaenops ocellatus*) and hybrid striped bass (*Morone chrysops* x *M. saxatilis*). In preliminary analyses, feed intake and growth does not seem to be impacted by feed pH in hybrid striped bass. Total feed consumed and total tank gain however, are both significantly higher with a lower pH feed in red drum. It can also be observed that although the digestive strategy of both target species does not reflect a drop in pH after feeding (pH in the stomach is always low), indications of the acid-base fluxes associated with the alkaline tide are present. Further analyses will compare variables such as carbonic anhydrase levels, microbiome composition, and digestive enzymes in tissues along the digestive tract.

Distribution, population status, and habitat assessment of crayfish in the Tickfaw, Tangipahoa, and Tchefuncte River basins, Louisiana

Frederick C. Davis IV* and Christopher P. Bonvillain

Department of Biological Sciences, Nicholls State University, Thibodaux, LA.

fdavis3@nicholls.edu

Crayfish are key components in freshwater ecosystems and trophic webs, fundamental in determining ecosystem structure and function, and are indicators of water quality and biodiversity. However, many crayfish species are currently imperiled due to anthropogenic activities and in Louisiana there is a paucity of basic ecological information on most crayfish, including species of greatest conservation need (SGCN). This lack of crayfish distribution, population status, and habitat preference information makes conservation actions difficult for scientists and resources managers. Therefore, the purpose of this project is to examine the distribution, population status, and habitat requirements for crayfish in the Tickfaw, Tangipahoa, and Tchefuncte River basins in Louisiana. Twenty-one 1st to 4th order streams were sampled from June to October 2020 using backpack electrofishing, kick seining, and dipnets. A total of 819 crayfishes representing 10 species were collected. Pinelands Creek Crayfish *Procambarus vioscai* was the most abundant and common species collected (547 individuals in 14 streams). The Pontchartrain Painted Crayfish *Faxonius hobbsi*, a SGCN, was found in all three river basins but was one of the rarest species encountered with only 20 individuals collected. Multivariate analysis of variance indicated a significant difference in water quality between streams with and without *F. hobbsi* (Wilks' Lambda = 0.03, $F_{1,19} = 6.76$, $P = 0.018$). Streams with *F. hobbsi* were characterized by sandy substrate and high in-stream woody debris and significantly higher dissolved oxygen ($P = 0.006$) and discharge ($P = 0.005$) and lower specific conductance ($P = 0.018$), turbidity ($P = 0.056$), and temperature ($P = 0.012$) compared to streams without *F. hobbsi*. Additionally, *F. hobbsi* did not co-occur in streams with Red Swamp Crayfish *Procambarus clarkii*. This information will aid state and federal resource managers with species conservation assessments and preservation efforts, help prioritize stream and watershed locations for future sampling efforts, and establish a robust, quantitative biological and ecological data set on crayfishes in Louisiana.

Movement and estuarine-coastal connectivity of southern flounder (*Paralichthys lethostigma*) in Rockefeller Wildlife Refuge

Gabrielle Fignar^{*,1}, Elsa Gutierrez¹, Phillip L. Trosclair III², and Michael A. Dance¹

¹*Department of Oceanography and Coastal Sciences, Louisiana State University, Baton Rouge, LA;* ²*Rockefeller Wildlife Refuge, Louisiana Department of Wildlife and Fisheries, Grand Chenier, LA.* Gfignar@lsu.edu

Southern flounder (*Paralichthys lethostigma*) support valuable recreational and commercial fisheries across the northern Gulf of Mexico. Unfortunately, recent stock assessments in Louisiana indicate that southern flounder recruitment and spawning stock biomass are in steep decline and the current stock status has been classified as overfished. Southern flounder have a unique life history and undergo annual inshore-offshore spawning migrations; however, our understanding of this migratory behavior remains limited, and a better understanding of environmental drivers, migration timing, and homing/dispersive behavior is necessary to improve management strategies. Here we use acoustic telemetry to characterize temporal and environmental patterns influencing estuarine-coastal connectivity of southern flounder in a model estuarine system, Rockefeller Wildlife Refuge, in coastal Louisiana. Data from this study will be used to assess variability in egress and ingress patterns and determine the importance of environmental cues (e.g., temperature, salinity, moon phase, cold fronts) to migratory behavior. Currently, several management scenarios (i.e., changes to size limits, bag limits, and/or seasonal closures) are being considered to recover southern flounder populations, and an improved understanding of migratory dynamics will provide critical information to inform management decisions.

Pass the salt!: environmental predictors of Brown Shrimp abundance in Louisiana

Caitlyn A. Fontenot^{*,1}, Abigail Bockus², and Terri J. Maness¹

¹*School of Biological Sciences, Louisiana Tech University, Ruston, LA;* ²*Louisiana Universities Marine Consortium, Chauvin, LA.* caf033@latech.edu

Shrimp are a crucial part of Louisiana's economy and ecosystems. Understanding the environmental factors that affect shrimp abundance can help us protect them and their habitats. It can also inform fisherman of the best times and places to catch shrimp. We assessed the influence of salinity, temperature, turbidity, oxygen, and trawling equipment on brown shrimp (*Farfantepenaeus aztecus*) abundance across coastal Louisiana. Louisiana Department of Wildlife and Fisheries trawling data were analyzed from January to August 2020 using general linear mixed models. The dataset included 1539 trawls from 194 different trawling sites with site treated as a random variable. Date and type of trawling gear both influenced how many shrimp were caught in trawls. The most important environmental predictor of abundance was salinity in that abundance declined rapidly when salinity dropped below 8 ppt. Temperature and turbidity were also important indicators explaining variability in number of shrimp caught. Our results allow us to examine how changes in estuaries and marshes will impact shrimp survival and abundance with important implications for coastal management, including the creation and management of river diversions.

Feeding patterns of sheepshead (*Archosargus probatocephalus*) during spawning aggregations in the northwest Gulf of Mexico

Elsa M. Gutierrez^{*,1,2}, Jeffrey D. Plumlee³, Derek G. Bolser^{4,5}, Brad E. Erisman^{4,6}, and R. J. David Wells^{2,7}

¹Department of Oceanography and Coastal Sciences, Louisiana State University, Baton Rouge, LA 70803; ²Department of Marine Biology, Texas A&M University at Galveston, Galveston, TX 77553; ³Institute of Marine Sciences, University of North Carolina at Chapel Hill, Morehead City, NC 28557; ⁴Department of Marine Science, University of Texas at Austin, Port Aransas, TX, 78373; ⁵Cooperative Institute for Marine Resources Studies, Oregon State University, Newport, OR 97365; ⁶Southwest Fisheries Science Center, National Marine Fisheries Service, National Oceanographic and Atmospheric Administration, La Jolla, CA 92037; ⁷Department of Ecology & Conservation Biology, Texas A&M University, College Station, TX 77843.
eguti12@lsu.edu jplumlee@live.unc.edu derekbolser@utexas.edu brad.erisman@noaa.gov
wellsr@tamug.edu

Feeding patterns of sheepshead, *Archosargus probatocephalus*, in the northwestern Gulf of Mexico were examined from samples collected at two known spawning aggregation sites in Texas. A total of 53 sheepshead stomachs were analyzed along with tissue samples from the muscle ($n = 20$) and liver ($n = 20$) for stable isotope analysis ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$). Amphipods made up the majority of prey items in sheepshead from both locations (Galveston, %IRI = 85.18; Port Aransas, %IRI = 65.05), with differing species, which may reflect differences in specific species present between locations. Stable isotope ratios in muscle and liver tissues differed significantly between locations, which may indicate a shift in foraging mode and location between spawning and non-spawning times. Sheepshead from Galveston had lower $\delta^{13}\text{C}$ values in tissue samples (muscle, -21.46 ± 2.81 ‰; liver, -21.60 ± 2.42 ‰) than those collected from Port Aransas (muscle, -15.49 ± 2.28 ‰; liver, -17.37 ± 1.57 ‰), but higher $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ (muscle $\delta^{15}\text{N}$, 18.00 ± 1.73 ‰; liver $\delta^{15}\text{N}$, 16.18 ± 1.49 ‰; muscle $\delta^{34}\text{S}$, 16.98 ± 1.46 ‰; liver $\delta^{34}\text{S}$, 17.26 ± 2.07 ‰), than those collected in Port Aransas (muscle $\delta^{15}\text{N}$, 12.57 ± 2.27 ‰; liver $\delta^{15}\text{N}$, 12.09 ± 2.31 ‰; muscle $\delta^{34}\text{S}$, 12.31 ± 3.47 ‰; liver $\delta^{34}\text{S}$, 13.71 ± 3.14 ‰). Strong location related differences in the foraging ecology were observed using core niche area (SEA_B) between the two locations. These results highlighted differences in trophic niche of sheepshead between the two locations, which was likely due to differences in salinity regime that affected the assemblage of prey organisms.

Developing a generalizable cryopreservation pathway for protecting the genetic resources of marine invertebrates

Jack C. Koch*, Allyssa M. Oune, and Terrence R. Tiersch

Aquatic Germplasm and Genetic Resources Center (AGGRC), School of Renewable Natural Resources, Louisiana State University Agricultural Center, Baton Rouge, LA.

jkoch@agcenter.lsu.edu aoune@agcenter.lsu.edu ttiersch@agcenter.lsu.edu

The safeguarding of economically relevant agricultural species has been advanced by storing genetic resources as cryopreserved germplasm in repositories. The shift to cryopreservation has been slow within the broader scientific community, especially for fish and shellfish. Aquatic biomedical model organisms and imperiled species are examples of groups with great need to begin widening the scope of germplasm repositories. The California sea hare, *Aplysia californica*, is a model organism largely used to examine neural development, behavior, and aging. In addition to the risks and costs of relying solely on maintenance of live individuals, the husbandry of *Aplysia* is complicated by their copious production of mucus and toxic ink expulsion, requiring flow-through aquarium systems or extensive filtration. Inbreeding is considered to have detrimental effects on the development in this species adding further costs and considerations. Repository storage of frozen material will provide opportunity for the research community to create and maintain mutant and transgenic lines. Our goal is to develop a generalizable cryopreservation pathway for this species in collaboration with the National Resource for *Aplysia* (NRA, University of Miami), with the intention of extending this pathway to other aquatic invertebrates such as oysters and imperiled corals (Figure 1).

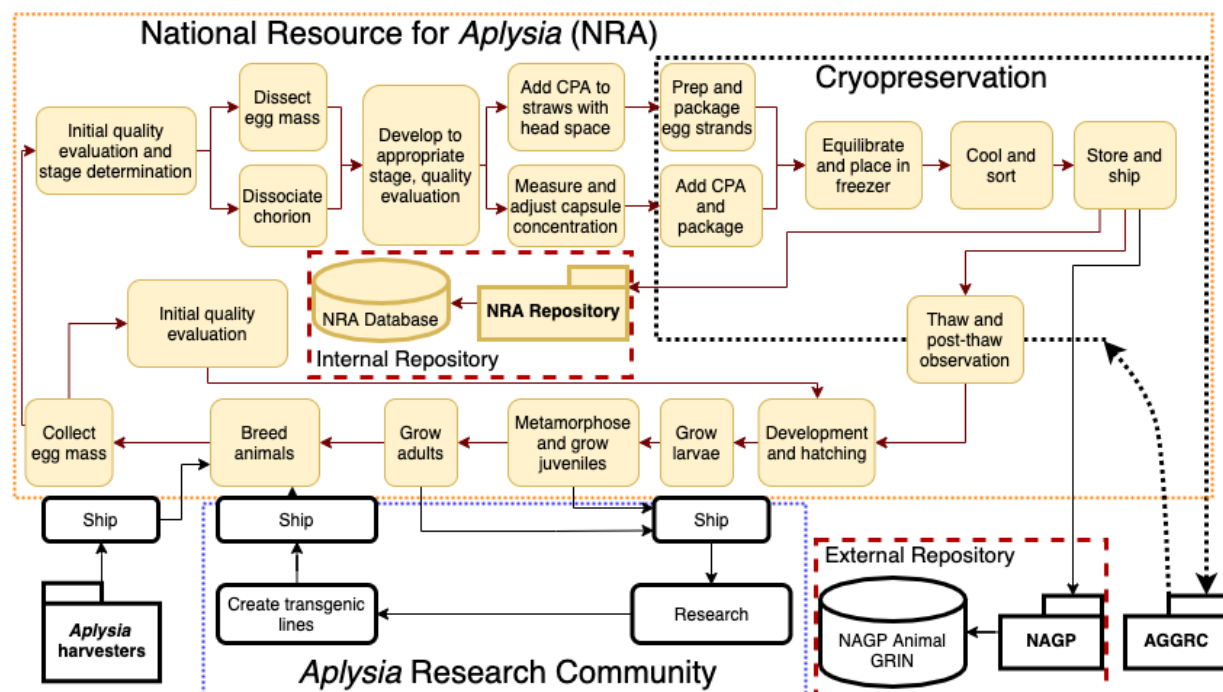


Figure 1. *Aplysia* resources currently follow a linear path, beginning with wild individuals and ending with research in the *Aplysia* community. With the addition of a cryopreservation pathway, the NRA could maintain genetic diversity stored in repositories (dashed lines) rather than relying on wild populations.

Decentralizing technology development with digital media and open-source platforms

Jonathan W. Lai* and Terrence R. Tiersch

*Aquatic Germplasm and Genetic Resources Center, Digital Media Arts and Engineering,
Louisiana State University, Baton Rouge, LA. jlai5@lsu.edu*

Working remotely became a universal standard in 2020 with COVID-19 shutdowns putting much of life on hold. Despite the challenges created by this internet-dependent lifestyle, a year's worth of adaptation has highlighted opportunities that might have been overlooked in the past. Video conferencing inadvertently equalized the voices at meetings: there is no longer a hierarchy between in-person attendees and those "calling in". Online Git repositories and cloud-based platforms gained even greater popularity. Thus, the move to remote collaboration has presented new challenges, but has also created opportunities to broaden participation. This is reducing reliance on industrial manufacturing as a sole source of innovation and production; ideation is no longer restricted by the length of a meeting, or the seating capacity of a room, or the output of a factory.

Decentralized technology development does not mean that ownership of the project is given up; indeed specific rights can be retained, but multiple individuals and groups can be actively involved in the process. A good example is open-source software (e.g., the Linux operating system) that is developed and improved by a global community of programmers, rather than behind the proprietary walls of a single company. Decentralization thus largely addresses the concerns about the ways that information is shared. Written descriptions can be enhanced with digital media like photography, animation, or narrated tutorials that improve comprehension. In the case of open-sharing platforms like Thingiverse or Git, this content is hosted online and can be accessed at any time. A video tutorial for example allows a viewer to analyze and digest information whenever and wherever it is needed. A comprehensive package that includes conventional documentation and supplemental digital media can make broad distribution of information easier, and more impactful. A practical extension of this thinking is the custom design of scientific hardware that can be distributed as editable 3-D models to user communities. In this way a research device that might cost thousands of dollars to develop and ship commercially, could be designed and distributed to researchers as data that is inexpensively modified, and 3-D printed in hundreds of separate laboratories. Open-source designs and supporting digital media would make research capabilities more accessible (for example in germplasm cryopreservation) and could provide a foundation for standardization of research approaches (Figure 1).

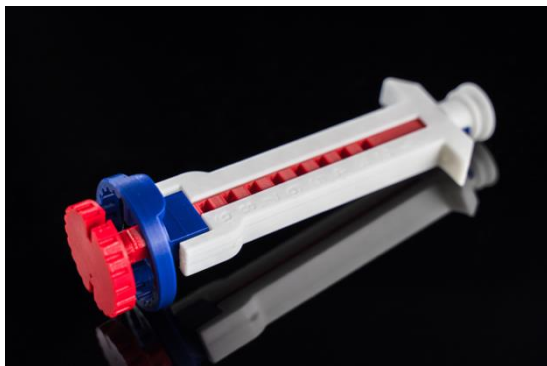


Figure 1. A 3-D printed cryopreservation device called the “Cajun Ejector” in which samples are frozen at specific cooling rates within a standard liquid nitrogen shipping dewar and are ejected when the freezing cycle is complete to allow loading of another batch. Data to 3-D print this device would be accompanied with assembly and operation instructions. Open projects empower community collaboration following their release and encourage development driven by the community’s needs.

Temporal shifts in the diet of an oceanic predator in a river-dominated marine ecosystem

Mitchell Lovell*, Michael J. Polito, and Michael A. Dance

Department of Oceanography and Coastal Sciences, Louisiana State University, Baton Rouge, LA. mlovel6@lsu.edu mpolito@lsu.edu mdance1@lsu.edu

Pelagic predators (e.g., tunas, billfish, sharks) play a significant role in the structure and function of marine ecosystems and a better understanding of the food web dynamics that influence predator distribution and movement is essential to improving spatial management and conservation of these species. Here, we employ complementary techniques (gut contents, DNA barcoding, & stable isotopes) to examine seasonal variability in the diets of yellowfin tuna (*Thunnus albacares*) from the northern Gulf of Mexico. Yellowfin tuna (n = 577) were sampled weekly from April 2019 – March 2020 from recreational charter landings and grouped into two size classes based on estimated size at sexual maturity (sub-adult: 70 – 100 cm & adult: 100 – 160 cm). While 114 unique taxa were documented in yellowfin tuna stomachs, prey assemblages varied by season and size class. Carangids (jacks), ommastrephid squids, exocoetids (flyingfishes), and hyperiid amphipods were among the most abundant and frequently encountered prey. Seasonal differences were characterized by squids and flyingfishes in the spring, juvenile fishes (carangids & scombrids) in the summer, coastal fishes during the fall, and an increased consumption of planktonic prey (amphipods & salps) in the winter. DNA barcoding proved to be an effective tool in reducing unidentified prey to < 8% by number. Furthermore, seasonal variability in bulk (white muscle) isotopic signatures ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, & $\delta^{34}\text{S}$) of yellowfin tuna were also observed. Sub-adult $\delta^{13}\text{C}$ values were highest during the spring and lowest in the fall, while adult $\delta^{13}\text{C}$ values were relatively consistent. Seasonal trends in $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ were observed in both size classes and inversely related, with low $\delta^{15}\text{N}$ (high $\delta^{34}\text{S}$) values from late summer to fall and high $\delta^{15}\text{N}$ (low $\delta^{34}\text{S}$) values during late winter/early spring. Finally, Bayesian mixing models were used to estimate the relative contributions of different prey sources (coastal fishes, oceanic fishes & squids, planktonic prey, and sargassum-associated prey) to yellowfin tuna isotopic signatures. Oceanic fishes and squid were the primary contributors to adult yellowfin tuna during all seasons (51.4 – 68.9%), while contribution estimates to sub-adults were seasonally variable. High contribution estimates from planktonic prey to sub-adult yellowfin tuna during summer (44.2%) was in agreement with observed winter peaks in amphipod consumption (~ 6 months for tissue turnover). Additionally, the contribution of coastal fish to sub-adult and adult yellowfin tuna was greatest during the spring (27.2% & 21.7%, respectively), which corresponded with heightened consumption of coastal prey during the fall. Our findings highlight the importance of a suite of seasonally abundant prey resources to yellowfin tuna populations in the northern Gulf of Mexico, and demonstrate the seasonal influence of migratory behavior, prey life cycles, the Mississippi River, and habitat (oil & gas platforms) to the utilization of both inshore and offshore prey resources by yellowfin tuna in this region.

American Eel (*Anguilla rostrata*) in Louisiana: ages, growth rates, diet, and emerging patterns

Robby Maxwell*,¹, Sean Kinney¹, and Kym Walsh²

¹Inland Fisheries Division, Office of Fisheries, Louisiana Department of Wildlife and Fisheries, Lake Charles, LA; ²Research and Development Division, Office of Fisheries, Louisiana Department of Wildlife and Fisheries, Baton Rouge, LA. rmaxwell@wlf.la.gov
skinney@wlf.la.gov kwalth@wlf.la.gov

Recently, there has been increasing interest in American Eel (*Anguilla rostrata*) populations in Gulf of Mexico drainages due to pressure on the species from markets in Europe and Asia, impacts from passage barriers, critical habitat loss, effects of *Anguillacolooides crassus* infections, and the lack of available life history information. Very little is known about Louisiana populations, so in 2017 LDWF began work on a two-year State Wildlife Grant to collect baseline data on American Eels found in state waters, primarily utilizing bycatch from standardized sport fish sampling efforts in the Department. Data collected from specimens includes length, weight, sex, age, gonad development, stomach contents, and presence of *A. crassus*. Of the 420 eels collected, otoliths were collected from 314 specimens. Ages ranged from zero up to 16 years old, with a mean age of five years. Eels aged at nine years and below made up 95.6% of samples. Sizes ranged from 106 to 940 mm total length. Notably reduced growth rates have been observed at one site, and various stomach contents have been detected with crayfish being the most abundant prey item across the state. *A. crassus* has been detected in 104 specimens. Results will be used to inform future methods and focus of eel stock research in Louisiana, and contribute to a growing body of knowledge regarding eel conservation and management in Louisiana and the Gulf South.

Effects of environmental hypoxia on red swamp crayfish *Procambarus clarkii* life history and fecundity characteristics in the Atchafalaya River Basin

Austin Ortman* and Christopher P. Bonvillain

Department of Biological Sciences, Nicholls State University, Thibodaux, LA.

aortman@nicholls.edu

The Atchafalaya River Basin (ARB) is the largest bottomland hardwood river-floodplain system in North America and produces approximately 90% of the wild crayfish harvest in Louisiana. However, anthropogenic modifications to the natural hydrology in the ARB have altered historic river-floodplain connectivity and reduced water circulation and flow patterns that facilitate extensive areas of hypoxia for several months throughout the annual flood pulse. Although red swamp crayfish *Procambarus clarkii* can tolerate relatively low dissolved oxygen concentrations, chronic environmental hypoxia can negatively affect *P. clarkii* population characteristics. The purpose of this project is to compare *P. clarkii* life history and fecundity characteristics between chronically hypoxic and normoxic areas in the ARB. Crayfish were sampled every two weeks at 14 sample sites in the eastern ARB during the 2020 crayfish season. Water quality and catch per unit (CPUE) effort were recorded at all sites on every sample date and sex and carapace length were recorded for all captured crayfish. Additionally, *P. clarkii* hemolymph samples were collected from ten intermolt individuals at all sample locations on every sample date to determine protein concentration. *Procambarus clarkii* oocyte number and maturation stage were compared between individuals from hypoxic and normoxic sites. Although overall CPUE for crayfish was low in 2020, hypoxic locations still had a lower CPUE (0.11 ± 0.03) compared to normoxic sites (0.41 ± 0.10). Mean *P. clarkii* hemolymph protein concentration was significantly higher ($F_{1,151} = 19.32$, $P = 0.001$) in individuals from normoxic areas (5.6 ± 0.2 g/100 mL) compared to chronically hypoxic areas (3.5 ± 0.3 g/100 mL). Although a significant difference was not detected, female *P. clarkii* produced less mean eggs (363 ± 37) compared to individuals from normoxic sites (444 ± 24). The information from this research can be applied to future ARB management decisions and is critical to stakeholders and resource managers as efforts to improve water quality and reduce the severity and duration of ARB hypoxia move forward.

Open fabrication in 3-D printing

Allyssa M. Oune*, Jack C. Koch, Nik Zuchowicz, and Terrence R. Tiersch
*Aquatic Germplasm and Genetic Resources Center (AGGRC), Louisiana State University
Agricultural Center, Baton Rouge, LA. aoune1@lsu.edu JKoch@agcenter.lsu.edu
nzuchol@lsu.edu tiersch@agcenter.lsu.edu*

Three-dimensional (3-D) printing is the process in which material is extruded, joined, and hardened to create various objects under the direction of a computer software, typically in a “G-code” language format. 3-D printing was first commercialized in 1986, but at the time, the cost of a 3-D printer was about \$300,000. Within the last few years, the price of consumer-level 3-D printers can cost from \$1,800 to as low as \$200, allowing more users to engage in 3-D printing and realize how useful and versatile it can be. Rather than spending money on devices that may or may not work, open-fabrication allows an individual to use freely shared files (e.g. STL and OBJ) to print devices themselves. Open fabrication allows use of computer-aided design (CAD) software to create new designs (such as tinkercad.com), or make adjustments to already existing open-source files. These design files are converted to G-code by software designed to program the printing process layer by layer (e.g. Ultimaker Cura). As a result, prints can be modified directly by the user. Additionally, the filament that is extruded to form the print costs between \$20 and \$40 per kg making it fairly inexpensive. For example, in a recent project, 12 bearings were needed to build a custom roller system for jars used to maintain larvae of a marine invertebrate (*Aplysia californica*). Packs of four bearings with mounts ranged from \$16-\$30 (on Amazon.com), not including shipping and handling, and did not guarantee that the bearing mounts would fit properly for the application. Alternatively, ten unmounted bearings can be bought for about \$10 and the mounts could be custom printed for less than \$5 total (Figure 1). Thus, the mounts were constructed specifically to fit the roller system instead of the roller system being made to fit the bearings. Overall open fabrication, based on free software and shared 3-D printing files, helps individuals have more control over devices by allowing customizable fabrication for a relatively low cost. The barriers to adopting such technologies are being reduced, granting the opportunity for individuals of various experience levels to grow and develop into large communities of users and developers of open-fabrication techniques in 3-D printing.

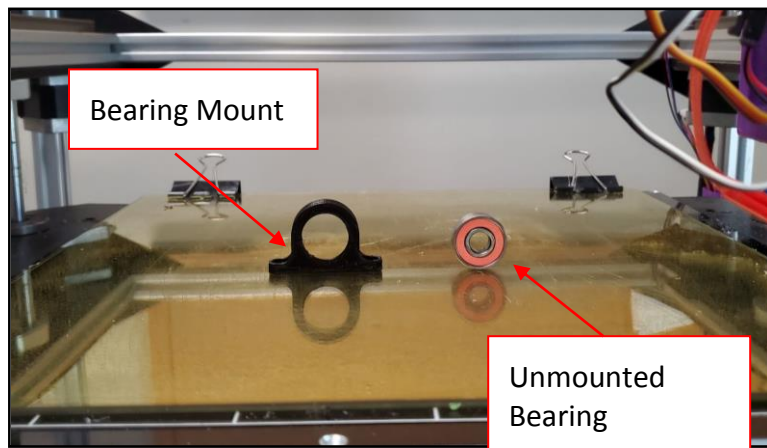


Figure 1: A custom 3-D printed mount and an unmounted bearing sitting on the print bed of a 3-D printer (Folger Technologies FT-5 v6) at the AGGRC.

Crabtivating behavioral analysis: the impacts of fipronil pesticides on Blue Crab (*Callinectes sapidus*) behavior

Sadie A. Rawls* and Jennifer M. Hill

Louisiana Tech University, Ruston, LA. sar055@latech.edu

Pesticides are used in agriculture, urban, and forest pest management to control weeds and insects. Through nonpoint source pollution these pesticides are carried to into rivers, lakes, and coastal habitats where low concentrations can impact organismal physiology, movement, and behavior. These alterations can reduce the fitness of animals by impeding their abilities to forage, avoid predators, and find mates. If pesticides accumulate in animal tissues, these toxins may continue to affect animal behaviors and fitness even after pesticides in the water have degraded or flushed from estuarine systems. Yet, there are few studies that examine how pesticides impact the behavior of coastal animals and fewer that examine if animals recover after pesticide exposure.

In coastal habitats, crustaceans, such as the blue crab, may be negatively impacted by pesticide exposure when polluted run-off enters estuaries. Blue crabs are ecologically important predators and scavengers that help to maintain estuarine habitats through trophic cascades and are also essential commercial fisheries species. Thus, impairments to blue crab movement or foraging could have significant economic and ecological impacts. To identify if blue crab behaviors and movements are impacted by pesticides, we examined a variety of blue crab behaviors before, during, and after exposure to fipronil pesticides (0, 0.5, 1, 5 ug/L). To assess how fipronil affects foraging behavior, we measured handling and total feeding time of crabs consuming periwinkle snails before and after exposure to fipronil. We also measured their righting time before, during, and after fipronil exposure.

To determine whether blue crabs recovered from pesticide exposure, we observed them for an additional eight days after exposure in which we measured their time to stop moving after being added into new tank environments, response to a dowel rod stimulus, and their time to stop moving after a disturbance. Video analysis of all variables is ongoing; however, we can report that crabs exposed to 5ug/L of fipronil began to show impairments which resulted in involuntary behavior such as, not being able to settle, spasms, and paralysis or stiffness along with longer times to stop moving after entering the tanks and after disturbance. However, crabs that were exposed to lower concentrations exhibited results similar to the controls.

Consequently, fipronil in higher concentrations is likely to impair crab foraging and other behaviors which could negatively impact blue crab populations resulting in economic losses in commercial fisheries as well as structure of ecological communities. These higher concentrations in estuarine environments may be less likely, but as we do not test for the presence of fipronil in estuarine waters, water quality monitoring should be performed to demonstrate fipronil does not contaminate estuaries and associated organisms.

***Callinectes sapidus* reovirus 1 (CsRV1) waterborne transmission in soft shell blue crab shedding systems**

Elizabeth M. Robinson* and Julie A. Lively

Renewable Natural Resources, Louisiana State University Agricultural Center, Baton Rouge, LA. ERobinson@agcenter.lsu.edu

Soft shell blue crab (*Callinectes sapidus*) aquaculture is one of the oldest domestic aquaculture industries along the East and Gulf Coasts of the United States. Soft shell blue crabs are produced by culturing pre-molt (peeler) crabs in shallow dockside or land-based shedding systems (floats or tables) until they shed their hard shell in order to grow. Diseases, such as *Callinectes sapidus* reovirus 1 (CsRV1), can play a significant role in crab mortality in shedding systems. The prevalence of CsRV1 in dead peeler crabs ranges from 22 – 75% in blue crab shedding facilities located in Maryland, Virginia, and Louisiana. We investigated the mode of transmission of CsRV1 among crabs in shedding systems using 4 recirculating aquaculture systems. Crabs that were either injected with CsRV1 or known to have been exposed to CsRV1 were held alongside crabs that did not have exposure to CsRV1. Crabs were kept in recirculating systems until death or for 27 days. Leg tissue was collected from each crab prior to the start of the experiment and when the crab died for Real time qPCR (RT-qpcr) analysis. RT-qPCR was used to determine the viral load of CsRV1 in the crabs. Our results support that CsRV1 can be transmitted among crabs in recirculating systems through waterborne contact.

Status of a recently established population of Cuchia (*Amphipnous cuchia*) in Bayou St. John, New Orleans

Victoria Rodrigues^{*,1}, Susan Thomassie², Olivia Guerra¹, and Frank Jordan^{1,2}

¹Environment Program, Loyola University New Orleans, New Orleans, LA; ²Department of Biological Sciences, Loyola University New Orleans, New Orleans, LA. jordan@loyno.edu

Cuchia (*Amphipnous cuchia*) is an air-breathing, synbranchiform fish native to Southeast Asia. Like other swamp eels, Cuchia is a nocturnal, opportunistic predator that inhabits dense vegetation, burrows into banks, can travel across land, and is tolerant of harsh environmental conditions. In June 2019, Cuchia was discovered in Bayou St. John, an urban waterway in New Orleans that is hydrologically connected to Lake Pontchartrain. Data on abundance, distribution, and ecology of Cuchia are needed to characterize early invasion dynamics and inform control and management of this recently introduced species. To help address this need, we used dip nets, seines, throw traps, leaf packs, minnow traps, and inspection of root mats to sample Cuchia and associated nekton at 12 sites in Bayou St. John, 3 sites in City Park, and 2 sites in Lake Pontchartrain during the summers of 2019 and 2020. We collected several age and size classes of Cuchia in both years, including young-of-year, which indicates this population of Cuchia is successfully reproducing. The distribution of Cuchia in Bayou St. John increased modestly between 2019 and 2020, but Cuchia has not yet been detected in City Park or Lake Pontchartrain. Leaf packs and minnow traps were ineffective for sampling Cuchia. Preliminary lab experiments indicate Cuchia is tolerant of salinities up to at least 10 ppt. The potential for Cuchia to spread into Lake Pontchartrain is troubling, as this provides an avenue for further dispersal into nearby drainages, including the Mississippi River.



Figure 1. Throw trap sampling for Cuchia (*Amphipnous cuchia*) in Lake Pontchartrain.

Fish community composition of two Mississippi River floodplain re-connectivity sites with a focus on the population demographics of gars (Lepisosteidae)

Derek C. Sallmann^{*,1}, KristieRae Ellis¹, Bryan P. Piazza², and Solomon R. David¹

¹Department of Biological Sciences, Nicholls State University, Thibodaux, LA; ²The Nature Conservancy Louisiana, Baton Rouge, LA. dsallmann@nicholls.edu kellis10@nicholls.edu bpiazza@tnc.org solomon.r.david@gmail.com

Connectivity of large rivers with their floodplains can benefit riverine fishes by providing access to food, additional habitat, spawning areas, and nursery refuge for juvenile fishes. However, due to anthropogenic modifications such as levees and channelization, floodplain habitats are often disconnected from their associated rivers, potentially limiting ecosystem function. To address this issue in the Lower Mississippi River Basin, the Nature Conservancy (TNC) (Site 1), Louisiana Department of Wildlife and Fisheries (LDWF) (Site 2), and partners have initiated projects to improve connectivity between the Lower Mississippi River and its floodplains. Species diversity and abundance among fish communities at these sites will be compared before and after floodplain connectivity is reestablished. A subsample of gars (Lepisosteidae) from each site visit will be retained for life history analysis. Baseline monitoring prior to restoration activities resulted in presence and absence data for the fish communities at these sites, including gars (Figure 2). There were 24 fish species found at Site 1, including four gar species, and young-of-the-year of three gar species, suggesting spawning activity in the floodplain. At Site 2, 16 species of fish were found, including three gar species. We expect fish diversity and abundance to increase after restoration efforts are completed at both sites.





Alligator Gar (<i>Atractosteus spatula</i>)	
	
3 (6)	0 (2)
Longnose Gar (<i>Lepisosteus osseus</i>)	
	
61 (6)	7 (2)
Spotted Gar (<i>Lepisosteus oculatus</i>)	
	
54 (6)	16 (2)
Shortnose Gar (<i>Lepisosteus platostomus</i>)	
	
96 (6)	23 (2)

Figure 2. Number of gars at Sites 1 and 2. Number of sampling trips in parentheses. Fish illustrations: Madhusudhan Gundappa @fish_lines.

Development of a low-cost sperm counting chamber

Ignatius Semmes*, Yue Liu, and W. Todd Monroe

LSU AgCenter, Louisiana State University, Baton Rouge, LA. isemmel@lsu.edu
yliu@agcenter.lsu.edu tmonroe@lsu.edu

Sperm cryopreservation allows banking of valuable genetic resources for biomedical research model animals such as zebrafish (*Danio Rerio*). Zebrafish are used in more than 2,000 laboratories worldwide and more than 500 zebrafish mutation lines have been banked via sperm cryopreservation. However, these efforts are hindered by a lack of standardized sperm concentration evaluation among laboratories, which is an essential step in the cryopreservation process. Many existing concentration evaluation devices have high cost, thus are unsuitable to many laboratories in which cryopreservation is not a focus. Current 3-D printing technology could offer a solution to this problem with high precision, reproducibility, and low cost. The goal of the present study was to explore the feasibility of fabrication of a sperm counting chamber with consumer-grade 3-D printers. Counting chambers were designed with computer-assisted design (CAD) to create different dimensions of chamber areas and heights. More than 30 prototypes (Figure 1) were fabricated by use of a commercially available stereolithographic (SLA) printer and near-transparent resin. Fabrication features of 10-200 μm in z-direction, and 35-385 μm in x-and y-directions were evaluated with profilometry (Figure 2). Fabricated dimension errors were approximately 10% from the designed geometries. Concentration measurement accuracy evaluated with microbeads showed it was feasible to count cell samples with this prototype. These prototypes could be further improved and standardized among user communities to improve counting accuracy for various aquatic species sperm.

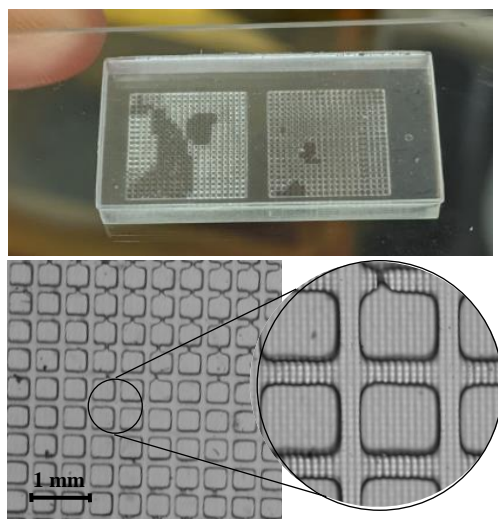


Figure. 1. A 3-D printed counting chamber fabricated (upper) with adequate visual clarity (bottom) was observed with bright-field microscope.

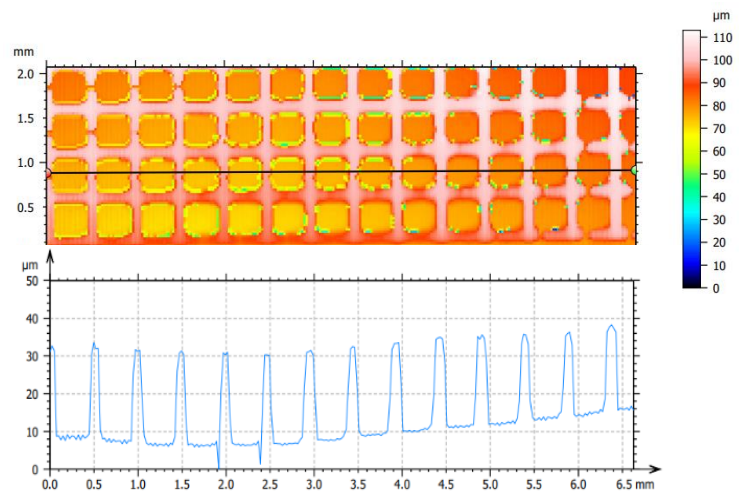


Figure. 2. Evaluation of dimensions and uniformity was analyzed with laser-profilometry. Maximum deviation in grid heights between fabricated prototypes and design was 6 μm .

Identifying ontogenetic shifts in primary energy pathways of invasive red lionfish (*Pterois volitans*) in the Florida Keys via stable isotope and otolith analyses

Emily E. Shallow*, Cassandra N. Glaspie, and Michael J. Polito

Louisiana State University, Department of Oceanography and Coastal Sciences, Baton Rouge, LA 70803. eshall2@lsu.edu cglaspie1@lsu.edu mpolito@lsu.edu

The invasive red lionfish, *Pterois volitans*, has been shown to considerably reduce reef biomass via predation and competition in non-native ranges. Primary maintenance of lionfish is through human removal (e.g., culling), which does not sufficiently control the population due to deep-water spawning, recolonization of recently culled reefs, and ontogenetic migrations to mesophotic waters. Here, we investigate the primary energy pathways and ontogeny of lionfish in the Florida Keys via otolith and stable isotope ($\delta^{13}\text{C}$ & $\delta^{15}\text{N}$) analyses to better understand their role in the trophic food web and find if age impacts isotopic composition. Additionally, we will examine isotopic overlap between lionfish and native reef predators to assess competition. Lionfish (n=48) and other community representatives were collected from a mosaic of habitats in the Florida Keys (i.e., mangroves, seagrass beds, and coral reefs) in August 2020. Preliminary findings suggest lionfish on shallow reefs obtain their basal carbon values from different sources than lionfish at greater depths. Furthermore, assuming length serves as a proxy for age, lionfish in the Florida Keys may not exhibit ontogenetic migration to deeper waters. Finally, lionfish show extensive isotopic overlap with native reef fish species, which may indicate resource competition extends beyond native predators. To further investigate our objectives, a Bayesian mixing model will be used to estimate the proportional contribution of each source to the energy pathway of lionfish. Additionally, we will assess community isotopic niche space using $\delta^{13}\text{C}$ & $\delta^{15}\text{N}$ to gain insight on the community structure. A better understanding of the role shallow water habitats play in the basal carbon transfer and ontogeny of lionfish in the Florida Key's ecosystem could lead to improved regional management strategies designed to target lionfish prior to deep-water spawning events.

Intracellular calcium signals as molecular fingerprints for assessing sperm quality in live-bearing fishes: an effort towards repository development for conservation

Amit Sharma^{*,1}, Terrence R. Tiersch², and Henrique Cheng¹

¹Department of Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA; ²School of Renewable Natural Resources, Aquatic Germplasm and Genetic Resources Center, Louisiana State University Agricultural Center, Baton Rouge, LA. Ashar55@lsu.edu ttiersch@agcenter.lsu.edu hcheng@lsu.edu

Xiphophorus (Poeciliidae) are live-bearing fishes native to Mexico that developed viviparity independently from neighboring fishes such as *Xenotoca* (Goodeidae). These fishes offer research, ornamental and ecological values. With increasing human encroachment on their habitats, the danger of extinction looms. Sperm cryopreservation is a strategy that can be used in conservation efforts to establish germplasm repositories to protect genetic diversity. Currently, there is a need to improve cryopreservation by evaluating spermatozoa damage at the molecular level. Our goal was to develop a methodology to study intracellular mechanisms in sperm of *X. helleri* to use calcium signals as a tool to compare cryopreservation conditions. The frequency and amplitude of these signals could be used as molecular markers “fingerprints” to determine spermatozoa viability. A range of sub-optimal (damaging) and suitable cryopreservation conditions was produced with slow (5°C/min), moderate (20°C/min) and fast (45°C/min) cooling rates. The loading time (15-60 min) and concentration (0.1-5 μ M) of a calcium-signaling dye, Fura-2 acetoxymethyl ester (Fura-2AM), were determined using a real-time imaging system, and 2 μ M and 30 min were selected. We found that extracellular alkalization and elevated calcium concentrations stimulated intracellular calcium signals in a concentration-dependent manner. Calcium channel gene expression was studied by qPCR analysis and potential calcium channels [melastatin (M7), canonical (C4), voltage-dependent (P/Q), vallinoid (V1), crac (Orai1), ankyrin (A1), polycystic (P2) and mucolipin (ML1)] were identified. These findings can provide a molecular basis to characterize calcium signals that control sperm cell activation and motility, which are required for fertilization, and to optimize conditions for cryopreservation.

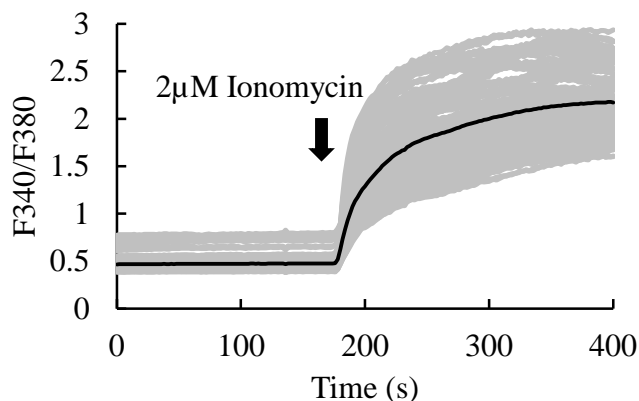


Figure. *X. helleri* sperm bundle loaded with 2 μ M Fura-2AM for 30 minutes gave sharp, detectable calcium signals under stimulation by 2 μ M Ionomycin

River continuums and dis-continuums: trophic relationships in Louisiana coastal watersheds

Erin E. Thayer*, William E. Kelso, and Michael D. Kaller

Freshwater Ecology Laboratory, School of Renewable Natural Resources, Louisiana State University Agricultural Center, Baton Rouge, LA. ethayer1@lsu.edu

Subtropical coastal plain (SCP) watersheds in the southeastern U.S. often exhibit minimal elevational gradients, long growing seasons, fine substrates, and substantial connectivity to the riparian zone. These attributes may alter applicability of the River Continuum Concept (RCC) as well as other temperate stream paradigms. Louisiana offers a range of SCP watersheds in terms of geology, landscape cover and flow regimes. We selected the similar-size Tickfaw River and Calcasieu River watersheds in Louisiana to investigate fish trophic relationships and potential carbon sources as per the RCC in headwater streams, intermediate sites and mainstem rivers based on stable isotopic analysis (carbon (^{13}C) and nitrogen (^{15}N)) of fish muscle tissue. This method accomplishes 3 of our hypotheses: 1) allochthonous energy sources throughout these two watersheds (lateral connectivity) will differ in the importance of autochthonous or allochthonous energy sources as evidenced by carbon signatures; ; 2) trophic levels will differ between these watersheds (longitudinal connectivity) as evidenced by nitrogen signatures; and 3) differences in trophic turnover as evidenced by species' and functional guilds' carbon and nitrogen signatures will differ between watersheds. Emphasis will be on Centrarchids because of their presence throughout and within each watershed. We predict that there will be obvious differences among these two watersheds in that the Tickfaw River will act more as a typical RCC system (i.e., allochthonous signatures in fishes will predominate in headwaters, and further downstream, signatures will transition to indicate more autochthonous energy sources, with taxa and functional guilds showing clear turnover from headwaters to mainstem). Conversely, the Calcasieu River, will exhibit more floodplain connectivity throughout the watershed (fishes will display similar carbon signatures throughout the watershed, with less taxa and/or functional guild turnover, but increased trophic levels downstream as more species are added. Results of this study will demonstrate the unique ecological requirements of these two watersheds, with the Calcasieu River potentially requiring more management/protection of the riparian zone relative to the Tickfaw River.

From Australia to Louisiana: a snapshot of germplasm repositories for amphibian conservation in Australia and how I came to be in Baton Rouge

Rose Upton^{*,1,2} and John Clulow²

¹Aquatic Germplasm and Genetic Resources Center (AGGRC), School of Renewable Natural Resources, Louisiana State University Agricultural Center, Baton Rouge, LA; ²Conservation Biology Research Group (CBRG), School of Environmental and Life Sciences, The University of Newcastle, NSW, Australia. rupton@agcenter.lsu.edu john.clulow@newcastle.edu.au

Amphibians face a wide suite of threats, from the global panzootic—chytridiomycosis—to the recent megafires that ravaged the east coast of Australia, affecting ~51 million frogs. In short, it's not a great time to be a frog. Innovative solutions are needed to increase the benefits of conservation actions already commonly used. Germplasm repositories have the potential to advance conservation efforts by preserving the genetic diversity of species, and allowing correction of inbreeding depression caused by habitat disruptions, such as fire. While application of such repositories in the conservation community has been slow, groups have begun to implement cryopreservation of sperm into existing programs, to a small extent. Outside of conservation, aquatic biomedical organisms, such as *Xenopus laevis* and *Ambystoma mexicanum*, are examples of groups that can benefit from development of germplasm repositories, which will allow creation of transgenic and mutant lines without the need to maintain high numbers of live organisms. We will give an overview of current programs and work being done in the laboratory and field (Figure 1) within Australia, and comment on how a generalised cryopreservation pathway for aquatic biomedical organisms can be extended to assist conservation efforts for imperilled amphibian species.



Figure 1. Bringing laboratory-based technologies to the Australian bush to cryopreserve sperm from endangered amphibians. (a) Sperm collection and cryopreservation in a temporary field-based setting; (b) *Helioporus australiacus*; (c) *Litoria subglandulosa*; (d) *Mixophyes iteratus*. Photo credits: (a) and (c) Samantha Wallace; (b) and (d) Rose Upton

Development of a low-cost 3-D printed ‘CryoArk’ for sperm cryopreservation

Rocky Vong^{*,1}, Yue Liu², and Terrence R. Tiersch³

¹Department of Industrial Engineering, Louisiana State University, Baton Rouge, LA;

²Department of Biological and Agricultural Engineering, Louisiana State University, Baton Rouge, LA; ³Aquatic Germplasm and Genetic Resources Center, School of Renewable Natural Resources, Louisiana State University Agricultural Center, Baton Rouge, LA. rvong1@lsu.edu yliu@agcenter.lsu.edu ttiersch@agcenter.lsu.edu

Sperm cryopreservation technology is used to assist clinical application, biomedical research, aquaculture, and conservation of imperiled species. However, commercial cryopreservation equipment can be expensive (e.g., >\$20,000). This high cost dissuades many laboratories from participating in germplasm banking. The goal of this study was to develop a low-cost 3-D printed device (‘CryoArk’) that can float on liquid nitrogen to achieve standardized sperm cryopreservation for aquatic species. Polylactic acid (PLA) filament was used to fabricate the CryoArk by use of fused deposition modeling (FDM) 3-D printers. Design of the CryoArk includes three major components (Figure 1). The first component is a rack that can hold multiple different types of cryopreservation containers, such as 0.25-mL French straws, 0.5-mL straws, 0.5-mL cryopreservation storage vials, and 2.0-mL cryopreservation vials. The second component consists of two pillars that support the top rack at different standard heights above liquid nitrogen. The third component is a base that provides buoyancy to float the device on liquid nitrogen without the need for additional foam accessories as used by the previous iteration



Figure 1. Testing of temperature profiles with thermocouples and 2-mL vials loaded on a 3-D printed CryoArk prototype shown floating on liquid nitrogen in a standard styrofoam container.

(‘CryoKit’). Fabrication time for a single complete device is ~6 hr and material costs are \$4.07. A total of six different prototypes were designed and fully tested for durability and flotation on liquid nitrogen. The final prototype could float for a recorded time of 7 hr but could float indefinitely if the liquid nitrogen did not evaporate. Temperature profiles of samples were recorded by placing thermocouples inside cryopreservation containers. Initial evaluations showed cooling rates generated by the CryoArk ranged from 13 to 25 °C/min, which is within the range to freeze sperm from aquatic species. Further directions include creating designs for vertical rack mounts, height-adjusting blocks, and components that can be printed more quickly. This device can be shared with users as open hardware files suitable for open fabrication to promote community-level standardization for repository development.

Patterns and drivers of introgression in Louisiana's Largemouth Bass (*Micropterus salmoides*) stocks

Colleen E. Walsh*, Michael D. Kaller, Sabrina S. Taylor, and William E. Kelso
School of Renewable Natural Resources, Louisiana State University, Baton Rouge, LA.
cwals11@lsu.edu

The Largemouth Bass (*Micropterus salmoides*; LMB) is one of the most popular sportfishes in North America, and populations in the southeast are often stocked in order to satisfy angler desires for a trophy fishery. Supplemental stocking programs of Florida Largemouth Bass (*Micropterus salmoides floridanus*; FLMB) have typically been developed to introduce and maintain FLMB genes into systems north of its native range of peninsular Florida. Stock enhancement through introgression of FLMB genes has been hypothesized to increase maximum potential size and growth of LMB, with suggestions that hybrid vigor is possible for intergrade subspecies. We quantified level of introgression of FLMB in six Louisiana lakes through the use of fragment analysis at 12 loci of microsatellites confirmed for subspecific identification. Bass were sampled with boat electrofishing with the assistance of Louisiana Department of Wildlife and Fisheries from 2018-2020 (N=360). The program STRUCTURE was used to assign percent FLMB genes to each sample fish, with 20 replicates at K=2. A detrended correspondence analysis (DCA) was used to reduce the dimensionality of the data, and linear components were modeled with beta regression to relate physicochemical variables to average percent Florida genes by lake. We then used generalized linear models (GLMs) to examine the effects of percent Florida genes and measured physicochemical variables on growth, mean length at age, W_r and log-length. The second, third, and fourth linear components of the DCA were significant in the beta regression model with average percent Florida genes, suggesting a positive relationship between Florida genes and highly vegetated lakes with increased turbidity. Models for W_r and mean length at age 1 models were better than the null models based on Akaike's Information Criterion (AIC) and likelihood ratio test (LRT). Percent Florida genes did not influence mean length at age 1 (parameter estimate = 1.15 (\pm 0.11 SE), p = 0.4). However, addition of percent Florida genes resulted in an increase in W_r to a certain point (parameter estimate = 1.32 (\pm 0.06 SE), p < 0.001), at which point the quadratic of this relationship became weakly negative (parameter estimate = 0.776 (\pm 0.08 SE), p = 0.02), indicating limited improvement in W_r with additional Florida genes past a threshold.

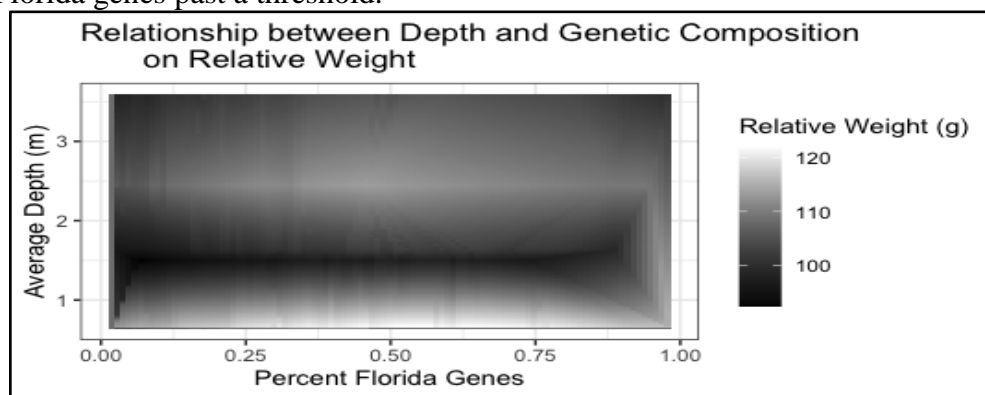


Figure 1. A heatmap displaying the relationship between average lake depth (m), percent Florida genes, and predicted relative weight (W_r ; g) from the best fit generalized linear model for Largemouth Bass sampled in Louisiana from 2018-20.

Genome-wide SNPs provide insight into migratory phenotypes of a partially migrant lake sturgeon population

Justine M. Whitaker^{*,1}, Darryl W. Hondorp², James C. Boase³, Charles Krueger⁴, Louis Bernatchez⁵, and Amy B. Welsh⁶

¹*Department of Biological Sciences, Nicholls State University, Thibodaux, LA;* ²*U.S. Geological Survey, Great Lakes Science Center, Ann Arbor, MI;* ³*U.S. Fish and Wildlife Service, Alpena Fish and Wildlife Conservation Office, Alpena, MI;* ⁴*Michigan State University, Fisheries and Wildlife, East Lansing, MI, 48823;* ⁵*IBIS (Institut de Biologie Intégrative et des Systèmes), Université Laval, Québec, Canada;* ⁶*West Virginia University, Division of Forestry and Natural Resources, Morgantown, WV.* justine.whitaker@nicholls.edu

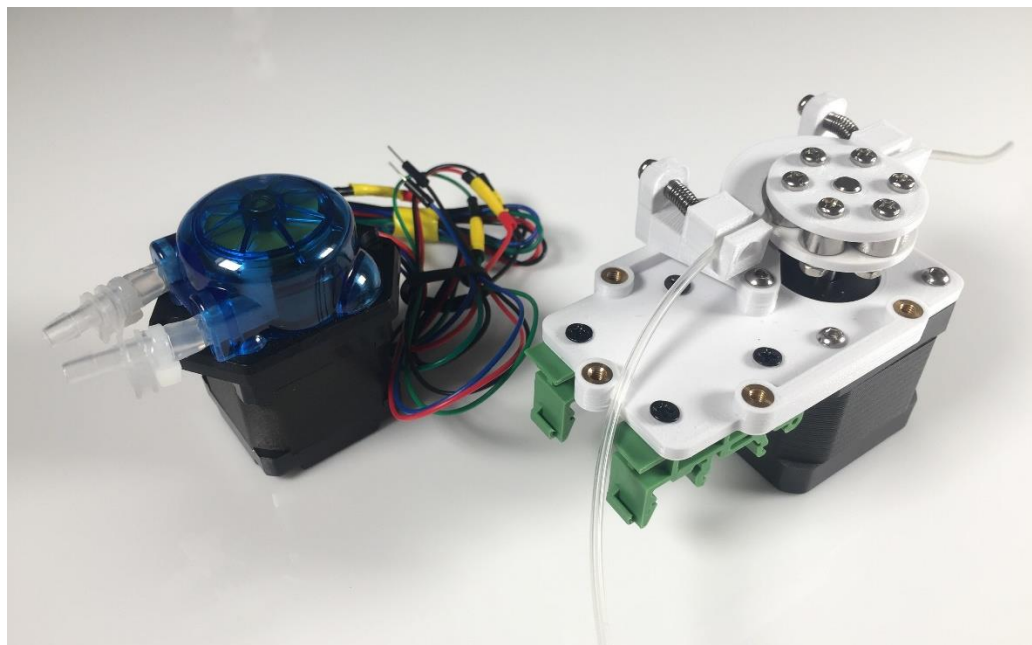
Lake sturgeon migratory patterns are not well understood but may be an important source of diversity for long-term management. In the St. Clair-Detroit River System (SCDRS), the lake sturgeon population is partially migrant and telemetry studies show that migration patterns may be even more complex. To further characterize migrant and resident phenotypes of lake sturgeon in the SCDRS, genome-wide SNPs were genotyped. Fish were categorized as migrant or river residents, but then were further categorized based on the river used by river residents or the river-lake combination used by migrants. F_{ST} analyses identified weak differentiation between resident, migrant, and several subgroups, with DAPC support for much of the differentiation. Outlier analysis (1,042 loci) resulted in fewer significantly differentiated phenotypes, but with higher F_{ST} values. By mapping outlier loci to the *Acipenser ruthensis* genome and identifying Gene Ontology annotations, 373 loci were identified in this study as potentially important gene regions for characterizing migratory phenotypes in lake sturgeon. Further investigation into migratory phenotypes may be useful for conserving a potentially underrated level of diversity in lake sturgeon populations.

Applying open technology concepts to aquatic research

Nikolas C. Zuchowicz* and Terrence R. Tiersch

Aquatic Germplasm and Genetic Resources Center, School of Renewable Natural Resources, Louisiana State University Agricultural Center, Baton Rouge, LA. nzuchol@lsu.edu

Open technology can support the aims of researchers, aquaculturists, and field biologists, especially those with specialized needs or modest resources. Using open hardware and software reduces costs, promotes do-it-yourself skill building, improves reparability, and combines the knowledge and skills of amateurs and professionals from around the world. Many useful projects can be found on internet platforms built for open distribution, including GitHub and Thingiverse. Open hardware designs may be built at low cost from components readily available from hardware stores or online retailers, or with a small investment in 3-D printing capabilities (e.g., ~\$250). For the more enterprising users, free and open software and hardware tools are available to create new, custom designs that may be shared easily with other professionals. One example is the Concentration Measurement and Adjustment System (CMAS) under development at the Aquatic Germplasm and Genetic Resources Center. This tool will automate measurement of the concentration of aquatic sperm samples and dilute the samples with cryoprotectant for cryopreservation. The CMAS serves here as a case study for the benefits that may be derived from an open-technology and file-sharing approach. Implementing open principles can save money, encourage creativity, and lead to custom designs that best answer specialized needs.



A commercially available peristaltic pump (MaschinenReich, model XP88) is shown on the left; a custom, largely 3-D printed peristaltic pump developed at the Aquatic Germplasm and Genetic Resources Center is on the right. Open technology enables custom solutions for unique problems. The custom pump at right offers compatibility with millimeter-bore tubing, easy tubing changeout, and mounting points for additional hardware – all features lacking in the commercial pump. The design files for this pump can be shared and modified by users.