LAKE MAUREPAS MONITORING (PHASE III)

ANNUAL REPORT: YEAR 1

(JAN 2023-DEC 2023)

Prepared by:

Kyle Piller, Chris Murray, Gary Shaffer, Fereshteh Emami, and Robert Moreau

With the assistance of:

Tyler Steven Coleman, Ariel Ebanks, Wray Gabel, Corwyn Hall, Alexander Mott, Nicholas Stevens, Maggie McGreal, and Anthony Johnson

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Executive Summary

The overall goal of this project is to conduct a monitoring study including the Lake Maurepas ecosystem and its surrounding watershed, specifically monitoring the abiotic and biotic components of this region. Southeastern Louisiana University will gather biological data within the aquatic and wetland realms to provide baseline abiotic and biotic data regarding the condition of Lake Maurepas prior to the initiation of the Air Products Carbon Sequestration Project. In particular, the aquatics team will monitor fish, crab, and shrimp populations to assess spatial and temporal variation throughout the lake. Scientific buoys will be deployed to monitor real-time water quality throughout the lake. The wetlands team will monitor wetland vegetation, elevation change, and update geographic information system habitat maps depicting ecosystem health of the Lake Maurepas wetland area. The physiology team will conduct an ecotoxicological assessment and monitoring survey to develop baseline levels of a variety of physiological parameters for a suite of target species. The chemical monitoring team will examine the impacts of dredging in Lake Maurepas and will monitor chemical particulates from both the water column and benthos. These chemical monitoring programs have been tasked with understanding the fate and transport of possible toxic chemicals in the environment. Finally, Southeastern will develop and maintain a project website that will highlight Southeastern's role as an independent monitoring entity in the project. Southeastern's Turtle Cove Environmental Field station will support the project researchers by providing access and transportation to the lake and wetland regions and will be responsible for the design, development, and implementation of education/outreach activities that combine our traditional transfer of ecosystem knowledge in the area with that of the scientific bio-monitoring findings from our research.

I. Aquatics Monitoring

Kyle R. Piller, PhD
Southeastern Louisiana University, Dept. of Biological Sciences
Hammond, LA 70402
kyle.piller@selu.edu

Overview

During the first year of the project, the aquatics team developed a sampling protocol to begin surveying spatial and temporal variation of the aquatic biotic community in Lake Maurepas. Aquatic surveys were conducted across six lake sectors on a quarterly basis. Sampling, using a variety of different gears—gillnets, trawls, electrofishing, crab traps, and dredges—focused on the fishes, shrimp, crabs, and other invertebrates in Lake Maurepas. During sampling, the abundance of each species was quantified and total lengths and weights of economically relevant species were recorded. To date, more than 5,900 fishes representing 14 species were collected during the first quarter of sampling (August-October 2023). Additionally, nine species of invertebrates have been identified. Crab traps were set out in the first quarter (15 traps/sector for a total of 90), and 352 blue crabs were collected. Our second quarter sampling period (November 2023–January 2024) is currently underway. The first round of environmental DNA (eDNA) sampling was conducted in December 2023 and water samples were taken from 24 localities around the lake—12 inshore and 12 offshore. The second round of eDNA data collection is currently underway. Lastly, four YSI monitoring buoys have been purchased, and were received in late-December 2023. These buoys will monitor aquatic parameters including water temperature, dissolved oxygen, pH, carbon dioxide, and turbidity. One of the four buoys will be outfitted with a weather station to provide information on atmospheric conditions including air temperature, relative humidity, barometric pressure, absolute humidity, wind speed, and wind direction. We anticipate deploying the buoys in early 2024.

Project Objectives

1. Monitor the biotic components of Lake Maurepas from a spatial and temporal perspective.

2. Deploy YSI buoys to monitor a suite of abiotic parameters in Lake Maurepas to help inform any changes we detect in the aquatic biodiversity component of the project.

Methods/Study Design

The aquatic biota (vertebrate and invertebrate organisms) of Lake Maurepas was sampled. The sampling methodology and sampling objectives varied based on the gear type and sampling design. These are discussed in detail in the following sections of this report.

Random Selection

The lake was divided into six sectors based on zones previous seismic testing of the lake (**Figure 1**). The new sectors were modified from the original seismic zones to be more similar in surface area (**Table 1**). A spatial grid of 800-m^2 was laid over the lake shapefile using ArcGIS software (version 10.0, Environmental Systems Research Institute, Redlands, California) and a centroid for each grid cell was generated and identified (**Figure 2**). This grid created a total of 313 points in Lake Maurepas. A series of points along the lake shoreline (referred to as "shoreline points" hereafter) spaced every 100m (n=652) were also created. Similarly, a second series of points 50m inshore from the defined lake shoreline (referred to as "inshore points" hereafter) that were spaced every 100m (n=601) were created and identified. Each of these points (grid centroids, shoreline points, and inshore points) were used to randomly select areas of the lake to focus different sampling efforts. The sampling objective is to sample the list of randomly selected points per sector for each gear type in each sampling period (**Table 2**). The number of random points selected per sector varies for each sampling method (**Table 3**). For bottom trawls and shrimp trawls, a direction to pull the trawl from the centroid of the grid cell was randomly selected. For each sampling method there were at least two extra randomized sampling points selected to account for obstacles (shipwrecks, dredge pipes, machinery, etc.) that would prevent sampling at the originally selected point.

Figure 1. Created sectors (left) used for the aquatic sample design, and (right) the seismic testing zones used by Exoduas during the seismic surveys in 2023.

Table 1. Surfacearea in square kilometers of each sector.

Sector	Area (km^2)
1	39
2	37
3	39
4	43
5	38
6	38

Table 2. Sampling period divisions.

Sampling Period	Start	End
Period 1	8/1/2023	1/31/2024
1a	8/1/2023	10/31/2023
1b	11/1/2023	1/31/2024
Period 2	2/1/2024	7/31/2024
2a	2/1/2024	4/30/2024
2b	5/1/2024	7/31/2024
Period 3	8/1/2024	1/31/2025
3a	8/1/2024	10/31/2024
3b	11/1/2024	1/31/2025
Period 4	2/1/2025	7/31/2025
4a	2/1/2025	4/30/2025
4b	5/1/2025	7/31/2025
Period 5	8/1/2025	1/31/2026
5a	8/1/2025	10/31/2025
5b	11/1/2025	1/31/2026

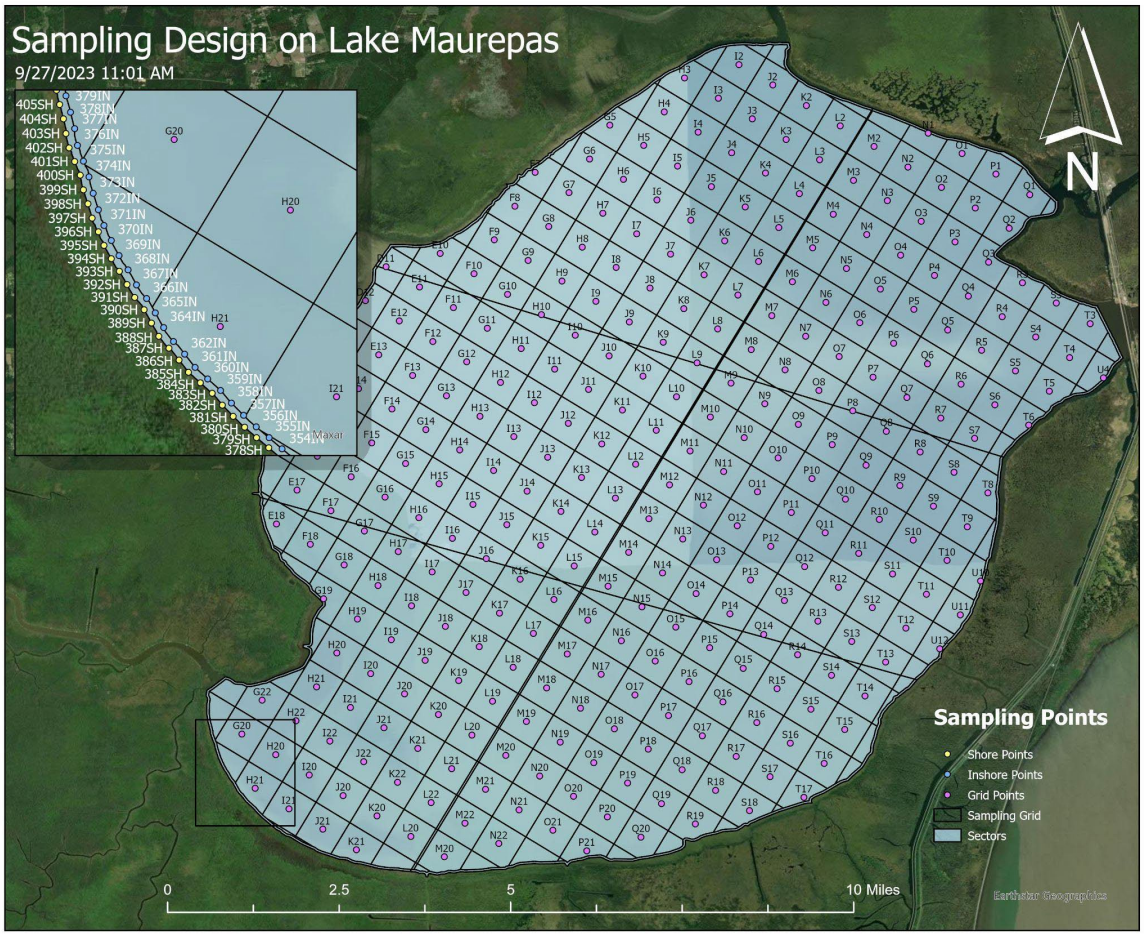

Figure 2. Points used to randomly select locations for sampling. The spatial grid cells with centroid points (letters D–U), inshore points (IN), and shoreline points (SH).

Table 3. Total number of random points sampled per sector and the type of point used for the sampling design of each gear type and sampling method.

Gear Type	Number of Points Per Sector	Type of Point
Dredge	3	Grid Centroid
Crab Traps	10	Grid Centroid
Gillnets	5%	Inshore Point
Bottom Trawl	3	Grid Centroid
Shrimp Trawl	3	Grid Centroid
Electrofishing	(undecided)	Shore Point

Note: For gillnets, 5% of the available points were sampled due to the variable amount of shoreline between sectors. Sectors 1, 3, 4, and 6 had 6 random points and sectors 2 and 5 had 3 random points.

Fishes

Fishes were collected using two methods in sampling period 1a (**Table 2**). A modified mini-Missouri bottom trawl (Herzog et al. 2009) was pulled at three sampling sites in each sector (**Table 3**), or 18 benthic trawls in each sampling period. Sampling began in the center of a selected grid if water was at least 1.0-m deep. If the center of the grid was not suitable for sampling (e.g., large coverage of vegetation, submerged structure), then a clockwise or counterclockwise direction was randomly selected and traveled until the grid was possible to sample. If the entire grid could not be sampled, the next randomly selected grid was sampled. Trawls were pulled in 3-minute intervals at approximately 1 m/s into the wind; if wind was absent, trawls were pulled in a randomly selected direction (Coleman 2023). The trawl was manually pulled from the water and the contents were placed into a livewell where fishes would be sorted to minimize mortality. Fish were identified to species and total length was recorded. For species where large quantities were captured, total length of the first 50 individuals were recorded and the rest were then counted. Economically relevant species were weighed. Catch per unit effort (CPUE) was determined as the number of fish caught per unit of effort (trawl time in minutes; Hubert & Fabrizio, 2007).

Gillnets were used to sample close to shore (i.e., shoreline points). Gillnets were set out perpendicular to the shoreline in pairs of two, one small mesh (⅜", ½", ⅝") 30' x 6' net and one large mesh (3", 3-½", 4") 30' x 6' net, at approximately 50 meters from the shore. Five percent of the shoreline was sampled in each sector of the lake, resulting in six sets in sectors 1, 3, 4, and 6, and three sets in sectors 2 and 5. Gillnets soaked for approximately one hour and were then pulled. Data were collected as stated above for bottom trawl sampling. CPUE was determined as the number of fish caught per unit of effort, defined as gillnet soak time in minutes (Hubert & Fabrizio, 2007).

Boat electrofishing is expected to begin in sampling period 1b. No electrofishing was conducted in sampling period 1a, as the electrofishing boat has not been delivered.

Invertebrates

Invertebrates were collected using two methods in sampling period 1a. Blue crabs *Callinectes sapidus* were collected using a 0.42 x 0.61 x0.61 m crab trap consisting of 4 open funnels for entry. One crab trap was deployed at each randomly selected grid point (15 points per sector, **Table 3**) for a total of 90 crab traps (one trap was not recovered). In future sampling periods, only 10 crab traps will be deployed per sector per sampling period (a total of 60 crab traps). Traps were baited with frozen Gulf Menhaden *Brevoortia patronus* and dropped to the bottom of the lake. After approximately 48 hours, traps were pulled and crab carapace length, width, and height (depth) and crab weight were all recorded. Crab sex, gravid status of female crabs, and overall health condition data were also collected. Crabs were then released if not kept for the physiological team.

Dredges were used to collect benthic aquatic invertebrates such as insects, polychaetes, snails, and of particular interest, *Rangia* clams. Dredges utilized the standard Petite Ponar Grab with a volume of 8.2 liters. Dredges were deployed at five points across each sector with three replicates at each point for a total of 90 benthic samples. The number of dredges was reduced to 3 points per sector per sampling period in future sampling periods beyond 1a. Samples were processed in the field first, mixing with water and straining through a 12 inch 500µm sieve. Non-living clams were discarded while all remaining organisms and soil were placed into a jar with 95% ethanol. In the laboratory, each sample was stained with Rose Bengal to stain and assist with sorting of the invertebrates within the sample. All aquatic invertebrates were removed from the sample, identified to the lowest taxonomic level, and recorded. All *Rangia* clams were also measured by total weight (g), wet weight of their internal tissue (g), shell weight (g), and sexed if able.

We also targeted clams by performing a series of benthic dredges at the areas where we assume they are most abundant—the mouths of the rivers that feed into Lake Maurepas. One grid centroid was randomly selected from the 10 points closest to each of the 4 river mouths (Amite, Blind, Tickfaw, and Pass Manchac; **Fig. 3**). At each point, three dredge samples were taken of the benthic environment using the standard Petite Ponar Grab and any live clams were placed into a jar with 95% ethanol. No other organic or inorganic materials were kept from these samples. This sampling methodology was only done during sampling period 1b and is unlikely to be continued due to lack of results.

Trawling for shrimp is expected to begin in sampling period 1b. No shrimp trawls were pulled in sampling period 1a. A 4.88 m otter trawl will be pulled at three randomly selected points per sector (**Table 3**). Like bottom trawls, shrimp trawling will begin at the randomly selected grid centroid and the trawl will be manually deployed and pulled into the wind at approximately 1 m/s for 3 minutes. If wind is absent, the trawl will be pulled in a randomly selected direction. After 3 minutes the trawl will be manually pulled from the water and any shrimp caught will be stored on ice and data will be recorded in the lab. Lab work includes identifying the specimen to species and taking the weight (g), total length (mm), carapace length(mm), and sex (if possible; Beukema, 1992; DeLancey et al., 2008; Mace & Rozas, 2015).

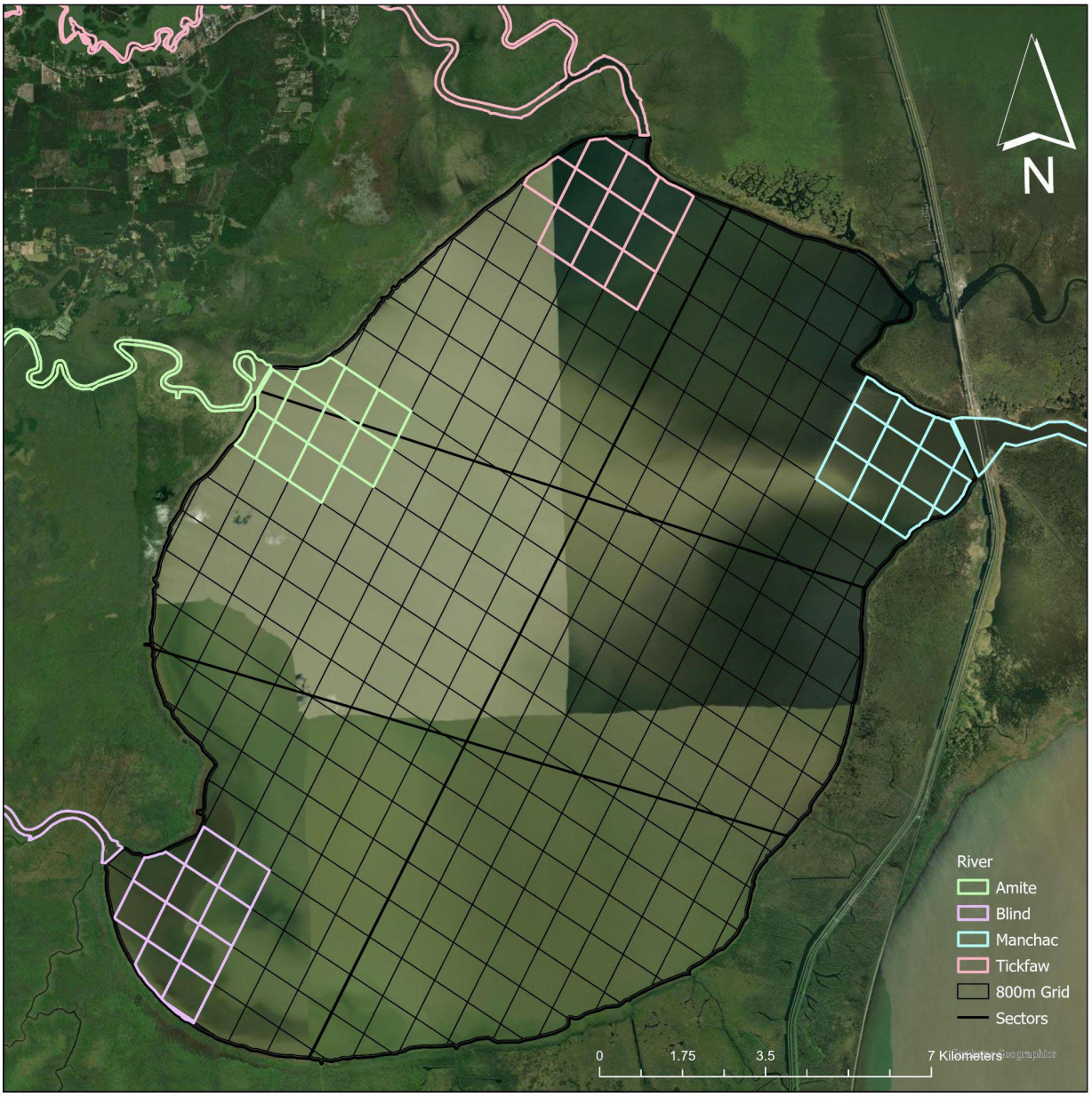

Figure 3. Sampling points used to randomly select sites for dredge sampling targeting clams at the mouths of the Amite, Blind, Tickfaw, and Pass Manchac rivers.

Buoys

Four YSI buoys were ordered July 2023 and at the time, we were informed that there was a 3-4 month build time. Unfortunately, due to supply chain issues there has been an additional delay in the delivery of these buoys. In the meantime, we have submitted the appropriate permit applications and have been granted approval to deploy the buoys from the Louisiana Department of Natural Resources (Coastal Use Permit) and US Army Corps of Engineers. Once deployed in early-2024, the buoy monitoring software will be integrated with

our Lake Maurepas Project Monitoring website https://www.southeastern.edu/acad_research/depts/biol/programs/lakemaurepas/index.html to publicly display the abiotic parameters (in real time) for each of the four buoys. These parameters include water temperature, dissolved oxygen, pH, carbon dioxide, and turbidity. One buoy will be fit with a weather station to monitor atmospheric conditions.

Preliminary Results/Progress to Date

Fishes

Data on fish biodiversity and assemblage structure of Lake Maurepas was collected using inshore gillnets and bottom trawls. Sampling objectives vary based on gear type (**Table 3**). Below are tables, figures, and maps depicting trends and summaries of the data collected during the current sampling period.

The greatest number of fishes caught were in sector 4 (2,055) and the least number of fishes caught were in sector 1 (158; **Fig. 4, Table 4**). The average number of fish caught per sampling effort (number of nets) during sampling period 1a was 75. The maximum number of species caught were in sectors 1 and 5 (12; **Fig. 4, Table 4**). Diversity was very similar between sectors. The least number of species caught were in sectors 2 and 3 (6; **Fig. 4, Table 4**). All sampling objectives for this sampling period (**Table 3**) were met, except for electrofishing, which was not done in Sampling Period 1a.

Table 4. Frequency summary of sampling events and fish species and total number of animals collected per sector for current sampling period.

Sector	Bottom Trawl	Inshore Gillnet	Electrofishing	Number of Species	Number of Fish
1	3	6	0	11	158
2	3	3	0	6	1062
3	3	6	0	6	820
4	3	6	0	7	2055
5	3	3	0	8	892
6	3	6	0	12	874

Figure 4. Number of fish (top) and number of species of fish (bottom) caught in each sector during each sampling period.

The total number of fish caught and the number of fish species collected (fish diversity) varied based on sampling method. The bottom trawl caught the greatest number of fish (5,723) and the inshore gillnet captured the least (136 fish; **Fig. 5, Table 5**). However, the inshore gillnet and bottom trawl caught a similar fish diversity (9 and 8, respectively). Total net time for this sampling period was 1,679 minutes (55.8 minutes of bottom trawl, 1,623 minutes of inshore gillnets; **Table 5**).

Table 5. Frequency summary for fish species sampled for current sampling period.

Species	Bottom Trawl	Inshore Gillnet	Electrofishing	Number of Fish
Alligator Gar	0	1	0	1
Bay Anchovy	4681	0	0	4681
Blue Catfish	334	36	0	370
Bluegill	13	0	0	13
Channel Catfish	65	14	0	79
Clown Goby	572	0	0	572
Hogchoker	54	0	0	54
Leather Jacket	0	1	0	1
Longnose Gar	0	1	0	1
Naked Goby	3	0	0	3
Redspotted Sunfish	0	1	0	1
Smallmouth Buffalo	0	1	0	1
Threadfin Shad	0	79	0	79
Western Mosquitofish	1	0	0	1
Yellow Bass	0	2	0	2
Totals	**5723**	**136**	**0**	**5859**
Net Time (min)	56	1623	0	
CPUE Number of Fish	102.93981	0.08884200	0	
CPUE Number of Species	1.37963	0.03522564	0	

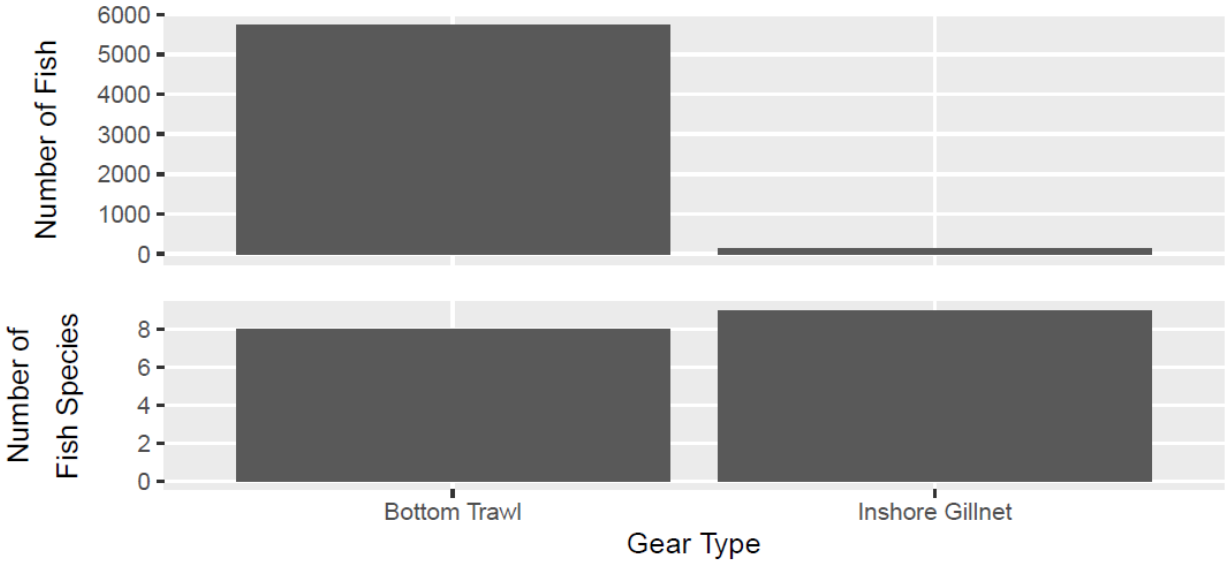

Figure 5. Number of fish (top) and number of species of fish (bottom) caught with each gear type.

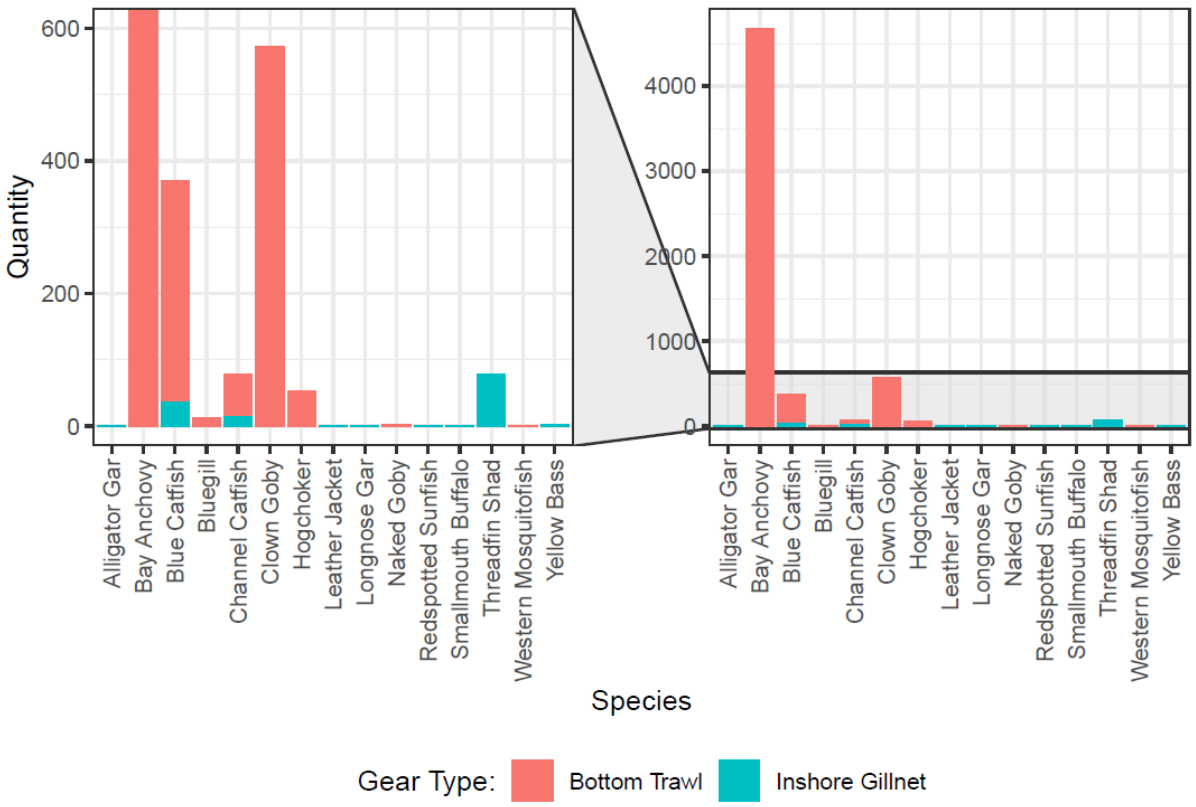

Figure 6. Number of fish of each species caught by gear type. The left figure (a) is a magnified portion of the right figure (b) to display quantities of rarer species (<600 individuals) without the influence of Bay Anchovy.

The most fish species collected were the Bay Anchovy (4,681), followed by the Clown Goby *Microgobius gulosus* (572) and Blue Catfish (370; **Fig. 6**). The fishes caught with the least frequency during this sampling period were Western Mosquitofish *Gambusia affinis* (1), Yellow Bass *Morone mississippiensis* (2), and Naked Goby *Gobiosoma bosc* (3). In terms of catfish, we caught two of the three expected species (Blue *Ictalurus furcatus* and Channel *Ictalurus punctatus*), with Blue Catfish being the most common (Blue Catfish: 370, Channel Catfish: 79). Also of note, in a single small-mesh inshore gillnet, several Threadfin Shad *Dorosoma petenense* (79) were caught. Blue Catfish and Channel Catfish were the only species caught by both bottom trawls and inshore gillnets.

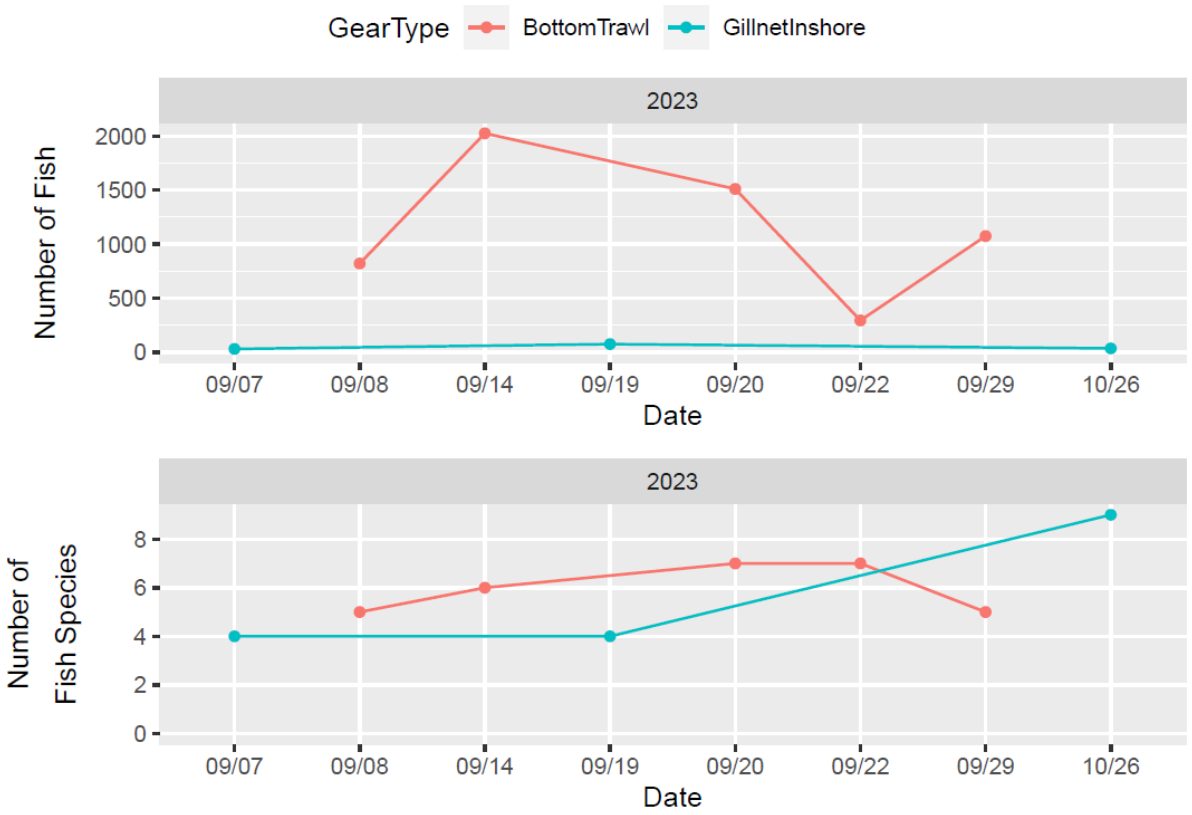

Figure 7. Number of fish caught (top) and number of fish species caught (bottom) with each gear type over time (year and date).

The number of fish caught over time qualitatively varied between gear types and throughout the two-month sampling period; however, this variation throughout time is likely due to the variation between sectors as opposed to time/date, and the fact this is only two months of sampling. We caught the greatest number of fish (2,026) on 14 September 2023 with a bottom trawl (**Fig. 7**). We caught the least number of fish (29) on 07 September 2023 with an inshore gillnet.

There is slight variation in fish length within a species based on the sector (**Fig. 8**). Blue Catfish had a couple individuals that were much larger than the median (**Fig. 8**). Overall, fish length within species appears to be uniform throughout the lake.

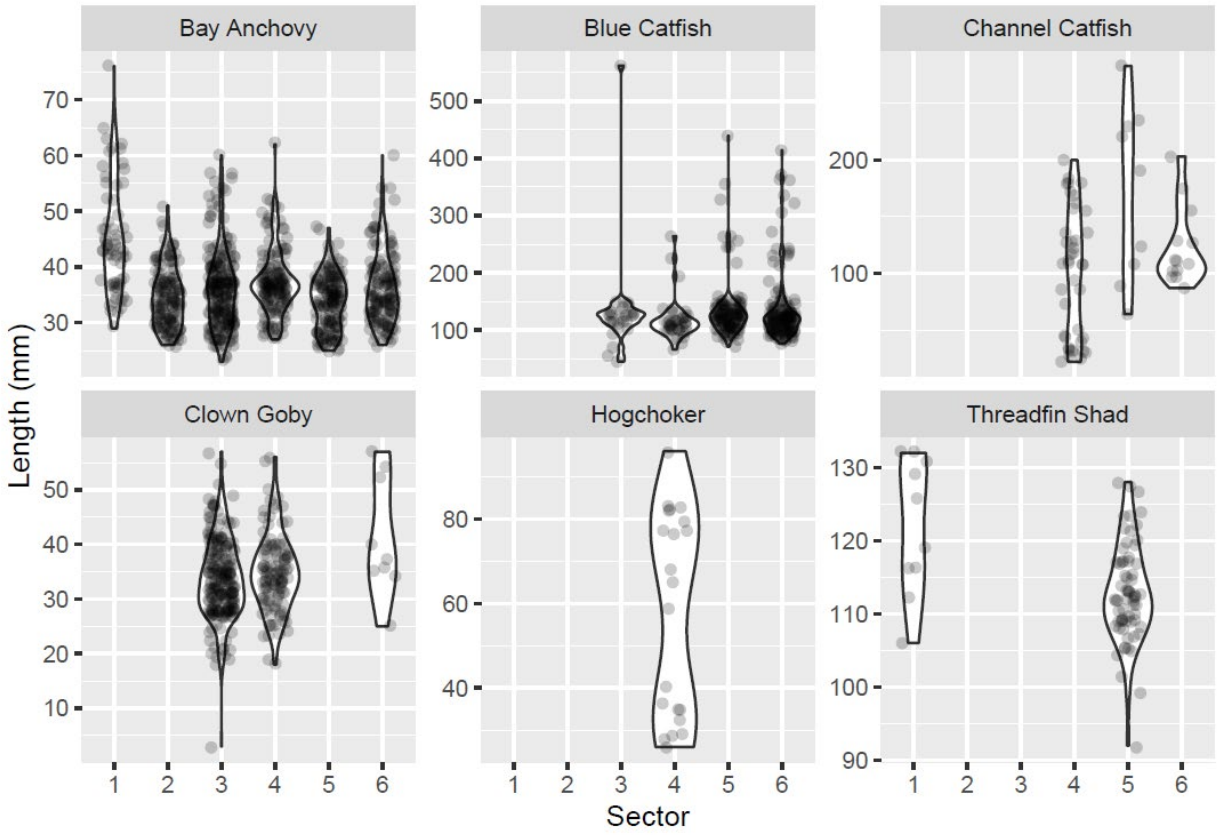

Figure 8. Fish length in each sector for species with >10 individuals measured. Note the different scales for each species.

Blue Catfish and Channel Catfish have similar observed lengths and weights on Lake Maurepas. There have been more Blue Catfish caught compared to Channel Catfish. We have caught larger sized (length and weight) Blue Catfish compared to Channel Catfish, which can be seen in **Figure 9**. The largest Blue Catfish caught was 562mm in total length and 1,580g in weight (**Fig. 9**). The largest Channel Catfish total length was 283mm and weighed 160g (**Fig. 9**). The average Blue Catfish length and weight was 132mm and 98g, respectively, and the average Channel Catfish length and weight was 115mm and 47g, respectively.

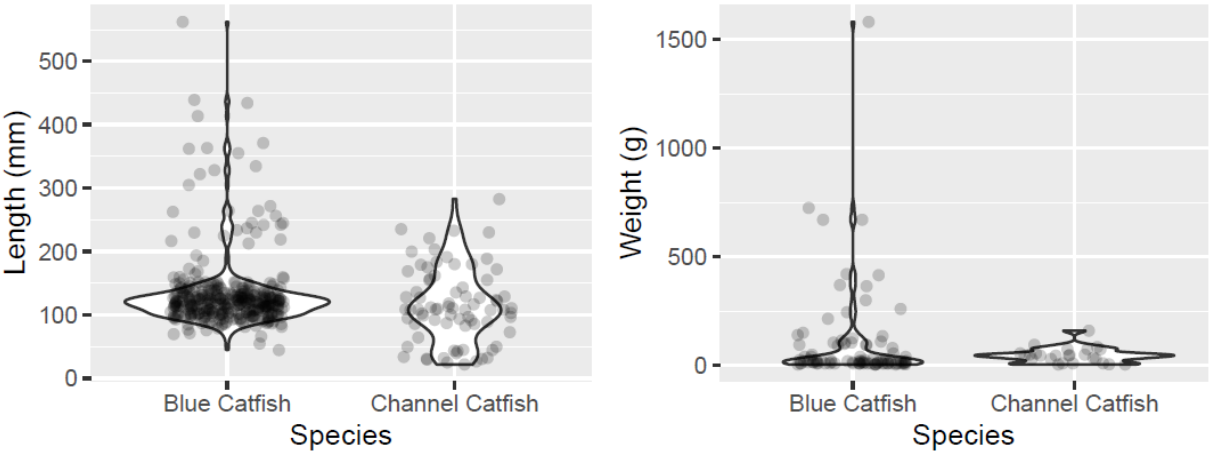

Figure 9. Lengths (left) and weights (right) of Blue Catfish and Channel Catfish.

Invertebrates

We have identified 9 species which make up most of the benthic invertebrate community, including 3 chironomids, 1 oligochaete, 1 *Gammarus* amphipod, 1 hirudinea, 2 gastropods, and 2 bivalves, including the focual species *Rangia cuneata*. Trawling for shrimp is expected to begin in sampling period 1b. Benthic samples are still being processed, but initial analysis of data suggests that *Rangia cuneata* are present in all sectors except Sector 6, with most being found in Sectors 1 and 2. No shrimp trawls were pulled in sampling period 1a.

Sampling objectives for blue crabs are to sample 10 randomly selected points per sector per sampling period (**Table 3**). Please note in sampling period 1a, the objective was to deploy crab traps at 15 randomly selected points per sector per sampling period which was dropped down to 10 for subsequent sampling periods. Below are tables, figures, and maps depicting trends and summaries of the data collected since the inception of the monitoring program.

The least and greatest number of crabs caught were in sectors 5 (28 crabs) and 3 (86 crabs), respectively (**Figs. 10-11**; **Table 10**). The average number of crabs caught per sector during sampling period 1a was 59. The ratio of male to female crabs caught during this sampling period was 336:11 (**Table 10**).

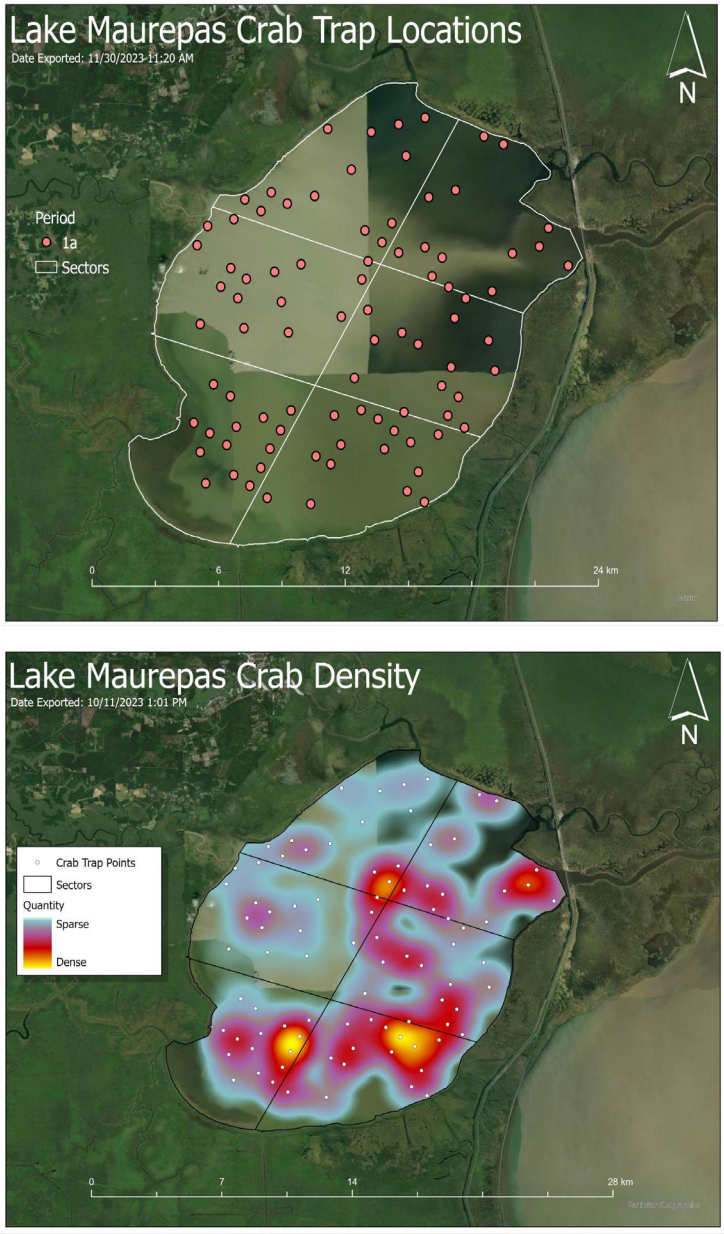

Figure 10. All crab trap locations on Lake Maurepas for each sampling period (left) and crab density based on crab trap data (right) from sampling Period 1a.

The total number of crabs caught in this sampling period did not change much (range: 92–147; **Fig. 11**).

Table 10. Frequency summary for crab sampling per sector for sampling period 1a.

Sector	Traps	Crabs	Males	Females
1	14	68	64	4
2	15	64	60	4
3	15	86	85	0
4	15	61	58	2
5	15	28	27	0
6	15	44	42	1

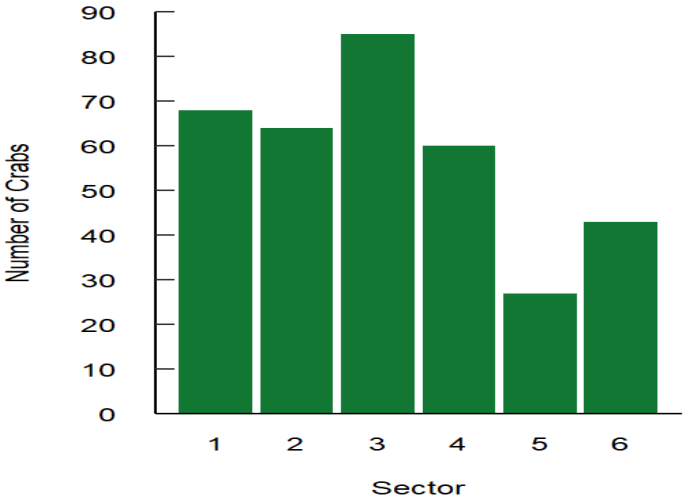

Figure 11. Number of crabs caught in each sector during sampling period 1a.

Observed crab carapace widths did not differ among sectors or between sexes; however, the sample size of female crabs is limited (n=11) (**Fig. 12**). Measured carapace widths ranged from 90mm to 194mm (**Fig. 12**). Observed crab weights varied more than carapace width, but were not different among sectors or between sexes. Blue crab weights ranged from 30g to 330g (**Fig. 12**).

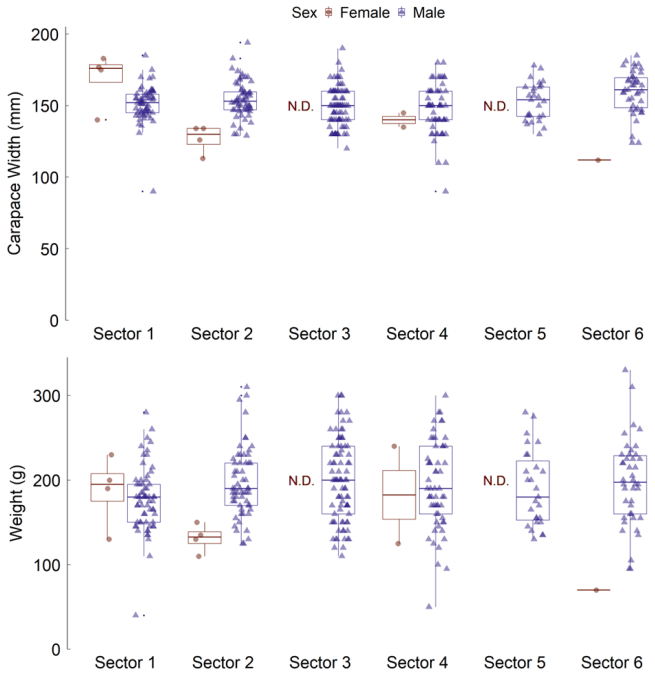

Figure 12. Blue crab carapace width (top) and weight (bottom) of both female (red) and male (blue) crabs in each sector. There were no female crabs collected in sectors 3 and 5, as indicated by N.D. (i.e., No Data).

Buoys

As described in the methods section of this report, there has been a significant delay in the deployment of the four YSI buoys that were ordered July 2023. The components of the buoy arrived in late-December and assembly and deployment is scheduled for late-January 2024. In the meantime, we have submitted the appropriate permit applications and have been granted approval to deploy the buoys from the Louisiana Department of Natural Resources (Coastal Use Permit) and US Army Corps of Engineers. The location of the buoys and their respective identifying names (Amite, Tickfaw, Maurepas, and Blind Buoys) are depicted in the map below (**Fig. 13**). Once deployed, the buoy monitoring software will be integrated with our Lake Maurepas Project Monitoring website to publicly display the abiotic parameters (in real time) for each of the four buoys.

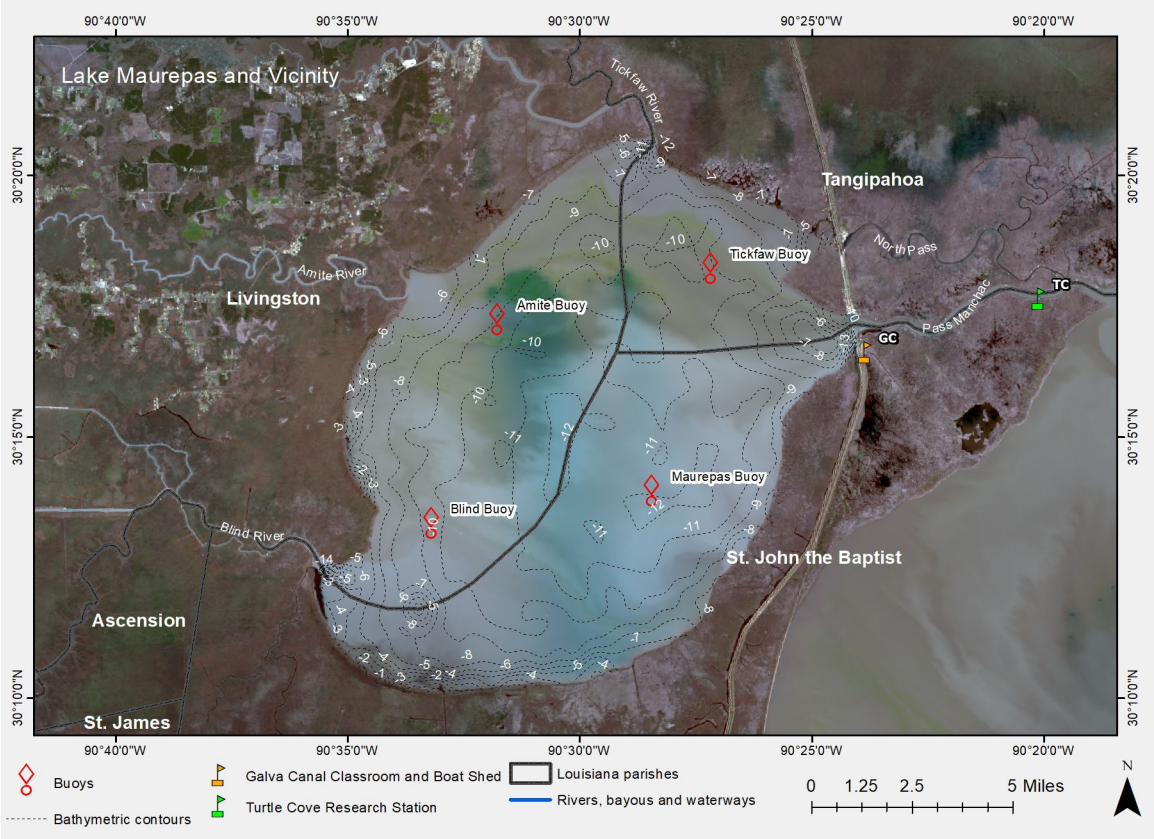

Figure 13. Proposed locations where the YSI monitoring buoys will be deployed in 2024.

Future Direction

We plan to continue to monitor the aquatic biodiversity of Lake Maurepas using the methods described above, with a few key changes to sampling design and protocol, and with the addition of several other sampling gear types and methodologies.

Fishes

Following the new arrival of our specialized Smith-Root electrofishing boat (November 2023) we aim to implement an electrofishing protocol along the shoreline of Lake Maurepas in Sampling Periods 1b and beyond. The exact sampling design is currently undecided and will require some trial and error once the boat is broken in based on the manual's specifications. We hope to start that process during sampling period 1b and be in full force electrofishing sampling during sampling period 2a.

Invertebrates

Our otter trawl has been delivered, so shrimp sampling will begin in early January using the methods described above. Based on bycatch we have collected using other sampling methods, we could identify White Shrimp *Litopenaeus setiferus*, Brown Shrimp *Farfantepenaeus aztecus*, Ohio Shrimp *Macrobrachium ohione*, Marsh Grass Shrimp *Palaemonetes vulgaris*, and Pink Shrimp *Farfantepenaeus duorarum*. In addition, we are

reducing the number of crab traps and benthic dredges in our sampling periods. For the former, we initially deployed extra crab traps as assurance to reach the target goal of ten crabs per sector for the physiological team, and we feel confident that we will still be able to reach our target even with the reduction of traps. This reduction also allows us to deploy traps in three sectors at once. For the latter, initial analysis of benthic samples show that invertebrate communities are relatively similar within each sector.

YSI Buoys

As stated above in the methods and results section of this report, the buoys were received in mid-December (**Fig. 14**). Tentatively, the buoys will be assembled and deployed in late-January 2024.

Figure 14. Unassembled YSI buoys currently housed in Southeastern's Receiving warehouse. The buoy components arrived in late December and will be assembled and installed in January 2024.

eDNA data collection/analyses

Environmental DNA sample collection was initiated in December of 2023. Twenty-four water samples will be taken from around the lake, each quarter from 12 inshore sites and 12 offshore sites that will be randomized for each sampling event (**Fig. 15**). Soil samples for invertebrate eDNA analysis will also be taken during each sampling event (6 inshore, and 6 offshore).

Figure 15: Lake Maurepas depicting the shoreline sampling points (green dots) around the perimeter of the lake and the open water sites (blue dots).

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II. Aquatic Physiology Monitoring

Chris Murray, PhD
Southeastern Louisiana University, Dept. of Biological Sciences
Hammond, LA 70402
christopher.murray@selu.edu

Overview

This report documents effort to monitor organismal physiological health as it pertains to the conservation, as well as human consumption, of organisms in Lake Maurepas during the 2023 procurement and field season. Physiological stress, endocrine disruption markers, heavy metal markers, and organismal gas gradients in blue crab, bullfrogs, catfish, and alligators are monitored twice annually in each of the six seismic testing quadrats in the lake. These organisms serve as model organisms for most other species present and chosen metrics elucidate population risk at a detail finer and more predictive than presence/absence data. All data will be compared spatially and across time during the duration of monitoring.

Data include:

- Physiological stress using contemporary leukocyte profiling (frog, catfish, and alligator) and hemolymph density (crab).

- Endocrine disruptor exposure quantified using sex steroid concentration monitoring and gonadal histopathology.

- Fecundity as a proxy for reproductive energy allocation (crab).

- Heavy metal exposure using histopathological metrics quantifying liver hyperplasia (frog, catfish, and alligator), hepato-pancreas hyperplasia and gill hyperplasia (crab).

- Blood or hemolymph pH monitored to quantify osmotic gas gradient stress.

- Alligator population viability using nest counts, egg viability and hatching success annually.

- Lastly, the Maurepas alligator population will be monitored demographically (population size, growth rate, survivorship) via mark-recapture analysis.

Project Objectives

Specific data objectives being collected are:

Crabs

- Hemolymph density
- Fecundity
- Sex hormone concentration
- Hepatopancreas histology
- Gill histology
- Hemolymph pH

Frogs

- Testosterone concentration
- Estradiol concentration
- Blood pH
- Liver histology
- Gonad histology
- Hepatosomatic Index
- Gonadosomatic Index
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Catfish

- Leukocyte profile
- Testosterone concentration
- Estradiol concentration
- Blood pH
- Liver histology
- Gonad histology
- Hepatosomatic Index
- Gonadosomatic Index

Alligator

- Leukocyte profile
- Testosterone concentration
- Estradiol concentration
- Blood pH
- Nest survivorship
- Population demography

The 2023 sampling protocol (sample period two; August to February) utilized a stratified random sampling design to capture alligators, catfish and crabs from each of the six sectors. Frogs targeted (*Lithobates sphenocelphalus*) are winter breeders and will be sampled in February 2024.

Methods/Study Design:

Alligators were captured opportunistically by hand or snare in each sector. Whole blood was drawn from the spinal vein using a heparinized 23 gauge needle and 3CC syringe within five minutes of capture (Murray et al. 2013). Each animal was sexed, total length and snout to vent length measured, and individually marked via caudal scute removal (Murray et al. 2013). All animals were released at the site of capture. Whole blood was smeared one cell layer thick on a microscope slide, fixed in methanol and stained using Geimsa Wright stain (VWR). For leukocyte profiles, the first 100 leukocytes were counted and the ratio of heterophils to lymphocytes calculated (Murray et al. 2013). Remaining whole blood used to quantify blood pH using an Orion Star A211 benchtop pH meter (Thermo Scientific) and was centrifuged and plasma supernatant removed for steroid hormone (T and E_2) concentration. Plasma samples were extracted for T using a methanol extraction protocol (Han and Liu. 2019) while E_2 samples were extracted using a 3:2 vol: vol ethyl acetate to hexane protocol (Murray et al. 2017). Samples are currently being analyzed for steroid hormone concentrations using low detection ELISA kits. Nest survivorship and population demography will be assessed when sample size permits. H: L thresholds for allostatic load (stress) were set at 1:1 H: L, whereby above this ratio indicates a chronic stress response and below this ratio indicates a lack there of (Lance et al. 2010).

Catfish were captured using trawl sampling in each sector in collaboration with the aquatics team. Whole blood was drawn from the caudal vein using a heparinized 23 gauge needle and 3CC syringe within five minutes of capture (Murray et al. 2013). Each animal was measured for total body mass (g), individually marked via Floy tag (Floy Tag Inc. Seattle, Washington, USA) and collected. All catfish were euthanized using a 300mg/L concentration of MS-222 (AVMA 2020), dissected for sexing and hepatosomatic and gonadosomatic indices. Leukocyte profiles, blood pH and plasma steroid samples were prepared and quantified as described above for alligators. Samples are currently being analyzed for steroid hormone concentrations using low detection ELISA kits. Liver and gonad samples were fixed in neutral buffered formalin and are being histologically processed to assess histopathology and gonadal malformations.

Crabs were captured via passive trapping in collaboration with the aquatics team. Each crab sampled was marked with a Floy tag and hemolymph drawn from the body cavity using a heparinized 27-gauge needle and 1CC syringe. Each crab was collected and brought back to the laboratory. Crabs were euthanized using ganglia puncture followed by freezing (Hatfield Science Center SOP) and dissected for gill and hepato-pancreas samples that were fixed in neutral buffered formalin for histological analysis. Hemolymph pH was quantified using an Orion Star A211 benchtop pH meter (Thermo Scientific). Hemolymph density was calculated by centrifuging whole hemolymph at 5,000 g for 5 minutes and cellular volume compared to remaining substrate volume. No females collected contained eggs so fecundity was zero for all present samples. Crustacean female sex hormone (CSFH) will be extracted and quantified from hemolymph samples using HPLC MS/MS techniques.

In summary, this report includes data for crab sampling and female fecundity; catfish leukocyte profiles, blood pH, gonadosomatic index (GSI) and hepatosomatic index (HSI); and alligator leukocyte profiles and blood pH. Simple summary statistics (averages with standard deviations and range) are reported here because no temporal and spatial comparisons are warranted after this first sampling event. These data serve as baseline for future comparison.

Results/Progress to Date

Alligators

Twenty-four alligators were captured between 8 November, 2022 and 29 September, 2023 across three sectors (1, 3 and 6; **Fig. 1**). Sex ratio was 11: 13 female to male that ranged between 15.1 and 102 cm snout to vent length and between 31 and 194 cm total length. Mean heterophil: lymphocyte ratio was 0.94 ± 0.4 (0.3 to 1.8) and mean blood pH was 7.45 ± 0.35 (7.05 to 7.71; **Table 1**).

Figure 1. Map of alligator sampling (n =24) from November 2022 through September 2023 from sectors 1, 3 and 6 in Lake Maurepas.

Table 1: Alligator sample sizes, sex ratios, mean heterophil to leukocyte ratios and mean whole blood pH by sector.

Sector	(n)	Mean H:L	Mean pH	Sex Ratio (F:M)
1	9	0.94	NA	4:5
3	11	0.93	7.05	5:6
6	3	0.98	7.65	2:1

Catfish

Thirty-nine catfish (*Ictalurus furcatus;* 27 and *Ictalurus punctatus;* 12) were captured between 27 October 2022 and 22 September 2023 across five sectors (1, 2, 3, 5 and 6; **Fig. 2**). The *Ictalurus furcatus* sex ratio was 10: 17 female to male that ranged between 10.45 and 1,968 g body weight. The *Ictalurus punctatus* sex ratio was 7: 5 female to male that ranged between 50 and 175 g body weight. Mean heterophil: lymphocyte ratio was 1.01 ± 0.9 (0.13 to 3.09) for *Ictalurus furcatus*. *Ictalurus punctatus* did not have a blood smear or whole blood pH sample size acceptable for summary because of small body sizes. Mean blood pH was 6.93 ± 0.63 (6.14 to 7.56; **Table 2**) for *Ictalurus furcatus*. Mean somatic indices for *I. furcatus* were 0.014 ± 0.011 (0.008 to 0.06) and 0.007 ± 0.01 (0.0007 to 0.06) for HSI and GSI, respectively. Mean somatic indices for *I. punctatus* were 0.016 ± 0.002 (0.013 to 0.02) and 0.003 for HSI and GSI, respectively.

Figure 2. Map of catfish sampling (n =39) from October 2022 through September 2023 from sectors 1, 2, 3, 5 and 6 in Lake Maurepas.

Table 2. Catfish sample sizes, sex ratios, mean HSI, mean GSI, mean heterophil to leukocyte ratios and mean whole blood pH by sector and species. *indicates metric of concern.

Sector	Species (n)	Mean H: L	Mean pH	HSI	GSI	Sex Ratio(F:M)
1	*I. furcatus* (3)	NA	NA	0.01	0.004	1:2
2	*I. furcatus* (2)	0.62	NA	0.01	0.03	1:1
	I. punctatus (10)	NA	NA	0.017	NA	6:4
3	*I. furcatus* (3)	0.54	NA	0.037	0.019	2:1
5	*I. furcatus* (9)	0.70	7.13	0.01	0.003	2:7
	I. punctatus (1)	0.29	7.3	0.01	0.003	0:1
6	*I. furcatus* (10)	1.94*	6.14	0.01	0.002	3:7

Crabs

Thirty-four Blue crabs (*Callinectes sapidus*) were captured between 6 December 2023 and 13 December 2023 across all six sectors (Figure 3). The sex ratio was 5: 29 female to male. Hemolymph density, pH and crab sex hormone concentration are currently under analysis. No females possessed eggs.

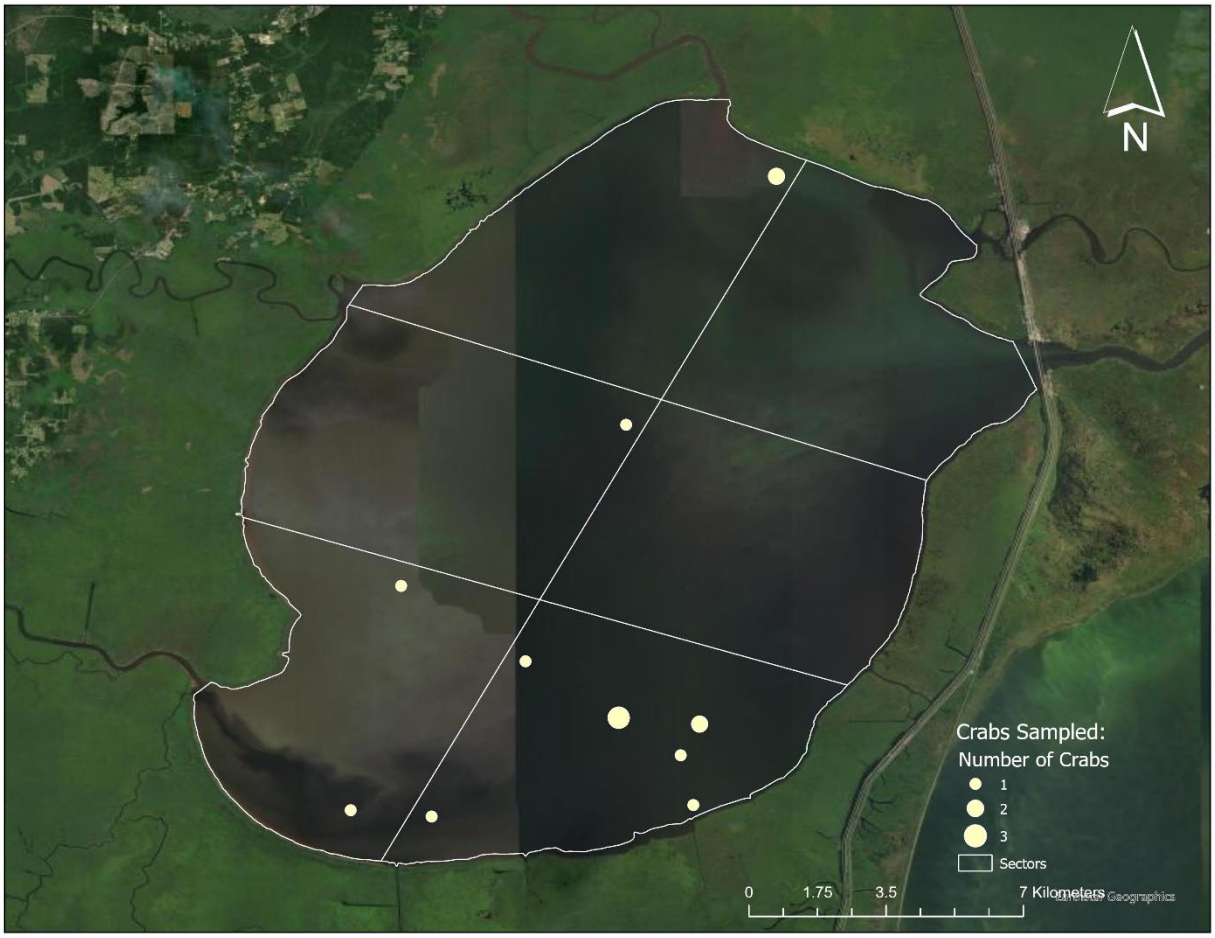

Figure 3. Map of crab sampling from 13 December 2023 as an example of stratified random design.

Table 2. Blue Crab sample sizes and sex ratios. Hemolymph density, pH and crab sex hormone concentration are currently under analysis.

Sector	N	Sex Ratio (F: M)	Date
1	10	1:9	12/13/23
2	10	4:6	12/13/23
3	7	0:7	12/6/23
4	4	0:4	12/6/23
5	1	0:1	12/6/23
6	3	0:3	12/13/23

Interpretation and Data Summary (to date):

Alligator, catfish and crab sampling protocols have proven effective during the first portion of the first sampling period. Collaborations, design and targeted specimen acquisition have been fleshed out. Alligator data reveal baseline H: L ratios that are consistent with the lack of an allostatic load (Lance et al. 2010). Data suggest no current chronic stressors of any note, nor any deviations from normal blood pH (Affonso et al. 2002) with minimal sampling to date. Catfish data reveal baseline data aside from sector six N: L ratios in *Ictalurus furcatus* and variation in GSI. Sector six presents no obvious anthropogenic threats (i.e. high fishing pressure, effluent, or impervious surface run off), so elevated N: L ratios are surprising and must be considered in further sampling. Further, GSI variation among sectors varied by orders of magnitude with high values indicating increased reproductive effort or gonadal hypertrophy not biased by sex ratio in sectors two and less so three. Sector three also exhibited the highest HSI.

Future Directions (Jan 2024-Dec 2024):

- Finish catfish and crab sampling for sampling period 1
- Sample frogs for sampling period 1
- Monitor sector 6 catfish N: L ratios in future sampling
- Monitor sectors 2 and 3 GSI variation relative to others sectors
- Complete all laboratory analyses for sampling period 1
- Finish acquiring and preparing ordered equipment
- Elevate research associate position to postdoctoral position to accommodate field and laboratory workload
- Begin sampling period 1, 2024

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III. Wetland Monitoring

Gary Shaffer, PhD
Southeastern Louisiana University, Dept. of Biological Sciences
Hammond, LA 70402
shafe@selu.edu

Overview

Working with Air Products personnel, we have chosen the locations of all ten of our permanent monitoring sites (**Fig. 1**), each of which will contain two 625m^2 permanent stations. For our yearly planting of baldcypress (*Taxodium distichum*) and water tupelo (*Nyssa aquatica*) seedlings, we will begin at the northern tip of West Jones Island (**Fig. 2**). We have grown well over 2,000 seedlings (our yearly planting goal) to a size of about 3' tall and planting is scheduled to begin in December 2023. We will begin installing our permanent stations in January 2024. Thus far, measurements of surface elevation, using our surface elevations tables (SETs) and marker horizons, has exceeded subsidence by about 19 cm, or over 1 cm per year of net elevation gain.

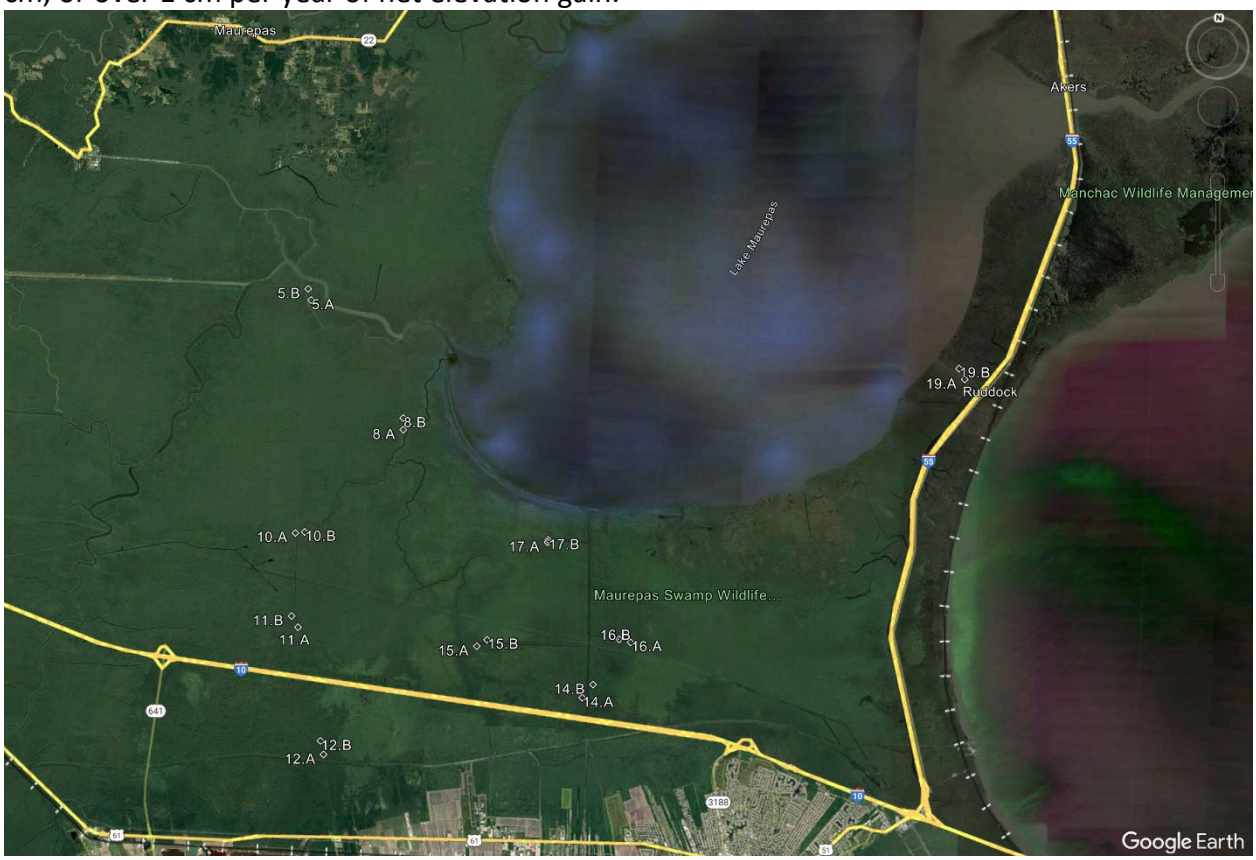

Figure 1. Locations of sites and stations in the Maurepas swamp.

Figure 2. Area encircled in white is the 10.2 acres on West Jones Island that will be planted with 2,000 baldcypress and water tupelo seedlings beginning in December 2023.

Project Objectives

Our first objective was to locate fourteen surface elevation tables (SETs, **Fig. 3**) established in the years 2000 and 2006 and measure elevation change over that period. We are also providing new marker horizons to all sites where SETs are found to monitor sediment accretion. Our second objective was to establish ten permanent sites with replicate stations for monitoring tree productivity. Our third objective was to plant and protect 2,000 baldcypress and water tupelo seedlings on West Jones Island. Our final objective is to update our 2011 habitat-state map (Shaffer et al. 2016, **Fig. 4**) to determine the amount of each type of habitat (degraded, relict, sustainable) that now exists.

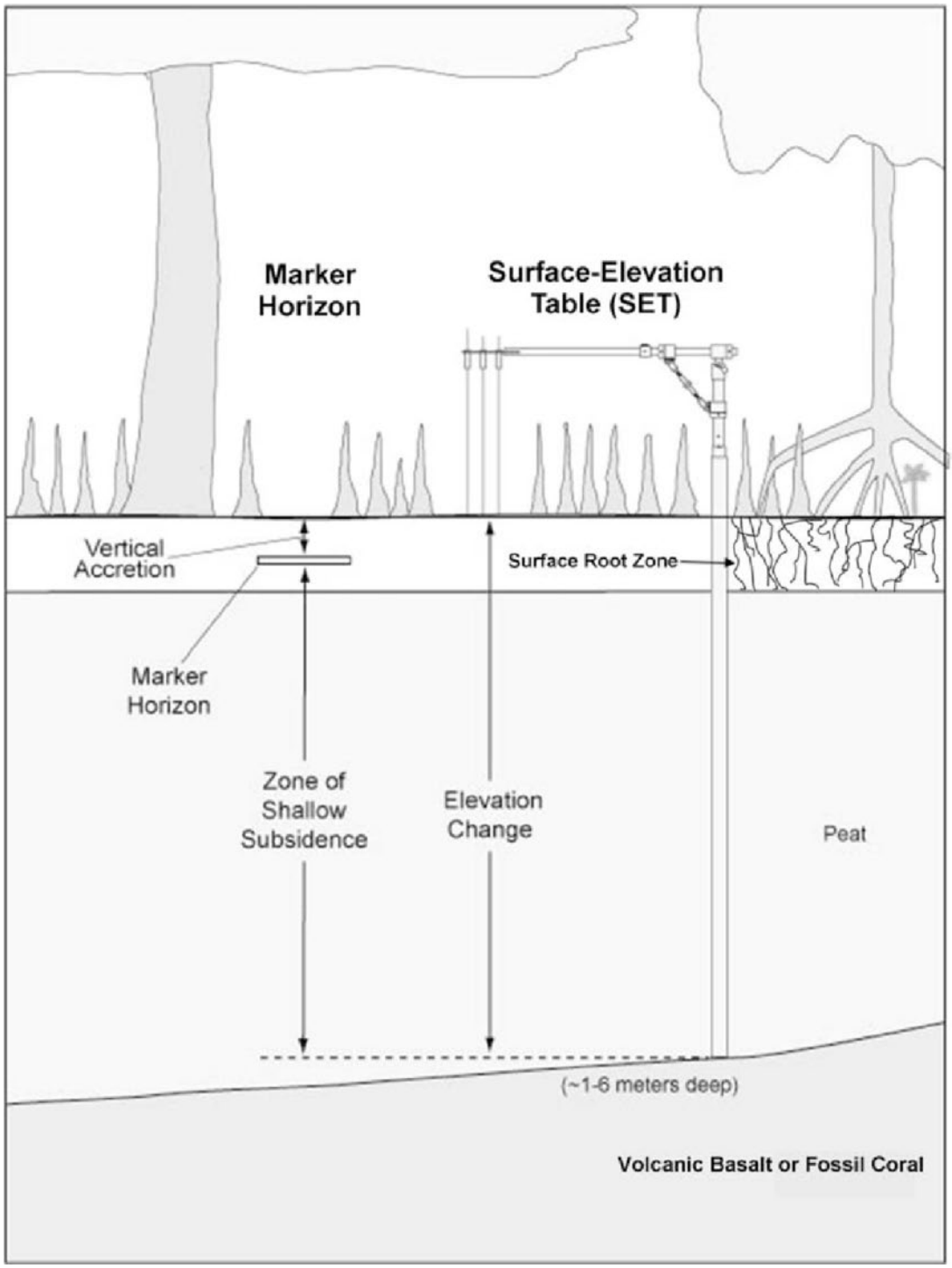

Figure 3. Surface elevation table and marker horizon (*redrawn from Krauss et al. 2010*).

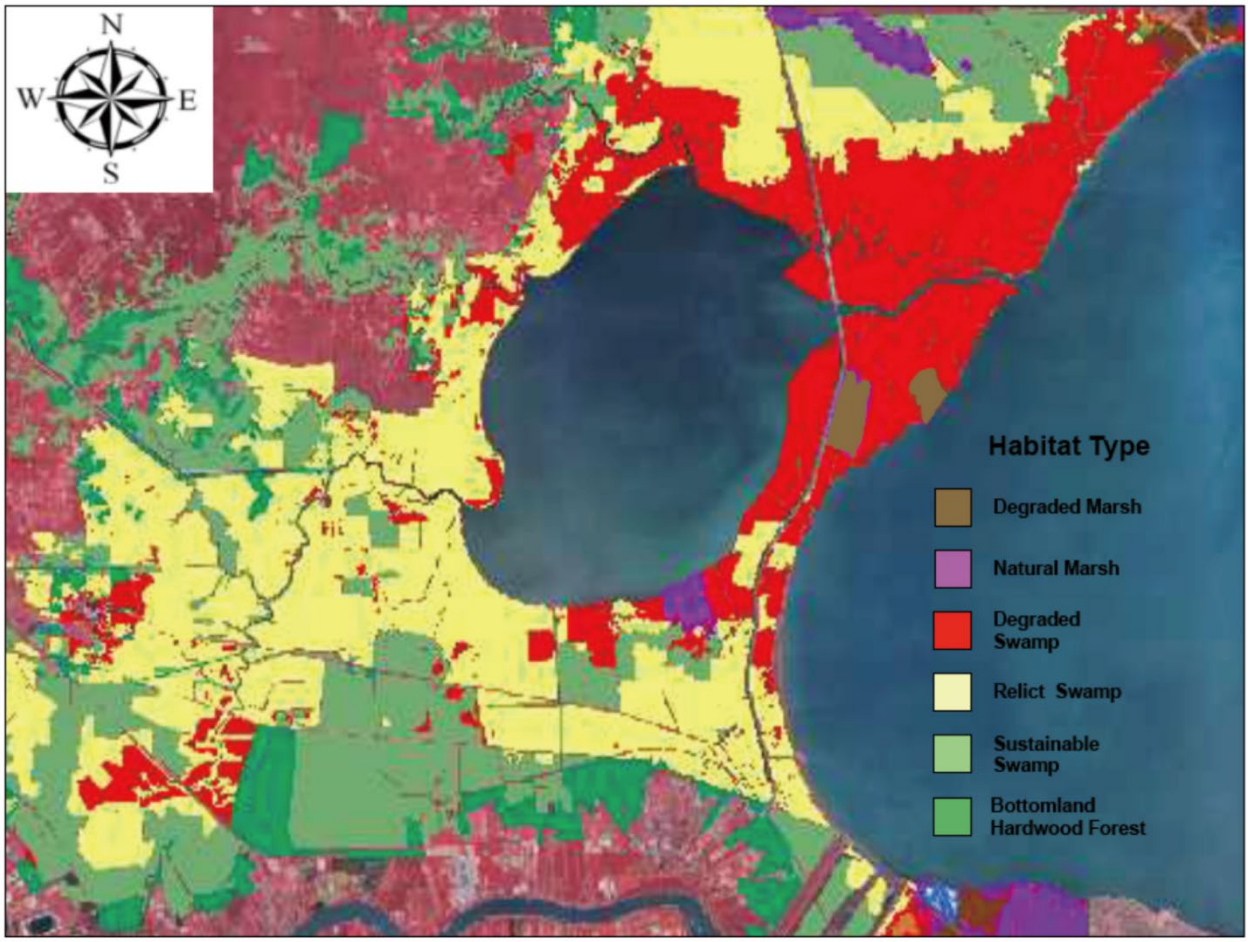

Figure 4. Amount of degraded, relict, and sustainable swamp habitat that existed in 2011 (*redrawn from Shaffer et al. 2016*).

Methods

Locating the surface elevation table pipes has become very difficult as the benches that are used to find the sites have largely decomposed. Moreover, accretion has been substantial enough that most of the pipes are underground. Thus far, we have located four SET pipes and will attempt to locate ten more beginning in January, 2024. Four each SET, nine measurements are taken at each of the four cardinal directions. Four marker horizons are placed halfway between each cardinal direction and are flagged for future accretion measurements.

Eight permanent stations will be established along Reserve Relief Canal where the Air Products pipeline will be placed. Eight others will be located along Hope Canal, Tent Bayou and Dutch Bayou. Four additional stations will be placed on Alligator Island and Ruddock Canal (**Fig. 1**). These stations are a subset from a study implemented in 2000 (Shaffer et al. 2009, 2016). Each station will have 625 m^2 of aerial coverage and will contain four 16 m^2 plots for measurement of herbaceous and canopy cover. Diameters will be measured for all trees greater than 4 cm in width. In addition, four 0.25 m^2 litterfall traps will be randomly located in each station and leaf litter will be collected at least every 2 months. Records of survival and recruitment are also maintained.

Over the past year we have grown several thousand baldcypress and water tupelo seedlings. These seedlings are started from seeds obtained from a local seed source.

Results

Our surface elevation and accretion measurements began in June and will continue over at least the next year. The surface elevation tables for this effort were installed in 2000 and 2006 and until now, no measurements have been completed for 17 years. All of the original marker horizons (used for measuring accretion) migrated through the very weak swamp soils, so we are inventing new measurement tools; thus far, plastic petri dishes with holes for flagging are working the best. While we are making the new set of measurements, we will also be obtaining new diameter and canopy cover measurements as well as obtaining new survivorship and recruitment data. Herbaceous vegetation cover, by species, will also be monitored. The SET measurements taken thus far demonstrate that accretion exceeds subsidence by a substantial margin (**Fig. 5A-D**). If this pattern holds up for the rest of the SETs, the Maurepas swamp will be one of the few areas in coastal Louisiana to have a net elevation gain. Interestingly, the variation between cardinal directions is as high as the variability between SETs (**Fig. 5A-D**).

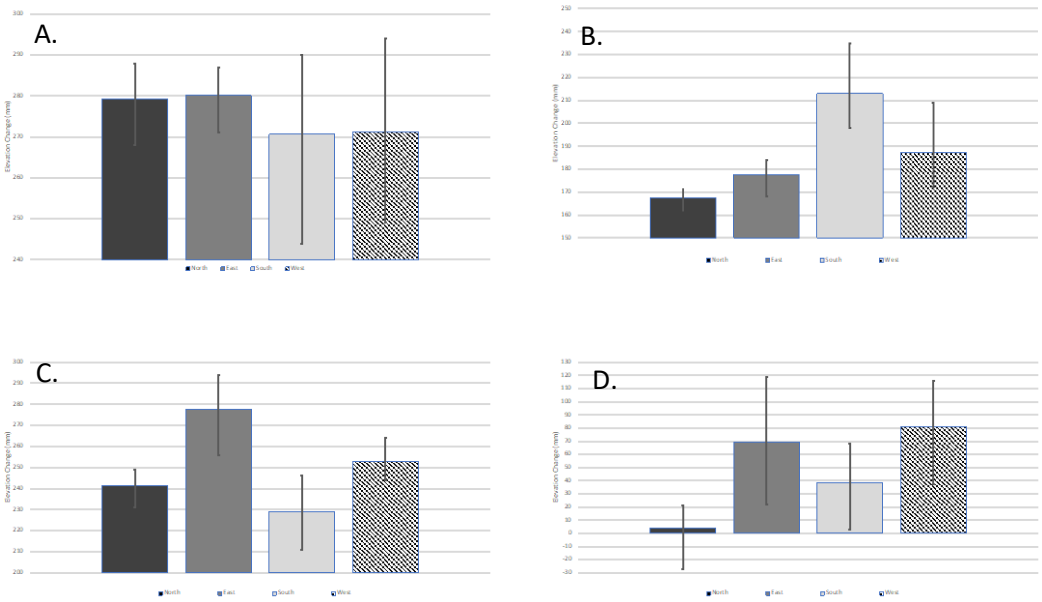

Figure 5. Results from four surface elevation tables demonstrating strong elevation gains for nearly all measurements (A-C=shallow pipe sets, D=deep rod sets).

Finally, our offices have been upgraded with new computers and monitors that are being used in our Geographic Information System (ArcPro) effort to build a new habitat-state map (Shaffer et al. 2009, 2016, **Fig. 4**). This effort will take about 2 years to complete. A boat with a drone will be used to ground truth the new map.

Summary

Our planting location has been chosen as have the ten site and 20 permanent station locations. To date, we have located four of our SET sites and measurements at these sites demonstrate a net elevation gain.

Future Direction

Beginning in December 2023 we will be planting and protecting 2,000 baldcypress and water tupelo seedlings on West Jones Island (**Fig. 6**). We will also continue to measure

subsidence and accretion rates at the remaining ten SET sites. We have begun working on the new habitat-state map and we will ground truth our final product.

Figure 6. Baldcypress and water tupelo currently growing at Southeastern's Sustainability Center that will be planted on West Jones Island starting in December 2023.

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IV. Chemical Monitoring

Fereshteh Emami, PhD
Southeastern Louisiana University, Dept. of Chemistry and Physics
Hammond, LA 70402
fereshteh.emami@selu.edu

Overview

In the context of the Air Products Carbon Sequestration/Exoduas seismic exploration project in Lake Maurepas and understanding the fate and transport of possible toxic chemicals in the Lake Maurepas ecosystem, we selected nine sampling sites based on recent dredging activities by Air Products. Of these sites, three were chosen from dredging (D) locations, and six were selected from non-dredged (ND) locations. Sediment and water samples have been gathered from these profiles and locations weekly using Ponar dredge and submersible monitoring devices. We have analyzed various vertical profiles and spatial distributions. These profiles include the surface layer (top), the middle layer, and the lake bed (bottom). We are currently conducting targeted analysis on the samples using Separation techniques and Atomic and Molecular spectroscopy to detect thirty-two heavy and non-heavy elements, total phosphorus (TP), total nitrogen (TN), ammonia-nitrogen (NH3-N), chemical oxygen demand (COD), and biochemical oxygen demand (BOD). To date, we have completed measurements on five sampling dates for TP, TN, COD, NH3-N in water, and mercury (Hg) in sediment samples. A preliminary study on two consistent sampling dates for these compounds found the highest TP and TN values in the ND locations compared to the dredging sites. TP was detected highest in the bottom layer of ND3 (0.64±0.08 mg/L), while the lowest amount was found in the middle layer of D3 (0.14±0.16 mg/L). Similarly, TN was detected the highest in the middle layer of the ND6 and the lowest in the middle of the D3. Similarly, for ammonia-nitrogen (NH3-N), chemical oxygen demand (COD), and mercury (Hg), the highest and lowest concentrations were found in the ND locations. The maximum NH3-N was found in the bottom layer of ND2 (0.38±0.57 mg/L), and the lowest amount was in the middle part of ND5 (0.04±0.03 mg/L). One notable finding from this study is that the COD level is higher in the surface level more or less at every point with the highest value was recorded in the top layer of ND3 (52.33±19.99 mg/L) and the lowest in the middle horizon of ND4 (11.67±4.88 mg/L). There was not any noticeable Hg in the water samples; however, the sediment samples carried a higher amount of Hg. The maximum level was detected in the ND2 (25.61±6.04 μg/kg), while the minimum was detected in the ND3 (7.53±1.09 μg/kg). A cluster analysis on the collected small data also indicated dissimilarities in contamination types and magnitudes throughout the lake.

Project Objectives

We are examining the impacts of dredging in Lake Maurepas prior to the initiation of the Air Products Carbon Sequestration Project. The big question is whether the process stirs up toxic chemicals sequestered in the sediments. As such, we are

1. Characterizing Water Quality by:
    a. Determining the physical and chemical parameters of the water, including temperature, pH, and nutrient concentrations.
    b. Assessing the presence of potential contaminants such as heavy metals, pesticides, and organic pollutants.

2. Evaluating Sediment Quality by:
    a. Analyzing sediment composition, and organic content.
    b. Identifying and quantifying pollutants in the sediment, including heavy metals and other contaminants.

Methods/Study Design

During the past year, the PI has purchased multiple instruments, chemicals, sampling tools, workstations, statistical software, and other supplies required to equip and maintain her lab to start the project. The PI has also hired two new graduate students, one post doctorate (in the process of visa approval), and one undergraduate student.
The project has started according to the following protocols:

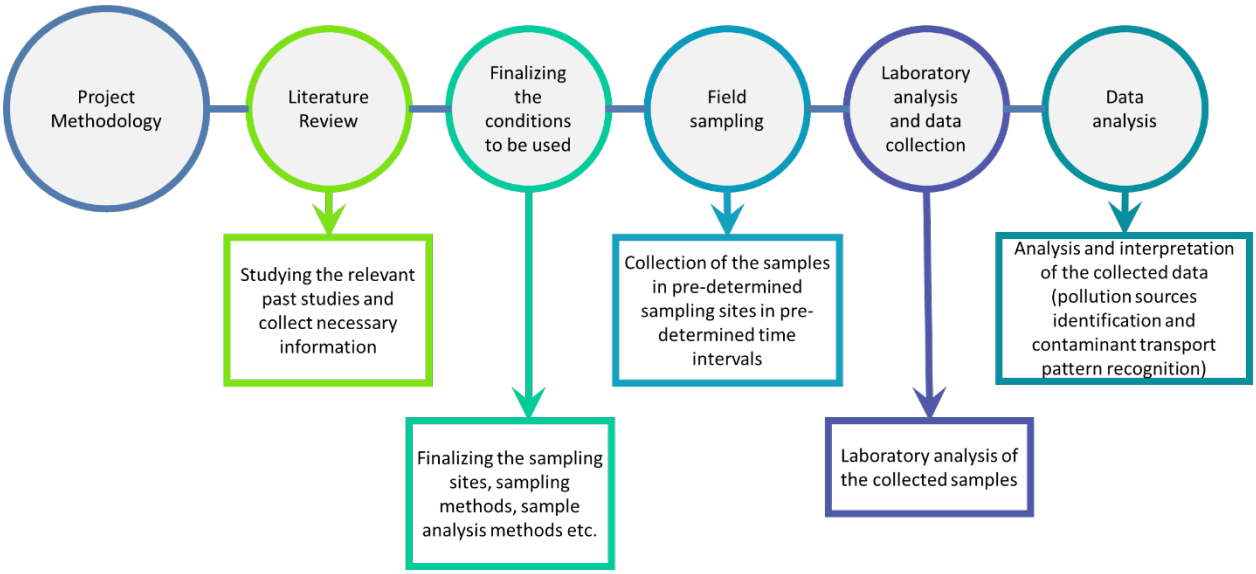

Figure 1. Schematic diagram of the project methodology

Field Sampling

Sites for sampling were selected based on factors such as discharges into the lake from rivers (proximity to human activities) and dredging locations. Additionally, we ensured that the sampling sites represented every section of the lake. Before use, the tools for sampling were properly rinsed and cleaned. Water samples from each location were collected at three different depths: surface level, middle level, and lake bed level, using clean, labeled 250 mL HDPE bottles to prevent contamination. Approximately 400 mL of water samples were collected from each depth level. Sediment samples were gathered from the lake bed at each sampling point using a grab sampling dredger (Wildco Ekman dredge, Cole Parmer). This method ensures proper sampling depth representation and avoids disturbing the sediment-water interface during collection. Sampling was conducted weekly, barring any weather restrictions such as rough wave conditions, rain, or high wind speed (**Figs. 2-4**).

Figure 2. Equipment used for field sampling and field-data collection (left) Water sampler, (middle) dredge, and (right) pH meter.

Figure 3. Collecting sediment samples from the lake bed.

Figure 4. (left) Water samples collection, and (right) taking pH and temperature readings.

Transportation and storage

After collection, samples were placed in an ice chest (Coleman) and covered with dry ice to preserve their integrity until transport to the laboratory (**Fig. 5**). Subsequently, they were

stored in a laboratory freezer at -20°C until analysis. Essential metadata, including the sampling date, time, and other physical conditions such as pH, temperature, and weather conditions, were recorded alongside the GPS coordinates of each sampling site. Additionally, any visible anthropogenic influences or unique characteristics observed during sampling were carefully documented.

Figure 5. (left) Sample bottles used for the collection of water and sediment samples, (middle) Dry ice, (right) Ice chest for safe transportation of the collected samples.

Laboratory analysis of the collected samples

The analysis of collected environmental samples, including water and sediment, is crucial for understanding the presence and concentration of various elements and compounds. To achieve this, we employ cutting-edge analytical techniques such as Microwave Plasma-Atomic Emission Spectroscopy (MP-AES), Direct Mercury Analyzer (DMA), and spectrophotometric methods. These techniques allow us to provide accurate and precise measurements of elemental composition and other contents in samples from Lake Maurepas. Altogether, we plan to identify around 50 compounds/contaminants.

Equipment used

Direct Mercury Analyzer (DMA-*evo* 80)

DMA (**Fig. 6**) is a specialized technique designed for the rapid and accurate determination of mercury content in various sample matrices. This method involves combustion of the sample, followed by the quantification of released mercury vapor. The key features of DMA include high sensitivity allowing for the detection of low concentrations of mercury in environmental samples and reduced sample preparation reducing the likelihood of contamination and ensuring quick results in compliance with Environmental Regulations. DMA is commonly used in environmental monitoring to meet regulatory requirements for mercury analysis in water and sediment samples.

Figure 6. Direct Mercury Analyzer (DMA-*evo* 80).

Microwave Plasma-Atomic Emission Spectroscopy (Agilent MP-AES 4210)

MP-AES (**Fig. 7**) is a powerful analytical technique for the simultaneous determination of multiple elements in a sample. This method utilizes microwave-induced plasma to generate excited atoms, whose emission spectra are then analyzed to quantify the concentrations of elements. The key features of MP-AES include the simultaneous Analysis which allows the MP-AES for the simultaneous analysis of multiple elements, providing high-throughput capabilities and efficiency. And the operational cost is lower unlike traditional methods as MP-AES operates without the need for expensive and consumable gases. This helps to reduce the overall operational expenses. Moreover, MP-AES has a wide elemental range. It covers a broad range of elements in the periodic table, making it suitable for the comprehensive analysis of environmental samples.

Figure 7. Agilent MP-AES 4210

Measuring biological oxygen demand using HACH BOD Track II

Biochemical Oxygen Demand (BOD) is a critical parameter in environmental monitoring that assesses the amount of oxygen consumed by microorganisms to decompose organic matter in water. The measurement of BOD is essential for water quality assessment as it is a key indicator of organic pollution in water bodies. High BOD levels suggest the presence of organic contaminants, indicating potential harm to aquatic ecosystems. Moreover, elevated BOD levels can deplete dissolved oxygen in water, adversely affecting aquatic life. The decline in oxygen levels can lead to fish kills and negatively impact the overall biodiversity of a water body. Furthermore, BOD levels are indirectly linked to public health. High organic pollution in water can create conditions suitable for the growth of harmful bacteria and pathogens, posing risks to human health when contaminated water is used for drinking or recreational purposes.

HACH BOD Track II (**Fig. 8**) instrument is used for this purpose as it is designed for the rapid and accurate measurement of BOD. The instrument streamlines the BOD determination process, offering several advantages such as automated measurement (automates the BOD measurement process, reducing the need for manual handling and minimizing the potential for errors), time efficiency, reduced sample volume, real-time monitoring of the microbial oxygen consumption process, allowing for continuous observation and data acquisition, employs advanced sensors and technology to ensure accurate and precise BOD measurements, enhancing the reliability of the data generated, and data storage and analysis.

Figure 8. HACH BOD Track II instrument.

Spectrophotometric methods to evaluate chemical oxygen demand (COD) and nutrient concentrations

The HACH DR3900 UV-Visible spectrometer was used to measure COD, concentrations of Total Nitrogen (TN), Ammonia-Nitrogen (NH_3-N), and Total Phosphorus (TP). The Hach DR3900 UV-Vis Spectrophotometer utilizes ultraviolet and visible light to analyze the absorbance and transmittance characteristics of liquid samples. It is equipped with advanced features to meet the demands of modern laboratories requiring accurate and efficient analytical capabilities.

The key features of the UV-Vis spectrometer include the coverage of broader wavelength range, typically from 190 to 1100 nm, allowing us to analyze a wide variety of compounds with different absorption characteristics. And it has a user-friendly interface, including a color touchscreen, making it easy to operate and navigate through various functions and applications. Also, this instrument comes with a range of pre-programmed methods for common analytical procedures, simplifying the analysis process and ensuring standardized testing procedures. The instrument is designed to support quality control and validation processes, including the ability to run calibration checks and verify the instrument's performance to ensure accurate and reliable results.

Fig. 9 shows the manufacturer-provided water quality analysis kits for the COD and nutrient measurements, HACH DRB 200 sample digester used for the digestion of the samples before measurements and the HACH DR3900 UV-Visible spectrometer.

Figure 9. (up) Water quality analysis kits for the COD and nutrient measurements, (down-left) HACH DRB 200 sample digester, (down-right) HACH DR3900 UV-Visible spectrometer

Results/Progress to Date

Sampling sites

Establishing sampling sites across the lake is a crucial step in gathering representative data that reflects the spatial variability of water quality parameters, contaminants, and ecological conditions. The selection of sampling points is guided by the need to capture diverse characteristics and potential sources of variability within the lake. Considering these factors, 6 non-dredging (ND) spots and 3 dredging spots (D) were selected as sampling sites namely; ND1, ND2, ND3, ND4, ND5, ND6, D1, D2 and D3. **Fig. 10** shows the locations of dredging and non-dredging sampling sites in Lake Maurepas.

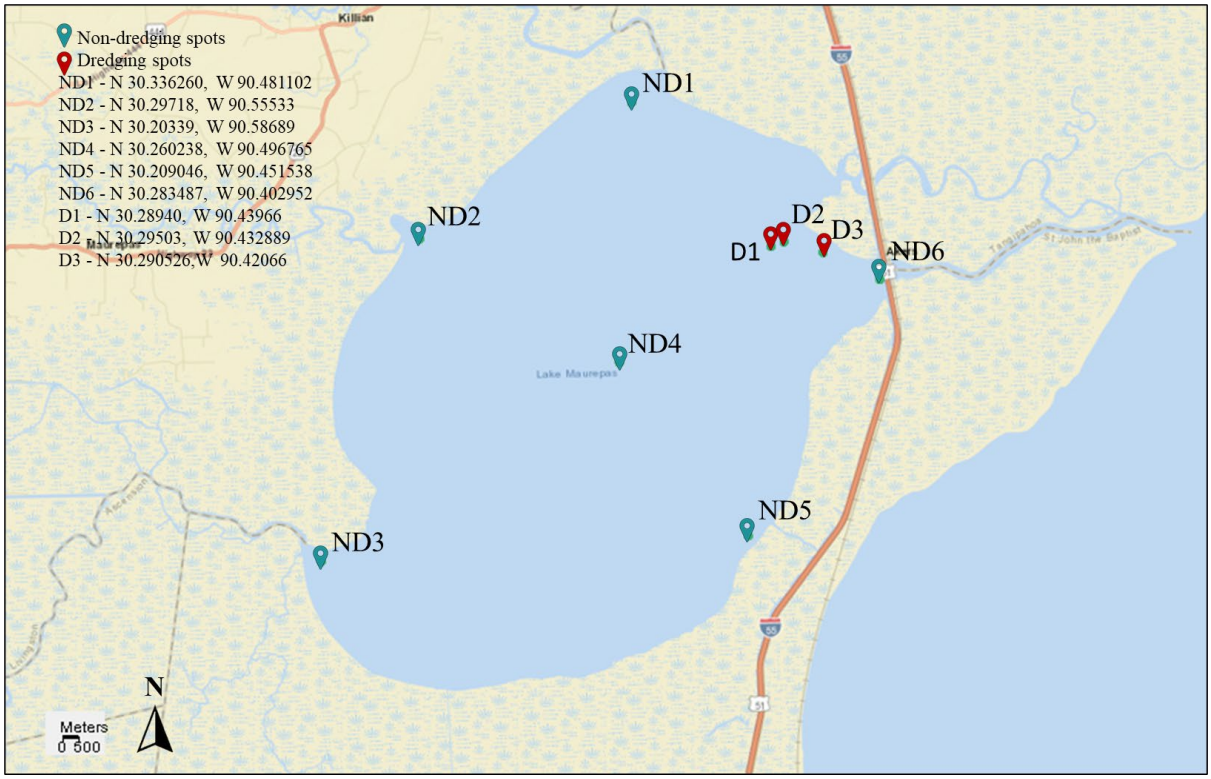

Figure 10. Sampling sites in the Lake Maurepas.

Statistical analysis

Statistical analysis and data visualization were conducted using R programming. Analysis of variance (One way- ANOVA) was used to determine the statistically significant differences of parameters magnitude among sampling stations. Various multivariate statistical techniques, including correlation coefficient analysis (CCA), cluster analysis (CA) and principal component analysis (PCA), are used to facilitate the interpretation of complex data matrices to identify the possible sources of pollutants and their influence on water quality (Dossou et al., 2021; Lee et al., 2001; Nagaraju et al., 2014; Reghunath et al., 2002; Simeonova et al., 2006; Wunderlin et al., 2001). All the multivariate analyses were carried out using RStudio desktop (V-2022.12.0-353).

Interpretation and Data Summary (to date)

Table 1 summarizes information on sample collection based on the date and sampling site. Out of the 18 sampling dates (**Table 1**), we measured concentrations of TP, TN, COD, NH3-N in water, and Hg in sediment samples on 07/20/2023, 08/18/2023, 08/25/2023, 09/15/2023, and 09/22/23. In our preliminary study, we analyzed concentrations collected on two consistent dates for all compounds, 08/18/2023 and 09/15/2023. Tables 2 and 3 indicate that contamination levels in non-dredged spots are generally higher than those in dredged spots. The assessment of mercury (Hg) element revealed that ND2 had the highest Hg concentration at 25.61 mg/kg, while ND3 had the lowest at 7.536 mg/kg. The minimum concentration for COD were detected at ND4, recording 15.83 mg/L. In evaluating TN, NH3-N, and TP, ND sampling spots consistently showed significantly higher concentrations compared to their dredged counterparts. Among the studied spots, ND3 spot emerged as a focal point for environmental concern, showing the highest average values for both TP and COD at 0.5639 mg/L and 41.28 mg/L,

respectively (**Table 2**). The elevated levels of TP and COD in the ND3 water indicate substantial nutrient content and organic contamination, potentially contributing to eutrophication and other ecological damages.

Table 1: Information on the sample collection up-to-date

Date	Sample collection spots	Number of samples collected	
		Water	Sediment
12/15/2022	ND1, ND2, ND3, D1, D2, D3	0	54
01/20/2023	ND1, ND2, ND3, D1, D2, D3	0	54
06/22/2023	ND1, ND2, ND3, D1, D2, D3	36	12
07/06/2023	ND1-D3*	54	18
07/13/2023	ND1-D3	54	18
07/20/2023	ND1-D3	54	18
07/27/2023	ND1-D3	54	18
08/18/2023	ND1-D3	54	18
08/25/2023	ND1-D3	54	18
09/01/2023	ND3, ND4, ND5	18	6
09/08/2023	ND1-D3	54	18
09/15/2023	ND1-D3	54	18
09/22/2023	ND1-D3	54	18
10/13/2023	ND1-D3	54	18
10/20/2023	ND1-D3 except ND2	48	16
10/27/2023	ND1-D3	54	18
11/17/2023	ND1-D3	54	18
12/08/2023	ND4	8	2
Total number of samples collected		**758**	**360**

* ND1-D3 means all designated sampling spots, ND1, ND2, ND3, ND4, ND5, ND6, D1, D2, D3

Table 2. Concentrations of the tested compounds in different sampling spots**.**

Sampling point	**pH Mean IQR**	**Temperature Mean IQR**	**TN Mean IQR**	**TP Mean IQR**	**COD Mean IQR**	**NH_3-N Mean IQR**	**Hg Mean IQR**
ND1	6.995 6.7-7.29	27.85 25-30.70	0.6839 0.5-0.8	0.5411 0.45-0.575	25.89 20-30.75	**0.1389** 0.095-0.17	16.18 1.91-18.39
ND2	6.75 6.7-6.8	27.35 24-30.7	0.4778 0.4-0.6	0.4528 0.402-0.51	33 25.5-39.5	0.2233 0.0725-0.2	**25.61** 21.01-31.80
ND3	6.97 6.74-7.20	28.5 25-32	0.5056 0.3-0.6	**0.5639** 0.53-0.597	**41.28** 28.50-49.75	0.1089 0.07-0.12	**7.536** 6.6-8.6
ND4	6.6 6.5-6.7	27 25-29	**1.193** 0.6-1.97	0.4789 0.35-0.61	**15.83** 12.25-20.75	0.08611 0.06-0.087	19.79 14.92-19.78
ND5	6.835 6.7-6.97	27.5 25-30	0.922 0.42-1.4	0.4072 0.30-0.51	22.89 15.25-27.75	0.095 0.02-0.08	20.16 13.03-27.13
ND6	8.15 6.9-9.4	27.5 25-30	1.172 0.52-1.65	0.3178 0.29-0.36	31.39 21.50-33.25	0.06 0.04-0.07	17.409 11.89-25.41
D1	6.95 6.9-7	27.5 25-30	0.6278 0.1-0.85	0.2089 0.01-0.37	31.06 23.5-34.75	0.0861 0.07-0.11	12.02 8.8-13.54
D2	6.98 6.9-7.07	27 25-29	0.722 0.22-0.67	0.1767 0.01-0.32	27.56 22.25-33.75	0.1044 0.07-0.12	13.71 13.27-14.02
D3	7.13 7.10-7.16	27.5 25-30	**0.3944** 0.2-0.58	**0.1761** 0.01-0.37	26.06 22-31.75	**0.07** 0.06-0.08	17.93 13.72-20.11

The minimum and maximum detected values were bolded. IQR=Interquartile range (First quartile-third quartile). The unit of the measured concentrations are mg/L for TN, TP, COD and NH_3-N and g/kg for Hg.

Table 3. Vertical distribution of the compounds in different sampling spots.

Sample ID	Vertical profile	pH	Temperature	TP (mg/L)	TN (mg/L)	NH_3-N(mg/L)	COD (mg/L)	Hg (□g/kg)
ND1	Top	6.99±0.32	27.85±0.32	0.49±0.03	0.8±0.25	0.13±0.05	33.33±9.41	16.18±4.58
	Middle			0.58±0.09	0.7±0.30	0.14±0.03	19±5.29	
	Bottom			0.54±0.05	0.55±0.27	0.14±0.04	25.33±6.91	
ND2	Top	6.75±0.05	27.35 ±3.66	0.46±0.06	0.55 ±0.21	0.13±0.05	39.83 ±11.54	25.61±6.04
	Middle			0.40±0.07	0.46±0.15	0.14±0.08	32.5±9.41	
	Bottom			0.49±0.07	0.41±0.29	0.38±0.57	26.67±12.83	
ND3	Top	6.97±0.25	28.5 ±3.83	0.56±0.02	0.56±0.20	0.08±0.04	52.33±19.99	7.53±1.09
	Middle			0.49±0.08	0.31±0.13	0.14±0.16	39.83±10.55	
	Bottom			0.64±0.08	0.63±0.45	0.09±0.02	31.67±17.44	
ND4	Top	6.6±0.1	27±2.19	0.47±0.13	1±0.79	0.13±0.06	21.17±6.79	19.79±5.96
	Middle			0.47±0.15	0.98±0.71	0.05±0.01	11.67±4.88	
	Bottom			0.49±0.18	1.6±1	0.06±0.01	14.67±9.17	
ND5	Top	6.83±0.14	27.5±2.73	0.4±0.12	0.93±0.59	0.19±0.31	29±25.48	20.16±7.69
	Middle			0.39±0.16	1.13±1.02	0.04±0.03	21.50±7.55	
	Bottom			0.42±0.13	0.7±0.35	0.05±0.04	18.17±8.61	
ND6	Top	8.15±1.36	27.5±2.73	0.29±0.06	1.11±0.56	0.07±0.04	34.67±23.16	17.4±7.30
	Middle			0.36±0.08	1.8±1.04	0.05±0.03	33±11.47	
	Bottom			0.30±0.05	0.6±0.42	0.05±0.01	26.5±7.23	
D1	Top	6.95±0.05	27.5±2.73	0.25±0.27	1.13±0.96	0.08±0.03	37±21.21	12.08±3.55
	Middle			0.2±0.21	0.41±0.47	0.08±0.01	27.33±9.79	
	Bottom			0.17±0.17	0.33±0.27	0.08±0.01	28.83±3.55	
D2	Top	6.98±0.09	27±2.19	0.14±0.15	0.35±0.21	0.1±0.04	27.67±6.47	13.71±0.44
	Middle			0.15±0.16	1.33±1.75	0.09±0.01	28±7.82	
	Bottom			0.23±0.24	0.48±0.29	0.11±0.02	27±7.42	
D3	Top	7.13±0.03	27.5±2.73	0.19±0.20	0.46±0.33	0.07±0.01	23.67±13.03	17.93±5.56
	Middle			0.14±0.16	0.25±0.28	0.05±0.02	27±5.56	
	Bottom			0.19±0.20	0.46±0.29	0.07±0.02	27.5±4.03	

Based on similarities in contamination types and magnitudes, cluster analysis (CA) grouped the nine sample locations into three clusters (**Fig. 11**). Cluster 1 includes five sampling spots; ND5, ND6, D1, D2, and D3. Meanwhile, cluster 2 consists of ND1, ND2, and ND3. Cluster 3 includes only one sampling spot, ND4. This cluster analysis suggests the potential presence of three dissimilarities in contamination types among the sampling spots.

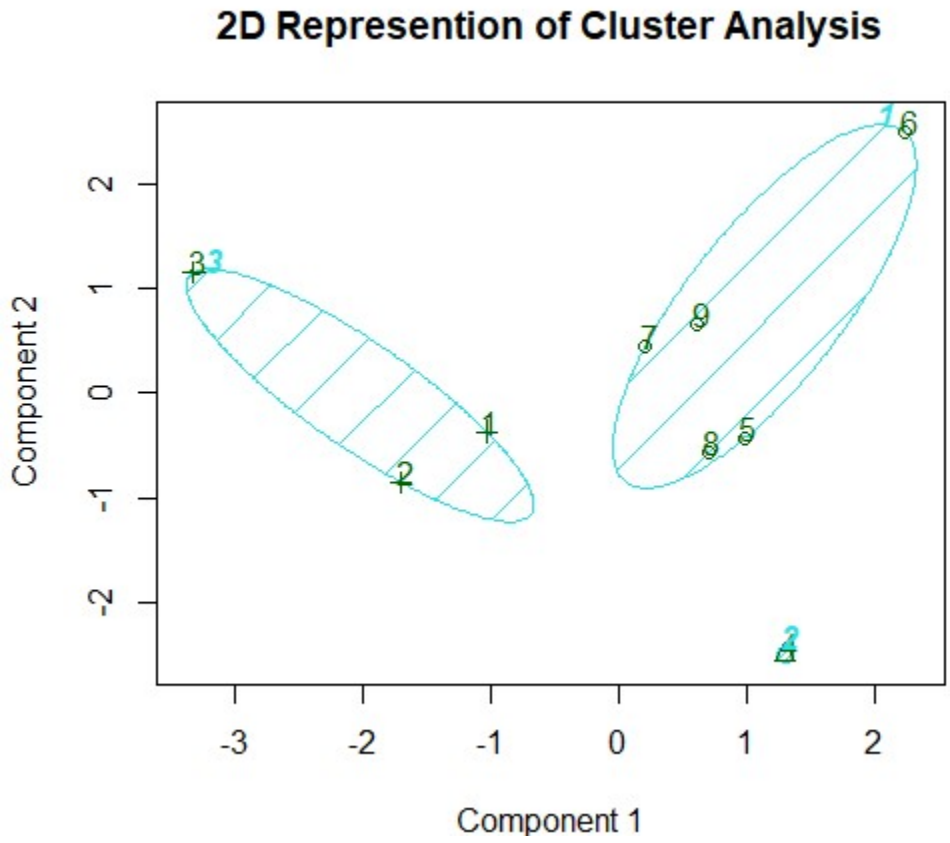

Figure 11. 2D representations of CA with the first two axes explaining 62.61% of the variance.

While this study does not claim to provide conclusive findings about the overall contamination levels in Lake Maurepas, it offers a preliminary glimpse into the potential contamination levels. Notably, concentrations of contaminants vary across different sampling spots, indicating spatial heterogeneity in pollution distribution. These differences may be influenced by factors such as proximity to pollution sources, local hydrodynamics, and the diverse nature of the lakebed. A notable trend emerges when comparing contamination levels between dredged and non-dredged locations. The majority of contaminants appear to be more concentrated in non-dredged areas than in dredged ones. While this observation provides a valuable starting point for understanding the impacts of dredging activities on contamination, it is essential to interpret this trend cautiously, considering the preliminary nature of our study.

Future Direction

- We will continue our measurement expanding to more compounds and continue sampling from the nine designated spots.
- We will conduct non-targeted analysis (NTA) on the waters, suspended sediments, and organisms to discover unknown chemicals.
- Upon completing the measurements for at least six months, we will start applying Bayesian Spatial Multivariate Receptor Modeling (BSMRM) or Positive Matrix Factorization (PMF) to pollutant source apportionment of water, sediment, and organism samples of Maurepas Lake. BSMRM/PMF decomposes the data matrix to water pollution source contributions and water pollution source profiles, which are interpretable in physical and chemical terms. This modeling aims to identify the number of pollutant sources, the species profile of each source, and the amount of mass contributed by each source. We will also assess the effect of any changes caused by dredging using the results obtained by BSMRM/PMF.
- We will continue assessing the changes in water quality of Maurepas Lake. Changes in surface water quality, spatial variability of water chemical composition, and possible impacts of different pollution sources will be analyzed using nonparametric regression methods. Using piecewise regression, significant changes in water pollution at different periods will be investigated.

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V. Education/Outreach

Robert Moreau, PhD
Southeastern Louisiana University,
Turtle Cove Environmental Research Station
Hammond, LA 70402
robert.moreau@selu.edu

Introduction

The Turtle Cove Environmental Research Station team is responsible for increasing public awareness of the Lake Maurepas Monitoring (LMM) project and facilitating the needs of LMM researchers utilizing field equipment and boats located at the Galva Canal Boatshed/Classroom Complex in Manchac, Louisiana. From January 2023 to December 2023 (CY 2023), Turtle Cove staff discussed the project and highlights of monitoring efforts primarily through Southeastern Louisiana University (SELU) courses and lectures, K-12 outreach events, and professional meetings, which consisted of visitors and attendees from several different parishes. Additionally, Turtle Cove maintained and provided boats, fuel and access to facilities for each team throughout the year. Our team also secured additional boat slips through our on-going 39-year partnership with the Louisiana Department of Wildlife and Fisheries (LDWF), and continued assisting the LMM project Director with the logistics of future monitoring buoy deployment.

Methods

All Turtle Cove-affiliated events that occurred in 2023 were recorded via our shared Google Calendar for all users. Each calendar entry included number of individuals, time, and location. These entries were input into a spreadsheet organized by overall category and type (**Table 1**), and used to calculate the total number of individuals and user days for the year. User days refers to the number of days a different individual visited or utilized Turtle Cove facilities and resources.

Table 1. Organizational scheme of categories and associated types for calendar entries.

Research	University Education	Public Outreach
Turtle Cove	SELU Courses/Lectures	General
SELU Biology Faculty	SELU-Related Field Trips	K-12
Lake Maurepas Monitoring	Outside University Field Trips	Professional Meetings
SELU Interdisciplinary	Workshops	-
Outside Universities	-	-

Any events that included LMM-related research, university education, or public outreach were analyzed separately and compared to overall user days and individual numbers

to identify events that could incorporate LMM-related material in the future. Additionally, all group types were organized by parish to visualize geographic extent of outreach.

Results

Approximately 1,860 individuals and 2,367 user days were recorded in CY 2023. Of these totals, 58 individuals contributed to 327 user days of research, 361 individuals contributed to 597 user days of university education, and 1,441 individuals contributed to 1,443 user days of public outreach (**Figs 1 and 2**).

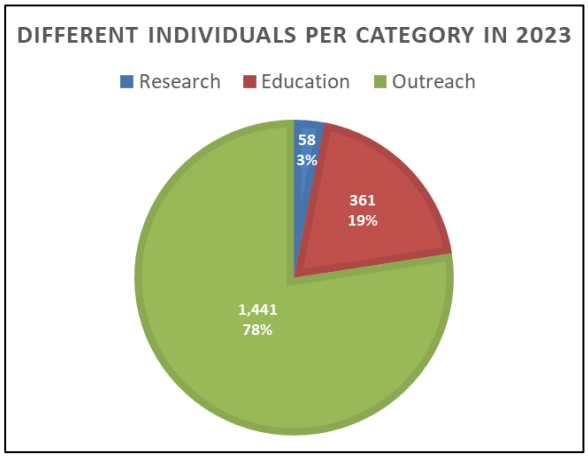

Figure 1. Pie chart showing totals and percentages of different individuals per category in CY 2023.

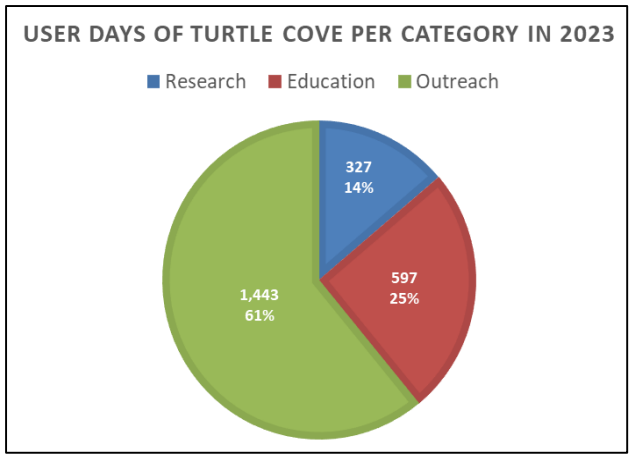

Figure 2. Pie chart showing totals and percentages of user days per category in CY 2023.

LMM was responsible for approximately 71% of the overall research category, which consisted of 21 individuals over 231 user days. Approximately 285 (79%) of the individuals recorded under the overall university education category and 956 (66%) of the individuals recorded under the public outreach category were provided with LMM-related information during tours and other events. SELU Courses and Lectures and K-12 events were the highest contributors to the university education and public outreach categories, with 112 (39%) individuals and 576 (61%) individuals, respectively (**Figs. 3 and 4**).

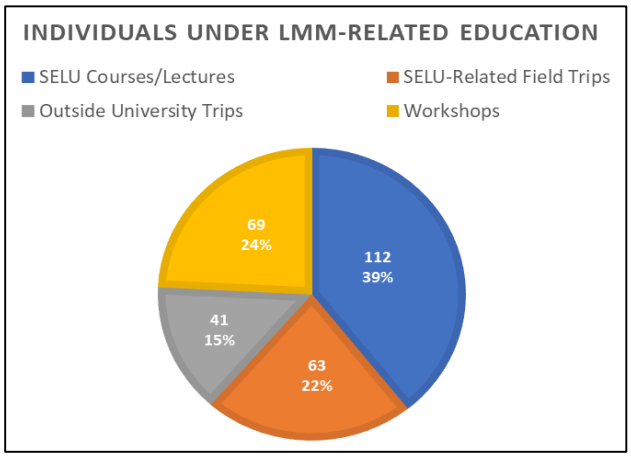

Figure 3. Pie chart showing totals and percentages of individuals recorded under the university education category that received LMM-related information in CY 2023.

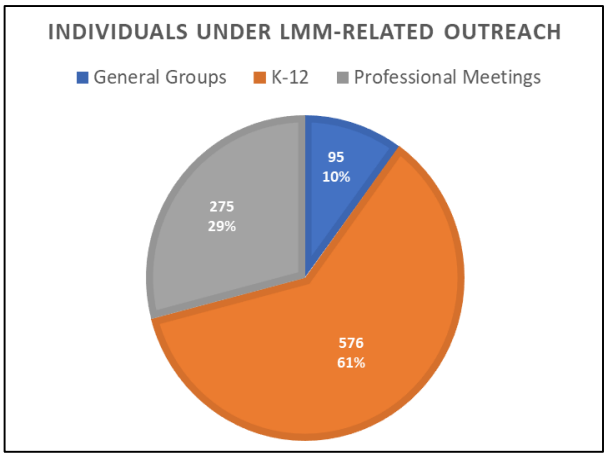

Figure 4. Pie chart showing totals and percentages of individuals recorded under the public outreach category that received LMM-related information in CY 2023.

Groups recorded under the university education and public outreach categories that received LMM information represented approximately seven (7) parishes in Louisiana (**Fig. 5**).

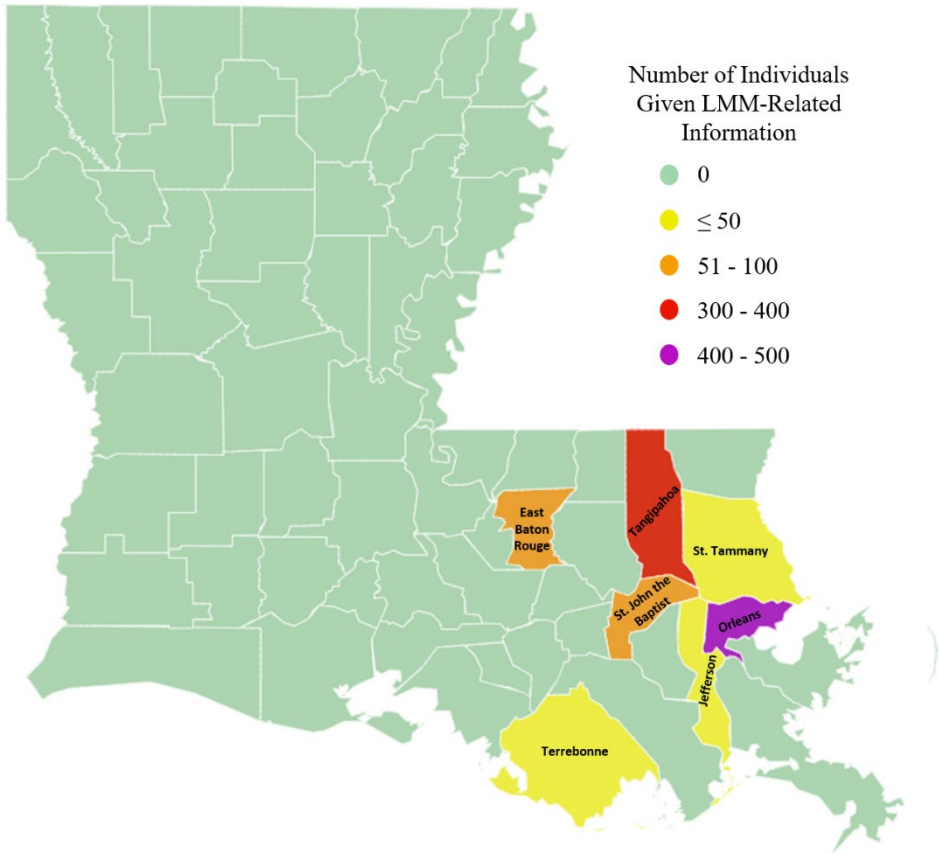

Figure 5. Map showing parishes of origin for university education and public outreach groups that received LMM information in CY 2023.

Discussion

Overall, Turtle Cove achieved its primary goals of LMM education and outreach and working with LMM researchers to ensure their field requirements at the Galva Canal facility were fulfilled. Our team provided LMM information to approximately 1,231 different individuals from 37 groups representing seven (7) parishes. We secured additional boat slips for the project (shared space in the brand new LDWF boatshed), oversaw the installation of the winch/hoist system underneath the Turtle Cove boatshed (for buoy management), and construction of a new 30ft Aluminum Cabin Workboat for Turtle Cove (funded by another external source) that was modified to assist in the forthcoming LMM buoy deployment (**Appendix A**). The Turtle Cove LMM budget also re-allocated $55,000 to the aquatics research team for the purchase of a new landing craft vessel that is needed for the project.

Future Goals

While we expect to continue on this successful trajectory, Turtle Cove is working toward several additional goals for LMM's CY 2024 activities.

Expansion of Media/Communication Methods Our analysis showed that many offsite K-12 outreach events involving Turtle Cove did not feature LMM-related material. We plan to incorporate LMM project descriptions and data into future events through brochures or handouts, designing hands-on activities based on LMM methods, and sharing monitoring buoy data with groups. We also plan to dedicate a monitor in the Galva Canal Classroom for showing real-time monitoring buoy data once deployment is completed. Ideally, these proposed actions will increase public attention to the project website and facilitate a better understanding of researcher's field techniques and overall health of Lake Maurepas.

Targeted Recruitment of Visitors and Partnerships Our team is planning to increase visitor numbers by contacting past visitors of Turtle Cove, growing our social media presence, and developing shared events through partnerships with other local organizations. Direct contact coupled with regularly updated social media profiles would allow us to share tour times and availability, regular updates on the monitoring project, weekly recaps of field trips, and other seasonal field observations with a larger audience. Additionally, we plan to continue working with local organizations such as the Lake Pontchartrain Maritime Museum, University of New Orleans' Coastal Education Research Facility, and others, to establish shared events and expand our LMM project outreach as appropriate.

Enhancement of Galva Canal Boatshed Resources and Vessel Capabilities Turtle Cove will also continue to support all LMM research teams by expanding our boat fleet and fuel access. We will assist the aquatics team with the purchase of a new vessel, and our team is currently finalizing the acquisition of a 110-gallon portable fuel (gasoline) tank for the Galva Canal Boatshed/Classroom complex. Finally, Turtle Cove plans to continue working closely with the LMM project Director on the management and deployment of monitoring buoys and construction of the new research boat, along with integrating the use of the new Turtle Cove 30ft Aluminum Cabin workboat.

All of these goals align with Turtle Cove's mission to facilitate a better understanding of the coastal wetland environment through supporting interdisciplinary environmental research, university education and public outreach activities for Southeastern Louisiana University and the surrounding regional community it serves, as well as our dedication to the LMM project.

Appendix A

Photograph Log

Appendix Figure 1. LSU Sea Grant Wetland Day with 80 Emily C. Watkins 6th and 7th graders hosted at the Turtle Cove Galva Canal Boatshed/Classroom Complex in Manchac.

Appendix Figure 2. Newly installed winch/hoist system for buoy deployment at the Galva Canal Boatshed/Classroom Complex.

Appendix Figure 3. Construction progress on new Turtle Cove 30’ Aluminum Cabin Workboat that will be used to assist in LMM buoy deployment, and which will be made available to all research teams as needed. Funds for the purchase of this Workboat came from an external source (non-LMM funds).

Appendix Figure 4. Aft/Stern view of construction progress of new Turtle Cove vessel. Funds for the purchase of this Workboat came from an external source (non-LMM funds).