PHASE II: BASELINE SAMPLING IN LAKE MAUREPAS (PHASE II, OCT-DEC 2022)

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Executive Summary

From October through December 2022, Southeastern Louisiana University undertook a rapid monitoring project to assess the current biotic and abiotic conditions in Lake Maurepas in the context of the Air Products Carbon Sequestration/Exoduas seismic exploration project. More than 90 man-hours were dedicated to biotic monitoring and data analysis, and another 120 man-hours to gather and summarize the abiotic data. The biotic data analysis indicates that the fish community is robust and healthy based on analysis on condition indices based on length and weight of the captured fishes (i.e., Fulton Condition Index). Baseline physiological data on many of these same samples also was gathered for a suite of fishes and alligators in Lake Maurepas. In general, although the sampling period was brief, a robust baseline data set was gathered that shows consistency in physiological conditions (i.e., low HSI low stress proxies and low liver toxin load) in Lake Maurepas vertebrates. Finally, multiple abiotic parameters were gathered from throughout Lake Maurepas. Turbidity showed the highest level of variability of any of the parameters, and tended to be associated with varying wind levels. The other parameters, including dissolved oxygen, salinity, and temperate were relatively stable throughout the study period and were within the ranges of what is typical of a freshwater ecosystem.

Morphological Assessment of Lake Maurepas Fish Health

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The Fulton Condition Index/Factor (K) (Fulton 1904, Bolger and Connelly 1989) is a measure that uses length and weight data to assess fish health or fish condition. In general, it is a measure of the robustness or well-being of an individual fish, as it is reflective of a fishes' nutritional status. The Fulton Index assumes isometric growth (Kimmerer et al. 2005). Although there is variation due to a fishes' age, species, and sex, a healthy fish is generally considered of good health when a Fulton Condition Index (K) nears a value of 1.0.

In the Fall of 2022 (November and December 2022), multiple 15-minute gill nets were set around Lake Maurepas as part of a larger study in which fishes were collected to assess the physiological health of the fish community using a variety of physiological metrics. Both standard length (cm) and weight (g) were gathered for each individual fish and recorded (Table 1). The results indicate every individual fish was at or above the 1.0 "healthy fish" threshold. These results suggest that the Lake Maurepas fish community, in general, is in good health and provides a small, but relevant baseline data set as the seismic survey project moves forward into 2023.

Table 1. Fulton Condition Index for a set of fishes collected in Lake Maurepas (Nov-Dec 2022).

Species	Length (cm)	Weight (g)	Fultons' Condition Factor	Collection Date
Largemouth Bass	32	800	2.44	Nov-22
Spotted Gar	53	2200	1.48	Nov-22
Skipjack Herring	20	120	1.50	Nov-22
Channel Catfish	18	90	1.54	Nov-22
Channel Catfish	23	170	1.40	Nov-22
Channel Catfish	18	60	1.03	Nov-22
Channel Catfish	19	100	1.46	Nov-22
Channel Catfish	21.5	100	1.01	Nov-22
Channel Catfish	16	45	1.10	Nov-22
Channel Catfish	17	50	1.02	Nov-22
Channel Catfish	18.5	70	1.11	Nov-22
Channel Catfish	17	53	1.08	Nov-22
Channel Catfish	18.5	65	1.03	Nov-22
Striped Mullet	28	225	1.02	Dec-22
Longear Sunfish	9.5	30	3.50	Dec-22
Ladyfish	20.5	96	1.11	Dec-22
Skipjack Herring	20.5	100	1.16	Dec-22
Skipjack Herring	18	90	1.54	Dec-22
Gizzard Shad	14.5	49	1.61	Dec-22
Spotted Gar	44	1000	1.17	Dec-22
Spotted Gar	41	900	1.31	Dec-22

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Baseline Physiological Indices in Vertebrates: Lake Maurepas Bio-Monitoring in the Context of Carbon Sequestration

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Goals: The goal of this bio-monitoring effort was to conduct repeated sampling in the Lake Maurepas ecosystem, specifically monitoring ecotoxicological indices of physiological allostatic stress and toxin load in vertebrates. This study aims to gather baseline data in the context of the Air Products Carbon Sequestration/Exoduas seismic exploration and prior to the initiation of the Air Products Carbon Sequestration Project. Specific project objectives are described below.

1). *Quantify leukocyte profiles for fishes and alligators across space and time in Lake Maurepas from mid-October to late December 2022.*

Leukocyte profiles that include granulocyte (heterophil or neutrophil depending on species) to lymphocyte ratios are a robust method to approximate relative chronic stress of an individual (Harris and Bird, 2000; Davis 2008; Murray et al. 2013; Goessling et al. 2015) using downstream immunological responses to changes in an otherwise metabolic axis (hypothalamus-pituitary adrenal axis). This metric avoids metabolic biases and does not attenuate prior to the removal of a stressor (Goessling et al. 2015). Here, we use this method to provide baseline stress proxy data for fishes and alligators in Lake Maurepas. Importantly, a ratio greater than 1 indicates elevated chronic stress in alligators (Murray et al. 2013), but such a threshold for fishes is not known. However, ratios *are* intraspecifically comparable across individuals, space and time.

2). *Quantify hepatosomatic indices for fishes across space and time in Lake Maurepas from mid-October to late December 2022.*

Additionally, we quantified the hepatosomatic index (HSI) for fishes in Lake Maurepas. This metric serves as a metric of relative toxin load impacting the liver (Pandit et al. 2019). Here, the weight of the liver compared to body weight is used to establish a distribution of HSI's for comparison across space, time, etc. and directly reflects the overall toxin load hepatically processed by an individual.

Methods: Three sampling events were performed between 27 October 2022 and 5 December 2022 totaling 20 sampling hours and 58 man-hours in the field. Fishes were captured using dual sets of 50m gill nets with 15-minute soak times. Fish were bled immediately from the renal portal using 22 or 27 gauge needles, depending on size, and a 1CC syringe. 5uL of whole blood was evenly spread on a microscope slide approximately one-cell layer thick. Fish were then kept on ice and transported back to the lab where they were dissected for HSI.

Alligators were sampled during sampling events 1 and 2 but data were only collected on sampling event 2 as a function of nighttime ambient temperature. Alligators were captured by hand or using a manual snare and bled within 5 minutes of capture using the methodology described above. Total length, snout to vent length and sex were recorded and each individual was marked for later mark-recapture study.

Leukocyte profiles slides were fixed in methanol and stained using Geisma-Wright stain. The first 100 leukocytes were counted and ratio assessed from that count. Trained technicians counted each slide independently. A paired two-sample T-test revealed that counts did not differ among technicians, so counts were averaged for each slide.

Table 1: Species, sampling event, and physiological data for HIS and H:L.

Species	**Date; Sampling Event**	**Sector**	**HSI**	**H:L**
Ictalurus furcatus	10/27/22; 1	2	0.010	0.27
Ictalurus punctatus	11/8/22; 2	6	0.017	0.12
Ictalurus punctatus	11/8/22; 2	6	0.015	0.23
Ictalurus punctatus	11/8/22; 2	6	0.016	0.19
Ictalurus punctatus	11/8/22; 2	6	0.017	0.20
Ictalurus punctatus	11/8/22; 2	6	0.017	0.22
Ictalurus punctatus	11/8/22; 2	6	0.019	0.23
Ictalurus punctatus	11/8/22; 2	6	0.016	0.17
Ictalurus punctatus	11/8/22; 2	6	0.019	0.17
Ictalurus punctatus	11/8/22; 2	6	0.015	--
Ictalurus punctatus	11/8/22; 2	6	0.014	--
Aplodinotus grunniens	10/27/22; 1	2	0.0009	0.21
Lepisosteus occulatus	10/27/22; 1	2	0.029	0.40
Lepisosteus occulatus	11/8/22; 2	6	0.020	0.25
Lepisosteus occulatus	11/8/22; 2	6	0.018	0.48
Lepisosteus occulatus	12/5/22; 3	6	0.023	0.34
Lepisosteus occulatus	12/5/22; 3	6	--	0.52
Micropterus salmoides	11/8/22; 2	6	0.009	0.06
Lepomis megalotis	12/5/22; 3	6	0.008	0.28
Mugil cephalus	12/5/22; 3	6	0.019	0.20
Elops saurus	12/5/22; 3	6	0.009	1.53

Elops saurus	12/5/22; 3	6	0.016	0.27
Elops saurus	12/5/22; 3	6	0.014	3.92
Alosa chrysochloris	11/8/22; 2	2	0.019	--
Alligator mississippiensis	11/8/22; 2	6	--	0.32
Alligator mississippiensis	11/8/22; 2	6	--	1.62

Results: Twenty-four fishes among nine species and 2 alligators were sampled. No species was sampled across all three sampling events enough to make temporal comparisons of data possible. HSI ranged from 0.009 in *Aplodinotus grunniens* to 0.029 in *Lepisosteus occulatus* with a mean of 0.015 (Figure 1). Similarly, N: L ratios ranged from 0.12 in *Ictalurus punctatus* to 3.92 in *Elops saurus* with a mean of 0.54 when all fish species are included (Figure 2).

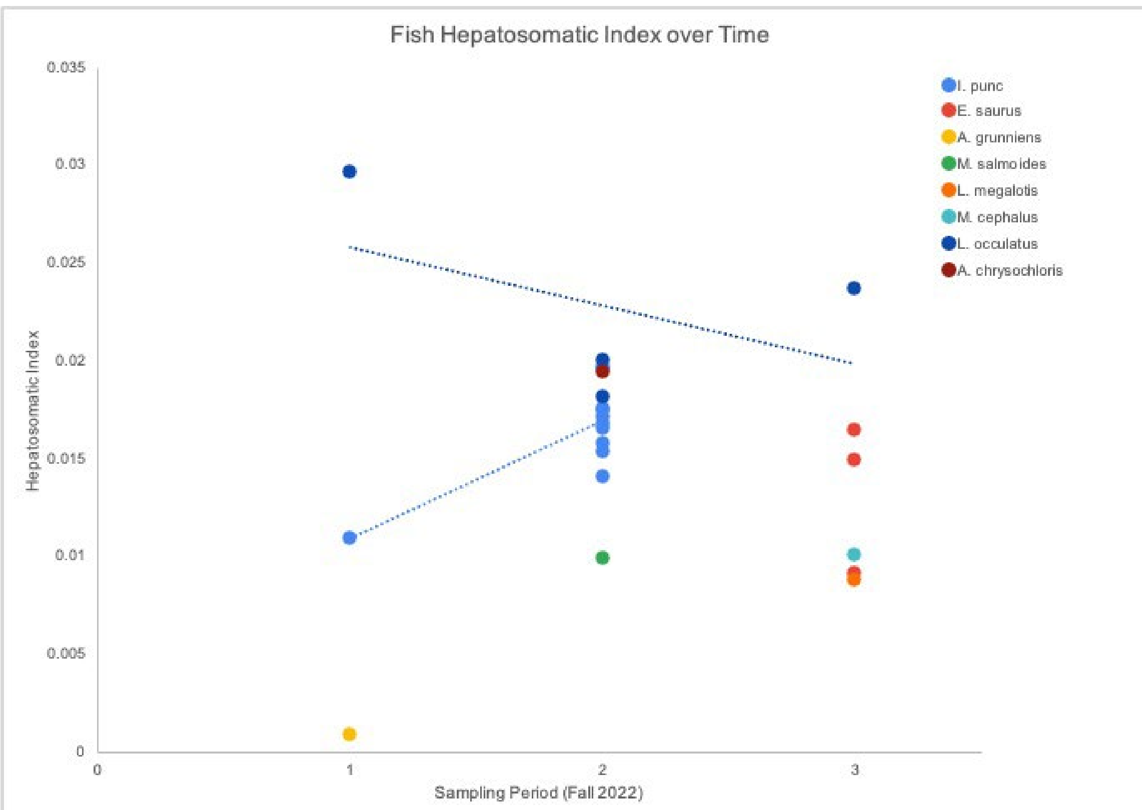

Figure 1: Plot of fish hepatosomatic indices across sampling periods in Fall 2022. Eight species are included exhibiting no temporal trends in HSI within or among species.

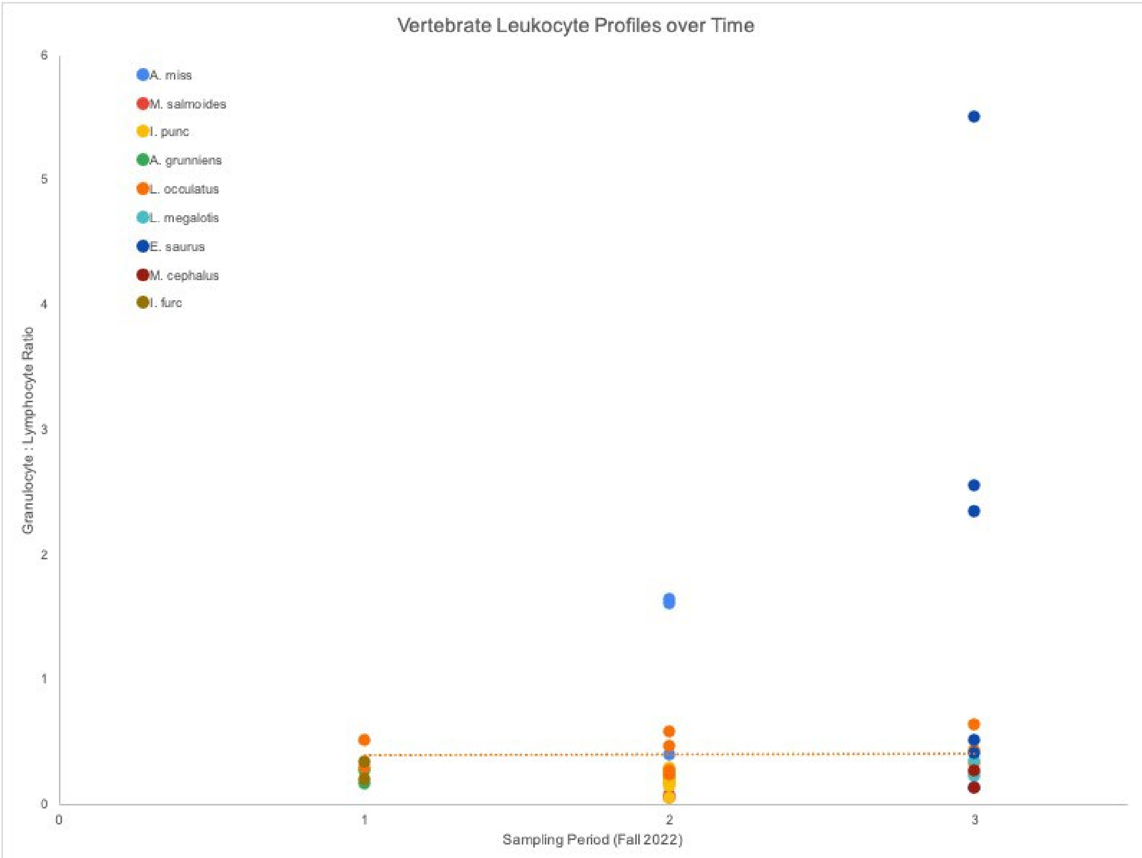

Figure 2: Plot of fish and alligator granulocyte (heterophil (or neutrophil) to lymphocyte ratios across sampling periods in Fall 2022. Eight species of fishes are included exhibiting no temporal trends within or among species.

Discussion: An overall short sampling period and lack of species consistency across sampling events inhibits the development of trends across Fall 2022. Robust baseline data, however, was acquired for long term temporal comparison. Interestingly, the majority of samples exhibited very low baseline granulocyte to lymphocyte ratios as well as HSI, indicating consistency in a healthy physiological condition in Lake Maurepas vertebrates. Only two individual spotted gar (*Lepisosteus occulatus*) exhibited elevated HSI. Similarly, the genus *Elops*, only sampled in sampling period 3, exhibited extreme granulocyte to lymphocyte ratios and one alligator exhibited a ratio indicative of physiological stress.

Sampling methods including gill nets do not present an ideal method of sampling fishes for leukocyte profiling. Blood must be taken before the HPA axis can alter leukocyte distributions as a result of the sampling itself, and gill nets offer little room for error and no standardized handling time across specimens. Rather, electrofishing should be used in future sampling to alleviate these sampling concerns.

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Water Quality of Lake Maurepas

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Introduction

Air Products will be injecting carbon dioxide underneath Lake Maurepas and has contracted the Department of Biological Sciences to conduct preliminary water quality monitoring. During Fall of 2022, Southeastern collected water quality data on five abiotic variables: turbidity, dissolved oxygen, salinity, water temperature, and pH. Herein, we analyze and interpret these data.

Methods

Air Products has broken Lake Maurepas into six sectors to monitor well-drilling activity (Figure 1). Our Sector 4 corresponds to Air Products Sector 5. Three replicates of each abiotic variable were measured in each sector over a ten-week sampling period covering November and December of 2022. Turbidity and water temperature were measured with an OTT Hydra 3LT meter and salinity, dissolved oxygen and pH were measured with a YSI Pro 60 meter.

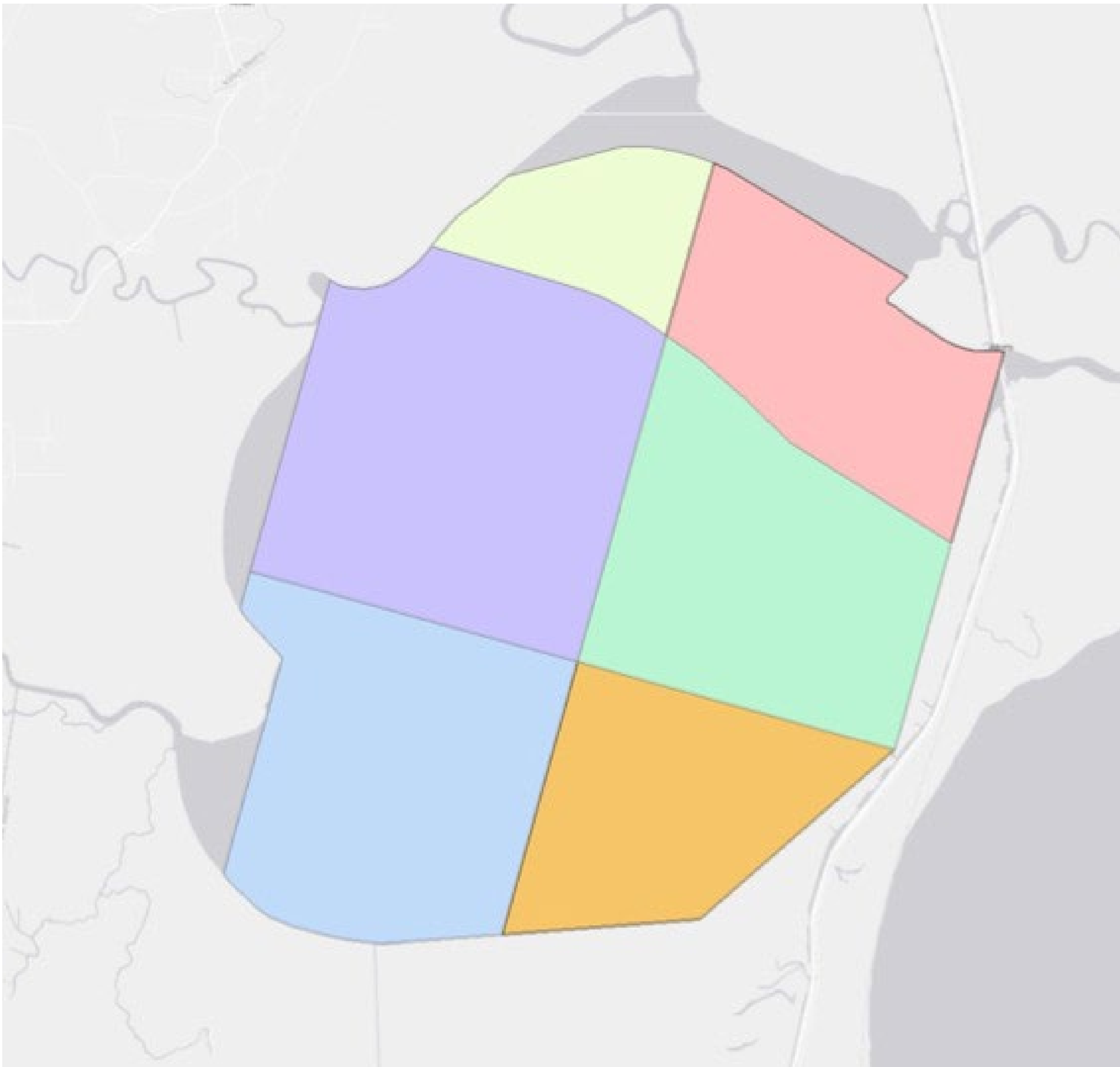

Figure 1. Map of Lake Maurepas broken into six sectors with Sectors 1 and 2 to the north and Sectors 5 and 6 to the south.

Results and Discussion

Turbidity

Turbidity varied widely across sectors ($F = 30.645$, $p < 0.001$) and time period ($F = 30.110$, $p < 0.0001$) and, within sector, was not consistent through time (interaction $F = 5.39$, $p < 0.0001$). Turbidity was highest during the beginning of December, especially for Sectors 3, 4, and 5 (Figure 2).

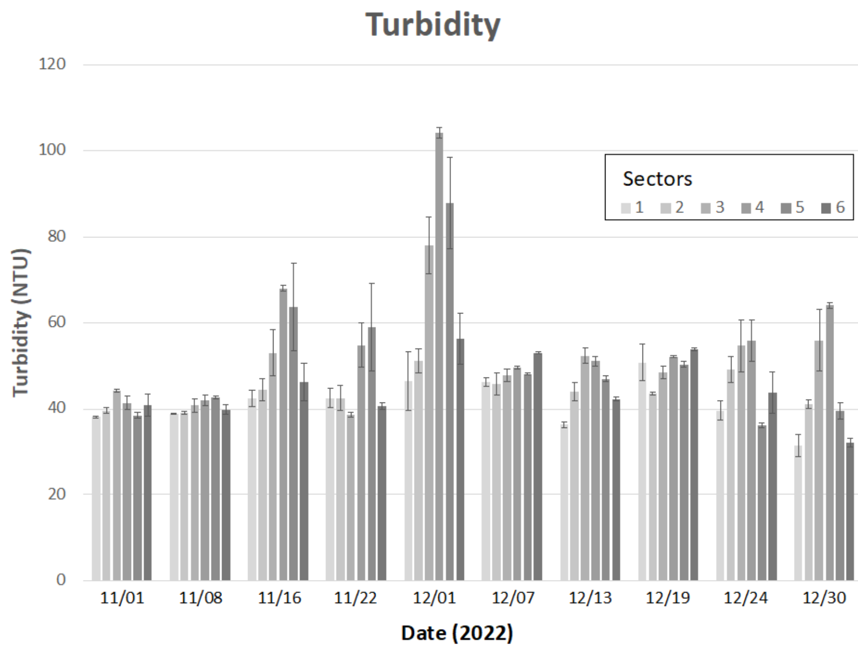

Figure 2. For each sector, turbidity variability was high within each time period and turbidities tended to be the highest in Sectors 3, 4, and 5.

Overall, Sector 4 (Air Products Sector 5) had the highest turbidity (Figure 3), almost certainly attributable to the extremely high turbidities of the Amite River Diversion Canal which exits through the Blind River. The lowest turbidities occurred for Sectors 1, 2, and 6 which were very similar to each other (Figure 3).

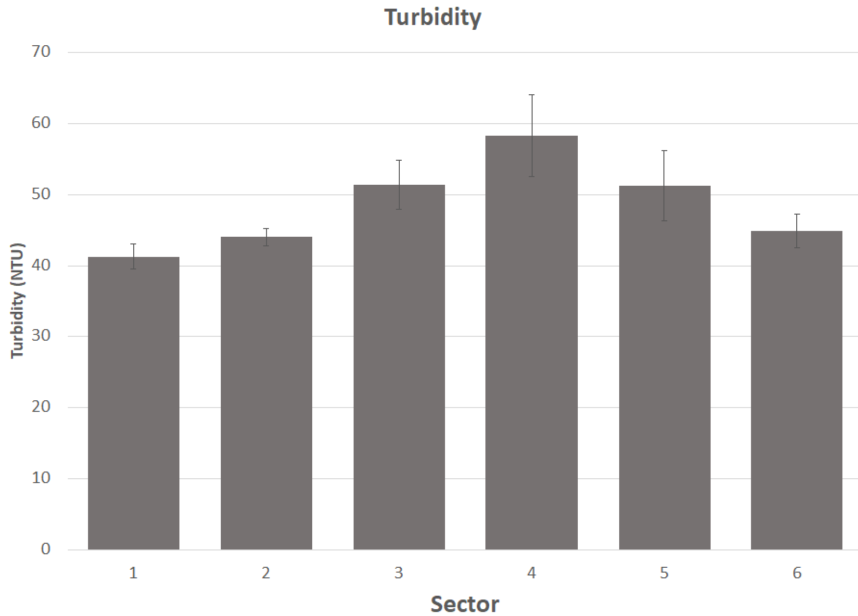

Figure 3. Main effect of sector showing highest turbidity at Sector 4 and lowest at Sectors 1, 2, and 6.

Across time the turbidity of the entire Lake was remarkably similar, with an average about 45 NTU (Figure 4). The only date that differed from all others was December 1, which had double the turbidity of other dates, presumably due to strong winds caused by passage of a cold front.

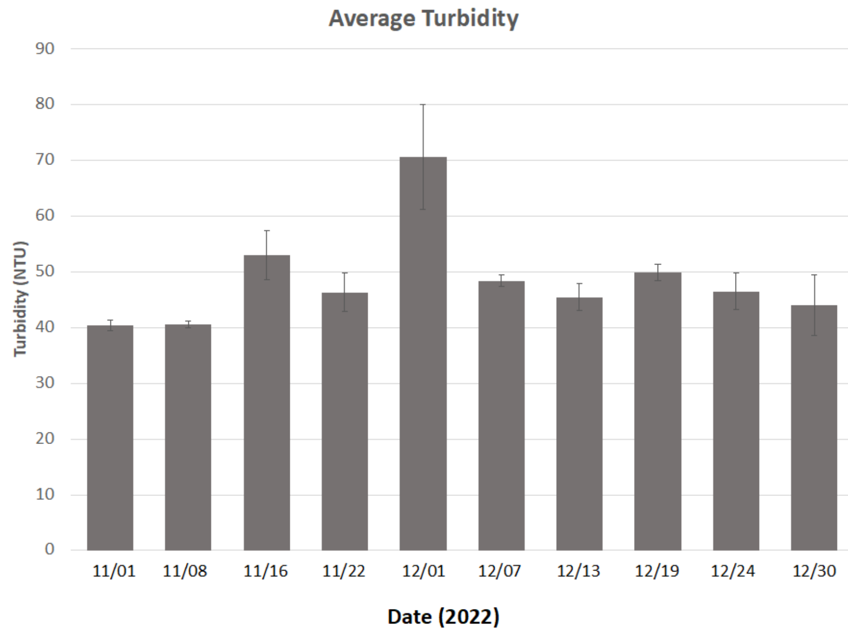

Figure 4. For the Lake as a whole, turbidity was remarkably similar with exception of December 1, 2022.

Dissolved Oxygen

Each sector across time tended to be at or above saturating levels (Figure 5; about 8 mg / L). The sectors averaged quite different oxygen levels ($F = 14.643$, $p < 0.0001$), as did the different time periods ($F = 43.930$, $p < 0.0001$). With regard to the different sectors, Sector 5 and 6 had the highest dissolved oxygen levels and did not differ from each other (Figure 6). Sectors 1 – 4 did not differ statistically.

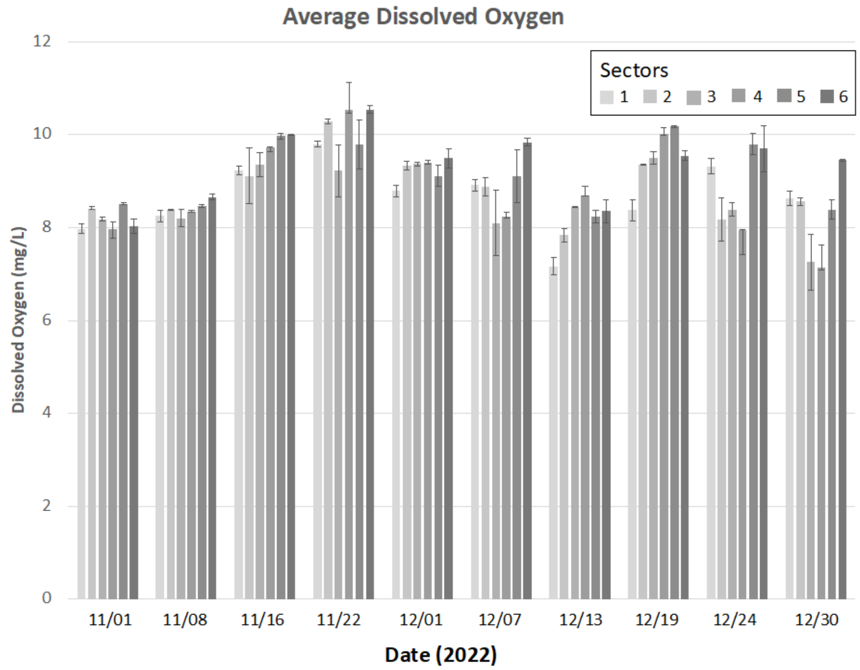

Figure 5. Lake Maurepas dissolved oxygen of each sector across time.

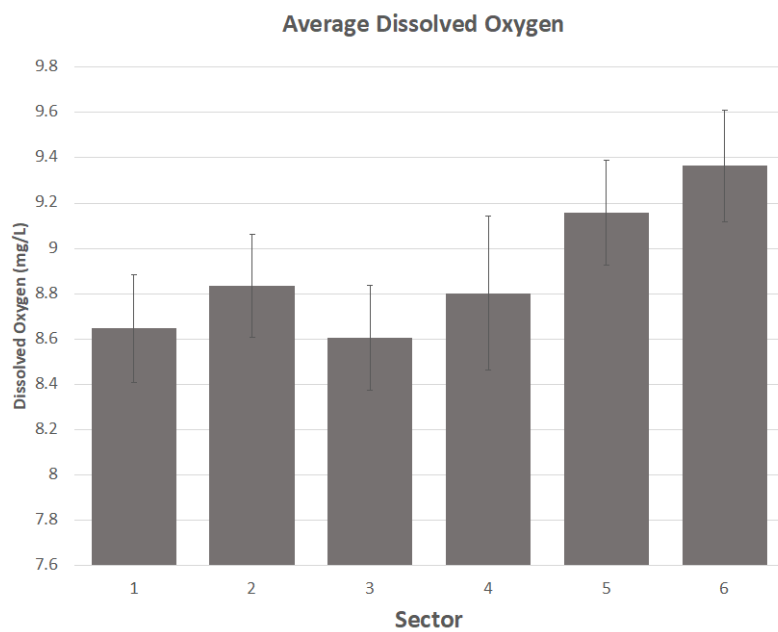

Figure 6. Overall, Sectors 5 and 6 had the highest dissolved oxygen levels.

For each time period, dissolved oxygen varied between 8 mg / L and 10 mg / L (Figure 7). In general, if the Lake were to undergo a lack of saturating levels, that would likely occur during the hottest months. Dissolved oxygen was statistically highest on November 22.

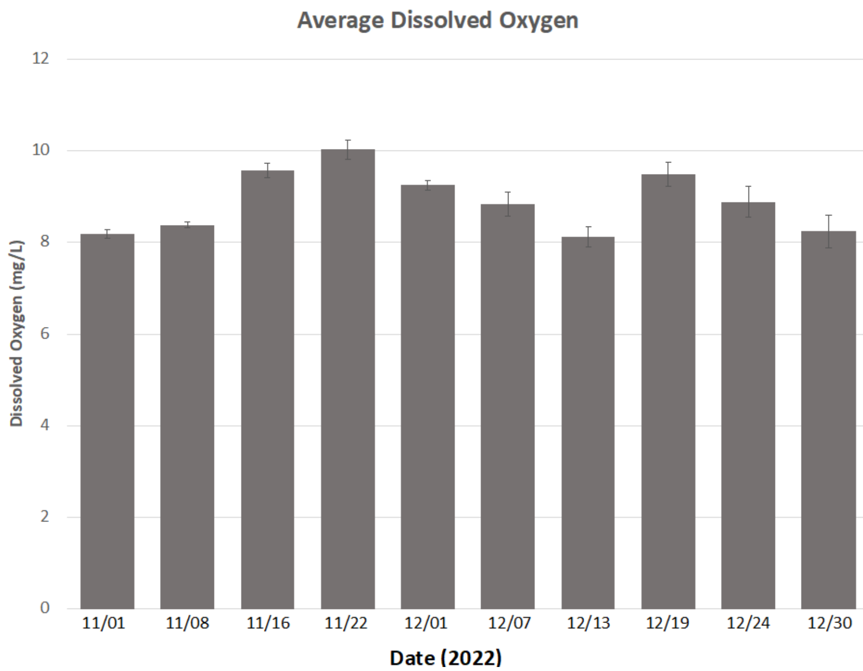

Figure 7. Dissolved oxygen across time periods.

Salinity

Surprisingly, on any one day, salinity can vary widely across the sectors ($F = 15.211$, $p < 0.0001$; Figure 8). There appears to be no trend concerning which sector will have the highest or lowest salinity (Figure 8). In general, salinities of about 0.5 ppt are not harmful to freshwater plants. During the year, Lake Maurepas has its highest salinities in August or September, which are associated with tropical storm activity.

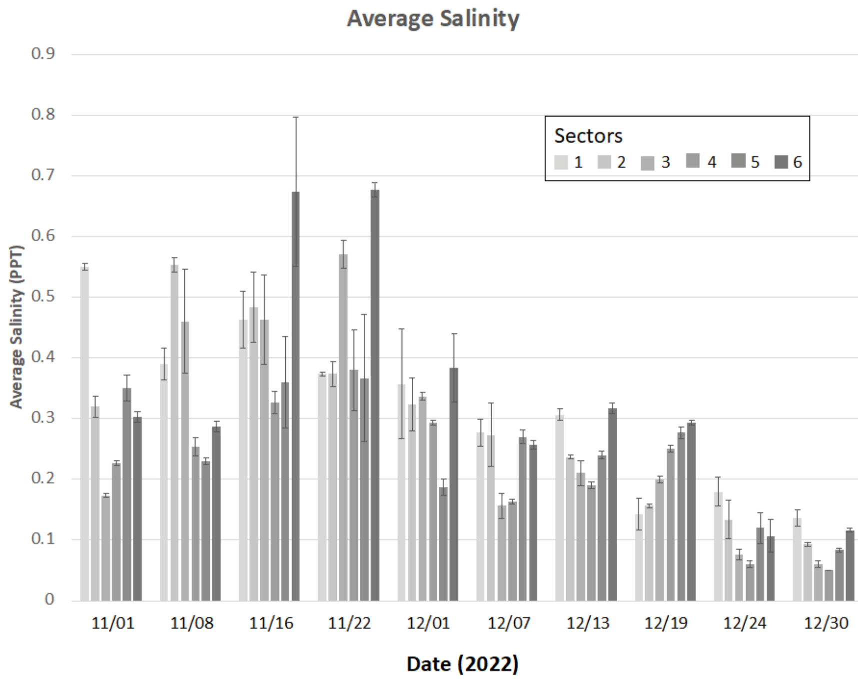

Figure 8. Salinity within each sector across time.

However, statistically salinity did differ by sector, with Sector 6 having the highest salinities and Sector 4 the lowest (Figure 9).

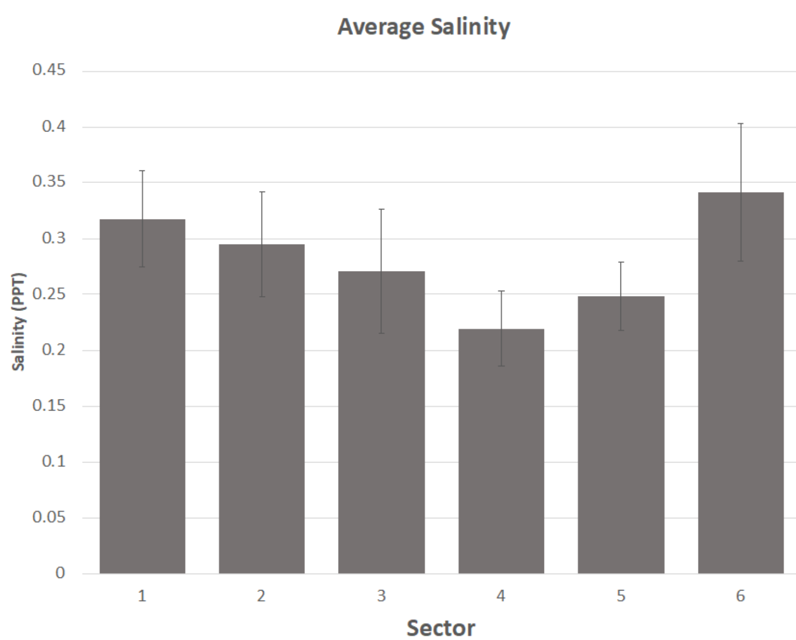

Figure 9. Water salinities for each sector.

With regard to time, salinity was widely variable (F = 72.232, $p < 0.0001$). The highest salinities occurred during the latter portion of November, whereas the lowest salinities occurred during the end of December (Figure 10).

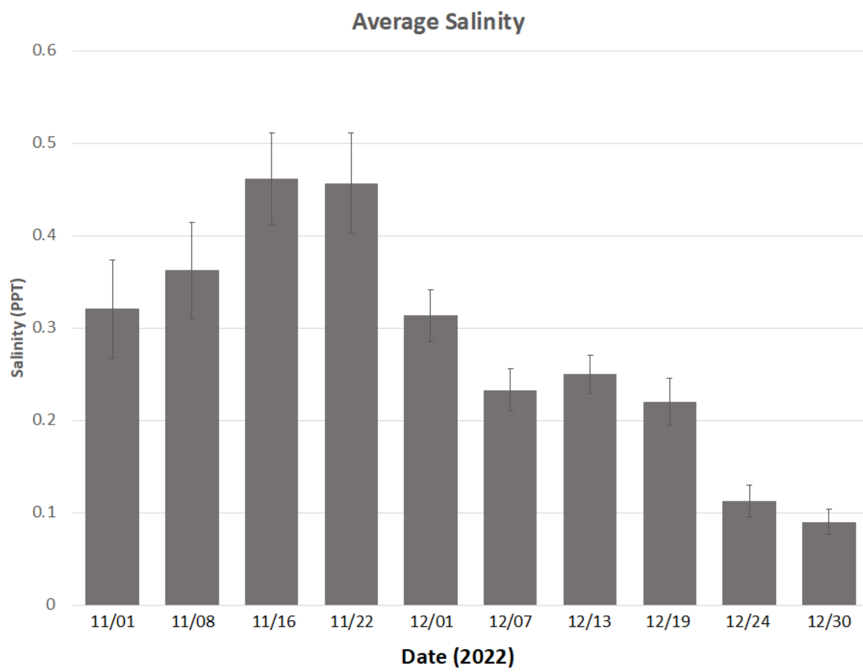

Figure 10. Water salinity across time showing statistically higher salinity during the latter part of November and very fresh conditions at the end of the year.

Water Temperature

On any one day the sectors are quite similar in water temperature (Figure 11).

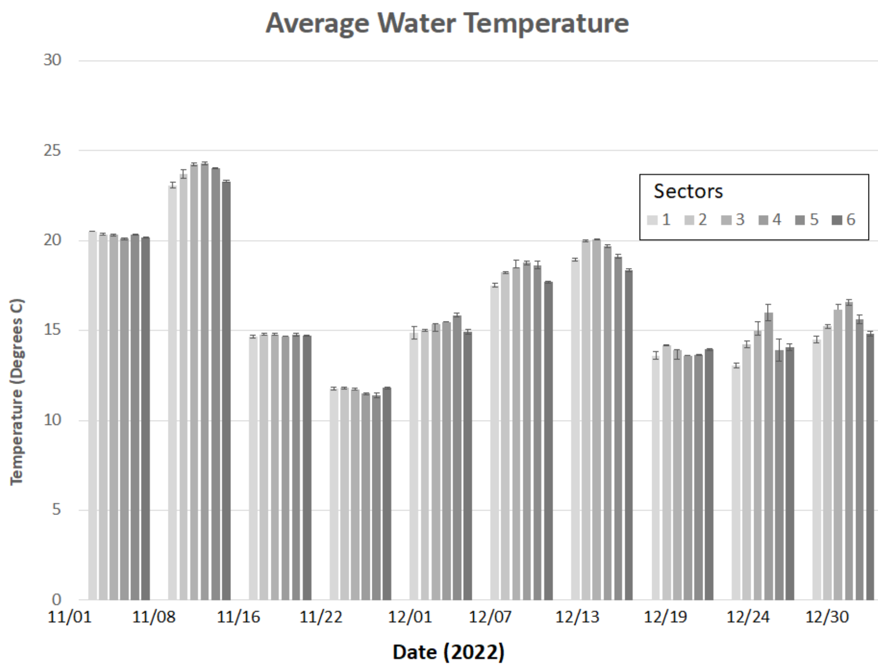

Figure 11. Water temperature within each sector across time.

As a whole the sectors varied widely from each other (F = 35.510, $p < 0.0001$), although this is not apparent by eye (Figure 12).

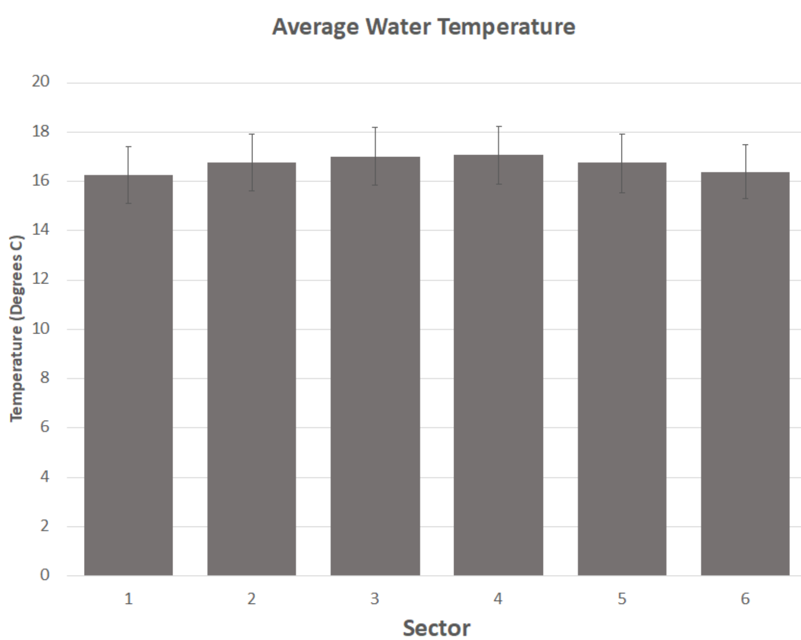

Figure 12. Water temperatures for each sector appear quite similar.

Across time, both the warmest and coolest water temperatures occurred in November and only differed by a 2-week period (Figure 13).

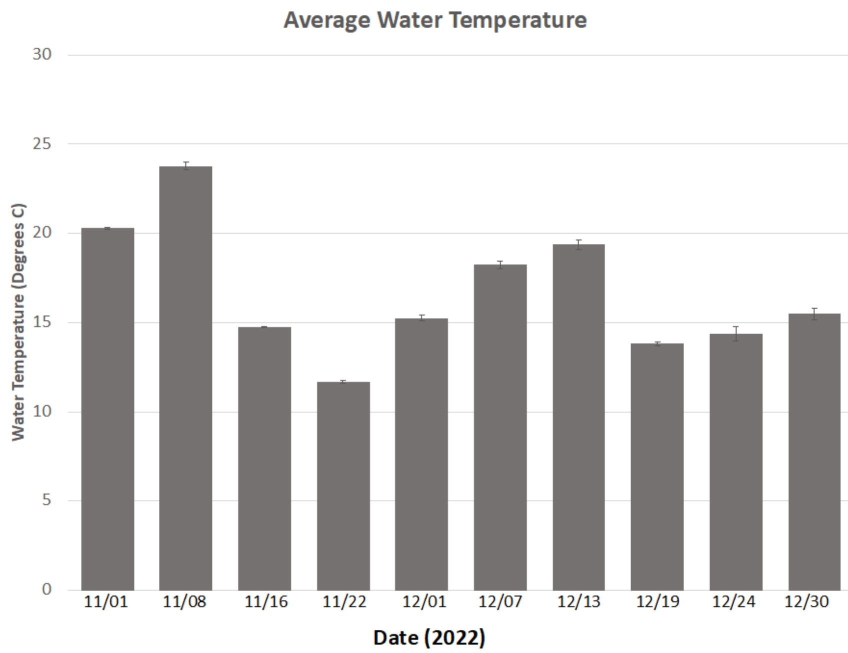

Figure 13. Water temperature for each time period averaged over sector.

pH

Of all of the abiotic variables, pH varied the least by sector across time (Figure 14) and was almost always near neutral.

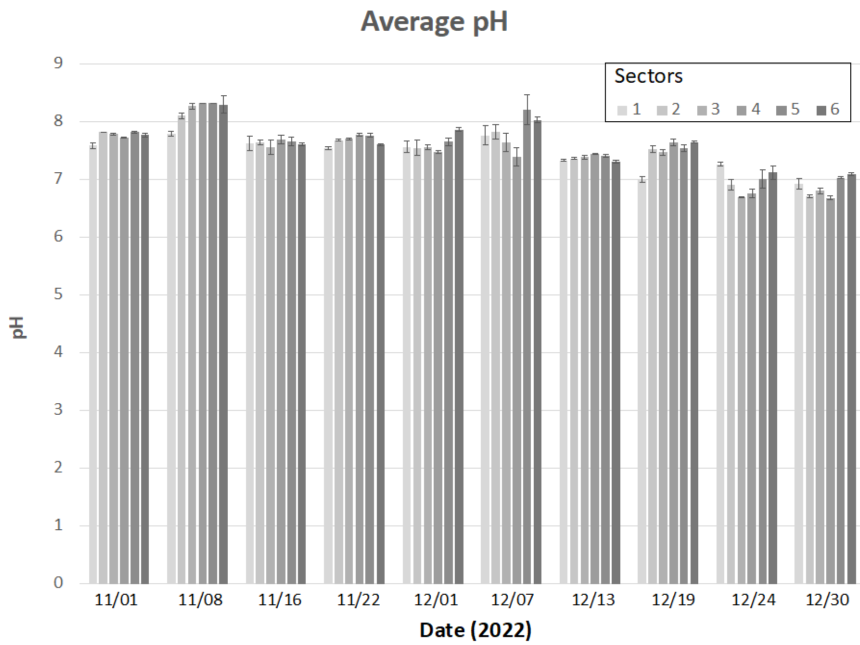

Figure 14. pH by sector and time.

Within each sector however, the replicates had high variation (see large standard error bars in Figure 15).

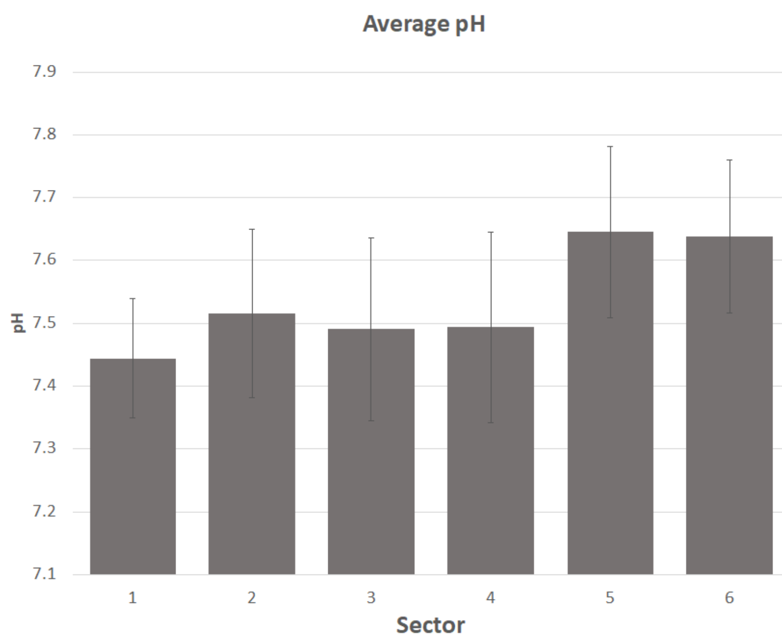

Figure 15. pH for each sector showing wide within-sector variability.

Unlike the wide within-sector variability of pH, sectors within a particular date varied little (Figure 16).

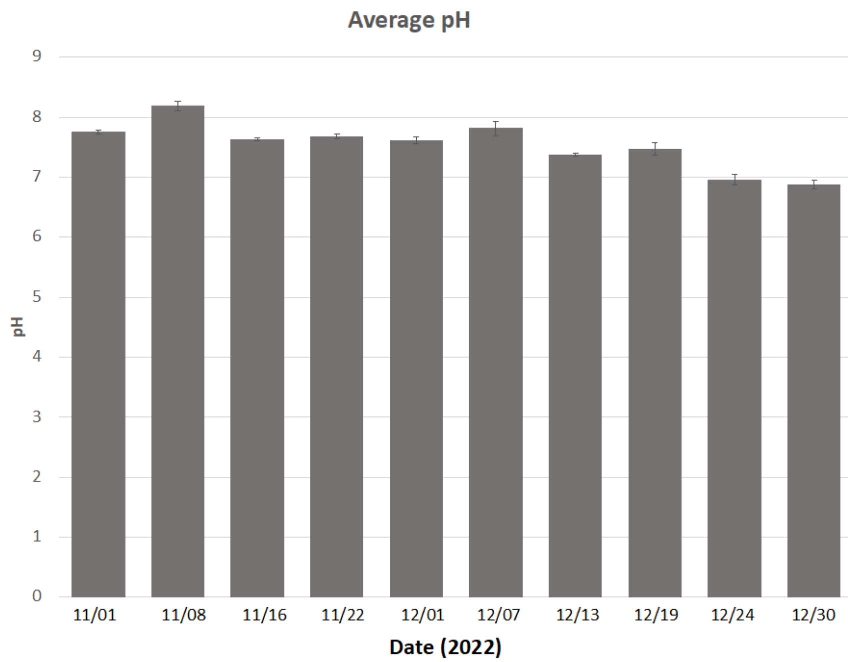

Figure 16. pH varied narrowly across time and even narrower within a particular time period.

Of the abiotic variables measured, turbidity showed the highest variability, averaging around 45 NTU, but displaying values higher than 70 NTU associated with high winds. Rarely does Lake Maurepas have lower turbidities than 40 NTU. The Lake was generally saturated with dissolved oxygen over the study period and the water was quite fresh, especially during December. Water temperature and pH were the least variable abiotic variables measured.