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DIFFERENCES IN THE GROWTH AND BIOENERGETICS BETWEEN CENTRARCHIDS IN A MIDWESTERN POWER PLANT LAKE

By

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ABSTRACT

Black Crappie *Pomoxis nigromaculatus* and Bluegill *Lepomis macrochirus* are popular sportfishes that co-occur in similar bodies of water throughout most of the Central and Eastern United States but have distinct thermal physiologies. *L. macrochirus* is often found in warmer regions of freshwater reservoirs, where *P. nigromaculatus* tends to exploit cooler regions, especially in power plant cooling reservoirs. The coal-fired Coffeen Power Station ceased energy production in October 2019, providing an ideal scenario to study the organismal effects of the sudden shift in thermal regime. Previous studies showed that *P. nigromaculatus* inhabited the less thermally-impacted areas of Coffeen Lake, whereas *L. macrochirus* were more broadly distributed across the lake. I hypothesized that more thermosensitive species (*P. nigromaculatus*) will be also reflected at the mitochondrial level, indicated by the properties of the energy transduction (how much oxygen and carbon substrates are coupled to ATP production) machinery. Both water quality and specimens were monitored between the fall of 2019 and the fall of 2020, with the objectives of assessing population structure, growth, and subcellular energetics, while simultaneously linking these traits to the changes in physicochemical properties of the reservoir after the thermal shutoff. Following the shutoff of thermal effluent in October of 2019, no values indicating unnatural stratification in the water column were observed. At the population and individual levels, this study found significant differences in organismal growth, with *L. macrochirus* displaying irregular, linear growth and were unable to have von Bertalanffy functions fit to them. Linear regressions run on fall 2019 Bluegill, spring 2020 Bluegill, and fall 2020 Bluegill showed r^2 values of 0.9845, 0.9961, and 0.9789 respectively, indicating a very high degree of fit to the data. Despite *P. nigromaculatus* and *L. macrochirus* seasonal fall data showing a statistically significant difference in distributions for length frequencies when compared using a Kolmogorov-Smirnov test ($p < 0.05$), both species displayed similar patterns in mortality. Total annual mortality values for fall Black Crappie, spring Bluegill, and fall Bluegill were 0.606, 0.774, and 0.732 respectively. When testing homogeneity of regression slopes on their respective catch curves, both species linear regressions for seasonal mortality proved to be similar to one another ($p > 0.05$). At the subcellular levels, this study found significant differences in mitochondrial function between species, assay temperatures, and seasons. Fall 2019 samples at 30˚C showed coupling ratios of ATP production divided by the Electron Transport System (ETS) capacity (P/E) of 0.96 for *P. nigromaculatus*, compared to 0.84 for *L. macrochirus*. Fall 2020 runs showed very similar significant findings, with noticeable interspecies differences in coupling efficiency and proton leakage also documented. These results show the Oxidative Phosphorylation System (OXPHOS) of *P. nigromaculatus* operating closer to the ETS capacity *in vitro*, compared to *L. macrochirus*. Interestingly, *L. macrochirus* mitochondria showed less coupling, indicating the species function less efficiently under a wider range of temperatures. Complex I OXPHOS during fall 2019 showed increased activity for Black Crappie (295.766 ± 69.241) when compared with Bluegill (79.616 ± 59.965; Two-way ANOVA, df = 17, $p = 0.033$). This increase in Complex I activity for Black Crappie could further solidify their energetic machinery as a mechanism for temperature tolerance, as this protein is known for its heightened sensitivity to temperature change. Results suggests that the lower occurrence of *P. nigromaculatus* in previously warmed sectors of the reservoir may be a consequence of the thermal sensitivity at the mitochondrial level.

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CHAPTER 1: GENERAL INTRODUCTION

Freshwater teleosts inhabiting lentic systems are often subject to challenging thermal regimes due to heated wastewater effluent release (Kesminas and Olechnovičienė 2008), nonnative introductions (Rooke et al. 2017; Whitney et al. 2021), and climate change (Stefan and Sinokrot 1993; Sharma et al. 2007; Mulhollem et al. 2016). Amongst the diverse fish community found in these bodies of water, the members of the sunfish family are of particular ecological and economical importance. The Teleost family Centrarchidae, more commonly known as the sunfishes, contains approximately 34 extant species that naturally occur throughout most of the eastern United States (reviewed by Cooke and Phillip 2009). Sunfishes often occupy lentic environments such as lakes or slower moving rivers around aquatic vegetation, generally having a deep or elongate bodied depending on the species (reviewed by Berra 2007). They are known to occupy separate ecological niches and differ in breeding habits and nesting behavior (Osenberg et al. 1992). Within the genus *Lepomis*, hybridization is common (reviewed by Etnier and Starnes 1993). Multiple sunfish species, including bass, crappie, and other sunfish, are considered predators in many systems, serving as important organisms for controlling the balance of lower trophic levels (reviewed by Carpenter and Kitchell 1993; reviewed by Cooke and Phillip 2009).

These species can also provide excellent angling opportunities due to their aggressive nature and body sizes (Near et al. 2003). Because of their appeal to the fishing industry, both recreational and tournament anglers result in strong economic incentives for the proper management of centrarchid populations (Ridgway and Phillipp 2002). These incentives are not just limited to gear purchases or related occupations, but encompass all the economic prospects (e.g. fuel, lodging, and other travel expenses) tied to the success of freshwater fisheries. Numbers taken in 1991 showed the total annual economic output from freshwater fishing alone was $46 billion (reviewed by Daily 1997).

Due to their popularity as sportfishes, many centrarchid species have been introduced into areas outside of their native ranges in North America, resulting in altered thermal regimes for individuals; these widespread transplants around the world have led to disputes as to the original ranges of various species (reviewed by Etnier and Starnes 1993; Copp et al. 2005; reviewed by Cooke and Phillip 2009). Introductions to unnatural thermal regimes have profound effects on the performance of individuals, and ultimately, the community structure of the species in the novel habitat. These introductions open the floor for an increased need to assess the physiological response of centrarchids to altered thermal regimes. Moreover, novel introductions, along with the pervasive effects of global climate change, pose unique challenges to these freshwater fishes and ectotherms in general, that can only be understood through integrative, multi-level studies assessing the ecology and physiology of each population.

Trends in data across multiple North American water bodies showed long-term increases in water temperatures for multiple sites, specifically near large metropolitan (Kaushal et al. 2010) and agricultural areas (Dai et al. 2022). Another notable example of thermally-altered habitats are power plant cooling reservoirs due to the heated discharge they release into aquatic systems. Heated effluent produces noticeable effects on the systems it discharges into, causing unnatural thermal regimes which can heavily influence water temperatures and stratification (Kesminas and Olechnovičienė 2008). The formation of thermal plumes in the water column, which can attract or repel fishes depending on the season, are frequent and persistent in these heated water bodies (Reutter and Herdendorf 1976). Previous studies have documented a strong link between laboratory and field preference of water temperatures in addition to avoidance responses of multiple fish species (Cairns and Cherry 1983). As thermally-impacted reservoirs being are subject to year-round discharge, the reproductive output, and growth rates of fishes inhabiting the reservoir are largely impacted (Barwick and Lorenzen 1984).

The passing of more stringent environmental policies by government agencies in addition to affordable, alternative energy sources have resulted in a reduction in the amount of coal-fired power plants still in use today. As more power plants decrease their electrical output and consequently their thermal loading (Johnson 2014; Russel et al. 2017), assessing the responses of aquatic species can provide a unique opportunity to examine a sudden change in their respective thermal regimes. Because these thermally impacted reservoirs simulate closed populations being continuously subjected to altered thermal regimes, results from these assessments serve as a sight glass of the organismal and physiological constraints placed by climate change for each species (Mulhollem et al. 2016).

Significant, species-specific studies have unveiled key physiological and ecological traits for centrarchids (reviewed by Cooke and Phillip 2009), although various knowledge gaps remain to be addressed, particularly in describing the mechanisms behind species-specific differences in thermal tolerances. While some sunfishes may appear similar in function and morphology, notable differences in performance have been documented even when comparing species within the same genus. Studies comparing Bluegill *Lepomis macrochirus* and Longear Sunfish *Lepomis megalotis* showed striking variations between the two species when comparing foraging success and thermal tolerance (Schaefer et al. 1999). Longear Sunfish had higher prey capture success in faster currents and higher variance in their loss of righting response (LRR) and the onset of opercular spasms (OS) compared to Bluegill, which exhibited higher values for both the LRR and OS measures. Data suggests although Bluegill may cope better with extreme water temperatures, Longear Sunfish showed greater phenotypic plasticity under thermal stress. Thermal tolerance is also influenced by hybridization, adding a complex factor to thermal physiology studies. For example, a study examining Bluegill, Pumpkinseed *Lepomis Gibbosus*, and hybrids between the two showed that the hybrids produced more reactive oxygen species (ROS) which damaged the mitochondria of hybrid individuals (Du et al. 2017). Additional studies on the aerobic capacity of Bluegill, Green Sunfish *Lepomis cyanellus*, and other centrarchids revealed significant differences in activity of a key metabolic enzyme, cytochrome c oxidase, between species, and across seasons (Bremer and Moyes 2011), suggesting that the molecular mechanisms behind their bioenergetics machinery may have a diverse set of regulatory pathways, influenced by environmental temperature. Results found in these studies emphasize that different species, despite being similar in taxonomy or ability to hybridize, have distinctive physiologies and operate very differently at the cellular and sub-cellular levels. Considering this, the next chapters of this thesis are aimed to describe the population structure of two important centrarchid species in a thermally impacted lake, and to describe the role of the sub-cellular bioenergetic machinery on the species-specific thermal performance.

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CHAPTER 2: POPULATION STRUCTURE AND GROWTH PERFORMANCE OF BLACK CRAPPIE AND BLUEGILL AS A FUNCTION OF PRE AND POST THERMAL EFFLUENT SHUTDOWN IN COFFEEN LAKE

INTRODUCTION

Environmental temperature is a pervasive influence on the distribution and abundance of freshwater fishes (Brett 1971; Dent and Lutterschmidt 2003; reviewed by Chung and Schulte 2020). Alterations of thermal regimes are reflected in the biology of fishes at multiple organizational levels, ranging from individual biochemical processes to the whole-organism level. Ultimately, habitat temperature influences the performance of individuals within a reproductive stock of a species, with the capacity of transforming the reproductive output and overall, the population structure of the species. In thermally-impacted cooling reservoirs, the population structure of fishes is subject to the year-round increment in temperature, which significantly alter the metabolic demands, growth and population structure of the species, if compared to conspecifics inhabiting non-thermally impacted reservoirs (Martinez et al. 2015)

Coffeen Lake is a 1,100 acre power plant cooling reservoir located in Southern Illinois that has been in operation and open for public fishing since 1986 (Figure 1). The reservoir's thermal plume effluent affects up to 75% of its water mass throughout the year since 1986. Recently, the power plant was scheduled to close operations by October of 2019, eliminating the thermal discharge immediately after closure and providing an opportunity to investigate changes in fish populations as the reservoir returns to a more natural temperature regime.

Previous studies assessed sportfish populations in Coffeen Lake, documenting the abundance of notable species inhabiting the lake including Black Crappie *Pomoxis nigromaculatus*, Bluegill, Redear Sunfish *Lepomis microlophus*, Channel Catfish *Ictalurus* *punctatus*, Largemouth Bass *Micropterus salmoides*, and White Crappie *Pomoxis annularis* (Porreca 2012). This study showed the average catch per unit effort (CPUE) (± Standard Error) for Black Crappie in the cooling loop and ambient zone were 0.8±0.3 and 4.2±1.7 respectively, while Bluegill had a CPUE of 214±39 and 132±15 respectively for the cooling loop and ambient zone (Porreca and Colombo 2012). Black Crappie, considered to be more thermally sensitive, were found more frequent in the ambient zone which was sheltered from the thermal effluent being released at the other end of the lake. Bluegill are thought to occupy the warmer end of the thermal tolerance spectrum and were found more frequently in areas of the cooling loop and transition zone, places more affected by heated effluent. Bluegill being frequently found in thermally impacted areas of power plant lakes has also been documented in the past, with individuals found concentrated near the effluent-outfall area (Neill and Magnuson 1974).

The objective of this study was to compare the population structures of Back Crappie and Bluegill found in Coffeen Lake pre and post thermal effluent shutoff. Since previous studies have shown a higher thermal tolerance in Bluegill with concomitant tradeoffs in growth performance (Martinez et al. 2015), the removal of the thermal effluent in Coffeen Lake was expected to improve size-at-age values for Bluegill. On the other hand, Black Crappie should not show as many significant growth differences, since they appeared to be more restricted to the less thermally impacted regions of the reservoir beforehand. Investigating two species that occupy different ends of the thermal sensitivity spectrum provide a more complete picture of how varying thermal regimes affect organismal growth and mortality.

METHODS

Field Sample Collection and Specimen Processing

I sampled Black Crappie and Bluegill from each zone (cooing loop, transition zone, and ambient zone) in Coffeen Lake (Figure 1) with DC electrofishing and modified-fyke nets. Fish sampled from both gears were combined for data analyses purposes when appropriate (Figures 8-15). Pulsed DC boat electrofishing was done with an ETS Boat Electrofishing System in 30 minute transects in each zone by following the Long Term Resource Monitoring Protocol (Gutreuter et al. 1995); this occurred in fall 2019, spring 2020, and fall 2020. During fall 2019 a total of eight modified-fyke nets were placed perpendicular to suitable shoreline across all zones at random locations. The dimensions of the nets used were 0.9 m by 1.8 m with 13 mm bar mesh and 15.2 m lead lines. I distributed them with three placed in the cooling loop, three in the transition zone, and two in the ambient zone. Nets were placed for one night and then recovered after 24 hours of deployment. Modified-fyke netting only occurred for the fall 2019 sampling to try and bolster Black Crappie sample size. Each individual sampled had their total length (mm) and total weight (g) taken, with some Black Crappie and Bluegill kept alive in aerated coolers for further lab work and the rest frozen for future dissection.

Water quality locations were selected based on previous research sampling points for comparative purposes (Porreca 2012); two water quality points were chosen in each of the three zones (cooling loop, transition zone, and ambient zone) and were monitored using a YSI Pro DSS (Figure 1). I documented variables that included water depth, water temperature, dissolved oxygen, specific conductivity, conductivity, pH, and Secchi depth; while water column profiles were created by using a 10-meter cord marked with one-meter increments starting from the YSI Pro DSS probe. Data for water quality was recorded during each individual sampling event during spring and fall, with additional outings occurring in summer and winter to gather habitat data. Coordinates taken for sampling were recorded on a Garmin GPSmap 62s before being transferred to ArcMap 10.8.1 for mapping and visualization purposes.

Laboratory Procedures for Collected Specimens

Frozen fishes were thawed and then had both sagittal otoliths extracted by cutting the isthmus and accessing the head cavity from the ventral side; the removed otoliths were then cleaned, dried, and placed in labeled micro centrifuge tubes. I counted growth rings under a Leica S8AP0 microscope with varying 10-30X zoom and used a black background for contrast. Annuli were tallied beginning at the nucleus and then counting prominent year marks to the extent of the otolith surface, and edge rings were only counted on spring otoliths. To ensure annuli judgements were consistent, counted rings were required to appear in three of four quadrants. Otoliths were aged separately by two readers, with disagreements resulting in a single consensus age. Otoliths from both species were weighed (to the nearest 0.01 milligram) on a Denver Instrument APX-200 Analytical Balance. This was done to investigate the strength of the relationship between age and average otolith weights, with the idea that fishes subjected to unnatural thermal regimes might be more easily aged with alternative methods if annuli become harder to read when constantly subjected to heated effluent. Any damaged or missing otoliths were not counted in this process, with each individual bone inspected to reduce error.

Data Analyses

Length frequency data for both species had Kolmogorov-Smirnov tests performed between fall Black Crappie, spring Bluegill, and fall Bluegill. This was done to compare their distributions and determine whether they were statistically similar or different from one another. The status of each species' population can come in the form of their Proportional Size Structure

(PSS), regarding both the PSS-Q and PSS-P values, with other terms like Proportional Stock Density (PSD) sometimes substituted instead. PSD is calculated to show the proportions of various size-classes of fish (Gabelhouse 1984a; Gustafson 1988). This allows fisheries managers to have a quantitative measurement for how certain populations are faring and distributed in terms of total lengths. PSS-Q stands for the number of fish greater than or equal to quality length divided by the number of fish greater than or equal to stock length, times one hundred. This can then be substituted out in the case of PSS-P, PSS-M, and PSS-T, where preferred (or any other size category) individuals are used instead of quality ones in order to find the specific ratio. By having multiple descriptive size categories for different species, a better understanding of stock structure can be obtained (Gabelhouse 1984a). PSS values for fall Black Crappie were then compared using a chi-square test for observed frequencies in Coffeen Lake versus expected frequencies considered to be desirable for Midwest impoundments, allowing experimental and theoretical values to be compared (Gabelhouse 1984b; Franke et al. 2012). Murphy et al. 1991 was also used for creating relative weight equations with corresponding figures.

Weighted catch curve analyses were performed on both species to look at seasonal survivorship, resulting in their sample size being natural log transformed as it related to their age class. This frequency distribution for fish age gives scientists vital information on the specific population being examined. The main assumption states there is a geometric decrease in population numbers as fish age, meaning that the target population is in a state of near equilibrium due to births and deaths balancing each other out (Chapman and Robson 1960). Homogeneity of regression slopes were tested between seasonal catch curves for Black Crappie and Bluegill, allowing for statistical differences in interaction for each group between covariate (age class) and dependent variable (natural log transformed sample size) to be compared.

When appropriate, a von Bertalanffy growth function was fit to seasonal Black Crappie data, with age classes containing an extremely low number of individuals excluded from the regressions. These growth functions convert the abstract characteristics of organismal growth into quantitative increases that can be formulated and expressed (von Bertalanffy 1938). Due to the irregular growth patterns seen in Bluegill, linear regressions were fit to their growth data as opposed to using other functions. Homogeneity of regression slopes were tested between seasonal regressions for Bluegill, allowing for statistical differences in interaction for each group between covariate (age class) and dependent variable (mean length) to be compared.

Otolith mass versus age class graphs for both species also contained regressions for the plotted data. Fall Black Crappie data had an exponential rise to maximum two parameter regression model, while Bluegill data was again fit with linear regressions in comparison.

RESULTS

Fall 2019 efforts collected 22 Black Crappie and 169 Bluegill from fyke netting as well as 10 Black Crappie and 50 Bluegill from electrofishing. 1 Black Crappie and 207 Bluegill were gathered from Spring 2020 electrofishing, with 138 Black Crappie and 200 Bluegill for fall 2020 electrofishing.

Water Quality

The first water quality sampling event was done in October 2019, immediately before the thermal discharge was shut down completely. When comparing the temperature and dissolved oxygen values of that outing to a post shut down state, the values were very different from one another (Figures 2 – 7). Having the thermal effluent present in the hot arm water mass caused the water body close to the discharge to remain more stratified in the fall months when vertical mixing should have been taking place (Figure 5, October 2019 Pre-shutdown). In terms of water quality profiles, fall 2019 pre shut down values most closely resembled the data observed in spring 2020, showing that there were substantial thermal impacts occurring in the southern portion of the reservoir due to the discharge. Despite these observations in other portions of the lake, both water quality points observed in the ambient zone remained largely unaffected by the loss of thermal effluent in Coffeen Lake (Figures 6 and 7).

Length Frequency

Length frequencies for Black Crappie and Bluegill sampled by electrofishing showed that the two species had much different distributions in terms of size categories (Figures 8-10). Black Crappie sampled via electrofishing surveys varied considerably in size (62 – 326 mm) and were sampled up to lengths considered memorable (300 mm). However, Bluegill sampled were more noticeably centered around stock lengths (80 mm). Fall 2019 and fall 2020 Black Crappie had a mean and SEM (Standard Error of the Mean) of 202.6 ± 5.3 mm with a median of 199 mm, while Bluegill had a mean and SEM of 100.6 ± 1.4 mm with a median of 101 mm. Kolmogorov-Smirnov tests performed for distribution similarity were run between fall Black Crappie and fall Bluegill, spring Bluegill and fall Bluegill, and fall Black Crappie and spring Bluegill. All of these came back as statistically significant ($p < 0.05$), meaning that the compared sets of samples were not drawn from the same distribution.

Fall 2019 and fall 2020 Black Crappie had a PSS-Q of 58 and PSS-P of 42, while spring 2020 Bluegill had a PSS-Q of 3 and PSS-P of 0.6 and fall 2019 and fall 2020 Bluegill had a PSS-Q of 1 and PSS-P of 0. Performing a chi-square test for fall Black Crappie between expected desirable PSS values (PSS-Q of 45 and PSS-P of 15) and actual values (PSS-Q of 58 and PSS-P of 42) showed a significant difference between the experimental and theoretical values compared against one another ($df = 1$, $p < 0.001$).

Catch Curves

Weighted catch curves for both species showed that fall Black Crappie were expected to live longer (at a theoretical maximum age of 5.9 years) and had a higher total annual survival (0.394) when compared with values seen from both seasonal bluegill data sets (Figures 11-13). Spring 2020 Bluegill had a theoretical maximum age of 4.8 years with a total annual survival of 0.226, while fall 2019 and fall 2020 Bluegill had a theoretical maximum age of 4.8 years with a total annual survival of 0.268. Despite both species displaying different data for mortality values, neither were observed to have many long-lived individuals past the age of 4 years. Homogeneity of regression slopes tests revealed that the linear regressions tied to the descending splines of the catch curves for fall Black Crappie, spring Bluegill, and fall Bluegill were all similar to one another, meaning no statistical significance was occurring among any of the comparisons ($p > 0.05$; Table 1).

Growth Functions

The fall 2019 sampling for Black Crappie did not have an adequate sample size to fit a growth curve, so a mean length at age graph was compiled for the individuals (Figure 14). Spring 2020 also had an issue with sample size for Black Crappie with only one individual sampled, so no plot was able to be constructed for that season. Fall 2020 Black Crappie were able to be fit with a von Bertalanffy growth function, showing older aged organisms on average were reaching a Memorable size (Figure 15). The calculated L infinity (L inf), or individual length at which growth equals zero, for fall 2020 Black Crappie was 297.020 mm.

Fall 2019 Bluegill were unable to have a von Bertalanffy growth curve fit to them due to their irregular, almost linear, growth (Figure 16). Spring 2020 and fall 2020 Bluegill also portrayed similar irregular growth patterns but were able to be fit with a von Bertalanffy growth function (Figures 17 and 18). However, the calculated L infinities for spring 2020 and fall 2020 Bluegill were 568.889 mm and 367.134 mm respectively. It should be noted that while these values were both calculated within a desired fit to their mean length at age data gathered, the probability of Bluegill attaining these sizes in Coffeen Lake is unlikely to occur. Because of this, all seasonal mean length at age graphs for Bluegill were fit with linear regressions that more appropriately displayed the data. Homogeneity of regression slopes tests for Bluegill revealed that each season (fall 2019, spring 2020, and fall 2020) had a statistically significant difference ($p < 0.05$) from one another when comparing their respective growth rates (Table 2).

Otolith Mass

Figures 18-21 show the sagittal otolith mass versus relationships for each species during the different seasons. Fall 2019 and fall 2020 Black Crappie had the following average values in otolith mass for age groups: age 0 – 6.07 ± 0.55 mg, age 1 – 28.55 ± 0.97 mg, age 2 – 72.05 ± 2.76 mg, age 3 – 85.01 ± 3.93 mg, age 4 – 71.65 ± 0 mg. Fall 2019 and fall 2020 Bluegill had the following values in otolith mass for age groups: age 0 – 5.53 ± 0.38 mg, age 1 – 8.40 ± 0.23 mg, age 2 – 13.57 ± 0.31 mg, age 3 - 21.66 ± 1.00 mg, age 4 – 21.4 ± 0, age 5 – 35.95 ± 0. Spring 2020 Bluegill had the following values in otolith mass for age groups: age 0 – 1.18 ± 0.14 mg, age 1 – 5.20 ± 0.22 mg, age 2 – 9.41 ± 0.22 mg, age 3 – 18.45 ± 1.08 mg, age 4 – 24 ± 0 mg, age 5 – 35.6 ± 3.3 mg. The fall Black Crappie otolith graph with an exponential rise to maximum two parameter regression had an equation of $y = 89.524*(1 – e^{-0.608x})$ with an r^2 of 0.8969. Linear regressions performed on spring Bluegill had an equation of $y = 27.175x + 47.941$ with an r^2 of 0.9961 and fall Bluegill had an equation of $y = 20.577x + 72.237$ with an r^2 of 0.9789. Regressions performed on otolith mass for both species showed high r^2 values, indicating a strong fit between the data and their fitted lines for all seasons.

DISCUSSION

Water Quality

Seasonal sampling of water quality values for Coffeen Lake showed there to be noticeable changes in pre versus post shutdown values. Profiles showed less severe stratification occurring during the cooler months of fall, likely as the result of more mixing occurring due to the loss of constant thermal discharge. This was seen primarily in the cooling loop and adjacent transition zone, with the ambient zone remaining consistent in values and staying largely unaffected by the loss of effluent in the reservoir.

Length Frequency

On average, Bluegill were sampled at sizes approaching stock lengths, while Black Crappie were found to have a much broader range of size categories, even reaching up to Memorable sized fish (300 mm). Interspecific size distributions and overall population structure were notable, showing distinctive growth patterns that supported my predictions based on differences in thermal tolerance.

Past research on crappie populations has shown that a PSS-Q of 30-60% and a PSS-P of 10-20% would be considered desirable standards in small, Midwest impoundments (Gabelhouse 1984b). Based off the fall 2019 and fall 2020 data taken from Coffeen Lake (PSS-Q = 58, PSS-P = 42), these recorded PSS values that exceed the desirable Gabelhouse values show that there is a diverse and desirable population of Black Crappie present in Coffeen Lake. This can be backed up by the statistically significant chi-square test which was performed to compare the expected and observed PSS values of fall Black Crappie just listed above. In comparison, Bluegill are thought to have balanced populations when achieving PSS-Q values between 50-80% (Anderson 1985). Bluegill for spring 2020 had a PSS-Q of 3 and PSS-P of 0.6 and fall 2019 and fall 2020 had a PSS-Q of 1 and PSS-P of 0. The size structure of a population has been suggested as one of the key factors influencing Bluegill growth within small impoundments (Neely et al. 2020); however, neither of these come close to the balanced population values described by Anderson. While studies have pointed towards overexploitation as a leading cause of undesirable size structures for Bluegill (Coble 1988; Rypel 2015), the lack of targetable individuals within Coffeen likely points to other factors. Because of the extremely low PSS-Q and PSS-P values seen in Bluegill, it is unlikely that anglers would be targeting fish such as those found in such small size categories.

Catch Curves

Both species have been documented displaying similar growth and survivorship patterns, with individuals possibly reaching up to eight years of age in balanced populations (Ellison 1984). Between the Black Crappie and Bluegill sampled, neither population contained large numbers of long-lived individuals despite their distinct differences in other population variables like organismal growth and length frequency distribution. Comparing all of the seasonal catch curves with homogeneity of regression slopes tests revealed that none of them statistically differed from one another. This similarity in mortality values could have multiple explanations. Based off numerous interactions with anglers at Coffeen Lake, it appeared that Black Crappie had more fishing pressure exerted on them compared with Bluegill, which agrees with previous findings (Porreca 2012). This could be a result of the Bluegill population failing to reach desirable sizes for harvest, likely resulting in very little angling mortality occurring. In addition, Bluegill showed distinct bioenergetics tradeoffs as a result of their thermal physiology which could help explain their shortened lifespans and will be further expanded on in the next chapter.

The survivorship curves for Bluegill show low mortality in younger aged fish, and a high mortality past the age of two years. Comparing this observation with previous studies shows that the trend for both spring and fall curves depict an unbalanced Bluegill population due to the lack of longevity (Anderson 1973). Truncated lifespans for Bluegill in the lake are not a new occurrence in Coffeen (White et al. 2020), with this trend occurring in heated environments with other sunfish species as well (Dembski et al. 2006). Desirable Crappie populations for harvest in Nebraska reservoirs were described as having consistent recruitment, a moderate mortality of intermediate-size fish, and fast growth rates up to 230 mm long during the third year of life (Ellison 1984). The Coffeen Lake Black Crappie population partially resembled this description, but also showed a high amount of mortality occurring after individuals reached age three, resulting in few older aged fish. High mortality after age three may likely be the result of fishing pressure on the Black Crappie population within Coffeen Lake, with research showing that angling can produce very noticeable effects on Black Crappie in impoundments (Willis et al. 1994). Since they are reaching size categories that would be much more appealing to anglers when compared to the sizes seen in Bluegill, it seems plausible that larger specimens are being targeted by hook and line.

Growth Functions

Bluegill growth curves across all three seasonal samplings showed irregular, linear growth occurring throughout the course of this study. The observed growth models for Bluegills pre and post thermal effluent shut off were statistically different, suggesting a direct effect of the change in thermal regime to the growth performance of the species. An irregular growth pattern in the Coffeen Lake Bluegill population has been documented before in 2015, where a constant growth rate across age classes was observed. In the study, it was hypothesized that shifts in prey selection, limited prey availability, or energetic drawbacks from the thermal regime were restricting growth rates (Martinez et al. 2015). Results from my study supports the possible effect of thermal effluent in the growth performance of the species in Coffeen Lake. Additionally, studies have shown size structure directly affecting the growth of Bluegills, suggesting this relationship may also provide possible answers for the population in Coffeen Lake (Tomcko and Pierce 2005; Neely et al. 2020).

While Black Crappie did experience rapid development in their first two years of life, growth performance was relatively low after passing that age mark. The growth curve displayed in Black Crappie fit the classic von Bertalanffy equation, suggesting that the species growth performance was unaltered by the thermal input in the reservoir. It should also be pointed out that the Black Crappie mean length at age data was able to be fit with a von Bertalanffy. A previous study in a Nebraska reservoir described a balanced Black Crappie population as having more growth past age three and higher longevity overall, with unbalanced populations having high mortality past age two (Ellison 1984). Black Crappie in Coffeen Lake appear to fit the latter description as they were never experiencing heightened growth during or after age 3 and up, as most individuals were removed from the population before reaching age 4. So, despite being able to reach desirable sizes within those growth patterns, Black Crappie displayed characteristics of what may be considered an unbalanced population.

Otolith Mass

A strong relationship between fish age and otolith mass for both species was also observed. Interestingly, otolith mass models parallel the growth models for each species; Black Crappie data better fit with a non-linear regression (exponential rise to maximum two parameter regression) versus Bluegill which better fit with a linear regression. Associations such as this have been documented in other studies, further emphasizing a relationship and possible advantages for using otolith weight to estimate fish age (Lepak et al. 2012; Pacheco et al. 2021). Doing so could provide a cheaper and easier route to aging individuals, even allowing for a streamlined process for the structures of difficult to read fishes; as some species at various life stages may prove harder to age by purely observing annuli rings. Other confounding factors that could lead to harder to age otoliths might include varying annual maturity cycles or inconsistent growth patterns. Additionally, species exposed to unnatural thermal regimes, as in the case of aquatic organisms under the stress of heated effluent, could benefit from this knowledge. Heated environments might see more consistent growth over the course of a year as opposed to slower growth during the winter in temperate reasons; making these, as well as otoliths from tropical environments, more difficult to accurately read (reviewed by Stevenson and Campana 1992). Since these metrics are obtained by objectively measuring structures as opposed to including human bias from readers, it could provide a more accurate understanding of the age groups in a specific population (Pawson 1990). Because the individuals taken from Coffeen Lake had distinct annuli markings, these could be used as an example for more complicated samples in future studies.

Black Crappie displayed a larger array of size categories when compared with that of Bluegill, which were mostly collected around stock sizes. Additionally, growth patterns for Bluegill more closely resembled an irregular, linear pattern when compared with the traditional von Bertalanffy function which was able to be fit to Black Crappie data. Both populations displayed high mortality values, although that is hypothesized to be stemming from two different causes. All these patterns point towards Black Crappie and Bluegill dealing with altered thermal regimes in different manners, further explored by examining their distinct thermal physiologies.

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Table 1. Homogeneity of slopes results for catch curve comparisons.

Fall Black Crappie versus Fall Bluegill					
	DF	SS	MS	F	P
Regression	2	12.7921501	6.39607505	3.318950355	0.096878366
Residual	7	13.48996537	1.92713791		
Total	9	26.28211547			
Spring Bluegill versus Fall Bluegill					
	DF	SS	MS	F	P
Regression	2	11.27934317	5.639671584	2.179271029	0.175586446
Residual	8	20.70296539	2.587870673		
Total	10	31.98230856			
Fall Black Crappie versus Spring Bluegill					
	DF	SS	MS	F	P
Regression	2	7.148157317	3.574078659	1.387775153	0.303810034
Residual	8	20.60321458	2.575401822		
Total	10	27.75137189			

Table 2. Homogeneity of slopes results for Bluegill growth linear regressions.

Fall 2019 Bluegill versus Spring 2020 Bluegill					
	DF	SS	MS	F	P
Regression	2	17465.83477	8732.917387	92.79749335	2.91597E-06
Residual	8	752.8580415	94.10725518		
Total	10	18218.69282			
Spring 2020 Bluegill versus Fall 2020 Bluegill					
	DF	SS	MS	F	P
Regression	2	16897.77197	8448.885985	161.3226982	3.42697E-07
Residual	8	418.9806433	52.37258041		
Total	10	17316.75261			
Fall 2019 Bluegill versus Fall 2020 Bluegill					
	DF	SS	MS	F	P
Regression	2	9472.415209	4736.207605	166.3528769	1.25598E-06
Residual	7	199.2959415	28.47084879		
Total	9	9671.711151			

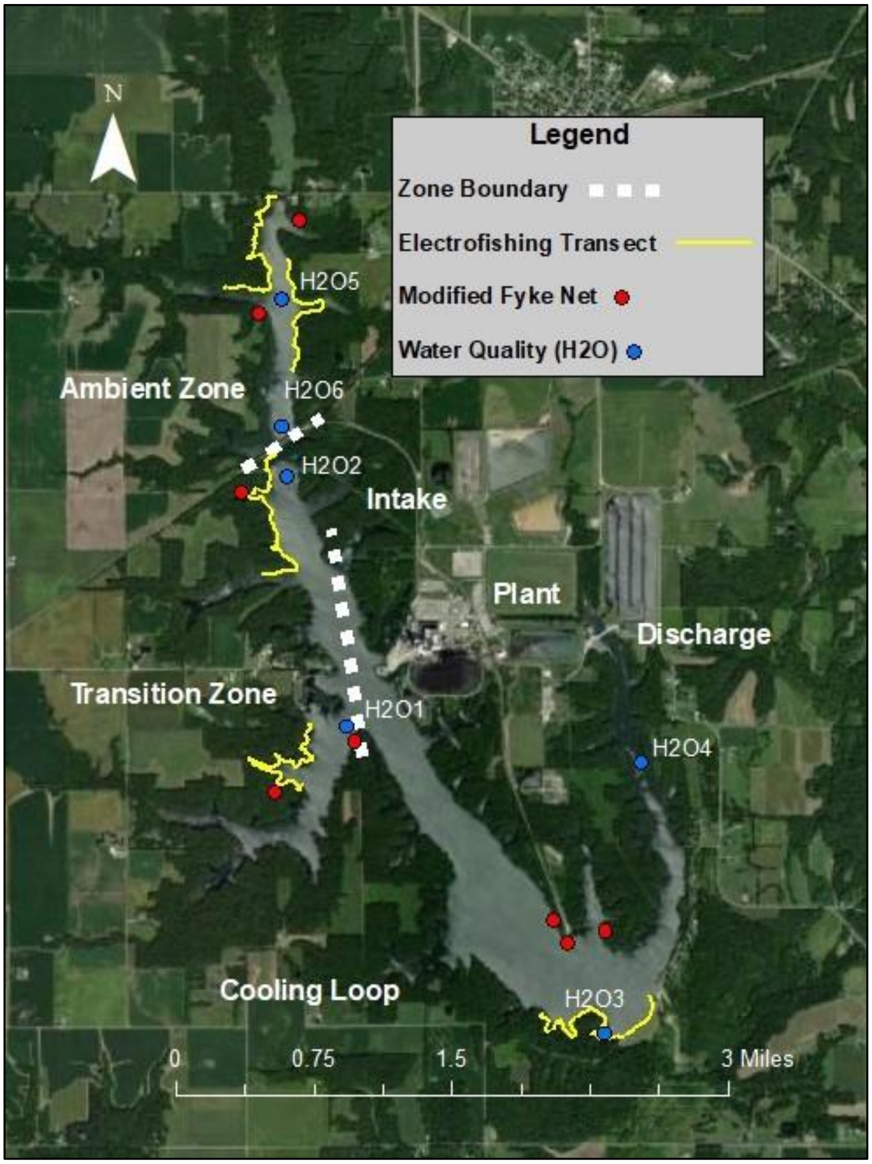

Figure 1. Labeled Map of Coffeen Lake located near Coffeen, Illinois.

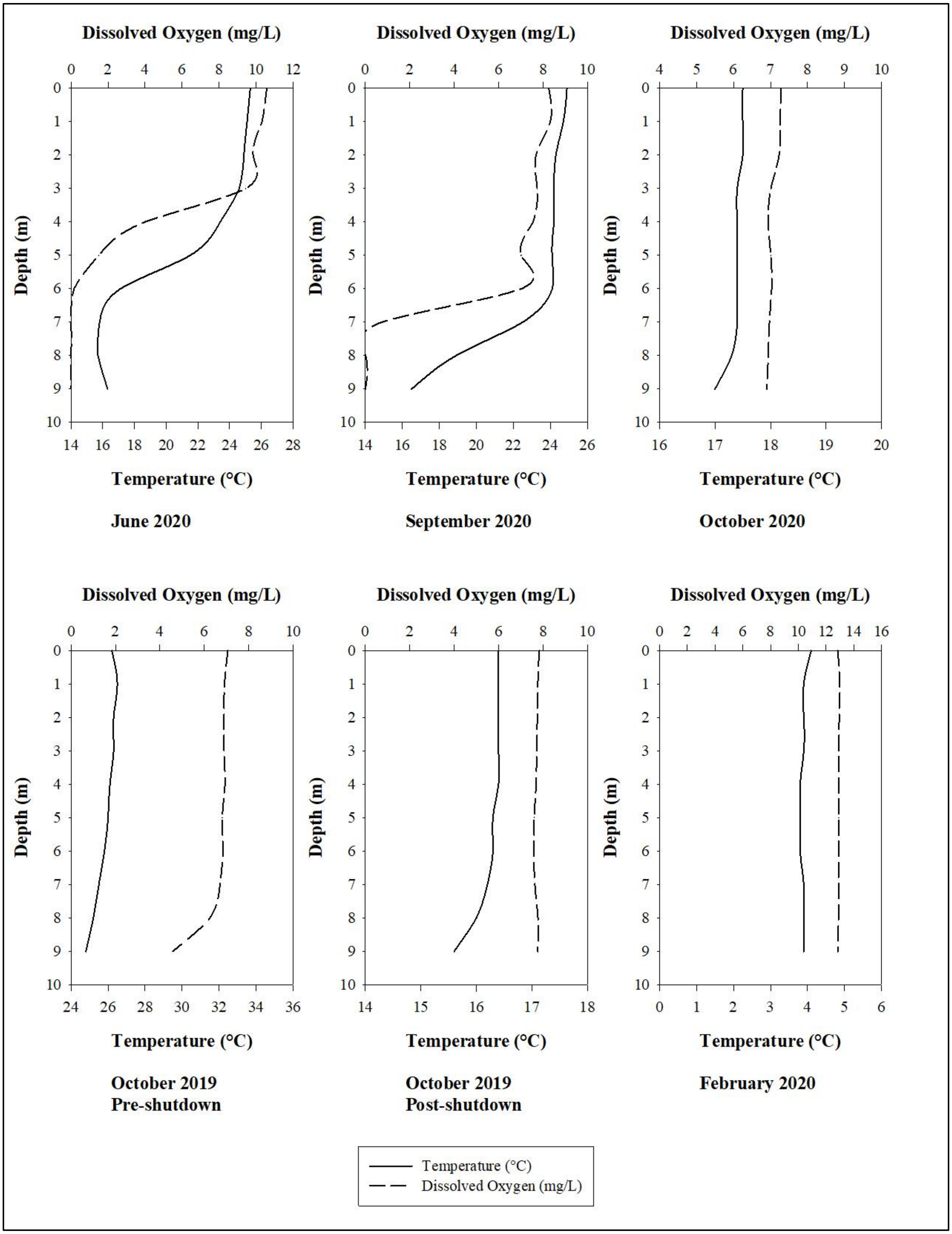

Figure 2. H2O1 depth profiles taken from the Coffeen Lake transition zone.

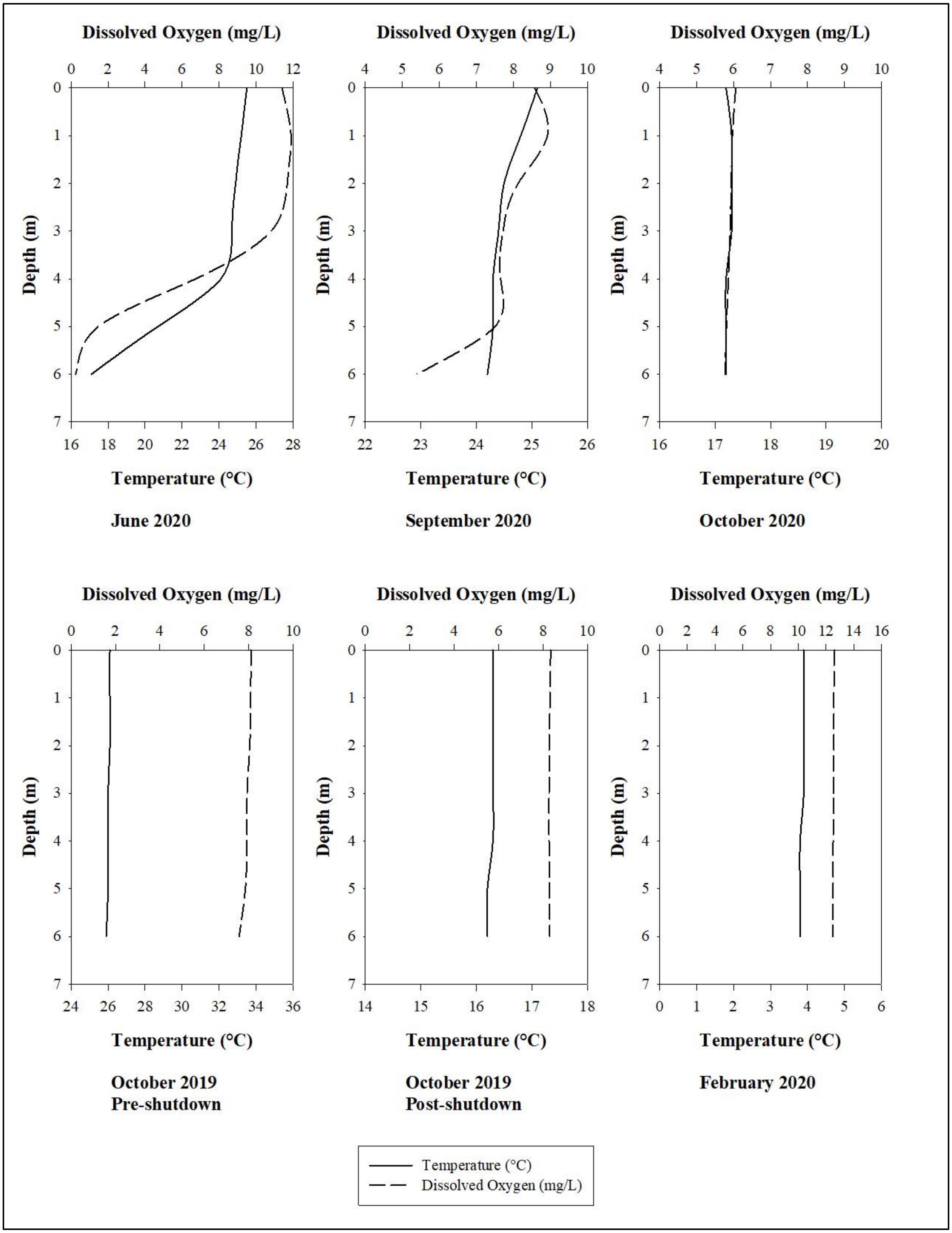

Figure 3. H2O2 depth profiles taken from the Coffeen Lake transition zone.

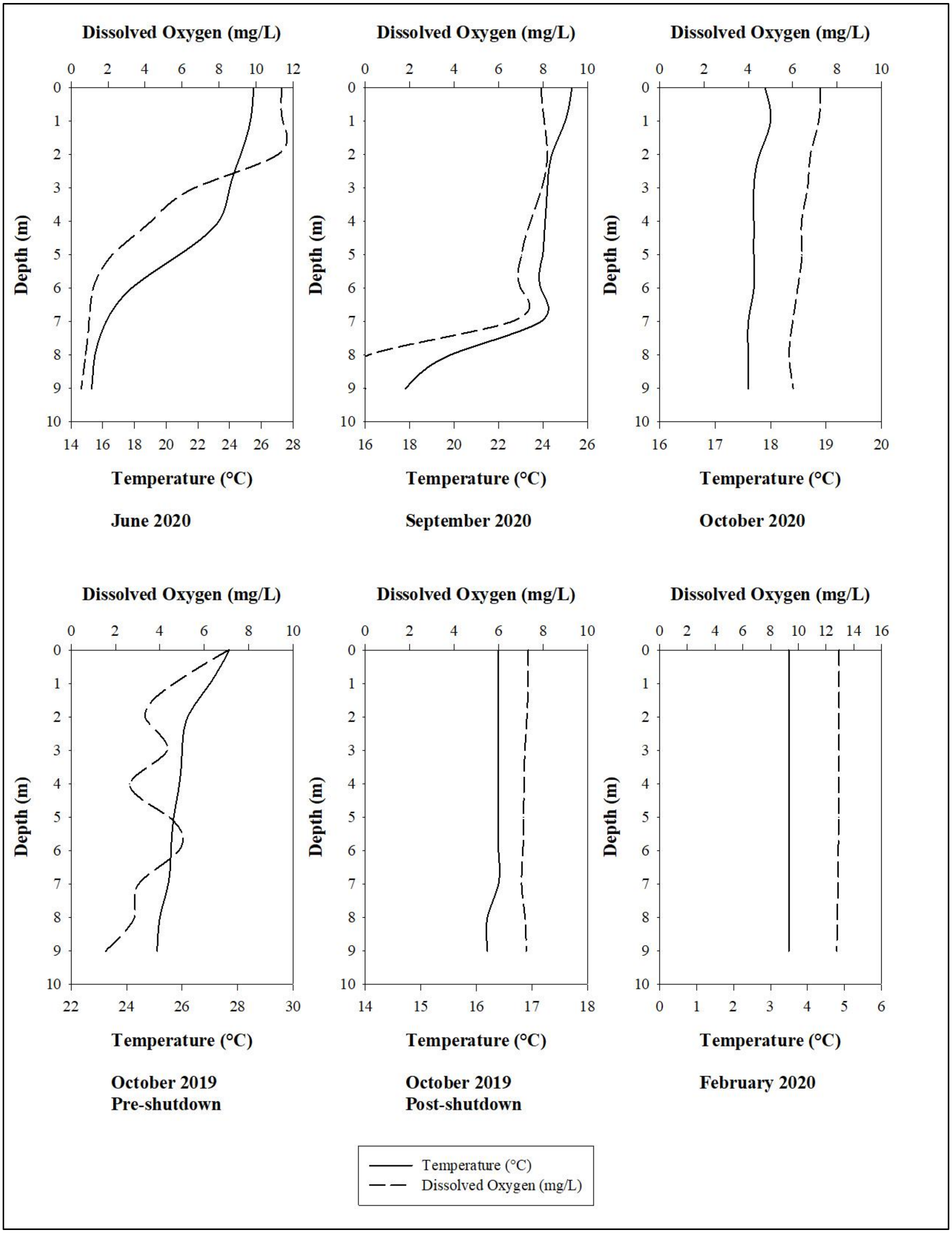

Figure 4. H2O3 depth profiles taken from the Coffeen Lake cooling loop.

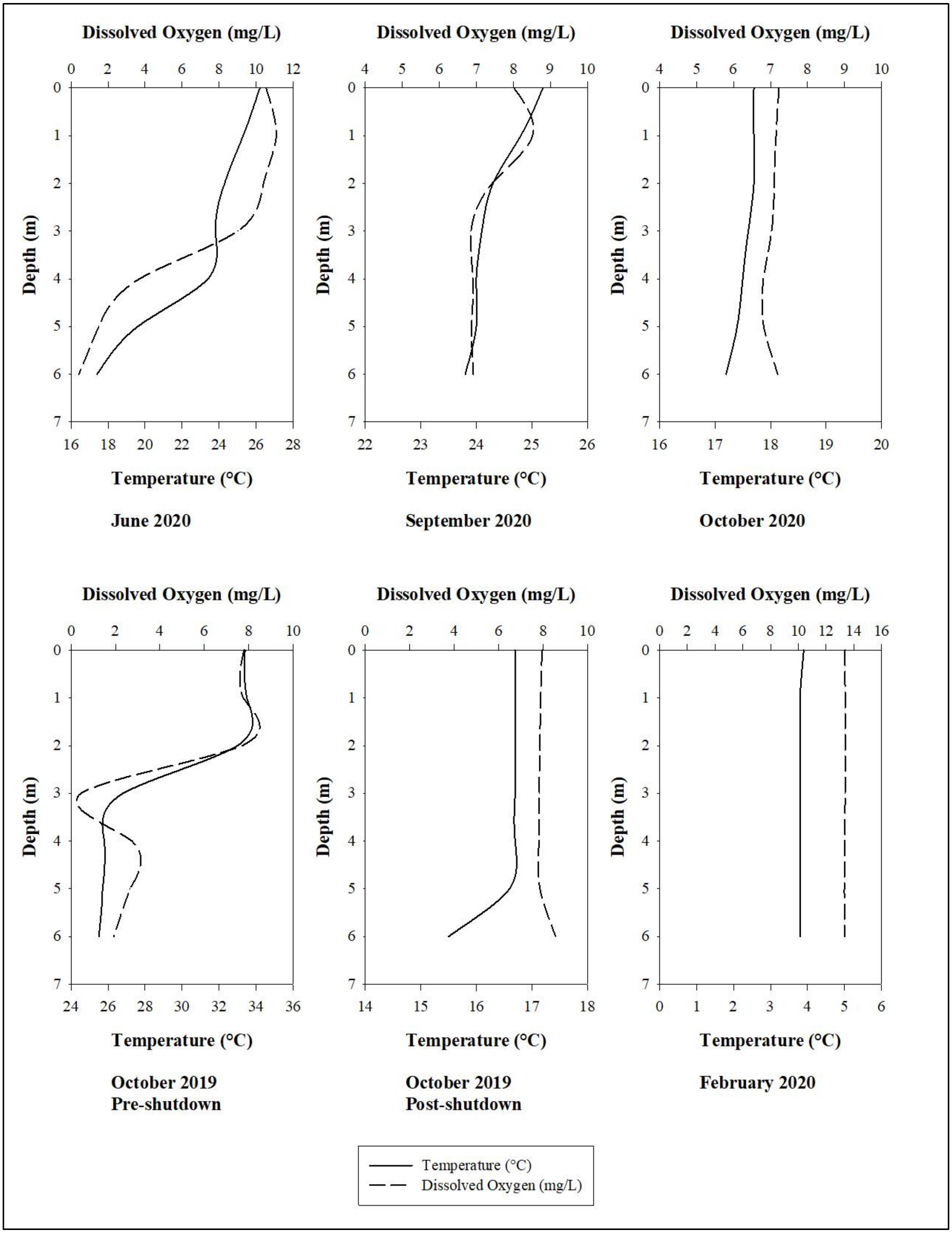

Figure 5. H2O4 depth profiles taken from the Coffeen Lake cooling loop.

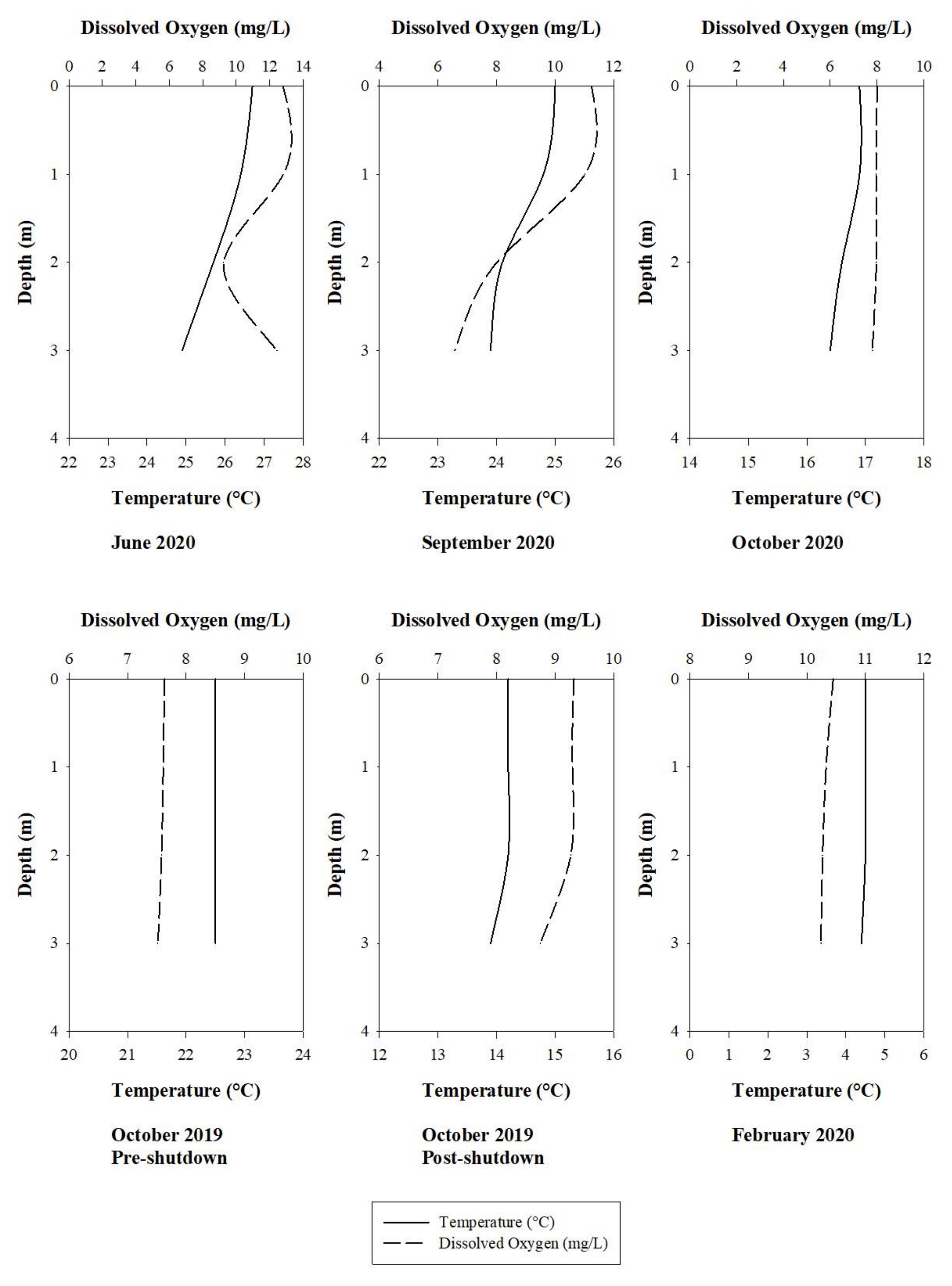

Figure 6. H2O5 depth profiles taken from the Coffeen Lake ambient zone.

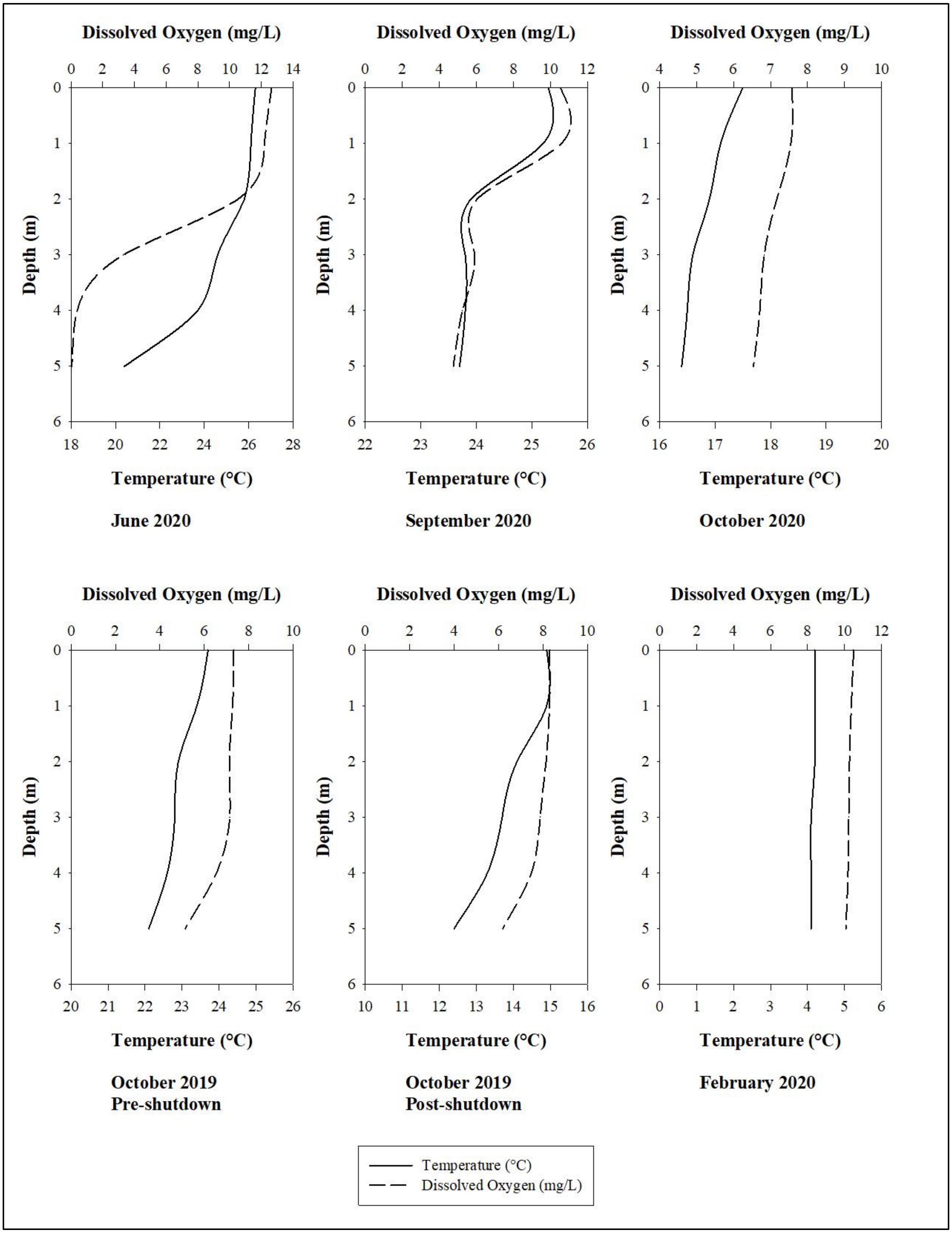

Figure 7. H2O6 depth profiles taken from the Coffeen Lake ambient zone.

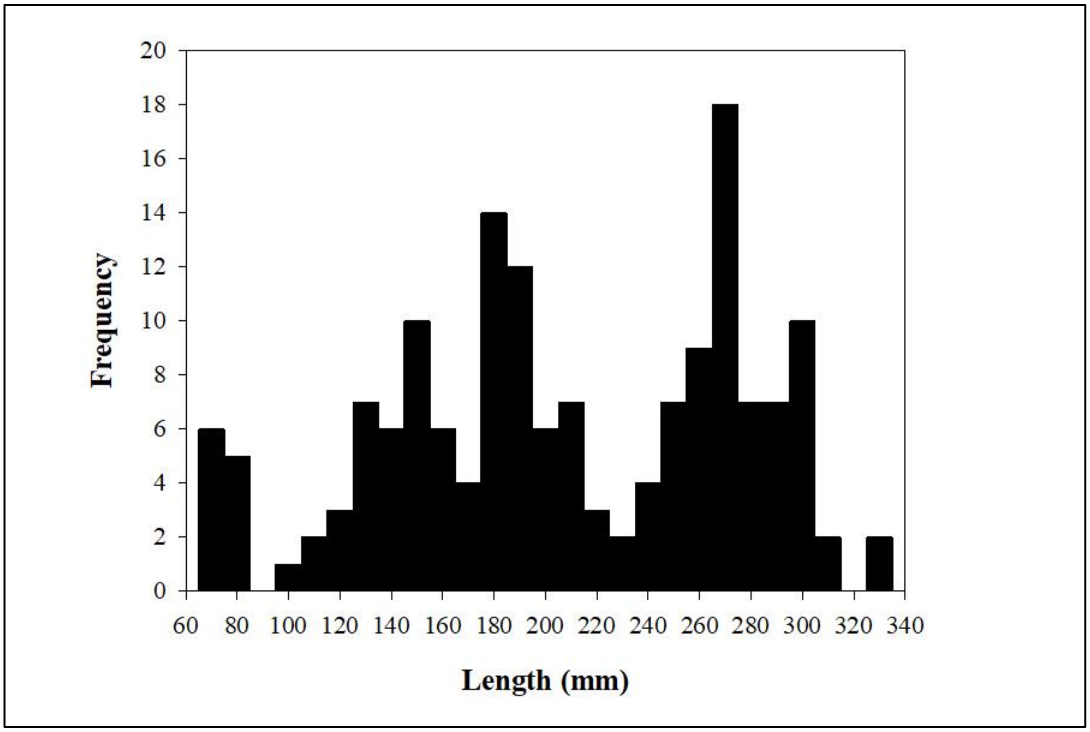

Figure 8. Length frequency distribution of Black Crappie sampled from Coffeen Lake using DC electrofishing during fall 2019 and fall 2020. n = 160, xbar = 202.6 mm ± 5.3, median = 199 mm, PSS-Q = 58, PSS-P = 42.

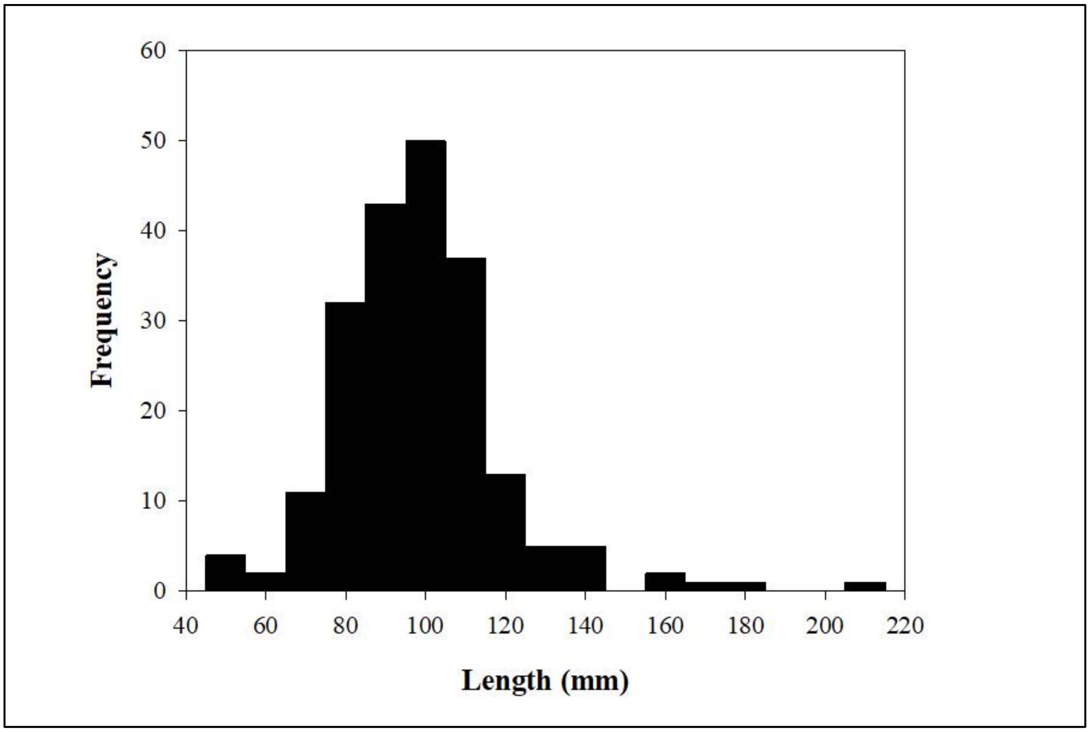

Figure 9. Length frequency distribution of Bluegill sampled from Coffeen Lake using DC electrofishing during spring 2020. n = 207, xbar = 94.1 mm ± 1.5, median = 93 mm, PSS-Q = 3, PSS-P = 0.6.

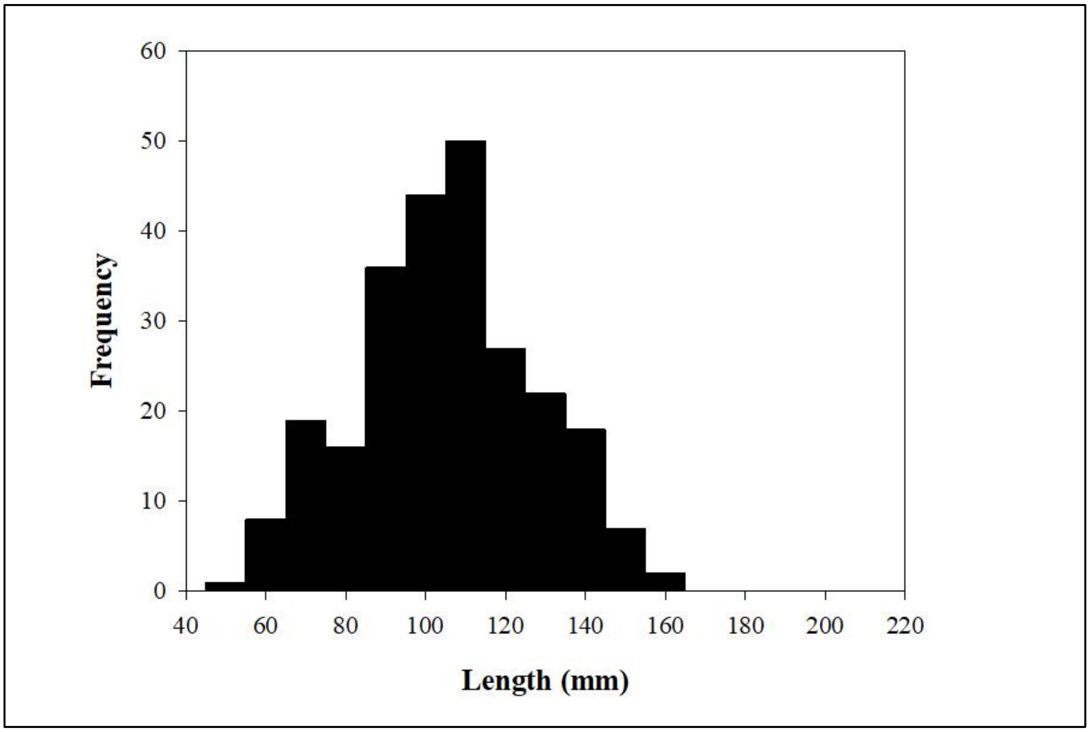

Figure 10. Length frequency distribution of Bluegill sampled from Coffeen Lake using DC electrofishing during fall 2019 and fall 2020. n = 250, xbar = 100.6 mm ± 1.4, median = 101 mm, PSS-Q = 1, PSS-P = 0.

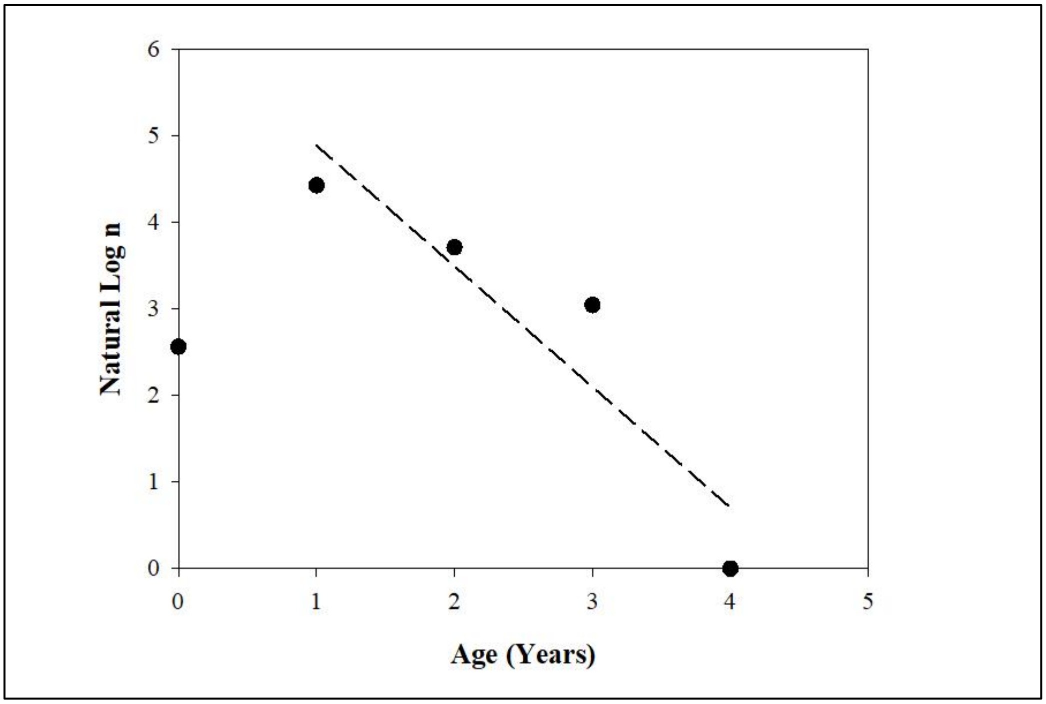

Figure 11. Weighted catch curve of Black Crappie sampled from Coffeen Lake using DC electrofishing during fall 2019 and fall 2020. n = 160, y = -1.3962x + 6.2876, r^2 = 0.9462, Total Annual Mortality = 0.606, Total Annual Survival = 0.394, Theoretical Maximum Age = 5.9 years.

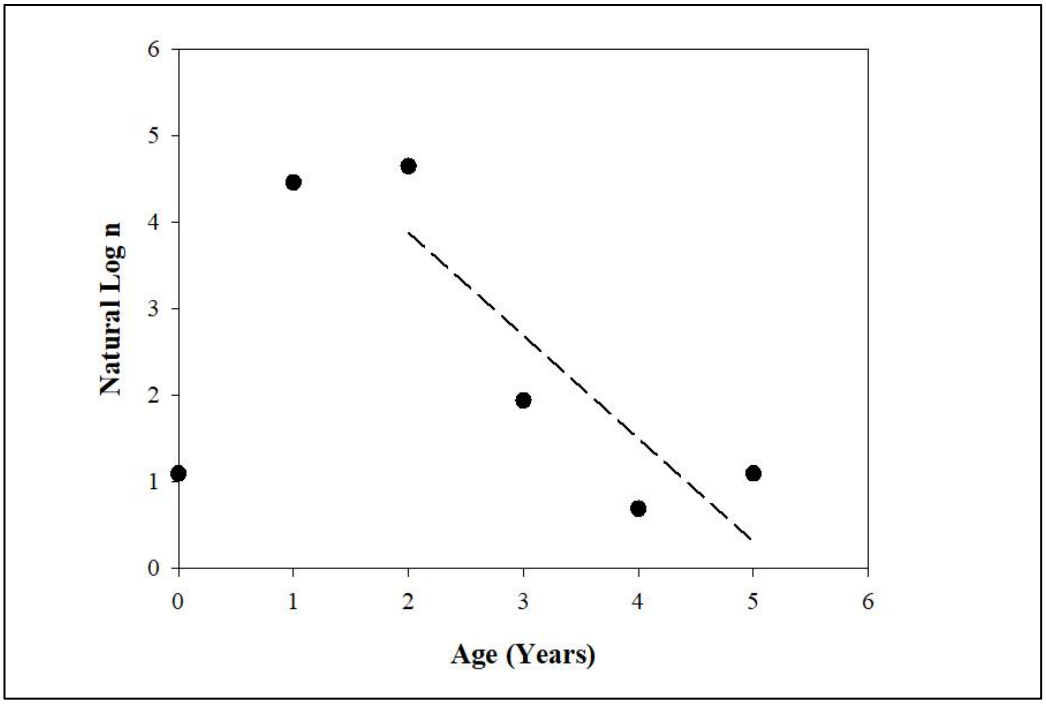

Figure 12. Weighted catch curve of Bluegill sampled from Coffeen Lake using DC electrofishing during spring 2020. n = 207, y = -1.1919x + 6.2695, r^2 = 0.8154, Total Annual Mortality = 0.774, Total Annual Survival = 0.226, Theoretical Maximum Age = 4.8 years.

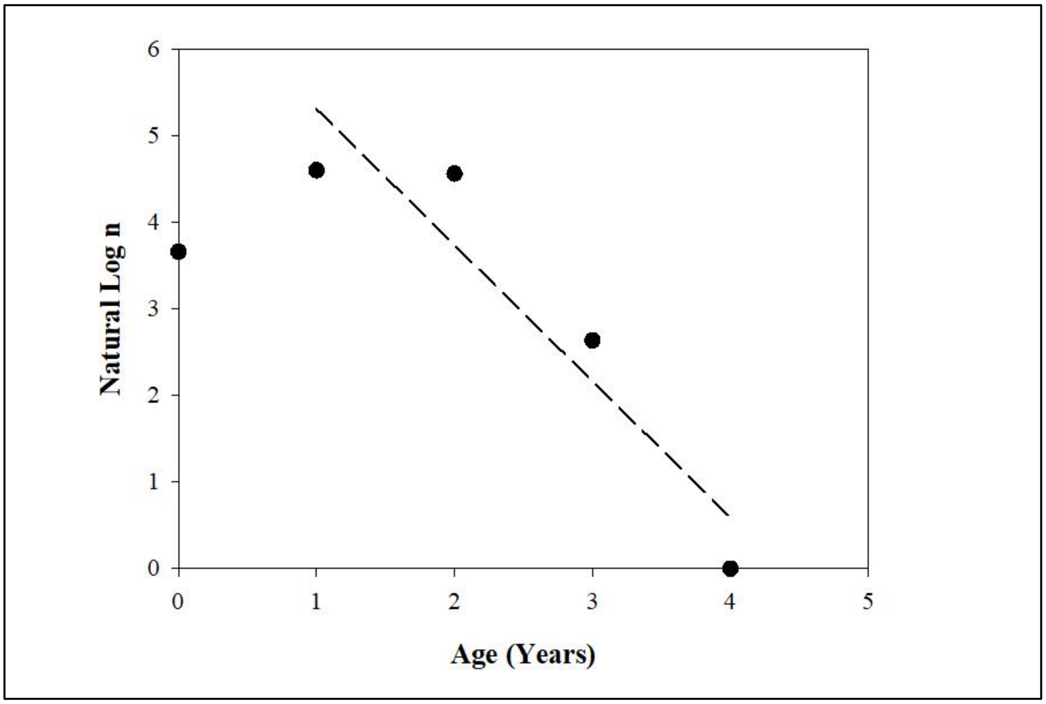

Figure 13. Weighted catch curve of Bluegill sampled from Coffeen Lake using DC electrofishing during fall 2019 and fall 2020. n = 250, y = -1.5741x + 6.8873, r^2 = 0.8060, Total Annual Mortality = 0.732, Total Annual Survival = 0.268, Theoretical Maximum Age = 4.8 years.

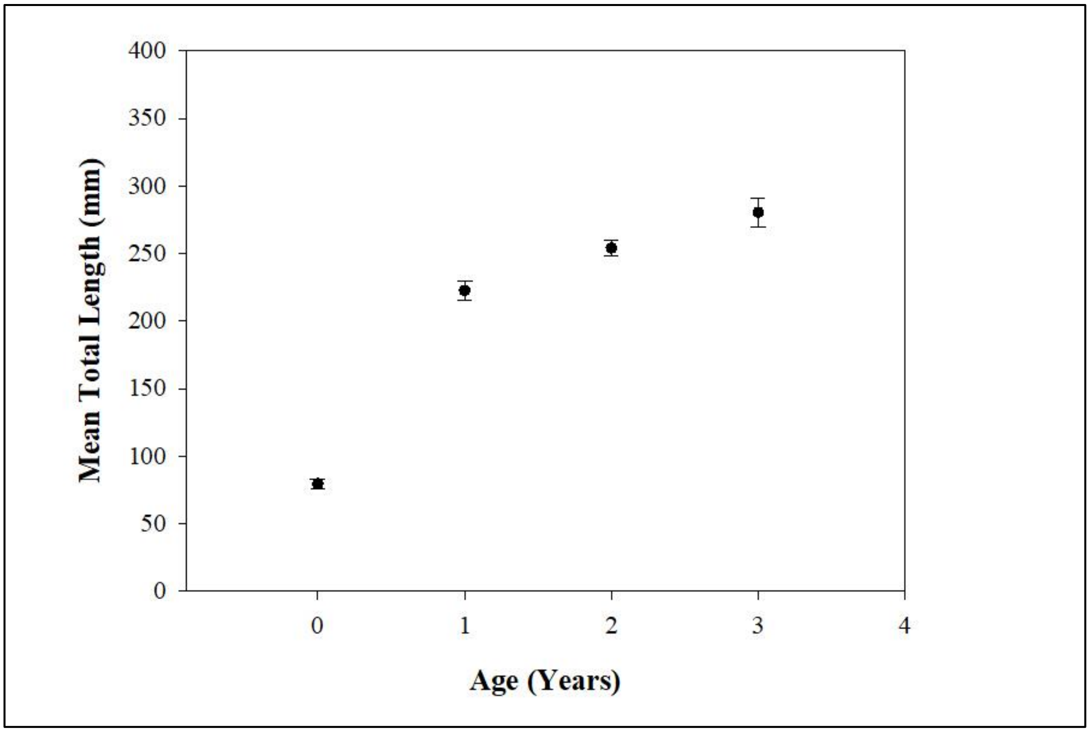

Figure 14. Mean length at age graph of Black Crappie sampled from Coffeen Lake during fall 2019. n = 35.

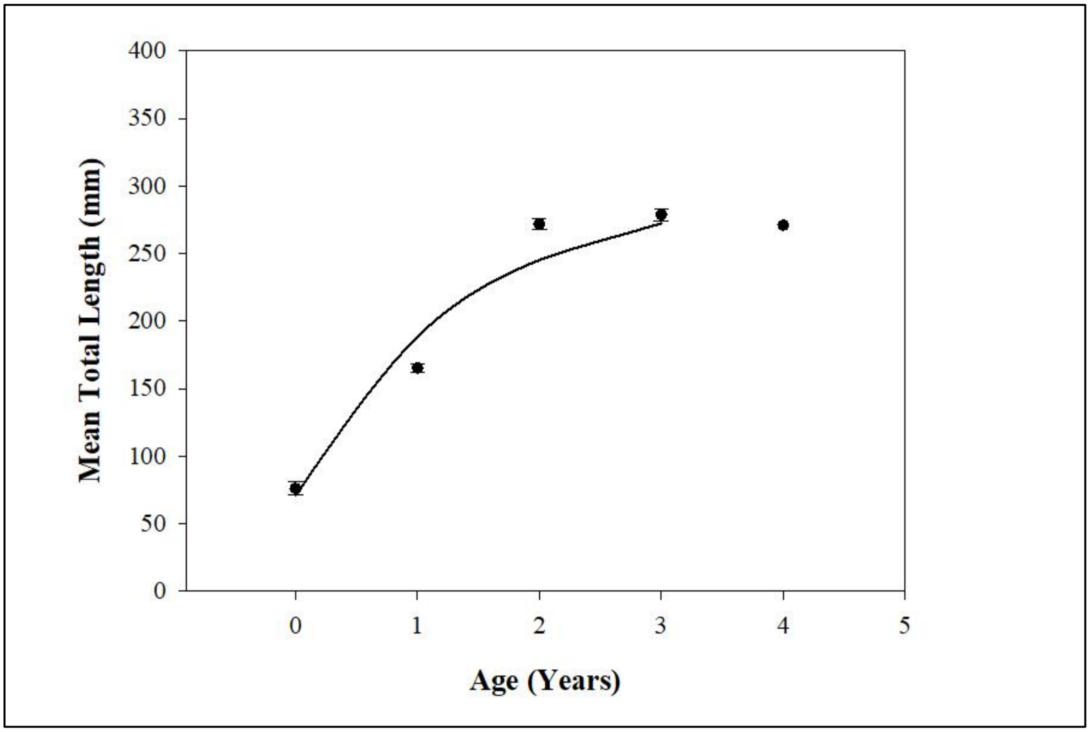

Figure 15. Mean length at age graph with von Bertalanffy function of Black Crappie sampled from Coffeen Lake during fall 2020. n = 138, r^2 = 0.9503, L inf = 297.020, K = 0.732, t0 = -0.372.

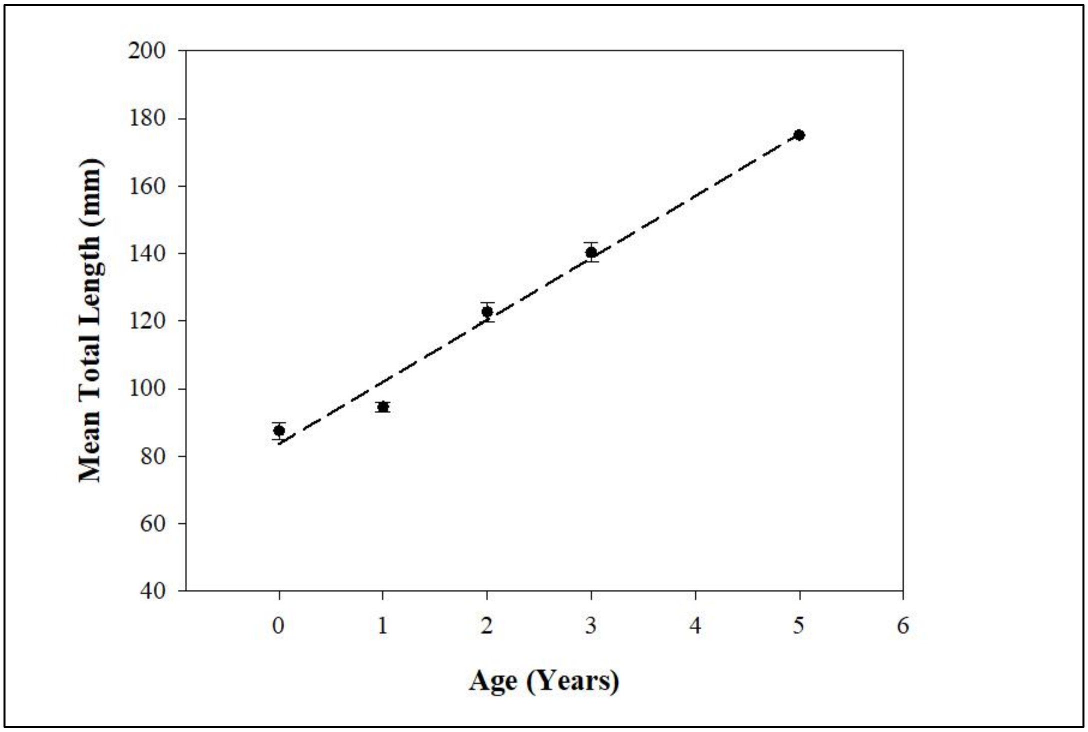

Figure 16. Mean length at age graph with linear regression of Bluegill sampled from Coffeen Lake during fall 2019. n = 219, y = 18.354x + 83.632, r2 = 0.9845.

Figure 17. Mean length at age graph with linear regression of Bluegill sampled from Coffeen Lake during spring 2020. n = 207, y = 27.175x + 47.941, r^2 *= 0.9961.*

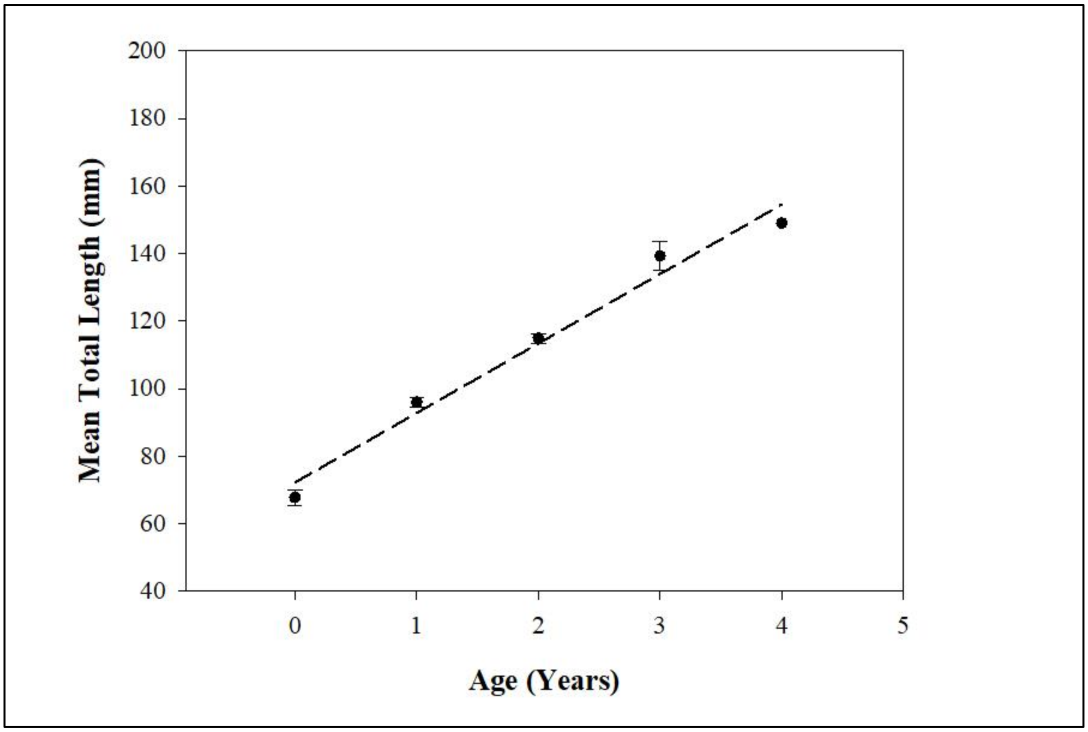

Figure 18. Mean length at age graph with linear regression of Bluegill sampled from Coffeen Lake during fall 2020. n = 200, y = 20.577x + 72.237, r^2 = 0.9789.

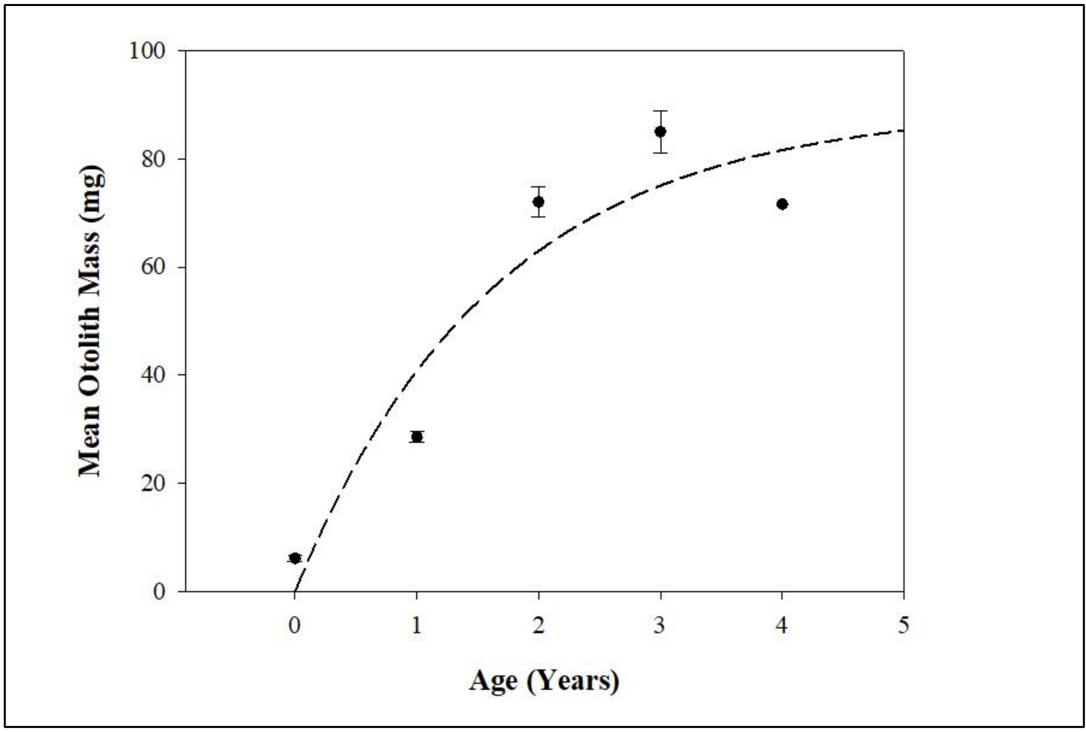

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Figure 19. Mean otolith mass versus age graph with exponential rise to maximum two parameter regression of Black Crappie sampled from Coffeen Lake during fall 2019 and fall 2020. n = 153, $y = 89.524*(1 - e^{-0.608x})$, $r^2 = 0.8969$.

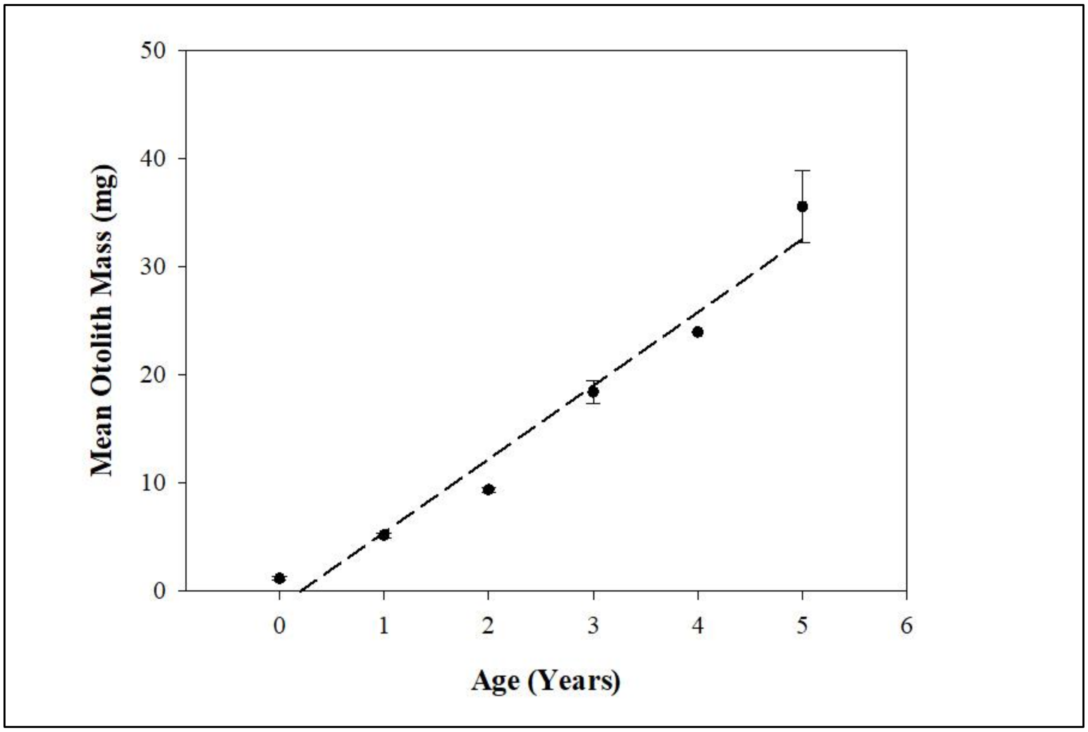

Figure 20. Mean otolith mass versus age graph with linear regression of Bluegill sampled from Coffeen Lake during spring 2020. n = 205, y = 6.787x – 1.327, r^2*.= 0.9675.*

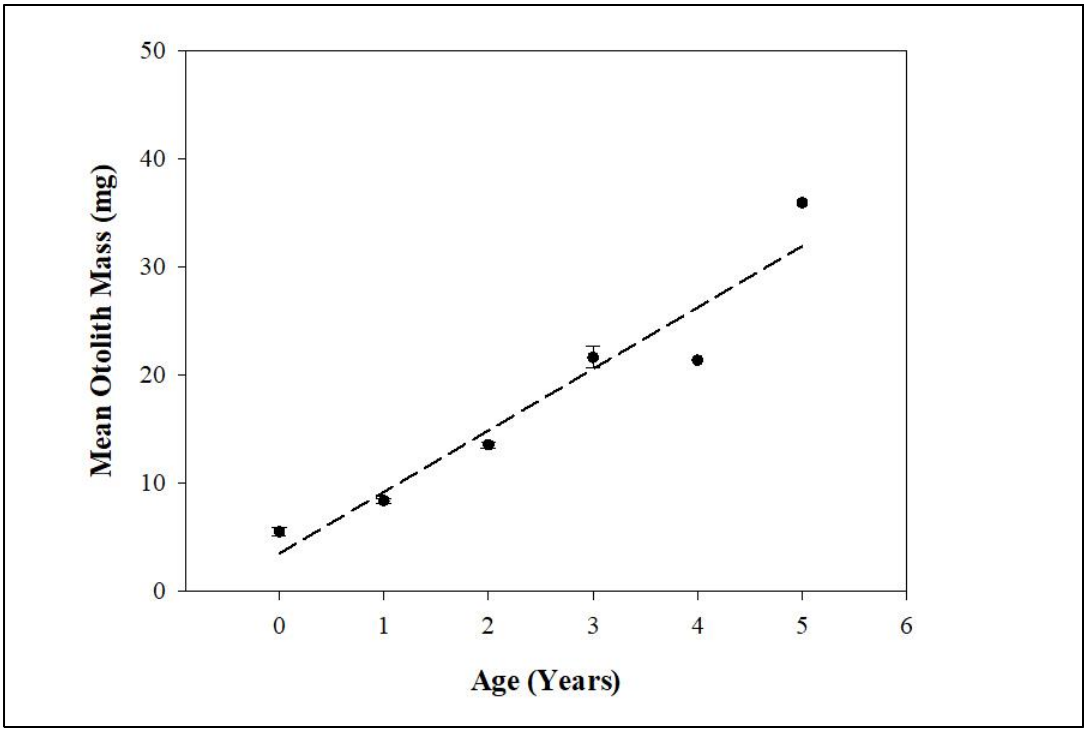

Figure 21. Mean otolith mass versus age graph with linear regression of Bluegill sampled from Coffeen Lake during fall 2019 and fall 2020. n = 396, y = 5.689x + 3.530, r^2*.= 0.9229.*

CHAPTER 3: MITOCHONDRIAL BIOENERGETICS OF BLACK CRAPPIE AND BLUEGILL AS A FUNCTION OF PRE AND POST THERMAL EFFLUENT SHUTDOWN IN COFFEEN LAKE

INTRODUCTION

Energy balance in ectotherms is considered a critical component of the species ecology, acting as a determinant of its niche requirements (Zimmerman 1965). With that much importance being placed on just one of any organism's many characteristics, obtaining a more thorough understanding of the influence of the environment upon organismal bioenergetics remains an active field of study for scientists (reviewed by Cooke and Phillip 2009; Kowalski et al. 1978).

The physiology of freshwater fishes, as with many other ectotherms, depends on a variety of environmental parameters, including habitat temperature. Understanding the influence of temperature on the energy balance of ectotherms could provide better predictions on how organisms will perform in challenging thermal regimes (Somero 2010). Although challenging thermal regimes can occur naturally over large spatial scales, these could also emerge from anthropogenic activities in a more regional scale, such as freshwater reservoirs affected by thermal effluent from water-cooled power plants. While some organisms may be able to cope with environmental challenges, others might choose to seek thermal refuges, when available. Within a given body of water these refuges may be scarce, but previous studies have shown that aquatic organisms are known to exploit thermal refugia where available (Westhoff et al. 2016).

For a Lake Eerie study, aquatic species that included Black Crappie *Pomoxis nigromaculatus* and Bluegill *Lepomis macrochirus* were shown to be attracted to higher water temperatures produced by thermal plumes in all seasons except for summer. However, tradeoffs in acute cold tolerance over winter when the warmed water mass meets the surrounding ambient water temperature (Reutter and Herdendorf 1976). Refugia have also been documented in stream systems, where cooler groundwater inputs were utilized by Atlantic Salmon *Salmo salar* and Brook Trout *Salvelinus fontinalis* to avoid higher summer temperatures (Power et al. 1999).

Extreme thermal regimes often result in adverse effects to the health and function of freshwater fishes. Cooling and heating the brain of a goldfish *Carassius auratus* lead to a defined change in behavior based on the duration and extent of the thermal change placed on it (Friedlander et al. 1976). Upper thermal exposure, brain activity of the zebrafish spontaneously decreases neural activity (Andreassen et al. 2022). All these effects have potential repercussion in thermoregulatory strategies and consequently, the survival of the individuals. Marked differences in the thermal regime change is associated with changes in the distribution and abundance of sunfishes, where the formation of thermal plumes attracted or repelled fishes depending on the season.

When surveying sunfishes from Centrarchidae in freshwater systems in Midwestern United States lakes and rivers, interesting distribution patterns arise. For example, Bluegill, are often found in the warmest regions of these freshwater bodies, whereas another Centrarchid, Black Crappie, are often occupying lower temperature profiles in the water column (Porreca and Colombo 2012). Previous research on Bluegill suggests this species is better suited to cope with high temperature extremes (Schaefer et al. 1999; Beitinger et al. 2000), judging from indicators such as the loss of righting response (LRR) and onset of opercular spasms (OS) values. Moreover, Black Crappie have been shown to have a low resistance to thermal stress (Baker and Heidinger 1996). Other studies on fishes point to behavioral thermoregulation being the key, as fishes should prefer environments that are optimal for physiological function (Neill and Magnuson 1974). Likely, since the two aforementioned species are thought to have distinct thermal physiologies, the bioenergetic machinery of the two species should show distinct thermal performance, as optimal functioning of energy production processes in the mitochondrion of fishes are also temperature dependent (Martinez et al. 2013; Martinez et al. 2015; Chung and Schulte 2015).

Routine metabolisms, as well as somatic and reproductive growth, largely rely on cellular energy. Since most energy production for ATP occurs under aerobic conditions, the thermal preferences at the sub-cellular level should reflect differences in organismal growth, thermoregulatory behavior, and whole organism energy balance. Changes in mitochondrial functions are thought to influence thermal tolerance limits, in addition to affect other energy-dependent processes including organismal growth and reproduction (Pörtner 2002). This effort to link mitochondrial and organismal performance together is not a new concept, with the mitochondrial energy transduction hypothesis (Martinez et al. 2017) and mitochondrial climatic adaptation hypothesis (Camus et al. 2017) continually being tested with different species. Largely, the current body of literature places mitochondrial energy transduction efficiency as not only being a temperature dependent process, but pivotal in shaping whole-organism performance (reviewed by Chung and Schulte 2020).

Over 90% of the cellular energy harnessed from carbon substrates and oxygen utilization under aerobic conditions occur in the mitochondrion (Salin et al. 2018). As both the energy generating processes in the mitochondrion as well as the energy consuming processes in organisms are thermally dependent (Guderley and St. Pierre 1999; reviewed by Guderley and St. Pierre 2002; Yan and Xie 2015), fishes inhibiting warmer waters tend to have increased energetic demands and poorly performing mitochondrial ATP production. The latter is caused by changes in the energy transduction efficiency, which leads to less ATP produced, an increased cost of maintenance, and a reduction in growth performance (Martinez et al. 2017). Thus, the present study aims to evaluate the role of mitochondrial energy transduction of these two Centrarchid species in shaping their distribution in a thermally-impacted lake.

METHODS

Field Sample Collection and Specimen Processing

I collected specimens needed for mitochondrial assays using either DC electrofishing in set shoreline transects or modified-fyke netting with overnight sets, and then transported them in aerated coolers from Coffeen Lake to Eastern Illinois University (EIU). Pulsed DC boat electrofishing was done with an ETS Boat Electrofishing System in 30 minute transects in each zone by following the Long-Term Resource Monitoring Protocol (Gutreuter et al. 1995); this occurred in fall 2019, spring 2020, and fall 2020. Individuals were then kept in 40 L holding tanks acclimated to appropriate water temperatures (according to lake water temperatures at the time of collection) and processed within 96 hours. All additional fishes not used for bioenergetics work were kept at -20°C for future dissection and otolith removal.

Laboratory Procedures for Collected Specimens

Fishes kept for mitochondrial testing were anesthetized via ice baths and euthanized via cervical dislocation and brain pithing (IACUC permit #18-001). I removed liver, heart, brain, and tissue samples and weighed them to the nearest 0.1 mg for each individual. This was done in order to create somatic indices for all specimens used. The liver samples were placed on ice-cold petri dishes for further processing, while the heart, brain, and epaxial muscle tissue samples were stored in -80°C for future use. Both sagittal otoliths were also removed from the dissected individuals before processing the fish carcasses.

Approximately 1.0 g of the liver was minced in ice-cold isolation medium (IM) at a ratio of 4:1 (1.0 g of liver: 4.0 mL of IM). IM consisted of 250 mM of sucrose, 0.5 mM NA_2EDTA, 10 mM Tris, and 1.0 mL of 100 mM potassium phosphate stock solution titrated with 1-5 M KOH to a pH of 7.4 at 0°C before adding 1.0 g of BSA (~ 1%) (Eigentler et al. 2012). The minced liver suspended in IM was transferred to a 7 mL Dounce smooth glass tissue homogenizer (Kontes®), where I used 5 passes of a loose-fitting pestle (A) and 2 passes of a tight-fitting pestle (B) to disrupt the tissue. Homogenate was kept on ice and then separated into 2.0 mL centrifuge tubes, which were then transferred to a pre-chilled centrifuge (HERMLE Benchmark Z 216 MK) for differential centrifugation. The first spin was done at 650 g (2,280 RPM at 5415 C) for 10 minutes, with the supernatant then transferred to new centrifuge tubes and kept on ice. The second spin cycle was done at 9,600g (2,280 RPM at 5415 C) for 15 minutes. Any lipid layer present was then removed with a Kimwipe and supernatant was removed using a pipette. All the separate microcentrifuge tubes for each specimen were then combined in order to obtain a single mitochondrial sample. This resulting pellet was then washed in 1.0 mL of fresh IM two consecutive times. The final pellet was resuspended in 200 µl of IM and placed on ice before being injected into an Oxygraph-2k (Oroboros Instruments) high-resolution respirometry system and recorded using Oroboros DatLab Version 4.3.2.7.

A select substrate-uncoupler-inhibitor titration protocol was followed for each run at the determined assay temperatures (20°C and 30°C) with the steps listed below (Table 3) (Eigentler et al. 2012). Both chambers had their polarographic O_2 electrodes standardized to the specified assay temperatures and were calibrated with sodium dithionite prior to performing any mitochondrial testing. Before each run, 2.0 mL of respiration medium (MiR05) were added to each oxygraph chamber before injecting the mitochondrial sample. MiR05 consisted of 110 mM sucrose, 20 mM HEPES, 20 mM taurine, 60 mM lactobionic acid, 3.0 mM $MgCl_2$, 0.5 mM EGTA, 10 mM potassium phosphate, and 1.0 gL^{-1} of BSA, titrated with KOH 1-5 M to a pH of 7.5.

I performed the titration process by adding 285-496 ug of mitochondrial protein into a single chamber; while respiration was attained by activating and deactivating electron convergent pathways of the ETS, with each substance added allowed to stabilize on the oxygraph for at least five minutes. Complex I Stage II (LEAK-1) was achieved with the addition of pyruvate (5 mM), malate (2 mM), and glutamate (10 mM). A 2.5mM dose of adenosine diphosphate (ADP) was injected to achieve Complex I Stage III (OXPHOS-I), and 10 mM of succinate were injected for Complex I and II Stage III respiration (OXPHOS I-II). Next, 20 mM of carboxiatractyloside (Cat) was added to observe the respiration rate under Complex I and Complex II activating substrates (LEAK I-II). Stepwise (0.5 μM steps) of FCCP were used to uncouple electron transfer from ATP production and thus assess maximum electron transfer by the ETS. Lastly, 0.5 μM of rotenone, 5 mM of malonic acid, and 2.5 μM of antimycin A were then added consecutively in order to inhibit Complex I, Complex II, and Complex II, respectively. To engage electron transfer directly to cytochrome *c*, 0.5 mM of the substrate TMPD were added to observe maximal electron transfer from cytochrome *c* to cytochrome *c* oxidase (COX). Each oxygraph chamber and cannula were soaked and cleaned with deionized water three times. The chambers were then filled with 70% for five minutes, three times. A final 100% ethanol soak was then done on each chamber for 15-20 minutes, before rinsing both chambers and stoppers with deionized water three times. This cleaning protocol was performed before and after each run to avoid contamination between runs.

I used the observed oxygen flux data to calculate three important control ratios: the coupling control ratio (P/E), the respiratory control ratio (RCR), and the leak control ratio (L/E). P/E is calculated by taking the oxygen flux observed under ATP production, then dividing this number by the maximum oxygen flux attributed to the Electron Transport System (ETS). This establishes how much of the ETS is controlled by the Oxidative Phosphorylation System (OXPHOS). The RCR provides an index of coupling between oxygen consumption and ATP production, and is calculated by dividing OXPHOS over proton leakage (LEAK). Higher RCRs indicate a more efficient ATP-producing mitochondria, offsetting the oxygen cost set by proton leakage (Salin et al. 2018). Finally, L/E, is calculated from the oxygen fluctuation observed when there's no ATP production, then dividing this number by the maximum oxygen fluctuation attributed to the ETS. This establishes how much of the ETS is controlled by LEAK. Differences in LEAK values for both species can be attributed to the energetic changes in metabolism to regulate the body's homeostasis (Divakaruni and Brand 2011). By understanding this index, the varying levels of coupling efficiency between Black Crappie and Bluegill can be explained.

Mitochondrial total protein content was quantified using a Bradford Coomassie Stain Assay (Coomassie Plus, Thermo Scientific, Rockford, IL). Samples and isolation media were diluted 100-fold and their absorption values were measured using a Thermo Spectronic Genesys 20 spectrophotometer at $\lambda = 595$ nm following 10 minutes of incubation at room temperature. Resulting protein content concentration was corrected by subtracting the protein content in the isolation medium, which was also measured using the aforementioned process.

Two-way ANOVAs were run on final values from the following flux control ratio and complex data, with the two factors used as a source of variation for these tests being species (Black Crappie or Bluegill) and assay temperatures (20°C or 30°C). All values reported for flux control ratio are shown fractionally, and all values reported for oxygen flux are expressed in nmol O_2 sec^{-1} mg mitochondrial $protein^{-1}$. Statistical significance was done by comparing mean values between the different factors and seeing if they were greater than would be expected by chance ($p < 0.05$). The values reported for each run described below will be expressed as the mean ± Standard Error of the Mean (SEM). Final statistical analysis of the collected values was performed using SigmaPlot Version 11.0, Fisheries Analysis and Modeling Simulator (FAMS) Version 1.64 and Microsoft Excel Version 15.0.5311.1000.

RESULTS

Tables 4 and 5 show the results for two-way ANOVAs performed on flux control ratio data, tables 6 and 7 show the data for complex specific oxygen flux under various respiratory states, and figures 22-27 plot the results from complex specific and flux control ratio data.

Complex Specific Data

Complex I OXPHOS during fall 2019 showed significant differences between species for Black Crappie (295.766 ± 69.241) and Bluegill (79.616 ± 59.965; Two-way ANOVA, df = 17, p = 0.033; Figure 23). Additionally, this trend was seen again in fall 2020 where there were significant differences between species for Black Crappie (286.308 ± 28.380) and Bluegill (60.100 ± 33.580; Two-way ANOVA, df = 23, p < 0.001), and between assay temperatures for 20°C (100.940 ± 31.089) and 30°C (245.468 ± 31.089; Two-way ANOVA, df = 23, p = 0.004). Fall 2019 showed significant differences in Complex I-II OXPHOS between assay temperatures for 20°C (344.200 ± 85.525) and 30°C (658.068 ± 74.067; Two-way ANOVA, df = 17, p = 0.015; Figure 24). Fall 2020 also showed this between assay temperatures for 20°C (222.487 ± 43.095) and 30°C (467.768 ± 47.208; Two-way ANOVA, df = 23, p = 0.002).

Complex I-II ETS during fall 2019 showed significant differences between assay temperatures for 20°C (585.820 ± 99.678) and 30°C (735.011 ± 86.324; Two-way ANOVA, df = 17, p = 0.043; Figure 25). This was seen again in fall 2020 where significant differences between assay temperatures for 20°C (265.514 ± 53.137) and 30°C (494.203 ± 53.137; Two-way ANOVA, df = 23, p = 0.006). Lastly, in fall 2020, statistically significant differences between species for Black Crappie (190.261 ± 31.360) and Bluegill (301.378 ± 37.106; Two-way ANOVA, df = 22, p = 0.033), and between assay temperatures for 20°C (179.120 ± 34.354) and 30°C (312.518 ± 34.354; Two-way ANOVA, df = 23, p = 0.012; Figure 26). Despite only being able to process one Black Crappie for bioenergetics during the spring 2020 season, complex data showed very similar trends to those seen in both the fall 2019 and fall 2020 data.

Flux Control Ratio Data

The P/E for fishes sampled during fall 2019 showed significant differences between species for Black Crappie (0.903 ± 0.027) and Bluegill (0.759 ± 0.024; Two-way ANOVA, df = 17, p = 0.001), and between assay temperatures for 20°C (0.765 ± 0.027) and 30°C (0.897 ± 0.024; Two-way ANOVA, df = 17, p = 0.003; Figure 22). When observing LEAK values with L/E statistical significance was only documented between temperatures for 20°C (0.132 ± 0.021) and 30°C (0.215 ± 0.018; Two-way ANOVA, df = 17, p = 0.01). Spring 2020 had a low sample size for Black Crappie, so statistical analyses between species were unable to be run for the season. However, despite only being able to process one fish for bioenergetics, ratio data from this season showed very similar trends to those seen in both the fall 2019 and fall 2020 data. P/E for fishes sampled during fall 2020 showed significant values between species for Black Crappie (0.938 ± 0.023) and Bluegill (0.943 ± 0.027; Two-way ANOVA, df = 23, p = 0.016), and between assay temperatures for 20°C (0.823 ± 0.025) and 30°C (0.958 ± 0.025; Two-way ANOVA, df = 23, p = 0.001). Values for RCR were significantly different between species in the fall 2020 samples, where Black Crappie displayed significantly higher RCR values across assay temperatures (Two-way ANOVA, df = 23, p = 0.001). Lastly, there were also significant values for L/E between species for Black Crappie (0.138 ± 0.019) and Bluegill (0.232 ± 0.022; Two-way ANOVA, df = 23, p = 0.004), and between assay temperature for 20°C (0.133 ± 0.020) and 30°C (0.238 ± 0.020; Two-way ANOVA, df = 23, p = 0.001).

Somatic Indices

Somatic indices were taken using the liver, heart, and brain masses (in grams) of all lab individuals tested, and then dividing them by the total body mass (in grams). The result of these values was then plotted for each species by season (Figure 27). Fall 2019 Black Crappie had an average liver ratio of 0.0083 ± 0.0006, spring 2020 Black Crappie had an average liver ratio of 0.0052, and fall 2020 Black Crappie had an average liver ratio of 0.0102 ± 0.0004. Fall 2019 Bluegill had an average liver ratio of 0.0127 ± 0.0007, spring 2020 Bluegill had an average liver ratio of 0.0099 ± 0.0004, and fall 2020 Bluegill had an average liver ratio of 0.0133 ± 0.0014. This trend showed a noticeable decrease for both species liver ratios when comparing the fall seasons to the spring season data.

DISCUSSION

Thermal thresholds of physiological traits are known to play an important role in an ectotherm's abundance, performance, and distribution (Pörtner 2002; reviewed by Angilletta 2009; Somero 2010). For example, traits such as the thermal performance of fish cardiac mitochondria can be a significant contributor to their thermal habitat range (Iftikar et al. 2014). The goals of this study were to compare the thermal performance of the bioenergetic machinery of two Centrarchids, with the purpose of establishing a link between their distribution and thermal physiology. Results suggest that although Black Crappie and Bluegill co-occur in small, freshwater systems, divergent mitochondrial bioenergetics of both species supports the observations indicating they occupy different thermal microclimates within Coffeen Lake.

Complex Specific Data

Maximal contribution to OXPHOS by Complex I of the ETS by Black Crappie showed a higher average when compared to that of Bluegill, especially when examining each species performance at 30°C. Complex I of the ETS is the site where most electrons enter the ETS and has been recognized the rate-limiting step when looking at overall respiration (Sharma et al. 2009), playing a crucial role in setting whole organismal limits (Strobel et al. 2013). This is also an important stage for energy metabolism, as Complex I contributes to the proton gradient that is eventually used for ATP production. CI-C II OXPHOS showed a similar trend but had a more negligible difference in values between the two. Based on these results, it seems as though the ETS capacity of Black Crappie relies heavily on Complex I electron transfer. Reliance on CI electron transfer at suboptimal and optimal temperatures has been found in insect mitochondria (*Drosophilia* spp.) and shifts in reliance on CI when exposed to supraoptimal temperatures have been observed (Jørgensen et al. 2021). These shifts are likely a response that influence the energy balance of the organism and allows for the control of the electron transfer capacity of the ETS.

Flux Control Ratio Data

Based on the P/E ratios, the Black Crappie liver mitochondrial OXPHOS assayed *in vitro* was approximate to the capacity of the ETS, contrary to OXPHOS values found for Bluegill. No significant differences in ETS capacity were observed between species. This data shows that Black Crappie liver mitochondrial OXPHOS operates closer to ETS capacity *in vitro*, suggesting an ETS-limited mitochondria at supraoptimal temperatures. Since the OXPHOS for Black Crappie was approaching maximal ETS capacity at higher assay temperatures (30°C), the behavior of avoiding thermally impacted regions of the reservoir may be linked to the supraoptimal ATP production at these warmer temperatures. Interestingly, the gap between Black Crappie and Bluegill P/E is reduced at 30°C, which suggests that warmer temperatures likely impinge limits to the mitochondrial ATP production to thermotolerant species like Bluegill. Changes in the mitochondrial ETS capacity with temperature have been shown for laboratory acclimated fishes (Chung and Schulte 2015), where cold-acclimated Mummichogs *Fundulus heteroclitus* usually exhibited higher ETS capacity, with little to no changes in their RCR thermal sensitivity. This observation is key when interpreting the differences between Black Crappie and Bluegill P/E and RCR, as Black Crappie tend to represent a colder adapted individual, which was found more abundantly in the cooler regions of the reservoir. Since this study contemplates the changes in P/E, RCR, and L/E pre and post shutoff for power plant thermal effluent, the bioenergetics framework between fall 2019 (pre shutoff) and fall 2020 (one year following the shutoff) shows emerging changes in both species' physiology. Overall, I observed a reduction in the ETS capacity on both species after the thermal cutoff. This difference does not result from changes in the nutritional state of the individuals, as both spring 2020 and fall 2020 CI-CII ETS values show a reduced ETS despite different HIS values. Changes in the ETS capacity could be a result of changes in the abundance of electron carriers, or regulatory processes modulating cytochrome *c* oxidase (COX) activity.

Preliminary data on COX expression for both species during the fall 2019 season (not shown) shows significant differences between species (lower COX expression in Black Crappie), however, this difference in expression does not result in a different ETS capacity measured through the respirometry assay. Research examining intraspecies thermal sensitivity with European Perch *Perca fluviatilis* taken separately from both heated and undisturbed ecosystems showed different local adaptation when it came to cardiac mitochondrial metabolism (Pichaud et al. 2019). Both intra and interspecific differences in mitochondrial function seem to provide the adaptive mechanisms in challenging thermal environments, however, the specific changes occurring in the mitochondria is still an active area of study. Further work including a more targeted enzyme specific expression and function will be necessary to reach a more conclusive answer for each species.

This difference in performance between Black Crappie and Bluegill could be explained by the Bluegill exhibiting a higher portion of their ETS being controlled by LEAK, indicating less efficient ATP production and lower RCR values compared to those in Black Crappie at the same temperature. A previous study involving Spotty *Notolabrus celidotus* showed that individuals exposed to acute heat stress resulted in lowered RCR values and increased LEAK values, which could help to explain this difference in bioenergetics between multiple species (Iftikar and Hickey 2013).

Higher LEAK values in Bluegill specimens could also be explained by the "uncouple to survive" hypothesis, which indicates that organisms that live in supraoptimal temperatures can reduce energy transduction efficiency in favor of lower membrane potentials and reduced production of reactive oxygen species in cells (Brand 2000). This "uncoupling to survive" strategy (Brand 2000) reduces the mitochondrial energy transduction efficiency and could explain, to a certain extent, the differences observed between species. The ability of Bluegill to perform under a wider range of temperatures could also be attributed to the species' phenotypic plasticity, specifically when examining their physiology and thermal tolerance (Dent and Lutterschmidt 2003).

Overall, it appeared that Bluegill were able to function at supraoptimal temperatures but did so in an inefficient manner. In a similar study, Bluegill mitochondrial energy production machinery was more uncoupled, resulting in reduced energy transduction efficiency as documented by their LEAK and RCR values (Das 2006). It is important to note that testing on other species has suggested mitochondrial coupling is not the sole mechanism playing a role in setting overall organismal thermal limits. Other factors including varying mitochondrial genotypes, ROS production, and hybridization of species still have much to be explored before a definite consensus can be reached (Du et al. 2017; reviewed by Chung and Schulte 2020).

Somatic Indices

The liver indices derived from lab specimens did show a modest increase in the ratios between the fall 2019 and fall 2020 samplings, agreeing with the initial hypothesis that the condition of both species should improve when thermal stress from the effluent was eliminated. Decreases in hepatosomatic indices for both Black Crappie and Bluegill during the spring 2020 season compared to the fall samplings could be partially explained by the use of lipid reserves towards reproductive growth. A study dealing with another Centrarchid, Largemouth Bass, showed that reductions in liver weight could be attributed to reproduction costs and energetic needs for dealing with increased thermal demands in their metabolic pathways (Adams et al. 1982). This knowledge of increased temperature affecting liver sizes could also help to explain why removing thermal effluent may be beneficial to the energy balance of both species in Coffeen Lake. Additionally, it has been documented that a peak in hepatosomatic indices after Largemouth Bass spawning should be recovered with other tissues by June (Brown and Murphy 2004), which could help insure accurate reporting of other Centrarchid species liver ratios.

This study compared two Centrarchid fishes that inhabited disparate regions of Coffeen Lake. Despite both being found in the same body of water, Black Crappie and Bluegill have unique physiologies and bioenergetics machinery that allows them to exploit their resources and survive varying thermal regimes. Black Crappie appear to engage mitochondrial oxidative phosphorylation at rates approaches maximal capacity of the ETS and showed more efficient energy transduction, typical of cold acclimated species, whereas Bluegill liver mitochondria is less thermosensitive, being more uncoupled and operating in a less efficient manner. Relating this bioenergetics data back to the fisheries component for this work can hopefully lead to more informed decisions by natural resource managers in the future. Some other work that could be beneficial to understanding Centrarchid bioenergetics in altered thermal regimes going forward could include studies examining age to maturity, looking at a broader range of temperature extremes, and incorporating more species into similar experiments.

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Table 3. Select substrate-uncoupler-inhibitor titration protocol performed in vitro for activating the ETS.

Substrates	**Event**	**Concentration in syringe (solvent)**	**Storage (˚C)**	**Titration volume**	**Final concentration in O2k chamber**	**Syringe µl**
Malate	M	0.8 M (H2O)	-20	5 µl	2 mM	25
Glutamate	G	2 M (H2O)	-20	10 µl	10 mM	25
Pyruvate	P	2 M (H2O)	fresh	5 µl	5 mM	25
Succinate	S	1 M (H2O)	-20	20 µl	10 mM	50
TMPD	Tm	0.2 M (H2O)	-20	5 µl	0.5 mM	25
ADP + Mg^{2+}	D	0.5 M (H2O)	-80	4 - 20 µl	1 - 5 mM	10
Inhibitors						
Rotenone	Rot	1.0 mM (EtOH)	-20	1 µl	0.5 µM	10
Anitmycin A	Ana	5 mM (EtOH)	-20	1 µl	2.5 µM	10
Carboxyatractyloside	Cat	5 mM (H2O)	-20	10 µl	5 mM	20
Malonic acid	Mna	2000 mM	NA	5 µl	5 mM	25
Uncoupler						
FCCP	F	1 mM (EtOH)	-20	1 µl steps	0.5 µM steps	10

Table 4. Two-way ANOVA flux control ratio results table for Black Crappie and Bluegill sampled from Coffeen Lake during fall 2019.

Season	Flux Control Ratio	Source of Variation	DF	SS	MS	F	P
Fall 2019	L/E	Species	1	0.00036	0.00036	0.109	0.746
BC 20° n: 3		Temperature (°C)	1	0.0296	0.0296	8.948	0.01
BC 30° n: 5		Species x Temperature (°C)	1	0.000951	0.000951	0.288	0.6
BG 20° n: 5		Residual	14	0.0463	0.00331		
BG 30° n: 5		Total	17	0.079	0.00465		
	RCR	Species	1	4.278	4.278	0.854	0.371
		Temperature (°C)	1	15.152	15.152	3.025	0.104
		Species x Temperature (°C)	1	0.432	0.432	0.0863	0.773
		Residual	14	70.135	5.01		
		Total	17	89.661	5.274		
	P/E	Species	1	0.088	0.088	15.673	0.001
		Temperature (°C)	1	0.075	0.075	13.344	0.003
		Species x Temperature (°C)	1	0.00291	0.00291	0.517	0.484
		Residual	14	0.0786	0.00562		
		Total	17	0.271	0.016		

Table 5. Two-way ANOVA flux control ratio results table for Black Crappie and Bluegill sampled from Coffeen Lake during fall 2020.

Season	Flux Control Ratio	Source of Variation	DF	SS	MS	F	P
Fall 2020	L/E	Species	1	0.051	0.051	10.691	0.004
BC 20° n: 7		Temperature (°C)	1	0.0645	0.0645	13.517	0.001
BC 30° n: 7		Species x Temperature (°C)	1	0.0184	0.0184	3.85	0.064
BG 20° n: 5		Residual	20	0.0955	0.00477		
BG 30° n: 5		Total	23	0.22	0.00956		
	RCR	Species	1	93.417	93.417	14.199	0.001
		Temperature (°C)	1	25.577	25.577	3.888	0.063
		Species x Temperature (°C)	1	0.165	0.165	0.0251	0.876
		Residual	20	131.584	6.579		
		Total	23	252.182	10.964		
	P/E	Species	1	0.052	0.052	6.971	0.016
		Temperature (°C)	1	0.106	0.106	14.205	0.001
		Species x Temperature (°C)	1	0.0093	0.0093	1.246	0.277
		Residual	20	0.149	0.00746		
		Total	23	0.309	0.0134		

Table 6. Two-way ANOVA complex results table for Black Crappie and Bluegill sampled from Coffeen Lake during fall 2019.

Season	Complex	Source of Variation	DF	SS	MS	F	P
Fall 2019	CI LEAK	Species	1	903.059	903.059	1.026	0.328
BC 20° n: 3		Temperature (°C)	1	5496.02	5496.02	6.246	0.026
BC 30° n: 5		Species x Temperature (°C)	1	386.151	386.151	0.439	0.518
BG 20° n: 5		Residual	14	12319.7	879.977		
BG 30° n: 5		Total	17	19709.2	1159.37		
	CI OXPHOS	Species	1	200232	200232	5.569	0.033
		Temperature (°C)	1	80237.1	80237.1	2.231	0.157
		Species x Temperature (°C)	1	34426.1	34426.1	0.957	0.344
		Residual	14	503408	35957.7		
		Total	17	870726	51219.2		
	CI-CII OXPHOS	Species	1	37421.9	37421.9	0.682	0.423
		Temperature (°C)	1	422199	422199	7.696	0.015
		Species x Temperature (°C)	1	4052.28	4052.28	0.0739	0.79
		Residual	14	768026	54859		
		Total	17	1273930	74937		
	CI-CII LEAK	Species	1	818.954	818.954	0.223	0.644
		Temperature (°C)	1	40432.2	40432.2	11.019	0.005
		Species x Temperature (°C)	1	313.733	313.733	0.0855	0.774
		Residual	14	51369	3669.21		
		Total	17	94036.2	5531.54		
	CI-CII ETS	Species	1	91.778	91.778	0.00123	0.972
		Temperature (°C)	1	369822	369822	4.963	0.043
		Species x Temperature (°C)	1	5821.75	5821.75	0.0781	0.784
		Residual	14	1043250	74517.8		
		Total	17	1419172	83480.7		
	CII ETS	Species	1	50035.6	50035.6	1.213	0.289
		Temperature (°C)	1	157906	157906	3.827	0.071
		Species x Temperature (°C)	1	56.502	56.502	0.00137	0.971
		Residual	14	577622	41258.7		
		Total	17	771488	45381.7		
	COX	Species	1	202178	202178	2.082	0.171
		Temperature (°C)	1	642174	642174	6.613	0.022
		Species x Temperature (°C)	1	8903.7	8903.7	0.0917	0.766
		Residual	14	1359491	97106.5		
		Total	17	2181535	128326		

Table 7. Two-way ANOVA complex results table for Black Crappie and Bluegill sampled from Coffeen Lake during fall 2020.

Season	Complex	Source of Variation	DF	SS	MS	F	P
Fall 2020	CI LEAK	Species	1	47.214	47.214	0.244	0.627
BC 20° n: 7		Temperature (°C)	1	4218.199	4218.199	21.774	<0.001
BC 30° n: 7		Species x Temperature (°C)	1	37.935	37.935	0.196	0.663
BG 20° n: 5		Residual	20	3874.483	193.724		
BG 30° n: 5		Total	23	8436.586	366.808		
	CI OXPHOS	Species	1	298491.128	298491.128	26.471	<0.001
		Temperature (°C)	1	121848.824	121848.824	10.806	0.004
		Species x Temperature (°C)	1	45949.598	45949.598	4.075	0.057
		Residual	20	225519.582	11275.979		
		Total	23	722257.943	31402.519		
	CI-CII OXPHOS	Species	1	31117.531	31117.531	1.197	0.287
		Temperature (°C)	1	350950.143	350950.143	13.498	0.002
		Species x Temperature (°C)	1	3628.132	3628.132	0.14	0.713
		Residual	20	520003.755	26000.188		
		Total	23	928064.623	40350.636		
	CI-CII LEAK	Species	1	5053.614	5053.614	6.753	0.017
		Temperature (°C)	1	27545.506	27545.506	36.809	<0.001
		Species x Temperature (°C)	1	1801.881	1801.881	2.408	0.136
		Residual	20	14966.903	748.345		
		Total	23	47790.931	2077.867		
	CI-CII ETS	Species	1	5629.717	5629.717	0.171	0.684
		Temperature (°C)	1	305076.646	305076.646	9.261	0.006
		Species x Temperature (°C)	1	4297.819	4297.819	0.13	0.722
		Residual	20	658832.862	32941.643		
		Total	23	995091.172	43264.834		
	CII ETS	Species	1	72024.567	72024.567	5.231	0.033
		Temperature (°C)	1	103804.721	103804.721	7.539	0.012
		Species x Temperature (°C)	1	4856.993	4856.993	0.353	0.559
		Residual	20	275371.807	13768.59		
		Total	23	451464.219	19628.879		
	COX	Species	-	-	-	-	-
		Temperature (°C)	-	-	-	-	-
		Species x Temperature (°C)	-	-	-	-	-
		Residual	-	-	-		
		Total	-	-	-		

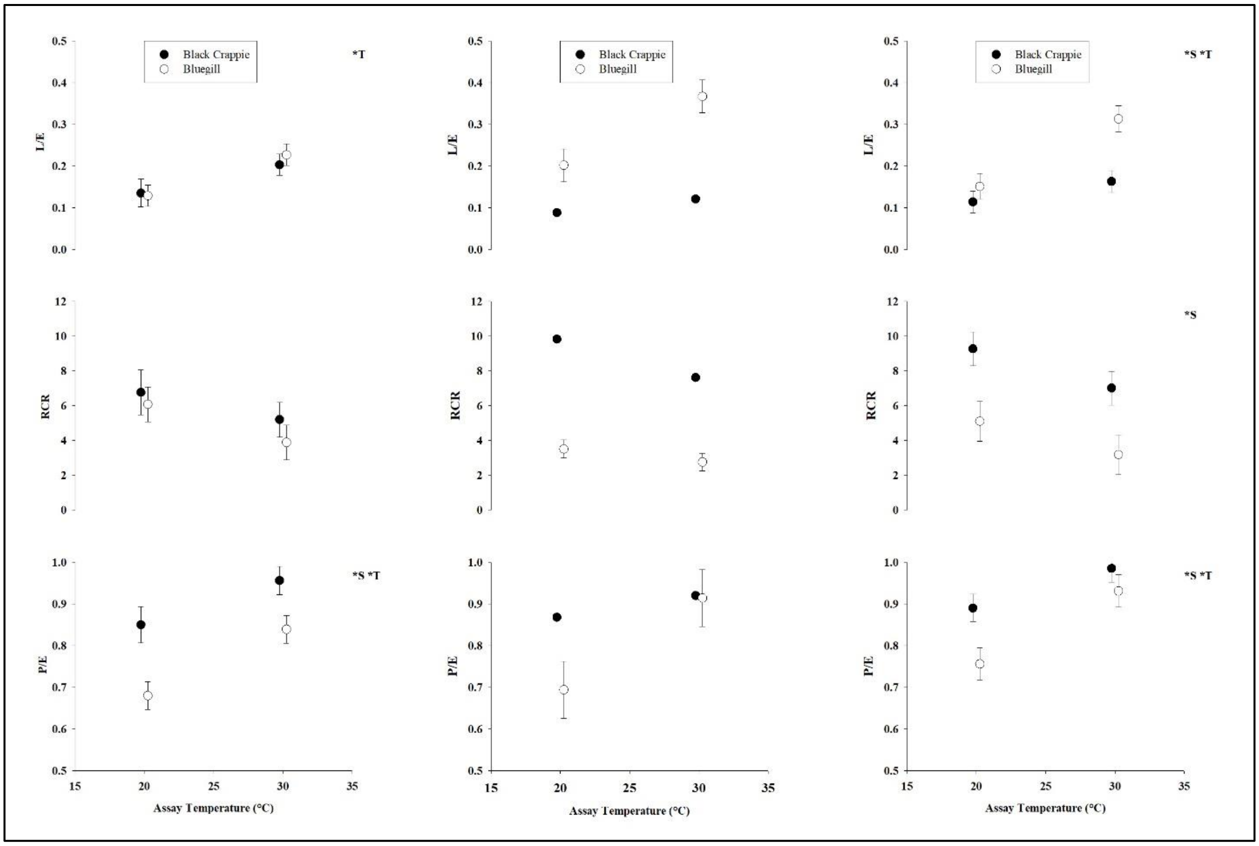

*Figure 22. Combined Flux Control Ratio Graphs for fall 2019 (left column), spring 2020 (middle column), and fall 2020 (right column) showing values for the coupling control ratio (P/E), respiratory control ratio (RCR), and leak control ratio (L/E) for Black Crappie and Bluegill. Sample sizes included the following; fall 2019 Black Crappie at 20°C = 3, fall 2019 Black Crappie at 30°C = 5, fall 2019 Bluegill at 20°C = 5, fall 2019 Bluegill at 30°C = 5, spring 2020 Black Crappie at 20°C = 1, spring 2020 Black Crappie at 30°C = 1, spring 2020 Bluegill at 20°C = 5, spring 2020 Bluegill at 30°C = 5, fall 2020 Black Crappie at 20°C = 7, fall 2020 Black Crappie at 30°C = 7, fall 2020 Bluegill at 20°C = 5, and fall 2020 Bluegill at 30°C = 5. If statistical significance occurred from the Two-way ANOVA, this is shown by the source of variation being species (*S) or temperature (*T).*

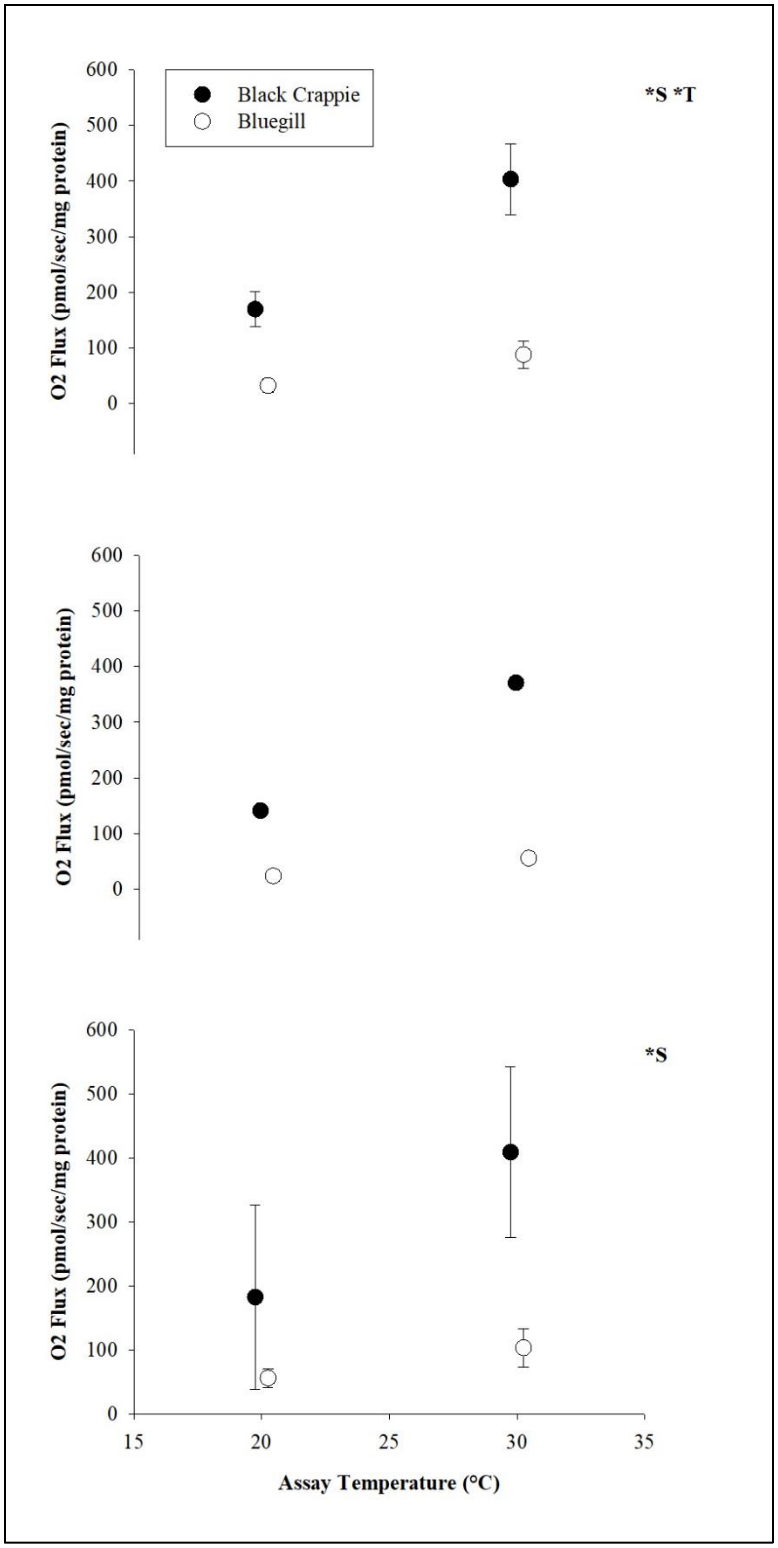

*Figure 23. Combined CI OXPHOS Graphs for Fall 2019 (bottom), Spring 2020 (middle), and Fall 2020 (top) for Black Crappie and Bluegill. If statistical significance occurred from the Two-way ANOVA, this is shown by the source of variation being species (*S) or temperature (*T). Data presented in mean ± SEM; n ranged from 1 – 7.*

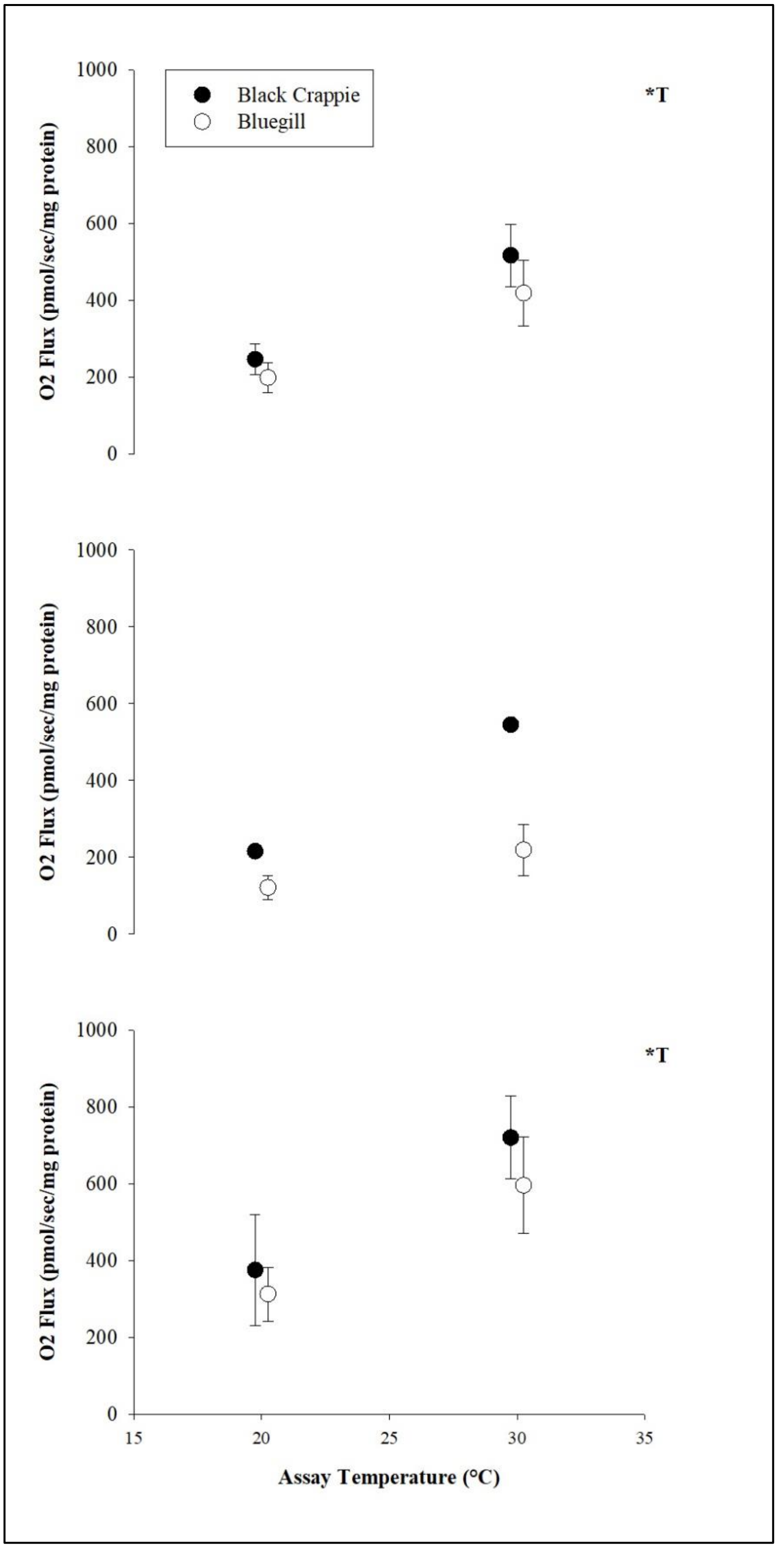

*Figure 24. Combined CI-CII OXPHOS Graphs for Fall 2019 (bottom), Spring 2020 (middle), and Fall 2020 (top) for Black Crappie and Bluegill. If statistical significance occurred from the Two-way ANOVA, this is shown by the source of variation being species (*S) or temperature (*T). Data presented in mean ± SEM; n ranged from 1 – 7.*

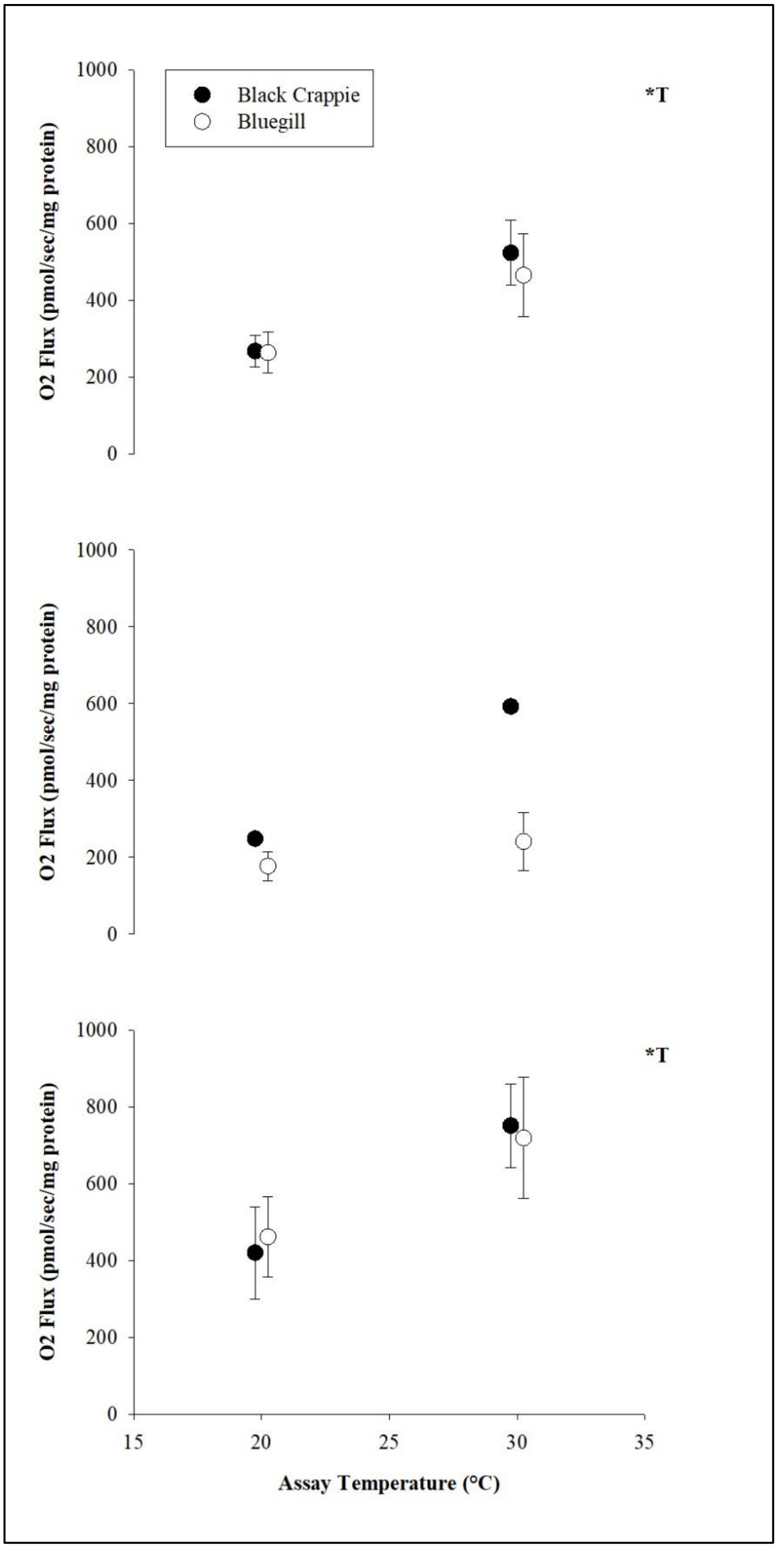

*Figure 25. Combined CI-CII ETS Graphs for Fall 2019 (bottom), Spring 2020 (middle), and Fall 2020 (top) for Black Crappie and Bluegill. If statistical significance occurred from the Two-way ANOVA, this is shown by the source of variation being species (*S) or temperature (*T). Data presented in mean ± SEM; n ranged from 1 – 7.*

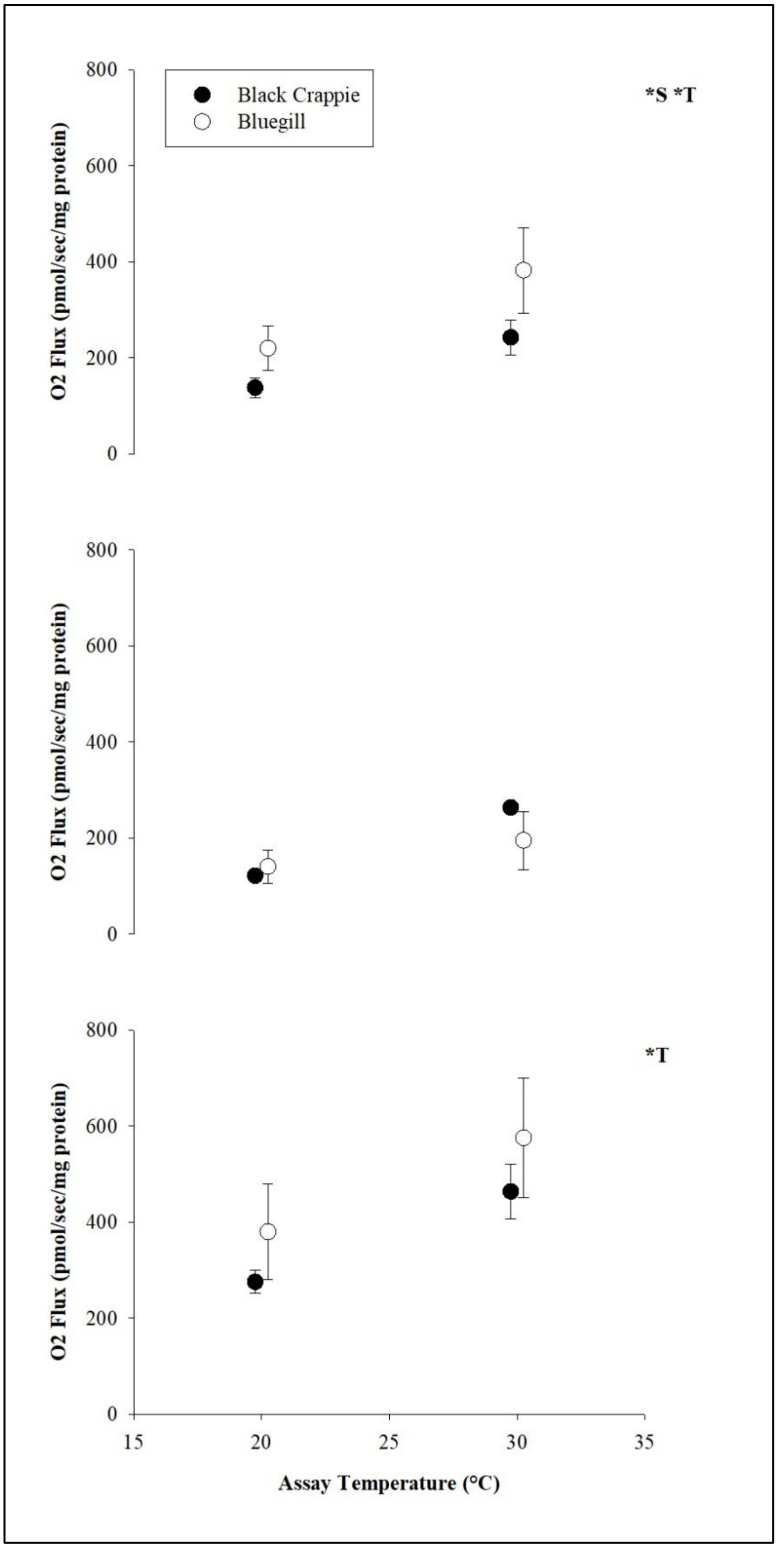

*Figure 26. Combined CII ETS Graphs for Fall 2019 (bottom), Spring 2020 (middle), and Fall 2020 (top) for Black Crappie and Bluegill. If statistical significance occurred from the Two-way ANOVA, this is shown by the source of variation being species (*S) or temperature (*T). Data presented in mean ± SEM; n ranged from 1 – 7.*

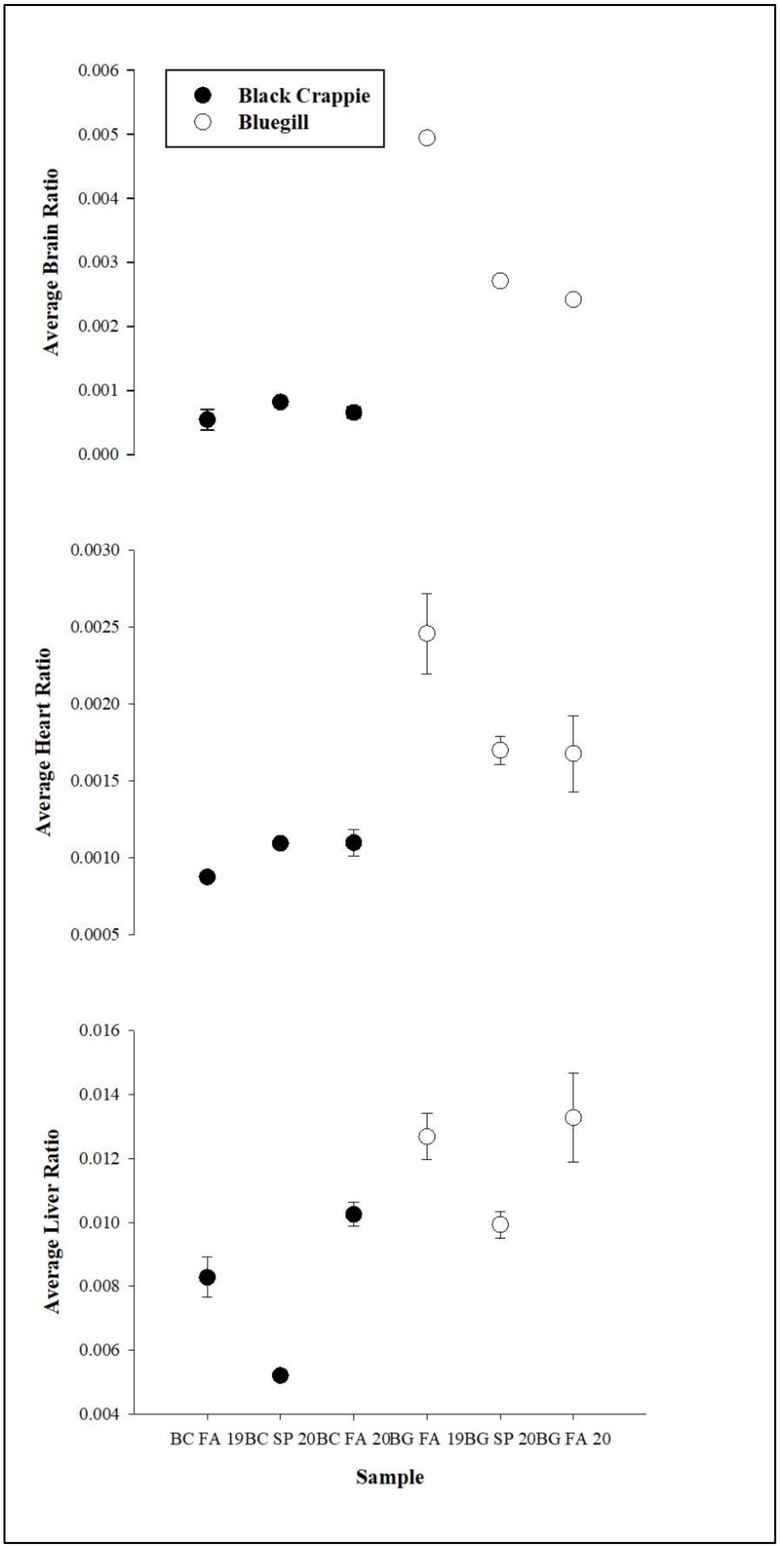

Figure 27. Combined somatic indices graphs for all seasons sampled, showing average liver, heart, and brain to body mass ratios for Black Crappie and Bluegill. Data presented in mean ± SEM; n ranged from 1 – 7.

CHAPTER 4: CONCLUDING REMARKS

A seasonal sampling of Coffeen Lake showed there to be noticeable changes in post shutdown values for not only environmental variables, but also for the various bioenergetics testing done between Black Crappie and Bluegill. Sampled water quality profiles showed less extreme stratification occurring during the cooler months of fall, likely as the result of more mixing due to the loss of thermal discharge. This was seen primarily in the cooling loop and adjacent transition zone, with the ambient zone remaining consistent in values and staying fairly unaffected by any effluent loss.

Bluegill showed irregular, almost linear, growth patterns throughout each of the seasonal sampling periods, and most individuals collected were below stock lengths. This irregular growth for Bluegill has been documented before on Coffeen Lake (Martinez et al. 2015) when the reservoir was receiving thermal discharge. The most apparent indicator of this was observed in the results of the fall 2019 sampling, immediately following the hot effluent shut off, where a growth curve was unable to be fit for the population in the reservoir due to the abnormal growth pattern occurring. Black Crappie on the other hand were sampled at a wider range of size classes and had a growth pattern that displayed a more standard logistic trend when compared to Bluegill in Coffeen Lake. Data for both species showed that comparing age class and mean otolith mass could provide useful correlations for interpreting difficult to age specimens.

Despite discrepancies in growth and size structures between the two species, both had high mortality rates for their respective species (Anderson 1973; Ellison 1984). High mortality in crappie was previously seen in Coffeen Lake, with angler harvest thought to be the prevailing cause (Porreca 2012). Bluegill on the other hand seemed to mostly reach sizes well below the desirable threshold for keep, pointing to a different, physiological explanation for their status in the lake.

Statistically significant results were observed when comparing the means by which the mitochondria of both species operate in relation to season and assay temperature, with complex-specific contributions to the ETS found to be species-specific. These trends can be seen in Black Crappie being more ETS limited by OXPHOS and having higher RCR values, resulting in more efficient ATP production occurring. Bluegill exhibited a higher portion of the ETS being controlled by LEAK, indicating less efficient ATP production. The higher LEAK rates seen in Bluegill could also be attributed to the uncouple to survive hypothesis, which indicates that organisms living in supraoptimal temperatures can reduce energy transduction efficiency in favor of lower membrane potentials and reduced production of reactive oxygen species in cells (Brand 2000). Because of the Bluegill being subject to a different thermal regime and operating their bioenergetics machinery in a less efficient manner, the tradeoffs in growth taken by each of the species could help to be explained.

Going forward, continuing to monitor and sample Coffeen Lake would be beneficial in documenting the reservoir’s reversion to a more natural state. Since it has been under the effect of thermal discharge for decades, documenting if the removal of the effluent has any effect on the organismal growth of the Bluegill should be a priority. Allowing them to reach desirable panfish sizes could be another draw for the public when fishing there. Additionally, keeping track of the Black Crappie distribution could open more areas where anglers can better target them for catch now that they are no longer being thermally impacted.

By combining fisheries and physiology data on Black Crappie and Bluegill, more efficient techniques can be employed for future Centrarchid management efforts. In a world that is constantly changing due to both anthropogenic impacts and naturally occurring seasonal cycles, addressing the challenges introduced by unnatural thermal regimes should remain a priority for researchers and stakeholders alike. Understanding that fishes can show their thermal tolerances at the whole organismal and sub-cellular level can be key in connecting scientific findings that have the potential to provide a more complete picture of the biomechanisms at play.

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APPENDIX A: ADDITIONAL FIGURES

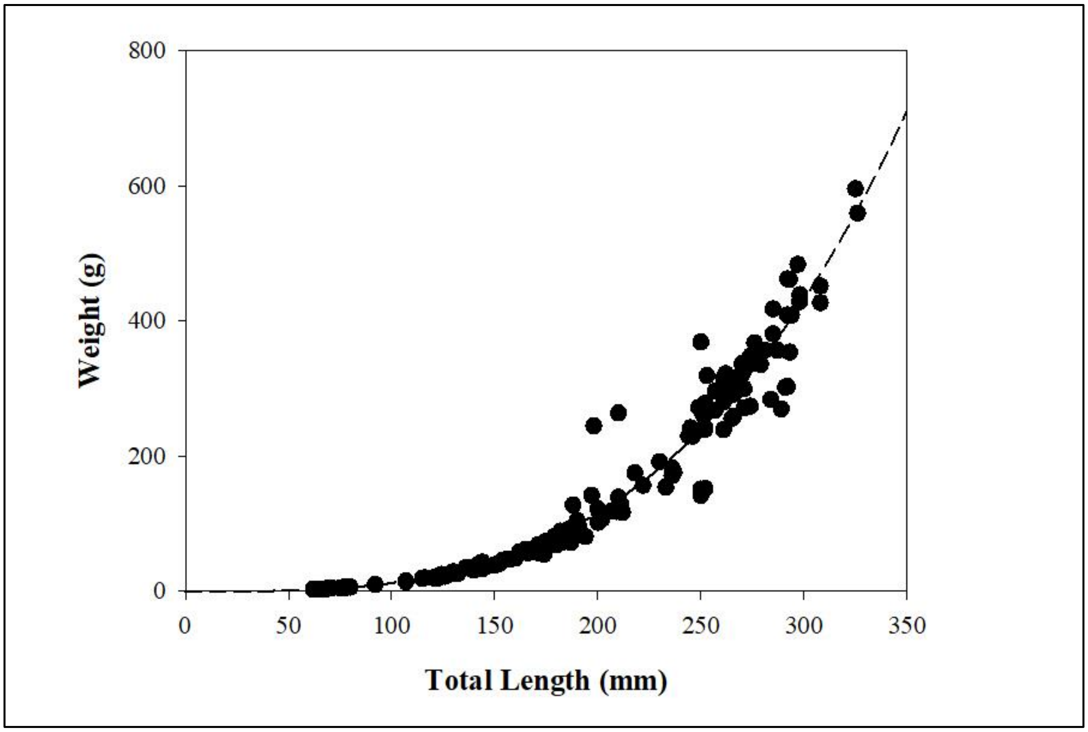

Figure A1. Length versus weight regression of Black Crappie sampled from Coffeen Lake using DC electrofishing during fall 2019 and fall 2020. n = 160, $y = 4E\text{-}06x^{3.2364}$, $r^2 = 0.9730$.

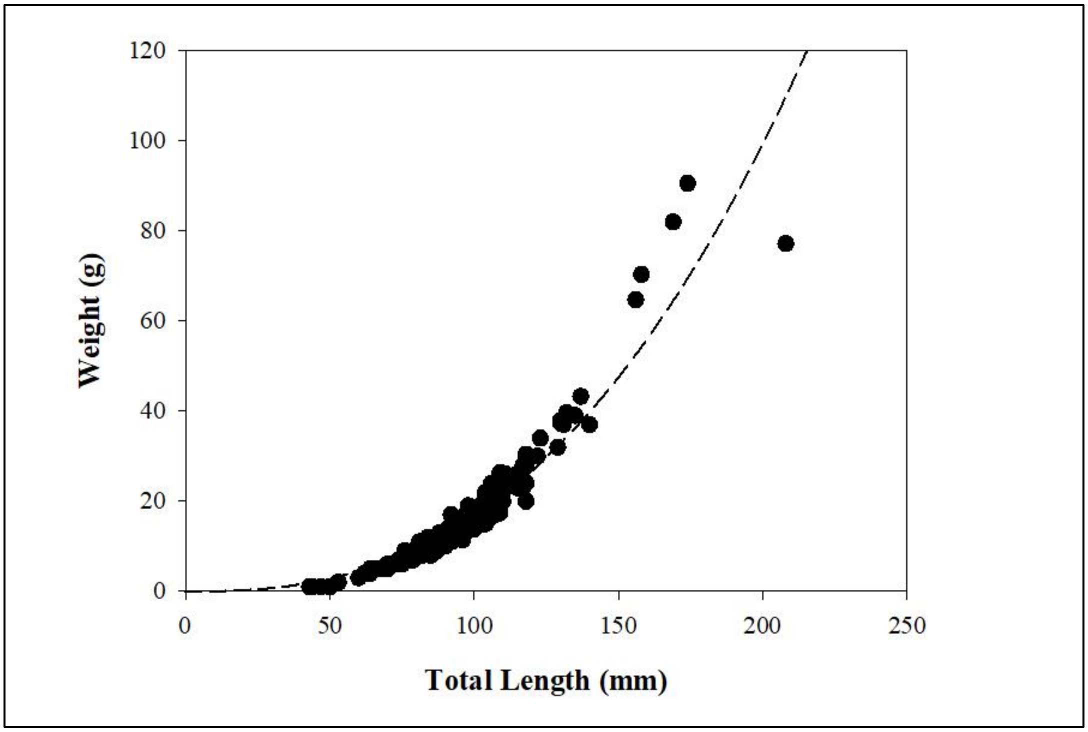

Figure A2. Length versus weight regression of Bluegill sampled from Coffeen Lake using DC electrofishing during spring 2020. n = 207, y = 1E-04x$^{2.5641}$, r^{2} = 0.9511.

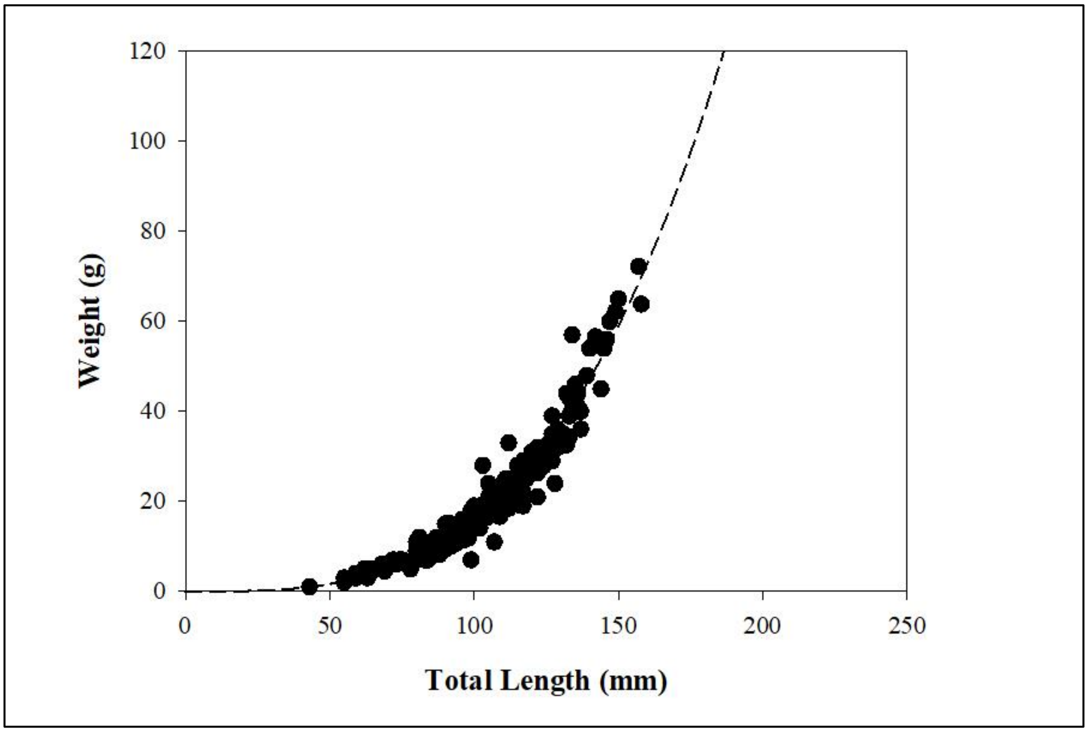

Figure A3. Length versus weight regression of Bluegill sampled from Coffeen Lake using DC electrofishing during Fall 2019 and Fall 2020. n = 250, y = 4E-06x$^{3.2532}$, r^{2} = 0.9760.

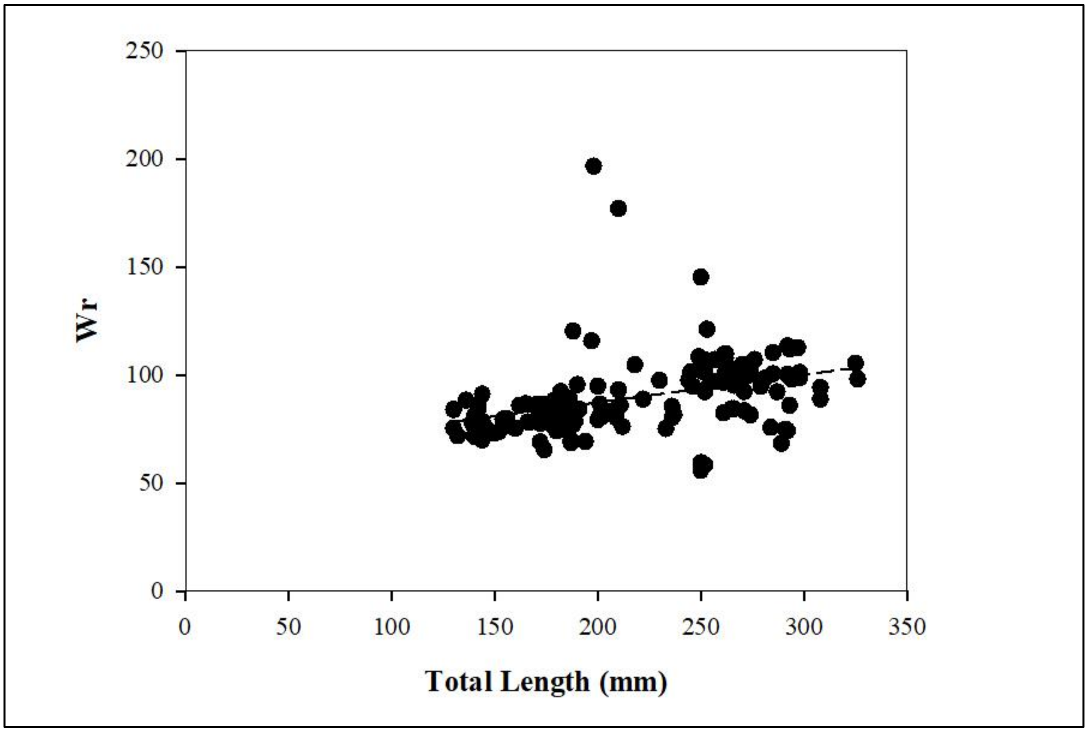

Figure A4. Length versus relative weight regression of Black Crappie sampled from Coffeen Lake using DC electrofishing during fall 2019 and fall 2020. n = 138, y = 3.3592x - 5.6828, r^2 *= 0.1381.*

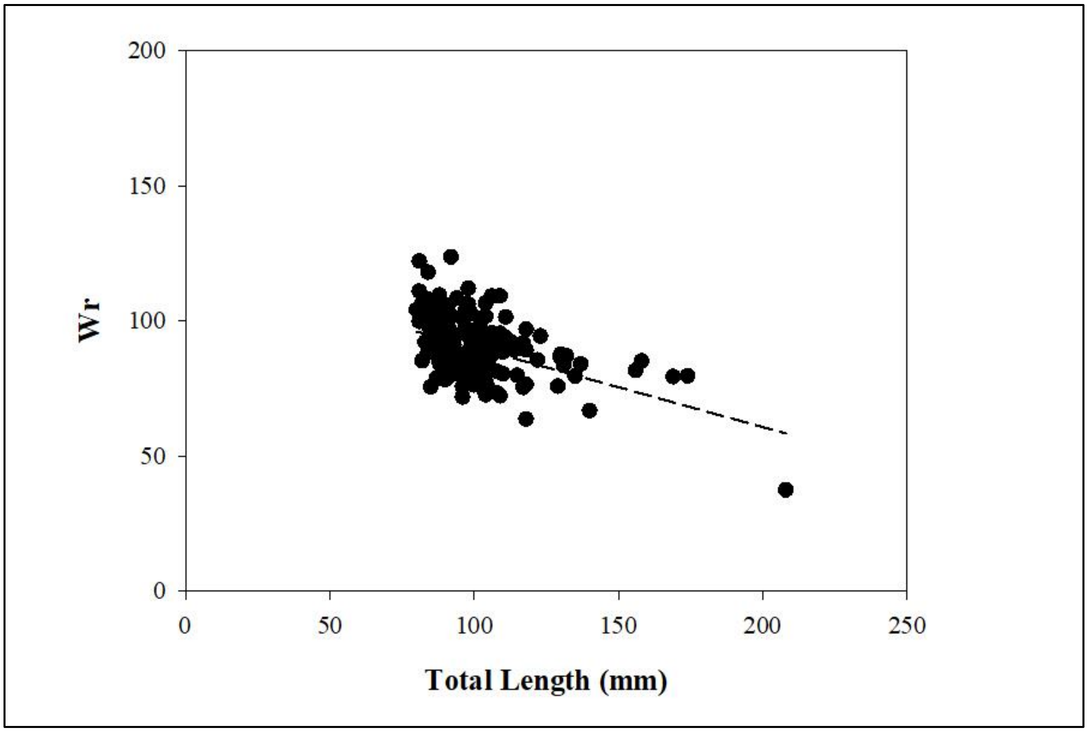

Figure A5. Length versus relative weight regression of Bluegill sampled from Coffeen Lake using DC electrofishing during spring 2020. n = 159, y = -0.2945x + 119.71, r^2 = 0.2146.

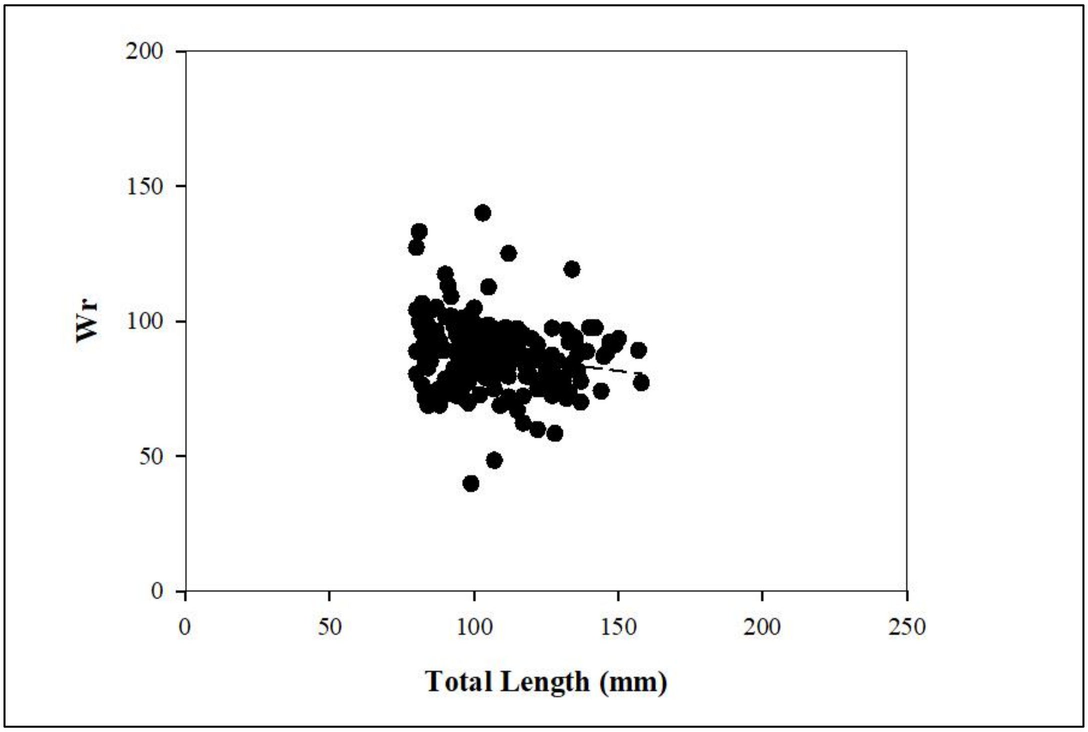

Figure A6. Length versus relative weight regression of Bluegill sampled from Coffeen Lake using DC electrofishing during fall 2019 and fall 2020. n = 211, y = -0.1375 + 102.31, r^2 = 0.0377.

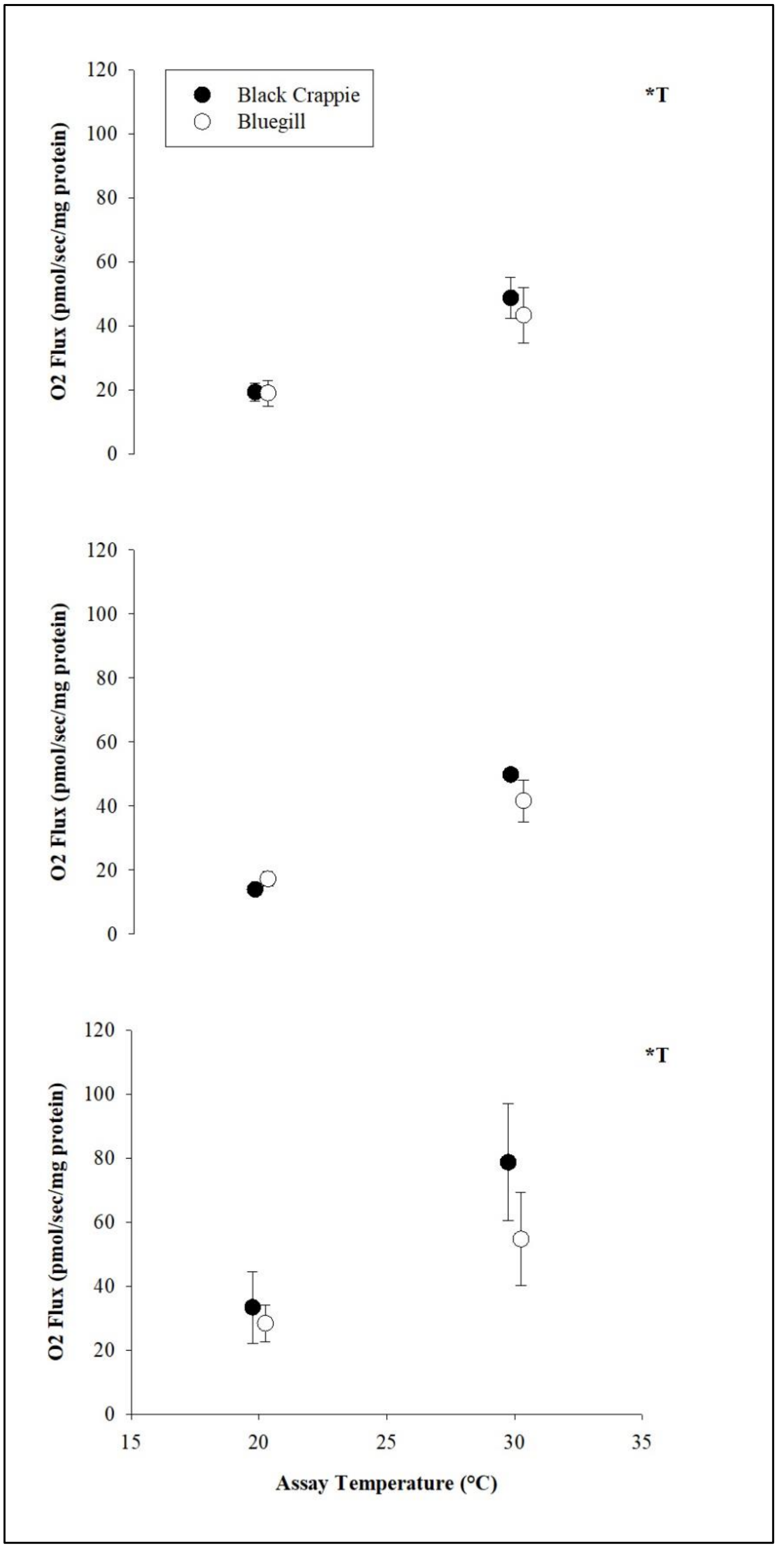

*Figure A7 Combined CI LEAK Graphs for Fall 2019 (bottom), Spring 2020 (middle), and Fall 2020 (top) for Black Crappie and Bluegill. If statistical significance occurred from the Two-way ANOVA, this is shown by the source of variation being species (*S) or temperature (*T). Data presented in mean ± SEM; n ranged from 1 – 7.*

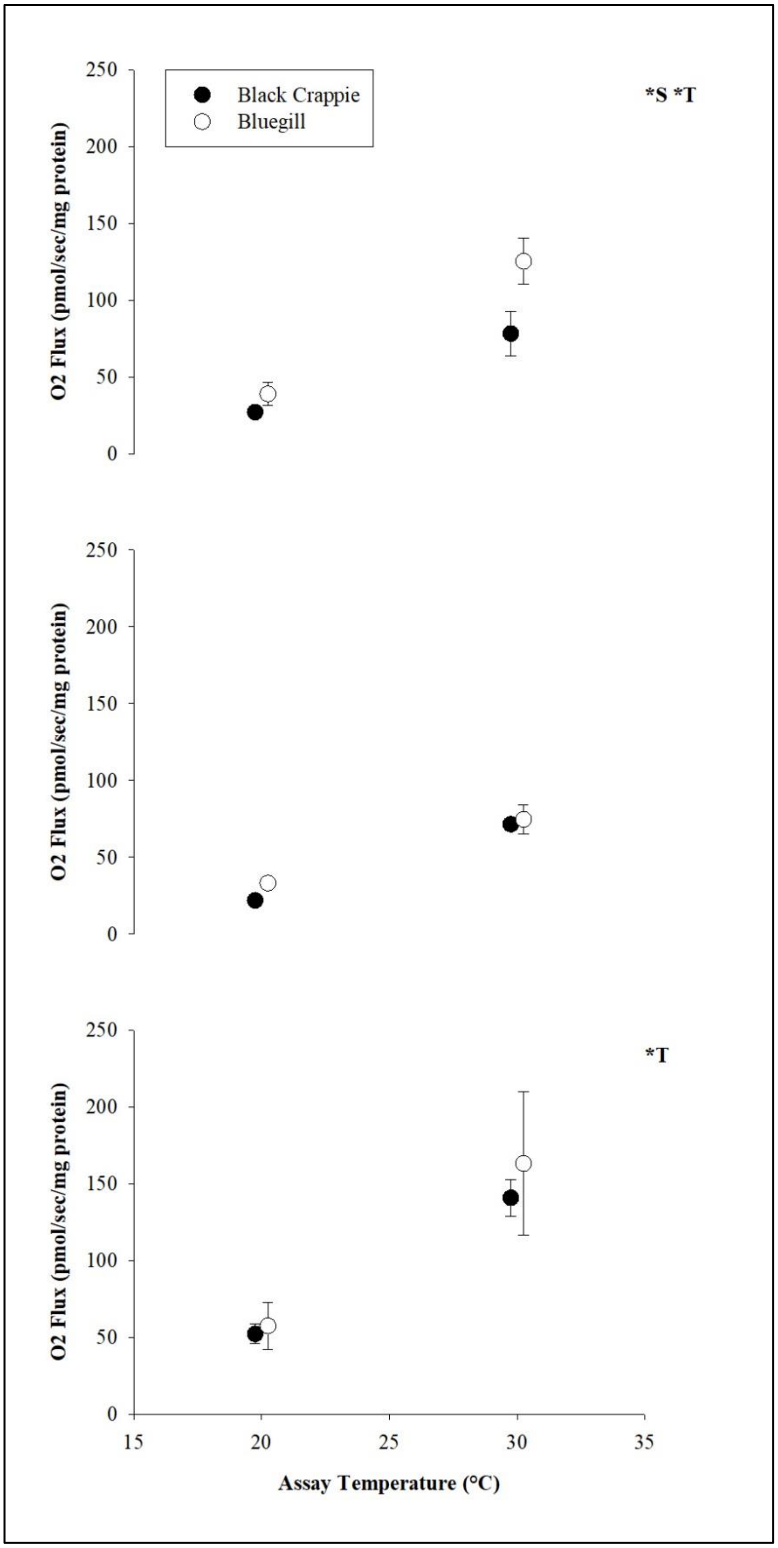

*Figure A9. Combined CI-CII LEAK Graphs for Fall 2019 (bottom), Spring 2020 (middle), and Fall 2020 (top) for Black Crappie and Bluegill. If statistical significance occurred from the Two-way ANOVA, this is shown by the source of variation being species (*S) or temperature (*T). Data presented in mean ± SEM; n ranged from 1 – 7.*

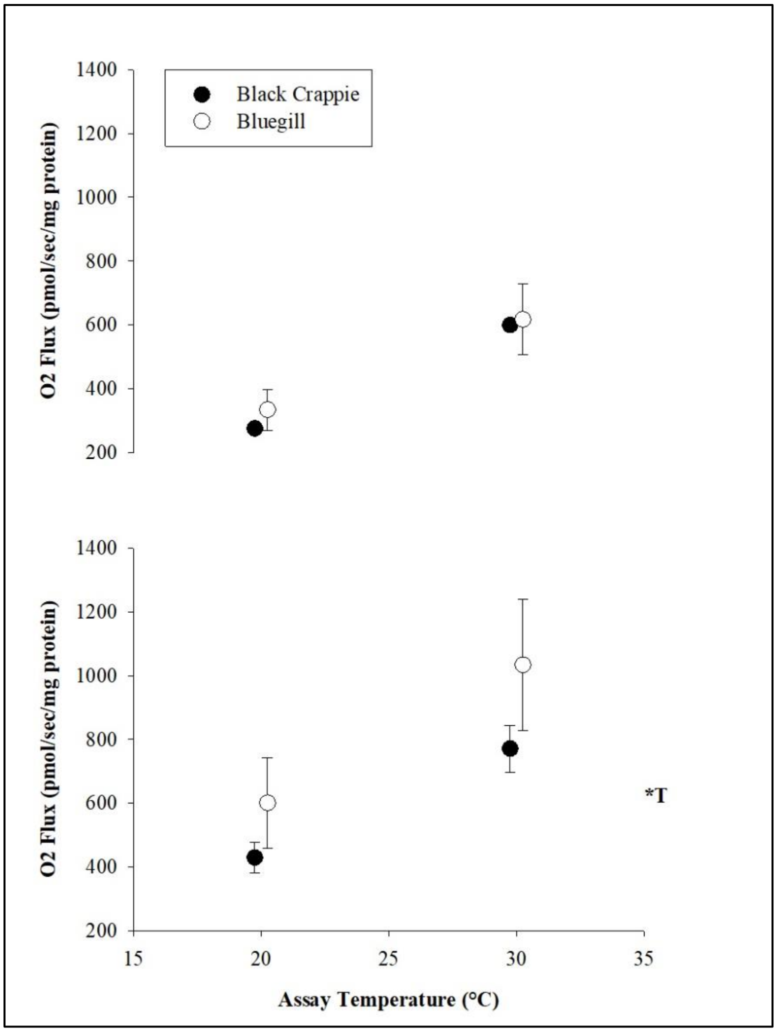

*Figure A11. Combined COX Graphs for Fall 2019 (bottom) and Spring 2020 (top) for Black Crappie and Bluegill. If statistical significance occurred from the Two-way ANOVA, this is shown by the source of variation being species (*S) or temperature (*T). Data presented in mean ± SEM; n ranged from 1 – 5.*