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The Biological Stress Response in Bluegill Sunfish (Lepomis macrochirus) to variations in environmental temperature and dissolved oxygen content.

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The Biological Stress Response in Bluegill Sunfish (*Lepomis macrochirus)* to variations in environmental temperature and dissolved oxygen content.

An Honors Thesis submitted in partial fulfilment of the requirements for Honors in the Biology Department.

By

Monique Kellman

Under the mentorship of Dr. Johanne Lewis

Abstract

With the predicted increase in global water temperature and acute hypoxic episodes, knowledge of the effects these stressors can have on local aquatic life is extremely valuable. This study thereby quantified the change in metabolic rate in Bluegill sunfish, in response to increased temperature and low dissolved oxygen concentration, by utilizing intermittent flow respirometry. Both maximum metabolic rate (MMR) and resting metabolic rate (RMR) were determined in response to variations in dissolved oxygen content, specifically > 95% O_2 and 40% O_2. Additionally, three temperature treatment groups were established, with temperatures of 20, 25 and 30°C in order to ascertain the effect of increased temperature on MMR and RMR. This data was then used to calculate aerobic metabolic scope (AMS) and extrapolate the effects these stressors have on energy availability. Decreases in dissolved oxygen content were determined to result in a decrease in AMS, due to the limiting of MMR. Additionally, increases in acclimated temperature, were shown to lead to an increase in AMS, until the optimum temperature for the species was attained, after which AMS decreased. Through this, it was determined that the bluegill sunfish are currently near or in their optimum temperature range in the summer months, leading to an increased urgency for mitigations to global warming. With the expected increases in temperature over the next eight decades, these fish may be pushed out of their optimum temperature range, leaving them more susceptible to the effects of additional stressors such as hypoxia.

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Introduction

With the effects of anthropogenic environmental change becoming increasingly evident, studies predicting the impacts of rising world water temperatures and decreasing dissolved oxygen content on aquatic organisms are becoming more relevant. U.S. coastal waters are expected to have increased in temperature by as much as 2.2°C by the end of the 21st century (USGCRP, 2009). Increased water temperature of 2-3°C has been shown to result in significant damage to both the reproductive and growth capabilities of some species of fish (Rummer et al., 2014, and references included within). Instances of increased temperature are also likely to co-occur with instances of hypoxia (low dissolved oxygen), this being a condition of the physical effects of temperature on the solubility of gases in water. As the temperature of the water increases, oxygen solubility decreases, leading to a greater susceptibility to hypoxic conditions (Diaz & Breitburg, 2009). An increase in temperature also results in an increased metabolic activity of the micro-organisms responsible for eutrophication, and thereby leads to an increase in hypoxia (McBryan et al., 2013). Not only is global warming dramatically affecting the stress response in aquatic animals, but aquatic hypoxia is also taking a toll on a global scale. Over the past 50 years, increases in both the intensity and the frequency of acute hypoxic episodes in coastal areas have been observed (Shaun et al., 2012, and references included within). These hypoxic episodes have been associated with large numbers of fish kills in various parts of the world (McBryan et al., 2013). Although hypoxia can occur naturally, acute hypoxic episodes can also be escalated as a result of anthropogenic stressors such as increased levels of nutrients and organic matter (Rosewarne et al., 2016, and references included within).

In order to determine the effects of both temperature and dissolved oxygen on aquatic animals, a measure of oxygen consumption rate must be taken, thereby allowing for an estimate of aerobic metabolic rate. Oxygen consumption rate consists of three metabolic variables; resting metabolic rate (RMR), maximum metabolic rate (MMR) and absolute aerobic metabolic scope, this being the difference between MMR and RMR. According to Rosewarne et al. (2016), RMR is defined as, "the minimum maintenance or resting metabolic rate of an unstressed, post-absorptive, and non-breeding ectotherm acclimated to experimental conditions, below which physiological function is impaired". RMR is thereby the basic cost of living, and if metabolic rate drops below this level, the organism would no longer be able to function normally. MMR, on the other hand, is usually measured at the point of exhaustion and represents the maximum possible metabolic rate experienced for an organism, at a particular temperature and dissolved oxygen content. Aerobic metabolic scope (AMS) thus represents the total amount of energy available to an organism to complete tasks unnecessary for everyday function, such as movement, growth, and reproduction. A study conducted by McBryan et al. (2013) showed an initial increase in aerobic scope as temperature increased, up to a point at which optimum temperature is attained, followed by a drastic decrease. This was determined to be a result of the parallel increase of both RMR and MMR, with MMR increasing at a greater rate, up to a point at which the optimum aerobic scope was reached. After this turning point, a rise in the rate at which RMR increased was observed, in conjunction with a decrease in MMR, causing a decrease in aerobic scope. This is because, as temperature increases, kinetic energy within the cells grows, leading to an increase in metabolic reactions within the cells.

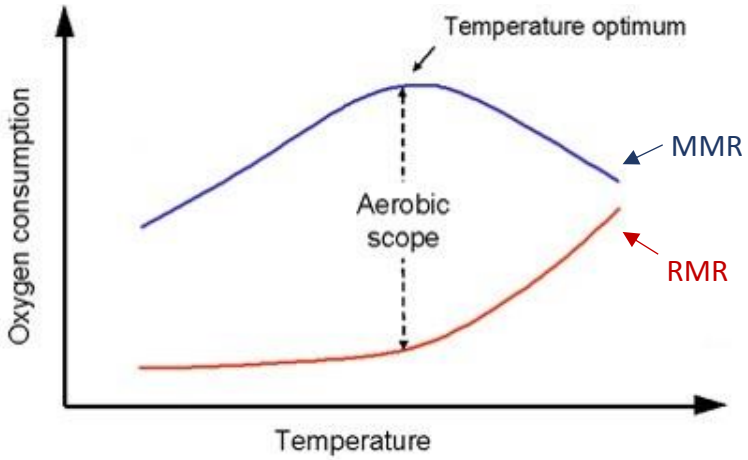


Figure 1. showing the effect of temperature on MMR and RMR, as well as aerobic scope ("Metabolic scope and temperature," n.d.).

Hypoxia, on the other hand, is expected to initially limit MMR, followed by a limiting of RMR as severity of hypoxia increases. This is because the dissolved oxygen in the water is not enough to support the maximum metabolic rate. Therefore, as dissolved oxygen decreases, the MMR is forced to decrease as a result. This occurs until the limits on MMR result in MMR having the same value as RMR. Further decreases in dissolved oxygen content lead to a limiting of RMR and thereby, a switch from aerobic respiration to anaerobic respiration. This leads to a decrease in aerobic scope as dissolved oxygen content decreases.

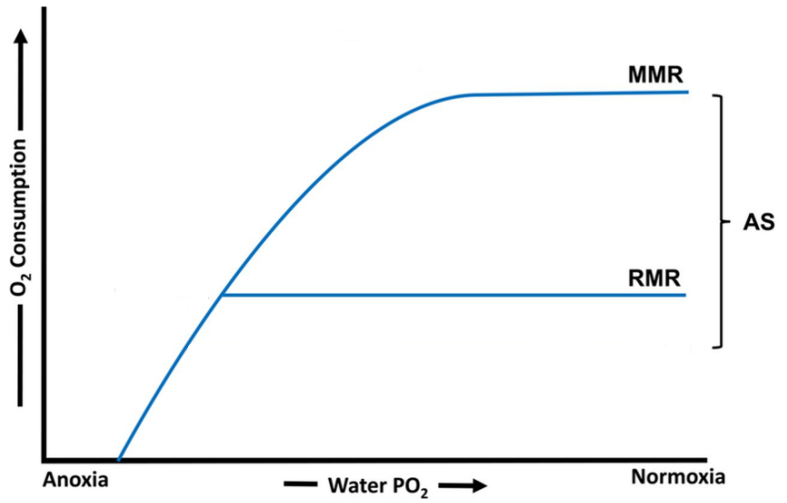


Figure 2. showing the effect of dissolved oxygen content on both MMR and RMR (Rogers et al., 2016).

The project's purpose is thus to determine the energetic costs of exposure to thermal and hypoxic stress in Bluegill sunfish (*Lepomis macrochirus*) by measuring MMR and RMR, thereby allowing for the determination of AMS and thus the relationship between dissolved oxygen availability and AMS. This will aid in the understanding of the influence of surrounding environmental conditions on physiological processes (Rosewarne et al., 2016).

This study species was chosen due to its abundance in the Ogeechee river, Georgia, as well as the lack of current research concerning this particular species and its biological stress response to environmental temperature and hypoxia. Also, due to the commonality of this species in the local Ogeechee River ecosystem, the data obtained from these experiments will be easily applicable to other species in similar habitats. Over the past few years, the Ogeechee River has experienced drought conditions, during the summer, consisting of low flow rate and high temperature, which has had multiple effects on the ecosystem in the area. Temperatures ranged from 20 to 30°C. These drought

conditions could possibly be attributed to changes in weather patterns due to global warming, and therefore justify the importance of determining the energetic costs on this local species of recreational importance, associated with changes in temperature and oxygen. With drought conditions and acute hypoxic episodes already occurring, possible predictions need to be made of how these changing conditions will affect the local species.

In conducting this research, for each temperature treatment group, the temperature of the water will be maintained in order to determine the effects of two dissolved oxygen contents on oxygen consumption, and thereby metabolism, by utilizing intermittent flow respirometry. Intermittent flow respirometry consists of short periods of closed respirometry in which the water is recirculated and measurements obtained, repeatedly followed by clean water flush periods in which the water in the respirometer is thoroughly replaced, thereby allowing the oxygen content to return to ambient levels, as well as the removal of excretory products (Svendsen et al., 2016; Rosewarne et al., 2016). Measuring oxygen consumption rate is an effective method for estimating the energetic costs of living in a particular environment with specific environmental stressors as oxygen consumption is an effective estimate of the metabolic rate of an organism. This is due to the demand for cell respiration in order to complete the biological processes necessary to sustain life.

The following hypothesis will be tested: - a decrease in dissolved oxygen content will result in a decrease in maximum metabolic rate, while resting metabolic rate remains constant, and thereby a decrease in aerobic scope. Also, as temperature increases by treatment group, both MMR and RMR will increase, with the increase in MMR being

greater than that in RMR, leading to an initial increase in aerobic scope, followed by a point at which the optimum temperature is reached and then a decrease. This decrease in aerobic scope will be due to the limiting of MMR and subsequent increase in RMR. This data will be most applicable in the context of climate change and global warming as once the effect of increased temperature and its synergistic effect with decreased oxygen availability are determined for *Lepomis macrochirus,* procedures and laws can be put in place to mitigate these effects as much as possible.

Materials and Methods

Animals

Juvenile Bluegill sunfish (*Lepomis macrochirus)* were acquired from the Richmond Hill Fish Hatchery (Georgia Department of Natural Resources Fisheries and Wildlife Division) in November 2016. Initially, the fish were placed in multiple tanks with recirculating dechlorinated fresh water and held at ambient temperatures (19 ± 1 °C). A constant supply of oxygen was maintained via an air stone in each tank. A 33% water change was performed every day for the first two months, followed by every other day for the remainder of the time. The fish were fed to satiation daily, however, fish were allowed to fast for 24 hours prior to experimentation. Approximately three weeks prior to the experimentation period, 30 fish, weighing 8.2 ± 2.3 g, were placed in three separate 20L holding tanks, with 10 fish in each tank. One tank was held at ambient temperature (19 ± 1 °C), while heaters were placed in the remaining two tanks and the temperature increased by 2 °C daily, until temperatures of 25 °C and 30 °C were obtained. These

temperatures were then held constant for two weeks to allow the fish to acclimate. Feeding and water change regimen for these experimental tanks were the same as described for the holding tanks.

Set-up for Intermittent Flow Respirometry

A medium sized respirometry chamber (Loligo Systems) was utilized in respirometry experiments. Two submersible pumps were connected to the respirometry chamber; the flush pump and the recirculation pump. The intake valve of the flush pump was exposed to the experimental tank water, and the output valve connected to the chamber via rubber tubing, thereby continuously flushing fresh oxygenated water through the chamber when the flush pump was turned on, as well as, providing the fish with oxygen and removing waste products from the chamber. During the flush period, the system is considered to be "open". The flush outlet tube, this being the tube running from the chamber and releasing water into the experimental tank, was secured to the tank a few centimetres above the water level to prevent backflow from the tank into the chamber. The intake valve of the recirculation pump was connected to the respirometry chamber, and the outtake valve connected to the flow cell, via rubber tubing, which then flowed back into the respirometry chamber, thereby forming a recirculation loop through which the dissolved oxygen content of the chamber could be determined.

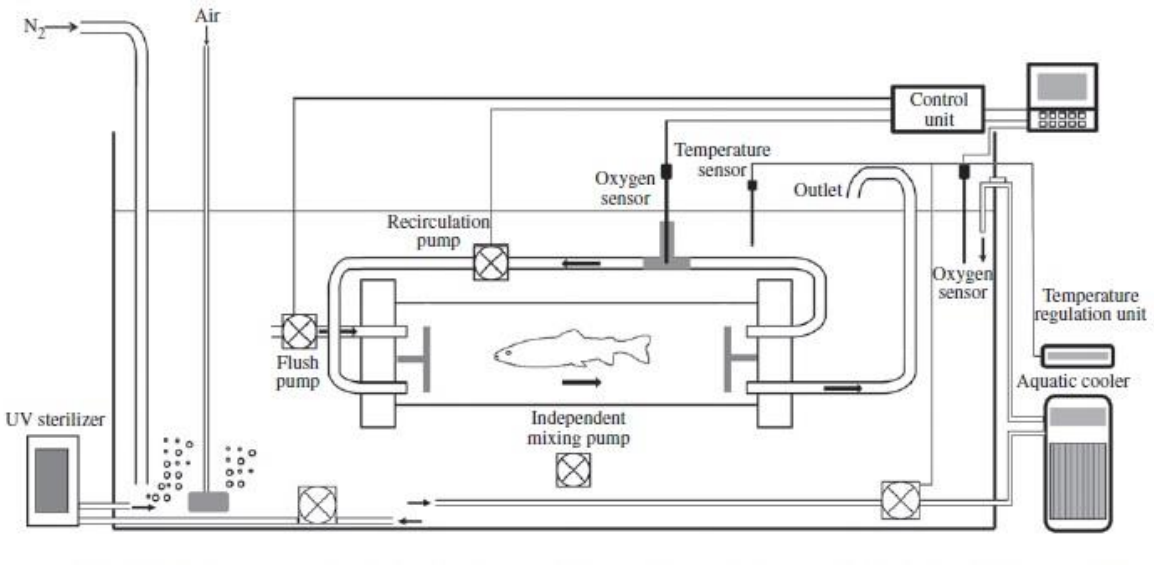


Figure 3. Setup of the intermittent flow respirometry system in the experimental tank, with block arrows denoting the direction of the flow of water, as well as N_2 and air denoting the input of both gases respectively, for use in hypoxic testing (Rosewarne et al., 2016)

Typically, intermittent flow respirometry consists of three timing periods for one measurement cycle, these being flush, wait and measure. In this experiment, the flush period was run for 4 minutes for the RMR readings, and removed for the MMR readings. This was followed by the recirculation cycle, consisting of the wait and measure periods, in which testing occurred. During the wait period, (2 minutes) and measure period (5 minutes), water was only being circulated from the chamber, past the flow cell containing the dissolved oxygen probe, and back through the chamber, making the setup a "closed" system. It was expected that dissolved oxygen concentration of the water within the chamber would begin to decline linearly as fresh oxygenated water was not being inputted. This linear decline is important for accurate measurement of oxygen consumption rates. During the 5-minute measure period, data was collected which was

then used for the calculation of the fish's oxygen consumption rate (units of O2 / kg body mass/ hr).

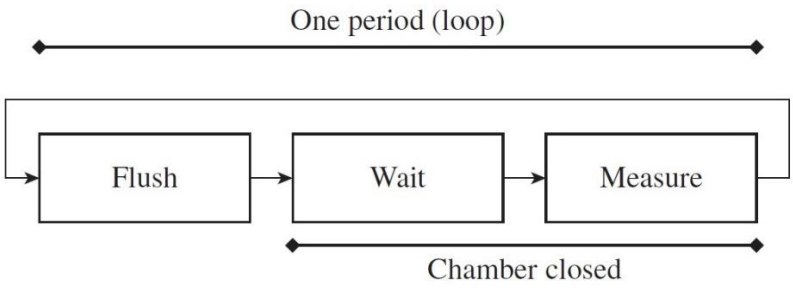


Figure 4. showing one measurement period for an intermittent respirometry system (Svendsen et al., 2016)

The total respirometry volume (520mL), consisting of the volume of water held in the chamber in addition to the volume of water held in the recirculation loop, was determined by filling the system, excluding the flush pump and tubing, with water and pouring that water into a measuring cylinder.

Method for varying environment temperature and oxygen

Water temperature was altered in the experimental tank by adding an external heater to the experimental setup. A pump was placed in the experimental tank to effectively pump water through the heating element before returning it to the experimental tank via the output tubing. These heaters enabled temperature to be maintained within ± 0.5°C of the desired experimental temperature.

Dissolved oxygen in the experimental tank was maintained at normoxic levels (>95% O_2sat) by vigorously bubbling air into the experimental tank water via an air

stone. Hypoxic conditions were achieved by bubbling N_2 gas into the experimental tank water at a rate that resulted in a decrease of 1-5% O_{2sat} per minute, as referenced in Rosewarne et al. (2016) and maintained at 40% ± 2% O_2sat by adding either air or N2 gas as needed. Following the hypoxic measurements, dissolved oxygen was restored to normoxic levels by vigorous bubbling of oxygen gas into the water for approximately 20 minutes. Oxygen content was monitored by using a previously calibrated oxygen sensor (YSI).

Measurement of Background Respiration

In all respirometry setups there will be low levels of oxygen consumption due to bacterial respiration (background respiration), that if not corrected for, can overestimate the oxygen consumption rate of the fish. To correct for this, rates of background respiration are thereby taken by measuring the oxygen consumption without a fish in the chamber. Background respiration was determined before the first (normoxic) measurement of MMR and after the second (hypoxic) measurement of MMR by running the respirometry cycle with a 4-minute flush phase, followed by a 2-minute wait phase, and finally a 10-minute measurement phase. Throughout this measurement phase, readings for oxygen concentration (mg/L) are taken in 30 second intervals.

Measurement of MMR

In order to accurately measure MMR, the fish was chased to exhaustion before measurements were made. Prior to chasing, the respirometry chamber was connected and the setup made ready to receive the fish, once exhausted. Exhaustion was stimulated by

placing the fish in an experimental MMR tank, where the fish was chased with both a net and the researchers hand in a glove, interchangeably, for 5 minutes. A pinching motion was made near the tail of the fish to ensure that the fish continued to swim away from the researcher's hand (Rosewarne at al., 2016). Temperature and oxygen content were kept constant during the 5-minute chase by placing a thermometer and an oxygen probe in the experimental MMR tank, as well as an air stone allowing for either oxygen or nitrogen to be pumped into the water as needed. Following the 5-minute chase, the fish was held in the air for 1 minute. In this time, the fish was gently blotted with a paper towel to remove excess water and weighed using a scale. Once the minute was complete, the fish was placed in the respirometry chamber, and the screws tightened, ensuring that no bubbles were left trapped in the chamber. In order to accurately get a measure for maximum metabolic rate, the 4-minute flush phase was omitted; instead the experiment began at the 2-minute wait phase. During the 5-minute measurement phase, measurements for oxygen content in the chamber were taken every 30 seconds. Once the 5 minutes were completed, the flush pump was turned on and the fish was left to recover in the chamber for 24 ± 1 hour before obtaining SMR measurements.

Measurement of SMR

According to Clark et al. (2013), in order to obtain an accurate measurement of SMR, a minimum of 24 hours must be allowed for the metabolic rate of the fish to drop from MMR to SMR. In this experiment, 24±1 hours were allotted for this acclimation. Also, to ensure that the rate obtained for SMR was at its lowest, disturbances such as changes in sound and light were minimized during the experimentation period by coating the experimental tanks in black plastic (Rosewarne at al., 2016). Also, to accommodate

for changes in the circadian rhythm of the fish, measurements for both MMR and SMR were only taken during daylight hours. SMR measurements consisted of a 4-minute flush, followed by a 2-minute wait, and a 5-minute measurement period. Once this measurement period was complete for normoxic conditions, the flush pump was turned on and the oxygen content of the experimental tank was decreased at a rate previously mentioned in order to obtain measurements for SMR in hypoxic conditions, followed by MMR in hypoxic conditions.

Formulas for Calculations

All calculations employed in this study were obtained from a study conducted by Rosewarne et al. (2016). In order to calculate oxygen consumption rate in $mgO_2\ kg^{-1}\ h^{-1}$ (represented by y), four values were required. These values consisted of K, the rate of decline in oxygen content in the respirometer over the 5-minute measurement period with the fish present in $kPa\ h^{-1}$, V, the total volume of water in the respirometry system, consisting of the respirometry chamber and the recirculation loop, minus the volume of the fish in L, β, the solubility of oxygen in water at a specific temperature, pressure, and salinity in $mgO_2\ L^{-1}\ kPa^{-1}$, and M, the mass of the organism in kg. The equation 1 is as follows:

$$y = KV\ \beta\ M^{-1} \qquad (1)$$

The rate of decline in oxygen content in the respirometer over the 5-minute measurement period was obtained by plotting a graph of $\%O_2$ ($mg\ L^{-1}$) versus time (s), as shown in

Figure 5 below. This rate was then converted to kPa h^{-1} using a pressure of 760 mmHg and salinity 0.

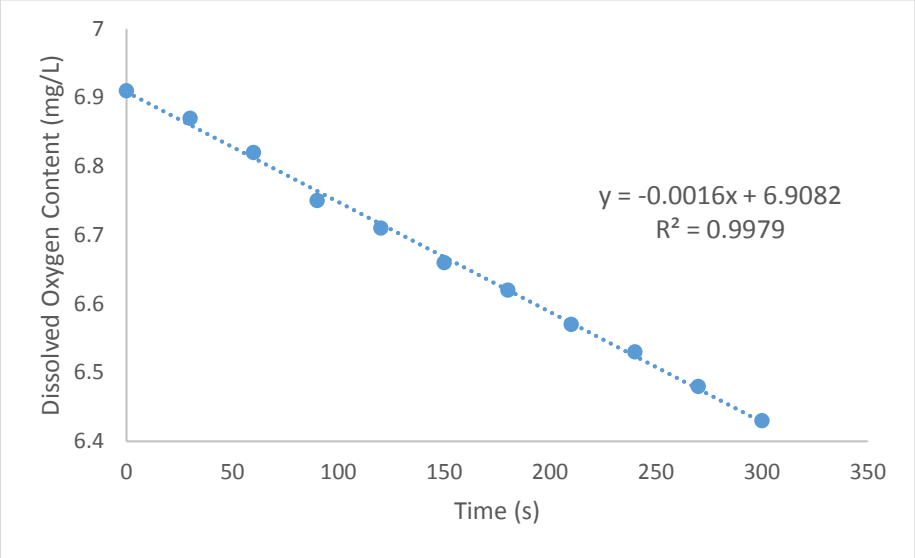


Figure 5. showing values obtained at 30 second intervals for dissolved oxygen content for fish 12 plotted against time. The slope acquired from this graph can then be used to determine the rate of decline in oxygen content in 300 seconds.

The value obtained for oxygen consumption rate was then corrected for background respiration by using the following equation:

$$y_{corrected} = [\beta (K_1 V_1 - K_2 V_2)]M^{-1} \quad (2)$$

In equation 2, $y_{corrected}$ represents the oxygen consumption rate in $mgO_2\ kg^{-1}\ h^{-1}$, corrected for background respiration, K_1 represents the rate of decline in oxygen content in the respirometer with the fish present in kPa h^{-1}, K_2 represents the rate of decline in oxygen content in the respirometer with the fish absent in kPa h^{-1}, V_1 represents the total volume

of water in the respiratory system minus the volume of the fish (L), and V_2 represents the total volume of water in the respiratory system (L). Both the value for β and the value for M remain the same as in equation 1.

Lastly, the value obtained for oxygen consumption rate, corrected for background respiration, was corrected for mass of the fish. This was done by using the following equation:

$$y_{Mkg} = y_{corrected}(M^{-1})^{(1-A)} \quad (3)$$

where $y_{M\ kg}$ represents the oxygen consumption rate ($mgO_2\ kg^{-1}\ h^{-1}$), corrected for both background respiration and rate, for a fish of mass M kg. $y_{corrected}$ is the oxygen consumption rate corrected for background respiration alone ($mgO_2\ kg^{-1}\ h^{-1}$), M is the mass of the organism (kg), and A is the mass exponent corresponding to the relationship between oxygen consumption rate and body mass. A value of 0.8 was used for A, based on previous literature.

In order to calculate background respiration for the measurements of RMR, a graph was made using the value for initial background respiration at time = 0 taken prior to the measurement of MMR in normoxic conditions, and the second value for background respiration at time = final time. This second value for background respiration was taken following the measurement of MMR in hypoxic conditions. Once a slope was

obtained, the value for background respiration for the time at which each value of RMR was taken was calculated by using the equation:

$$y_{background} = y + (m * t) \quad (4)$$

Where $y_{background}$ represents the value for background rate of decline in oxygen content in the respirometer with the fish absent at the time of the specific measurement for RMR (kPa h^{-1}), y represents the rate of decline in oxygen content in the respirometer with the fish absent at time = 0 (kPa h^{-1}), m represents the slope of the graph plotted, and t represents the time(h) after the first measurement of background respiration.

Statistical Analysis

A single-factor one-way ANOVA was used in statistical analysis of the data collected. This statistical analysis was applied to variance between values of MMR under normoxic conditions and MMR under hypoxic conditions within the same temperature treatment group with a confidence interval of $p \leq 0.05$. This was repeated for RMR as well. Single-factor one-way ANOVA ($p \leq 0.05$), was also applied to variance between average RMR values between temperature treatment groups, under both normoxic and hypoxic conditions.

Results

Effect of temperature and hypoxia on RMR

Resting metabolic rate (RMR) was determined for 12 fish belonging to the species *Lepomis macrochirus,* each fully acclimated to one of three test temperatures, these being 20, 25 and 30 °C. In reference to data displayed in Figure 6, detailing the comparison of RMR for fish acclimated to temperatures of 20, 25 and 30 °C under both normoxic and hypoxic conditions, a clear increase is observed under normoxic conditions with the RMR increasing with each 5°C increase in acclimation temperature, with the rate of increase becoming greater as temperature rose (378.5 ± 96.5, 420.3 ± 55.2, 621.5 ±85.4 $mgO_2\,kg^{-1}\,h^{-1}$ for fish acclimated to 20, 25 and 30 ºC, respectively; Figure 6). However, although a visible increase was noticed, this increase was not statistically significant ($p = 0.55$) as determined by a one-way ANOVA.

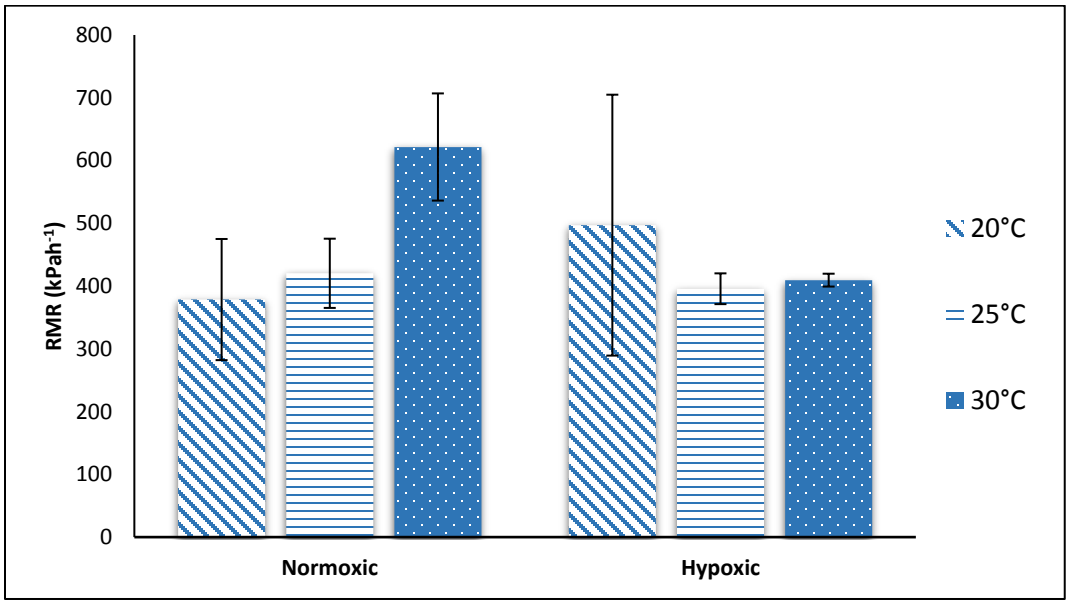


Figure 6. showing the effect of temperature and hypoxia on RMR in both normoxic and hypoxic conditions. Data presented as mean ± SE (n=12 for normoxic RMR at 20, 25 and 30°C, n=2 for hypoxic RMR at 20°C and n=4 for hypoxic RMR at 25 and 30°C)

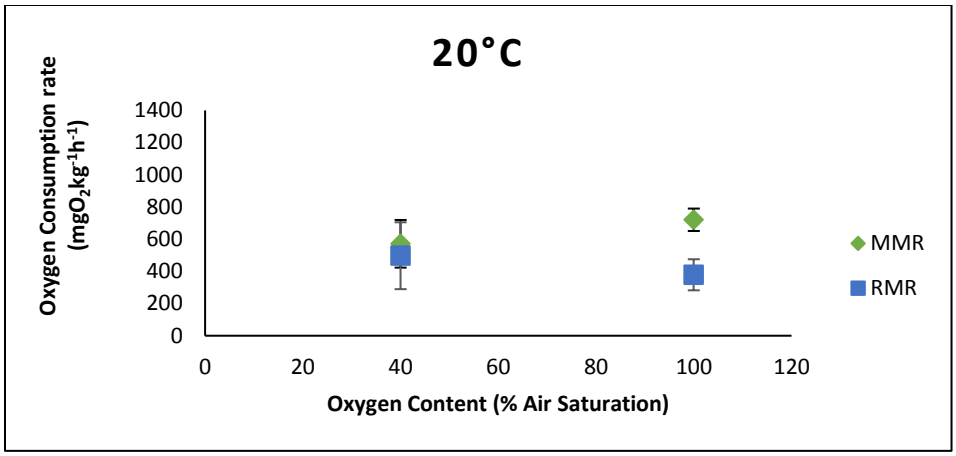
The effect of hypoxia on RMR, as acclimation temperature was increased, was found to be such that RMR initially decreased from 497 ± 207.8 to 395.7 ± 24.5 $mgO_2\,kg^{-1}\,h^{-1}$ as acclimation temperature increased from 20 to 25°C, after which RMR remained fairly constant with a slight increase to 409.4 ± 10.2 $mgO_2\,kg^{-1}\,h^{-1}$ as temperature increased to 30°C (Figure 6). The variations between these values of RMR under hypoxic conditions however, were not found to be statistically different, as determined by a one-way ANOVA ($p = 0.43$).

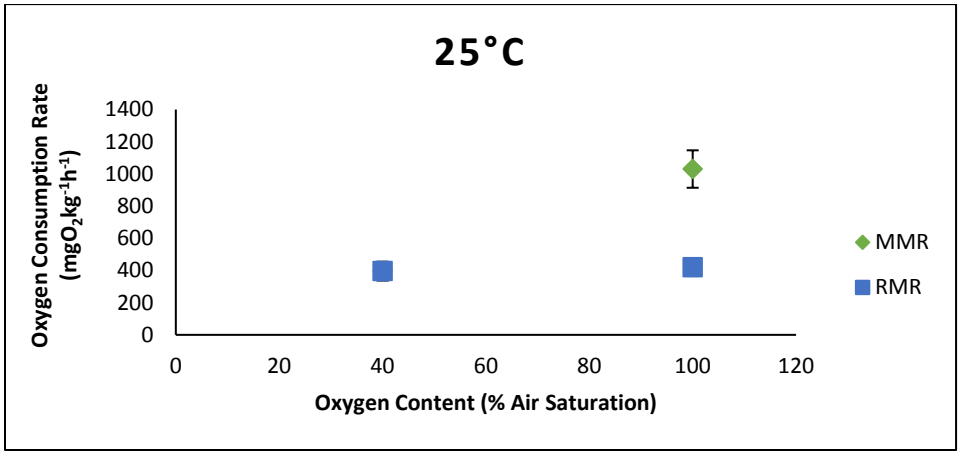
In reference to Figure 7, RMR was found to remain fairly constant as dissolved oxygen content decreased from 100% to 40%, with RMR increasing slightly from 378.5 ± 96.5 to 497 ± 207.8 $mgO_2\,kg^{-1}\,h^{-1}$ at 20°C, decreasing slightly from 420.3 ± 55.2 to 395.7 ± 24.5 $mgO_2\,kg^{-1}\,h^{-1}$ at 25°C and decreasing from 621.5 ± 85.4 to 409.4 ± 10.2 $mgO_2\,kg^{-1}\,h^{-1}$ at 30°C. Variation between points was found not to be statistically significant as determined by a one-way ANOVA ($p = 0.75, 0.85, 0.26$ for fish acclimated to 20, 25 and 30°C respectively).

Effect of hypoxia on MMR

For all three temperature treatment groups (20, 25 and 30°C), MMR was observed to decrease with a decrease in dissolved oxygen content (Figure 7). For fish acclimated to 20°C, MMR decreased from 720.3 ± 69.7 to 571.2 ± 148.1 $mgO_2\,kg^{-1}\,h^{-1}$ as dissolved oxygen content decreased from 100% O_2 to 40 ± 1% O_2. Although a clear trend was observed, a one-way ANOVA statistical analysis determined that these values were not

statistically different ($p = 0.61$). For fish acclimated to 25°C, a significant decrease was observed in MMR as determined by a one-way ANOVA ($p = 0.05$). MMR decreased from 1030.2 ± 116.3 to 396 ± 58.6 $mgO_2\,kg^{-1}\,h^{-1}$, as dissolved oxygen content decreased from 100 to 40 $\%O_2$. A clear decrease in MMR was also observed for the 30°C treatment group, with MMR decreasing from 1159.4 ± 134.7 $mgO_2\,kg^{-1}\,h^{-1}$ at 100% O_2 to 814.34 ± 33.8 $mgO_2\,kg^{-1}\,h^{-1}$ at 40% O_2. These values however were not found to be different, based on a one-way ANOVA ($p = 0.26$).





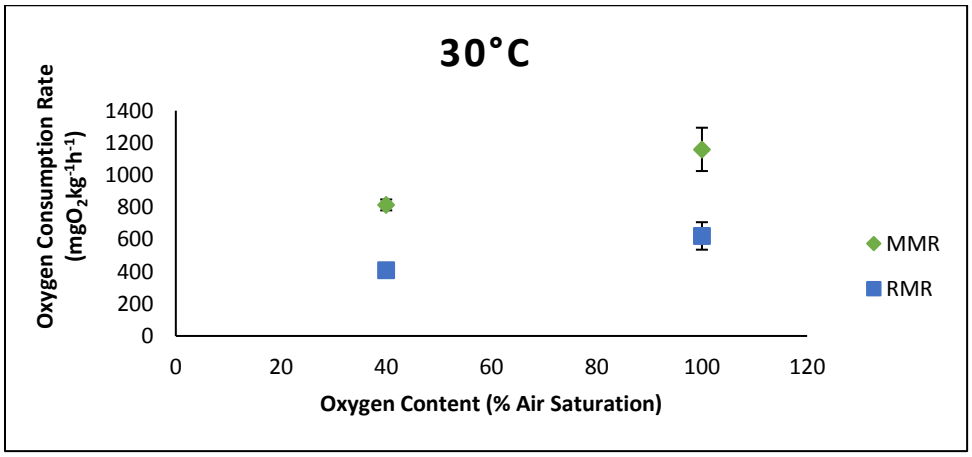


Figure 7 showing average MMR and RMR values with associated standard error obtained at both normoxic (>95% O_2 sat) and hypoxic (40% O_2 sat) conditions for three experimental temperatures (20, 25 & 30°C). (n=4 for normoxic RMR and MMR at 20, 25 and 30°C, n=2,3 for hypoxic RMR and MMR respectively at 20°C and n=4 for hypoxic RMR and MMR at 25 and 30°C).

Effect of temperature on MMR

As acclimation temperature increased from 20-30°C, MMR was determined to increase from 720.3 ± 69.7 to 1030.2 ± 116.3 $mgO_2\,kg^{-1}\,h^{-1}$, followed by a decrease to 1159.4 ± 134.7 $mgO_2\,kg^{-1}\,h^{-1}$ (Figure 8). These values for MMR however, were not found to be statistically different by a one-way ANOVA ($p = 0.62$).

Effect of temperature on Aerobic Scope

In reference to Table 1, under normoxic conditions, an initial increase in aerobic scope from 515.5 ± 168 to 609.9 ± 54.9 $mgO_2\,kg^{-1}\,h^{-1}$ was observed as acclimation temperature increased from 20 to 25°C, followed by a decrease to 537.9 ± 131.1 $mgO_2\,kg^{-1}\,h^{-1}$ as temperature increased to 30°C. Under hypoxic conditions, AMS decreased from 208.6 ± 32.9 to 134.9 ± 30.4 $mgO_2\,kg^{-1}\,h^{-1}$ as temperature increased from 20 to 25°C, followed by a decrease to 404.9 ± 30.7 $mgO_2\,kg^{-1}\,h^{-1}$ for fish acclimated at 30°C. There was no statistical difference in the mean AMS values as temperature increased (one-way ANOVA).

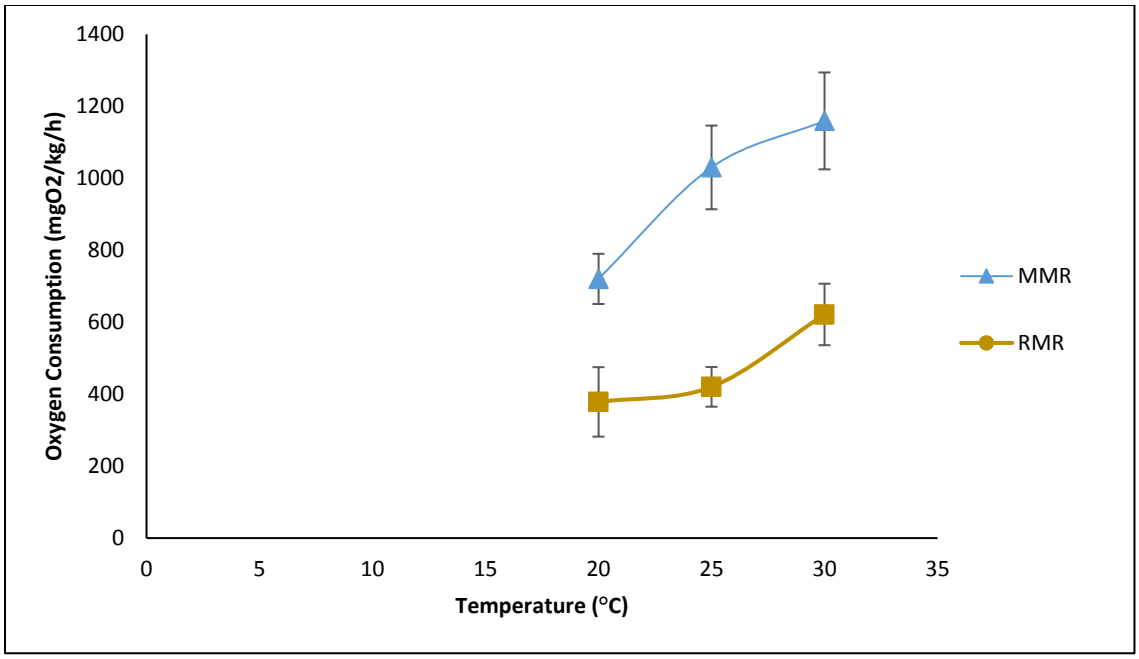


Figure 8. showing the effect of temperature on both MMR and RMR. Data presented as mean ± SE (n=4)

Table 1 showing values calculated for Aerobic Metabolic Scope (AMS) under both normoxic and hypoxic conditions.

	Aerobic Metabolic Scope (AMS)	
Temperature (°C)	**Normoxia**	**Hypoxia**
20	515.46	208.60
25	609.92	134.90
30	537.89	404.91

Discussion

Effects of Hypoxia on MMR, RMR, and AMS

In reference to Figure 6, results showed a 20% decrease in RMR from 20 to 25°C under hypoxic conditions followed by a 3% increase from 25 to 30°C. These results, although visibly evident, were determined not to be statistically different ($p > 0.05$), and with standard error for hypoxic RMR at 20°C being very large (±207.8), hypoxic RMR can therefore, be considered constant as acclimation temperature increases. Results also show that the values for RMR under hypoxic conditions, for fish acclimated to each temperature, are less than those under normoxic conditions, excepting the RMR recorded at 20 °C. This can be attributed to the role of oxygen in the body. Oxygen is required as part of the electron transfer chain in the mitochondria to produce energy that can be used by the various cells in the body. If there is a decrease in available oxygen, then there should also be a subsequent decrease in the energy available to carry out everyday functions.

In reference to Figure 7, a clear trend can be observed in MMR as dissolved oxygen content decreased (21, 62, and 30% for 20, 25 and 30°C respectively), such that, a decrease in MMR was observed to coincide with a decrease in dissolved oxygen. This follows the expected results shown in Figure 2, based on a study conducted by Rogers et al. (2016), as MMR was expected to initially remain constant, as dissolved oxygen content decreased, until MMR could no longer be supported by the oxygen present in the water and MMR began to decrease. RMR however, remained constant in both previous studies and this study (one-way ANOVA, $p = 0.75, 0.85, 0.26$ for fish acclimated to 20,

25 and 30°C respectively), until RMR could no longer be supported by the percentage of dissolved oxygen present, after which aerobic respiration could not be maintained and a switch to anaerobic respiration was made.

With respect to hypoxic conditions, RMR remained fairly constant as temperature increased. A decrease in MMR of 31% was observed as temperature increased by 5°C, followed by a larger increase in MMR of 106% as temperature approached 30°C. This does not follow what is expected, as, according to McBryan et al. (2013), MMR is expected to increase as temperature increases, until optimum temperature is reached, after which MMR decreases, and yet MMR is also expected to decrease as dissolved oxygen content decreases. This would lead to the expectation that under hypoxic conditions, MMR would increase until limited by hypoxia as a larger intake of oxygen cannot be facilitated. This limiting may result in the optimum temperature occurring in a lower temperature range, demonstrating the balance between increasing temperature and decreasing oxygen content. Also, these differing in results, as compared to expectations based on previous literature, could be attributed to an incorrect measurement of MMR, as a result of an oxygen bubble being trapped in the respirometer apparatus or the apparatus not being completely air tight. This could lead to an inaccurate measure of MMR, as oxygen is released into the water of the closed respirometer, counteracting the decrease in oxygen as the fish respires, and causing the slope of oxygen uptake to be less steep. In all temperature treatment groups, however, values for MMR under normoxic conditions were higher than those under hypoxic conditions. This corresponds to results obtained from a study conducted by Tiffany et al. (2010), such that a decrease in oxygen consumption was observed as dissolved oxygen content decreased.

Effects of Temperature on MMR, RMR and AMS

In reference to Figure 8, a visible increase in RMR under normoxic conditions was observed as temperature increased, as expected based on previous studies (Figure 1). Only a slight increase in RMR of 11% was observed under normoxic conditions as temperature increased from 20 to 25°C. However, as temperature approached 30°C, a larger increase in RMR of 48% was observed. This could be attributed to the increased metabolic demand under increased temperatures, due to the fact that heat increases the kinetic energy in cells, leading to an increase in the rate of metabolic reactions in the body. Therefore, cells require more energy to sustain body functioning in higher temperatures. Also, as determined by a colleague in the same lab, $CT_{max,}$ this being the upper thermal limit, of the bluegill sunfish is 37 ± 0.5 °C. This may explain the larger increase between 25 and 30°C, as opposed to that between 20 to 25°C, as with the temperature rising closer to CT_{max}, a larger metabolic strain is expected.

Changes in MMR and RMR for both oxygen concentration and temperature were recorded in Figure 7. A clear increase in MMR is observed under normoxic conditions as temperature increases, when compared across temperature treatment groups, although the increase in MMR is greater from 20 to 25°C, as compared to that from 25 to 30°C (43 and 13% respectively). These results correspond to the expected results, as seen in Figure 1, as MMR is observed to increase with a very steep slope initially, followed by a decrease in the gradient as the curve approaches a pivoting point, this being the point of optimum temperature. This can be explained through the concept of metabolic reactions, as, the rate of enzymatic reactions increases rapidly as temperature increases, up to a

point where the rate reaches its maximum and optimum value, after which enzyme denaturing occurs and the rate of reaction decreases drastically.

As the increase in MMR from 20 to 25°C was larger than the increase in RMR, an increase in AMS of 18% was observed. However, as the increase in RMR was greater than that of MMR as temperature rose from 25 to 30°C, the AMS decreased by 12%. These results reinforce those discussed in McBryan et al. (2013), as well as Rogers et al. (2016) (Figure 1), as in these previous studies, an increase in aerobic scope was observed, up to a maximum optimum temperature, followed by a decrease in AMS. These results show the optimum temperature for aerobic scope to be near 30 °C.

Implications for Climate Change

Anthropogenic stressors, such as hypoxia, as well as climate change, are believed to have a large influence on AMS (Rosewarne et al., 2016). The effect of hypoxia on the function of a species can thereby be determined by the change in AMS. A decrease in AMS implies that there is less energy available for the organism to complete tasks such as movement, growth and reproduction. Therefore, when an organism is under hypoxic conditions, and there is an increased metabolic demand, growth may be reduced due to the lack of energy available for digestive processes. Also, both recovery from anaerobic metabolism, and aerobic swimming performance may be limited in hypoxic conditions due to the decrease in AMS (Rosewarne et al., 2016, and references included within).

As the effects of increased temperature and decreased oxygen are studied together, it is important to consider the effect these two variables can have on each other.

One such effect can be due to hemoglobin's oxygen affinity. An increase in temperature usually leads to a rightward shift in the haemoglobin oxygen saturation curve, meaning, as temperature increases, haemoglobin's affinity for oxygen decreases. This could lead to a reduction in the tolerance for hypoxia (McBryan et al., 2016). This is reinforced by the data showing that the bluegill sunfish are currently in or near their optimum temperature range during the summer months in the Ogeechee River. Therefore, as climate change causes continuing increases in temperature, these native fish could be pushed past their optimum temperature range into the pejus range, this being the range of temperature at which fish begin experiencing thermal stress. This could lead to the possible immunocompromising of their systems and an increased sensitivity to changes in dissolved oxygen and other stressors.

Future research could include an expansion of the sample size, as well as the number of varying temperature points, in order to allow for a greater estimate of the changes in AMS, due to temperature, in the bluegill sunfish.

Conclusion

The effect that rises in global temperature and increases in acute hypoxic episodes have on the bluegill sunfish is a topic of great importance, due to the many uses of the bluegill sunfish in various parts of Georgia. This study has shown both increased temperature and instances of hypoxia to be considerable stressors to the bluegill sunfish, with these stressors impacting their aerobic scope negatively. Also, the bluegill sunfish were determined to currently be functioning in their optimum temperature range during

the summer months in the Ogeechee River, leading to a greater urgency for the increased regulation of activities contributing to global warming, such as, the implementation of laws that could hold persons more accountable for their contribution to global warming and thereby decrease the rate of global warming overall. Other mitigations can consist of monitoring the water temperature and oxygen content of local rivers and coastal areas, utilizing various methods to control the microorganisms present in the water that could result in hypoxia with an increase in temperature, as well as controlling additional stressors such as setting new limits for concentrations of toxins that are allowed to be released into the rivers and coastal areas, as these could act synergistically with increased temperature and hypoxia.

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