Aerobic Scope in Kentucky Stream Fishes and Their Offspring in Response to Temperature Fluctuations.

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Introduction

Temperature fluctuations brought upon by climate change may have direst consequences on fish fitness and metabolism, or aerobic scope (AS). Aerobic scope is found by taking the difference between standard metabolic rate [SMR] and maximum metabolic rate [MMR] (Clark et al. 2013). The standard metabolic rate of an organism is the minimum amount of energy used to sustain bodily functions. An organism's maximum metabolic rate is the measure of oxygen intake at a state where the organism reached the greatest amount of tolerable activity. Environmental variation has shown to have an effect on metabolic rates; thus, variation in temperature is relevant to the observed differences in the metabolism of organisms (Norin and Metcalf, 2019).

We chose three common Kentucky indigenous fish to monitor their metabolic processes and subjected them to different temperatures. Bluntnose Minnows are found in mid-lower water columns and are native to the streams of the north-eastern part of America. They can range 40-100 millimeters in length. Rainbow darters are native to North American streams and range in length from 30-65 millimeters. Due to their lack of a gas bladder, they are commonly found on stream bottoms. Mosquitofish are also native to Northern American streams and range in lengths from 20-60 millimeters. Being a topminnow, they spend much of their time in the higher water columns. Since these three fish are unique and belong to different parts of the stream, we believed that they would be suitable fish to evaluate under various thermal conditions. Due to environmental conditions changing globally at quicker rates than expected, we wanted to determine if a range of temperatures may impact the physiology of Kentucky stream fish. We hypothesize that as the water temperatures (°C) increase, then the standard metabolic rate, maximum metabolic rate, and aerobic scope of the fish will also increase showing a direct relationship between metabolism and temperature. We measured the aerobic scope of adult Bluntnose minnows (*Pimephales notatus*), Rainbow darters (*Etheostoma caeruleum*), and Mosquitofish (*Gambusia affinis*) at four temperatures (28°C,23°C, 18°C, 13°C).

Image 1: Male Bluntnose minnow (*Pimephales notatus).*

Image 2: Male Rainbow darters (*Etheostoma caeruleum*),

Image 3: Female Mosquitofish (*Gambusia affinis*)

Methods

All three species of fish were collected from Kentucky streams (Banklick creek, 4-mile creek, and teg UK Ecological Research and Education Center). Fish were checked for any signs of diseases before testing. For each species, a total of 64 fish were separated into four groups of 16. For the three species, each group of 16 fish were placed in a 38 Liter (10 gallon) and acclimated to one of 4 temperatures (13, 18, 23, and 28°C). The temperatures of tanks 13°C and 18°C were regulated using environmental chambers and 28°C was regulated using aquatic heaters. All fish fasted for 48-hours before any metabolic trials were conducted.

A static flow respirometry system consisting of fiber optical dissolved oxygen sensors (4 channel FireSting O2 Optical Oxygen Meters, PyroScience™) was utilized to monitor fish respiration. The system was submerged in approximately 38 liters of water within a 76-liter (20 gallon) glass tank. Fish were chased at maximum speed for 4 minutes or till exhausted in a circular tank. Immediately after the 4-minute chase, the fish were placed into one of the four 50mL chambers within the respirometer to record the maximum respiration (R_m). The respirometer recorded fish respiration for an minimum of 9 hours, while oxygen recharge periods occur every 15 minutes for 6 minutes. Oxygen consumption was calculated with linear regression coefficients (slopes) for oxygen concentration in the fish chamber per time using the *respR aquatic respiratory analysis* package (Harianto et al. 2019) using RStudio version 1.4.1106 (Rstudio Team 2021). The mass (g) and length (mm) of the fish was measured and the formula $MO_2 = \frac{\Delta O_2 X (v_{chamber}) - v_b}{\Delta t X M_b}$ was used to calculate the mass specific metabolic rate. The change of oxygen concentration in the respirometer is represented by ΔO_2, $v_{chamber}$ is the chamber volume of the respirometer (mL), v_b is the fish body volume (assumes density of 1kg L^{-1}), Δ is the change in time, and M_b is the mass of the fish (kg).

Breeding was attempted in all three species of fish. Rainbow darters are gravel spawners, the female partially buries herself in gravel before spawning begins. Plastic trays filled with sloped gravel were used in each of the darter tanks as a spawning chamber. Gravel was checked daily for eggs, when eggs were found they were placed in the floating tumblers. Bluntnose minnows lay eggs underneath flat surfaces such as stones or logs. The bluntnose tanks were set up with rocks caves and pvc pipe halves to replicate nesting sites. Clutches were found on the underside of rocks and placed in the floating tumblers. Mosquito fish are live-bearers and pregnant females were kept in floating tumblers with net bottoms to allow offspring to swim free of adults.

Results

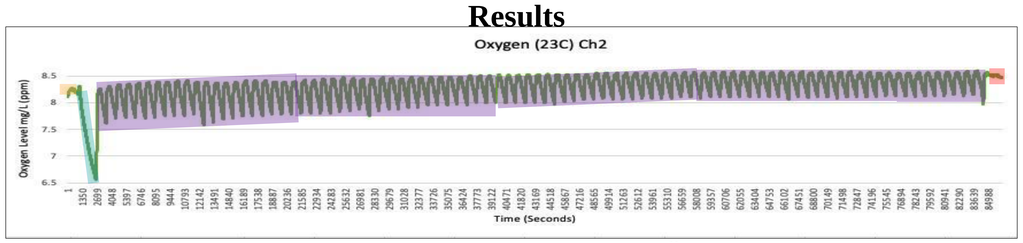

Figure 1: Bluntnose minnow respiration at 23C. Oxygen respiration (mg/L) per time (sec).

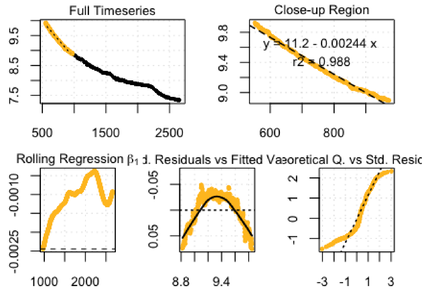

Figure 2: In the above graphs, the MMR is highlighted. The fish is recovering after being chased and takes in more oxygen to meet the energetic demands of this high activity level. The steepest point of this regression is the MMR.

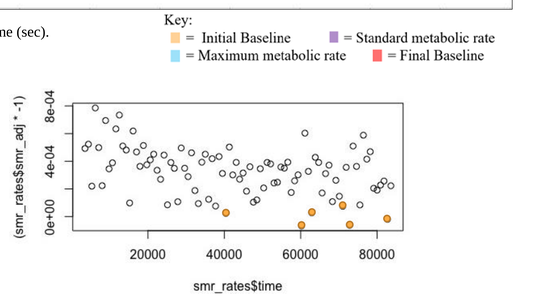

Figure 3:
This shows all 88 regressions as the fish respire.The standard metabolic rate represents the lowest 10% of these values (colored dots). This is the lower metabolism of the fish as it is resting.

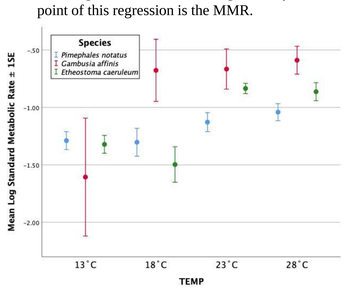

Figure 4: Standard metabolic rate, Log transformation to normalize the data, were found to be significantly different among species and temperatures with a 2-way ANOVA($F_{species,\ 2,116}$= 4.124, p=0.019, $F_{temp,\ 3,116}$= 11.172, p<0. 001), where mosquitofish differed from bluntnose minnows and rainbow darters. And 13˚C-18˚C temperatures differed from 23˚C-28˚C temperatures based on Tukey post-hoc tests.

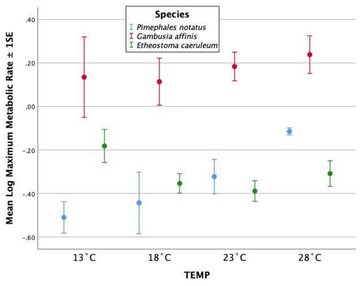

Figure 5: Maximum metabolic rate, Log transformation to normalize the data, were also found with a 2-way ANOVA to be significantly different among species ($F_{species,\ 2,116}$= 23.909, p<0.001), but not among temperatures $F_{temp,\ 3,116}$= 1.822, p=0. 147).

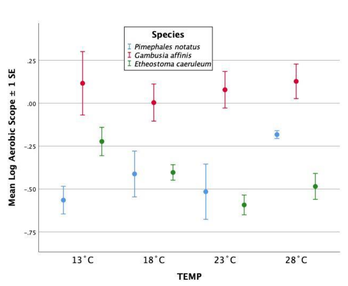

Figure 6: Aerobic Scope, Log transformation to normalize the data, were also found with a 2-way ANOVA to be significantly different among species ($F_{species,\ 2,116}$= 17.521, p<0.001), but again not among temperatures $F_{temp,\ 3,116}$= 1.822, p=0. 34).

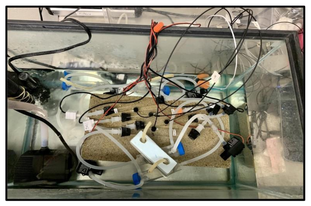

Image 4: The 20-gallon tank, where the chambers with fish, and the fiber optical dissolved oxygen sensors are located.

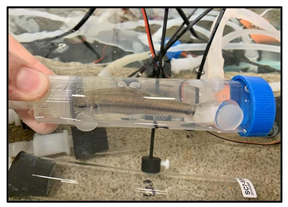

Image 5: The 50mL chambers used for each individual fish.

Image 6: Dr. Durtsche (left) and Students: Mackenzie Danker, Hannah Taulbee, Sam Bauer and conducting fish sampling in the field.

Conclusion

- Metabolic Rates were measured at 13C, 18C, 23C, and 28C among the three species of stream fishes. Data were $\log_{10}$ transformed to normalize for statistical analyses.
- Standard metabolic rates (SMR), Maximum metabolic rates (MMR), and Aerobic Scope (AS) for these fish were all found to be significantly different from each other for the species of fish tested. Post-hoc Tukey tests indicate that *Gambusia affinis* , the mosquito fish was different in metabolism than the other species of fish, showing very high metabolisms especially at higher temperatures. Mosquito fish can be found in the tropics, and possibly have adaptations for higher metabolism at warmer temperatures.
- Rainbow darters had trends for higher SMR at warmer temperatures, but the higher Aerobic Scope at lower temperatures is a result of the larger MMR at the lower temperatures.
- Offspring were maintained at current (cold) and +3°C (warm, future climate) stream temperatures. During the breeding season, spawning was successful for the Bluntnose minnows and Rainbow Darters, however once the breeding season ended there has been little spawning. While we had some eggs, many of the offspring have not survived to a full juvenile stage. Maintaining and raising the egg clutches to maturity was difficult, many eggs were lost to fungus. Four bluntnose offspring hatched from the egg clutches. The Mosquitofish spawn live young rather than lay eggs, which eliminates the challenge of raising eggs to maturation. Pregnant Mosquitofish were placed in floating tumblers with a screen at the bottom large enough for the hatchlings to get out but not the mothers. Mothers must be separated from the juveniles because Mosquitofish exhibit filial cannibalism. Approximately 40 hatchlings have spawned. The juveniles are fed brine shrimp and fish meal. Further work and research is being done on the effects of altering the photoperiod and water temperatures to induce breeding and increase survivorship of juveniles.
- Once the offspring are fully developed standard metabolic rate and maximum metabolic rates will be tested to determine the fish response to embryogenesis under climate change conditions.

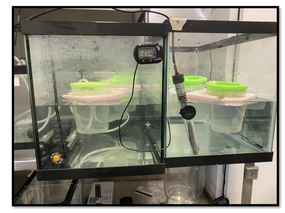

Image 7: The hatching and egg growth tumblers acclimated to breeding temperature.

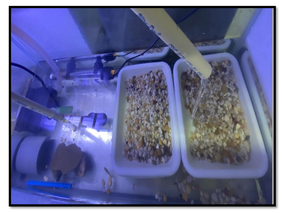

Image 8: Set-up of Rainbow Darter breeding chamber.

Citations

Clark, T. D., Sandbloom, E., & Jutfelt, F. (2013). Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *Journal of Experimental Biology*, 216, 2771-2782. doi: 10.1242/jeb.084251.

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Acknowledgements

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