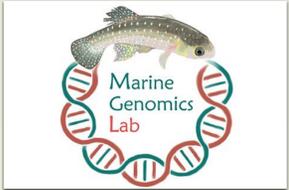


Effects of mitochondrial haplotype on critical thermal maximum under hypoxic and normoxic conditions in the common killifish, *Fundulus heteroclitus*

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Introduction

- F. heteroclitus* are great models for biological and ecological responses to environmental changes because they live in highly variable estuarine systems [1].
- Fluctuating temperature can impact a fish's susceptibility to disease [2], reproduction rates [3], and even growth rates [4]. Therefore, temperature is an important factor to study to further understand its impacts on *F. heteroclitus*.
- The method used in this study to measure thermal tolerance in *F. heteroclitus* is Critical Thermal Maximum, the maximum temperature at which fish lose their ability to maintain equilibrium and turn belly up.



Figure 1. Critical Thermal Maximum set up using *F. heteroclitus* as the model organism.

Study Aims

Assess the impacts that the hypoxic and normoxic conditions have on the thermal tolerance of the population of *F. heteroclitus* collected while also taking into consideration other factors including their mitochondrial haplotype and sex.

Methodology

- F. heteroclitus* were collected in Mantoloking New Jersey and acclimated to 28°C and 15 ppt.
- 20 fish were measured using CTMax under the hypoxic condition and 20 under the normoxic condition.
- DNA was isolated, amplified, and digested with the restriction enzymes to determine mitochondrial haplotype.

Results

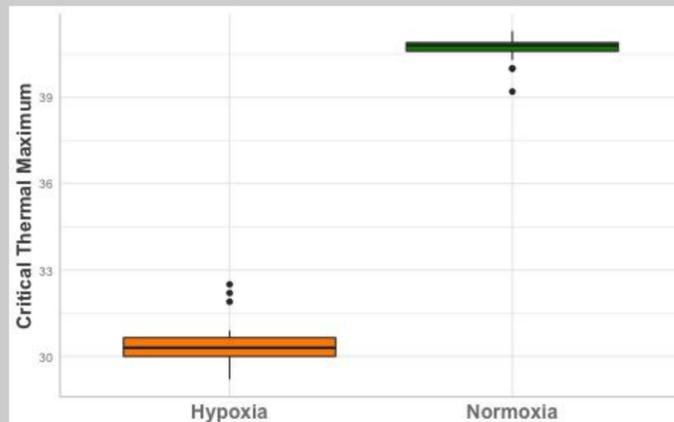


Figure 2. Thermal Tolerance measurements under the hypoxic vs the normoxic condition for the *F. heteroclitus* observed. CTMax is much higher for fish under normoxic conditions in comparison to fish under hypoxic conditions.

Table 2. Average CTMax measurements for both hypoxia and normoxia.

Hypoxia	Normoxia
29.6±0.8	40.7±0.4

Table 3. The same method of using the Akaike information criterion (AIC) model selection was used for the male only data. Table 3 shows the best model, to represent the male data, was CTMax with Hypoxia/ Normoxia alone with no interaction terms (AIC=43.70).

CTMax ~ Hypoxia/Normoxia	
Variable	Hypoxia/Normoxia
P-value	2.2e ⁻¹⁶ ***

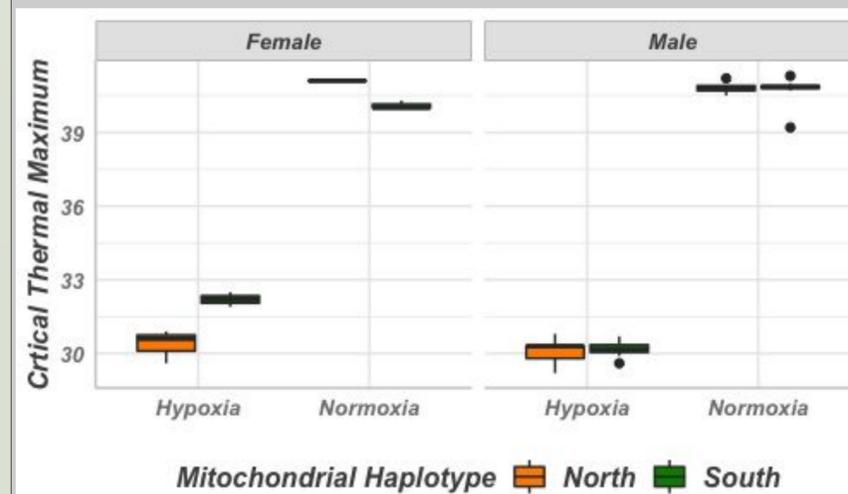


Table 1. The best model for the data, listed at the top of the table, was determined using Akaike information criterion (AIC) model selection where there were three interactions, as shown using a colon, combined with the original variables, except for mass (AIC=74.69).

CTMax~ Sex + Hypoxia/Normoxia + Haplotype + Sex:H/N + Sex:Hap: + H/N:Hap						
Variables	Sex	H/N	Hap	Sex:H/N	Sex:Hap	H/N:Hap
P-value	0.0255 *	2.2e ⁻¹⁶ ***	0.0040 **	2.2e ⁻¹⁶ ***	0.0587	0.0382 *

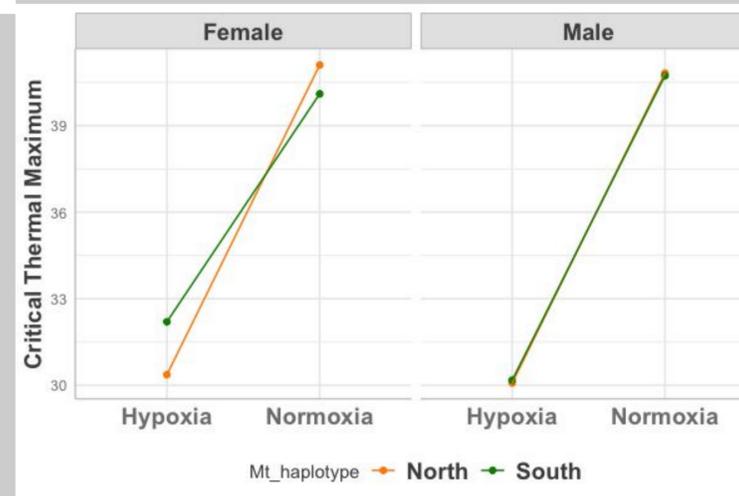


Figure 3(above): Thermal Tolerance measurements were separated by hypoxia/normoxia and the northern and southern haplotypes along with males and females. Parallel lines represent no interaction.

Figure 4 (left): Similar representation to figure 3, but in this figure, significant difference between the northern and southern haplotypes for the females is evident, where as for the males, no significant difference in haplotype was found for neither the hypoxic nor the normoxic condition.

Table 4. Finally, the same method of using (AIC) model selection was used for the female data. This table shows the best model, listed at the top of the table, includes two interaction terms, as shown using colons, combined with the original variables (AIC=13.57)

CTMax~Mass + Hypoxia/Normoxia + Haplotype + H/N:Hap + Mass:Hap					
Variable	Mass	H/N	Hap	H/N:Hap	Mass:Hap
P-value	0.1107	3.07e-05 ***	0.0499 *	0.0063 **	0.2392

Conclusion

- Critical thermal maximum for the *F. heteroclitus* collected is much higher under the normoxic condition when compared to the hypoxic condition supporting the idea that oxygen has a significant effect on thermal tolerance.
- Body mass does not have a significant effect on the thermal tolerance of *F. heteroclitus*.
- Mitochondrial haplotype did not have a significant effect on CTMax for either the hypoxic or normoxic condition when measured for males and females combined.
- While mitochondrial haplotype was not significant for males in either measurement condition, there was a significant relationship between thermal tolerance and mitochondrial haplotype for females for the hypoxic and normoxic condition.



Figure 5. *F. heteroclitus*; A) Male B) Female

Future Research

To further investigate this relationship between CTMax, mitochondrial haplotype, oxygen, and sex for *F. heteroclitus*, a larger sample size should be collected and used for this particular study. Another possible direction for this data is to investigate these relationships at different temperatures.

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