The influence of age on metabolic and thermal tolerance traits in New Jersey populations of Atlantic killifish (*Fundulus heteroclitus*)

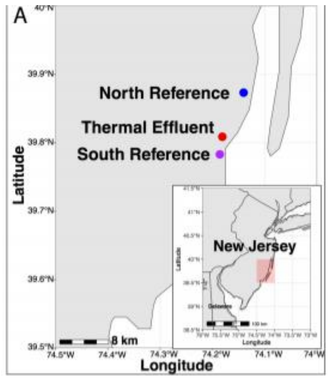
Meghan Roberts[1], Agatha Freedberg[1], Melissa Drown[1], Amanda DeLiberto[1], Moritz Ehrlich[1], Douglas Crawford[1], Marjorie Oleksiak[1]

University of Miami Rosenstiel School of Marine and Atmospheric Science[1]

UNIVERSITY OF MIAMI
ROSENSTIEL SCHOOL of MARINE & ATMOSPHERIC SCIENCE

Background

Study Site



The TE population experienced water that was 4°C warmer than the NOC and SOC populations due to thermal effluent from The Oyster Creek Nuclear Generating Station, (1960-October 2018).

Critical Thermal Maximum

Definition: the temperature where a fish displayed a loss of equilibrium for 5 seconds.

To test: Acclimate the fish to either 12°C or 28°C and then slowly raise water temperature until CTMax is observed.

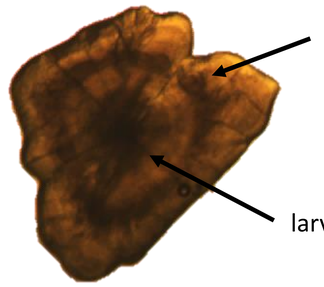
Whole Animal Metabolism

Definition: the amount of oxygen used by an individual over time.

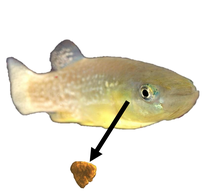
To test: Use intermittent flow respirometry to measure oxygen consumption.

Otoliths for Aging

Otoliths are the ear bones of fish. Every day, a new layer is deposited. When the fish are growing rapidly in the summer, the rings are deposited far apart. This can be seen by the lighter bands. Whereas the darker bands represent the fish growth in the winter.

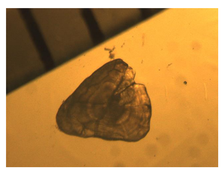


Methods



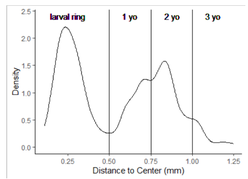
1) Remove otolith from fish and store in 70/30 glycerol ethanol solution.

2) Take picture of otolith under microscope.



3) In ImageJ, calculate the distance of rings from center.

4) Determine age by calculating number of rings.



5) Create age cutoff distances (corrected age) and compare with calculated age

Results

Whole Animal Metabolism

Variable/Interaction	In Final Model?	P-Value
Sex	yes	0.254
Age	no	NA
Log Mass	no	NA
Population	yes	0.74
Acclimation Temperature	yes	$<2.2 \times 10^{-16}$
Sex:Population	yes	0.23
Sex:Acclimation Temp	yes	0.336
Log Mass: Population	yes	0.168
Log Mass: Acclimation Temp	yes	0.091

Figure 1: Variables and second-order interaction effects included in the backwards elimination of the linear model for WAM at the beginning along with which factors stayed in the final model and their p-values. The final model had the lowest AIC.
*all second-order interaction effects were tested in the linear model

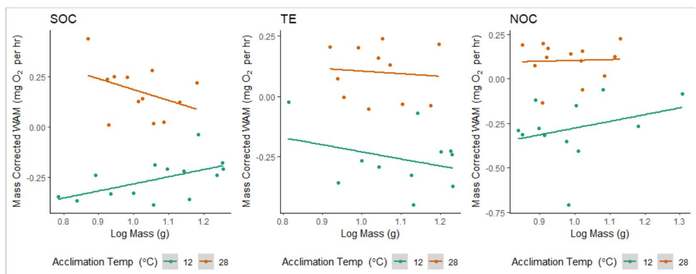


Figure 2: Linear regression of the log of mass as compared to mass corrected WAM (residuals from log(WAM)~log(Mass)) at each acclimation temperature separated by population.

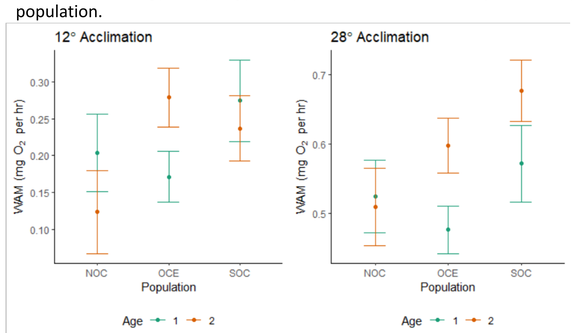


Figure 3: Mean WAM per population per age with standard error bars.

Critical Thermal Maximum

Variable/Interaction	In Final Model?	P-Value
Sex	no	NA
Age	yes	5.49×10^{-14}
Log Mass	yes	1.10×10^{-7}
Population	yes	1.16×10^{-6}
Acclimation Temperature	yes	$<2.2 \times 10^{-16}$
Age: Population	yes	0.256
Log Mass: Acclimation Temp	yes	0.057
Population: Acclimation Temp	yes	0.044

Figure 4: Variables and second-order interaction effects included in the backwards elimination of the linear model for CTMax at the beginning along with which factors stayed in the final model and their p-values. The final model had the lowest AIC.
*all second-order interaction effects were tested in the linear model

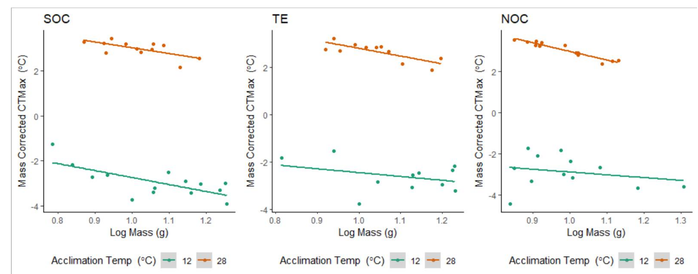


Figure 5: Linear regression of the log of mass as compared to mass corrected CTMax (residuals from CTMax~Mass) at each acclimation temperature separated by population.

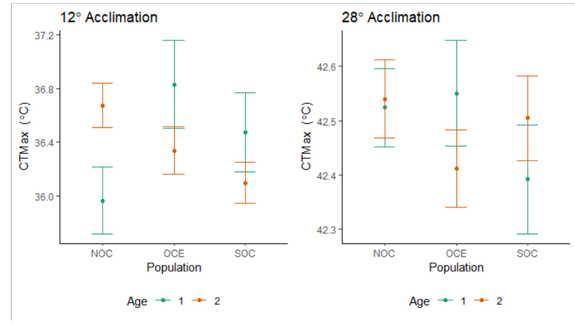


Figure 6: Mean CTMax per population per age with standard error bars.

Conclusions

- Ages calculated were as expected since the fish were expected to only have lived one year before being collected and one year at the lab (so most would be 2 years old with a few being younger)
- Age significantly impacted CTMax but not WAM
- Acclimation temperature and population were important in both CTMax and WAM models
- No evidence for age specific selection, which would lead to differences between age groups independent of mass

Contact:
mnr68@miami.edu

Acknowledgments. Thank you to graduate students Melissa Drown, Amanda DeLiberto and Moritz Ehrlich and to PIs Douglas Crawford and Marjorie Oleksiak. Undergraduates Agatha, Joe, Liam, Rebecca, Angel. National Science Foundation NSF/105 1556396 and NS/105 1754437.