UNIVERSITY OF MIAMI
ROSENSTIEL SCHOOL of MARINE & ATMOSPHERIC SCIENCE

Comparing Frequency of Aquatic Surface Respiration Among *F. heteroclitus* from Distinct Estuarine Microhabitats

Liam A. Dorsey, Moritz A. Ehrlich, Douglas L. Crawford, Marjorie F. Oleksiak

Rosenstiel School of Marine and Atmospheric Science

Marine Genomics Lab

Background

- *Fundulus heteroclitus* (the mummichog) can tolerate wide ranges of environmental conditions, including chronic hypoxic and anoxic events
- Aquatic Surface Respiration (ASR) is used by *F. heteroclitus* to respirate highly-oxygenated surface water under hypoxic conditions.
- However, using ASR puts individuals at an evolutionary disadvantage, as they can more easily be preyed upon by shorebirds, especially in enclosed habitats such as marsh ponds.
- Marsh ponds frequently experience hypoxia during the summer months at night due to an increase in respiration and lack of primary production
- Previous research has found that individuals residing in distinct estuarine microhabitats (marsh Ponds and Basins) have significant differences in allele frequencies
- We believe that Pond fish have evolved adapted phenotypes to tolerate more frequent and acute anoxic events in order to avoid predation; thus, we expect Pond fish to utilize ASR less frequently than in Basin fish, reflecting divergence between local populations over short time periods.

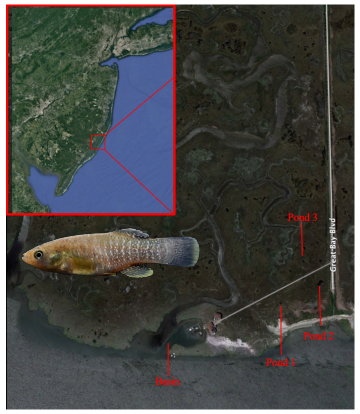

Figure 1: Location of sampling sites near Rutgers University Marine Field Station (RUMFS), Little Egg Harbor, NJ

Methods

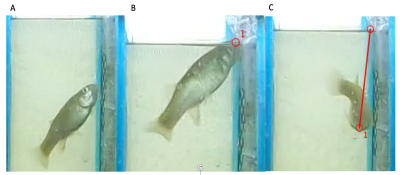

Figure 2: Fish were observed (A) below surface interval, (B) entering the surface interval, and (C) leaving the surface interval

- All fish were acclimatized in the same recirculating aquarium system for 9 months prior to measurement
- ASR was observed in 150 wild individuals using video recording while depleting the dissolved oxygen (DO) in an observation tank from fully oxic to anoxic conditions
- The duration and DO at every surfacing event was extracted using ImageJ software
- DO at first major ASR event and the duration of events were compared between Pond and Basin fish using a linear mixed model, t-test, and linear regression analysis

Results

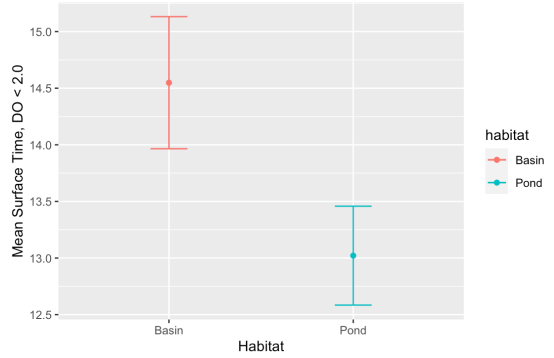

Figure 3: Mean Surface Time
Mean and standard error of mean surface interval duration in Pond versus Basin fish, DO < 2mg/L (t = 2.096, df = 13285, p-value = 0.0361). Pond fish surfaced for significantly less time on average than Basin fish

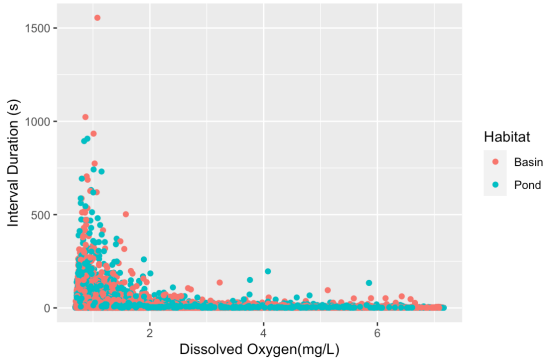

Figure 4: Aggregate data of ASR events and dissolved oxygen
All ASR events between microhabitats from fully oxic conditions (7.0mg/L saturation) to anoxic conditions (0.7mg/L saturation). Threshold DO value found to lie between 1.5 and 2.0 mg/L

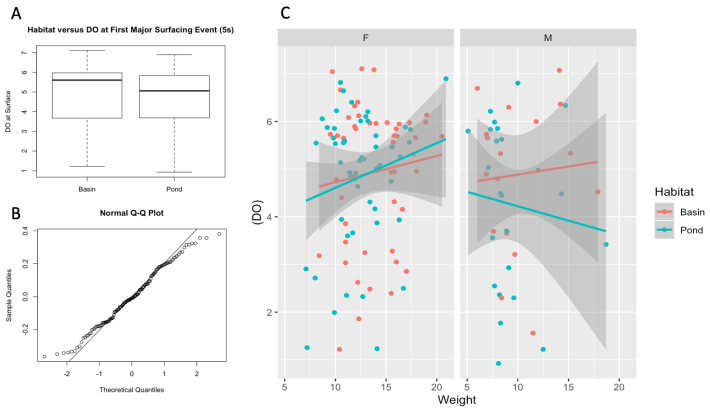

Figure 5 Dissolved oxygen at first major surface (>5s): (A) DO concentration of first major ASR event (>5s) in Pond versus Basin fish, (B) Q-Q plot created from linear mixed model versus the sample quantities provided from the data. (C) Scatter plot of each individual's first major surfacing event in male versus female fish, stratified by microhabitat.

Conclusions

- Pond fish surface for significantly less time than Basin fish per instance of ASR even after acclimation, suggesting that the trait is selected against in Pond habitats and that this difference is not due to short-term physiological response.
- The threshold value of DO in which a majority of fish utilize ASR as their primary mode of respiration lies between 1.5 and 2.0 mg/L
- Given the difference in allele frequency between microhabitats, this phenotypic divergence suggests natural ecological conditions may drive selection for adaptive traits over shorter time scales than previously believed
- These adaptive phenotypes may become more represented in the population over time as climate change and other anthropogenic factors such as nutrient loading exacerbate hypoxic events in estuarine habitats
- A genome wide association study (GWAS) and heritability study could in the future provide insight on the heritability of ASR and alleles associated with its differential use

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Contact:
lad173@miami.edu