Mitochondrial Influence of Thermal Tolerance in the European Green Crab (*Carcinus maenas*)
Jared Lynch, 2024

The European green crab (*Carcinus maenas*) is an invasive species that was introduced from Europe to the East Coast of the United States on two separate occasions—first in 1817 from Portugal, then in the 1980s from Norway (Roman 2006). These brought about "warm-adapted" and "cold-adapted" variants, respectively, allowing the green crab to thrive as far south as Virginia and as far north as Newfoundland (Tepolt and Somero 2014). This is part of a greater concern, as this species has now invaded every continent except Antarctica and threatens eelgrass beds, clam fisheries, and native shellfish (Howard et al. 2019, Walton et al. 2002). In order to better understand its expansiveness and future trajectory in the face of climate change, it is essential to investigate the physiology of cold- and warm-adapted variants.

As such, my research has focused on thermal tolerance in relation to mitochondrial haplotype (that is, a specific set of mutations in the mitochondrial DNA). Intriguingly, haplotypes deriving from Portugal possess superior heat tolerance while those from Norway possess superior cold tolerance (Coyle et al. 2019). Mitochondrial haplotypes encompass a wide range of nuclear variation, yet they continue to show a consistent link with thermal tolerance. This begs the question of whether the mitochondria itself is driving this correlation, setting the foundation for my research question: *what mechanism mediates the link between the mitochondria and thermal tolerance?*

To investigate this, my original plan was to assess copy number variation in the mitochondria (the number of copies of a particular gene). However, additional funding from INBRE and the Bowdoin Faculty Scholarship allowed me to pursue a more thorough technique known as RNAseq where all RNA transcripts in an individual are sequenced and quantified. By comparing RNA expression between haplotypes, this could reveal which genes are differentially expressed. This was applied to 48 samples following a stress experiment.

The stress experiment involved subjecting three haplotypes (one warm-adapted, two cold-adapted) to one of three temperatures for a period of 3 hours. Crabs were collected from Harpwell, ME and Pomquet, Nova Scotia, and haplotypes were identified using a restriction digest. Individuals were acclimated at 13.5°C for 3-4 weeks, then representatives of each haplotype were subjected to 5°C, 13.5°C, or 32°C. The first and last were used as stressors while 13.5°C was a control. After the 3 hour period, the heart tissue and resulting RNA was extracted and sent to MDI Biological Laboratory for sequencing. The sequencing and analysis is still ongoing, but the effort will be completed during the fall semester.

This data may help us to better understand the mitochondria as not simply a neutral genetic marker, but as a powerful force that shapes populations. Its genome may very well have a selective pressure that results in phenotypic differences. As this organelle is better understood, it will not only answer questions about the green crab invasion but also about its significance in broader contexts from evolution to biomedicine.

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