

Powering an invasion? Testing for an association between cytochrome oxidase 1 and thermal tolerance in the invasive European green crab *Carcinus maenas*

Sam Walkes '18

European green crabs (*Carcinus maenas*) have been among the most impactful marine invaders, and are now established in every continent except for Antarctica (Grosholz and Ruiz 1996). Green crabs first invaded the North American east coast in 1817, before spreading north of Cape Cod and into the Gulf of Maine in the 20th century, and into the Canadian Maritimes between 1980 and 2000 (Carlton and Cohen 2003). A [series of genetic studies strongly suggests two invasions at different times](#) (Reviewed in Carlton & Cohen 2003; Roman 2006; Tepolt & Palumbi 2015). Roman (2006) showed that lineages based on mitochondrial DNA (mtDNA) were identical to those collected from its native European range. Interestingly, he found crabs from Nova Scotia and Newfoundland generally aligned with lineages from Norway, while crabs from the Gulf of Maine southward tended to align with lineages from southern Europe. Additionally, Roman (2006) found more mixed frequencies of each lineage, or “haplotypes,” along the coast of Nova Scotia and in the Bay of Fundy.

[Aidan Coyle's recent A-recent honors project in from the Carlon lab has identified genes in the mitochondrial genome that are related to behavioral performance under cold stress in affect cold tolerance in *C. maenas*. Crabs with Northern European haplotypes showed greater tolerance for cold water temperatures, compared to crabs with Southern European haplotypes. and noted continued persistence of haplotype diversity in the northern extremities of the North American range including some Southern Europe haplotypes.](#) My project looks to expand upon these findings and address the following questions: 1) Is there also a link between the mitochondrial genome and heat tolerance? 2) ~~ie.~~ Can haplotype diversity be preserved by plastic responses to thermal stressors? Currently, increasing genetic admixture of populations (Pringle *et al.* 2011) may and haplotype diversity in the Gulf of Maine in the future and allow northern haplotypes to enter the Gulf of Maine and allow southern haplotypes to enter the Canadian Marities. -Understanding the genetic links to physiological responses will further our understanding of *C. maenas* population dynamics associated with a rapidly changing Gulf of Maine.

To address these questions, I collected 150 crabs from four different sites in the North Atlantic; from Harpswell Sound ME, Machias ME, Kent Island NB, and Pomquet NS. I measured physiological performance in cold temperatures after a two-week period of acclimation in 10°C seawater by turning the crabs on their back in seawater chilled to 10, 8, 6, 4, 2, 0°C in an incubator and assessed whether the crabs could successfully right themselves. After another two-week acclimation at 20°C, I repeated the same process in water heated to 26, 28, 30, 32, 34, 36°C. After I finished these experiments, I sacrificed the crabs by freezing, extracted DNA from leg tissue, and amplified the mtDNA gene cytochrome oxidase I (COI) with a polymerase chain reaction (PCR). PCR products were sequenced using Sanger sequencing, and mapped back to known European haplotypes using the bioinformatics software Geneious.

I integrated COI haplotype, sampling location, crab size, sex, and whether it was missing legs into a generalized linear model to determine if any of these variables had a significant effect on righting response. At 2°C, the only factor that had an effect on righting response was sampling site, while at 36°C, only COI haplotype had a significant effect on righting response. We found that crabs from Nova Scotia were most likely to right themselves at 2°C, while crabs from Harpswell were the least likely to. This finding suggests that consistent, long-term acclimation to cold water may increase cold tolerance regardless of whether the crab is of northern haplotype or southern haplotype. This finding contrasts Coyle's (2017) results. However, I used a colder acclimation temperature, which likely improved righting response and obscured links to the mitochondrial genome. Moreover, these results indicate that both long term and short-term acclimation may impact righting response. We also found that crabs with southern haplotypes, and haplotypes mapped back to sequences found across Europe were most likely to right themselves at 36°C, indicating that thermal tolerance can be linked to genes in the mitochondrial genome. Given that the Gulf of Maine is one of the fastest changing marine ecosystems (Pershing *et al.* 2015), our results suggest that green crabs may have the evolutionary tool kit to respond to rapid ecosystem change.

Funded by the Grua/O'Connell Fellowship
Faculty mentor: Dave Carlon

References:

- Carlton, J. T., & Cohen, A. N. (2003). Episodic global dispersal in shallow water marine organisms: the case history of the European shore crabs *Carcinus maenas* and *C. aestuarii*. *Journal of Biogeography*, 30(12), 1809-1820.
- Coyle, A.F (2017). Some like it cold: the relationship between thermal tolerance and mitochondrial genotype in an invasive population of the European green crab, *Carcinus maenas*. *Bowdoin College Special Collections*
- Tepolt, C. K., & Palumbi, S. R. (2015). Transcriptome sequencing reveals both neutral and adaptive genome dynamics in a marine invader. *Molecular ecology*, 24(16), 4145-4158.
- Pringle, J. M., Blakeslee, A. M., Byers, J. E., & Roman, J. (2011). Asymmetric dispersal allows an upstream region to control population structure throughout a species' range. *Proceedings of the National Academy of Sciences*, 108(37), 15288-15293.
- Roman, J. (2006). Diluting the founder effect: cryptic invasions expand a marine invader's range. *Proceedings of the Royal Society of London B: Biological Sciences*, 273(1600), 2453-2459.

Graphs/images/figures (if applicable)

Faculty Mentor: Insert professor's name here in bold

Funded by the (insert fellowship name, e.g., Rusack Coastal Studies Fellowship, Kibbe Science Fellowship here in bold)

References (if applicable)