Plasticity of thermal tolerance and its relationship with the accumulation of taurine in juvenile mussels (*Mytilus californianus*)

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Introduction

- The California mussel (*Mytilus californianus*) lives in the rocky intertidal zone, a temporally and spatially variable environment that ranges from fully terrestrial to fully aquatic conditions.
- Thermal tolerance can be affected by the presence of thermoprotectants such as organic osmolytes. For instance, previous work has identified a positive correlation between tissue contents of taurine, a thermoprotectant, and thermal history.
- However, little is known regarding the roles of developmental plasticity vs. physiological constraint in the thermal tolerance of juveniles or the levels of accumulation of thermoprotectants that correlate with thermal tolerance.

Objectives

1. To determine whether fixed genetic differences or developmental phenotypic plasticity (in response to environmental experience) determine juvenile mussels' thermal tolerance.
2. To compare the levels of accumulation of taurine in juveniles across the four treatment groups.

Methods

Thermal Tolerance:

- Juvenile mussels (7-14 mm) were collected from low-intertidal and high-intertidal mussel beds, at both a wave-protected (warm) and a wave-exposed (cool) site.
- Mussels from each of these 4 origin sites were then divided into four treatment groups (n = 8 per origin site per treatment per assay temperature):

  1. Field-acclimatized mussels tested immediately;

  2. Common-garden mussels were kept submerged in flow-through seawater tanks for 1 month in an attempt to erase recent environmental influences;

  3. Outplant-exposed and **4. outplant-protected** mussels were placed in cages at the exposed or protected sites, respectively, for 1 month (Fig. 1).

Figure 1. Stainless steel outplant cages at a) **exposed** and b) **protected** field sites. Note the difference in wave splash.

Methods (continued)

- Mussels from each treatment group were exposed to an acute heat stress in air at one of three peak temperatures (35.8°C, 37.7°C, or 38.6°C) using a programmable water bath (Fig. 2).
- Adult mussels (60-70 mm) were also collected from the same wave-protected and wave-exposed sites and divided into 2 treatment groups: **field-acclimatized** and **common-garden**. Adults in these two treatment groups were exposed to the same heat stress temperatures used for the juveniles (n = 8 per site per treatment per assay temperature).

Figure 2. Juvenile mussels before being exposed to acute, aerial heat stress in individual microcentrifuge tubes.

- Survival for both juveniles and adults was determined after 7 days.

Accumulation of Taurine:

- Standard PCR and 1.2% Gel Electrophoresis were used to isolate single nucleotide polymorphisms (SNPs) in the coding region of the below genes that could account for different functions under thermal stress.
  - Transmembrane taurine transporter (TAUT) gene
    - involved in intracellular accumulation of taurine
  - Cysteine sulfinate decarboxylase (CSAD) gene
    - a key step in the taurine synthesis pathway within mussel tissues

Results

Thermal Tolerance:

- Origin site, outplant location, and their interaction all significantly influenced survival 7 days after heat stress.
- After 1 month, juveniles outplanted to the **protected** site exhibited higher survival following thermal stress than those in the **common-garden (CG)** or at the **exposed** site, regardless of the origin site (the 4 panels in Fig. 4).
- Treatment differences were most pronounced at 37.7°C.

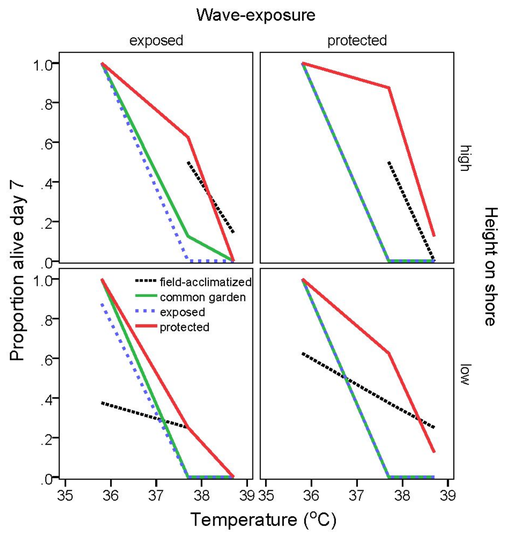

Figure 3. Survival data for juvenile mussels exposed to each of three different acute heat stress temperatures. In each panel (site of origin), mussels were assayed for thermal tolerance following each of 4 treatments. Field-acclimatized mussels from high sites were not tested at 35.8°C. Data were analyzed with a binomial GLM, with origin site and outplant site as factors and assay temperature as a continuous covariate. $p_{temperature} < 0.001$; $p_{origin} = 0.006$; $p_{treatment} = 0.007$; $p_{temperature*origin} = 0.006$; $p_{origin*treatment} = 0.023$.

Results (continued)

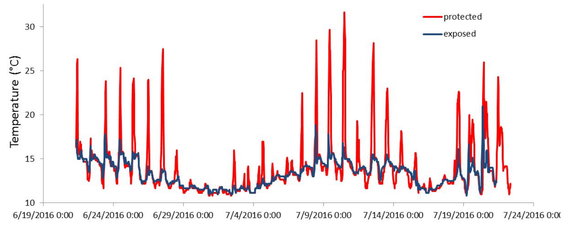

Figure 4. Estimates of mussel body temperatures were collected using iButton dataloggers embedded in silicon-filled adult mussel shells during the 1-month outplant period at each field site.

- Thermal regime varied dramatically between the **protected** (warm) and **exposed** (cool) outplant sites (Fig. 3).
- Temperature in the common garden ranged from 12.4 to 15.2°C.

Accumulation of Taurine:

- Results were inconclusive due to continuous wet-lab troubleshooting of PCR and gel electrophoresis.
- Evidence of the presence of taurine was found, but not in enough volume or consistency to be of great significance.

Conclusions and Future Work

- Our results imply substantial, environmentally driven, developmental plasticity in both thermal tolerance and growth rate among recent mussel recruits. However, there is no evidence for analogous plasticity in adults from the same locations.
- Thermal tolerance and growth rate were inversely correlated; juvenile mussels from all origin sites were more thermally tolerant but grew more slowly at the protected location.
- Although currently inconclusive, the project to investigate whether candidate genes from juvenile RNAseq analysis show sequence divergence between individuals that died vs. survived heat stress is still promising.
- Future work would include: further wet-lab protocol improvements to yield clean results regarding concentrations of taurine within mussel tissue, and long-term studies ranging from six months to one year.
- There is great potential in investigating the role of gene (genetic) and gene expression (epigenetic) differences that are involved in thermal tolerance and phenotypic plasticity.

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