Plasticity of thermal tolerance and its relationship with growth rate 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.
- Previous work has demonstrated substantial adult plasticity in sublethal physiological metrics, such as defense against oxidative stress, that varies between intertidal sites (Jimenez et al. 2016).
- However, little is known regarding the roles of developmental plasticity vs. physiological constraint in the thermal tolerance of juveniles.

Objectives

1. To determine whether fixed genetic differences or developmental phenotypic plasticity (in response to environmental experience) determine juvenile mussels' thermal tolerance.
2. To examine potential tradeoffs between thermal tolerance and growth rate of juveniles.
3. To compare plasticity of thermal tolerance in juveniles and adults from the same field sites.

Methods

- 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).

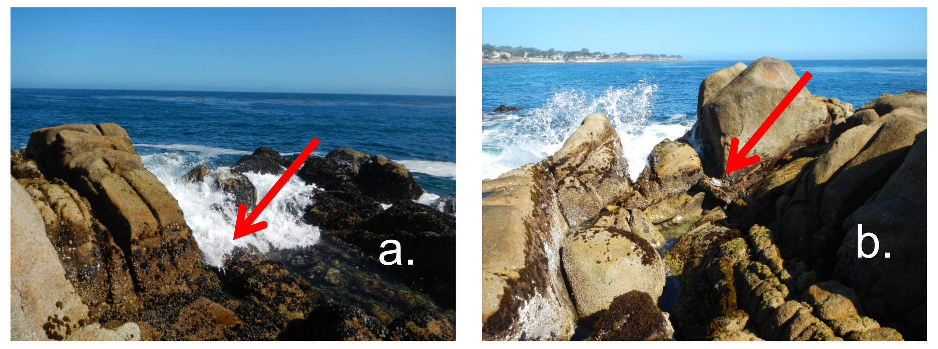

Figure 1. Stainless steel outplant cages at a) exposed and b) protected field sites. Note the difference in wave splash.

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

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

Results

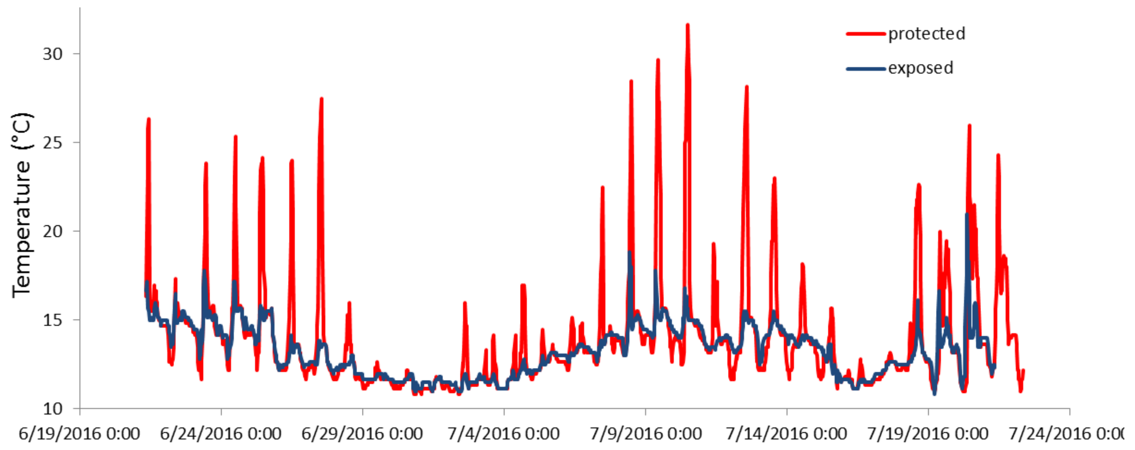

Figure 3. Estimates of mussel body temperatures were collected using iButton dataloggers embedded in silicon-filled adult mussel shells during the 1-month outplant period at each field site.

- 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.

- 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.

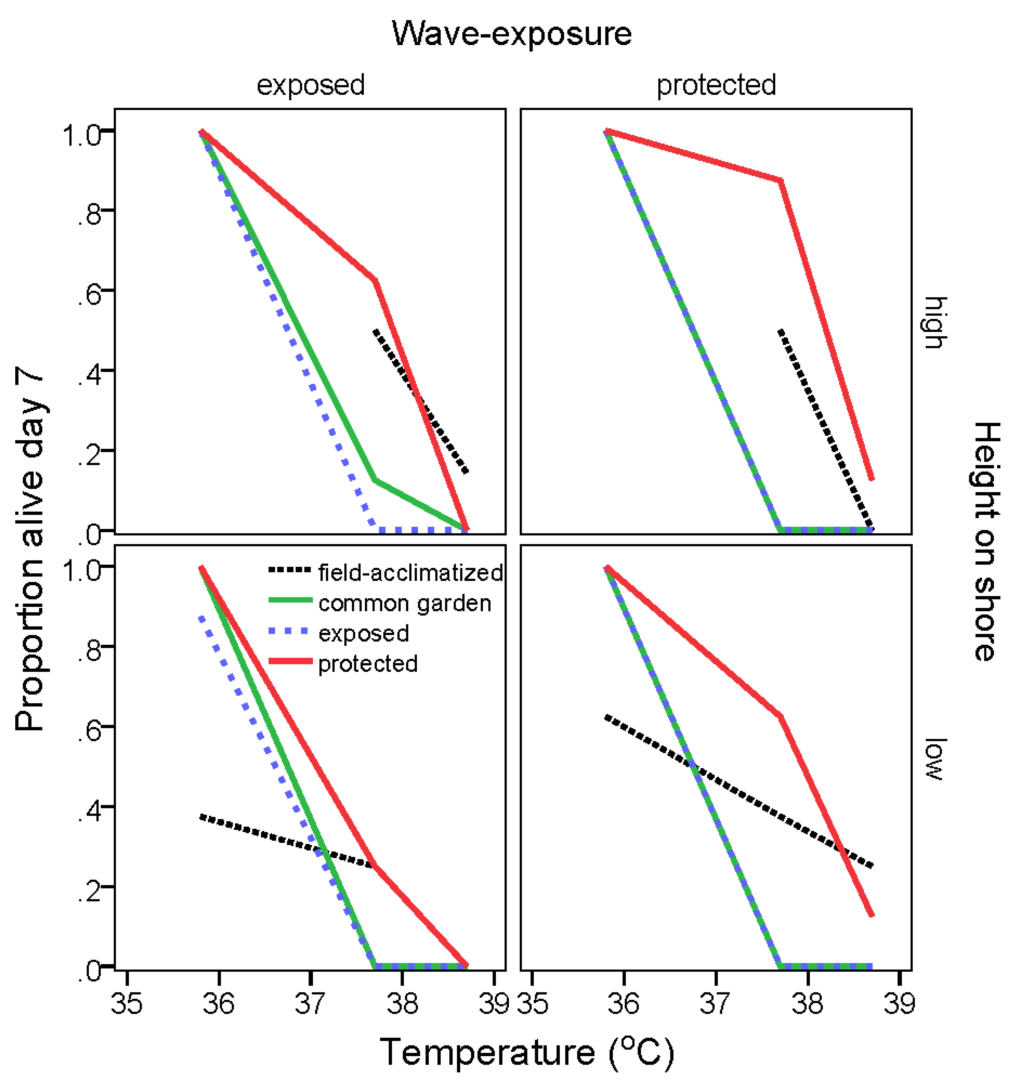

Figure 4. Survival data for juvenile mussels exposed to each of three different acute heat stress temperatures. In each panel (site of origin), mussels were assayed for thermal tolerance following each of 4 treatments. Field-acclimatized mussels from high sites were not tested at 35.8°C. Data were analyzed 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)

- For growth rate, there was a significant interaction between origin and outplant site. High-site juveniles grew faster at the **exposed** site (Fig. 5). All juveniles grew slowly in the **common garden**.

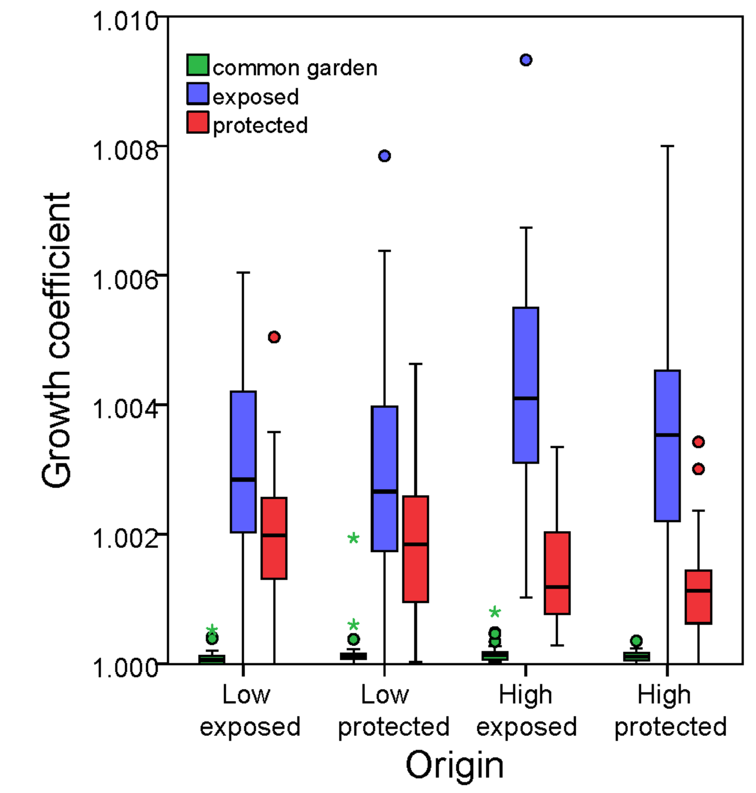

Figure 5. Daily growth coefficients of juvenile mussels from each of the 4 origin sites (x-axis). Mussels were measured immediately after collection from the field and after 1 month in each treatment. $p_{origin} < 0.001$; $p_{treatment} < 0.001$; $p_{length} < 0.001$; $p_{origin*treatment} < 0.001$.

- In contrast with juveniles, adult mussels from exposed and protected sites exhibited no shift in thermal tolerance following common-garden acclimation for 4 weeks (Fig. 6).
- Adults from the protected site were more tolerant at the intermediate assay temperature.

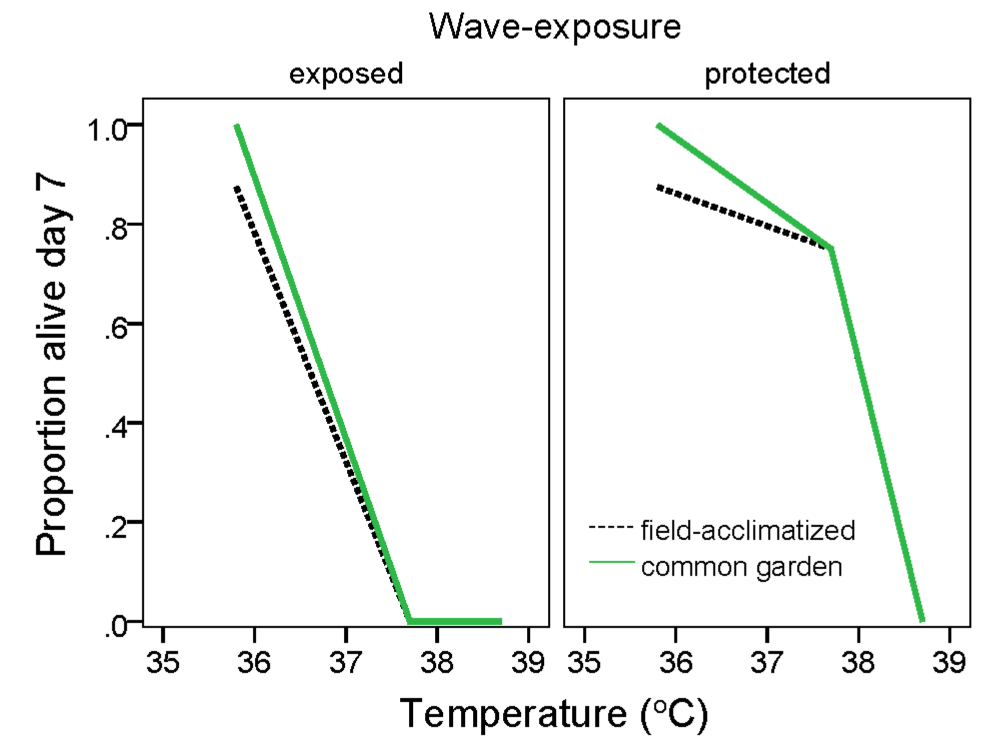

Figure 6. Survival of adult mussels from wave-exposed and wave-protected low sites, directly from the field or after common-garden acclimation. $p_{temperature} < 0.001$; $p_{origin} = 0.004$; $p_{treatment} = 0.43$.

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.
- Future work will 1) investigate whether candidate genes from an adult RNAseq analysis show sequence divergence between individuals that died vs. survived heat stress, and 2) measure growth and thermal tolerance of juveniles after being outplanted to the same field sites for 6 months.

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Reference

Jimenez, A. G., Jayawardene, S., Alves, S., Dallmer, J. and Dowd, W. W. (2015). Micro-scale environmental variation amplifies physiological variation among individual mussels. *Proc. R. Soc. B* 282, 20152273.