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Connecting the Physiological and Behavioral Response to Heat Stress on a Warming Planet

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Introduction

The intertidal zone proves to be a very good model of the biological effects of climate change, as its resident organisms are subjected to heat spells during daytime low tides (2). As the planet warms, these heat spells are predicted to become more frequent and intense, making their effects on marine organisms more pronounced (6). The response of these intertidal species may predict wider biological responses to global warming.

Exposure to increased temperature induces a physiological and behavioral "heat-shock" response.

There is a growing body of research on the physiological effects of heat-shock (5), but much less is known about behavioral responses. Previous work in Dr. William Wright's lab has shown that **heat shock can reduce feeding by the blue-banded hermit crab, *Pagurus samuelis*.** Current literature considers *Pagurus samuelis* to be an ideal model of heat-shock responses; the animals reside in highly variable thermal environments and are thus more likely to express **heat-shock proteins** due to heat stress (5).

A previous student in the Wright lab (1) attempted to correlate the temperature-induced cessation of feeding with the expression of a heat-shock protein, HSP-70 in the crabs' tissues. **HSP-70** is an evolutionarily conserved and ubiquitous chaperone molecule, mitigating protein denaturation caused by elevated heat (6). Changes in its expression can reveal information about the physiological response of single- and multi-cellular organisms to heat stress (3). Feck et al. (2015) found that a 1.25-h exposure to elevated water temperature (29 °C) completely blocked feeding, but did not increase HSP-70 levels. In the present study, we used **longer heat-shocks of 2.5-h**. Such heat-shocks have been shown to increase expression of HSP-70 in intertidal invertebrates (6).

We subjected experimental crabs (N=8) to a 2-h heat treatment in a 29 °C water bath, followed by a 30-min feeding assay at the same temperature. Control crabs (N=8) were left at ambient temperature of 18 °C. Feeding behavior was quantified and HSP-70 levels were analyzed using immunostaining techniques.

Hypothesis

A 2.5-hour exposure to a 29°C heat-shock should inhibit feeding behavior. Such a heat shock should also cause increased expression of HSP-70 relative to unheated controls, and this increase should be positively correlated with feeding.

References

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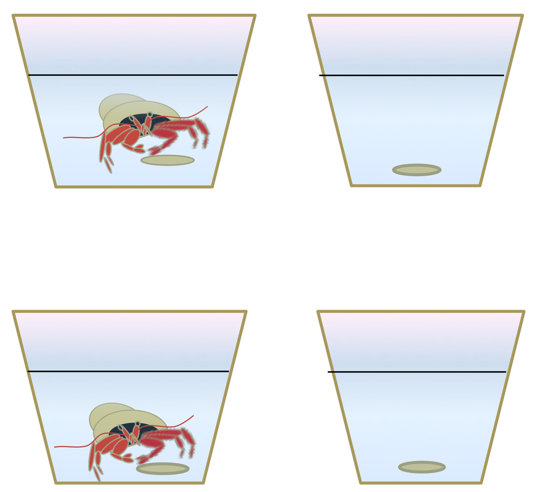
Methods

I. Feeding Assay

We tested the effect of a 2.5 h exposure to elevated temperature on feeding behavior in *Pagurus samuelis*.

- We collected hermit crabs from Inspiration Point (Corona del Mar, Newport Beach).
- Standard squid pellets made in Wright lab were used.
- Pre-test: each crab was presented a squid pellet and observed for 10 min at ambient temperature to verify normal feeding
- We placed each individual crab into a plastic cup for a 2-h incubation.
  - Experimental crabs were placed into an artificial sea water water (ASW) bath at 29 °C
  - Control crabs were placed into ASW at ambient temperature of 18 °C
- After incubation, we placed a pre-weighed squid pellet into each cup for 0.5 h while maintaining incubation temperature



Control n=8
Kept at 18°C
Blank Cups = 3

Experimental n=8
Kept at 29°C
Blank Cups = 3

II. Western Blot Protein Analysis

We used standard immunostaining techniques to measure relative HSP-70 concentration in each crab.

- We removed the large cheliped from each animal.
- Chelipeds were ground with a pestle and treated with protein isolation buffer ($MgCl_2$, DTT, 25 mM Tris,, 1% TritonX-100, 15% glycerol, 1x protease inhibitor and dH_2O).
- Bradford assay to measure total protein concentration.
  - Western blot loading volumes were based on Bradford results.
- Loaded samples into 7.5% polyacrylamide gel.
- Transferred gel onto membrane
- We stained the membrane with Rabbit HSP-70 and mouse beta-actin antibodies
- We quantified HSP-70 levels using chemiluminescence and ImageJ analysis, and normalized by dividing by beta-actin concentration.

Results

Feeding behavior: **Unlike the strong effect of previous experiments with short heat-exposure (1.25 h), prolonged (2.5 h) heat failed to significantly inhibit feeding relative to unheated control animals.**

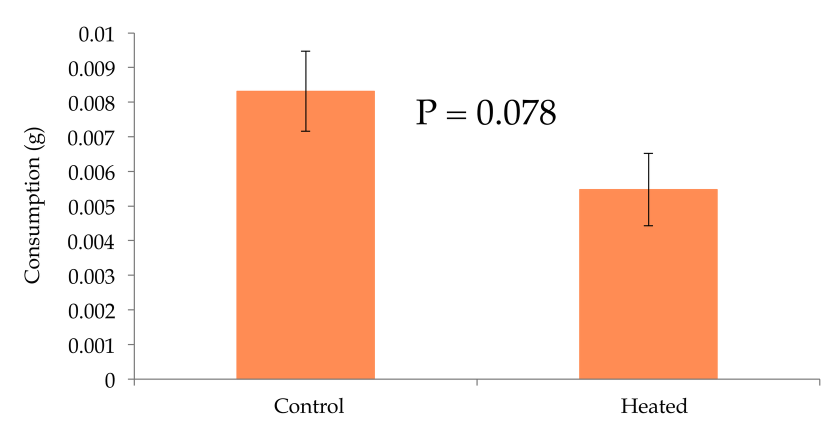


Figure 1: Consumption of standard squid pellet by heated (29 °C) versus control (18 °C) animals. Heating for 2.5 h did not significantly inhibit feeding, although a non-significant trend was apparent.

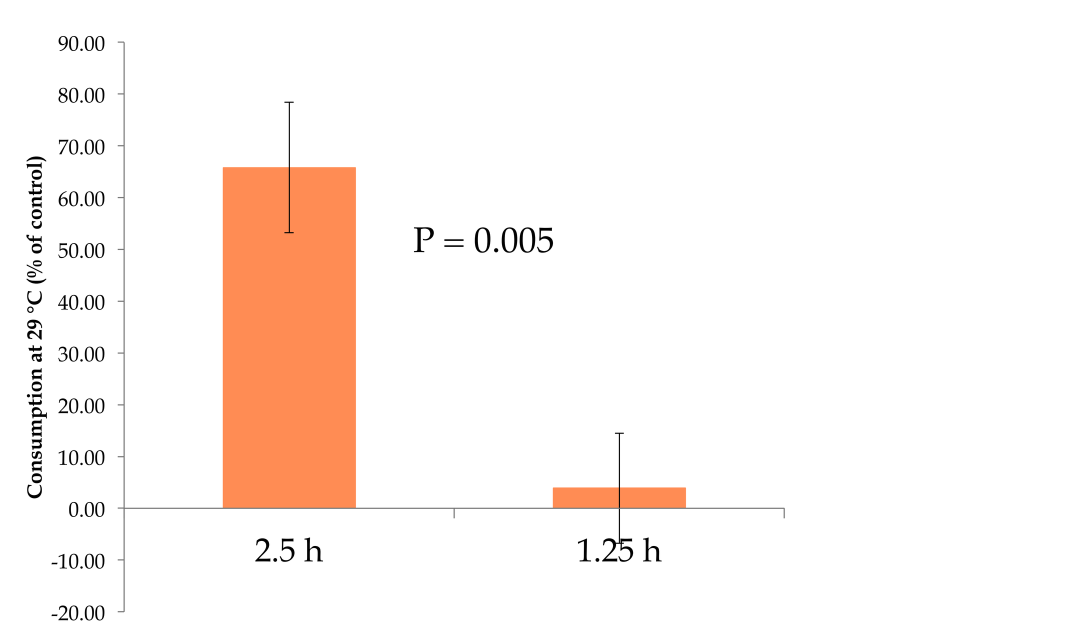


Figure 2: Consumption after 2.5 h in 29 °C ASW was significantly recovered, relative to consumption after 1.25 h incubation. Shown is pellet consumption (relative to control-crab consumption) after 2.5 h (data from Figure 1) vs after 1.25 h (data from Feck et al. 2015). Hermit crabs appeared to acclimate after 2.5 h of heated water: they ate significantly more than crabs heated for only 1.25 h.

The significant increase in feeding after a longer incubation time in heated water suggests that crabs can behaviorally adapt to heat shock after a certain time period.

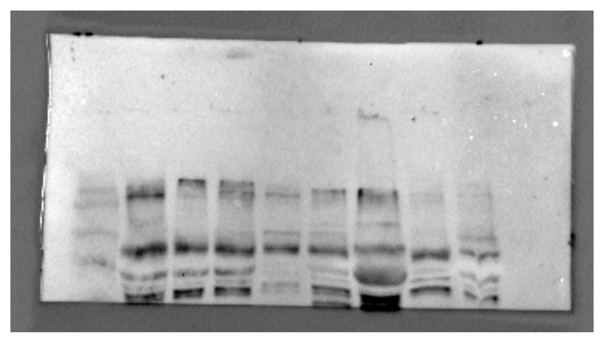


Figure 3: The western blot gel showed three unique bands that we believe to be isoforms of HSP-70.

HSP-70 expression: **There was no significant difference in HSP-70 levels between experimental and control animals.**

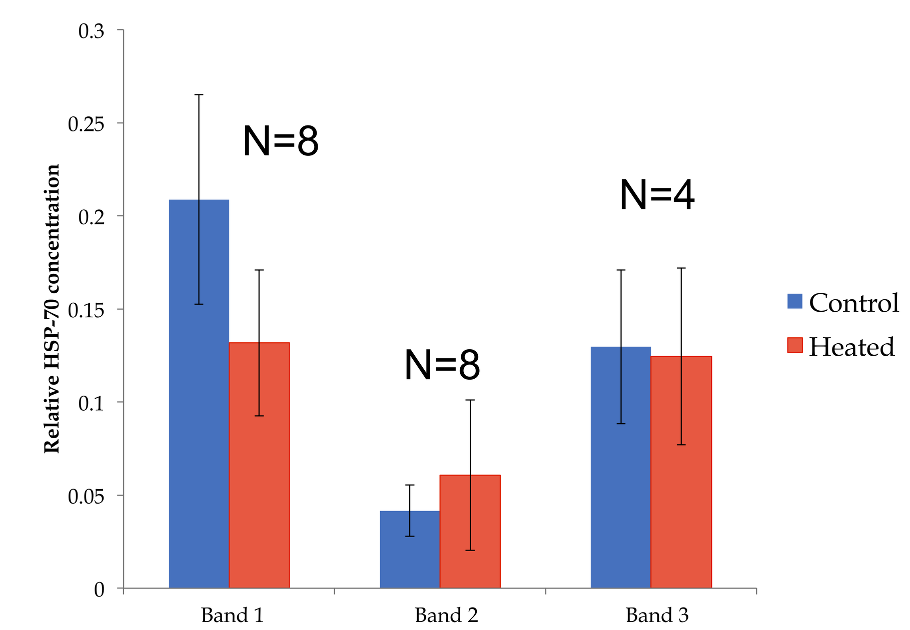


Figure 4: HSP-70 concentrations for the three HSP-70 isoform bands observed. No significant differences were observed between control and experimental animals for any band.

Band 1: Opposing correlation with behavioral consumption in heated vs control animals: **Pellet consumption was positively correlated with HSP-70 expression (P=0.01) in unheated control animals, yet negatively correlated with HSP-70 in heated animals (P=0.03). There were no significant correlations of consumption with expression of HSP-70 bands 2 and 3.**

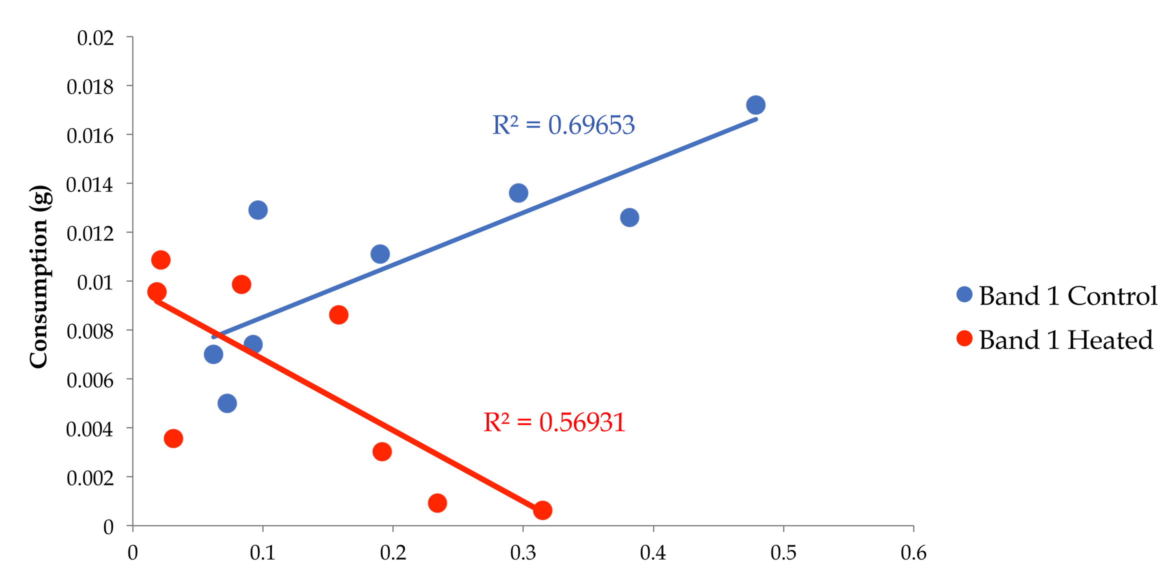


Figure 5. Squid pellet consumption vs relative HSP-70 concentration for band 1, in control (18 °C) and heated (29 °C) hermit crabs. N=8 for each group. HSP-70 levels based on one of three HSP-70 bands seen in western blot of crab tissue.

Conclusion

Lengthened heat exposure may elicit adaptive recovery of feeding behavior, taking at least 2-h to develop. Feeding is positively correlated with HSP-70 levels in control animals but negatively correlated in heat-exposed animals.

Discussion

While a 1.25-h heat exposure completely inhibited feeding, our 2.5-h exposure resulted in significant recovery, suggesting an adaptive response
- This response likely takes at least 2 h to develop

2.5 h in heated water did not significantly change HSP-70 expression.

Feeding was negatively correlated with HSP levels in heated animals.
- Animals with higher HSP-70 expression consumed less food.
- HSP-70 is a measure of how "heat-stressed" the animal is.
- One possible explanation is that increased stress reduces feeding.

Feeding was positively correlated with HSP levels in control animals.
- No heat stress allows normal feeding to continue

Future Research
- These results strongly suggest that the behavioral biology of heat-shock protein expression is more complex than our initial hypothesis.
- We tentatively hypothesize that animals with greater initial expression of HSP-70 levels may:
  - have a previous history of stress, and be less able to adapt to the heat shock.
  - have greater metabolic need for calories and thus consume more food in cool control conditions
- We will continue experiments applying a new "pre-post" method, measuring the HSP-70 levels of each animal before subjecting them to heat.
  - This will allow us to isolate the physiological response caused by our induced heat-shock from the apparently large inter-animal variation in constitutive expression of HSP-70.

Acknowledgements

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