Do Cnidarians remember previous foe attacks?

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Abstract

Background: Immune priming has traditionally been viewed as a trait of the adaptive immune system identified in higher metazoans and thus not found in invertebrates such as cnidarians. However, recent studies suggest a role of immune priming in some invertebrates. In this study we found evidence of immune priming in the sea anemone *Aiptasia pallida* challenged with the coral pathogen, *Vibrio coralliilyticus*.
Methods: All *A. pallida* challenge experiments were conducted at virulent conditions (i.e. elevated temperature of 30˚C) and a concentration of 10^8 CFU/ml of *V. coralliilyticus*. *A. pallida* (n=22) individuals were primed by receiving a 4-day challenge followed by a 4-day recovery period. Control anemones (n=22) only experienced increased water temperature and did not include the bacterial pathogen. The anemones were then challenged again with the same concentration of *V. coralliilyticus* under thermal virulent conditions and monitored for ten days. They were also compared to a treatment where anemones (n=18) were challenged for the first time with the pathogen. Half of the heated control *A. pallida* from the first experiment (n=16) were also treated in the 2nd challenge with bacteria to identify if a temperature effect on the pathogenic tolerance response was occurring.
Results: Significant differences in *A. pallida* survival were detected among the treatments (Kaplan-Meier p=0.003). Bacterially primed anemones showed a nearly two-fold increase in survival rate when exposed to a second challenge compared to those anemones that were not primed (heat control-challenge and non-primed challenge).
Conclusions: The findings from this study show evidence that supports the hypothesis for the existence of immunological priming in a lower metazoan, a sea anemone. Cnidarians such as anemones and corals are long-lived organisms, and immune priming provides a potential mechanism for increased resistance to re-ocurring pathogens over their extensive lifespan. Additional studies are needed to establish the molecular mechanisms underlying this immunological memory in cnidarian organisms.

Figure 1: Clonal culture of *Aipasia pallida*, implemented as a model system to study immune response of cnidarians in the lab.

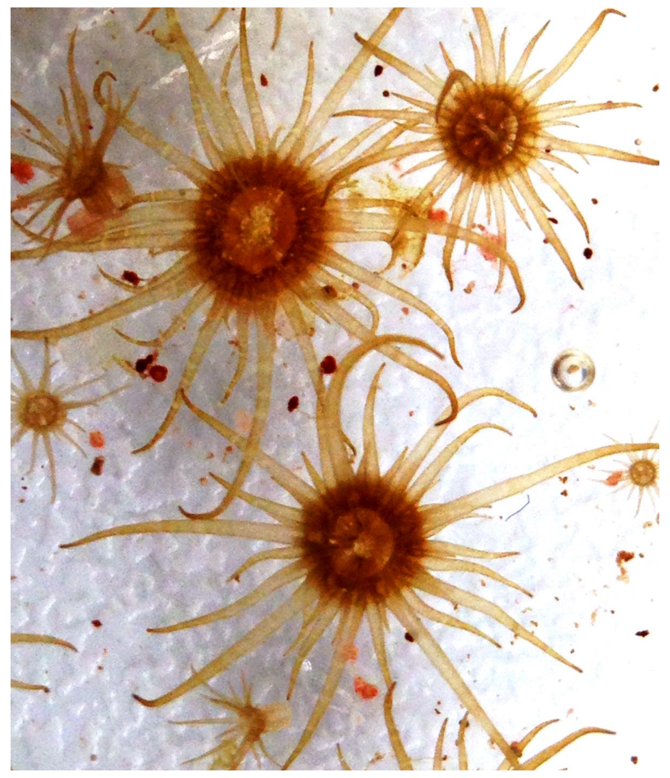

Photo By Anthony Bellantuono

Methods

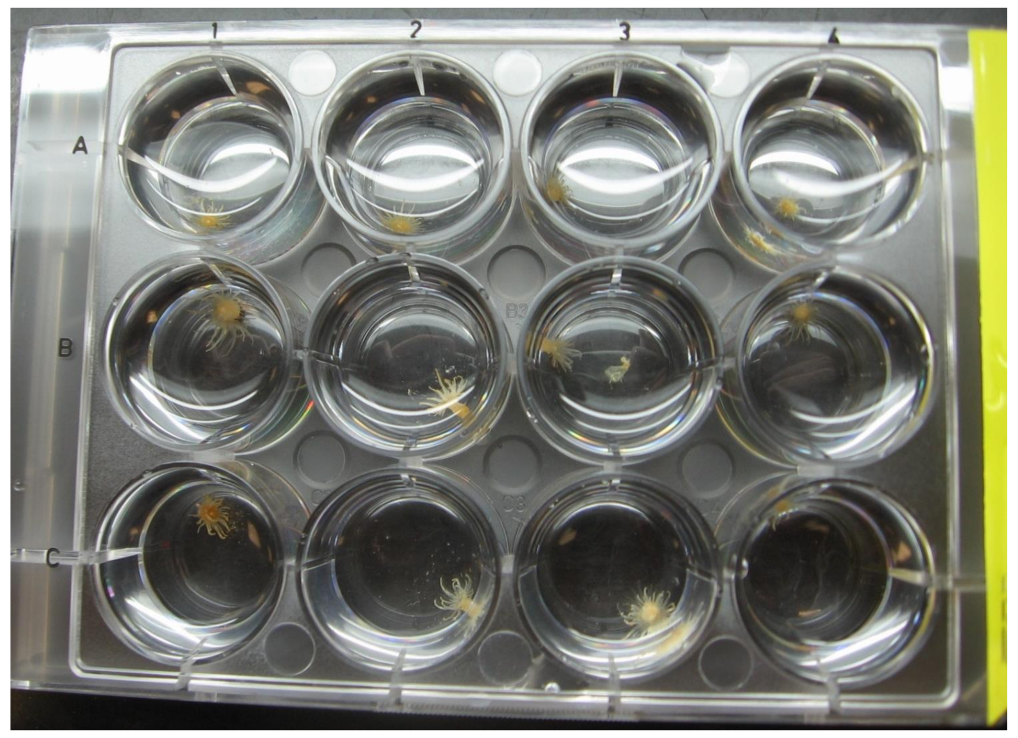

Figure 2: The experimental set up for the priming experiments. One *A. pallida* was placed into each well of a 12 well plate. Sea anemones and plates were placed in a water bath heated to 30˚C.

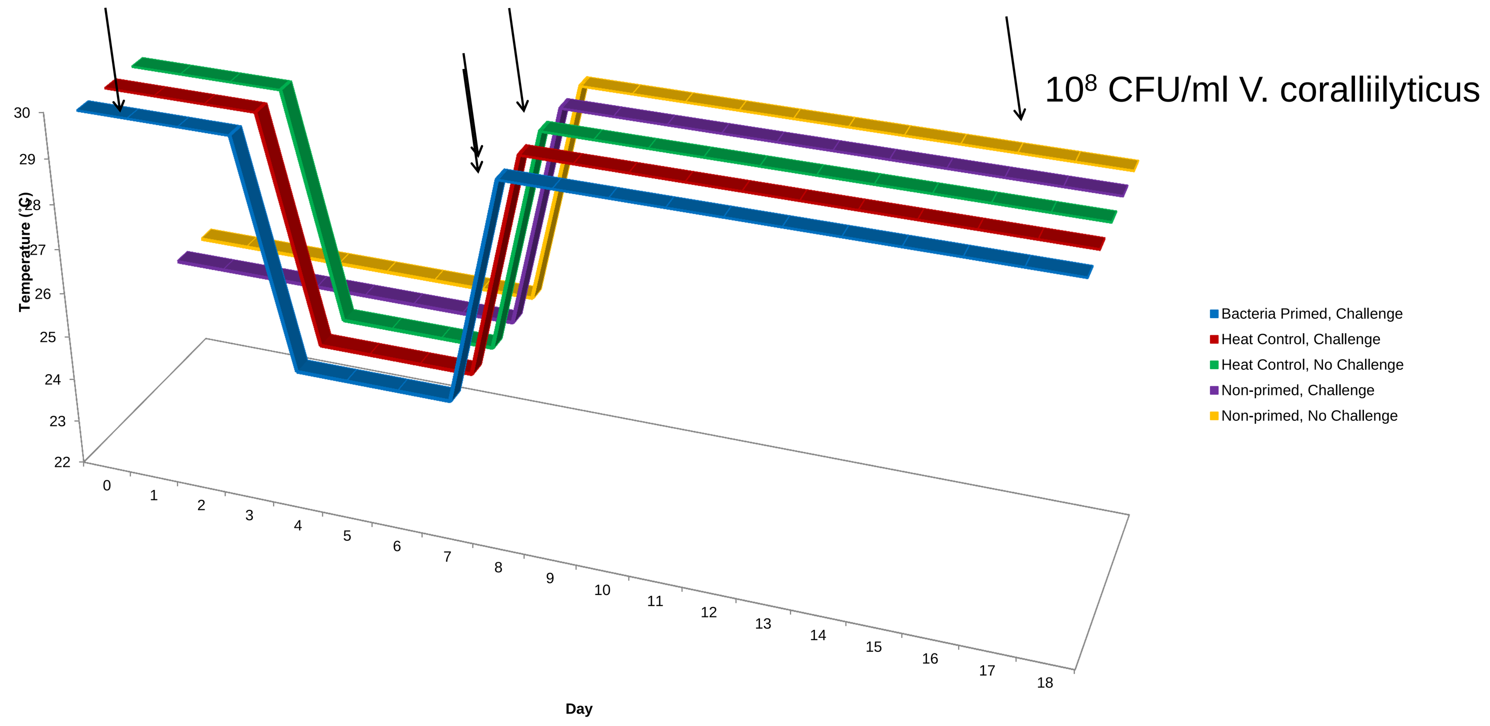

Figure 3: Experimental design depicting the temperature profile and time points of bacterial inoculation (indicated by arrows). Anemones were assessed for death and retractness every 24 hours.

Results

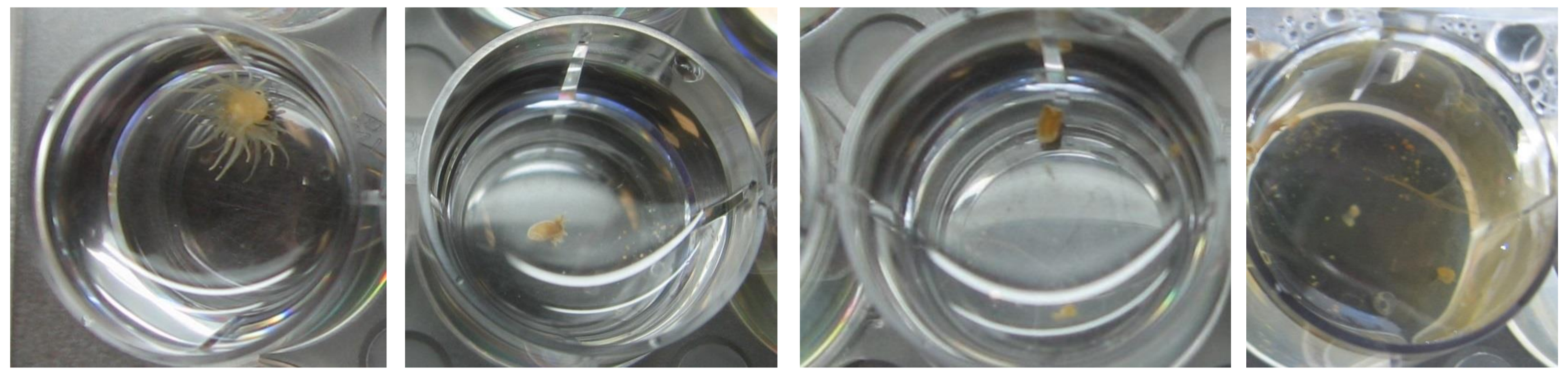

Figure 4: Photographs of *A. palliida* showing the three tentacle retraction states. This retractness classification, including the anemone death state, was used to assess the anemones through the course of the experiment.

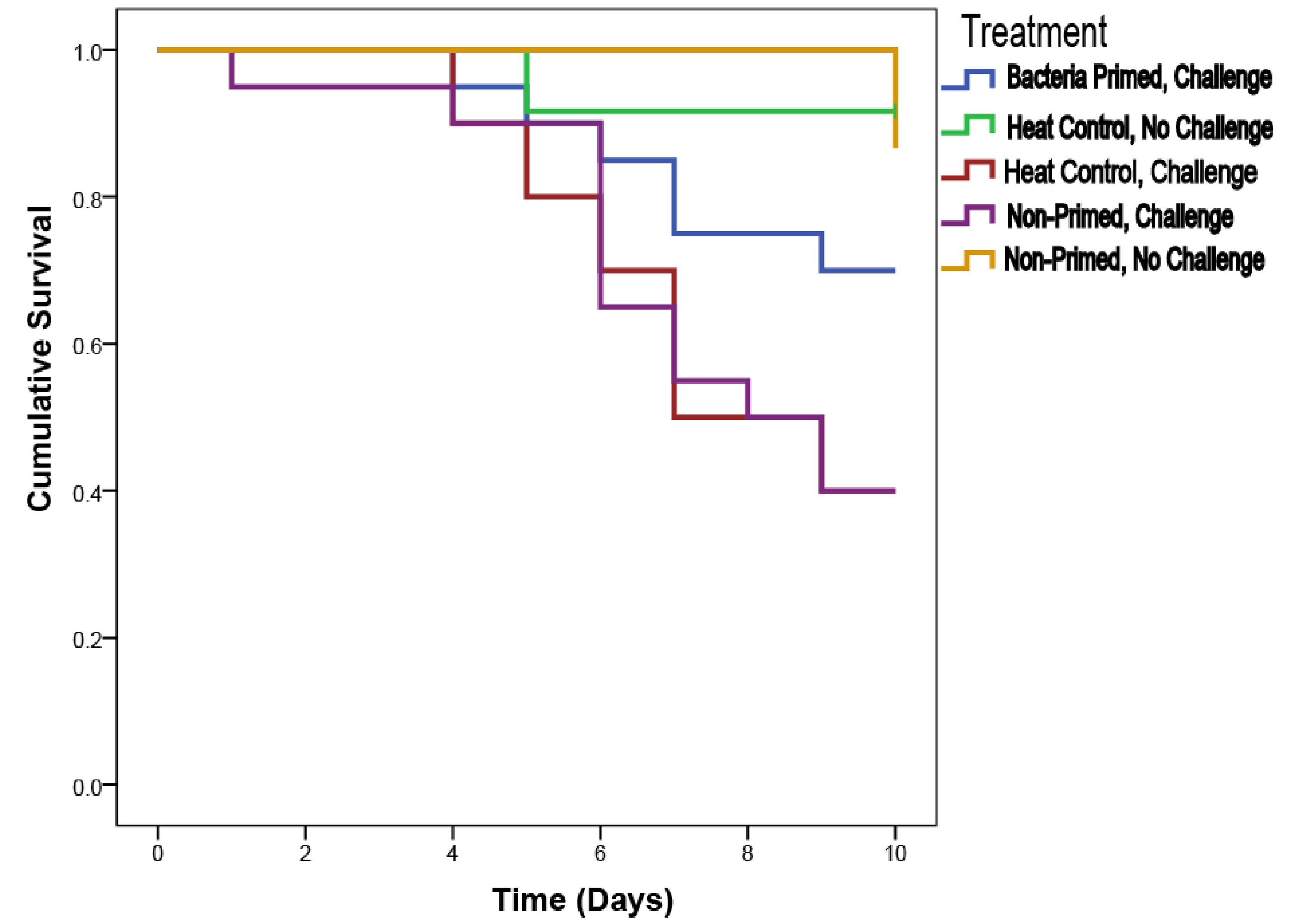

Figure 5: Survival plot for the 10-day challenge experiment. Deaths were noted and plotted on the survival plot. Kaplan Meier Analysis of the data dictates that there was a significant difference among treatments versus controls (p=0.003). The difference between bacterially primed challenged and non-primed challenged treatments were on the border of significance (p = 0.06).

After 10 days of bacterial challenge:

- 30% of the bacterially primed, challenged individuals died
- 60% of the heat controlled, challenged individuals died
- 60% of the non-primed, challenged individuals died

Conclusions

- After 10 days of bacterial challenge, there is a two fold increase in survival of primed individuals in comparison to heat controlled, challenged individuals and non-primed, challenged individuals.
- The data presented here indicate for the first time evidence of immunological priming in *A.*.
- Further studies are needed to determine if priming is sustained over longer periods of time.
- Preliminary results indicate that immune priming still occurs after 2 weeks of recovery.

Acknowledgements

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