Microparasite challenge and subsequent energy trade-offs in an invasive crab species

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Background

Invasive populations often show phenotypic distinctions in relation to their native-range populations. A topic of current interest in populations of invasive species is their immune systems and functions, and whether costs of immune function are altered given the differences in selective pressures they experience in the invasive range. In general it is assumed to be costly in terms of energetic resources for hosts to mount an immune response[4]. Are the subsequent effects of mounting said immune response costly on other necessary immune functions for the host? These imbalances in resource allocation in parasitized hosts can be described as trade-offs[5], in which key resources are redirected to different functions in the host in response to a foreign parasite. Diseases in the ecological sense exert a selective pressure on populations and can sometimes force hosts to use resources in different manners, oftentimes shaping the life histories of species and their innate immune responses[6]. It is important to understand resource allocation and immune response and function in wild populations, so our study focuses primarily on whether a microparasite challenge would induce an immune response and subsequent alterations in energy expenditure in a novel host, *Hemigrapsus sanguineus*.

Hemigrapsus sanguineus

- An omnivorous[2] Asian shore crab originating from Japan which has established an invasive population on the New England coast for approximately 30 generations
- Native-range *H. sanguineus* are often faced with a variety of macroparasites, but invasive-range species are not; however, it is still infected by microparasites such as viruses and bacteria, which are ubiquitous in the marine environment.
- The constant threat of microparasite infection may compromise the crab's resources and redirect them into immune defense, so it is useful to know if these types of challenges are costly.

Objectives

- Establish a protocol to measure the microparasitic killing ability of crab hemolymph using *Vibrio alginolyticus*
- Investigate short-term energy demand (via respiration rate) in response to microparasite challenge
- Investigate if there is a relationship between supposed immune stimulation and bacterial killing ability
- Investigate if there is a relationship between bacterial killing ability and respiration rate

Methods

LPS/PBS injections[3]

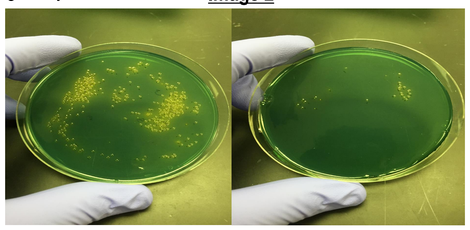
In order to induce an immune response, we injected crabs with lipopolysaccharide (LPS), a component of gram-negative bacterial cell walls and potential immunostimulant. Control crabs were injected with phosphate-buffered saline (PBS). For standardization purposes, we carried out injections and subsequent immune and energy assays across four time blocks, with 3-4 crabs per treatment for a total of 14 crabs per treatment. Crabs picked from the NE coast 1 month prior were consistently fed green algae 2-3 times/week. We used a pipette to inject 5µL of 0.25 mg/mL LPS (in PBS) into a sterilized puncture wound in the walking leg membrane. We repeated for crabs with PBS control also. We then incubated crabs in an aerated humid chamber for 20 minutes, and then replaced into appropriate cages.

Image 1

Bacterial killing assay[1] & colony counts

In order to measure bacterial killing ability of pre- and post- injection crabs, we utilized a bacterial killing assay using *V. alginolyticus*. We used a pipette to withdraw 20 µL of hemolymph from a sterilized puncture wound in the walking leg membrane. We added to 500 µL of PBS aliquots and froze at -80ºC for 1 hour to lyse the hemocytes. We made 1:100, 1:1,000, and 1:10,000 serial dilutions of a centrifuged and washed 3 hour culture of *V. alginolyticus*. Different overnight cultures were used for each block of 3-4 crabs per treatment, which contributes to the variability of this assay. We thawed frozen hemolymph and added to aliquots of measured culture dilutions, then incubated in a 30° C shaker for 1 hour. We then plated onto TCBS plates, incubated overnight at 30° C, and then counted individual colonies on each plate. An ANCOVA analysis of log-transformed killing ability was run.

Image 2



Control plate Culture + hemolymph

Respirometry

A Qubit Respirometer was utilized to measure oxygen consumption in pre- and post- injection crabs. The crabs were allowed to acclimate for approximately 9 minutes, and then the rate of oxygen consumption was measured for three replicate 4.5 minute periods with flushing between data collection periods. An ANCOVA analysis was run to determine the effect of treatment on oxygen consumption.

Results

- Microparasite challenge had no effect on energy expenditure in LPS crabs but injection had a significant effect on PBS crabs, with PBS control crabs having significantly higher oxygen consumption than LPS crabs (Figure 1, Table 1)
- Microparasite challenge had little to no effect on bacterial killing ability (Figure 2, Table 2)
- There is no significant relationship between bacterial killing ability and respiration rate (Figure 3, Table 3)

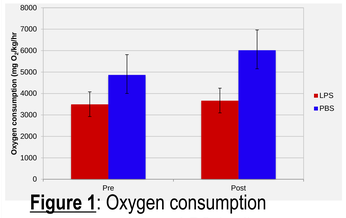


Figure 1: Oxygen consumption for pre- and post- LPS (red) & PBS (blue) injected crabs using mean and standard error

Table 1: ANCOVA analysis output of the effect of treatment on oxygen consumption (size/ starting O2 consumption/block = covariants)

	DF	Sum of Sq	F value	Pr(>F)
Treatment	1	0.68	7.06	0.02
Block	3	1.24	4.29	0.02
V Pre	1	0.13	1.33	0.26
CW	1	0.44	4.56	0.05

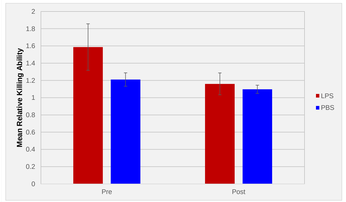


Figure 2: Bacterial killing ability for pre- and post- LPS & PBS injected crabs using mean and standard error

Table 2: ANCOVA analysis of log-transformed bacteria killing ability

	DF	Sum of Sq	F value	Pr(>F)
Treatment	1	0.31	0.16	0.69
Block	2	4.85	0.40	0.32
logBKA1	1	0.95	0.51	0.49
CW	1	0.00	0.00	0.98

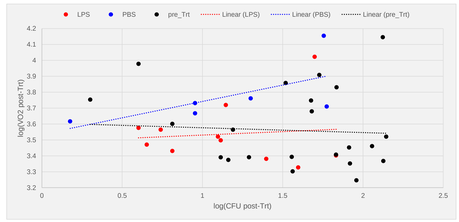


Figure 3: Relationship between bacterial growth and energy expenditure

Table 3: Results of linear mixed effects regression for the relationship between bacterial growth and O_2 consumption (block included as random effect)

	Estimate	Std. Error	T Value	pr(>|t|)
(Intercept)	-0.30	10.28	-0.03	0.98
log(VO_2post)	0.30	1.05	0.29	0.78
Trtmt PBS	-9.24	13.38	-0.69	0.50
CW	0.02	0.14	0.17	0.87
log(VO_2post):TrtmtPBS	1.09	1.58	0.69	0.50

Discussion

The most reasonable explanation for these results stems from procedural practices: in terms of bacterial killing assays, not alternating plating between LPS and PBS individuals (LPS individuals always came first, then PBS), although we assume that this would not yield a significant difference. In terms of respiration rates, there is a small increase in oxygen consumption in the PBS control individuals, but it is mostly negligible; these results also indicate that it was likely not the LPS or even being injected that stimulated the immune response, but the PBS control that stimulated an immune response. This could most likely be caused by either an insufficient concentration of LPS (although this was pulled from another assay that shows 0.25 mg/mL is effective), or a possible sickness response in LPS crabs or increased activity levels in PBS crabs. To improve this study, there would have to be a significantly larger sample size with alterations in methodological practices. The null hypothesis is supported in relation to experimental conditions, but the opposite hypothesis is supported in relation to microparasite effect on PBS control crabs. While our study is a first step in investigating the immune response in the invasive range, further experiments comparing native and invasive parasite response would allow us to infer if our results are due to the altered selective regime in the invasive range.

Future Directions

For a comprehensive study of this invasive model's immune response, future directions would include assessing the following:

1.Is there a relationship between bacterial killing ability and encapsulation?
2.Is there a relationship between encapsulation and
   a. Hemocyte density?
   b. Respiration rate?
3.Is there a relationship between hemocyte density and respiration rate?

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