Body-region-specific effects of ocean acidification on exoskeleton properties in the snow crab, *Chionoecetes opilio*

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Introduction

- Ocean acidification (OA) has caused ocean pH levels to decrease by 0.1 units since the beginning of the industrial revolution
- Observed negative effects of OA include disproportionally reduced microhardness (resistance to permanent or plastic mechanical deformation) in the claws (chela) in relation to the carapace of decapods; this could compromise the "crushing" abilities of the claws, potentially diminishing defense and foraging abilities
- Although the entire ocean is absorbing atmospheric CO_2 and experiencing acidification, high latitude regions, like the East Bering Strait, are more likely to be impacted by OA than lower latitudes regions due to higher solubility of CO_2 in colder waters
- The snow crab (*Chionoecetes opilio*) is one of the many valuable commercial species that inhabit the Bering Sea
- The goal of this study was to systematically assess the effects of ocean acidification on exoskeletal properties of the snow crab, *C. opilio*. Specifically, we evaluated microhardness and thickness of the two major structural layers of the cuticle, the endocuticle and exocuticle, within five different body regions, the carapace, left and right claws, and left and right 3rd walking legs

Hypothesis

Long-term exposure to decreased pH levels (7.5 and 7.8) will significantly reduce microhardness and alter elemental composition in the exoskeleton of adult snow crabs

Animal Collection & Exposure

- Adult, female Alaskan snow crabs were collected from the East Bering sea and were individually housed in 68-L basins that received filtered seawater from one of three head-tanks with pH levels of 8.0 (ambient), 7.8 (intermediate), or 7.5 (extreme)
- At the end of the 2 year-long exposure period, surviving crabs were sacrificed and kept frozen at -80°C until micromechanical assessments and elemental composition analyses were conducted.

Microhardness & Cuticle Thickness Testing

- Vickers microhardness of the carapace, left and right claws, and left and right legs were assessed using a microindentation hardness tester (Mitutoyo HM-200). The hardness tester measured individual indents at 100 X magnification in two dimensions, and Vickers microhardness values were automatically calculated. Mean microhardness was calculated by taking the mean of indentations within the same samples
- Samples were imaged under a reflected light microscope (Zeiss AxioScope A1 with a Zeiss AxioCam 105 color camera). The camera's analysis software (ZeissZen) was used to take digital images and collect total thickness measurements, which included thickness of the endocuticle, exocuticle, and epicuticle.

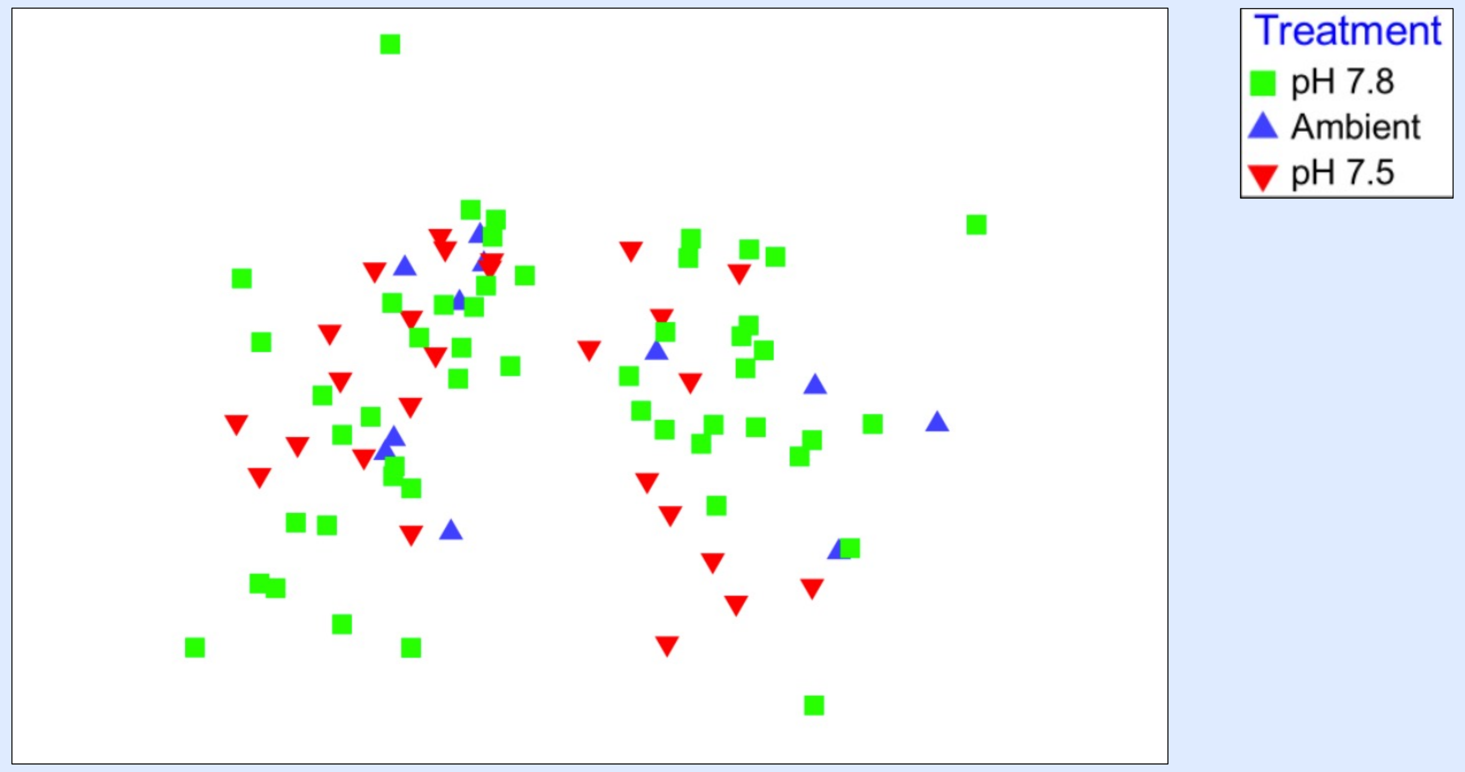
Elemental Composition Analysis

- Inductively coupled plasma optical emission spectrometry (ICP-OES) was used to measure calcium, magnesium, and strontium content of the five body-regions.
- Elemental weight-percentages were calculated for each sample by multiplying concentration by the volume of HNO_3 added prior to ICP-OES analysis, and then dividing by the total dry weight of the sample using the conversion 1 ppm = 1 mg/L

Statistical Analysis

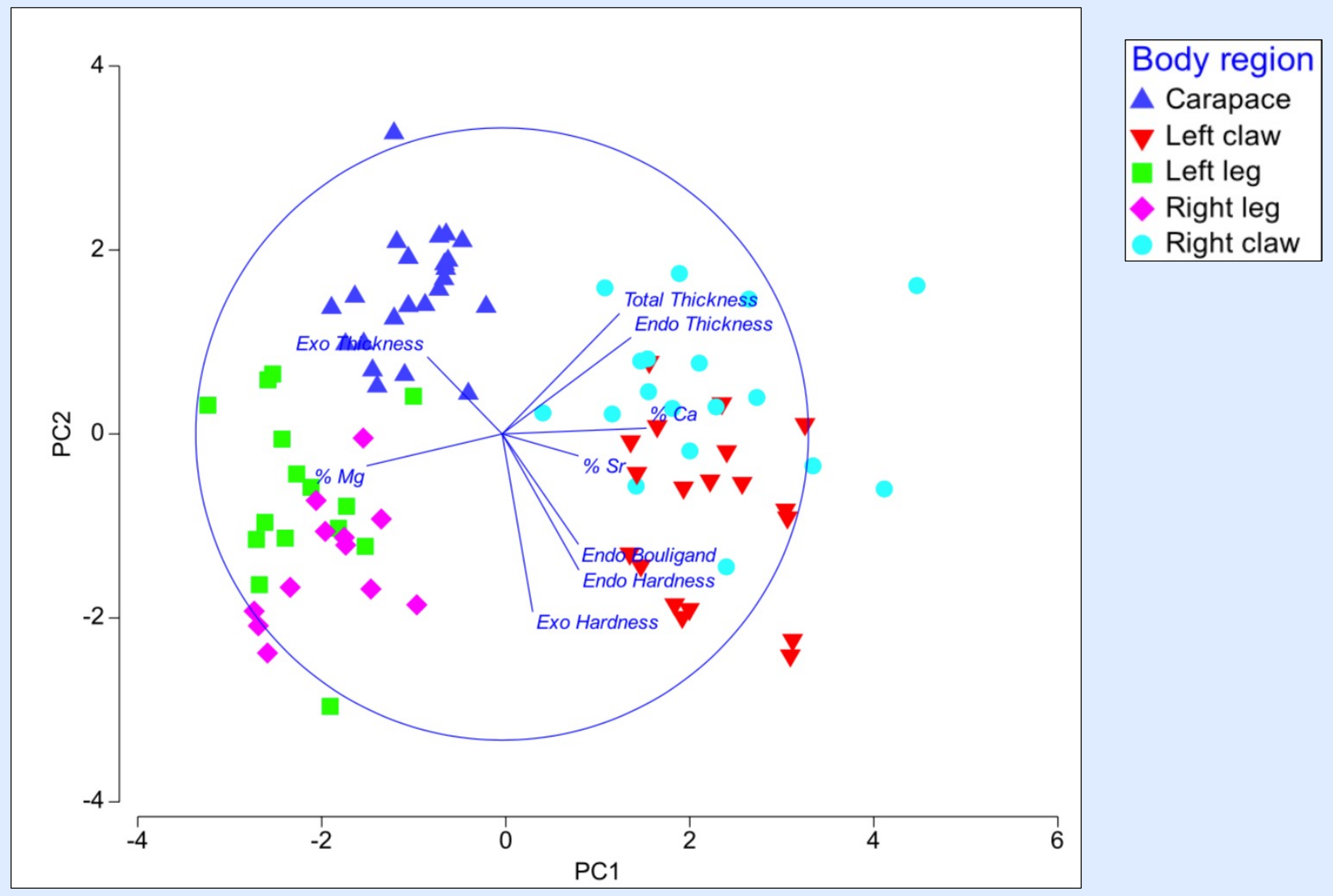
Variables were normalized prior to multivariate analysis and visualized with a non-metric multidimensional scaling plot (nmMDS). Differences among treatments were analyzed with a permutational analysis of variance (PERMANOVA), with treatment fully crossed with body region. Differences in dispersion were analyzed with a permutational analysis of dispersion (PERMDISP). Principle component analysis (PCA) was used to visualize what factors drove the differences among body regions

Differences Among Treatment Levels



This nmMDS illustrates the level of similarity between individual crabs that were sampled in this study. Clear clusters of individual treatments levels would be indicative of significant differences between ambient, intermediate, and extreme pH levels. As no clear clusters are observed, it appears that pH treatment level had no significant effect on the measured properties of snow crabs.

Differences Among Body Regions



This PCA shows data points representing individual body-region samples and plots them in a way that displays which response variables drive overall differences between the different body regions. Claws showed higher thickness, hardness, and calcium weight percentage values than the carapace and legs. The carapace had the highest exocuticle thickness, and the legs had the highest magnesium content.

Summary & Conclusions

- Seawater pH did not affect microhardness or cuticle thickness in any of the body regions examined in this study
- The left and right claws and left and right third walking legs appear to be asymmetrical in terms of micromechanical properties
- Chelal asymmetry points to behavioral bias in claw preference in the snow crab, implying handedness, or heterochely, in this species
- Snow crabs appear to be resistant to exoskeletal dissolution consequent to long-term pH reduction, indicating resilience to future ocean conditions
- These findings indicate that snow crab populations in the East Bering Strait will not be drastically affected by future seawater chemistry changes and will continue to be a viable source of revenue to the Alaskan commercial fishing industry.

Acknowledgements

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