Ocean Acidification and Increased Temperature Impacts on California Spiny Lobster Defenses

Keywords climate change; exoskeleton; crustacean; calcification; gene expression

Introduction Predicted ocean acidification levels (OA) have been shown to change calcium carbonate structures of taxa ranging from corals to oysters, many of which experience decreases in calcification.[1,2] Surprisingly, crustaceans appear to increase calcification, likely because of their osmoregulatory capacity.[3] Increased temperature can also hinder internal pH regulation,[4] so multivariate experiments are integral to understanding real-world responses of crustaceans to future ocean conditions.

The California spiny lobster, *Panulirus interruptus*, is the fifth most important fishery in Mexico and is fished recreationally by 30,000 people yearly in California alone.[5,6] As urchin predators, they play an important role in kelp forest ecology by reducing urchin disease and macroalgae overgrazing.[7] *P. interruptus* rely on their calcified exoskeletons as armor to prevent predation and as a tool for sound production to warn away predators.[8] Predicted changes in ocean conditions could alter the integrity of the lobsters' exoskeleton, ultimately affecting its predation and, given the species' key role in the ecosystem, producing far-reaching trophic effects.

Proposed Research To determine the potential impact of OA and increased temperature (hereafter referred to as multiple stressors) on *P. interruptus* exoskeletons, I will integrate tools from biomechanics, genetics, and ecology. I aim **to understand how multiple stressors potentially affect (1) exoskeleton morphology, (2) gene expression, and (3) defensive sound production.**

Experimental Design Juvenile *P. interruptus* are readily collected along the Southern California coast. Sixty-four animals will be maintained at Scripps Institution of Oceanography (SIO) in an existing flow-through, experimental aquarium system for six months. In four header tanks that feed to individual animals, I will maintain four combinations of pH and temperature: ambient conditions, and pH and temperatures that are adjusted to reflect changes predicted by the year 2100 (pH reduced by 0.3; temperature increased by 3°C).[1] All equipment is available in my advisor Dr. Jennifer Taylor's lab or open for use on campus.

Aim I: Exoskeleton morphology I will test the hypothesis that lobsters will respond to multiple stressors with increased calcification. I will examine exoskeleton ultrastructure using scanning electron microscopy (SEM) and then quantify elemental composition using both inductively coupled plasma mass spectroscopy and energy dispersive x-ray analysis. Results will establish potential changes in mineralization and thickness of the lobster exoskeleton.

Aim II: Regulation of calcification Spiny lobsters will likely respond to multiple stressors by regulating genes like those that concentrate and bind Ca^{2+} near the calcification site.[9] With assistance from Dr. Ron Burton's lab (SIO), tissue from all treatments will be combined and RNA-seq will be used to assemble a reference transcriptome. After its annotation, I will compare transcriptomes of three animals per treatment against the reference to look for up- or downregulation of epidermal genes. Results will establish the important link between environmental conditions and morphology in *P. interruptus*.

Aim III: Anti-predation strategies Increases in calcification due to multiple stressors tend to confer hardness but also brittleness.[10] Attacks may require greater bite forces, yet successful bites may be more catastrophic. I will measure the hardness, stiffness, and brittleness of the exoskeleton using materials testing machines. Results from this study will reveal how multiple stressors affect the integrity of the calcified exoskeleton. These data will be compared to published analyses of the bite mechanics of common predators like horn sharks.[11]

In addition to armoring, the exoskeleton is critical for other anti-predation strategies. Spiny lobsters rub their antennae across a file near the eye to produce a sound that startles away potential predators.[12] Individuals will be stimulated with a predator model while behavior and sounds are recorded using video and acoustic techniques. The file will then be excised and examined using SEM for any ultrastructural changes that may influence ability to produce sound.

Alternative hypothesis It is possible that the natural range of temperature and pH experienced by *P. interruptus* makes them more adaptable to forecasted changes in environmental conditions. Thus, if my hypothesis in Aim I is not supported and there are no morphological responses to multiple stressors, I will expand this research to include multiple spiny lobster species that inhabit different environments. Conducting this experiment across multiple species within a phylogenetic framework will provide robust information about how this important group of animals will respond to climate change.

Intellectual merit Studying potential morphological changes and the underlying mechanisms will help us understand how these animals may adjust to predicted ocean conditions and determine if these responses are robust or if they leave them vulnerable to other threats like predation. Examining morphological changes that may impact anti-predation mechanisms elucidates how OA may not only affect individual species, but potentially have much larger community impacts if predation rates change. Spiny lobsters must contend with fishery pressure too, and it is important that managers anticipate any changes in population structure due to a changing environment. This study focuses on juvenile lobster that are not sexually mature until age 4-6, so results will help us understand how potential changes in predation will impact recruitment of mature and legal-size spiny lobster.[13]

Broader impacts *Expanding opportunities:* I will involve undergraduate lab volunteers and recruit students in the Scripps Undergraduate Research Fellowship (SURF) program, which provides summer research experience to undergraduates that do not otherwise have access to ocean science opportunities.

Promoting education: Given their recognition in Southern California, spiny lobsters create an avenue to communicate climate change impacts with the general public. As such, I will work with SIO's Birch Aquarium to expand their OA and spiny lobster education programs and develop the results of this research into a SEA day at Birch Aquarium, a half-day event where children learn science through hands-on activities. I will plan a similar event for Expanding Your Horizons San Diego, an annual conference designed to interest young girls in STEM fields.

Fishery impacts: I will complete a report for the CA Dept. of Fish and Wildlife's Spiny Lobster Advisory Committee that clearly details how my results may show changes in juvenile survival, especially via potentially increased predation. I will plan a forum meeting through my interdisciplinary program, which includes graduate students from UC San Diego and El COLEF in Tijuana, Mexico, and invite fishery managers to discuss this regional issue.

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