<u>INTRODUCTION</u>: In combination with ocean warming, marine ecosystems are projected to experience shifts in carbonate chemistry due to ocean acidification (OA), the diffusion of anthropogenic CO_2 into surface oceans. With a 26% increase in seawater acidity since the Industrial Revolution [1], OA is expected to have a large impact on marine ecosystems [2,3], but the consequences for many ecologically significant species are still unknown [1]. Thus, important research priorities are to determine whether marine organisms can survive in an acidifying ocean through migration, acclimation (phenotypic plasticity) or adaptation [4]. Here, I propose to examine the balance of plasticity and adaptation in the acorn barnacle *Balanus glandula*, a cornerstone of Pacific intertidal food webs [5]. *B. glandula* inhabits coastal regions that experience a natural mosaic of pH conditions, and so it is ideally suited to investigating organismal responses to environmental variation in pH.

In the California Current System (CCS), coastal upwelling creates a dynamic mosaic of low pH (high upwelling) and high pH (low upwelling) sites [6]. Upwelling has occurred in this region for the past 12-15 million years [7]; however, the compounding effects of seasonal upwelling and anthropogenic OA are expected to produce under-saturation of aragonite (a form of calcium carbonate) in most nearshore habitats over the next 20-30 years [3]. This will make it progressively more difficult for calcifying organisms to construct and maintain their shells.

One important research question is whether organisms have already adapted to current variation in pH. *B. glandula* is an ideal study organism from this standpoint, due to distinct genetic differentiation between populations in Southern California and Oregon [8]. This population structure, in light of the pH mosaic, suggests a potential for local adaptation in populations of barnacles that have different capabilities to withstand future low-pH conditions. These areas may be 'hotspots' for adaptation [9] – fostering traits that will allow *B. glandula* to survive future OA [10]. <u>I propose to examine effects of future climate change on *B. glandula* by identifying resistant populations and genotypes, as well as evaluating the effect of OA on *B. glandula* on a community level.</u>

In the face of OA, an important tool will be next-generation sequencing technologies, which have been extended to non-model organisms and present an innovative opportunity for identifying sources of adaptive capacity [11]. Such studies have been conducted on marine invertebrates and have identified population structure, indicating the potential for local adaptation [12,13]. However, there is the possibility that an organism's tolerance is governed not only by genetics, but also by phenotypic plasticity. Furthermore, "carry-over" effects have been observed, which show that stresses during earlier life stages can impact the later stages of the organism as well [14]. It is important to analyze a wide range of processes to understand the effect of decreasing pH on a population's overall adaptive capacity [15], as the response of each species to variable pH in the environment is a combination of plasticity and genetics.

<u>PROPOSED RESEARCH</u>: My specific research aims are:

1. Do intertidal recruitment and community structure vary along the pH mosaic?

2. Will *B. glandula* in more acidic regions show better shell growth in low pH conditions?

3. Can we measure the heritability of traits that govern low-pH tolerance in *B. glandula*?

I hypothesize that populations of *B. glandula* may already have adapted to local low-pH conditions, having a significant impact on community structure, and that organisms without this tolerance will perform poorly in acidic waters.

<u>METHODS</u>: Field collection sites will be at Strawberry Hill, OR (~44˚N) and Lompoc Landing, CA (~34˚N). Each site is instrumented with pH recording sensors, called SeaFETs, deployed by the Ocean Margin Ecosystems Group for Acidification Studies (OMEGAS) which cover

~1400km of the U.S. west coast from Southern California to Oregon [7]. I will have access to the time series data recorded by these sensors, which is crucial in correlating my results to future projections of OA. The CO_2 manipulation experiments and corresponding genomic analyses will be supported by existing infrastructure in the Hofmann Lab at UC Santa Barbara.

1. *Intertidal communities* – Using settlement plates (*n*=10 at each site + 4 within-site replicates), I will allow communities to develop at intertidal OMEGAS sites with different degrees of upwelling, then conduct a reciprocal transplant of these settled plates between sites to observe community-level calcification rates and community structure dynamics. Recruitment tiles have been successfully used in the past to observe community-level calcification near CO_2 seeps [16].

2. *Physiological tolerance* – I will collect *B. glandula* at the same sites and incubate them in the lab at pH and temperature levels representing the range of conditions at sites. I will use an ICP Atomic Emission Spectrometer (UC Santa Barbara) to observe Mg/Sr impurities in shells, which develop under high pCO_2 conditions and cause weak shell structure [17]. This will allow me to compare the strength of shells that grew in the field and in the lab. I will also conduct lipid analysis on the barnacle tissue to observe oxidation state as an indicator of stress using an FPD-Iatroscan [18], and will assess shell growth rates over time in the different treatments.

3. *Heritability* – I will conduct single nucleotide polymorphism (SNP) analysis on *Balanus*, paired with a selection experiment for low-pH tolerance. This will allow for an understanding of genetic variation between populations under different stressors [12] to pinpoint the genetics responsible for OA adaptation.

INTELLECTUAL MERIT: Physiological responses to ocean change can be highly varied between species [19]. My proposed research will allow for a multidisciplinary, multi-tiered approach to the potential threat of OA on an ecologically important species. By analyzing *B. glandula* from the community level down to the genetic level, I hope to understand the big picture surrounding this key calcifier as the pH conditions in the California and Oregon coastal oceans decline. Understanding the tolerance of key organisms to changes in seawater chemistry will be a valuable contribution to the study of OA and global climate change biology.

BROADER IMPACTS: Ocean acidification is a pressing scientific issue that needs to be addressed through interdisciplinary, collaborative research. I will work across disciplines with other researchers through the OMEGAS group and at UC Santa Barbara. My findings will be published in open-access journals such as *PLoS One* in order to make my work as accessible as possible to researchers and for use in STEM education [20]. In addition, certain parts of my research, such as the community structure analysis, are accessible to undergraduates. The NSF Graduate Research Fellowship will allow me to mentor and teach undergraduate and high school students to increase the representation of minority groups in academia and STEM careers and to foster the next generation of global change biologists.

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