MIT Sea Grant preproposal

Working title: Carbon dioxide uptake by kelp (*Saccharina latissima*) and biogeochemical feedbacks to blue mussel (*Mytilus edulis*) growth

This project aims to assess whether the presence of kelp indirectly influences the expression of key shell formation genes by locally ameliorating coastal acidification in a Massachusetts estuarine system.

This proposal supports MIT Sea Grant's strategic plan to integrate biological studies that will aid in CO2 sensor placement in the Gulf of Maine.

a. Jarrett Byrnes, PhD Marine Ecologist UMass Boston- Jarrett will serve as the Principle Investigator and has an on-going research project monitoring sites within the study area that we will use for this project. He will be responsible for Objective 1 and for coordination of the entire project.
b. Nichole Price, PhD Senior Research Scientist Eco-physiologist, Bigelow Laboratory for Ocean Sciences, Boothbay Harbor, ME. Nichole is a kelp physiologist and has on-going research monitoring acidification in shallow marine ecosystems; she will be responsible for Objective 1.
c. John Bucci, PhD Senior Marine Scientist, Gloucester Marine Genomics Institute (GMGI), Gloucester, MA. John is a marine scientist specializing in shellfish genomics to understand ecosystem impacts from nutrient and carbon pollution. He will be responsible for Objective 2, including the gene expression study, which will be conducted at the Blackburn facility in Gloucester.

Project Narrative

The goal of this 2-year field study will focus on linking *in situ* measurements of kelp metabolic activity and bulk seawater carbonate chemistry dynamics with in the growth response of mussels in a Massachusetts estuarine system.

Ocean acidification (OA) is a term that refers to a water quality condition that occurs as a result of excess atmospheric CO_2 absorbed into surface water, which decreases carbonate saturation and pH at a rate detrimental to marine organismal physiology, particularly to calcification processes.[1] Ocean acidification is happening at a global scale, but seawater pH can be highly variable across space and time particularly in coastal systems,[2] and is already impacting wild and farmed shellfisheries, including the blue mussel.[3] Secondary drivers of OA that contribute to nearshore variability include natural processes (e.g., tidal flux and diurnal cycling) and anthropogenic factors, such as land-use modification and wastewater inputs (nutrient loading) that impact estuarine environments.[4] Scientists are beginning to understand the factors

that influence the quality of fisheries habitat exposed to OA conditions; although more field research is essential.

The blue mussel is a hearty species that exists in estuarine and coastal waters of the Gulf of Maine. It is threatened by exposure to ocean acidification and management of commercially and ecologically valuable shellfisheries, such as the blue mussel, will be challenged in the coming decades. These shellfish depend on this critical habitat and experience non-lethal but deleterious effects when exposed to acidified conditions, especially in Gulf of Maine[5], which delay time to market size and potentially fecundity. However, it might find some refuge from species present in tts prime nursery habitat. Juveniles and some adults are often located within aquatic plant beds that include brown habitat forming kelp species, such as *Saccharina latissima*. Our team of Massachusetts and Maine researchers are well positioned to lead the effort to address whether kelps may buffer mussels from the effects of OA.

The proposed study will directly address two key knowledge gaps outlined in the 2016 MIT Sea Grant strategic plan, which include species-response studies of OA impacts on shellfish coupled with an *in situ*, sensor-based examination of multi-stressors in critical macro algal habitat. For example, a key task will to understand which mechanisms, potential impacts, and biogeochemical feedbacks affect blue mussel and kelp habitat at long term monitoring sites.

As an outcome, we will generate times series of carbonate chemistry data and quantify the indirect species interactions kelp and blue mussel growth in response to estuarine acidification dynamics. This project will also provide biological data to support the development of a Gulf of Maine carbonate chemistry monitoring system. Given that co-PI Price is on the steering committee of NECAN (northeast coastal acidification network), we will contribute our data and insights directly to this effort.

Background: Research suggests that OA impacts to coastal shellfisheries may be more complicated in Gulf of Maine coastal systems than previously thought.[6] For example, the rate of temperature rise in the GOM is nearly five times faster than elsewhere in the US[7] and the complex coastline and range of land-use and anthropogenic inputs to the coastal system make it difficult to predict the rate of coastal ocean acidification. Mussels are already being lost at the southern end of their range due to warming waters[8]. Data from limited discrete sampling and extrapolated modeling efforts have revealed that acidic conditions are likely driven by a combination of low pH, oceanic surface water mixed with riverine dominated nutrient runoff.[9,10], but habitat specific trends should be verified with autonomous instrumentation dedicated to key sites. Shellfish such as the blue mussel, *Mytilus edulis*, may be at risk from coastal acidification because they make their shells out from a calcium carbonate matrix material, which is directly derived from the water column.[11] The blue mussel has a bio-mineralized shell composed of a calcite outer shell and an aragonite inner shell. The focus of this proposed research is to better understand the process of this complex shell formation and the key bio mineralization genes (related proteins) that may regulate the health of the blue mussel in a natural setting and how biogeochemical feedbacks from key habitat forming species may influence this biological process. It has been hypothesized that photosynthesis rates by macrophytes (macroalgae and seagrasses) may be sufficient to locally decrease or "draw down" excess dissolved carbon levels in the water and alter calcification[12], but confirmation of the ability for kelp to increase calcification potential *in situ* has yet to be shown.[13] Exposure to poor water quality conditions may be minimized with the presence of estuarine macrophytes such as kelp and serve to mitigate the negative impacts on shellfish stocks.

General approach and experimental design: For each of the 2-years, we will work at two study sites (naturally with and without kelp) that experience similar hydrodynamic conditions (i.e. , bathymetry, tidal exchange, flow, nutrient concentration, residence time) within a Massachusetts nearshore coastal embayment using existing kelp monitoring programs. Carbonate chemistry sensor packages recording high resolution time series paired with regular discrete sampling for remaining carbonate parameters that

cannot be measured autonomously will provide measurements of the temporal variability throughout the kelp growing season. At each site, mussels will be outplanted next to living kelp or kelp mimics (at the site without kelp) to control for small hydrodynamic alterations the blade can create and isolate the impact to mussel physiology as a result of kelp productivity. More detailed methods of these kelp mimics will be described in the full proposal.

- Objective 1 is to determine if primary productivity from a dominant macroalgae species can alter carbonate system dynamics and reduce seawater *p*CO2 and raise pH in a Massachusetts estuary. Question to be addressed are: 1) are conditions less acidic on average at a site dominated by macroalgae and 2) does temporal variation in seawater pCO2 relate to light availability and rates of primary production in kelp?
    - To accomplish this objective, we will deploy sensor packages (SeapHOxes, CTDs, EcoPAR, and ProOceanus pCO2 sensors) measuring PAR, pH, O2, CO2, S, and T every 30 minutes at our study sites from May to October of each year. Biweekly, discrete samples will be taken to analyze DIC, TA, chorophyll a (as a proxy of food availability for shellfish), and nutrients. The data will be combined to estimate temporal variability in carbonate saturation state and general water quality at each site.
    - We will simultaneously measure monthly kelp biomass, growth and metabolic processes (using non-destructive PAM fluorometry) related to the uptake rates of water column *p*CO2 levels).[14] Changes in morphometrics of kelp blades (to estimate biomass) and rapid light curves created with a Walz diving PAM fluorometer (to estimate photosynthetic efficiency) will be made for the duration of the mussel outplants (see Objective 2). Drs. Price and Byrnes will lead this effort as well as coordinate mussel collections with Dr. Bucci.
    - To evaluate the effect of kelp on carbonate conditions, we will evaluate how carbonate metrics (namely biweekly summary statistics of aragonite saturation, including mean, minimum, maximum, and frequency and magnitude above thresholds determined to be

critical for calcification) correlate with kelp biomass and photosynthetic efficiency using multiple regression. To establish the extent to which these relationships are driven causally by kelp, we will compare fluctuations in carbonate variables at each site with light intensity (PAR), as kelp-mediated changes in chemistry should disappear when kelps are not photosynthesizing.

- Objective 2 is to characterize a selection of the blue mussel genes expressed that are associated with shell formation processes at the kelp-dominated site as compared to relatively 'acidified' conditions anticipated at the site without kelp.. A goal is to identify those genes that regulate the complex process of shell growth when exposed to a range of conditions (e.g., throughout a tidal cycle or immediately following a precipitation event) within and without kelp habitat. . Water quality parameters and kelp physiology, as described in Objective 1, will be recorded throughout of mussel growth period.
    - Experimental mussels at the same life stage (i.e. juveniles) originating from a local shellfish farm will be planted and rope grown within cages at field sites. A sufficient number (~90) of out-planted blue mussels from local growers will be deployed at each study site to ensure adequate sample size. Deployment will overlap with the prime growth season (i.e. April – July). A subset of adult mussels will be included in the study for comparison.
    - A morphometric analysis of shells (collected for genetics) will be employed to estimate shell growth.[15]
    - To compare gene expression levels (regulation) and shell growth response variables by site, a sufficient sample of mussels (10) will be collected at 3 time points (before, during and at the completion of the study).
    - In the laboratory, mantle tissue will be dissected from each individual sample for subsequent gene expression study. Extracted RNA from mantle tissue will be synthesized to cDNA for comparative genetic analyses. Assays will be designed using primers and probes developed

from previous transcriptome studies that reference those genes most associated with the production of bio-mineralization processes and subsequent proteins.[16] Once the assays have been validated, reverse transcriptase PCR will be conducted to detect and quantify gene expression levels from cDNA samples collected across all sub-sampling field sites and time points.

- Gene expression level differences between and within reference genes and between tissue samples exposed at each site and across a range of water conditions will be evaluated.
- Statistical analyses will include an ANOVA model to compare the dependent (e.g., gene expression levels, shell length) to the explanatory variables (e.g. water chemistry) by site (kelp presence). In addition, potential relationships between carbon dioxide absorption (uptake) by marine macroalgae with blue mussel shell growth processes will be evaluated.
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Implication / Potential outcome: The proposed study will provide results that fulfill MIT's strategic goal to develop a Gulf of Maine sensor buoy network to monitor ocean acidification impacts to estuarine habitat. Results will add to the knowledge gap on how shell growth responds to acidic water conditions in the presence of kelp habitat. Furthermore, our work will begin to answer whether growing kelp in polyculture with mussels may mitigate future OA impacts on shellfish aquaculture. This project will contribute to the need for experimental data on how a valuable shellfish species in Massachusetts coastal systems responds to variable OA conditions. For example, results will provide new information on whether key blue mussel shell formation genes influence growth in a dynamic estuarine system exposed to changing OA conditions. Results will enable more in-depth transcriptomic analyses to discover an array of blue mussel biomineralization proteins. In terms of community support, this project will offer valuable student training in shellfish genetics at the Gloucester Biotechnology Academy (gmgi.org) with supervision from Dr. Bucci and the entire science team.

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