Garden Club of America Coastal Wetlands Studies Scholarship
David S. De La Mater

Effects of elevated temperatures and eutrophication on plant-herbivore interactions and impacts on a salt marsh foundation species

Proposed Research Project *(Statement of Work)*

Climate change and anthropogenic disturbances are altering environmental conditions and impacting ecosystems across the globe [1–3]. It is imperative that we understand how these impacts, in present day and in the future, will affect ecosystems and the crucial services they provide to humanity. Doing so will allow us to address and, where possible, mitigate these impacts. The conservation and restoration of ecosystems in the face of large-scale environmental change necessitates a mechanistic understanding of how those systems are structured, how they function, how they are maintained and, especially, how changing conditions will affect these systems and the services they provide.

Impacts of climate change and anthropogenic disturbances are rampant in coastal ecosystems [4–6]. Coastal wetland ecosystems, generally, and salt marshes, specifically, are among the most valuable and productive systems in the world [7,8]. Salt marshes provide myriad crucial ecosystem services: they attenuate wave action; stabilize and protect shorelines from erosion; mitigate storm surges and flooding; purify water and intercept nutrient runoff; maintain fisheries by providing important shelter and nursery habitat for both finfish and shellfish while boosting productivity; sequester carbon; and provide important tourism, recreation, education, and research benefits [7,8]. Despite their value, salt marshes are being degraded at an unprecedented rate; globally, about half of all salt marshes have already been lost [9,10]. Among the many salient threats facing salt marshes are eutrophication, increasing air temperatures, and runaway grazing caused by loss of predators [6,11,12]. If our goal is to protect, conserve, and restore crucial coastal ecosystems, like salt marshes, it is imperative that we learn how such threats will influence salt marsh ecosystem structure and function.

In the salt marshes of the eastern US, smooth cordgrass (*Spartina alterniflora*) is a “foundation species”; its physiological adaptations endow it with the ability to withstand harsh abiotic conditions, allowing it to colonize unproductive mudflats and attenuate abiotic stressors such as salinity, heat, wave action, and hypoxia, thus creating a habitat for other species to exist. Foundation species, like cordgrass, are essential to the systems which are based upon them; the structure and function of the ecosystem and maintenance of the communities within it are all very much tied to the dynamics of the foundation species itself [13–15]. If cordgrass is eliminated from a salt marsh system, the whole system, and all the services it provides, are lost [15]. It has been shown that cordgrass populations in eastern US saltmarshes are commonly controlled from the top-down by snail grazers (the saltmarsh periwinkle snail; *Littoraria irrorata*), that these effects are impacted by nutrient availability, and that if left unchecked (via predator exclusion / overfishing), snail grazers can decimate a salt marsh, turning it into an unproductive mudflat in a relatively short amount of time [11,12]. What is not clear is how eutrophication, rising temperatures, and herbivory may interact to impact salt marsh structure and function.

A 2015 meta-analysis indicated that eutrophication in salt marshes increases herbivory and that the strength of this effect is greatest at higher latitudes [16]. In the eastern US, much work has been done to discover what seems to be a latitudinal gradient in herbivory and plant palatability, both in salt marshes generally and in *S. alterniflora* specifically [17–21]. Little work has been done to uncover the mechanistic underpinnings of those latitudinal patterns in herbivory and palatability in salt marshes or to link specific plant traits to observed patterns [19]. Recent research has revealed that temperature variables, such as mean annual temperature and temperature variability, are believed to drive patterns in predation by ectothermic predators, such that there is a global pattern of increasing ectothermic predation closer to the equator [22,23]. Following this finding, it would not be a huge conceptual leap to hypothesize that ectothermic herbivores are driven by temperature variables as well. Temperature drives key aspects of ectothermic herbivore behavior and demography, but the effects are varied, may change between systems, and have not yet been investigated in salt marshes [24–27]. A logical progression of these disparate findings would indicate that as climate change alters both the mean and the variability of temperatures across the globe, and as humans continue to alter the nutrient dynamics of salt marshes, we should expect to see massive changes in how these systems are structured and function.

In order to uncover the mechanistic underpinnings of observed patterns, and to link recent findings on the effects of temperature and eutrophication on herbivory to salt marsh structure and function, I propose a trait-based manipulative field study in salt marshes in order to address the question: **“How do rising temperatures and eutrophication interact to affect herbivory and traits of cordgrass in salt marshes?”** Some researchers have had success measuring effects of herbivore exclusion and nutrient addition on cordgrass primary production [12,17]. Few studies have used a trait-based approach in a salt marsh system [19], while none have used manipulative field experiments in conjunction with a trait-based approach. The advantage of using a trait-based approach is that it allows us to better understand the mechanistic processes underlying patterns of system response to environmental drivers, thereby providing clearer avenues of approach when it comes to designing conservation and restoration interventions. Additionally, if we are able to both 1) link abiotic variation in key environmental variables, like temperature and nutrient availability, to plant traits and 2) link variation in key plant traits to herbivore abundance and activity, which is known to heavily influence salt marsh structure and function, we will have made significant progress towards understanding how environmental changes mechanistically impact coastal ecosystems, which will enable us to make smart, ecologically-informed decisions in the realms of conservation and restoration.

Methods

I will test how the effects of elevated temperatures and eutrophication interact to influence herbivory and traits of cordgrass by conducting a factorial field experiment, using treatments of warming and nutrient addition, while measuring responses in herbivory and plant traits. Nutrient addition will be achieved through fertilization [12,17]. Warming treatments will be achieved in the same way as in previous studies of warming in salt marshes [28,29]: open-topped warming chambers (OTCs) will be used to warm plots. OTCs are five-sided, are made of corrugated panels of polycarbonate (1 m diameter X 2 m height), and are capable of increasing ambient air temperatures by around 1.5°C and soil temperatures by about 2.5°C. OTCs will be constructed such that 6” gaps below panels will permit snail movement into and out of the warming chamber, thus allowing herbivory while still achieving sufficient warming – this has been shown to be effective in past studies using OTCs to study herbivory [26,30]. The open tops, as well as the gaps below chambers, will be sufficient to allow predators unhindered access to plots. Experiments will be conducted at the Rachel Carson preserve near Duke University Marine Lab, in Beaufort NC.

An estimated 12 plots (depending on available funding) will be constructed in the Rachel Carson preserve, and will be 0.5m X 0.5m. These 12 plots will be divided into 3 blocks of 4 different treatments (Figure 1). Each block will be placed in such a way as to maximize distance between blocks while also maximizing coverage of the Rachel Carson preserve. There will be a total of 4 plots in each block, 3 with a unique treatment and 1 control, with at least 15 meters between each plot to prevent nutrient additions from bleeding between plots. Order of treatments in blocks will be varied to further mitigate any possible effects of plot treatments on neighboring plots. Nutrient plots require the addition of fertilizer while warming plots require the construction of open-top warming chambers. Treatments will be placed at the start of the growing season (May 2019) and measurements will be taken at the start of the experiment and once a month throughout the growing season (until September 2019).

I will measure cordgrass growth with methods adapted from Morris and Haskin (1990): a subset of ramets in each plot will be marked with bird bands, and the height of those stems will be tracked throughout the growing season. I will measure foliar traits that are known to be important for growth and herbivory: toughness, specific leaf area (SLA), nitrogen content, carbon content, and carbon:nitrogen ratios (C:N). To measure foliar traits, a subset of ramets that are not marked for growth measurements will be destructively harvested each month. Methods of trait quantification will be based on those used in Niinemets (1997) and Pérez-Harguindeguy et al. (2013). Briefly, toughness will be assessed with a penetrometer, SLA will be derived from mass and area measurements, and nitrogen and carbon will be measured via elemental analyzer. Carbon:nitrogen ratios will be determined by dividing carbon concentrations by nitrogen concentrations. Destructive harvesting of neighbors may have an effect on the growth of ramets marked for growth measurements, but because every plot will experience an equal amount of destructive harvest, and because each plot is compared to an adjacent control plot, destructive harvesting should not influence comparisons between plots. I will measure herbivory in two ways: herbivore density and grazing damage. Herbivore density will be quantified by counting the number of periwinkle snails that are present in each plot and dividing the number by the area of the plot. To quantify grazing damage, both the length and the number of snail radulations (a telltale sign of snail grazing) will be measured and counted for a subset of ramets in each plot.

I will test the effects of treatments (and their interactions) on each plant trait and herbivory with factorial ANOVAs. I predict nutrient addition will increase herbivory, increase plant growth, decrease toughness, increase SLA, increase nitrogen content, have no effect on carbon content, and decrease C:N. I predict that warming will increase herbivory, increase plant growth, decrease toughness, increase SLA, decrease nitrogen content, have no effect on carbon content, and increase C:N. I predict that treatments will have both additive and synergistic interactions, depending on the combination and variables measured. I will use simple linear regression models to test for relationships between plant measurements and herbivory measurements. I predict that plant growth will have a negative relationship with amount of herbivory; that nitrogen content will have a positive relationship with herbivory; and that toughness, SLA, carbon and C:N will all have negative relationships with herbivory.

Progress to date *(what portions of work have already been completed?)*
No progress for this project is possible until funding is secured, due to the cost of supplies.

Broader impacts & implications *(how the study benefits coastal wetlands)*
This proposed work is directly relevant to the Garden Club initiative of promoting wetlands conservation. If this project is funded, it will elucidate the mechanisms by which serious threats impact salt marshes, which are crucial coastal ecosystems that furnish us with incredibly valuable services [7,8], yet are being destroyed at an unprecedented rate [9,10]. If we know more about how these threats take hold and induce changes to ecosystem structure and function, we will be better equipped to design effective, evidence-based interventions. Furthermore, this project has the potential to link disparate findings involving plant-herbivore interactions, biogeography, and environmental change, through the elucidation of mechanistic underpinnings uniting those findings. If this project is successful, future plans involve conducting similar experiments at multiple latitudes to further explore the biogeography of interactions and the role that environmental change will play in influencing those interactions and the ecosystems to which those interactions are integral.

Budget

Item	Justification	Subtotal
Supplies	1mX2m corrugated polycarbonate paneling, to build open-top warming chambers *($106 per panel x 5 panels x 9 plots = $4770)* 8-lb bag of fertilizer *($22)*	$4792
Travel	Transportation, once a month for 5 months *(Mileage: 360 miles round trip x 5 months = 1800 miles)* *(Fuel: 1800 miles / [27mpg / $2.80 per gallon] = $187)*	$187
	Total	**$4979**

Dissemination Plan *(plans or opportunities for sharing research results with a larger audience)*
I will make every effort to communicate my science to a broad and diverse audience. I will begin to do this as soon as possible, by using preliminary analyses done on my freshly-acquired data set and making a poster presentation to communicate my progress and immediate findings at the 104th Annual Meeting of the Ecological Society of America (ESA) in Louisville KY, in August 2019. I will use that opportunity to communicate with ecologists from different institutions and areas of expertise, taking their feedback to refine and improve upon my research. Following this, I will build a manuscript, in collaboration with my advisors, and attempt to publish in an open-access journal that will disseminate my findings as broadly as possible. Upon publication, I will make my trait measurement data available upon request, as well as submit all of my gathered plant trait data to the TRY Plant Trait Database (https://www.try-db.org/TryWeb/About.php). I will publish my poster and a summary of this work on my website, which I will attempt to disseminate through various social media platforms. All R code used for analyses will be hosted on my personal website and all data will be made available upon request. In February 2020, I will present my findings at the annual meeting of the American Association for the Advancement of Science (AAAS), in Seattle WA. AAAS meetings are geared towards multidisciplinarity and communication of research to broad audiences, especially the general public, and often hold workshops on how to do this more effectively. In August 2020, I will present my findings in an oral presentation format to ecologists at the 105th Annual Meeting of the Ecological Society of

America in Salt Lake City UT. Throughout the development of this project, I will make continuous efforts to communicate my findings through both formal (publications; conference presentations; Duke Nicholas School of the Environment website, newsletter, and social media platforms) and informal (personal website and social media platforms) modes of communication in an attempt to reach scientists, policymakers, and the general public. Given the relevance of my proposed research to local stakeholders, I will make every effort reach out and share my data and findings with as many interested parties as possible, including but not limited to the NC Department of Environmental Quality, Coastal Reserve and National Estuarine Research Reserve, US Fish and Wildlife Service, NC Division of Parks & Recreation, NC Wildlife Resources Commission, NC Sea Grant, and local media outlets. By reaching out to these various outlets, I hope to be able to disseminate my findings to as broad an audience as possible.

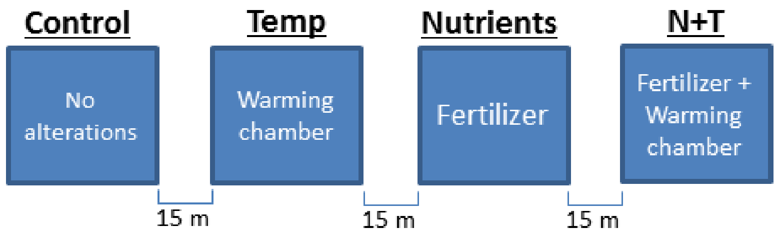

Figure 1: Visual representation of experimental layout.

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