2024

Shannon Point Marine Center Research Intern

Ben (Benjamin Gregory) Molenhouse

Recommended Citation

Molenhouse, Ben (Benjamin Gregory), "Shannon Point Marine Center Research Intern" (2024). *College of the Environment Internship Reports*. 183.
https://cedar.wwu.edu/cenv_internship/183

COLLEGE OF THE ENVIRONMENT

Internship Title: Research Assistant

Organization Worked For: Shannon Point Marine Center

Student Name: Ben Molenhouse

Internship Dates: 3/20/2023 6/3/24

Faculty Advisor Name Manuel Montano

Department ESCI

STUDENT SIGNATURE

DATE: 6/11/24

Shannon Point Marine Center Research Assistant Internship: Final Report

Ben Molenhouse

College of the Environment, Western Washington University

ESCI 498B: Internship

Manuel Montano

June 18, 2024

Introduction: Academic Goals

Before beginning my work as a research assistant, I set various academic goals for myself that I intended to achieve throughout my time at work. One of my first goals was to implement the skills I have gained at university and apply them to real world scenarios. I wanted to learn about the intricacies of *Zostera marina* (eelgrass) populations in Puget Sound as well as the critical factors that seagrasses play within the Salish Sea. I particularly wanted to research how local conditions effect phenotypes of eelgrass populations. Lastly, I set a goal to use the opportunity to conduct research to help further my knowledge of the effects that climate change has on eelgrass populations in marine ecosystems.

My experience working as a research assistant helped contribute to my educational goals. I was able to utilize the invaluable knowledge that I acquired through the College of the Environment to help aid my research, field work, data collection, data analysis, and science communication during my work as a research assistant. I believe throughout my time working on the eelgrass crew I not only succeeded in achieving the goals I set for myself prior to starting my internship, but surpassed the goals I set and ended up achieving more than I could have imagined at the start.

Background: Eelgrass & Estuaries

Eelgrass is an herbaceous flowering aquatic-marine grass that grows in estuarine ecosystems. Eelgrass is part of a lineage of flowering plants that migrated into water (S. Yang, personal communication, June 2023) after the initial plant colonization of land in the late Precambrian era (Knauth & Kennedy, 2009). In the Salish Sea, our native species of eelgrass is *Zostera marina,* but there is also a non-native species, *Zostera japonica* (Shull, 2014). This non-native species from Japan is not considered invasive as it grows shorter, smaller, and higher in the intertidal zone compared to the native *Z. marina,* with our native species not having any trouble outcompeting the non-native species in its niche (Bohlmann et al., 2018).

Eelgrass can reproduce asexually via a creeping rhizome and vegetative shoots, as well as sexually via flowering and seed production (Yang et al., 2013). Eelgrass rhizomes produce above ground vegetative leaf biomass that can range from a few centimeters to over a meter in length. Leaf width can range from a few millimeters to over a centimeter. Sexual reproduction of eelgrass occurs via flowering shoots or inflorescence (De Cock, 1980). Flowering shoots consist of spathes that send out styles to be pollinated, then the spathe may release its own pollen (Figure 1). After releasing pollen, the spathe can start developing seeds. Eelgrass restoration is often implemented employing one of these reproductive strategies by transplanting vegetative shoots or by direct seeding into sediment (Katwijk et al., 2016).

Lastly, eelgrass is also unique in that it has two distinct life history strategies; eelgrass is most often perennial, but also can exhibit an annual life history strategy in stressful environments that consist of frequent disturbance (Gagnon et al., 1980). Eelgrass is an important component of estuarine ecosystems as meadows provide important refuge, habitat, and food for a wide variety of organisms such as jellyfish, crabs, and estuarine dwelling birds (O’Connor, 2016). Eelgrass

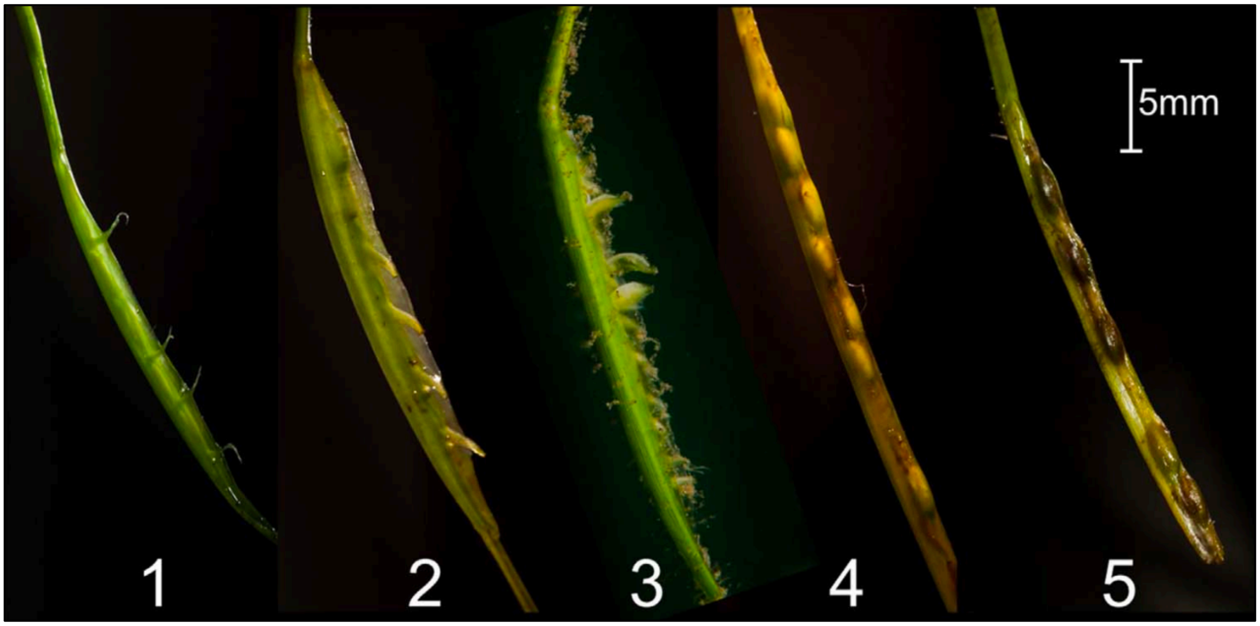


Figure 1. Eelgrass spathe development divided into categories 1-5 as outlined by De Cock (1980). Categories show: (1) styles erect, (2) styles bending post-pollination, (3) dehiscence of anthers, (4) ripening of fruit, and (5) developed and dehiscence of fruit. Image: Heinicke et al 2018.

harbors diversity in shorelines by providing such resources to the ecosystem (O'Connor, 2016). Furthermore, eelgrass can filter pollutants, sequester carbon, and prevent erosion of shorelines by anchoring sediment (Gaeckle, 2016).

Spring 2023: Experiment 1

At the start of my research position, an experiment was already being conducted at Shannon Point Marine Center (SPMC) in Anacortes, WA. The experiment that I joined on to was studying the effects of heightened thermal regimes on eelgrass populations that were sourced from various locations throughout the Puget Sound region. It is important to research the topic of heighted thermal regimes on eelgrass populations as marine waters are expected to increase in temperature as the global biosphere experiences climate change caused by anthropogenic vectors (Bohlmann et al., 2018). Furthermore, studying how different eelgrass populations react to stressful physical environmental factors may give us insights into which populations are best suited and most hardy as donor populations for future restoration efforts.

The first experiment consisted of 8 mesocosm tanks that were fed seawater from a water pump located in the Strait of Juan de Fuca (Figure 2). The seawater pump diverted flow to inputs into each of the 8 mesocosm tanks, 4 of these tanks were then warmed above ambient seawater temperatures via a pump that circulated heated freshwater through a closed tubing system. The 4 heated tanks thereby operated under convection and conduction to warm the surrounding water in tanks. The heating system at that time warmed the seawater 5˚C above ambient seawater temperatures. The other 4 tanks remained at ambient seawater temperatures consistent with that

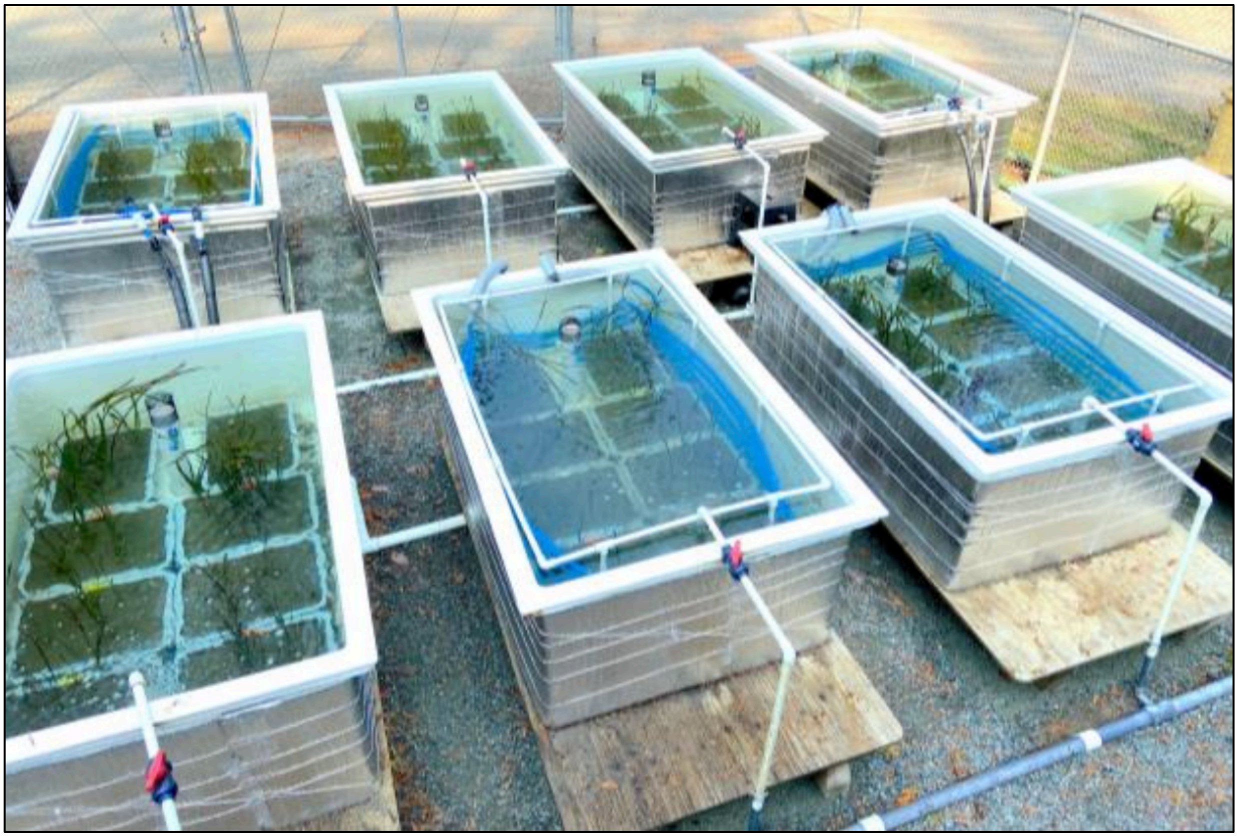


Figure 2. SPMC mesocosm tanks containing eelgrass gardens. Each tank being fed sea water, with only half of the tanks containing blue heating element tubes.

of the conditions within the Puget Sound. Within each mesocosm tank were 8 total tubs containing sediment holding 4 eelgrass plant community phenotypes with 2 replicate pairs from each location from the Sound. From here we had the baseline for our experiment where we could cultivate, maintain, and survey our eelgrass gardens to assess differences in populations under heightened thermal regimes versus populations under untreated conditions.

Our job at this time primarily consisted of general maintenance of the mesocosms, pump system upkeep, surveying of growth, as well as remote work including research and reading to familiarize ourselves with the topic of eelgrass in the environment. Maintenance ensured smooth operation of the tank pump system and ensured optimal growth of eelgrass plants within the cultivated garden. Without weekly maintenance, detrital, algal, and epiphytic growth would have enveloped the plants and prevented primary production as individuals would have been shaded. In the natural environment, there are many more detritivores and herbivores present in eelgrass meadows that keep the plants free from this issue such as sea cucumbers, bubble shells, eelgrass isopods, as well as Taylor sea hares (O'Connor, 2016). With many of these natural feeders absent from our experiment, it was up to us to care for the plants. In doing so this also enabled us to have better access to the plants such that we may more easily survey growth and collect measurements in the tanks.

During surveying of the eelgrass mesocosms, we assessed various aspects of growth as well as presence of eelgrass wasting disease (EWD). EWD is caused by a microscopic pathogenic protist called *Labyrinthula zosterae* which infects cells of eelgrass and destroys chloroplasts within the plant, resulting in a transparent, sometimes black appearance in shoots (Short, 2014). This disease is relevant because we want to assess if there is any relation to the treatment of our experiment, that is heightened seawater temperatures to EWD. This has important implications for eelgrass meadows in the Puget Sound, as the pathogen can cause mass die offs of plant populations (Short, 2014). If die offs occur, it has negative consequences for bay and estuarine ecosystems from the resulting impacted ecosystem services.

Summer 2023: Field Work

General maintenance, upkeep, and data collection of this experiment continued from spring throughout the end of summer in 2023. Yet, much of our work in the summer of 2023 was concentrated in Padilla Bay National Estuarine Research Reserve rather than SPMC. During the summer field work season, we were tasked with various goals to achieve before the end of the season. Our primary goal was to eventually set up a new experiment at SPMC. The new experiment would have a focus on seed germination, seed viability, seedling survival, growth, morphology, branching, flowering, gene expression, as well as phenology of eelgrass. Our new experiment would also utilize the current experimental set up in studying the effects of different thermal regimes on eelgrass populations. Lastly, the new experiment would also compare differences in perennial versus annual populations of eelgrass as an annual eelgrass meadow was discovered in Padilla Bay during the 2022 summer field season.

Routine quadrat surveying of the eelgrass meadows within the bay were completed at the beginning of summer, to assess the health of the meadows. Our surveys of meadows in Padilla Bay were spread across 3 survey sites including Bay View (BV), Interpretive Center (NI), as well as Joe Leary (JL) (Figure 3). Each survey site consisted of 3 transects that ran parallel to the shore. Survey site JL was the site of our annual eelgrass meadow. While surveying the meadows, our crew kept an eye on the development of flowering shoots of eelgrass within the bay as well. Assessment of flowering shoot development would later help us to correctly time flowering shoot harvest, with a goal of collecting seeds from Padilla Bay for our future experiment.

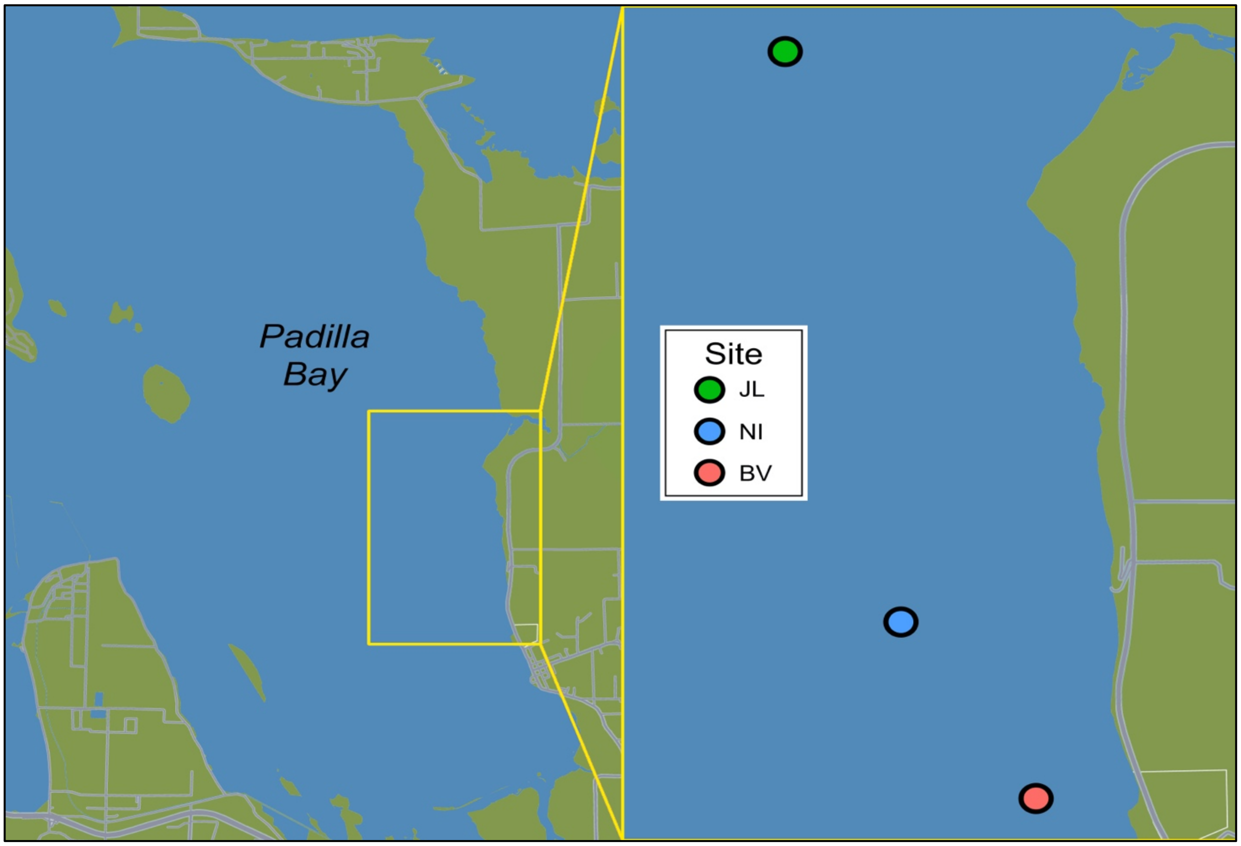


Figure 3. Map of Padilla Bay survey site locations from north to south including JL (green), NI (blue), and BV (red).

We would assess flowering shoot development by analyzing a subsample of flowering shoots from each transect to measure the proportion of spathes in each category of development (Figure 1). When a transect had a majority proportion of spathes at category 4 of development, then that meadow was ready to be harvested for seeds. A subsample of flowering shoots from each survey site were assessed to estimate the average number of seeds per flowering shoot within the meadow (Figure 4). Next, we utilized primary literature of past studies which assessed seed viability and germination rates of eelgrass seeds. We could then apply these values to estimate the correct number of flowering shoots that we would need to harvest such that we would have enough seeds for the new experiment. Harvested inflorescence were transported the

Figure 4. Padilla Bay field crew surveying flowering eelgrass shoots at the JL survey site to assess spathe categories for flowering shoot harvest.

day of to be held in seawater-tables at SPMC where they would be vernalized and ripened over the coming months in preparation for the new experiment.

Various other side projects were also completed during our summer field season at Padilla Bay, some of these projects included helping College of the Environment graduate student Yuki Wilmerding with her master's project of delineating an annual eelgrass meadow. For this project we delineated the newly discovered JL annual meadow within Padilla Bay. We also helped with eelgrass biomonitoring in the northern parts of Padilla Bay that were part of a study to assess long-term changes in eelgrass habitat in the bay. I personally helped researcher and Western Washington University (WWU) graduate Shayla Ferrero wrap up her experiment studying EWD near the NI survey site in Padilla Bay. I also personally had the amazing opportunity to help the Padilla Bay crew complete their semi-annual Multi-Agency Rocky Intertidal Network (MARINe) biomonitoring of local sea star *Pisaster ochraceus* (purple sea star). Lastly, we helped complete surveys of efficacy of native *Ostrea lurida* (Olympia oyster) restoration in Padilla Bay.

Fall 2023: Wrap Up and New Experiment Preparation

While the harvested seeds were ripening in sea-tables, we spent much of the fall of 2023 taking our last measurements from the first experiment. We wanted to gather as much data as we possibly could from the first experiment before we took it down and overhauled the mesocosms and pump system. This process of taking down and setting up a new experiment took the most planning and preparation. During this time of my internship, it helped me hone my skills as a scientist in being able to utilize the resources available to me and get real world experience setting up an experimental research project outside of the classroom environment. We concluded our first experiment during fall quarter 2023.

The conclusion of the first experiment consisted of a full day doing our last major census of the mesocosm eelgrass populations. We then took measurements of every single plant in each mesocosm tank. After this, we carefully removed every plant from its tub, being cautious to preserve the above and belowground biomass of each individual. This would then allow us to separate and dry the above and belowground biomass of each tub population to assess how populations differed in total biomass, above ground photosynthetic biomass, as well as below ground biomass which could give us indicators of different populations' rhizome branching patterns.

Around the same time, we had much to do to prepare the new experiment. We needed to order new tubs and sediment. We also had to order supplies and create mesh seed bags that were to be used to enclose seeds from each eelgrass population. This was done to contain distinct populations from getting mixed up during our experiment which would cause error. On a bi-weekly basis we also had to wash out and care for the flowering shoot bags that were being held in sea-tables. The vegetative matter of flowering shoots in the sea-tables naturally rotted away during the seed ripening process, but this presented a potential issue as anoxic conditions promote seed germination in eelgrass plants. This is a natural strategy of eelgrass seeds as in the environment, once they are buried under sediment, they experience natural anoxia which is a chemical signal to germinate. Furthermore, we had to overhaul the previous experiment tank and pump system. This consisted of removing any last algal growth that remained on tank walls as well as tubs. We had to remake covers to go over each mesocosm tank that prevent terrestrial matter and detritus from interfering with our experiment. Lastly, we had to finally go through and separate and sieve all the seeds from the detritus contained in the flowering shoot bags (Figure 5). After separation of all matter except for seeds, we counted seed yield from each survey site location. Once we had our ripened and processed eelgrass seeds, we completed tetrazolium seed viability tests on subsamples of 20 seeds from each survey site location.

Winter 2024: Experiment 2

At the end of fall 2023 and beginning of winter 2024, we were starting our new experiment. We had successfully wrapped up the previous experiment, processed the seeds from our summer field work, overhauled the mesocosm tanks and pump system, created an experimental design for the new project, and ordered supplies for the new experiment. We were primarily studying the effects of heightened thermal regimes on seed germination and seed viability of perennial and annual eelgrass populations. We also decided that our new experiment would only raise the ambient sea water temperatures by 3˚C in treatment groups as opposed to the previous experiment which had ambient temperatures raised by 5˚C in treatment groups. This was done primarily because temperature increases of 5˚C is unlikely to be observed in the natural environment during a marine heat wave. Additionally, we had suspicions in the previous experiment that the prolonged increase of 5˚C over ambient sea water temperatures may have caused an unnecessary amount of stress for eelgrass plants in our mesocosms.

The process of starting the experiment was simple as much of the work had already been completed. All we had to do was assemble all the pieces together; label tubs and seed bags, put

Figure 5. SPMC laboratory eelgrass seed processing and sorting. Eelgrass seeds have been sieved and separated from flowering shoot detritus.

sediment in each tub, turn on the seawater pump and fill the mesocosm tanks with seawater, put the seed bags into each tub, put the tubs into their respective mesocosm tanks, turn on the hot water pump system, and our new experiment had begun. From here it would mostly be a waiting game. The tubs had seeds in them, but it would likely not be until spring till we would observe any germination in the tubs. In the future this new experiment will give us insights into how heat plays a role in seed germination and viability of annual and perennial eelgrass. Similarly to the last experiment, we may also use the data acquired from this experiment to assess which populations and life history strategies are best suited for restoration efforts in the future. Especially under the implications of climate change where conditions in the Salish Sea may be more stressful than historically due to increased frequency and intensity of marine heat waves.

Spring 2024: Data Analysis & Presentation

The past quarter has consisted mostly of data analysis and preparation for WWU's Scholars Week. Each member of the eelgrass crew chose a specific concentration for conference season and we utilized data we had collected over the past year to make these projects come to fruition. For my project I decided to focus on the site-specific development and yield of eelgrass seeds in Padilla Bay. I analyzed the data collected from the 3 field sites at Padilla Bay that we had surveyed throughout the summer. We had a plethora of data that we had not used from the summer season that tied well into the academic goals that I had established at the beginning of my internship.

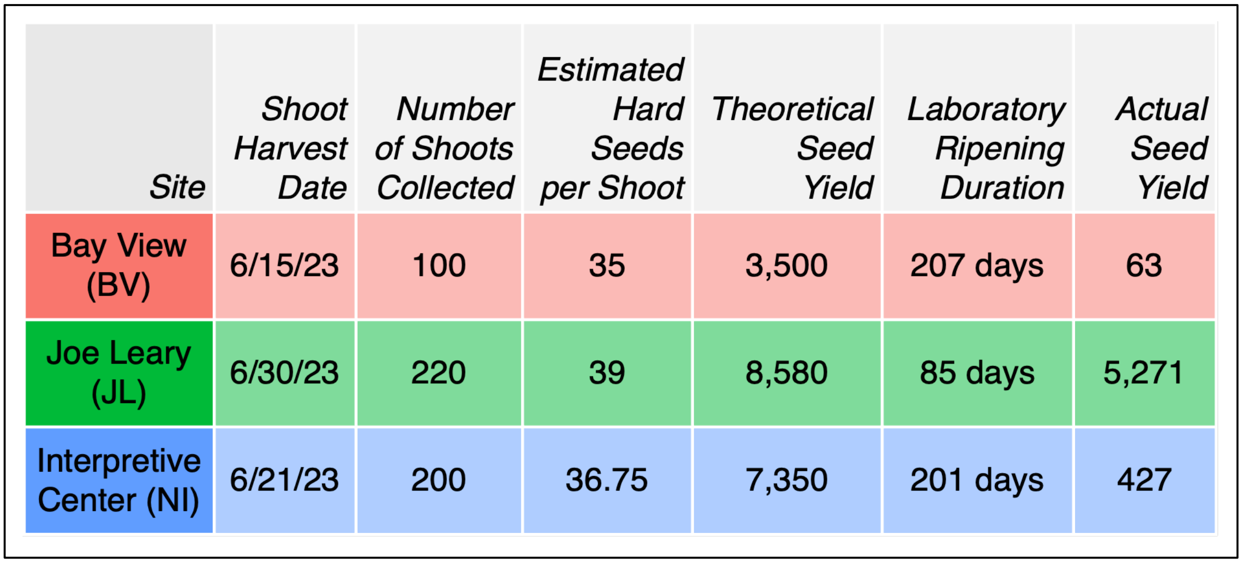
My topic focused on how seed yield may be maximized from donor sites in efforts of direct seeding restoration. My objectives were to assess seed yield at each site and identify the factors that influence seed yield. The factors I was most interested in analyzing were harvest timing, laboratory ripening of seeds, flowering shoot morphology, spathe maturation, seed development, and environmental conditions. I chose to only assess differences in perennial populations of eelgrass as JL was the only site that had both annual and perennial plants. I compiled data such as shoot harvest date, number of shoots collected, estimated hard seeds per shoot (collected in situ), laboratory ripening duration, actual seed yield, and extrapolated theoretical seed yield values from hard seed estimates (Table 1).

I utilized the software package RStudio to create various graphs and conduct statistical analyses on these data. I analyzed the differences in flowering shoot morphology at each site by plotting flowering shoot length and spathes per flowering shoot in respective boxplots. I ran a 1-way ANOVA on these data with site as the explanatory variable and flowering shoot length and spathes per flowering shoot as response variables (Figure 6). A Tukey Honest Significant Difference post-hoc test was executed as well. I created a bar graph depicting spathe maturation and seed development with the data of spathe proportions throughout the summer (Figure 7). Lastly, I assessed environmental conditions at each site by creating a standardized graph of mean deviation from average temperature at each site (Figure 8).

I found that there was a large source of bias in our data as flowering shoots were stored and seed were ripened for significantly different amounts of time that resulted in error in our data set. I suspect that BV and NI bags had gone anoxic during extended laboratory ripening time. Furthermore, variation in the data suggest that a maximum seed yield may be achieved with a better understanding of the site specific variables that influence development of seeds. These data suggest there is likely an optimal; duration of laboratory seed ripening, number of spathes per flowering shoot, duration of time between spathe categorization and harvest, and range of temperatures that maximize seed yield. Yet, higher resolution data is necessary to definitively conclude if variation in seed yield was affected by site-specific conditions. I concluded that assessment of seed germination and viability of collected seeds is necessary to validate any results. How seeds are affected by the storage and ripening process need to be studied further. Future studies would benefit from assessing morphology and spathe categorizations just before harvest to more accurately determine how these variables effect seed yield. Lastly, more data is needed to accurately predict how temperature effects morphology, spathe maturation, seed development, and seed yield. Since experiment 2 is currently underway, it is still unclear whether our efforts of flowering shoot harvest, seed ripening, seed processing, and direct seeding in our mesocosm tanks was successful.

While I was unfortunately unable to complete my poster presentation in time for the Pacific Estuarine Research Society annual conference, I was still able to attend and absorb the information presented there from studies that are being conducted across the Salish Sea. I did on the other hand have the great opportunity to present my poster at WWU's Scholars Week.

Table 1. Data from 2023 Padilla Bay perennial eelgrass meadow survey sites BV (red), JL (green), and NI (blue). Table includes flowering shoot harvest date, number of shoots collected from each survey site, in situ estimated number of hard seeds per shoot, theoretical seed yield extrapolated from estimated hard seeds per shoot, laboratory ripening duration length, and actual seed yield.



Site	*Shoot Harvest Date*	*Number of Shoots Collected*	*Estimated Hard Seeds per Shoot*	*Theoretical Seed Yield*	*Laboratory Ripening Duration*	*Actual Seed Yield*
Bay View (BV)	6/15/23	100	35	3,500	207 days	63
Joe Leary (JL)	6/30/23	220	39	8,580	85 days	5,271
Interpretive Center (NI)	6/21/23	200	36.75	7,350	201 days	427

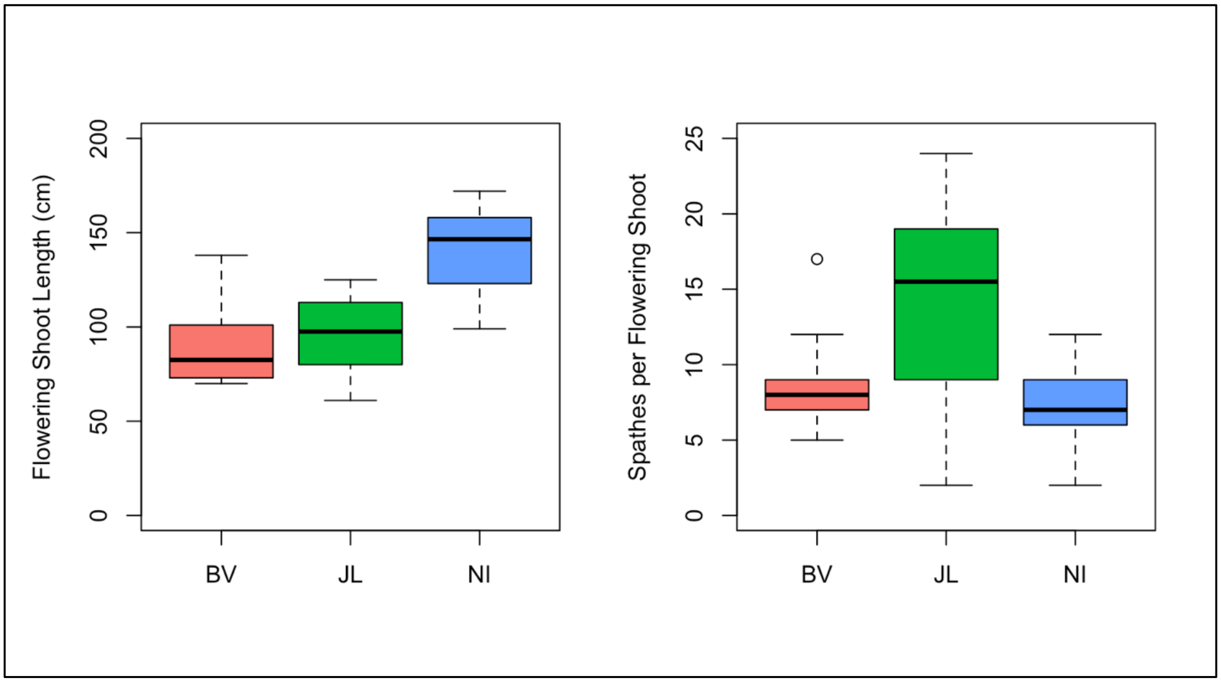


Figure 6. Flowering shoot morphology data from 2023 Padilla Bay perennial eelgrass meadow survey sites BV (red), JL (green), and NI (blue). Boxplots include flowering shoot length and spathes per flowering shoot.

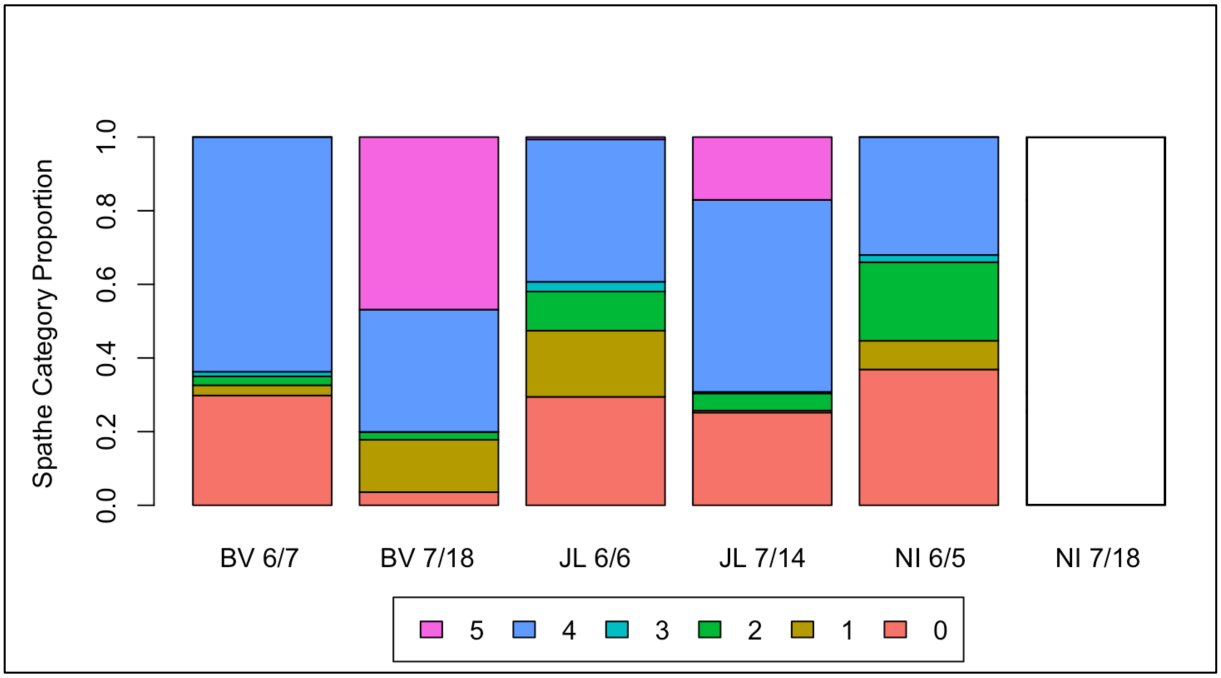


Figure 7. Spathe maturation and seed development data from 2023 Padilla Bay perennial eelgrass meadow survey sites. Surveys were completed between June and July 2023. Spathe categories are divided utilizing methods described in De Cock (1980). Categories are color coded by: (1) magenta, (2) blue, (3) cyan, (2) green, (1) orange, (0) red.

Figure 8. Environmental conditions from Padilla Bay eelgrass meadow survey sites BV (red), JL (green), and NI (blue).

Conclusion: Final Remarks

My research internship provided me the opportunity to implement skills that I acquired from courses at WWU's College of the Environment. I was able to research and assess the various important ecological roles that eelgrass meadows play in the Salish Sea. The first experiment we conducted helped me learn about the intricacies of different eelgrass populations and estuarine ecosystems. I was able to analyze how local conditions and different phenotypes of eelgrass populations differ throughout Padilla Bay through my research poster presentation. Throughout my internship I was able to assemble a greater picture of the environment and assess how climate change will impact eelgrass, marine ecosystems, and the biosphere as a whole. I was able to gain invaluable hands-on experience working on experiment upkeep and maintenance, field surveying, data collection, experimental design, data analysis, and scientific communication. My courses at WWU’s College of the Environment played an essential role in my ability to succeed in this position by properly preparing me with the foundational knowledge and skills to be a competent research assistant.

Literature Cited

Bohlmann, H., Apple, J. K., Burnett, N., Shull, S., & Salish Sea Ecosystem Conference (2018 : Seattle, Wash.). (n.d.). *Warm water temperature regimes in eelgrass beds (Z. marina and Z. japonica) of Padilla Bay, WA*. Digital content made available by University Archives, Heritage Resources, Western Libraries, Western Washington University.

De Cock, A.W.A.M. (1980). Flowering, pollination, and fruiting in Zostera marina L. *Aquatic Botany,* 9: 201-220. https://doi.org/10.1016/0304-3770(80)90023-6

Gaeckle, J., & Salish Sea Ecosystem Conference (2016 : Vancouver, B. C.). (n.d.). *Assessment of nutrient, metal, and organic contaminant concentrations in eelgrass (Zostera marina L.) in Puget Sound, WA (USA)*. Digital content made available by University Archives, Heritage Resources, Western Libraries, Western Washington University.

Gagnon, P.S., Vadas, R.L., Burdick, D.B., May, B. (1980). Genetic identity of annual and perennial forms of Zostera marina L. *Aquatic Botany,* 9: 157-162. https://doi.org/10.1016/0304-3770(80)90047-9

Katwijk, M. M., Thorhaug, A., Marbà, N., Orth, R. J., Duarte, C. M., Kendrick, G. A., Althuizen, I. H. J., Balestri, E., Bernard, G., Cambridge, M. L., Cunha, A., Durance, C., Giesen, W., Han, Q., Hosokawa, S., Kiswara, W., Komatsu, T., Lardicci, C., Lee, K., … Österblom, H. (2016). Global analysis of seagrass restoration: the importance of large-scale planting. *The Journal of Applied Ecology*, *53*(2), 567–578. https://doi.org/10.1111/1365-2664.12562

Knauth, L. P., & Kennedy, M. J. (2009). The late Precambrian greening of the Earth. *Nature (London)*, *460*(7256), 728–732. https://doi.org/10.1038/nature08213

O'Connor, M. I., & Salish Sea Ecosystem Conference (2016 : Vancouver, B. C.). (n.d.). *Biodiversity and food webs supported by BC eelgrass meadows*. Digital content made available by University Archives, Heritage Resources, Western Libraries, Western Washington University.

Short, F., & Salish Sea Ecosystem Conference (2014 : Seattle, Wash.). (n.d.). *Eelgrass Wasting Disease: an Overview*. Digital content made available by University Archives, Heritage Resources, Western Libraries, Western Washington University.

Shull, S., & Salish Sea Ecosystem Conference (2014 : Seattle, Wash.). (n.d.). *Distribution of Zostera japonica 2004 to 2013 in Padilla Bay, Washington*. Digital content made available by University Archives, Heritage Resources, Western Libraries, Western Washington University.

Yang, S., Wheat, E. E., Horwith, M. J., & Ruesink, J. L. (2013). Relative Impacts of Natural Stressors on Life History Traits Underlying Resilience of Intertidal Eelgrass (Zostera marina L.). *Estuaries and Coasts*, *36*(5), 1006–1013. https://doi.org/10.1007/s12237-013-9609-0