1. **Title Page (Proposal Cover Sheet**)

Project title: Land-sea metabolic coupling in temperate eelgrass beds: The role of watershed connectivity and environmental gradients on carbon sequestration of seagrass meadows

Reserve: Padilla Bay National Estuarine Research Reserve

Management need: Ecology of eelgrass in waters of the Salish Sea – What is the carbon storage and sequestration capacity of PNW eelgrass and how does this vary among eelgrass species, tidal elevation, and across different temporal scales (e.g. long term vs short term sequestration)?

Project period: August 1, 2020-July 31, 2022

Applicant:

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Student’s University and Degree Sought:

University of Washington

PhD

Anticipated Graduation Year:

2022

Faculty Advisor:

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Requested Funding:

Year 1: $60,000

Year 2: $55,836

1. **Statement of Interest**

To the National Oceanic and Atmospheric Administration,

I am writing to apply for the Margaret A. Davidson Graduate Fellowship to collaborate with the Padilla Bay National Estuarine Research Reserve (NERR). As a MS student in the School of Aquatic and Fishery Sciences at the University of Washington, my ultimate goal in attending graduate school is to prepare for a career as a research ecologist and to conduct research relevant to coastal management in the Pacific Northwest. The intent of my graduate work is to strengthen the depth with which we understand aquatic biogeochemistry and ecosystem function in the Puget Sound, while broadening connections across disciplines. I am excited to move forward in this endeavor and continue my graduate research as I bypass from a MS to a PhD student this winter. I feel strongly that a partnership with the Padilla Bay NERR would greatly improve my ability as a PhD student to conduct research that integrates scientific knowledge with policy and coastal management.

My interest in biogeochemistry began in the small streams and ponds of Rhode Island where I first studied watershed nitrogen cycling as an undergraduate. This interest has since expanded, first in the rivers of Vermont, and then in southwestern Greenland where I worked as a recent graduate studying the carbon cycle. My career as an ecologist and biogeochemist truly became defined, however, during my time at the Ecosystems Center in Woods Hole, Massachusetts. For close to three years, I worked studying estuarine biogeochemistry, specializing in climate and human-derived impacts to coastal ecosystem function and water quality as a collaborating scientist with the Waquoit Bay NERR. The culmination of my years at the Ecosystems Center and Waquoit Bay resulted in a wide-ranging skillset in research ecology and applied science. I managed multiple research projects that challenged me to think critically, while teaching me important skills in laboratory chemistry, scientific writing and data analysis. I successfully co-authored multiple manuscripts, with one as the first author. Additionally, through the applied aspects of my work, I engaged in regular collaboration with the stewardship and education programs at Waquoit Bay, an experience that sparked the interest for this application. Through this relationship, I learned the importance of advancing ecological knowledge through effective scientific partnerships.

Complementing my past research experience, this year I entered my third year of graduate school where I have focused on building my quantitative and interdisciplinary skills. My graduate coursework has emphasized the mastery of advanced statistical and data skills for ecological analysis, including generalized linear modeling, multivariate statistics, and process-based modeling. This newly training has challenged me to think critically as a data analyst and has provided with me a new toolbox with which to approach my work as a biogeochemist. This past year, I was also a fellow of the Northwest Climate Actionable Science Center (NW CASC). The NW CASC fellowship program focuses on equipping graduate students with tools to produce actionable science through cross-disciplinary partnerships. Through this, I learned the importance of matching scientific questions with on the ground applications. As I have described in my proposal, one of my goals for this project is to bring cutting-edge statistical analyses and modeling to the question of carbon transfer and ecosystem function in estuaries. The skills gained from my graduate coursework and the NW CASC Fellowship are directly transferrable to my proposed work and will support my ability to create accessible science that is both decision-relevant and statistically robust.

Looking forward in my career as an ecologist, I intend to conduct scientific research with clear objectives to improve coastal science and management. I feel strongly that the Margaret A. Davidson Graduate Fellowship will not only allow me to explore my interests in estuarine science but will also strengthen the trajectory of my professional development in the Pacific Northwest. Having recently moved to Washington state, I am just beginning to develop relationships with local agencies. A piece of Woods Hole that I loved so much was the integration of my research with the NERR System. I am excited for the potential to broaden my experience in estuarine science with Padilla Bay NERR, as I feel it would allow me to reconnect with the invaluable resource of the NERR system locally here in Washington.

Thank you for your consideration. I look forward to hearing from you.

Sincerely,

Elizabeth Elmstrom

1. **Project Summary**

Project Title: Land-sea metabolic coupling in temperate eelgrass beds: The role of watershed connectivity and environmental gradients on carbon sequestration of seagrass meadows

Reserve: Padilla Bay National Estuarine Research Reserve

Project Summary: Seagrass meadows form highly productive ecosystems, with important implications for ecosystem function and carbon cycling in coastal environments1–3. Due to their high rates of productivity and effective burial of organic matter, seagrasses are known for their capacity to store carbon in both their living biomass and within sediments below1,4,5. This has prompted growing scientific interest in seagrass meadows as ‘blue carbon’ sinks3,4,6–8. Studies of ecosystem metabolism, the balance of productivity and respiration, are a means to evaluate whether biological productivity within seagrass meadows represents a source or sink of carbon5,9–12. However, seagrass metabolism dynamics are not fully understood13. Net ecosystem metabolism – the balance of gross primary productivity and whole ecosystem respiration – differs across different estuaries based on local physical and biological conditions14. Individual species of seagrass can also vary in their photosynthetic efficiency, affecting rates of overall productivity15. Understanding of how seagrass metabolism is shaped by different biological and physical processes is key to developing fundamental principles of how ecosystem metabolic regimes are established, maintained, and ultimately support ecosystem services such as carbon sequestration1.

In this proposal, we address these questions in a group of estuaries in the Puget Sound of Washington state, a relatively understudied area in terms of seagrass metabolism4,8. The Skagit, Samish and Padilla Bay estuaries represent a natural gradient of watershed influence and generate predictable variation in biological and physical conditions, such as light, organic matter inputs, and species composition. Both native (*Z. marina*) and non-native (*Z. japonica*) seagrass species exist in the nearshore environment of the Puget Sound, and the distribution of the two shifts distinctly from high to low tidal elevations15. Within this setting, this project seeks to understand how seagrass metabolism varies with watershed connectivity and with local species and environmental gradients. We propose to apply a series of metabolic studies to elucidate the capacity of seagrass meadows to store carbon in the PNW. Ultimately, the proposed work seeks to address the management need: *What is the carbon storage and sequestration capacity of PNW eelgrass and how does this vary among eelgrass species, tidal elevation, and across different temporal scales (e.g. long term vs short term sequestration)?*

This project leverages a wealth of existing data from the National Estuarine Research Reserve (NERR) system and seeks to bolster collaborations between the University of Washington and the Padilla Bay NERR. To identify drivers of metabolism, the proposed work will study changes in DO, temperature, and irradiance within and across the Padilla, Samish and Skagit Bays. A Bayesian statistical model of diel oxygen in aquatic ecosystems will be applied to simultaneously estimate ecosystem metabolism9. The expectation is that 1) metabolism will vary with contrasting watershed influence due to differences in light, turbidity, and allochthonous inputs and 2) production rates will differ between *Z. japonica* and *Z. marina* across an elevational gradient due to increasing light limitations. Despite expected differences in species production rates, we expect overall net autotrophic conditions in Padilla Bay (little watershed influence). In Samish and Skagit Bays (moderate to high watershed influence), we expect overall net heterotrophic conditions. This will highlight the degree of watershed connectivity as an important driver of ecosystem metabolism and carbon sequestration in eelgrass meadows. This work is intended to benefit managers concerned with the carbon storage capacity of PNW eelgrass meadows through the creation of new metabolic data and modeling frameworks. An active collaboration with Padilla NERR and the Puget Sound Partnership will support the incorporation of new insights into estuarine, watershed and coastal management plans.

1. **Project Description**

**a. Problem Statement and Background Information:** Seagrass meadows are widely recognized as key ecosystems in the coastal zone, with important implications for marine ecosystem function and the global carbon cycle1–3. These foundational habitat-forming species develop highly productive ecosystems that support diverse and economically important food webs16,17. Expansive meadows in subtidal and intertidal areas create locally stable conditions, conducive to the deposition and burial of organic matter5,18,19. Due to their high rates of primary productivity and carbon burial, seagrasses are known for their capacity to store carbon in both their living biomass and within sediments below1,3. Their position at the land-sea interface allows seagrass ecosystems to receive inputs of nutrients and organic matter from terrestrial and oceanic sources14,20,21. The carbon storage of capacity of different seagrass meadows is likely to vary as a function of nutrients, sediment and organic carbon supply4,5, yet the overwhelming potential of seagrasses to store carbon has prompted growing scientific interest in the of seagrass meadows as ‘blue carbon’ sinks, coastal areas known for their contribution to global carbon sequestration3,4,7,8.

Central to understanding the carbon balance of seagrass meadows is determining whether carbon fixed within the ecosystem (autochthonous carbon) exceeds what is respired, indicating there is a pool of organic carbon that can be sequestered6. Aquatic ecosystem metabolism is the net effect of gross primary productivity (GPP) and ecosystem respiration (ER) in an ecosystem6,9,10. GPP is a fundamental metabolic rate that describes the total fixation of inorganic carbon (CO2, HCO3-) to organic carbon via photosynthesis10. In contrast, ER describes the total rate of mineralization of organic to inorganic carbon by all heterotrophic organisms (plants, animals, microbes)10. The difference of these two fluxes is net ecosystem productivity (NEP), with positive values of NEP indicating more carbon is being fixed than respired, providing conditions for a potential sink of autochthonous carbon as long as it is buried either within the meadow or elsewhere for a sufficiently long period of time6. Alternatively, when mineralization exceeds carbon fixation and NEP is negative, the ecosystem is either receiving external carbon inputs or respiring stored organic carbon10. The net import or export of carbon from seagrass meadows complicates the evaluation of carbon budgets based on NEP alone6,22. If carbon is exported as nearshore ‘wrack’ (dead biomass) or dissolved organic carbon (DOC) elsewhere, its fate is crucial to determine whether the meadow can be considered a carbon sink6,22.

Observation and experimental studies of how seagrass metabolism is shaped by biological and physical processes is key to developing fundamental principles of how ecosystem metabolic regimes are established, maintained, and ultimately support ecosystem services such as carbon sequestration14,23. Seasonal variations in light, temperature, and nutrients drive fluctuations in GPP, while nutrients and organic matter inputs from the terrestrial or marine environment can contribute significantly to ER14,20,24. Individual species of seagrass can also vary in their photosynthetic efficiency under varying environmental conditions, affecting rates of NEP15. The combined effects of these biologic parameters differ across ecosystems with varying physical characteristics such as depth, tidal forcing, and degree of watershed connectivity5,14,25. Even within a single ecosystem, metabolism rates vary spatially and temporally with irradiance, depth, and species composition or density6. Ultimately, the magnitude of primary production and respiration varies widely across latitudes, within habitats from deep to shallow, and on multiple timescales (daily, seasonally, annually), complicating estimates of the role of seagrass meadows in the global carbon budget8.

Untangling the biotic and abiotic factors that influence GPP and ER in seagrass meadows has been the focus of previous studies, mostly in Australian or Mediterranean meadows of *Posidonia* species8. In the temperate region of the Pacific Northwest (PNW), metabolic studies on the local seagrasses *Zostera marina* (native eelgrass) and *Zostera japonica* (introduced eelgrass) are distinctly lacking4. Estuarine ecosystems in the Pacific Northwest are diverse in physical structure and hydrology, and include fjords (e.g., Puget Sound), broad embayments with little riverine influence (e.g., Padilla Bay), moderate river influence (e.g., Samish Bay), and river-dominated systems (e.g., Skagit River estuary)26. Previous work suggests the carbon sequestration in Padilla Bay is low, but to understand the relative contribution of autotrophy vs. heterotrophy of eelgrass species in PNW estuaries, it is important to gain metabolic data from several ecosystem types26.

PNW seagrass ecosystems are relatively understudied when compared to the literature4,8,27. Furthermore, while trends in overall eelgrass abundance appear stable, certain areas in the Puget Sound have experienced noticeable declines since the year 200028. Scientists and environmental planners in the PNW are currently seeking indicators and monitoring tools to help them to better understand and manage their estuaries to promote the stability of eelgrass habitat29. Eelgrass is a critical component of the Puget Sound ecosystem, creating productive habitat for juvenile salmon, Dungeness, crabs and numerous other species as well as stabilizing the seafloor, filtering nearshore waters and contributing to the food web30,31. Recognizing the importance of eelgrass to Puget Sound, the Puget Sound Partnership established eelgrass as a “Vital Sign” for assessing the status and health of the Sound and identified eelgrass habitat as a primary indicator of ecosystem recovery28,29. A 20% increase in eelgrass area by the year 2020 is one of the PSP’s goals to improve the health of the Sound28,29.

Within this setting, this project seeks to understand how seagrass ecosystem metabolism differs across north Puget Sound estuaries as a means to better understand ecosystem productivity and the carbon storage capacity of eelgrass meadows in the PNW. We first propose a cross-system comparison of Padilla, Samish, and Skagit Bay estuaries to understand how varying degrees of watershed connectivity affect rates of GPP, ER, and NEP. We then seek to explore variance across species (native vs. non-native) and environmental gradients (light and depth) within Padilla Bay. This project leverages a wealth of existing data from the National Estuary Research Reserve (NERR) system and seeks to bolster collaborations between the School of Aquatic and Fishery Sciences at the University of Washington and the Padilla Bay NERR.

**b. Project Approach:**

***Research Questions and Objectives:***

The key objective of this research is to improve our understanding of the carbon fixation and sequestration capacity of PNW eelgrass through a study of net ecosystem metabolism across estuaries with varying degrees of riverine/marine influence. Seasonal measurements of ecosystem metabolic rates (GPP, ER, NEP) in Padilla (most marine-influenced), Samish, and Skagit (most river-influenced) bays will elucidate: 1) the magnitude and ecosystem drivers of eelgrass carbon fixation (GPP) and whole ecosystem carbon mineralization (ER), and 2) whether there is a net carbon gain through *in situ* productivity exceeding *in situ* respiration. Ultimately, this will provide critical new information on how eelgrass meadows fit into regional carbon cycle assessments of Puget Sound. This goal will be realized through two separate, but linked project questions and activities described below.

**R1)** This research element aims to understand how eelgrass metabolism differs across Puget Sound estuaries with varying degrees of marine/watershed connectivity. Diel oxygen measurements and ecosystem metabolic modeling in Padilla, Samish, and Skagit Bay eelgrass meadows will reveal cross-system differences in GPP, ER, and NEP, seasonally, annually, and across years. The initial focus will be to quantify whether each system is net autotrophic or net heterotrophic in order to identify whether each system is a relative carbon sink or source to the atmosphere. Subsequent regression analyses will elucidate the key biological and physical drivers (e.g., allochthonous inputs, light, turbidity, hydrology/tidal inputs, and vegetation) that define the net balance of GPP, ER, and NEP across different systems of eelgrass meadows specific to the Salish Sea. The expectation is that allochthonous inputs and light will be the primary controls of NEP, and this effect will be variable across systems, seasons, and hydrologic years. Specific hypotheses are:

**H1)** Metabolic regime will vary with contrasting watershed influence characteristics due to differences in light, turbidity, and allochthonous inputs, specifically sediment supply. Sediment inputs will decrease GPP relative to ER, while GPP rates will increase in sediment starved areas.

**H2)** In terrestrially-unconnected systems, I expect average rates of GPP and ER to be low, but *slightly* positive NEP (GPP > ER). In terrestrially-connected systems, I expect low rates of GPP, high rates of ER, and negative NEP. Low magnitudes of NEP in terrestrially-unconnected systems will highlight controls of watershed inputs on the carbon storage capacity of seagrass meadows.

**R2)** Specific to Padilla Bay, this second research element seeks to identify how seagrass community metabolism may shift with respect to species and environmental gradients. Metabolic measurements from stations placed along established vegetation monitoring transects that form elevational and species gradients within Padilla Bay will capture variance in GPP, ER, and NEP with light and depth, *and* among native vs. non-native species. Metabolic rates quantified outside of the reserve’s established vegetation monitoring transects will span the upper and lower limits of the seagrass depth distribution of the meadow. This captures a transition from non-native (*Z. japonica*) to native (*Z. marina*) eelgrass species4. Comparisons of GPP/ER to existing biomass estimates will elucidate controls of light and tidal elevation on eelgrass productivity. Photosynthesis to irradiance relationships of the two species (P-I curves) will provide measurements of species-specific productivity rates, allowing us to infer whether one species has greater capacity to store carbon than the other. The expectation is that elevation will govern metabolic rates in seagrass species, due to the control of depth on light attenuation. Specific hypotheses are:

**H1)** Rates of eelgrass metabolism will be strongly correlated with estimates of biomass, and there will be an apparent shift to lower rates of both GPP and ER with increasing tidal depths, due to the effect of lower light attenuation on eelgrass growth. Rates of GPP will be high in areas of high eelgrass density.

**H2)** The magnitude of GPP and ER will be different between the two eelgrass species. This will be a factor of both location along the elevation gradient, and differences in photosynthetic capacity. Photosynthetic rates will be higher in *Z. japonica* than *Z. marina*. However, this will be met with lower rates of ER in *Z. marina*, due to minimal subsidies of organic matter. This suggests carbon storage capacities between the two species will be comparable.

***Study Design:***

*Study Sites:* The Padilla Bay NERR in Washington state offers a unique opportunity to study drivers of metabolism due to existing research infrastructure, years of data, and its location in proximity to three estuaries of varying degrees of watershed connectivity (Padilla, Samish and Skagit Bays). Located in the northern reaches of greater Puget Sound, Padilla Bay estuary is a shallow, inactive delta, isolated from riverine influence4,26. In the absence of freshwater inputs, the eelgrass meadow has thrived, and Padilla Bay currently supports the largest eelgrass meadows in the lower United States4. The Samish Bay estuary is a slightly smaller bay, located north of Padilla Bay. Samish Bay is moderately influenced by the 40-km Samish River which has an average annual discharge of 246 ft³/s32. Skagit Bay is located south of Padilla Bay and receives freshwater inputs from the Skagit River, the third largest river that drains into the Salish Sea. At 240-km with an average annual discharge of 16,570 ft³/s, inputs from the Skagit River highly influence the marine environment33. Eelgrass beds grow on tidal flats in both Samish and Skagit Bays28.

*Ecosystem Metabolism Methods:* In aquatic systems, time series of dissolved oxygen (DO) have been widely used to compute estimates of ecosystem metabolism because it is directly related to GPP and ER1,6,9,11–13,34,35. Measurements of whole-ecosystem GPP, ER, and NEP using diel O2 data integrate all aerobic organisms (autotrophs, heterotrophs) and habitats (benthic, planktonic, and hyporheic zones) that contribute to the ﬁxation, transformation, and availability of organic matter10. More recently, dual (16O, 18O) stable isotope measurements of dissolved oxygen have been used as secondary and independent natural tracers of metabolic processes in ocean and large-river ecosystems that approximate steady-state conditions9,10,36,37. A key assumption of the open-water method is that the DO time series is a Lagrangian speciﬁcation of the ﬂow ﬁeld (characterizes a moving parcel of water)9,11,34. However, most DO time series are collected at ﬁxed locations and changes in DO are assumed to reﬂect metabolism with minimal effects of tidal mixing11. In estuarine systems, a weighted regression approach can create dynamic predictions of DO as a function of time and tidal height, which are then used to ﬁlter, or de-tide, the DO signal11. The idea is based on the recognition that daily ﬂuctuations in DO caused by metabolism are associated with the solar cycle, whereas other ﬂuctuations in estuaries are likely associated with cyclical water movements that do not have the same period or phase as the light and dark hours of the day11.

*R1 Sampling approach:* While previous research suggests carbon storage is low in Puget Sound estuaries4, ER, GPP, and NEP rates, and their respective variability among and within locations, are not yet well quantified. For Padilla Bay, I will leverage existing data collection from the NERR System-wide Monitoring Program (SWMP). YSI data sondes from continuous water quality monitoring stations will provide long-term oxygen data and temperature measurements from the reserve’s eelgrass meadow. The reserve also has a weather station on site that will provide measurements of incident light for calculations of irradiance. Additional high-resolution, low-cost dissolved oxygen sensors will be deployed in Samish and Skagit Bay eelgrass stands at sites with conditions comparable to those in Padilla Bay. By matching the existing experimental design from Padilla Bay, I can leverage existing data from the SWMP monitoring water quality station with newly derived oxygen data from Skagit and Samish Bays to compare metabolism across the three ecosystems. Data from each estuary will be supplemented with hourly sampling for dissolved oxygen isotopes, and O2 + Argon concentration, taken four times over a 24-hour period during fall, winter, spring, and summer seasons.

*R2 Sampling Approach:* Measurements across depth in Padilla Bay will be taken during the annual biomass maximum to correspond with the reserve’s ongoing vegetation monitoring sampling, which takes place annually during the summer months. Metabolic rates will be quantified outside of the reserve’s established vegetation monitoring transects (n=3), located in the northern portion of Padilla Bay. These transects include six zones based on local topography and dominant vegetation characteristics. The zones are categorized as bare, *Z. japonica*, mixed *Z. japonica*, mixed *Z. marina*, *Z. marina*, and subtidal *Z. marina*. Each transects spans the upper, middle, and lower limits of seagrass depth distribution in the meadow. Daily diel DO will be measured *in situ* in each zone as will temperature and irradiance using sensors described above. Existing biomass estimates from the reserve’s vegetational monitoring plots will be linked to estimates of productivity using regression analyses. A comparison of photosynthetic efficiency between *Z. marina* and *Z. japonica* will provide measurements of species-specific productivity rates and insight into how this biological invasion has altered ecosystem function.

*Ecosystem Metabolism Modeling* *and Data Analysis*: Dissolved oxygen concentration and stable isotope data will be analyzed by a version of the Bayesian Metabolic Model that will be extended to incorporate the effect of tidal advection; creation of this model will be a significant work product. Using the daily-scale data, I will compare the diel balance of GPP and ER across estuarine replicates to understand how metabolic rates differ on a system vs. system scale. The slope of the photosynthesis–irradiance curve is a measure of photosynthetic efficiency that can be derived from DO time-series data using inverse modeling techniques9. On an individual study site basis, I will look for seasonal shifts across systems using dynamic factor analysis, a likelihood-based multivariate timeseries methodology. I will then use principal components analyses (PCAs) to summarize variation among estuaries in their chemical and physical conditions. To evaluate the sensitivity of net seasonal metabolic rates to different controls, I will use linear mixed effects modeling, with the PCA axes as fixed effects and a random effect of estuary. PCA axes will be used to evaluate how variation among metabolic rates is explained by composite variation in estuary characteristics.

**c. Expected Outcomes:** The expected outcomes of this work include: 1) generation of new continuous DO data within the Padilla Bay NERR and nearby eelgrass ecosystems, 2) creation of a dynamic Bayesian model of diel DO dynamics for calculating estuarine ecosystem metabolism, 3) regular updates of progress and results to Padilla Bay NERR to inform specific management priorities regarding carbon sequestration in the PNW, 4) the development of K-12 educations modules and undergraduate mentorship training in estuarine science, and 5) the development of collaborative links among scientists, managers, and educators across a broad range of disciplines through a series of outreach and feedback sessions. The project will be evaluated for success through the following metrics: 1) At least one full year of continuous DO data suitable for metabolism modeling from Padilla, Samish, and Skagit Bays; 2) A generalizable Bayesian metabolism model for estuaries programmed in R and available on GitHub; 3) Outreach and feedback sessions with the Padilla Bay NERR; and 4) The successful integration of education modules into the reserve’s education program.

This work will have direct relevance to managers such as the Padilla Bay NERR, the Washington DNR, and the Puget Sound Partnership (PSP) who are tasked with creating strategies to preserve seagrass ecosystem function and their ecosystem services in the PNW. Formal semiannual meetings with the Padilla Bay NERR staff will facilitate the delivery of results and will provide a platform to gain feedback on how research can best inform the reserve’s management plans. Training sessions on ecosystem metabolism models with Padilla Bay NERR staff/students will bolster future efforts in metabolic research at the reserve. Understanding the connection between watershed inputs and eelgrass ecosystem function in native and non-native species will directly inform the PSP and WA DNR’s restoration goals for eelgrass in the Puget Sound by highlighting downstream effects in watershed planning. Elmstrom will host an outreach and feedback session at the Washington Department of Ecology, where she has previous collaborations from her Northwest Climate Actionable Science Center Fellowship. This will provide a yet another avenue for cross-disciplinary collaboration from scientists to state agencies.

Direct mentorship of students from the University of Washington by Elmstrom will provide field research, analytical, and authorship training and opportunities to young scientists. Elmstrom has successfully mentored 10 undergraduates through a series of independent research projects and will offer this experience to engage students in ecological research at the Padilla Bay NERR. Additionally, Elmstrom will customize lessons and a project-based learning activity that demonstrates the basic concepts of photosynthesis and respiration in seagrass meadows as an introduction into ecosystem metabolism, targeted for use as a STEM based learning module. Throughout this process, Elmstrom will work closely with the stewardship and education coordinators at Padilla Bay for feedback and evaluation of education modules. Once complete, these lessons will be offered for integration into the reserve’s education program. This reusable lesson kit will also be presented to visiting students at Padilla Bay and at UW’s Aquatic Science Open House, an annual event geared towards K-12 students and their families. Through Padilla Bay’s existing stewardship and education program, Elmstrom will apply her skills as a collaborator, mentor, and ecologist to strengthen the ties between scientific research, education, and natural resource management.

**d. Milestone Schedule: Year 1 Year2**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *Research Activities:* | F | W | S | S | F | W | S | S |
| Calibrate and (re)deploy sensors | X | X | X | X | X | X | X | X |
| Diel oxygen sampling | X | X | X | X | X | X | X | X |
| Build statistical models |  | X | X |  |  | X | X |  |
| Submission of publication for peer review. |  |  |  |  |  |  |  | X |
| Padilla Bay research/management meetings | X |  | X |  | X |  | X |  |
| *Education components:* |  |  |  |  |  |  |  |  |
| Mentorship of two undergraduates. |  |  | X | X | X | X | X | X |
| Creation of education lesson plans. |  |  |  |  |  |  | X | X |
| UW’s Aquatic Science Open House |  |  | X |  |  |  | X |  |
| Stewardship and education meetings | X |  | X |  | X |  | X |  |
| *Dissemination of results:* |  |  |  |  |  |  |  |  |
| WA Ecology, Puget Sound Partnerships Presentations |  |  |  |  |  | X | X |  |
| Present results at professional research conference |  |  |  | X |  |  | X |  |
| NERRS/NERRA Annual meeting | X |  |  |  |  |  |  |  |

1. **Budget Narrative and Justification**

***Salaries***

Twelve months of graduate student stipend is requested for year one ($2,650/month; $32,314 total). In year two, we are requesting nine months of graduate student stipend for a total $24,720. This includes an anticipated 2% increase in student stipend rates. The student, Elizabeth Elmstrom, is a current Master’s student in the University of Washington School of Aquatic and Fishery Sciences (UW SAFS) and is on track to bypass in the PhD program in academic year 2019/20. The activities proposed herein will constitute a significant part of her PhD dissertation. PI Holtgrieve will not receive salary.

***Fringe Benefits***

Fringe benefit rates in 2019 are 27.1% for graduate student fellows. Fringe benefits in year 1 are $8,757 and $6,699 in year 2. Rates are unchanged over the two years of the project as the UW does not provide estimates of future fringe benefit rates for budgeting.

***Equipment***

None

***Travel***

In year one, a total of $714 of domestic travel funds ($400 airfare, $150 per diem, $164 lodging) are requested to support student travel. Year two travel funds are requested at $7,000 ($3,500 airfare, $1,500 per diem, $2,000 lodging), for a project total of $7,714. We anticipate this will be sufficient to cover student attendance at the required meetings, particularly since the NERRS/NERRA Annual meeting is being held in Seattle and thus will not require significant travel support. In the event there is a shortfall in either a given year or in total, the difference will be cover by a combination of UW travel awards or discretionary funds form the PI.

***Other Direct Costs***

*Graduate Operating Fees*

Graduate Operating Fees (i.e., tuition less student and technology fees) at the University of Washington are currently $18,215 for four quarters each year ($5,266 per academic quarter; $1,550 per summer quarter). This is an integral part of UW graduate student support and is required by University of Washington. This fee is currently projected to increase 5% annually for a total of $35,632 over the two years of the project.

***Total Direct Costs***

Direct costs for the project are $60,000 in year 1 and $55,836 in year 2, for a project total of $115,836.

***Indirect Costs***

This proposal is not subject to indirect cost recovery.

1. Appendices

**References**

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**Elizabeth Elmstrom**

Curriculum Vitae

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University of Washington, Seattle, Washington 98105 elmstrom@uw.edu

**Education**

**M.S. University of Washington**, Seattle, Washington

*August 2017 – Present*

School of Aquatic and Fishery Sciences

**B.S., University of Vermont,** Burlington, Vermont

*August 2009 – May 2013*

Rubenstein School of Environment and Natural Resources

Environmental Science: Concentration in Water Resources

**Research Experience**

**Masters Student,** School of Aquatic and Fishery Sciences, University of Washington

Seattle, Washington September 2017 – present

Project:  Nitrogen dynamics in rivers of the Puget Sound watersheds

* Researched sources and fates of nitrate in rivers and receiving coastal waters, finding seasonal shifts in magnitudes and drivers with hydrology
* Mentored two undergraduates; Teaching assistant: Limnology, Fall 2019
* Approval of bypass application from MS to PhD by supervisory committee, Winter 2020

**Research Assistant II,** The Ecosystems Center, Marine Biological Laboratory

Woods Hole, Massachusetts *November 2014-August 2017*

* Primary research assistant to distinguished scientist, Dr. Ivan Valiela. Aided in the management of multiple long-term research projects in the coastal estuaries and salt marshes of Cape Cod and in the mangrove swamps of Trinidad and Panama.
* Conducted field work, laboratory chemical analysis, and data analysis, including statistical analysis and ecosystem modeling. Supervised undergraduate students in the field and laboratory.
* Worked closely with Dr. Valiela to produce scientific publications, academic texts, and grant proposals for continuing work.

**Research Assistant I,** Pennsylvania State University, Eissenstat Root Ecology and Eric Post Laboratories   
Kangerlussuaq, Greenland *May-August 2014*

* Gathered data to support a long-term warming experiment examining the effects of climate change on Arctic tundra plant phenology and net ecosystem CO2 exchange in southwestern Greenland.
* Photographed Arctic tundra plant roots, measured and kept a detailed record of weekly carbon flux from soils, soil moisture, soil temperature, and leaf area index.

**Research Assistant I,** University of Vermont, Bowden Watershed Research Laboratory

Burlington, Vermont *May-October 2013*

* Provided assistance on a project to calibrate an Acoustic Doppler Current Profiler against manual total suspended sediment samples from an automated water sampler (ISCO).
* Collected and processed stormwater samples for total dissolved phosphorus, particulate phosphorus, total suspended solids and particle size distribution analysis.

**NSF EPSCoR SURF Research Assistant,** University of Rhode Island, Watershed Hydrology Laboratory

Kingston, Rhode Island *May-August 2011*

* Assisted graduate students and staff on all stream and pond projects related to watershed nitrogen cycling. Gathered, prepped and analyzed water samples for nitrogen content. Collected sediment cores and used mesocosm studies to extract and analyze gases.
* Conducted an independent research project examining biogeochemical cycling and greenhouse gas emissions within aquatic anaerobic sediments.

**Publications**

**Elmstrom, E.**, G. Holtgrieve, K. Leazer, and A. Schrauer. *Manuscript in Prep*. The climate sensitivity of water quality in the Pacific Northwest: Linking anticipated shifts in hydrologic regime to riverine nitrogen sources in Puget Sound watersheds.

Valiela, I., R. Juman, H. Asmath, D. Hanacek, J. Lloret, E. **Elmstrom**, K. Chenoweth, and E.N.J.

Brookshire. *Submitted*. Water quality, nutrients and stable isotopic signatures of particulates and vegetation in Caroni Swamp, Trinidad, a mangrove ecosystem exposed to past anthropogenic perturbations. *Estuaries and Coasts*.

**Elmstrom**, E., I. Valiela, and S. Fox. 2018. Watershed-estuary coupling in Pacific Panama: Isotopic evidence of forest and pasture land covers in mangrove estuary suspended particulate matter. *Estuaries and Coasts* 1-9*.*

Valiela, I., E. **Elmstrom**, J. Lloret, T. Stone, and L. Camilli. 2018. Tropical land-sea couplings: Role of watershed deforestation, mangrove estuary processing, and marine inputs on N fluxes in coastal Pacific Panama. *Science of the Total Environment* 630, 126-140*.*

Martin, R.M, C. Wigand, E. **Elmstrom**, J. Lloret, and I. Valiela. 2018. Long-term nutrient addition increases respiration and nitrous oxide emissions in a New England salt marsh. *Ecology and Evolution* 8, 4958-4966.

Valiela, I. J. Lloret, T. Bowyer, S. Miner, D. Remsen, E. **Elmstrom**, C. Coggswell, and R. Theiler. 2018. Transient coastal landscapes: Rising sea level threatens salt marshes. *Science of the Total Environment* 640-641, 1148-1156*.*

Valiela, I., C. Owens, E. **Elmstrom**, and J. Lloret. 2016. Eutrophication of Cape Cod estuaries: Effect of decadal changes in global-driven atmospheric and local-scale wastewater nutrient loads. *Marine Pollution Bulletin* 110, 309-315.

**Research Fellowships**

Northwest Climate Adaptation Science Center Graduate Fellowship 2018-2019

School of Aquatics and Fishery Sciences Graduate Research Fellowship 2017-2018

**Scientific Presentations**

Northwest Climate Conference, Portland Oregon, “The climate sensitivity of water quality: Linking climate, hydrology, and nitrogen sources in Puget Sound rivers”, October 2019

Vermont EPSCoR Student Research Symposium, Saint Michael’s College, Colchester, Vermont, “Beaver ponds as potential sources of greenhouse gas emissions”, Spring 2012

Rhode Island Summer Undergraduate Research Fellows Symposium, University of Rhode Island, Kingston, Rhode Island “Beaver ponds as potential sources of greenhouse gas emissions”, Summer 2011

**Data Sharing Plan**

***Types of Data and Other Products***

*Stable Isotope Data*: The Thermo Finnigan and Picarro mass spectrometer software creates methodological, configuration, and raw data files. Analysis of samples also creates comma delimited text data files with summarized results from the raw data. These files are post-processed (reduced) to account for working standards, instrument drift, and error checking. The reduced data outputs are summary tab delimited text data files via Matlab and R script files. Each mass spectrometer produces approximately 200 kB of data per day.

*Sensor Data*: All sensors for this project log data internally. These data are transferred from the instruments to Holtgrieve Ecosystem Ecology Lab (HEEL) computers as text files (see below regarding archiving). At time of transfer, HEEL students and employees are required to fill out standardized hardcopy forms that documents critical metadata including site information (latitude, longitude, site names, channel location, etc.), deployment information (start and end times), personnel, and details about accompanying data collections. Sensors data for this project is estimated to be < 1 GB in total.

*Hardcopy Data*: Hardcopy data include field notebooks, lab data sheets/notebooks, diagrams and plans for instrument or peripheral configurations will also be produced.

*Model Code, Results, and Project Outputs*: Data analysis and models will be constructed in the open source statistical programming language R using simple text files.  HEEL project members frequently work on projects through the online version repository GitHub, which facilitates both documentation and sharing of computer code. Final outputs from the data analysis and modeling will be archived as a combination of text (both .txt and .csv), image, and word processing files.

***Data and Metadata Standards***

The summarized output files of stable isotope data created from Δ\*IsoLab and UW CSIA Matlab and R scripts are designed to be a stand-alone file containing all appropriate reference material information and descriptions that adheres to the guidelines put forth by the Commission on Isotopic Abundances and Atomic Weights. Summarized data files include full metadata including analytical uncertainties, analytical techniques, analysis dates, instrument settings and sample descriptions that are required in peer-reviewed, international journals for publication.

Metadata related to field collections, modeling, and data analysis outputs will follow detailed guidelines developed for the HEEL that are based on USGS metadata standards. As condition of employment, all HEEL students and employees are required to follow metadata and data archive standards documented in detail and routinely updated on the HEEL intranet. Metadata information is designed such that the data can be understood, reused, and integrated with other datasets and must include the following four components:

1. *Workflow Capture*– A formal description of how the data have been processed to get to the current state, which includes a description of the researcher's method for experimental data.
2. *Data Dictionary* – A repository of information which defines and describes the data resource with the goal of making it useable by someone unfamiliar with its collection.
3. *Data Citation* – A suggested way this data set should be cited going forward including reference to other data sets incorporated into the current dataset.
4. *Access Controls* –  Defines who “owns” the data and allowable uses for the data.

***Data Archiving and Preservation of Access***

Both the Δ\*IsoLab and UW CSIA lab have a system of incremental backups of daily data changes, including electronic metadata, performed using a network-based backup service to backup databases and workstations for the purpose of protecting against the loss or corruption of individual files. In the Δ\*IsoLab, data and electronic metadata are deposited for long-term storage via the UW archival system called the *lolo* Archive. Similarly, all computers within the UW CSIA facility and HEEL are dynamically backed up in real time to an 8 TB Network Attached Storage (NAS) system (Synology 16 Bay RackStation RS4017xs+) running RAID 6 doubly parity. RAID 6 allows for two disk failures within the RAID set before any data is lost. This system is backed up to an identical system situated in a separate on-campus location four times daily. Δ\*IsoLab, UW CSIA Lab and the HEEL therefore maintain, in pertpertuity, all the raw data neecesary to vailadate and reanalyze specific results.

The HEEL has established a strict protocol for data management based on standards set forth by the USGS. All students and employees are required to follow these protocols as a condition of their employment. In particular, all HEEL project data must be 1) adequately described via metadata, 2) managed for data quality, 3) backed up in a secure manner, and 4) archived in an easily reproducible format. Specifically, this means that each member of the project team is required to do the following:

* Save and curate all data generated during their work in an easily readable/understandable format.  All sensors and instruments mush be initially downloaded to one the HEEL computers, which are automatically integrated into the HEEL data backup system.
* All HEEL project data must have, from the outset, accompanying metadata that includes at a minimum the four components given above: workflow, data dictionary, data citation, and data access.  Metadata should be in a text file or similarly readable format.
* Data in hard copy form must be scanned into digital form and curated along with any associated digital data.  Hard copies should remain in the HEEL.
* All personnel computers for HEEL members that contain project data must be backed up on a daily basis and data stored in a least two findable locations.
* At the completion of a project, the researcher must upload to the HEEL NAS system a final project folder that includes, at a minimum, compiled and generated data, metadata, computer code, manuscripts, and any visualizations.

***Dissemination Methods***

The primary method of dissemination of our results and data will be through peer-reviewed journals.  Whenever possible, the primary data used in analyses and accompanying model code will be published with the manuscript on the journal’s website. A secondary means of disseminating data will be through FigShare. FigShare is a digital repository where researchers can preserve and share their research outputs, including figures, datasets, images, and videos, in adherence to the principles of open data. FigShare is advantageous because data and results can be assigned DOIs for permanent and efficient searching and identification. For completed projects not under embargo (see below), data will be directly available for download; requests can be made to the PI for embargoed data.

***Policies for Data Access, Sharing, Re-Use, or Re-distribution***

Sharing of data and samples will follow the NSF policy on data sharing as described in the Grant Proposal Guide as well as the guidelines outlined in points 1 through 6 of the NSF-EAR memo of September 2010. Data from this project will remain under embargo (i.e., within the research group) until publication or upon one year of the grant period end date, whichever is first. Access to the data by outside groups prior to the end of the embargo period will be considered individually upon request. Such requests will be granted unless they directly overlap with the goals of the proposed project.

***Roles and Responsibilities***

Andrew Schauer has responsibility for data management of *Δ\*IsoLab* generated data (i.e., raw results and instrument configurations). Terry Rolfe has the same responsibility for UW CSIA facility. Elmstrom is ultimately responsible for managing compiled data sets, model code, and project results, however specific tasks are delegated to project students and staff as defined in the HEEL Data Management Protocol described above. Elmstrom will be the primary point of contact regarding data access and sharing.

**Facilities, Equipment and Other Resources**

***University of Washington and the College of the Environment***

The University of Washington (UW) is a Tier 1 research university and the flagship higher education institution for the state of Washington. College of the Environment (CoEnv) is the hub of environmental research, education, and outreach across the UW and will serve as the administrative home for this project. The CoEnv is an engine of scholarship, innovation, and education to advance our understanding of the environment and our interactions with it. The CoEnv includes six Schools and Departments (Aquatic & Fishery Sciences, Atmospheric Sciences, Earth & Space Sciences, Environmental & Forest Sciences, Marine & Environmental Affairs, and Oceanography), the Program on the Environment, and multiple research institutes and labs (Joint Institute for the Study of the Atmosphere and Ocean, Friday Harbor Labs, Quaternary Research Center, Climate Impacts Group). There are strong interdisciplinary connections among units within the CoEnv; particularly relevant to this proposal is the 15 years of collaboration between PI Holtgrieve in Aquatic and Fishery Sciences and faculty in the School of Oceanography.

The CoEnv is fully staffed with administrative personnel to administer and coordinate the activities of this proposal, including purchasing, personnel, billing, and reporting. Multiple undergraduate courses, including directed research, provides a means for student involvement in research associated with this project.

***School of Aquatic and Fishery Sciences***

The School of Aquatic and Fishery Sciences (SAFS) is the premier inland aquatic sciences research entity at the UW with both Master’s and PhD granting authority. The school has long-standing collaborations with federal, state, and local agencies and is widely regarded for pioneering research in quantitative ecology and natural resource science. As such, the school has the necessary course offerings, computing resources, and technical expertise to effectively implement the modeling components of the project. Office space is available in UW-SAFS for all project participants. SAFS also employs three financial administrative staff who will administer the financial aspects of this grant, a director of computing who will facilitate computing and data needs, and a building manager who is in charge of maintaining lab facilities.

***UW Facility for Compound-Specific Isotope Analysis of Environmental Samples***

The UW Facility for Compound-Specific Isotope Analysis of Environmental Samples (UW CSIA) facility housed in 600 ft2 in the Ocean Sciences Building. This facility is closely aligned with the UW Oceanography Stable Isotope Lab directed by Professor Paul Quay sharing staff, resources, and istrumentation. PI Holtgrieve directs the facility, which is centered around a new Thermo Finnigan Delta V Plus isotope ratio mass spectrometer. The instrument has four inlet devices including a gas chromatograph-combustion interface for analysis of 13C:12C or 15N:14N of organic compounds (fatty acid, amino acids, sterols), and elemental analyzer for 13C:12C or 15N:14N or bulk organic material, a dual inlet for purified gas samples, and a custom “Exetainer Interface” for automated sampling of headspace gases from water samples collected in 12 mL Exetainer vials; it is this interface that will be utilized during this project. The instrument has a custom Faraday cup configuration that allows for the simultaneous quantification of mass to charge (m/z) 28, 29, 30 (14N & 15N of N2), 32, 33, 34, 40 (16O, 17O, 18O of O2 and Ar), and 44, 45, 46 (12C & 13C of CO2). Each set of gases have their own working standards and can be calibrated against accepted international isotope standards. Also available through the SIL are high-purity vacuum lines for isolation of biogenic gasses dissolved in water for high precision analysis by dual inlet and a high automated system for 17O-O2 analysis, which is the current gold standard for in suit estimates of gross primary productivity in oceanographic settings.

***Holtgrieve Ecosystem Ecology Lab***

Holtgrieve is a PI in the School of Aquatic and Fishery Sciences at the UW. The Holtgrieve Ecosystem Ecology Lab (HEEL) employs a half time lab manager and two part-time student technicians. It is housed in 800 ft2 of analytical laboratory space with an additional 800 ft2 of shared wet lab space. There are established protocols and the necessary equipment to prepare biological, sediment, and water samples for isotope and chemical analysis, including a lyophilizer, grinding apparatus, acid washing systems, microbalances, and sample storage (-20 °C and -80 °C freezer space).

Analytical instruments in the HEEL include three gas chromatorgraphs (GC) with both flame ionization and electron capture detectors. One GC is configured for the analysis of organic macromolecules (amino acids and fatty acids, primarily). A second GC is configured for small gas sample volume (< 4 mL) analysis of CH4, CO2 and N2O. This GC can also measure sulfur hexafluoride as part of stream reaeration studies. The last GC is a field portable unit for the analysis of CH4 and CO2. The HEEL also has one LI-COR 840 infrared gas analyzed for CO2 that can be configured for wither large (20 mL) or small (1 mL) sample volumes and a Hewlett Packard 8453 UV/Vis spectrophotometer. Finally, the HEEL has shared ownership of a Picarro G2201-i for simultaneous measurement of concentration and 13C:12C isotopes of both CO2 and CH4.

The HEEL is equipped with a Reacti-Vial heating block, water bath, sonicator, centrifuge, and in-house N2 generation system for preparation of organic solids for compound-specific stable isotope analysis. There is a high-purity helium and nitrogen line for preparation of Exetainter sampling vials to sample dissolved gas ratios.

Field gear owned by the HEEL includes one YSI 6600V2 data sonde equipped with optical dissolved oxygen, turbidity, chlorophyll-a fluorescence, pH, conductivity, pressure, and temperature sensors. There are also two PME miniDOT optical dissolved oxygen and temperature sondes and one YSI 600OMSV2 sonde with optical dissolved oxygen, conductivity, pressure, and temperature sensors. Additional field equipment includes an AquaFluor field portable fluorometer for in situ and extracted chlorophyll analysis, field balances, and a suit of water sampling gear including nets, van dorn bottles, filtering apparatus, and red inflatable pack boat (R/V Predator). The HEEL also manages a set of field instruments owned by the UW student technology fund and are dedicated to student projects (including graduate student projects). This includes two next generation YSI EXO2 sondes equipped with optical dissolved oxygen, turbidity, chlorophyll-a fluorescence, DOM fluorescence, pH, conductivity, pressure, and temperature sensors, a Los Gatos Research Ultraportable Greenhouse Gas Analyzer for continuous methane, carbon dioxide, and water vapor, and two Swoffer stream flow meters.

***Δ\*IsoLab***

The University of Washington Δ\*IsoLab (http://depts.washington.edu/isolab) is a multi-investigator lab founded in 2001 by Eric Steig and Roger Buick; Steig is lab Director and Andrew Schauer manages the day-to-day operations. Schauer has extensive experience with mass spectrometry, laser spectroscopy, vacuum system design, etc., and has authored several recent methodological papers, including recent advances with the nitrate triple isotope method (Costa et al. 2011, Schauer et al. 2012, Geng et al. 2013). Δ\*IsoLab also employs a part-time technician, a part-time administrative assistant, and from one to three undergraduate assistants. The facility can operate as a cost center and routinely works with investigators and students from across the University. Δ\*IsoLab also accepts samples from outside the University as time and resources allow and collaborates with both academic and governmental agencies (i.e., NOAA, USGS, Washington Department of Ecology).

The Δ\*IsoLab instrumentation laboratory is 890 ft2 and currently has five Thermo Finnigan isotope ratio mass spectrometers and three cavity-ring-down spectroscopy lasers devoted to the isotopic analyses of hydrogen, carbon, nitrogen, oxygen, and sulfur. This project will primarily utilize a Thermo Finnigan Delta XL dedicated to the nitrate triple isotope method (15N:14N, 18O:16O and 17O:16O) and a Picarro cavity-ring-down spectroscopy instrument designed for triple isotopic measurements of water (D:H, 18O:16O and 17O:16O). Currently there is ample time available on these machines to process our samples.

Each instrument is computer controlled with Windows based software. Three additional desktop computers are housed for data reduction, storage, and backup. This lab also has a chemical fume hood and compressed gas cylinder rack. The sample preparation laboratory is 780 ft2 and has a carbonate digestion / CO2 purification vacuum line, a glass multipurpose vacuum line, a water fluorination vacuum line, two microbalances, three coarse balances, three drying ovens, one muffle furnace, a refrigerator, two fume hoods, a reciprocal shaker, and flexible lab bench space.

1. **Permits and Approvals**

No permits or approvals are required to perform the proposed work.