RESEARCH/PD ANNUAL REPORT - PROGRESS REPORT

2015 annual report - progress
David Shull
[full] - Effects of sediment porewater sulfide on eelgrass health, distribution and population growth in Puget Sound
R/HCE-7
Submitted On: 04/21/2016 09:36:49 PM

METRICS & MEASURES

Metric/Measure	Value	Note
Acres of coastal habitat	0	The purpose of this project was not to directly restore habitat.
Fishermen and seafood industry personnel	0	Fishing and aquaculture was not the purpose of this project.
Communities - economic and environmental development	0	
Stakeholders - sustainable approaches	0	
Informal education programs	0	
Stakeholders who receive information	124	As part of our project, we organized a workshop on eelgrass in August 2015 at the Shannon Point Marine Center. It was attended by eelgrass researchers and members of the public. In addition to our workshop, members of our research group have given seminars and made presentations at conferences. I am estimating the number of people who attended these events last year.
Volunteer hours	0	
P-12 students reached	25	Classroom presentation by Melissa Ciesielski on eelgrass research at Anacortes High School
P-12 educators	4	Four P-12 educators attended our eelgrass workshop in August 2015.

REQUESTED INFORMATION

Publications

No **Publications** information reported

Students Supported

Alexandra Simpson (Continuing Student)
simpsoa6@students.wwu.edu
WWU, Environmental Sciences

Field of Study: Environmental Science
Advisor: David Shull

Degree Type: MS
Degree Year: 2016

Student Project Title: Sulfide concentration and distribution increased from added detritus in Zostera marina sediments

Involvement With Sea Grant This Period (capstone, fellow, intern, etc.): Graduate research assistant

Post-Graduation Plans (employer, grad school, etc.): None

Was this thesis/dissertation supported by Sea Grant?: Yes

Thesis / Dissertation: Sulfide concentration and distribution increased from added detritus in Zostera marina sediments

New or Continuing?: continuing

Degree awarded this reporting period?: No

Financially supported?: Yes

Melissa Ciesielski (Continuing Student)
ciesiem@students.wwu.edu
WWU, Environmental Sciences

Field of Study: Environmental Science
Advisor: David Shull
Degree Type: MS
Degree Year: 2015

Student Project Title: Effects of hypoxia and sulfide intrusion on eelgrass (Zostera marina)

Involvement With Sea Grant This Period (capstone, fellow, intern, etc.): Graduate research assistant, summer public outreach specialist

Post-Graduation Plans (employer, grad school, etc.): Peace Corps, marine resource conservation specialist

Was this thesis/dissertation supported by Sea Grant?: Yes

Thesis / Dissertation: Effects of hypoxia and sulfide intrusion on eelgrass (Zostera marina)

New or Continuing?: continuing

Degree awarded this reporting period?: Yes

Financially supported?: Yes

Michael Adamczyk (Continuing Student)
mcadamcheck@gmail.com
WWU, Biology

Field of Study: Biology
Advisor: Sylvia Yang
Degree Type: BS
Degree Year: 2016

Student Project Title: Abiotic factors and donor site influence growth and development of

Zostera marina seeds

Involvement With Sea Grant This Period (capstone, fellow, intern, etc.): Intern

Post-Graduation Plans (employer, grad school, etc.):

Was this thesis/dissertation supported by Sea Grant?: No

Thesis / Dissertation:

New or Continuing?: continuing

Degree awarded this reporting period?: No

Financially supported?: Yes

Narratives

Effects of sediment pore water sulfide on eelgrass health, distribution and population growth in Puget Sound Year two report
Uploaded File: Year_two_report_-_WSG.docx

Partners This Period

No **Partners This Period** information reported

STANDARD QUESTIONS

Impacts and Accomplishments

(1)

Type	accomplishment
Title	Washington Sea Grant researchers seek to better understand the relationship between the presence of sulfides and eelgrass health
Relevance	Washington's native eelgrass (Zostera marina) is considered an indicator species for the health of Puget Sound, and it plays a key role in maintaining a healthy coastal habitat. But its abundance has declined significantly in certain areas where high concentrations of pore water sulfide are present. Sulfide appears to be an important stressor for eelgrass but is difficult to measure because it rapidly oxidizes when exposed to air and its varied distribution in sediment is challenging to track.
Response	The Washington Sea Grant-supported research team set out to identify the naturally occurring relationship between eelgrass distribution and sediment pore water sulfide concentration. Investigators tracked tolerance limits of different eelgrass life stages to various levels of sulfide, light and water column-dissolved oxygen. The goal was to determine how much eelgrass ameliorates sediment

	sulfide conditions and to predict eelgrass population trajectories, given different sulfide conditions and restoration strategies.
Results	The researchers concluded that eelgrass growth and photosynthetic efficiency is reduced in the presence of high sulfide concentration, but it requires multiple stressors before eelgrass is negatively impacted. They constructed diffusive gradient thin-film (DGT) sensors that provided better measurement of sulfide and its distribution in sediment pore water. They also constructed tanks that could be filled with eelgrass, sediment and the DGT sensors for testing. In the field, researchers observed higher sulfide concentrations near eelgrass leaves than near eelgrass root systems, which explained the variable distribution of sulfide in the sediment.
Recap	Washington Sea Grant researchers made progress toward understanding the relationship between pore water sulfide and eelgrass health and distribution.
Comments	
Primary Focus Area	Healthy Coastal Ecosystems
Secondary Focus Areas	
Goals	Ocean and coastal habitats are protected, enhanced and restored.
Partners	Western Washington University
PI Draft	* Type accomplishment * Title DGT sulfide sensor adapted for use in eelgrass research * Relevance Sulfide is an important stressor for eelgrass but is challenging to measure because it is rapidly oxidized when exposed to atmospheric oxygen and it has a fine and complex spatial distribution in sediments. Our new DGT (diffusive gradient thin-film) sensors enable better measurement of sulfide and its 2D spatial distribution. * Response We constructed sensors large enough to measure sulfide near eelgrass root systems and in the surrounding sediment. We also constructed aquaria that could be filled with eelgrass, sediment and the DGT sensors. * Results We observed that sulfide in the field varied with the presence of eelgrass leaves and root systems. We then manipulated these in the laboratory using our DGT aquaria and observed that the presence of eeglrass leaves increases sulfide concentration whereas root tips decrease the concentration of sulfide. * Recap We created new sensors to better measure the two-dimensional distribution of sulfide in the sediment and discovered how eeglrass roots and leaves influences the two-dimensional concentration profile of this environmental stressor. Comments Primary Focus Area Healthy Coastal Ecosystems Secondary Focus

	Areas Goals Ocean and coastal habitats are protected, enhanced and restored. Partners

Tools, Technologies, Information Services / Sea Grant Products

(1)

Description	Two-dimensional diffusive-gradient thin-film sulfide sensor. Developed from published studies, our sulfide sensors were refined and built to determine fine-scale distributions of sulfide in the sediment. Although these have not been used by managers in Washington State, they have the potential to improve sulfide measurements by managers.
Developed (in the reporting period)?	Yes
Used (in the reporting period)?	Yes
Used for EBM?	No
ELWD product?	No
Number of managers	0
Description/Names of managers	0

Economic Impacts

No **Economic Impacts** information reported

Community Hazard Resilience

No **Community Hazard Resilience** information reported

Meetings, Workshops, Presentations

(1)

Type of Event	Sea Grant-sponsored/organized event
Description	Eelgrass workshop at the Shannon Point Marine Center. The workshop included a keynote address by Joel Elliot of the University of Puget Sound, a lecture and video on eelgrass, created by graduate student Melissa Ciesielski, a presentation on sulfide chemistry by graduate student Allie SImpson and undergraduate student Laura Tripp, a talk on the effects of sulfide on seed germination by undergraduate students Tyler Tran and Katelyn Wright, and a hands-on science presentation by Melissa Ciesielski and Sylvia Yang. The workshop was attended by members of the Anacortes community including four P-12 teachers, and researchers from WWU, UPS, Northwest Indian

	College and the Padilla Bay National Marine Research Reserve.
Event Date	08-20-2015
Number of Attendees	24

(2)

Type of Event	Public or professional presentation
Description	Classroom presentation on eelgrass and sulfide by D Shull's ESCI 491 Oceanography class
Event Date	06/01/2015
Number of Attendees	30

(3)

Type of Event	Public or professional presentation
Description	Adriana Sepulveda poster for PERS Conference
Event Date	03/01/2015
Number of Attendees	20

(4)

Type of Event	Public or professional presentation
Description	Chesley Ekelem poster for PERS Conference
Event Date	03/01/2015
Number of Attendees	20

(5)

Type of Event	Public or professional presentation
Description	Classroom presentation by Melissa Ciesielski on eelgrass research at Anacortes High School
Event Date	05/01/2015
Number of Attendees	25

(6)

Type of Event	Public or professional presentation
Description	Melissa Ciesielski citizen event at Shannon Point Marine Center, Anacortes, WA
Event Date	08/01/2015
Number of Attendees	20

Leveraged Funds

(1)

Purpose	Effects of sediment porewater sulfide on eelgrass health, distribution and population growth in Puget Sound - undergraduate student support to develop a diffusive equilibrium thin film device for measuring dissolved iron in sediment porewater
Source	NSF
Amount	5130
Start Date	06-23-2015
End Date	08-20-2015

(2)

Purpose	Effects of sediment porewater sulfide on eelgrass health, distribution and population growth in Puget Sound - undergraduate student support to develop a stage-based population dynamics model of Zostera marina (eelgrass)
Source	NSF
Amount	5130
Start Date	06-22-2015
End Date	08-20-2015

Effects of sediment pore water sulfide on eelgrass health, distribution and population growth in Puget Sound Year two report – April 2016

Submitted by David H Shull and Sylvia Yang

The objectives of our project were to elucidate the naturally-occurring relationship between eelgrass (*Zostera marina*) distribution and sediment pore-water sulfide concentration, discern tolerance limits of different eelgrass life stages to experimental levels of sulfide, light, and water column dissolved oxygen, determine the degree to which eelgrass ameliorates sediment sulfide conditions, and predict eelgrass population trajectories given different sulfide conditions and restoration strategies. In our second year, we tested the impacts of wood pollution on sediment pore water sulfide production and eelgrass seed germination. We described and development of bacterial communities and sulfide in sediments enriches with sucrose, wood waste, or phytoplankton. We employed new sensors for measuring sulfide and iron(II) using diffusive gel thin films based on the work of Robertson et al. (2008). We deployed these sensors in the field and in manipulative laboratory experiments to better understand the relationships between eelgrass and sulfide. We also developed a stage-based model of eelgrass population growth and are currently monitoring a manipulative field experiment to assess how sulfide affects eelgrass reproduction. Finally, we held an eelgrass workshop in August that was attended by researchers and members of the community in Anacortes. Attendees listened to presentations on eelgrass, the eelgrass-sulfide work we have been doing, and participated in hands-on exercises about how to do science.

Summary of progress to date

Wood pollution on sediment pore water sulfide production and eelgrass seed germination
Historically, sawmills released wood waste onto coastal shorelines in many locations in the Salish Sea, enriching marine sediments with organic material. The increase in organic carbon in the marine sediment boosts the bacterial reduction of sulfate from seawater and results in the production of hydrogen sulfide. Our previous work indicates that sulfide can decrease growth of adult shoots. But, little is known about the effects of sulfide and wood pollution on the survival and germination of eelgrass seeds. The objective of this study was to determine the effects of wood debris on sediment pore water hydrogen sulfide concentrations and eelgrass seed germination. We conducted a laboratory mesocosm experiment, adding sawdust to marine sediments and measuring the sulfide levels weekly. We subsequently planted eelgrass seeds collected from two natural populations into the mesocosms and measured germination rates. Concentrations of sawdust $\geq$ 10% led to higher levels of pore water hydrogen sulfide and drastically lower eelgrass germination rates. The results can potentially be used to set thresholds for remediation efforts and guide seed distribution in wood-polluted areas.

Microbial communities influenced by sediment organic enrichment and hydrogen sulfide concentrations
Hydrogen sulfide is produced by sulfate reducing bacteria and sediments with high levels of sulfide can harbor mats of sulfur oxidizing bacteria as well. Better understanding of the relationship between organic matter enrichment, bacterial mats, and hydrogen sulfide levels can lead to improved predictions of the effects of organic enrichment on sediment chemistry and its effects on eelgrass health. Sawdust, phytoplankton, agar, and sugar were added to marine sediment mesocosms. Bacterial growth was observed for 48 days, and final total and free sulfide concentrations were measured. Two main bacterial communities flourished: white (possibly dominated by *Arcobacter*) and orange (possibly dominated by

Chromatium). Phytoplankton and sugar treatments had higher microbial mat cover and white bacterial mats prevailed over orange mats over time. Orange mats were associated with a narrow range of sulfide levels (~0.0-0.1 mM H_2S), which was lower than for white mats (~0.1-2.0 mM H_2S). Phytoplankton and sugar treatments yielded the highest H_2S levels, with 1.2 and 0.8 mM, respectively. Phytoplankton's high sulfide levels could indicate it was more accessible to bacterial degradation compared to the other sources of organic matter. *Arcobacter* is known for quickly colonizing and dominating high sulfide sediments over other species such as *Chromatium*, which may explain the white microbial mat prevalence over orange microbial mat through time. These results imply that algal blooms may result in toxic levels of H_2S, promoted by bacteria whose activity hinders sulfide oxidation, as *Arcobacter* creates a sulfur veil that excludes oxygen from sediments. Wood waste pollution leads to delayed sulfide development and may result in slower H_2S release for a longer duration.

Diffusive gradient thin films for measuring sulfide and iron(II)

We constructed 10-cm by 15-cm diffusive gradient thin films (DGTs) for measuring sulfide concentrations in two dimensions. We also built diffusive equilibrium films (DETs) for measuring dissolved iron(II), which reacts with sulfide to produce iron-sulfide, which is not toxic. We deployed these sensors in the field and observed that sulfide concentration varied strongly with the presence of buried eelgrass leaves. The DGTs at some sites displayed images of sulfide concentration that actually traced the shapes of buried eelgrass leaves. Iron(II) increased with depth in the sediment but then dropped in concentration in the presence of high sulfide concentration due to the formation of iron sulfide, which removes iron from solution. We tested the effects of buried eelgrass leaves and the presence of an eelgrass rhizosphere on sulfide concentration by growing eelgrass in sediment with and without buried eelgrass leaves. Average sulfide concentrations were higher in the presence of eelgrass, perhaps due to eelgrass production of dissolved organic carbon that might stimulate sulfate reduction. Sulfide concentration at a given depth was higher in the presence of eelgrass leaves. And, sulfide concentration was lower near eelgrass roots (Figure). Sulfide and iron(II) were negatively correlated. These data indicate that measurements of average sulfide concentration do not necessarily represent eelgrass exposure to this toxin.

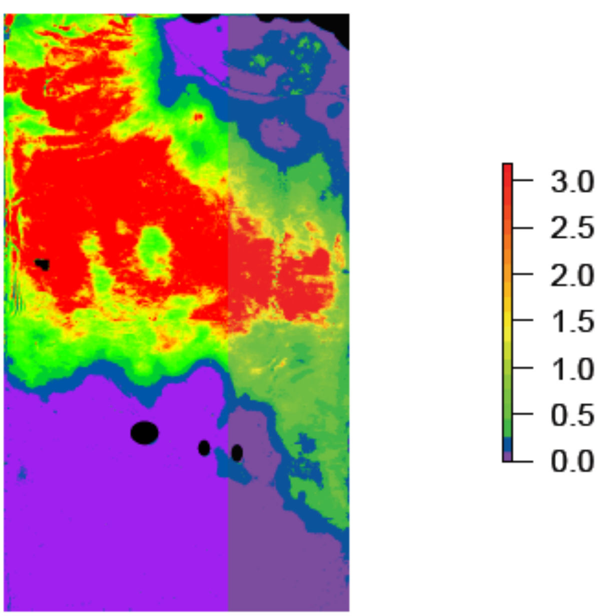

Figure. Two-deimnsional map of sulfide concentraion (in mM) from the aquarium experiment. The red color indicates high sulfide concentration. The purple section in the upper right corresponds to the location of eelgrass. The purple section at the bottom has low concentration of dissolved sulfide due to precipitation of iron sulfide.

Eelgrass stage-structured population model

Variable sulfide tolerance may ultimately dictate the ability of an eelgrass population to resist or naturally recover from sulfide stress. However, the population-level repercussions of these individual effects of sulfide are unknown. To adress this issue, we developed a stage-based matrix population model, parameterized by stage-specific individual effects of sulfide. The eelgrass lifecycle was quntified via three stages (vegetative shoots, flowering shoots, and seeds) and vital rates describing transitions between stages (branching rate, flowering rate, fecundity, germination rate, seedling survival rate). The stage-based matrix model was constructed with population growth and stage composition defined as functions of the vital rates on an annual time scale. Data were collected for each vital rate in response to hydrogen sulfide concentrations and defined as functions of sulfide where correlation existed or at low-medium-high intervals where no correlation was observed. The model was run for a range of sulfide concentrations and for all vital rates, and the population growth (λ), population composition, and λ sensitivity to changes in vital rates were extracted for each set of conditions. Branching rate had the largest effect on population growth, seedling survival rate had a moderate effect, and fecundity had a negligible effect. Population growth was positive (>1) for the majority of conditions. Further data collection and refinement of vital rates as well as the addition other environmental conditions will increase the accuracy of the model and help inform management and conservation strategies. To that end, we are currently conducting a field experiment in which we increased organic matter in sediment plots to stimulate sulfide production. We will continue to monitor this experiment to assess the effects of sulfide and organic-matter enrichment on eelgrass branching and flowering rates.

Eelgrass workshop

We invited local scientists and residents from Anacortes, WA to attend an eelgrass workshop at the Shannon Point Marine Center on August 20, 2015. Approximately 20 Anacortes residents participated along with scientists with an interest in eelgrass. Joel Elliott from the University of Puget Sound made the keynote presentation. Graduate students Melissa Ciesielski and Allie Simpson and undergraduate students Laura Tripp, Tyler Tran, Mike Adamczyk, and Katelyn Wright gave presentations. PI Sylvia Yang led the workshop participants in a hands-on science investigation addressing the scientific method.

Areas of focus for year three

We will report our findings for 2015 at the upcoming Northwest Algal and Seagrass Symposium (May 2016). This summer we will repeat some of our early experiments examining sulfide and eelgrass growth using the DGTs as sampling sulfide sampling devices to better understand those early results. We will also continue to monitor our manipulative field experiment and assess whether sulfide influences the production of flowering shoots in eelgrass.

Reference

Robertson, D., Teasdale, P.R. and Welsh, D.T., 2008. A novel gel-based technique for the high resolution, two-dimensional determination of iron (II) and sulfide in sediment. Limnol. Oceanogr. Methods, 6, 502-512.