Update Report

Period: 2/1/2014 - 1/31/2015

Project: R/HCE-7 - *[full] - Effects of sediment porewater sulfide on eelgrass health, distribution and population growth in Puget Sound*

STUDENTS SUPPORTED

Adamczyk, Michael, mcadamcheck@gmail.com, Western Washington University, Biology, status: new, field of study: Biology, advisor: Sylvia Yang, degree type: BS, degree date: 2016-06-01, degree completed this period: No
Student Project Title:
Tolerance and effects of sulfide on mortality and germination of eelgrass seeds
Involvement with Sea Grant This Period:
Lab technician
Post-Graduation Plans: *none*

Ades, Kathryn, adesk@students.wwu.edu, WWU, Biology, status: new, field of study: Biology, advisor: Sylvia Yang, degree type: BS, degree date: 2016-06-01, degree completed this period: No
Student Project Title:
Environmental cues of eelgrass seed germination
Involvement with Sea Grant This Period:
intern
Post-Graduation Plans: *none*

Blatt, Ari, blatta@students.wwu.edu, Western Washington University, Environmental Science, status: new, field of study: Environmental Science, advisor: Sylvia Yang, degree type: BS, degree date: 2016-06-01, degree completed this period: No
Student Project Title: *none*
Involvement with Sea Grant This Period: *none*
Post-Graduation Plans: *none*

Ciesielski, Melissa, ciesiem@students.wwu.edu, Western Washington University, Environmental Sciences, status: new, field of study: Environmental Sciences, advisor: David Shull, degree type: MS, degree date: 2015-06-01, degree completed this period: No
Student Project Title:
A study on the effects hypoxia and sulfide intrusion on eelgrass (Zostera marina)
Involvement with Sea Grant This Period:
Research Assistant
Post-Graduation Plans:
None yet.

Ekelem, Chelsey, ekelem@college.harvard.edu, Harvard College, Oranismic and Evolutionary Biology, status: new, field of study: Biology, advisor: Sylvia Yang, degree type: BS, degree date: 2016-06-01, degree completed this period: No
Student Project Title:
The effects of wood pollution on sulfide levels and eelgrass germination
Involvement with Sea Grant This Period:
MIMSUP intern
Post-Graduation Plans: *none*

Felling, Clarisa, fellinc@students.wwu.edu, Western Washington University, Biology, status: new, field of study: Biology, advisor: Sylvia Yang, degree type: BS, degree date: 2017-06-01, degree completed this period: No Student Project Title: Sediment characteristics correlated with porewater sulfide Involvement with Sea Grant This Period: Intern Post-Graduation Plans: *none*
Mayer, Miles, mayerm2@students.wwu.edu, Western Washington University, Environmental Science, status: new, field of study: Environmental Science, advisor: Sylvia Yang, degree type: BS, degree date: 2016-06-01, degree completed this period: No Student Project Title: Environmental cues of eelgrass seed germination Involvement with Sea Grant This Period: intern Post-Graduation Plans: *none*
Sepulveda, Adriana, adriana.sepulveda@upr.edu, University of Puerto Rico, Biology, status: new, field of study: Biology, advisor: Sylvia Yang, degree type: BS, degree date: 2017-06-01, degree completed this period: No Student Project Title: Sediment microbial diversity depends on source of organic enrichment Involvement with Sea Grant This Period: MIMSUP intern Post-Graduation Plans: *none*
Simpson, Alexandra, simpsoa6@students.wwu.edu, Western Washington University, Environmental Sciences, status: new, field of study: Environmental Sciences, advisor: David Shull, degree type: MS, degree date: 2016-06-01, degree completed this period: No Student Project Title: Effects of eelgrass (Zostera marina) on the distribution of sediment pore water hydrogen sulfide Involvement with Sea Grant This Period: Research Assistant Post-Graduation Plans: None yet
Walser, Annie, walser.annie@gmail.com, Western Washington University, Environmental Sciences, status: new, field of study: Environmental Sciences, advisor: David Shull, degree type: MS, degree date: 2014-06-01, degree completed this period: Yes Student Project Title: A study of pore-water sulfide and eelgrass (Zostera japonica and Zostera marina) in Padilla Bay, Washington Involvement with Sea Grant This Period: Research assistant Post-Graduation Plans:

Nautilus Environmental Consulting, San Diego, CA

Woodrish, Daniel, woodrid@students.wwu.edu, WWU, Biology, status: new, field of study: Biology, advisor: Sylvia Yang, degree type: BS, degree date: 2016-06-01, degree completed this period: No
Student Project Title:
Environmental cues of eelgrass seed germination
Involvement with Sea Grant This Period:
intern
Post-Graduation Plans: *none*

CONFERENCES / PRESENTATIONS

Workshop: Organized by Yang, S., and Shull, DH. (2015) Effects of sediment porewater sulfide on eelgrass (Zostera marina) health, distribution, and population growth in Puget Sound, Friday Harbor Laboratories, Jan 17-19., SG-sponsored, 24 attendees, 2015-01-17

ADDITIONAL METRICS

P-12 Students Reached:	0	**P-12 Educators Trained:**	0
Participants in Informal Education Programs:	0	**Volunteer Hours:**	0
Acres of coastal habitat protected, enhanced or restored:	0	**Resource Managers who use Ecosystem-Based Approaches to Management:**	0
Annual Clean Marina Program - certifications:	0	**HACCP - Number of people with new certifications:**	0

ECONOMIC IMPACTS

No Economic Impacts Reported This Period

SEA GRANT PRODUCTS

No Sea Grant Products Reported This Period

HAZARD RESILIENCE IN COASTAL COMMUNITIES

No Communities Reported This Period

ADDITIONAL MEASURES

Number of stakeholders modifying practices:	Sustainable Coastal Development **# of coastal communities:**

PARTNERS

No Partners Reported This Period

IMPACTS AND ACCOMPLISHMENTS

Title: **Washington Sea Grant research considers whether sediment porewater sulfide is harmful to eelgrass health, distribution, and growth in Puget Sound**
Type: accomplishment
Description:
Relevance: Eelgrass is a critical resource for coastal ecosystems and an indicator of healthy Puget Sound estuaries. But its abundance has declined significantly in certain areas, particularly those with high levels of porewater sulfide, which is toxic to plants under low-oxygen conditions. It is unclear how eelgrass can be restored in such areas and whether high porewater sulfide levels are the primary cause of its absence. Better understanding of the relationship between porewater sulfide and eelgrass health would assist in shaping eelgrass restoration strategies.
Response: Washington Sea Grant researchers leveraged the skills of 20 undergraduate students through Western Washington University’s science and minorities program. Working with the students, researchers completed nine Puget Sound field studies that measured profiles of porewater sulfide and eelgrass abundance. Students also conducted a survey of potential new sites in Puget Sound where conditions might support eelgrass growth. The team convened a workshop assembling internationally recognized eelgrass experts and Washington coastal resource managers.
Results: Workshop participants discussed eelgrass health and restoration, which led to new insights and a report on the relationship between eelgrass and sediment chemistry. The students and researchers discovered some growth impacts but no significant relationship between sulfide concentrations and eelgrass at any of the sites surveyed. Finally, the students found no Puget Sound sites that were suitable for further eelgrass expansion.
Recap:
Recap: Washington Sea Grant-supported research investigates the relationship between eelgrass and porewater sulfide and engages resource managers, scientists, and students to better understand the role of porewater sulfide in eelgrass health.
Comments:
Primary Focus Area: HCE
Associated Goals: Ocean and coastal habitats are protected, enhanced and restored. (HCE)
Partners:
Western Washington University
Related Partners: *none*

PUBLICATIONS

Title: **Effects of sediment pore water sulfide on eelgrass (Zostera marina) health, distribution, and population growth in Puget Sound. Eelgrass-Sulfide Workshop Report**
Type: Workshops, Proceedings, Symposia Including Highlights/Summaries of (please note: document number reflects the year the proceedin Publication Year: 2015
Uploaded File: Sulfide_Workshop_Repor....t.pdf, 246 kb
URL: *none*

Abstract:

The purpose of the Eelgrass-Sulfide workshop was to develop a deeper understanding of seagrass-sulfide interactions in Puget Sound and worldwide. To accomplish this we:
1. Synthesized the current state of research through discussions and presentations;
2. Developed new seagrass-sulfide research ideas through collaboration;
3. Determined the implications and applications of sulfide research for seagrass restoration.

Throughout the workshop we considered the interaction of seagrass and sulfide worldwide and how this applies to the range of sulfide conditions throughout Puget Sound. The workshop participants reviewed seagrass-sulfide interactions, discussed new research ideas and management implications and initiated the creation of a database of sulfide ranges in Puget Sound and around the globe.

Citation:

Simpson, A., D.H. Shull, and S. Yang. 2015. Effects of sediment pore water sulfide on eelgrass (Zostera marina) health, distribution, and population growth in Puget Sound. Eelgrass-Sulfide Workshop Report. Friday Harbor Laboratories. January 17-18, 2015.

Copyright Restrictions + Other Notes:

Journal Title: *none*

Title: **A study of pore-water sulfide and eelgrass (Zostera japonica and Zostera marina) in Padilla Bay, Washington**

Type: Full theses / Dissertations Publication Year: 2014

Uploaded File: A_study__of_pore-water....d.pdf, 1467 kb

URL: http: //cedar.wwu.edu/wwuet/350/

Abstract:

Two species of eelgrass can be found in Padilla Bay, Washington (Zostera japonica and Zostera marina) and act as a bioindicators of ecosystem health. Many factors can contribute to the status of an eelgrass bed, including light, temperature, salinity, and nutrients. However, following several cases of seagrass die-off events worldwide, another factor is suspected to contribute to eelgrass health: pore-water sulfide. This study examined the relationships between Z. japonica, Z. marina, and pore-water sulfide in Padilla Bay and the effects of elevated pore-water sulfide concentrations on eelgrass. Forty sites were surveyed for eelgrass shoots and sulfide concentration profiles were measured at depths of 0 to 12 cm. A correlation was expected between eelgrass and the inventory of sulfide during August and September due to increased temperature and increased bacterial respiration as a result of higher quantities of organic matter accumulation. While the data hinted at patterns between eelgrass density and sulfide, there were no significant correlations found between Z. japonica and Z. marina and the inventory of sulfide from June 2013 through September 2013. This is perhaps due to relatively low concentrations of sulfide at the study sites and documented eelgrass tolerance to the concentration range, as well as the overall health of eelgrass in this location. To further examine the relationship between eelgrass and sulfide, Zostera japonica and Zostera marina were grown in sediment amended with sulfide in an outdoor laboratory tank to study growth response and photosynthetic yield. Eelgrass shoots were grown for four weeks under different

sulfide manipulations and shoot growth was recorded weekly. Quantum efficiency of PSII in eelgrass shoots was measured by PAM fluorometry at the conclusion of the experiment. The growth rates of Z. japonica and Z. marina were significantly reduced in treatments with elevated sulfide concentrations. Manipulated concentrations of pore-water sulfide resulted in significantly lower growth rates among Z. japonica shoots treated with moderate and high levels of sulfide. The decrease in growth in both species suggests that elevated levels of pore-water sulfide have an impact on eelgrass in Padilla Bay. The average photosynthetic yield of the shoots for Z. japonica and Z. marina was lower in shoots treated with sulfide, although this difference was not statistically significant, suggesting the drop in growth was not due to chloroplast damage.

Citation:

Walser, A (2014) A study of pore-water sulfide and eelgrass (Zostera japonica and Zostera marina) in Padilla Bay, Washington, MS Thesis, Western Washington University, Bellingham, WA

Copyright Restrictions + Other Notes:

Journal Title: *none*

OTHER DOCUMENTS

No Documents Reported This Period

LEVERAGED FUNDS

Type: influenced Period: 2015-01-01: : 2015-01-31Amount: $5000

Purpose:

Research funds were awarded to Chelsey Ekelem and Adriana Sepulveda to study eelgrass and sulfide and salary was provided to Sylvia Yang to supervise their work.

Source: NSF MIMSUP (Minorities in Marine Sciences Undergraduate Program)

Type: influenced Period: 2014-06-23: : 2014-08-22Amount: $14102

Purpose:

Research stipends and subsistence funds awarded to undergraduate students Lowell Iporac and Socheata Lim along with salary and fringe for D Shull and S Yang. Students completed research on eeglrass and sulfide interactions at the WWU Shannon Point Marine Center.

Source: NSF Research Experience for Undergraduates program

UPDATE NARRATIVE

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Effects of sediment pore water sulfide on eelgrass health, distribution and population growth in Puget Sound Year 1 report – March 2015

Submitted by

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and

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Effects of sediment pore water sulfide on eelgrass health, distribution and population growth in Puget Sound Year one report – March 2015

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 first year, we addressed the first four of these goals through a series of field and laboratory studies. We held a workshop at the University of Washington Friday Harbor Laboratories to bring together researchers and managers to discuss this relationship and consider the implications for resource management and eelgrass restoration.

Summary of progress to date

Field surveys

To date we have completed nine field surveys in which we measured profiles of pore-water sulfide and eelgrass abundance. Sediment pore water was collected from three depths in the sediment (3, 6, and 12 cm) using sippers (Howes *et al.* 1985, Fuller 1994). Pore water samples were preserved in sulfide anti-oxidant buffer and sulfide was measured using an ion-selective electrode (Brouwer and Murphy 1994). Eelgrass was counted within either randomly or hap-hazardly positioned 0.25-m^2 quadrats. Eight surveys were conducted in Padilla Bay and one was conducted in Hood Canal. These surveys enabled us to determine the range of sulfide concentrations found within eelgrass beds at our study sites (0.032 µM to 0.74 mM) and to assess whether there is a relationship between sulfide concentrations and eelgrass abundance. To date we have found no significant spatial relationships between sulfide and eelgrass at any of the sites surveyed.

Laboratory experiments

Eelgrass experiments were carried out at the Shannon Point Marine Center in Anacortes, WA. Experiments with seeds were conducted in indoor flowing seawater tables. Seedling and adult shoot growth experiments were conducted in outdoor tanks under natural light. Eelgrass shoots were maintained in 470-ml plastic cups of sediment collected from the field. One experiment examining the interactive effects of sulfide and dissolved oxygen was conducted indoors in tanks with artificial lighting (14h:10h light:dark cycle).

Effects of sulfide on growth of adult eelgrass shoots

In our initial studies on the effects of pore water sulfide on growth of eelgrass shoots, we manipulated sulfide concentrations in cups of sediment containing eelgrass shoots over four weeks. Sulfide concentrations were manipulated by “reverse-sipping”. The sediment pore-water sippers were used to inject sulfide directly into the sediment after allowing the concentrations to equilibrate for two days, sediment pore-water samples were collected to determine sulfide concentrations in control and treated sediments. More sulfide was added as necessary to maintain sulfide concentrations at experimental levels. Eelgrass growth was measured using the pin-pick method (Short and Coles 2001) and photosynthetic efficiency was measured using PAM fluorometry (Krause and Weis 1991, Maxwell and Johnson 2000). Initial experiments were conducted on both *Zostera marina* and the invasive eelgrass species *Zostera japonica*. In both species, growth rate declined with increased sulfide concentration up to a concentration of approximately 0.3 mM at 6 cm depth for *Z. marina* (Fig. 1) and up to 1.0 mM at 6 cm depth for *Z. japonica* (Fig. 2). The reason for the difference in the range of sulfide concentration over which growth declines in these two species is not known, but it is likely due to differences in the depth of root penetration. *Z. marina*, with deeper roots, would be exposed to higher sulfide concentrations than *Z. japonica* because sulfide in nature and in our experimental treatments increased with depth in the sediment.

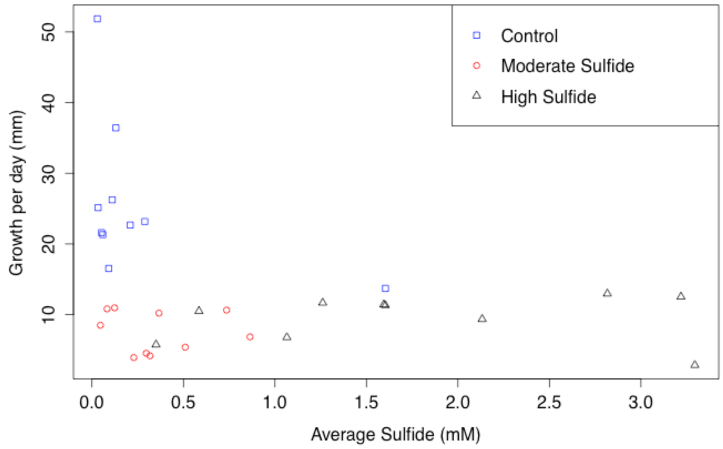

Figure 1. The average pore-water sulfide concentration (mM) versus the growth per day of *Z. marina* shoots (n=30) treated with moderate levels of pore-water sulfide, high levels of pore-water sulfide, and no added pore-water sulfide over four weeks.

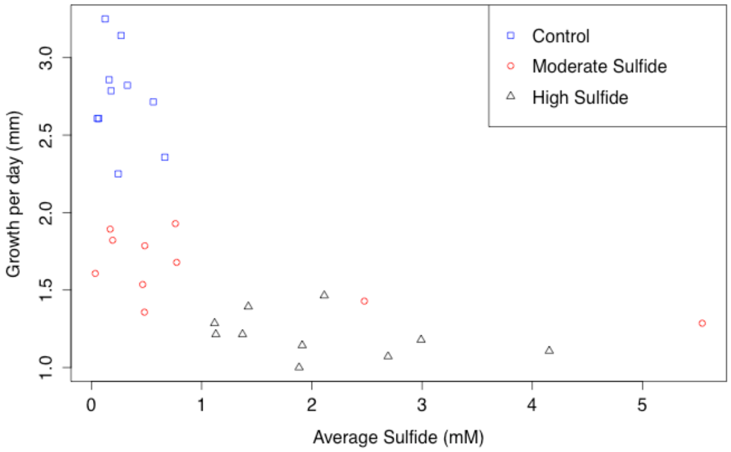

Figure 2. The average pore-water sulfide concentration (mM) versus the growth per day (mm) of *Z. japonica* shoots (n=30) treated with moderate levels of pore-water sulfide, high levels of pore-water sulfide, and no added pore-water sulfide over four weeks.

One curious aspect of these results is that sulfide at concentrations higher than 1 mM had little apparent effect on growth. No mortality was observed even at the highest manipulated sulfide concentrations. In order to explore the effects of even higher sulfide concentration on eelgrass growth and mortality, we repeated our experiments but manipulated sulfide concentration by placing disks of sulfide-impregnated agar in the bottom of the experimental cups. This enabled us to create a greater range of sulfide concentration in experimental containers than the reverse-sipping method. We observed the same trend of no significant effect of sulfide on eelgrass growth up to 24 mM. And, we observed no eelgrass mortality even at sulfide concentrations as high as 39 mM (Fig. 3).

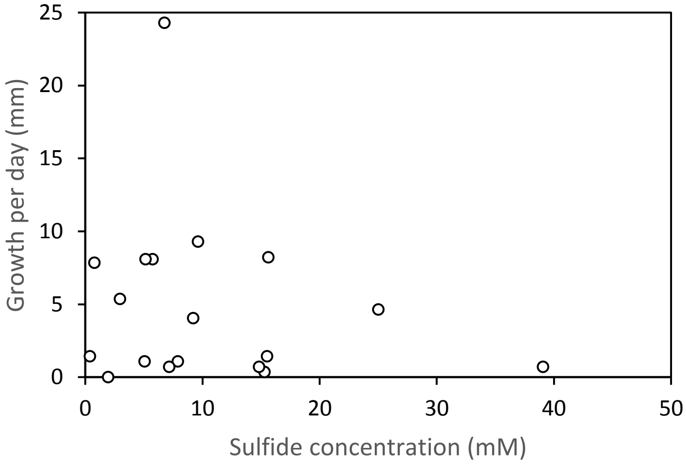

Figure 3. The average pore-water sulfide concentration (mM) versus the growth per day of *Z. marina* shoots treated with sulfide ranging from 0.4 to 39 mM.

These results indicate at concentrations less than 1 mM, sulfide has a significant effect on growth of eelgrass collected at our study site in Padilla Bay. However, higher concentrations of sulfide had little effect on growth or mortality. This result was surprising since previous reports have indicated that eelgrass are absent at sites in Puget Sound with very high concentrations of sulfide (> 3mM, Elliott et al. 2006). One explanation of this odd result is that most of the sulfide was in the form HS^- rather than the more toxic H_2S. We are now studying how pH varies with different sulfide treatments to determine if pH affected our results.

Other factors that might interact with sulfide include light and dissolved oxygen. Eelgrass produce oxygen during photosynthesis and some of this dissolved oxygen diffuses from root tips and can oxidize pore water sulfide to harmless sulfate (Frederiksen and Glud 2006). As a result, both low light levels (which would reduce the rate of photosynthesis) and low levels of water column dissolved oxygen (which would reduce diffusion of oxygen from the water column) might exacerbate the effects of pore water sulfide. To examine the interaction between sulfide and light availability we conducted experiments in which we manipulated sulfide using the agar method and manipulated natural light availability by the use of screens. Although high sulfide concentrations decreased eelgrass growth rate, there was no significant interaction between light and sulfide (Fig. 4).

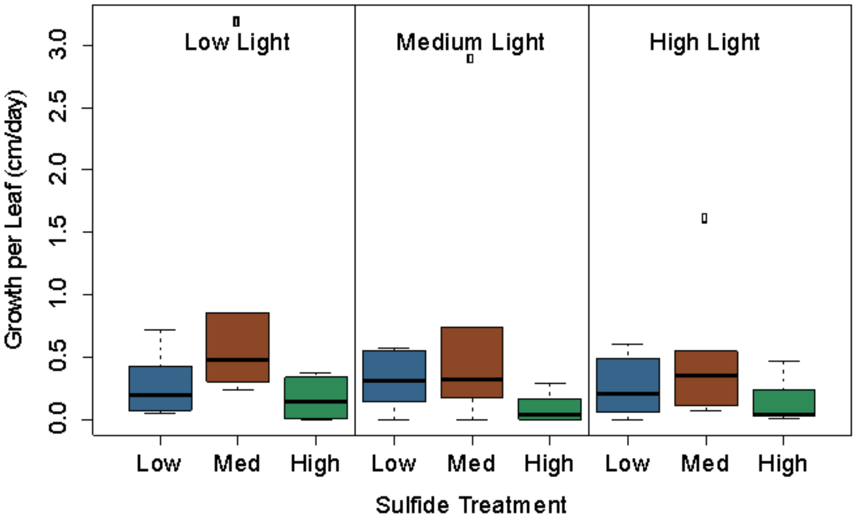

Figure 4. Growth rate per leaf versus sulfide and light treatments. Standard deviation is shown in the bars. The symbol (°) represents outliers. A 2-way ANOVA indicated that sulfide reduced eelgrass growth rate (p=0.01)

We also conducted an experiment to determine the interaction between sulfide and dissolved oxygen concentration. We conducted these experiments in tanks with artificial light (14:10 h day:night cycle). We manipulated sulfide as in previous experiments using sulfide-impregnated agar and manipulated water column dissolved oxygen by bubbling the tanks with nitrogen + 400 ppm CO_2. Eelgrass growth was monitored over six weeks. During this time the sulfide concentration slowly increased in the experimental treatments due to slow diffusion of sulfide from the agar disks. Eelgrass growth was lower in treatments with hypoxic water compared to tanks in equilibrium with atmospheric oxygen. However, there was not a significant interaction between sulfide and oxygen (Fig. 5).

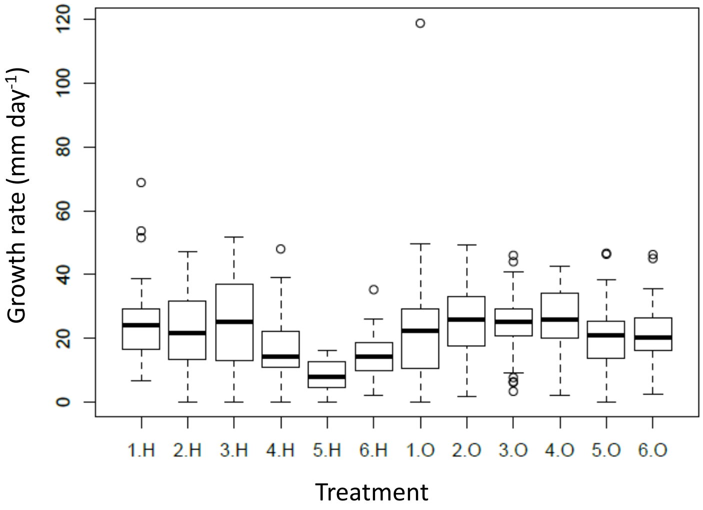

Figure 5. Average eelgrass shoot growth rate in hypoxic tanks (H) over six weeks versus oxic tanks (O) over the same time period. Growth rates for weeks 1 through 6 are plotted using a box and whisker plot showing major quartiles, ranges and extreme points.

Effects of sulfide on seed survival and germination

Sulfide intrusion may be necessary to break seed dormancy, as germination rate is higher under anoxic, high organic-content sediment conditions (Marba et al. 2006, and for two north Puget Sound sites, Monreal and Yang 2013). In a survey of 17 sites encompassing Hood Canal, Padilla Bay and Puget Sound north to Lummi Bay, early season seedlings were only found at sites with organic-rich sediments (Yang et al. 2013), which are associated with high sulfide conditions. To test whether anoxia and varying concentrations of sulfide act as triggers to break seed dormancy, we conducted experiments to determine the roles of anoxia and sulfide on seed germination.

We collected flowering eelgrass shoots were in summer 2013 from two different field locations, Padilla Bay National Estuarine Research Reserve (July) and near the San Juan Island ferry landing at Ship Harbor (two collections made: July and August. Flowering shoots were stored in mesh bags in running seawater tanks, and when seeds had fallen, they were sorted and sieved from the decaying shoot material.

We conducted these experiment using 20-ml glass scintillation vials. Vials were filled with one of 5 treatments: oxic seawater, anoxic seawater, or anoxic seawater with added sulfide to reach concentrations of 0.1 mM, 0.5 mM, and 1 mM. The range of sulfide concentrations mimicked what we had observed in the field in Padilla Bay as well as concentrations found to impact eelgrass, as tested experimentally in previous studies (e.g., Korhonen et al. 2012, Goodman et al. 1995). To create the anoxic sulfide treatments, nitrogen gas was bubbled through seawater and calculated amounts of a 22 mM sulfide solution was added. All air was displaced from anoxic treatment vials. For the oxic treatment, head space was left as a reservoir of oxygen.

Five replicates of each treatment were randomly placed in trays in a running seawater table with ambient indoor lighting. Germination was visually censused weekly from Feb 4, 2014 – May 20, 2014 and vials re-randomized to minimize any effect of local environmental differences (such as light levels). Seeds were censused for emergent roots and shoots at every time point. At the end of the experiment, ungerminated seeds were tested for viability using 0.5% tetrazolium chloride staining for 24 hours at room temperature (Conacher et al. 1994). Metabolic activity causes living seeds treated with tetrazolium to become pink. Sulfide measurements were not taken throughout the experiment so as not to expose the treatments to oxygen. We found that by 93 days, the sulfide treatments had become indistinguishable in this experiment, but follow-up studies demonstrated that sulfide concentrations created using the same method were maintained through 6 weeks. Interestingly, it was after this time point in this experiment that the seeds in the vials began to decompose, possibly changing sulfide concentrations.

The number of seeds that germinated increased over time up until approximately 6-weeks, when the seeds began to deteriorate and rot. Treatment had no effect on germination over time for Padilla or Ship Harbor August, but did for Ship Harbor July (ANOVAR for SH-July, treatment effect $p = 0.008$) (Fig. 6 a-c). The census of days 51, 72, and 93 from Ship Harbor July consistently showed a significantly higher rate of germination in 0.5 mM sulfide than in oxic conditions with no added sulfide (Tukey Honest Significant Difference, $p < 0.05$, Fig. 6 d). This result suggests that germination from this seed source may not be cued by anoxia alone, but sulfide must be present at a certain concentration.

Tetrazolium tests indicated that a portion of ungerminated seeds were still viable, regardless of sulfide or oxygen treatment. Instead, variation in ungerminated seed viability depended on seed source (2-way ANOVA, site effect, $F = 6.9$, $p = 0.002$) (Figure 7). Interestingly, the proportion of ungerminated seeds that were still alive was higher for Padilla Bay than for either Ship Harbor collection, despite Padilla Bay having the lowest germination rate (Figure 1). Thus, the Padilla seeds were viable but appear to be less sensitive to sulfide as a cue to germinate. These results imply that germination response to anoxia and sulfide may depend on seed source and collection timing. Further studies are necessary to tease apart the effects of donor population and phenology (such as timing of seed maturity/ripeness).

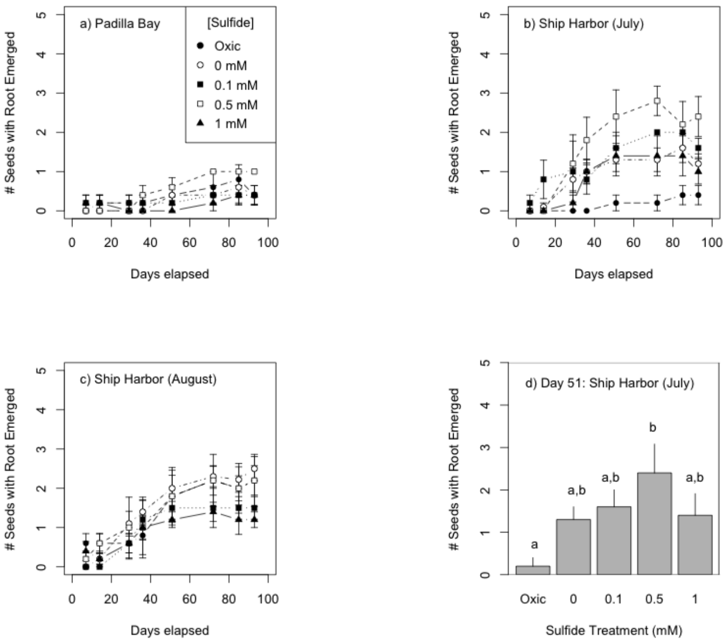

Figure 6. Number of seeds with emergent roots (germinated) during the 8-week experiment for three seed sources: (a) Padilla Bay, (b) Ship Harbor collected in July, (c) Ship Harbor in August. (d) Variation in germination due to sulfide treatment arose after 6 weeks in Ship Harbor (July) seds. Data shown are from day 51.

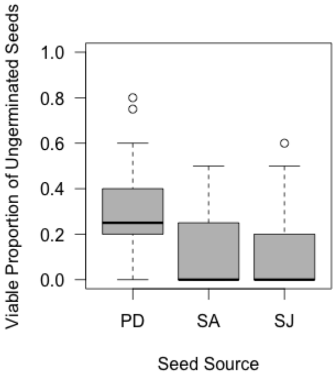

Figure 7. The proportion of ungerminated seeds that were still viable depended on seed source. Padilla Bay (PD) was higher than Ship Harbor July (SJ) and Ship Harbor August (SA).

To test the effects of varying concentrations of sulfide and duration of exposure on eelgrass seed viability, we conducted two follow-up experiments using seeds collected in July 2014 from Padilla Bay. The two experiments were similar to the first seed germination experiment, but we used a larger number of seeds and we used a greater range of sulfide concentrations. In an experiment to test duration of exposure, for each of 6 sampling weeks, 5 replicate vials of 6 seeds each were prepared approximately 0, 1, 5, and 10 mM (Fig. 8a). Each week, a new set of 5 replicate vials were opened, sulfide concentration measured, and seed viability tested using tetrazolium staining. In a second experiment, eelgrass seeds were exposed for a short duration (2 weeks) to extremely high concentrations of sulfide: 0, 0, 1, 2.5, 5, 10, 15, 20, 25 mM. The experiment was repeated in a second trial to examine germination at 50 mM;

Sulfide concentrations were consistent over the course of the 6 week experiment (Figure 8a), but did not affect rates of unstained seeds (definitely nonviable) (43% unstained, Figure 8b). Duration of exposure also had no effect, even at levels of sulfide much higher than found in nature (5 mM & 10 mM). For both trials of this experiment, eelgrass seeds exposed to sulfide concentrations ranging from 0 mM - 50 mM for 2 weeks all had similar rates of unstained (definitely nonviable) seeds (42% unstained, Fig. 9). This suggests that even extremely high levels of sulfide did not cause additional seed mortality in a 2-week period. However, partially stained seeds were not included in this count and may also represent mortality. Observations of partial staining patterns revealed a frequent pattern: just the radicle's outer surface stained brown while the rest of the radicle (enclosed in the endosperm) was stained pink. Whether these seeds have the potential to germinate is unknown since the tetrazolium staining procedure kills the seed. Further investigation is necessary to assess germination rates of seeds exhibiting different partial staining patterns. Alternatively, seeds exposed to sulfide should be tested for viability through germination trials, not tetrazolium staining.

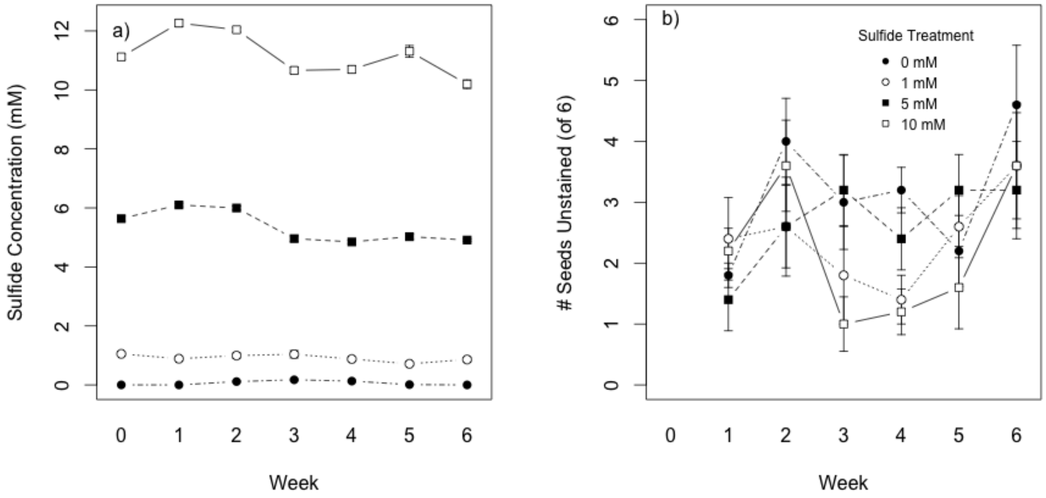

Figure 8. (a) Sulfide concentration in experimental vials were maintained over the duration of the experiment (error bars show standard error, legend same as for b). (b) The number of seeds that remained unstained after soaking in tetrazolium (suggesting nonviability) did not vary with length of time exposed nor sulfide concentration.

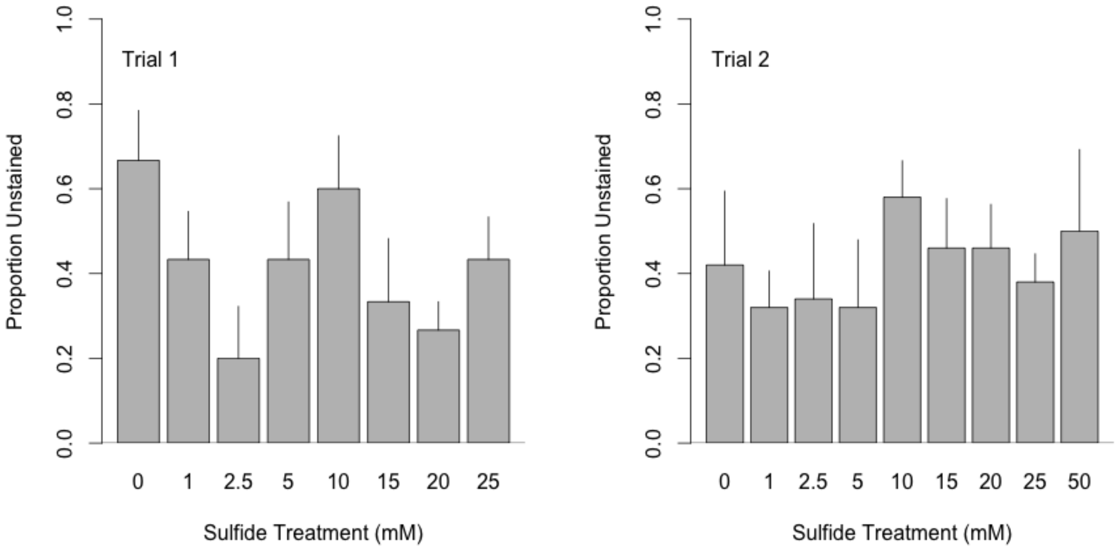

Figure 9. The proportion of seeds that remained unstained after soaking in tetrazolium was 42% for both trials of 2-week exposures, regardless of sulfide treatment.

Summary of findings from year one

- Eelgrass densities and sulfide concentrations do not covary at our study sites
- Sulfide concentration does reduce rates of growth in *Z. marina* and *Z. japonica*.
- Hypoxic conditions reduce growth rate in *Z. marina*.
- However, reduced light and reduced concentration of dissolved oxygen (at levels we examined) do not exacerbate the effects of sulfide on growth.
- Although growth rates were reduced, little eelgrass mortality was observed even at extremely high levels of sulfide.
- Sulfide had little effect on seed germination except for one site where germination rate was higher with elevated sulfide.

Publications

Walser, A. 2014. A study of pore-water sulfide and eelgrass (*Zostera japonica and Zostera marina*) in Padilla Bay, Washington. MS. Thesis. Western Washington University.

Areas of focus for year two

- Repeat experiments, controlling for and measuring pore water pH.
- Better understand the effects of eelgrass on sulfide concentration and sediment chemistry
- Examine the effects of sulfide on vegetative growth in *Zostera marina*
- Explore implications for coastal resource management and restoration
- Develop stage-based population dynamics model for *Zostera marina*

References

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