Impacts of the Control of Exotic Eelgrass on Native Eelgrass in Willapa Bay, Washington. Evaluation of Property Buffer Width One-year Post-application

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April, 2016

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1.0 EXECUTIVE SUMMARY

In April 2014 a general NPDES permit was issued by the Washington State Department of Ecology (Ecology) authorizing application of the herbicide Imazamox to aid in the control of the class C Noxious weed Japanese eelgrass (*Zostera japonica*) within commercial clam beds (excluding geoduck) in Willapa Bay, Washington (Ecology 2014). A permit condition prohibits the application of Imazamox after the third year, and based on a study set forth in Appendix B to the Fact Sheet of the NPDES permit and monitoring results, Ecology will make a determination to modify the permit to allow continued application of Imazamox or terminate the permit after the third year. The study in Appendix B to the Fact Sheet requires monitoring impacts to adjacent native eelgrass (*Zostera marina*) following protocols developed in 2013 (Grue et al 2013) to statistically detect a 20% reduction in native eelgrass stem density or cover index ($p \leq .10$, power=.80). During development of the study protocols in 2013, control and treatment plots were selected in collaboration with the Willapa Grays Harbor Oyster Growers' Association (WGHOGA), WDFW, WDNR, Washington State University, and the University of Washington (Figure 1). Study transects were placed at least 10 meters from herbicide application areas and aligned to the landward and seaward margins of the study plots. Stem density and cover index of native eelgrass were collected in each of 540 quadrats along these transects on May 24-26, 2013 corresponding to the anticipated time of year that herbicide applications would occur. Thirty days after initial data were collected, surveyors returned to the site to collect density and cover data. Data collection during this survey was conducted in only half of the quadrats based on the determination (using the previously-collected data set) that only half the number of samples was necessary to provide results meeting the statistical requirements of the project (Grue et al 2013). These two datasets were used to determine if the proposed study design could detect a 20% change in native eelgrass stem density and native eelgrass cover index at the prescribed levels of certainty ($p \leq .10$, power = .80). Power analysis and analysis of variance of the data led investigators to conclude that, assuming the measured metrics will go down, a 20% change was detectable at a $p \leq .10$ and a power of .80 (using a one-tailed t-test and three study plot pairs).

The study design was subsequently applied in May-June 2014 when eelgrass stem density and cover index data were collected along the same study transects where data were collected in 2013. Data were collected immediately prior to, and approximately 30 days after, Imazamox application inside the test plots. A one-tailed t-test was applied to seaward transects and a two-tailed t-test was applied to landward transects to determine the significance level of the detected changes. Analysis of the resulting data was presented in a report submitted in October, 2015 (Grue et al. 2015) and showed that there were no statistically significant reductions in native eelgrass stem density or cover index. Appendix B to the NPDES Permit Fact Sheet states: "The study will be complete when a studied buffer width returns a determination of non-significance for the stem density metric."

The October, 2015 report states the cover index along the lower elevation transect in one of the study plots showed a measured 22.6% reduction, but the measurements did not meet the *a priori* requirement that the p-value must be less than .10 for the change to be considered significant. Nonetheless, the authors presented the following hypothesis: "Although the observed reduction in percent cover on the lower elevation transects was not statistically significant, the fate of the affected plants (reduced green [live] shoots) is not known, and one should be careful in assuming that the observed reduction in percent cover would not ultimately translate to a reduction in stem density."

Surveys were repeated in May 2015, one year after the application of Imazamox, to test the hypothesis that reduction of cover index 30 days after application of Imazamox might lead to measurable reductions in stem density one year later. No statistically significant reductions in native eelgrass stem density were detected. This report presents the methods and findings of the 2015 surveys with a comparison to 2014 findings presented in Grue et al 2015.

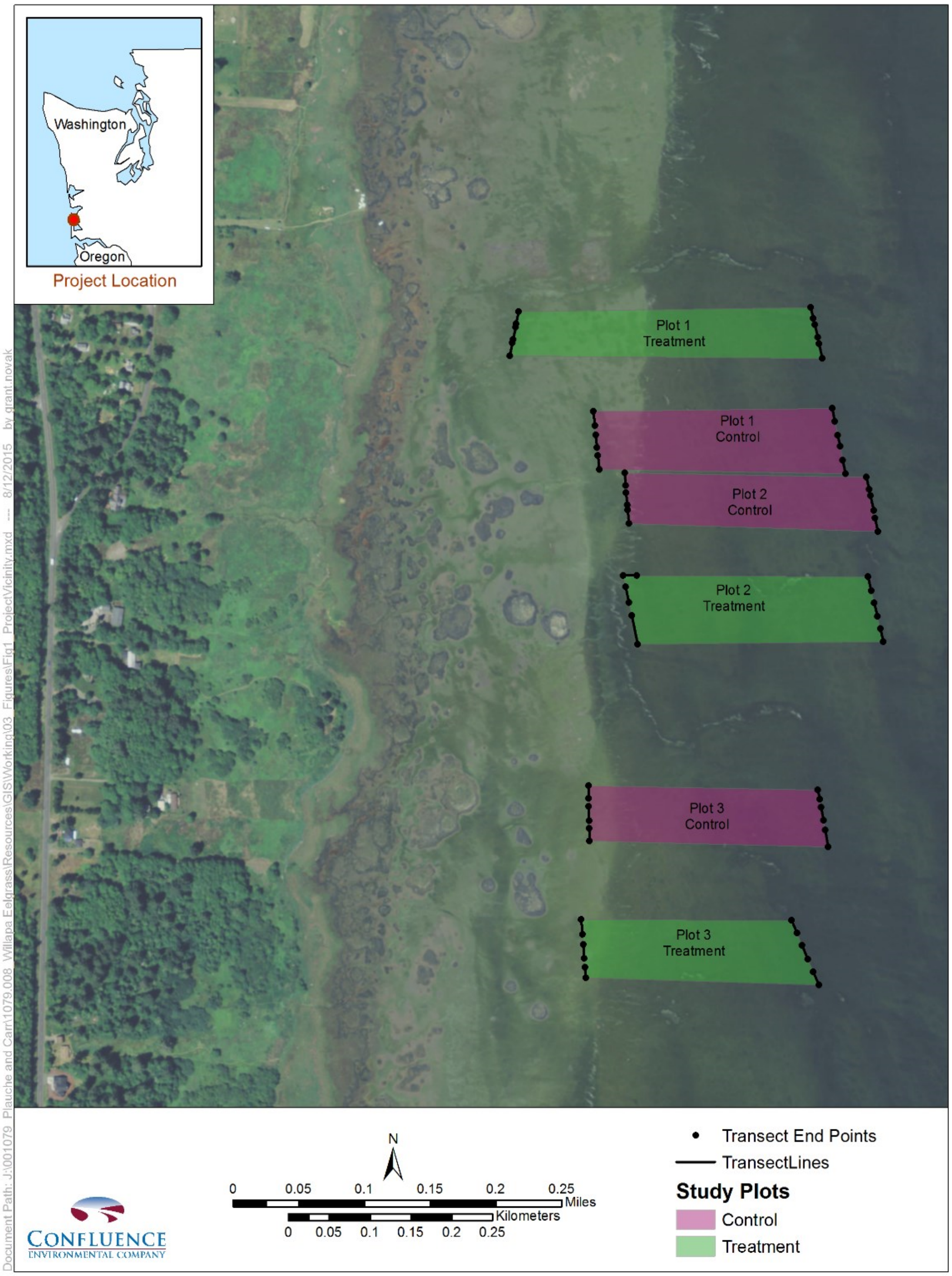

Figure 1. Study Site Vicinity

2.0 STUDY OBJECTIVES

This study is intended to investigate the hypothesis that leaf coverage reductions detected approximately 30 days after Imazamox application could ultimately translate to a reduction in stem density.

3.0 METHODOLOGY

Methods of the 2015 surveys followed those outlined in Appendices B and C to the Fact Sheet of the NPDES permit issued by Ecology for the treatment of *Zostera japonica* in commercial clam beds in Willapa Bay, Washington (Ecology 2014). The methods repeated those that had been previously applied at the site in 2013 and 2014 (Grue et al 2013, 2015).

On May 19-20, 2015 a team of professional biologists familiar with the marine ecosystems of Washington State counted eelgrass stem density at each of the study plots that had been surveyed in 2013 and 2014. PVC pipe that had been used to mark transect end points in 2014 was still present making identification of study plots and survey transects a straightforward endeavor. Three transects are located along each of the landward and seaward margins of the study plots. One transect, at the landward side of Treatment Plot 2, was laid out perpendicular to the other transects in order to be perpendicular to tidal flow (Grue et al 2013, 2015) (Figure 1). A tape measure was stretched along each transect between the transect end points to guide the placement of fifteen evenly-spaced .25m^2 quadrats. Native eelgrass stems emerging from the substrate within each quadrat were counted to quantify stem density.

Digital photographs were taken of at least three quadrats along each transect to provide photo-validation of at least 20% of the sampled locations.

4.0 RESULTS

Stem density data are categorized by study plot pair (1, 2, or 3), position relative to the test plot (i.e. seaward vs. landward), and plot type (test or control). The following tables report a comparison between data collected immediately prior to the application of Imazamox in 2014 (t_0) to data collected in 2015 (t_{365}), approximately one year after the application of Imazamox.

Positive percent values indicate an increase in that metric from 2014 to 2015 while negative percent values represent a decrease.

4.1 *Stem Density*

4.1.1 Landward

Table 1. Landward Transect Results: *Mean Stem Density, and Percent Change Between Time₀ (May 14-17, 2014) and Time₃₆₅ (May 19-20, 2015).*

	Plot Pair	Control Mean t_0	Control Mean t_{365}	Difference (%) (from t_0)	Treatment Mean t_0	Treatment Mean T_{360}	Difference (%) (from t_0)	Difference Between Treatments	Mean Difference/ p-value*
Stem Density	1	32.2	12.2	-62%	50.2	21.6	-57%	4.9%	
Stem Density	2	32.2	15.8	-51%	23.7	12.0	-49%	1.5%	
Stem Density	3	19.7	10.3	-48%	24.3	17.4	-28%	19.4%	8.6% / .257

* two-tailed t-test

One year after herbicide application, changes in stem density within the landward transects ranged from -48% to -62% in the three control plots and from -28% to -57% in the three treatment plots. The difference in change between control and treatment plots one year after Imazamox application ranged from 1.5% to 19.4%. The mean difference in change from t_0 to t_{365} between control and treatment plots was 8.6% (Table 1) (p-value = .257, two-tailed t-test). This indicates that, while stem density went down in all landward study plots, the reduction within the treatment plots was not as great as the reduction within the control plots. This represents a minor (less than 20%), statistically insignificant (p greater than .100) increase in eelgrass stem density index along landward transects.

4.1.2 Seaward

Table 2. Seaward Transect Results: *Mean Stem Density, and Percent Change Between Time₀ (May 14-17, 2014) and Time₃₆₀ (May 19-20, 2015).*

	Plot Pair	Control Mean t_0	Control Mean T_{365h}	Difference (%) (from t_0)	Treatment Mean t_0	Treatment Mean T_{360}	Difference (%) (from t_0)	Difference Between Treatments	Mean Difference/p-value *
Stem Density	1	18.0	17.6	-2%	27	21.2	-21%	-19.2%	
Stem Density	2	24.1	17.1	-29%	30.4	19.1	-37%	-8.1%	
Stem Density	3	18.2	17.6	-3%	20.8	21.5	3%	6.5%	-6.9% / .450

* two-tailed t-test

One year after herbicide application, changes in stem density within the seaward transects ranged from -2% to -29% in the three control plots and from 3% to -37% in the three treatment plots. The difference in change between control and treatment plots one year after Imazamox application ranged from 6.5% to -19.2%. The mean difference in change from t_0 to t_{365} between control and treatment plots was -6.9% (Table 2) (p-value = .450, two-tailed t-test) indicating that, while both increases and decreases were measured along seaward transects, the ultimate change was a reduction in stem density of 6.9%. This represents a minor (less than 20%), statistically insignificant (p greater than .100) reduction in eelgrass stem density index along landward transects.

5.0 DISCUSSION

Surveys conducted in 2014 concluded that no significant decrease in stem density had occurred in treatment plots 30 days after Imazamox application. Stem density appeared to have increased in landward treatment plots relative to control plots (+4%) but decreased in seaward treatment plots relative to control plots (-.8%) (Grue et al. 2015).

The results of 2014 surveys did not measure a significant decrease in stem density, and Appendix B to the NPDES Permit Fact Sheet states: "The study will be complete when a studied buffer width returns a determination of non-significance for the stem density metric." Nonetheless, the authors of the October, 2015 report (Grue et al. 2015) hypothesized that a reduction in leaf cover may lead to future reduction in stem density.

Surveys in 2015, one-year after the application of Imazamox, indicate that the 22.6% reduction of cover index measured in seaward plots in 2014 did not result in significant stem density reduction in seaward plots in 2015. A 6.9% reduction in seaward stem density was measured in 2015 (Table 2). This reduction is neither above the 20% permit threshold nor is the result statistically significant as the p-value is well above the .100 permit threshold (p-value = .450). Additionally, the 13.1% reduction of cover index measured in landward plots in 2014 did not result in reduced stem density in landward plots in 2015. In fact, an 8.6% increase in stem density was measured in landward plots in 2015. This 8.6% increase is not statistically significant as the p-value is well above the .100 permit threshold (p-value = .257).

2014 results did not show statistically significant reductions of a high enough magnitude to warrant additional studies. However, in order to test the hypothesis that cover index reductions might lead to stem density reductions, shellfish growers implemented further studies of their own accord in 2015. The results of those additional studies, presented above, clearly indicate that cover index reductions did not lead to statistically significant stem density reductions of a large enough magnitude to warrant additional studies.

6.0 REFERENCES

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