Final Report

Impacts of Imazamox on Native Eelgrass Following Application to Control Exotic Eelgrass in Willapa Bay, Washington: An Evaluation of Buffer Width

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Executive Summary

In 2012, the Washington State Noxious Weed Control Board listed Japanese eelgrass (*Zostera japonica*) as a Class C weed allowing the shellfish growers within Willapa Bay to request a NPDES permit to use herbicide to control the grass on commercial clam (*Ruditapes philippinarum*) beds. A NPDES Permit allowing the use of the herbicide for this purpose was issued by the Washington Department of Ecology in April 2014. A condition of the permit was the monitoring of impacts to adjacent native eelgrass (*Zostera marina*). The specified monitoring protocols were developed in 2013 to statistically detect a 20% reduction in either shoot density or percent cover ($p \leq 0.10$) based on assumptions of the presence and characteristics of the plant. We applied the proposed monitoring design in May-June 2014, prior to and ca. 30 days after operational applications of the herbicide to the same clam beds on which the monitoring design was tested. We found that only the percent cover of native eelgrass on the lower elevation transects was reduced in excess of 20% (-22.6%) compared to controls and this difference approached statistical significance (p=0.122). Reductions in percent cover among the three treated beds varied between -4 and -50%. This difference was also apparent when only 7 of the 15 quadrats per transects on the treated and control transects were used in the analysis (-26.7%, p=0.110) with reductions among treated beds of 6 to 56%. The maximum distance of overt visual off-site impacts to native eelgrass from the

lower elevation borders of the treated beds exceeded the 10-m buffer zone on two of the three treated beds (14.3 and 20.1 m). 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.

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Background and Justification

In 2012, the Washington State Noxious Weed Control Board listed Japanese eelgrass (*Zostera japonica*) as a Class C weed allowing the shellfish growers within Willapa Bay to request a NPDES permit from the Washington Department of Ecology (WDOE) to use an herbicide to control the grass on commercial Manila clam (*Ruditapes philippinarum*) beds. A NPDES Permit allowing the use of the herbicide imazamox for this purpose was issued by the Washington Department of Ecology in April 2014 (WDOE 2014a). A condition of the permit is monitoring of impacts to adjacent native eelgrass (*Zostera marina*). The specified monitoring protocols were developed in 2013 to statistically detect a 20% reduction ($p \leq 0.10$, power = 0.80) in either percent cover or shoot density (Grue et al. 2013). We applied the proposed monitoring design in May-June 2014, prior to and ca. 30 days after operational applications of the herbicide to the same clam beds on which the monitoring design was tested.

Objectives

Using the same study beds on which the monitoring protocols were tested, determine the impacts of operational applications of imazamox on shoot density and present cover of *Z. marina* on the upper tidal elevation and lower tidal elevation ends of the beds outside of a 10 m buffer as prescribed in the NPDES permit (WDOE 2014a,b).

Study Site Selection

Study sites were selected on 25 and 26 April 2013 among ca. 1,000 ac of clam beds in Willapa Bay managed by Taylor Shellfish Farms on the Long Beach Peninsula near Oysterville, WA. Study site selection criteria included (1) commercial clam beds of similar size, tidal elevation, and sediment characteristics in need of removal of *Z. japonica*, (2) operational/commercial size (5-20 ac), (3) significant cover by *Z. marina* 10 m from the beds on both the lower and upper elevation ends, (4) tidal flow (ebb and inundation) that moved in the direction of the lower and upper ends of the beds increasing the potential for off-site impacts of herbicide application on non-target *Z. marina*, and (5) assignment of treatments (control, treated [herbicide]) that minimized the potential for cross contamination (i.e., movement of herbicide onto control plots). The ability to use the selected study plots (beds) for at least 2 years to study the ecosystem impacts of *Z. japonica*, including effects on Manila clam culture, on a commercial scale was an additional factor in selecting the acreage managed by Taylor Shellfish Farms.

Within the acreage available, 6 paired plots (3 control + 3 treated) were selected that were ca. 5 ac in size (Fig. 1, Appendices 1 and 2). Although a systematic assignment of treatments was desired, water flows within swales that were associated with the desired presence of native eelgrass on the lower and upper ends of the plots prevented this from occurring (Fig. 1). Growers, agency representatives (WDOE, WDFW, and WDNR), and researchers from the University of Washington, Washington State University, and USDA-ARS met on site on 30 April 2013 to review the concerns associated with *Z. japonica* in the Bay, the monitoring design in the draft NPDES permit, and the selection of study plots.

Figure 1. Location of paired study plots near Oysterville, WA (upper left). Markers indicate the GPS locations of the corners of the plots and the location of each transect. T = treatment, C = control. Sizes of the plots were between 4.5 and 5.7 ac.

Monitoring Design

The design provided in the NPDES permit to quantify the off-site impacts of herbicide application to control *Z. japonica* included the placement of three 50-m transects 10 m from the upper and lower elevation ends of the study plots (control and treated). Each transect contained 15 0.25 m^2 quadrats in which the cover and shoot density of *Z. marina* were to be determined (Fig. 2). Transects were to be of equal distance from each other such that the array began or ended at a corner of the bed. On 23 May 2013, transects were permanently marked with 0.75 inch PVC pipe. Dye tests were conducted to ensure flow onto the transects should the herbicide move off-site post application. In one case, the transect array was adjusted to be in line with the flow of inundation water (Fig. 1; T2, upper elevation).

In establishing the study plots, it was not apparent that some of the upper elevation boundaries were not on property owned by Taylor Shellfish Farms (Appendices 1 and 2). Permission was obtained from the owners of property on the treatment beds other than Taylor Shellfish Farms. However, in one case, the first transect on the upper elevation NW corner of T3 was moved to be perpendicular to the upper bed boundary and 10 m away from the property of Connie Underwood because permission was not received in time before the scheduled application of the herbicide (Appendix 1).

Study Plot Characteristics: Native Eelgrass Cover and Shoot Density

Cover and shoot density of *Z. marina* were determined in each of the 540 quadrats on 24-26 May 2013 corresponding to the time herbicide applications to control *Z. japonica* would be conducted in 2014 should the NPDES permit be approved (Grue et al. 2013). The objectives

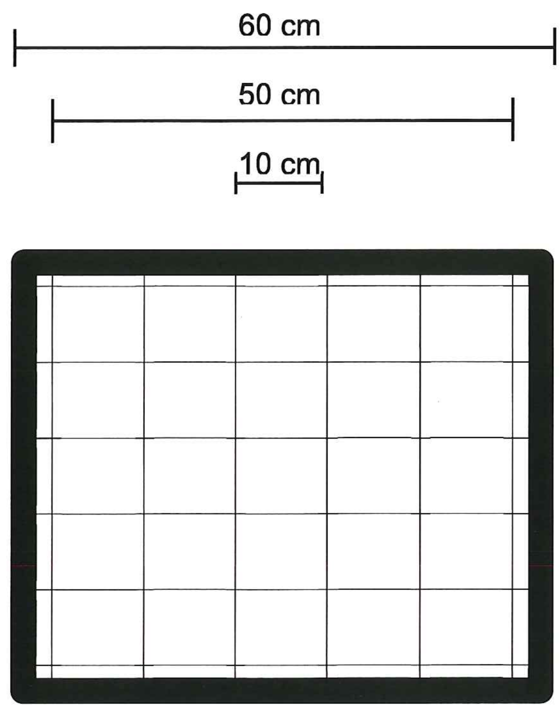

Figure 2. Sampling frame (0.25 m^2) used to determine percent cover and shoot density of *Zostra marina*. Cover was quantified by counting the number of line intersections (n = 36) formed by the 25 10-cm cells under which live (green) *Z. marina* was present (potential values = 0-36 with 36 = 100% cover). Shoot density (number live shoots) was determined by counting all present within the 0.25 m^2 frame (outer line boundary).

of this pre-sampling were (1) evaluate the feasibility of conducting the monitoring including effort required (cost to the growers), (2) demonstrate the presence and extent of *Z marina* within the transects on the ends of each bed, and (3) allow for a determination of change in the two endpoints, percent cover and shoot density, between pre-herbicide application (end of May) and 30 days post application, when off-site impacts to *Z. marina* would be expected to have occurred according to the monitoring plan in the then draft NPDES, and (4) confirm that the monitoring design would meet the specified statistical requirements (Appendix 3). In addition, sediment was collected from the center of each plot to ensure similarity among the study plots (Grue et al. 2013). Sediments were very similar among the study plots with greater than 93% of the samples in the "sand" size category (75-4750 microns) by dry weight. Further breakdown of the "sand" category was also similar among plots (Grue et al. 2013).

As in 2013, cover was quantified by counting the number of line intersections (n = 36) formed by the 25 10-cm cells within each 0.25 m^2 sampling frame (Fig. 2) under which live (green) *Z. marina* was present (potential values = 0-36 with 36 = 100% cover). Shoot density (number live shoots) was determined by counting all present within the 0.25 m^2 frame (outer line boundary; Fig. 2, Appendix 4). Digital photographs were taken of three randomly selected quadrats (20%) on each transect to provide photo-validation. Measurements were conducted on 14-17 May 2014 (pre-spray) and again on 12-14 June 2014 ca. 30 days post spray. Average initial values for each endpoint across the three

Table 1. Descriptive statistics (mean [Av], SD) for stem density and percent cover for the top (upper elevation) and bottom (lower elevation) ends of the control and treatment clam beds (ca. 5 ac each) near Oysterville, WA. Values are from 45 0.25 m^2 quadrats at the ends of each bed on 14-17 May 2014.

			Stems Av	Stems SD	Cover Av	Cover SD
Control	1	Top	32.2	11.3	23.3	5.2
Control	2	Top	32.2	11.1	27.8	3.9
Control	3	Top	19.7	10.0	15.1	6.4
Treatment	1	Top	50.2	16.7	29.7	4.3
Treatment	2	Top	23.7	9.7	22.3	7.4
Treatment	3	Top	24.3	11.0	19.9	6.6
Control	1	Bottom	18.0	7.5	10.9	3.9
Control	2	Bottom	24.1	8.5	21.2	6.2
Control	3	Bottom	18.2	4.6	11.3	4.9
Treatment	1	Bottom	27.0	10.5	15.1	4.9
Treatment	2	Bottom	30.4	8.2	26.6	6.3
Treatment	3	Bottom	20.8	6.4	18.1	5.8

transects at the ends of each bed are given in Table 1; comparable values for 30 days post application are given in Table 2.

Imazamox Applications

Imazamox (Clearcast®, SePRO Corporation, Carmel, IN) was applied to the treatment beds at 11.5-11.7 oz active ingredient per acre on 18 May 2014. The application was conducted by Washington State University (WSU) – Long Beach personnel using an ATV (and hand application as needed) with a 10-foot (3.0 m) spray swath and 10-15 in overlap leaving a 10 m (32.8 foot) buffer along the edges of the beds as required by the NPDES permit. Swales (areas with standing water) were not sprayed accounting for ca. 0.5-0.75 ac total that were not treated across the three beds (K. Patten, WSU-Long Beach, personal communication). The application rate used was less than the maximum allowed by the NPDES permit (16 oz ai per ac), but was similar to what the growers were using elsewhere (10-12 oz ai per ac, K. Patten, WSU-Long Beach, personal communication).

Imazamox Concentrations in Sediment

Sediment (3 pooled 10-cm deep cores) was collected at the center of each treated bed by WSU-Long Beach personnel 48 h after application following the methods specified in the NPDES permit. Whole sediment samples were kept on ice and shipped to Pacific Agricultural Laboratory (PAL, Portland, OR) where imazamox was extracted from the whole

Table 2. Descriptive statistics (mean [Av], SD) for stem density and percent cover for the top (upper elevation) and bottom (lower elevation) ends of the control and treatment clam beds (ca. 5 ac each) near Oysterville, WA. Values are from 45 0.25 m^2 quadrats at the ends of each bed on 12-14 June 2014.

			Stems Av	Stems SD	Cover Av	Cover SD
Control	1	Top	30.2	9.0	24.9	4.3
Control	2	Top	26.4	9.0	25.0	4.0
Control	3	Top	19.5	9.0	16.5	6.6
Treatment	1	Top	47.7	15.1	22.1	4.8
Treatment	2	Top	24.4	8.8	22.4	3.8
Treatment	3	Top	21.6	10.8	18.2	6.6
Control	1	Bottom	18.0	7.5	12.0	4.7
Control	2	Bottom	21.8	8.5	20.8	5.3
Control	3	Bottom	16.2	5.5	14.1	5.4
Treatment	1	Bottom	25.3	9.6	14.4	5.5
Treatment	2	Bottom	27.9	8.3	25.0	5.8
Treatment	3	Bottom	19.1	5.6	13.7	7.2

sediment and quantified using the American Cyanamid Method for HPLC-MS with a limit of quantification of 0.50 ppb (ug/kg). Concentrations within the sediment ranged between <0.50 ppb (non-detect) to 3.2 ppb wet weight (Appendix 5).

Changes in Cover and Shoot Density of Native Eelgrass Post Application

All comparisons between pre- and post-treatment values were separated for upper and lower elevation transects because (1) movement of the herbicide and the magnitude of effects on off-site *Z. marina* may vary between elevations (i.e., ebb water movement on lower elevation transect, inundation on upper elevation transects) and (2) the separation does not affect the degrees of freedom associated with the paired t-test (Grue et al. 2013).

Upper Elevation Transects – Changes in percent cover among the three control beds varied between -10.2% and 9.7% with comparable values among the three transects on the treated beds varying between -25.4% and 0.4% (Table 3). The average difference between the changes on the upper elevation control and treated transects was -13.3% (-32.1-10.6%).

Changes in stem density among the three control beds varied between -17.8% and -1.1% with comparable values among the three transects on the treated beds varying between -10.9% and 2.8% (Table 3). The average difference between the changes on the upper elevation control and treated transects was 4.3% (-9.8-20.6%).

Table 3. Difference in percent change in measurements of cover and shoot density of native eelgrass on the upper elevation end of study plots between $Time_0$ (14-17 May 2014) and $Time_{30}$ (12-14 June 2014).

Plot Pair	Control Mean t_0	Control Mean t_{30}	Difference (%)	Treated Mean t_0	Treated Mean t_{30}	Difference (%)	Difference Between Treatments
Cover							
1	23.3	24.9	6.7	29.7	22.1	-25.4	-32.1%
2	27.8	25.0	-10.2	22.3	22.4	0.4	10.6%
3	15.1	16.5	9.7	19.9	18.2	-8.2	-17.9%
Stem Density							
1	32.2	30.2	-6.3	50.2	47.7	-5.0	1.3%
2	32.2	26.4	-17.8	23.7	24.4	2.8	20.6%
3	19.7	19.5	-1.1	24.3	21.6	-10.9	-9.8%

Lower Elevation Transects – Changes in percent cover among the three control beds varied between -2.3% and 25% with comparable values among the three transects on the treated beds varying between -24.6% and -4.6% (Table 4). The average difference between the changes on the upper elevation control and treated transects was -22.6% (-49.6--3.6%).

Changes in stem density among the three control beds varied between -11.2% and 0% with comparable values among the three transects on the treated beds varying between -8.4% and -6.5% (Table 4). The average difference between the changes on the lower elevation control and treated transects was -0.8% (-6.5-3.2%).

Table 4. Difference in percent change in measurements of cover and stem density of native eelgrass on the lower elevation end of study plots between $Time_0$ (14-17 May 2014) and $Time_{30}$ (12-14 June 2014).

Plot Pair	Control Mean t_0	Control Mean t_{30}	Difference (%)	Treated Mean t_0	Treated Mean t_{30}	Difference (%)	Difference Between Treatments
Cover							
1	10.9	12.0	10.0	15.1	14.4	-4.6	-14.6%
2	21.2	20.8	-2.3	26.6	25.0	-5.9	-3.6%
3	11.3	14.1	25.0	18.1	13.7	-24.6	-49.6%
Stem Density							
1	18.0	18.0	0.0	27.0	25.3	-6.5	-6.5%
2	24.1	21.8	-9.2	30.4	27.9	-8.4	0.8%
3	18.2	16.2	-11.2	20.8	19.1	-8.0	3.2%

Statistical Analyses – The effectiveness of the monitoring plan was based on a paired design that maximizes statistical power with the prescribed number of paired plots through the comparison of changes through time in the variables of interest between treatment (imazamox) and control plots. Using a paired design between the treatment and control plots allows one to take advantage of the fact that prior to actual treatment, two paired beds are more likely to yield similar results (as compared to beds from different pairs). This helps to ensure comparability when actual treatment occurs, and also requires fewer pairs (and thus fewer total number of plots) in order to detect a minimum percentage difference between controls and treatments with a prescribed statistical power. An objective of the design was to evaluate off-site impacts to native eelgrass at an operational/commercial scale – in this case, ca. 5 ac, which decreased the likelihood of obtaining a large number of comparable plots.

The initial proposed criteria for determining an ecologically significant effect of the herbicide on off-site native eelgrass was a 20 percent reduction in either metric at an alpha of 0.10 and a power of 0.80 using a one-sided paired t-test (Grue et al. 2013). A one-sided test was selected because of an *a priori* expectation of a decrease in the two eelgrass metrics should exposure to imazamox occur. Comparisons in 2013 among the plots to be treated and those selected as controls between May and June (ca. 30 days apart conforming to the times when pre- [t_0] and post [t_{30}] application monitoring would occur) confirmed that the design would allow for the detection of a 20 percent reduction in either metric at a power of 0.80 (Grue et al. 2013).

On the upper elevation transects, *p* values associated the mean difference between the changes in percent cover and stem density greatly exceeded 0.10 (mean difference percent cover = -13.13%, t=-1.046, *p*=0.203; stem density = 4.03, t=0.454, *p*=0.694 [two-sided t-test]). We used a two-sided t-test for stem density on the upper elevation transects because the *a priori* assumption of a decrease in the metric was not met. In both cases, differences between the imazamox treated and the control beds were not considered statistically significant.

On the lower elevation transects, the *p* value associated with the mean difference between the changes in percent cover was 0.122 (mean difference = -22.6%, t=-1.63). In comparison, the *p* value associated mean difference between the changes in stem density was 0.401 (mean difference stem density = -0.833%, t=-0.286). Although not statistically significant, given the small sample size, the inherent and observed variability in the responses of the two metrics (Tables 3 and 4), and the uncertainty in the fate of the plants suffering shoot loss, one may consider the reduction in percent cover on the lower elevation transects to be biologically significant.

Analysis of the 2013 data for the upper and lower elevation transects indicated that the number of quadrats necessary to stabilize the variance (expressed as SD) for both metrics, shoot density and cover, could be reduced by ca. 50% (7 *vs* 15 quadrats per transect) and still meet the prescribed statistical power associated with a the 20 percent reduction criterion (Grue et al. 2013). We re-analyzed the 2014 data for changes in percent cover on the lower elevation transects between the treated and control beds. Results of the paired t-tests were similar to that for all 15 quadrats per transect (mean difference = -26.73%, t=-1.76, *p*=0.110).

Table 5. Average differences in cover of native eelgrass from $Time_0$ (14-17 May 2014) To $Time_{30}$ (12-14 June 2014) on control and treatment beds 1 and 3 (both top [T] and bottom [B]) separated by transect (A, B, C). Plot pair: T=upper elevation, B=lower elevation.

Plot Pair	Transect	Control Mean t_0	Control Mean t_{30}	Difference (%)	Treated Mean t_0	Treated Mean t_{30}	Difference (%)
1T	A	22.7	24.0	5.57	29.5	20.5	-30.32
	B	20.9	22.7	8.28	29.5	23.8	-19.23
	C	26.3	28.0	6.33	30.1	22.1	-26.61
1B	A	11.7	14.6	24.43	16.2	14.6	-9.88
	B	11.8	10.2	-13.56	14.5	12.6	-13.30
	C	9.1	11.1	22.06	14.7	16.1	10.00
3T	A	19.3	18.9	-2.07	23.7	23.3	-1.97
	B	16.2	18.4	13.58	18.3	17.5	-4.36
	C	9.7	12.3	26.90	17.5	13.9	-20.53
3B	A	9.2	11.5	25.36	22.0	6.7	-69.39
	B	9.3	12.1	30.00	19.1	21.0	10.14
	C	15.3	18.7	21.74	13.3	13.3	-0.50

We examined the change in percent cover between the control and treated beds on the individual upper and lower elevation transects on paired plots 1 and 2 (Table 5) to highlight the variability in differences among transects. Comparisons indicate that reductions in eelgrass cover were not restricted to lower elevation transects (e.g., see 1T, Table 5) and that impacts were associated with primary movements of water off the treated beds during and/or after application of the herbicide irrespective of tidal elevation. Whether or not differences were statistically significant depended on the variability in direction and magnitude of the changes in percent cover among the individual transects as well as the averages across the ends of the individual beds.

Additional Measurements of Off-site Impact to Native Eelgrass

To further quantify the magnitude of impacts on native eelgrass beyond the 10-m buffer, we used dual hand-held GPS units (GPSMap 78, Garmin, Olathe, KS) to mark the boundary of visual effects (loss and browning of shoots) along the perimeter of each imazamox-treated plot (Table 6). Of interest was the maximum distance of visual impact and the total area affected outside the 10-m buffer for each of the three treated beds based on averages of the positions from the two GPS units. Maximum distances varied between 0 and 66 feet (20.1 m) from the spray boundary and were greatest on the lower elevations (east) ends of the beds (Table 6). The total area of impact (acres) outside of the 10-m buffer ranged from 0 (no visible impact outside of the buffer) to 0.04 ac (Table 6). We note that in some cases, pre-application adjustments to the boundary of the area to be sprayed by the applicator resulted in buffer widths less than 10 m (32.8 feet). Based on the GPS measurements,

Table 6. Distances (feet) and areas (ac) associated with visible impacts to native eelgrass outside of the spray boundaries of study beds treated with imazamox (T1-3). Distances (feet) were based on the averages from two hand-held GPS units operated concurrently. Measurements were taken on 12 June 2014, ca. 30 days after the application of imazamox.

Measurement	T1	T2	T3
Total Study Bed Area (ac)	5.24	5.96	4.84
Total Area Sprayed (ac)	4.91	5.59	4.65
Area of Overspray Outside of Buffer (ac)	0	0.001	0.037
Maximum Distance of Impact			
North	18	21	0
South	2	15	16
East	18	47	66
West	0	3	0
Maximum Distance from Spray Boundary to Transects	21	25	37

maximum distances from the spray boundary to the monitoring transects varied between 21 and 37 feet (6.4 and 11.3 m). Whereas distances less than the specified buffer width (10 m) may have resulted in greater potential for observed impacts, the transects most impacted (T3 East, Appendix 6) had the greatest maximum distance to the spray boundary (11.3 m), and impacts were visible for an additional 10 m. The total area sprayed on each of the three beds varied between 4.84 and 5.96 ac (Table 6).

Permit Criterion for Ecological Impact: A Cautionary Note

In the documentation associated with the NPDES Permit (WDOE 2014b:66), the criteria for a determination of "significant" impact to native eelgrass is a greater than 20% reduction in stem density ($p \leq 0.10$) on either the upper or lower elevation transects across the treated beds. This criterion was not exceeded on either elevation. The average change in stem density on the upper elevation transects of the treated beds relative to the controls was positive (4.03%, t=0.454, p=0.694 [two-sided t-test]). On the lower elevation transects, the mean difference between the changes in stem density was -0.8% (t=0.286, p=0.401), far below the -20% criterion. Whereas these data suggest that reductions in stem density outside a 10-m buffer are unlikely, uncertainty exists about the fate of the plants suffering a reduction in the number of shoots due to breakage and changes in coloration (browning) following exposure to imazamox (Appendix 7). This was particularly true on the lower elevation treated transects and where off-bed flows post application were concentrated, irrespective of tidal elevation. If a reduction in shoots translates to death of the affected plants, it may be premature to conclude little if any impact on native eelgrass outside of the 10-m buffer.

The results of the present analysis apply only to the subject study plots. Monitoring in 2013, 1 year prior to the applications confirmed the sites and the monitoring design met the statistical criteria (Grue et al. 2013). The variances in the metrics between t_0 and t_{30} at other sites will dictate the ability to detect at least a 20% change following application of the herbicide.

Conclusions and Recommendations

The criteria for a determination of "significant" ecological impact to native eelgrass of a greater than a 20% reduction in stem density ($p \leq 0.10$) on either the upper or lower elevations of the treated beds were not realized. The average change in stem density on the upper elevation transects of the treated beds relative to the controls was slightly positive (4.03%) and on the lower elevation transects, slightly negative (-0.8%). Whereas these data suggest that reductions in stem density outside a 10-m buffer are unlikely, uncertainty exists about the fate of the plants suffering a reduction in the number of shoots (cover) due to breakage and changes in coloration (browning) following exposure to imazamox. This was particularly true on the lower elevation treated transects and where off-bed flows post application were concentrated, irrespective of tidal elevation. Percent cover of native eelgrass on the lower elevation transects was reduced in excess of 20% (-22.6%, p=0.122) compared to controls and this difference may be biologically significant. Reductions in percent cover among the three treated beds varied between -4 and -50%. The maximum distance of visual off-site impacts to native eelgrass from the lower elevation borders of the treated beds exceeded the 10-m buffer zone on two of the three treated beds (14.3 and 20.1 m). If a reduction in shoots translates to death of the affected plants, it may be premature to conclude little if any impact on native eelgrass outside of the 10-m buffer. To address this question, we proposed to monitor the same sites 1-year post spray (May 2015) at no cost to the growers. We believe this was the only way to determine if stem density was ultimately impacted, given the plants would have begun senescing naturally by the end of June 2014. Although there may have been difficulties in interpretation due to the effects of removing *Z. japonica* on the treated beds (e.g., changes in elevation due to sediment loss), results of monitoring 1-year later could have been considered a measure of the "total" direct and indirect effects of the herbicide applications. Unfortunately, although initially agreed upon, access to the study sites was ultimately denied by the growers.

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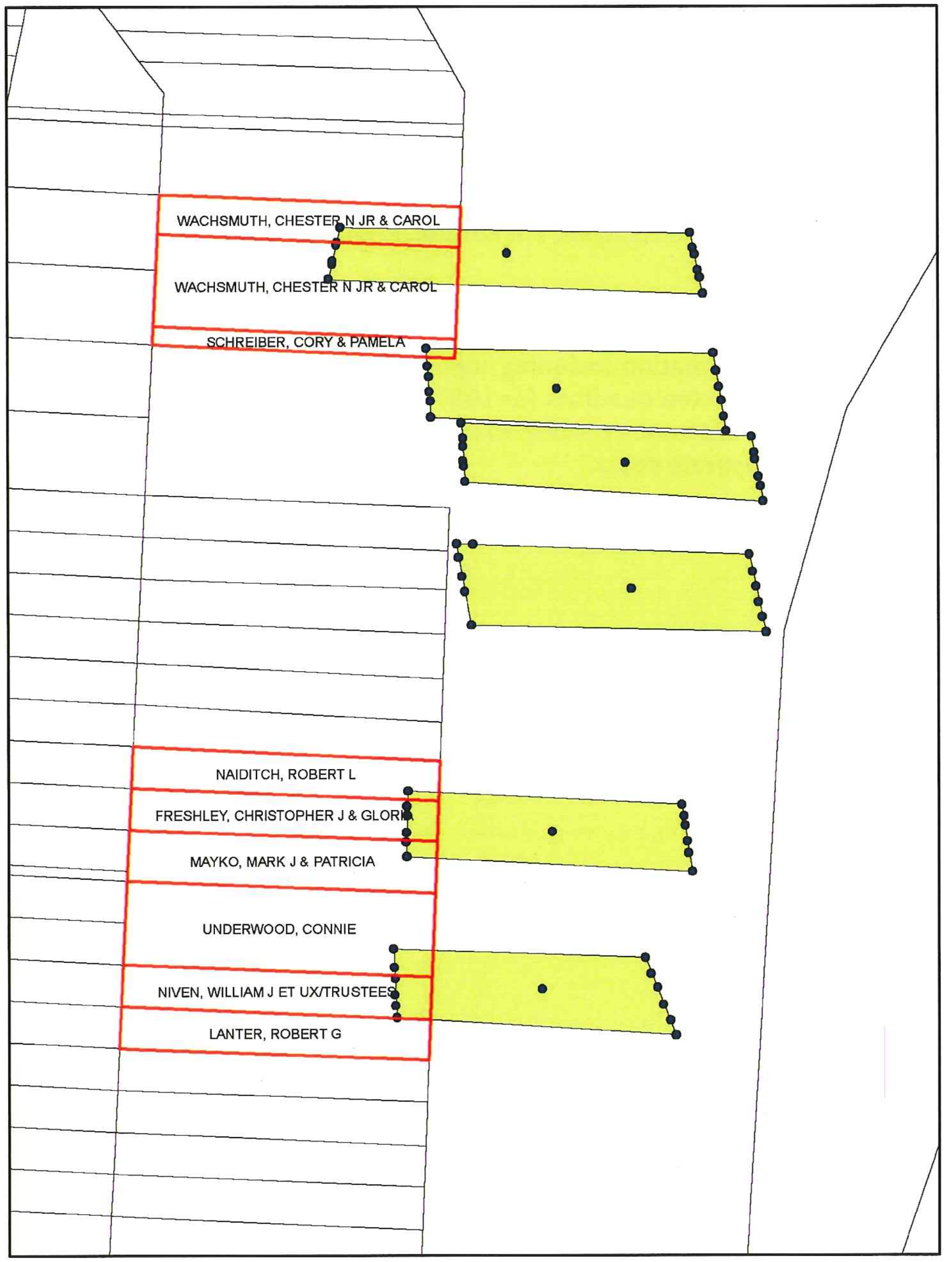
Supplementary Documentation

Supplementary documentation including digital files with all of the monitoring data, the photographs of the selected quadrats (n=108) taken during the T_0 and T_{30} sampling, and a copy of the sediment imazamox residue analysis report from PAL are provided in electronic format (CD) under separate cover.



Appendix 1. Ownership of study plots other than Taylor Shellfish Farms. The upper and lower plots were both to be treated with imazamox and have multiple ownerships and were of greatest concern.

Appendix 2. GPS locations for the initial corners of each of the study plots near Oysterville, WA used to evaluate the impacts of imazamox on off-site native eelgrass.

Treatment			Control		
Bed	Latitude	Longitude	Bed	Latitude	Longitude
1	46.545078	-124.018539	1	46.543293	-124.021881
	46.544529	-124.018341		46.543285	-124.017952
	46.544510	-124.023331		46.543991	-124.018173
	46.544991	-124.023201		46.543926	-124.021988
2	46.542149	-124.021477	2	46.543259	-124.021477
	46.542171	-124.017570		46.542713	-124.021400
	46.541470	-124.017311		46.542660	-124.017418
	46.541409	-124.021233		46.543251	-124.017616
3	46.538418	-124.022079	3	46.539867	-124.021988
	46.537788	-124.021996		46.539856	-124.018326
	46.537743	-124.018272		46.539242	-124.018150
	46.538441	-124.018723		46.539268	-124.021965

Appendix 3. Initial criteria from the WDOE draft monitoring plan and meeting with stakeholders at study sites on 30 April 2013.

Paired analysis

One-sided paired t-test *vs* time series analyses as the latter was not necessary with one time point post application and a reduction in endpoints expected.

End points = difference in percent change between paired control and treated sites between the two time points (T_0 and T_{30}).

End points = shoot density (number per m^2) and percent cover (not estimated); shoot length was eliminated as an endpoint.

Alpha = 0.10.

Power = 0.80.

Sensitivity = ability to detect a 20% reduction in either end point.

Analysis separated for upper and lower elevation transects.

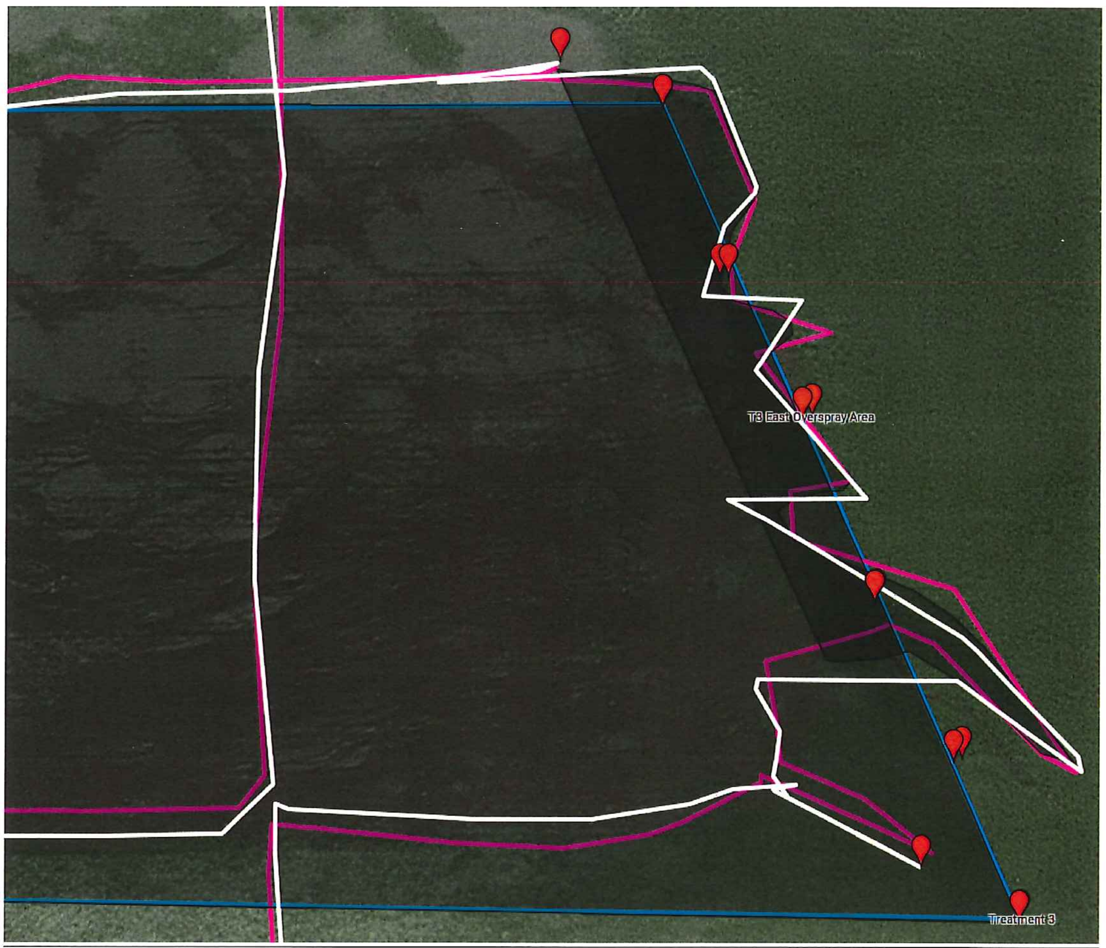
Photo validation = 20% of quadrats on each transect at Time 0 and Time 30.

Appendix 4. Photographs of a line transect and quadrat used to determine percent cover and shoot density of native eelgrass (*Zostra marina*) near Oysterville, WA in May 2013.

Appendix 5. Imazamox concentrations (ppb wet weight) in whole sediment 48 h post application at the center of the three treated beds near Oysterville, WA in May 2014. Percent recoveries = 79 and 133%. Values given are not adjusted for percent recovery. Limit of quantification = 0.50 ppb.

Treatment Bed 1	Treatment Bed 2	Treatment Bed 3
3.2	<0.50	1.5

Appendix 6. Imazamox impact zone (loss or browning of shoots, black shading) on the lower elevation of Treatment Bed 3 30 days post application (12 June 2014) mapped using two GPS units (white and pink lines) overlaid onto the boundary of the treatment bed (blue) with the 10 m buffer indicated by the area between the left black line and the bed boundary (blue line). See Table 6 for quantification of the impact zone outside of the buffer.



Appendix 7. Visible impacts of imazamox treatment on native eelgrass (*Zostera marina*) 30 days post application to control Japanese eelgrass (*Z. japonica*): A. Band of brown discolored shoots of *Z. marina* showing impact zone, B. Boundary of off-site impact on *Z. marina* within a drainage from a treated bed, C. Deformed/discolored meristem of *Z. marina* only observed on and outside of the treated beds (not quantified), D. brown discolored shoots of *Z. marina* adjacent to treated beds.