Final Report

Evaluation of Sampling Design for Monitoring Impacts of the Control of Exotic Eelgrass on Native Eelgrass in Willapa Bay, Washington

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Executive Summary
In 2012, the Washington State Noxious Weed Control Board listed Japanese eelgrass (*Zostra 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. Assuming the proposed permit is adopted, treatments would begin in May and June 2014. A condition of the permit will be the monitoring of impacts to adjacent native eelgrass (*Zostra marina*). The specified monitoring protocols were developed *a priori* to detect a 20% reduction in either shoot density, shoot length, or percent cover based on assumptions of the presence and characteristics of the plant. However, whether or not this level of sensitivity will be able to be achieved in the field with the study sites available was not known. We applied the proposed monitoring design to selected study sites in May-June 2013, during the time period monitoring would occur in 2014, to ensure that monitoring objectives would be met. Specifically, we selected appropriate study sites, performed the specified monitoring with modifications agreed upon *a priori*, and conducted a power analysis based on measurements of percent cover and stem density of native eelgrass. We found that, for the study sites monitored, the monitoring design would be able to detect a 20 percent reduction in the two metrics at an alpha of 0.10 and a power of 0.80. Surprisingly, both metrics indicated that the eelgrass had begun to senesce by the end of June; a finding that the Agency may wish to consider in deciding whether or not post treatment monitoring should occur at the prescribed 30-days post application and/or 365 days later. We note that our findings are specific to the study sites selected and suggest that the growers and the Agency utilize the same sites in 2014 should the NPDES permit be granted.

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Ecology Publication No. 13-10-054

Please cite this report as:

Grue, C.E., J.M. Grassley, and L. Conquest. 2013. Evaluation of Sampling Design for Monitoring Impacts of the Control of Exotic Eelgrass on Native Eelgrass in Willapa Bay, Washington. Washington Cooperative Fish and Wildlife Research Unit, University of Washington, Seattle, WA. 27 pp.

Background and Justification

In 2012, the Washington State Noxious Weed Control Board listed Japanese eelgrass (*Zostra japonica*) as a Class C weed allowing the shellfish growers within Willapa Bay to request a NPDES permit from the Washington State Department of Ecology (WDOE) to use an herbicide to control the grass on commercial Manila clam (*Ruditapes philippinarum*) beds. Assuming the proposed permit is granted, treatments would begin in May and June 2014. A condition of the permit will be the monitoring of impacts to adjacent native eelgrass (*Zostra marina*). Preliminary draft monitoring protocols were developed by WDOE through discussions with the Washington State Departments of Fish and Wildlife (WDFW) and Natural Resources (WDNR) with input from the growers. Protocols were designed *a priori* to detect a 20% reduction in either shoot density, shoot length, or percent cover based on assumptions of the presence and characteristics of the plant (Appendix 1). However, whether or not this level of sensitivity would be able to be achieved in the field with the study sites available was not known. WDOE suggested that study sites be selected in April and May 2013 in collaboration with the Willapa Grays Harbor Oyster Growers' Association (WGHOGA), WDFW, WDNR, Washington State University, and the University of Washington (Washington Cooperative Fish and Wildlife Research Unit, WACFWRU). The objective of the present study was to apply the proposed monitoring design to the study sites in May-June 2013, at the time monitoring would occur in 2014, to ensure that monitoring objectives would be met. This would have benefits to the growers who will be paying for the monitoring in 2014, and the State agencies that will evaluate the resulting data.

Objectives

Select appropriate study sites, perform the monitoring as described in the draft monitoring plan with modifications agreed upon *a priori*, and conduct a power analysis based on measurements of native eelgrass to ensure criteria will be met when the actual monitoring is conducted before and after herbicide applications in 2014.

Study Site Selection

Potential study sites were examined 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 *Zostra japonica*, (2) operational/commercial size (5-20 ac), (3) significant cover by *Zostra 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, Appendix 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

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.

design in the draft NPDES permit, and the selection of study plots. As a result of this meeting, shoot length was removed as a monitoring endpoint (Appendix 1).

Monitoring Design

The design provided in the draft NPDES 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).

Study Plot Characteristics

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

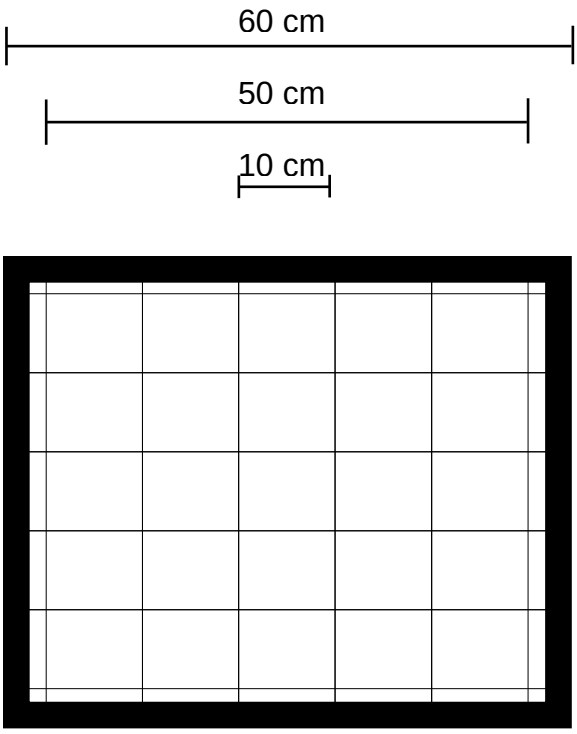


Figure 2. Sampling frame (0.25 m^2) used to determine percent cover and shoot density of *Zostra marina*. Cover was quantified by counting the number of line intersections (n = 36) formed by the 25 10-cm cells under which live (green) *Z. marina* was present (potential values = 0-36 with 36 = 100% cover).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

this report). In addition, sediment was collected from the center of each plot to ensure similarity among the study plots.

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 3). Digital photographs were taken of three randomly selected quadrats (20%) on each transect to provide photo-validation. Average initial values for each endpoint across the three transects at the ends of each bed are given in Table 1.

Frequency distributions were generated for each endpoint across the lower and upper ends of the control and treated beds (Figs. 3-6). The normality of these data and subsequent data relative to the robustness of the paired analysis is addressed later in this report.

Of interest was the strength of the relationship between shoot density and percent cover (cover index), as it may be possible to measure one of the two endpoints *vs* both as prescribed in the draft monitoring plan. The results of linear regression analyses between the two endpoints for the upper and lower elevation ends of the beds across treatments are shown in Figure 7. Correlation coefficients were 0.83 and 0.72 for the upper and lower

Table 1. Descriptive statistics (mean [Av], SD) for shoot density and percent cover for the top (high elevation) and bottom (low 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 24-25 May 2013.

			Shoots Av	Shoots SD	Cover Av	Cover SD
Control	1	Top	34.1	12.5	25.1	5.6
Control	2	Top	33.8	13.0	20.6	5.5
Control	3	Top	17.9	11.5	8.5	5.0
Treatment	1	Top	48.0	17.2	29.3	4.1
Treatment	2	Top	22.0	9.1	12.7	5.4
Treatment	3	Top	21.5	15.1	15.2	10.0
Control	1	Bottom	35.7	12.2	22.2	5.7
Control	2	Bottom	36.1	11.1	29.2	6.1
Control	3	Bottom	26.5	7.8	20.8	6.4
Treatment	1	Bottom	36.5	11.2	22.6	5.8
Treatment	2	Bottom	50.4	11.6	32.8	2.6
Treatment	3	Bottom	34.3	9.2	25.4	6.1

elevation transects, respectively; values probably too low to eliminate one of the two endpoints in the monitoring plan.

Sediment from the center of each study was collected on 26 May 2013 and characterized by Analytical Resources Inc. (ARI), Tukwila, WA using ASTM Protocol D422. Three cores (7 cm wide and 10 cm deep) were collected within 1 m of the center of each plot and combined into a single Nalgene® sample jar for each plot. The samples were kept on ice until delivered to ARI for analysis on 29 May 2013. Results of the sediment characterization are presented in Table 2. 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 (Table 2A). Further breakdown of the "sand" category was also similar among plots (Table 2B).

Effort to Conduct Monitoring

Time to determine native eelgrass cover and shoot density was collected for each transect (15 quadrats) for each of the four persons involved in the initial sampling on 24-26 May 2013 (Table 3). Upper and lower elevation ends of the six study plots (12 total) were randomly assigned such that each team member was responsible for three transect arrays (3 transects in each array, each containing 15 quadrats = 135 quadrats per person). Average time to measure both end points within 45 quadrats (across three transects on one end of a study plot) was ca. 2.75-3.0 h and varied between elevations with the upper elevation transects taking more time (Table 3).

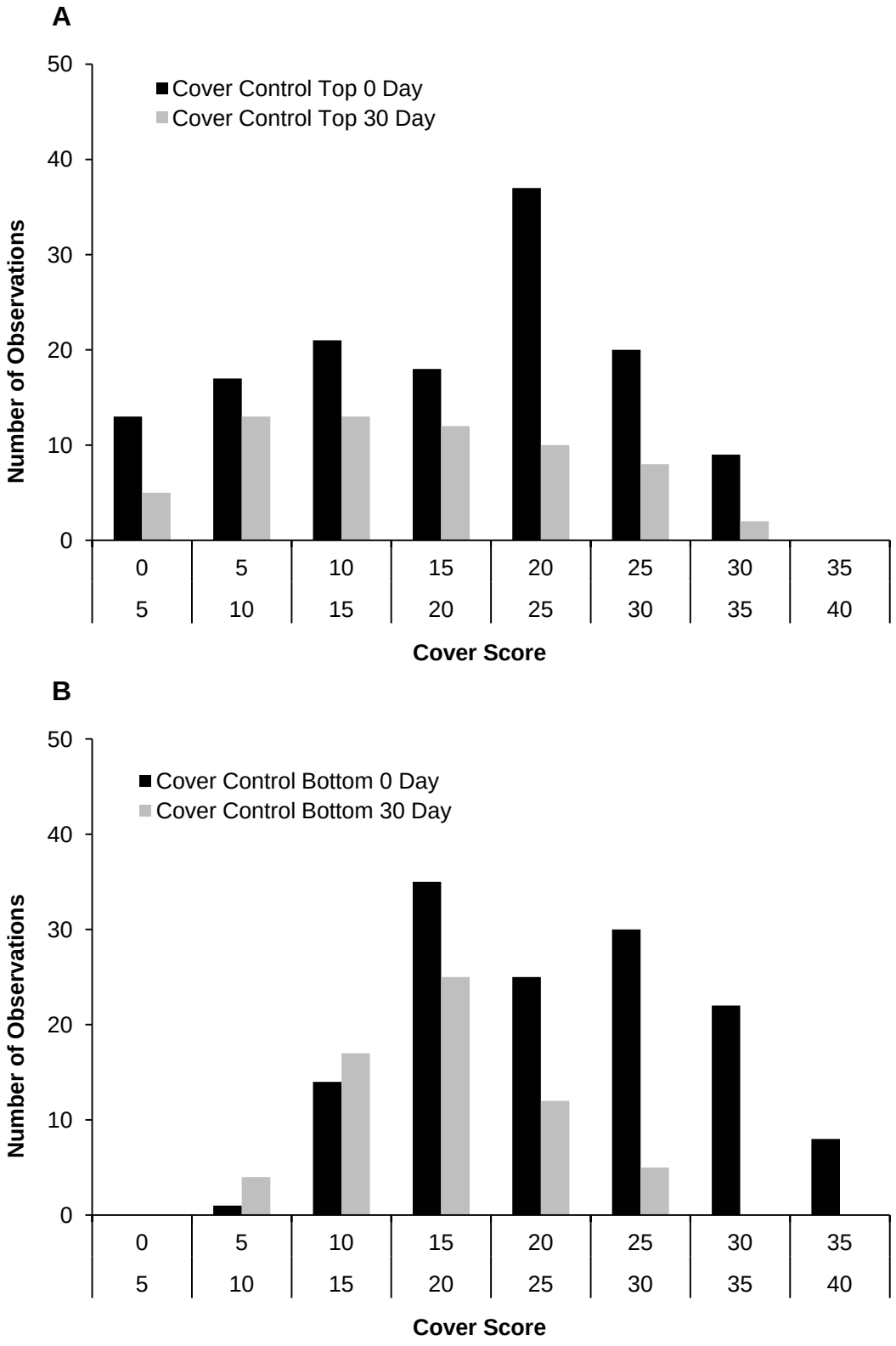
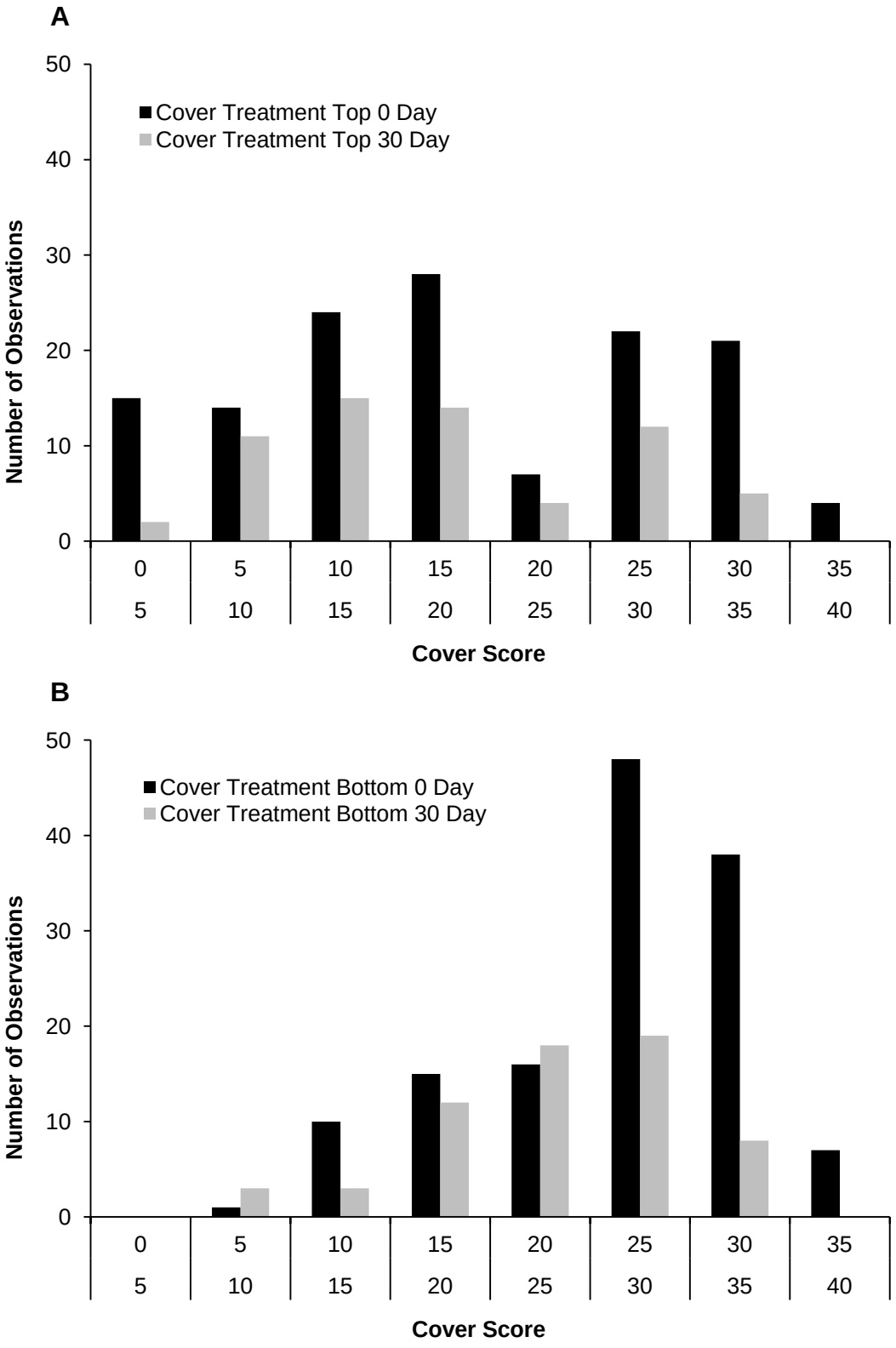


Figure 3. Distribution of cover scores (0-36) for *Z. marina* on the upper (A) and lower (B) elevation ends across the three control beds at Time 0 (black bars, n=135) and 30 days later (grey bars, n=63).



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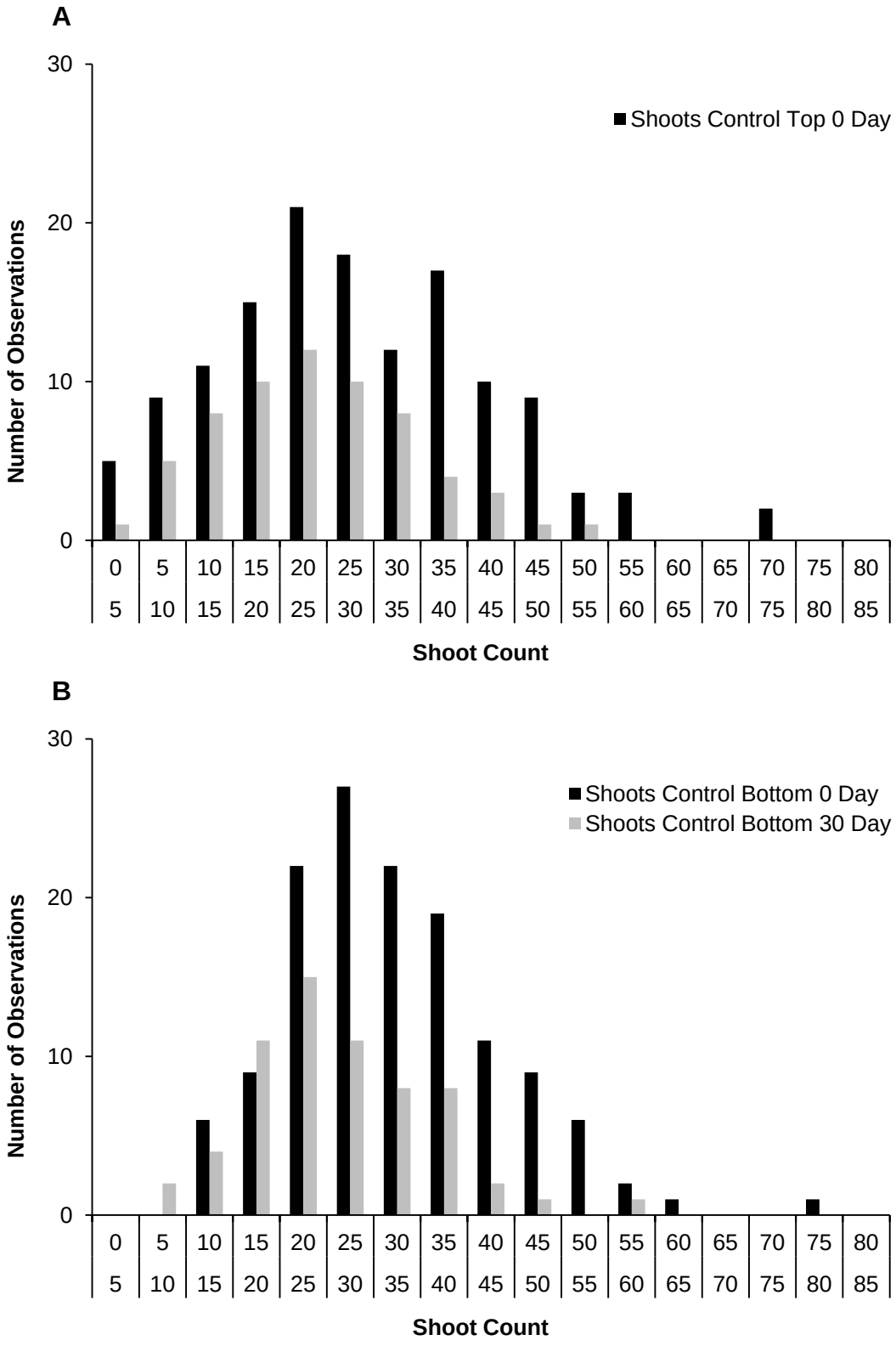

Figure 5. Distribution of shoot densities (number/m^2) for *Z. marina* on the upper (A) and lower (B) elevation ends across the three control beds at Time 0 (black bars, n=135) and 30 days later (grey bars, n=63).

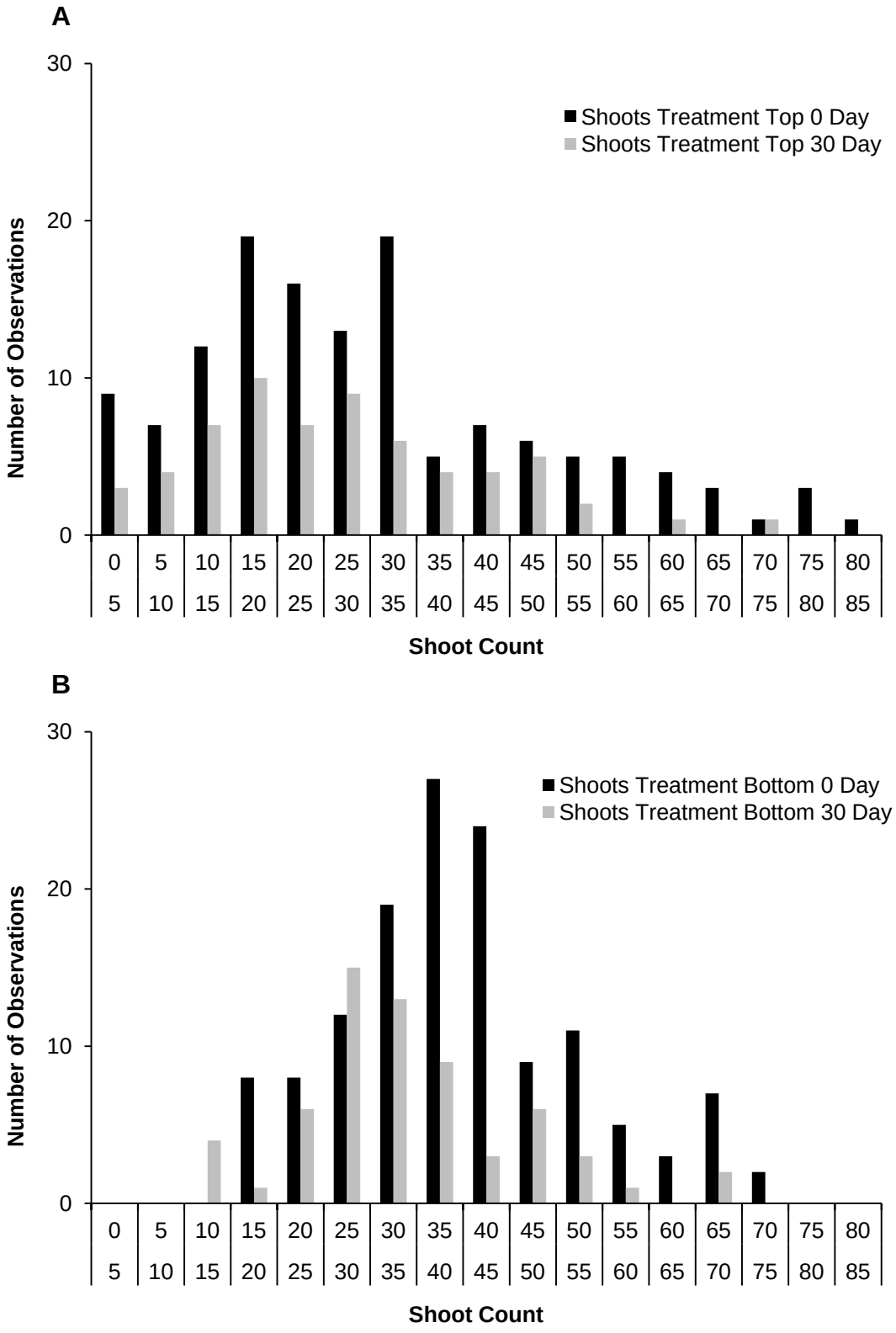

Figure 6. Distribution of shoot densities (number/m^2) for *Z. marina* on the upper (A) and lower (B) elevation ends across the three treatment beds at Time 0 (black bars, n=135) and 30 days later (grey bars, n=63).

Second Sampling 30 Days Later

Estimates of the variance associated with the percent change in each endpoint on the end of each study plot is required to calculate the statistical power of the paired analysis (paired t-test, n = 3 [paired plots]). The difference between $Time_0$ (t_0) and $Time_{30}$ (t_{30}) for each endpoint expressed as a percent of t_0 for upper and lower elevation ends of the control and treated beds would be compared. We recommend a separate analysis for the upper and lower elevations 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. In order to determine the number of quadrats among the three transects that would need to be resampled to provide a variance comparable to that in the t_0 values (45 quadrats per end of study plot), we plotted the standard deviations (SD) associated with different sample sizes (number of quadrats) for each of the endpoints for the ends of each study plot using the t_0 values for each quadrat (Fig. 8). Quadrats within a three transect array (n=45) were randomized and a SD calculated for the first two values, the first three, … to a total of 45. This process was repeated five times and each time the number of transects required to stabilize the variance was recorded. The resulting averages (Table 4) indicated that 21 quadrats were necessary for re-sampling shoot density and 15 were necessary for estimating cover. We then systematically selected 7 quadrats (i.e., number 2, 4, 6, 8, 10, 12, 14) of the 15 within each transect for a total of 21 per transect array at the end of each study plot. Transects were resampled by three of the four original team members with each sampling the ones they did at t_0 and one of the three transect arrays conducted previously by the fourth team member.

Descriptive statistics from the 30-day resampling effort are given in Table 5. Frequency distributions for each endpoint on the lower and upper elevation transects at t_{30} are presented in Figures 3-6. Surprisingly, in nearly all cases there was a decrease in percent cover and shoot density from T_0. Average changes are summarized in Table 6. Cover decreased on the upper and lower elevations across the study plots 8.6 and 18.8 percent, respectively. Comparable values for shoot density were 12.9 and 16.1 percent. Visual inspection of the quadrats suggested an increase in dead (brown) leaves and broken seed (flowering) stems. Each of the members of the team commented on the observed decrease in both endpoints at the end of the 2-day monitoring period, observations that were subsequently borne out in the data analysis.

The decrease in the two endpoints suggests *Z. marina* is already senescing by the end of June when 30-day post herbicide application monitoring is scheduled to occur on these study sites. It may be that monitoring 365 days post application would be better to assess any "net loss" of native eelgrass associated with the control of *Z. japonica* on commercial clam beds in Willapa Bay. This is discussed further in a subsequent section of the report.

Power Analysis

The effectiveness of the proposed monitoring design 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 (shoot density and percent cover) 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 ensures comparability when actual treatment occurs, and also requires fewer pairs (and thus fewer total number of plots) in order to detect a stated

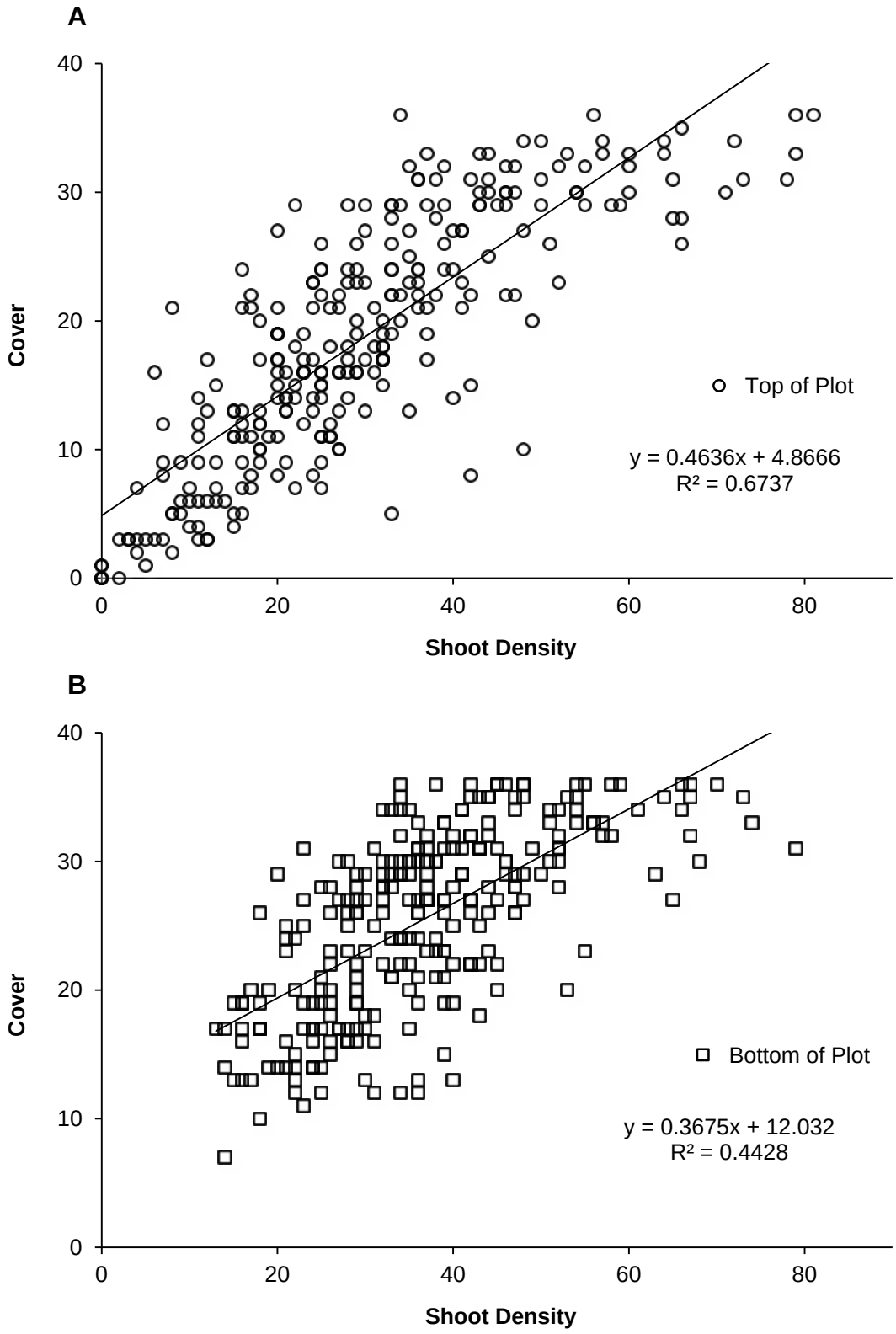

Figure 7. Correlation between shoot density and cover of *Z. marina* on the upper (A) and lower (B) elevation transects across treatments (n=270) on 24-26 May 2013.

Table 2. A. Characterization of sediments at the center of each study plot using ASTM Protocol D422: percent in particle size categories (microns) by dry weight. B. Additional breakdown of the "sand" category by particle size (microns) among study plots. C = Control, T = Treated; 1-3 = Control/Treated paired plot.

(A)

Whole Sample	**Gravel**	**Sand**	**Silt**	**Clay**	**Fines**	
Size Class (microns)						
Start	76200	4750	75	3.2	<1.3	
End	4750	75	3.2	1.3		Total
Percent Within Class						
C1	0.0%	94.3%	2.9%	0.0%	2.6%	99.8%
C2	0.0%	94.3%	2.4%	0.9%	2.2%	99.8%
C3	0.0%	93.3%	3.4%	0.9%	2.2%	99.8%
T1	0.0%	94.4%	2.8%	0.4%	2.2%	99.8%
T2	0.0%	93.1%	4.2%	0.0%	2.7%	100.0%
T3	0.0%	94.6%	3.1%	0.4%	1.7%	99.8%

(B)

Sand Fraction	**Coarse**	**Medium**		**Fine**		
Size Class (microns)						
Start	4750	2000	850	425	250	150
End	2000	850	425	250	150	75
Percent Within Class						
C1	0.0%	0.1%	0.3%	5.0%	78.2%	10.7%
C2	0.0%	0.2%	0.6%	5.6%	76.6%	11.3%
C3	0.0%	0.2%	0.4%	4.4%	77.0%	11.3%
T1	0.0%	0.1%	0.3%	4.6%	76.4%	13.0%
T2	0.0%	0.2%	0.5%	6.9%	72.9%	12.6%
T3	0.0%	0.1%	0.5%	5.4%	77.6%	11.0%

percentage difference between controls and treatments with 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, decreasing the likelihood of obtaining a large number of comparable plots.

The proposed criteria for determining a biologically 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. To test this, we compared pre-treatment values for each variable at Time 0 (t_0; shortly before herbicide would be applied should the NPDES be approved) on the paired plots treatment and control plots with comparable measures 30 days later (t_{30}; when post treatment monitoring would occur). Initial analysis of the pre-treatment data indicated that using percent change between controls and treatments (where the control and treatment values are themselves percent differences from t_0 to t_{30}) as the response variable is a reasonable approach. For both shoot density and cover, plots of t_{30} values against t_0 values

Table 3. Average time needed to determine percent cover and shoot density of *Z. marina* within 0.25 m^2 quadrats on the lower (bottom) and upper (top) elevation ends of each of the six study plots, three control and three treatment. Ends of each plot contained three transects each containing 15 quadrats for a total of 45 quadrats on each end. Values are in hours and minutes unless specified otherwise. Four persons conducted the monitoring, each sampling a total of 135 quadrats over three morning low tides (24-26 May 2013).

		Bottom	Top
Control	1	3:13	4:15
	2	2:32	3:38
	3	2:18	1:35
	Average	2:41	3:09
Treatment	1	2:51	2:34
	2	3:01	2:34
	3	2:35	3:23
	Average	2:49	2:50
Overall Average Time per Plot (h:min)		2:45	2:59
Number of Quadrats Counted		45	45
Average Quadrat Count Time (min:sec)		3:40	4:00

showed positive trends, as would be expected (a larger/smaller t_0 value tends to be associated with a larger/smaller t_{30} value). Furthermore, the treatment and control data points were intermixed, as would be expected for pretreatment data. Plots of log-transformed data displayed exactly the same phenomenon; hence we stayed with the original response variable of percent change (Fig. 9). We note that the paired t-test is highly robust to deviations from normality (Zar 2010, pp. 136-137, 181).

As previously noted, a paired design between the treatment and control beds 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 ensures comparability when actual treatment occurs, and also requires fewer pairs (and thus fewer total number of beds) in order to detect a stated percentage difference (here, 20%) between controls and treatments with 80% power. Using 3 pairs, a sample size and power analysis computation (SSPA, see Equation 1 below) was used to estimate how small a percent change could indeed be detected with 0.80 power (1-beta), using a t-test with a 1-sided significance level (since decreases are expected to be observed) of alpha = 0.10. (Zar 2010, pp. 115, 182):

[1] delta = (s/sqrt(n))*(t.alpha + t.beta), where:

delta = minimum detectable difference from control to treatment, here expressed in terms of percent change (note delta is always > or = zero)

s = estimated standard deviation associated with the differences between the control and treated plots from t_0 and t_{30} (Table 7)

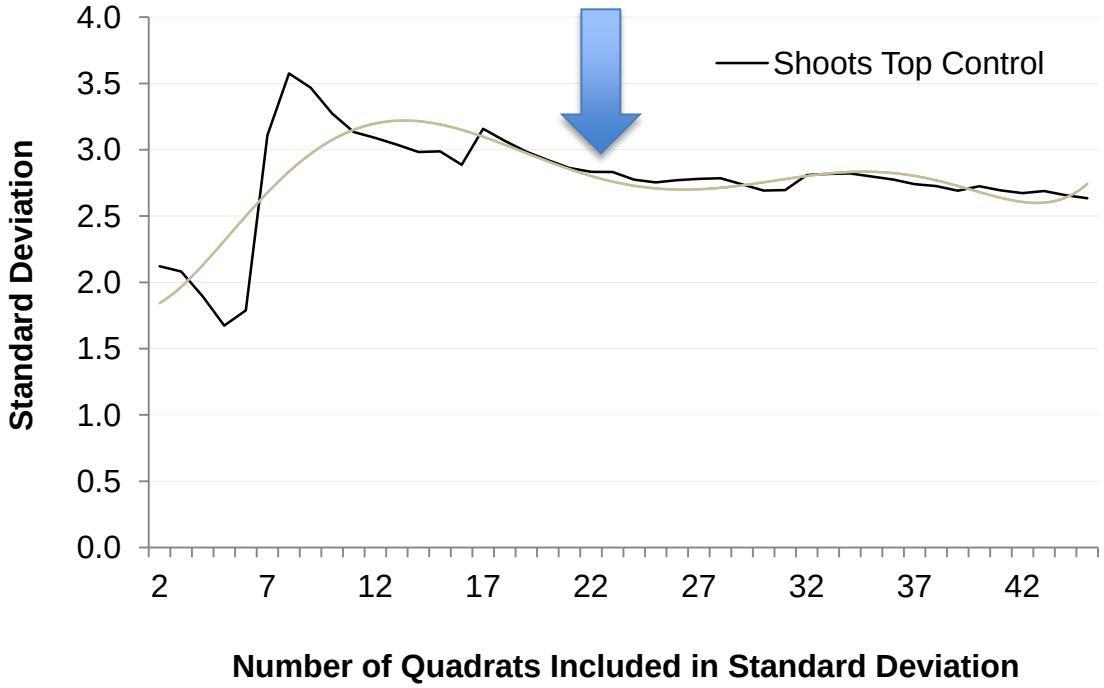

Figure 8. Example of the identification of the number of quadrats necessary to stabilize the standard deviation associated with shoot density on the end of one of the study plots. The lighter line represents a smoothing of the data. Standard deviations were generated from a unique randomization of the 45 quadrats within a transect array. In this example, the variance stabilizes at an *n* of 22.

t.alpha = t-value for alpha(1) = 0.10, df = # of pairs – 1 (t.alpha = 1.89 for n = 3 and df = 2)
t.beta = t-value for beta(1) = 0.20, df = # of pairs - 1 (t.beta = 1.06 for n = 3 and df = 2; t.beta values are always 1-sided, Zar 2010).

Response	Std. Deviation	delta (percent change) for n=3 pairs
Upper Elevation Cover	7.41	12.61
Upper Elevation Shoot Density	11.54	19.63
Lower Elevation Cover	8.51	14.48
Lower Elevation Shoot Density	7.77	13.21

Thus, with the present design of n=3 treatment/control pairs, all four of the computed values of delta are below 20%, the criteria stipulated by the agency. If one expects either increases or decreases away from the controls, then this is a more general situation, and a 2-sided statistical test is called for. (This then uses a different t.alpha value, depending upon the number of pairs). Under the same conditions as above, this will increase the minimum detectable percentage change between the controls and the paired treatment beds as follows:

Response	Std. Deviation	delta (% change), n=3 pairs	4 pairs	5 pairs
Upper Elevation Cover	7.41	17.04	12.34	10.18
Upper Elevation Shoot Density	11.54	26.52	19.22	15.85
Lower Elevation Cover	8.51	19.56	14.17	11.69
Lower Elevation Shoot Density	7.77	17.85	12.93	10.67

From the above results, if 2-sided statistical tests were called for, and the goal is to detect changes of at least 20% in either direction, then 4 pairs of study plots rather than 3 pairs are needed.

Cautionary Note

The results of the present analysis apply only to the subject study plots. Differences in the variances in the metrics at other sites will dictate the ability to detect at least a 20% change following application of the herbicide in 2014. In addition, it is assumed that the variances associated with shoot density and cover will not change between the measurements in 2013 and those 1 year later. With the exception of shoot density on the upper elevation transects, the delta's were substantially below the prescribed 20% criteria.

Landownership of Study Plots and Future Monitoring

In an effort to situate the study plots such that there was significant cover of native eelgrass on the upper elevation transects, some study plots inadvertently extended onto the property of landowners other than Taylor Shellfish Farms. This may result in owner-imposed restrictions on the application of imazamox on their property in spring/summer 2014. Two of the treatment plots (T1, T3) are impacted involving three property owners other than Taylor Shellfish Farms (Fig. 10, Appendix 4). In most cases, the acreage involved is small relative to the size of the plots, but options to shift the plots are extremely limited as they were selected to include significant native eelgrass on the upper elevation transects, which in itself is difficult to obtain. Any change will likely alter the variances and ultimately the ability to meet the prescribed statistical criteria. Permission from these landowners will need to be obtained before herbicide is applied to their lands. Because the proposed NPDES permit is restricted to "commercial" clam beds, a temporary lease of the portions of the private lands affected may have to be obtained. We suggest the growers develop a strategy for contacting the affected landowners and obtaining the necessary permissions that would include a description of the importance of the proposed monitoring to the protection of the Bay's environment, a fact sheet on imazamox, and an emphasis on the fact that the herbicide would be applied only once.

Recommendations

Based on the present analysis, the study plots selected in 2013 on lands primarily owned by Taylor Shellfish Farms near Oysterville, WA will meet the prescribed statistical criteria for documenting reductions in the endpoints of shoot density and cover of native eelgrass on upper and lower tidal elevations of Manila clam beds of commercial acreage following application of imazamox in 2014. This conclusion is based on the assumption that the variance in the endpoints will not change between the 2 years, and that monitoring will be conducted in the same manner by experienced personnel and at the same time points. To ensure this, the WACFWRU in collaboration with the WGHOGA is prepared to conduct the prescribed monitoring in 2014 before and after the application of the herbicide, conduct the necessary analyses, and report the results. Additionally, the UW (WACFWRU) is willing to work with the growers to obtain the necessary permission from the three landowners other than Taylor Shellfish Farms, on whose property two of the treatment plots impinge.

The Agency may want to consider two changes to the existing protocol. Analyses indicate 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% and still meet the prescribed

Table 4. Number of quadrats necessary to stabilize the standard deviation associated with each end point (shoot density and percent cover) for the upper (top) and lower (bottom) elevation transects of each of the six study plots. Each number represents a unique randomization of the 45 quadrats within each transect array (see Fig. 8) with the process repeated five times. C = Control, T = Treatment. Numbers (1-3) = Control/Treatment paired plot.

SHOOTS

Treatment	C	C	C	T	T	T	C	C	C	T	T	T
Pair	1	2	3	1	2	3	1	2	3	1	2	3
Position	Top	Top	Top	Top	Top	Top	Bot	Bot	Bot	Bot	Bot	Bot
	23	23	18	25	25	21	18	20	18	15	15	19
	23	24	24	29	28	25	22	25	18	12	8	19
	17	26	17	9	12	25	28	21	18	18	23	30
	18	26	18	13	9	13	23	19	23	25	20	28
	27	16	28	9	11	28	22	23	30	28	15	27
Averages	21.6	23.0	21.0	17.0	17.0	22.4	22.6	21.6	21.4	19.6	16.2	24.6
			21.9			18.8			21.9			20.1
						20.3						21.0
												20.7

COVER

Treatment	C	C	C	T	T	T	C	C	C	T	T	T
Pair	1	2	3	1	2	3	1	2	3	1	2	3
Position	Top	Top	Top	Top	Top	Top	Bot	Bot	Bot	Bot	Bot	Bot
	15	7	9	18	19	18	10	17	8	11	9	8
	20	11	18	20	7	22	11	18	14	12	9	15
	22	23	11	14	10	25	17	13	20	20	13	23
	18	20	15	19	10	18	9	22	12	10	9	28
	12	9	17	15	13	18	9	15	18	13	20	18
Averages	17.4	14.0	14.0	17.2	11.8	20.2	11.2	17.0	14.4	13.2	12.0	18.4
			15.1			16.4			14.2			14.5
						15.8						14.4
												15.1

Table 5. Descriptive statistics (mean [Av], SD) for shoot density and percent cover for the top (high elevation) and bottom (low elevation) ends of the control and treatment clam beds (ca. 5 ac each) near Oysterville, WA. Values are from 21 0.25 m^2 quadrats at the ends of each bed on 25-26 June 2013.

			Shoots Av	Shoots SD	Cover Av	Cover SD
Control	1	Top	25.9	10.4	19.5	5.2
Control	2	Top	29.2	9.0	21.4	6.2
Control	3	Top	17.4	9.2	8.6	3.6
Treatment	1	Top	41.4	13.7	24.2	5.6
Treatment	2	Top	21.7	8.6	14.1	6.3
Treatment	3	Top	19.0	10.7	14.5	8.9
Control	1	Bottom	26.9	8.1	17.9	5.0
Control	2	Bottom	32.0	10.5	17.6	3.7
Control	3	Bottom	21.6	7.7	17.7	6.1
Treatment	1	Bottom	27.0	7.8	19.5	6.9
Treatment	2	Bottom	44.4	11.8	24.1	5.0
Treatment	3	Bottom	32.3	8.3	27.4	4.4

Table 6. Average shoot density and cover of *Z. marina* on the upper (top) and lower (bottom) elevations across the six study plots on Day 0 and Day 30 and percent change between the two time points. Day 0 measurements were conducted on 24-26 May 2013; Day 30 measurements were conducted on 25-26 June 2013.

		Day 0	**Day 30**	**% Change**
Shoot Density	Top	29.5	25.7	-12.9%
	Bottom	36.6	30.7	-16.1%
			Average	-14.7%
Percent Cover	Top	18.6	17.0	-8.6%
	Bottom	25.5	20.7	-18.8%
			Average	-14.5%

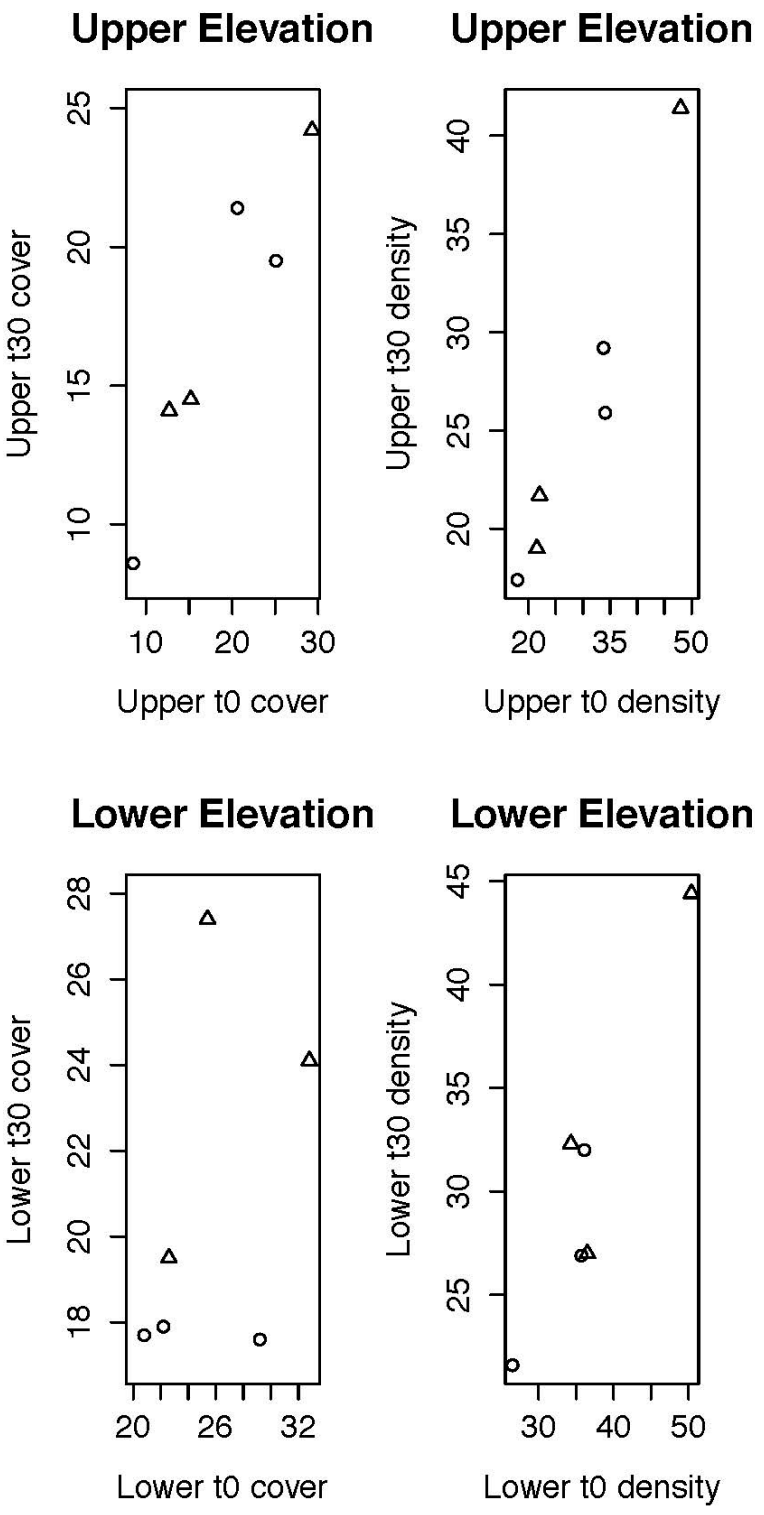

Figure 9. Correlation between Time 0 (t0) and Time 30 (t30) values for cover and shoot density of *Z. marina* on the upper and lower elevations of control (o, n=3) and treatment (Δ, n=3) study plots.

Table 7a. Difference in percent change in measurements of cover and shoot density of native eelgrass on the upper elevation end of study plots between $Time_0$ and $Time_{30}$.

Plot Pair	Contol Mean t_0	Control Mean t_{30}	Difference (%)	Treated Mean t_0	Treated Mean t_{30}	Difference (%)	Difference Between Treatments
Cover							
1	25.1	19.5	-22.4	29.3	24.2	-17.3	-5.1%
2	20.6	21.4	4.0	12.7	14.1	11.3	-7.3%
3	8.5	8.6	1.5	15.2	14.5	-5.0	6.5%
Shoot Density							
1	34.1	25.9	-24.1	48.0	41.4	-13.8	-10.3%
2	33.8	29.2	-13.4	22.0	21.7	-1.6	-11.8%
3	17.9	17.4	-2.8	21.5	19.0	-11.7	8.9%

Table 7b. Difference in percent change in measurements of cover and shoot density of native eelgrass on the lower elevation end of study plots between $Time_0$ and $Time_{30}$.

Plot Pair	Contol Mean t_0	Control Mean t_{30}	Difference (%)	Treated Mean t_0	Treated Mean t_{30}	Difference (%)	Difference Between Treatments
Cover							
1	22.2	17.9	-19.3	22.6	19.5	-13.6	-5.7%
2	29.2	17.6	-39.8	32.8	24.1	-26.4	-13.4%
3	20.8	17.7	-14.7	25.4	27.4	8.0	-22.7%
Shoot Density							
1	35.7	26.9	-24.8	36.5	27.0	-26.0	1.2%
2	36.1	32.0	-11.2	50.4	44.4	-11.9	0.7%
3	26.5	21.6	-18.5	34.3	32.3	-6.0	-12.5%

20 percent reduction criteria. This would mean that 7 or 8 *vs* 15 quadrats per transect could be sampled. Resulting standard deviations for the two endpoints were, in most cases, similar between the two sampling intensities at the two time points, T_0 (n=15 quadrats per transect) and T_{30} (n=7 quadrats per transect) (Table 8). [It important to again emphasize that a reduction in quadrats pertains only to the study sites used in the present evaluation, as variances associated with the endpoints at other locations may differ.] In either case, we recommend that the pre-treatment sampling be conducted shortly before treatment instead of immediately post treatment as prescribed in the draft monitoring plan. This is important as it eliminates the potential for physical disturbance to the eelgrass on the transects pre-treatment associated with treatment activities, as well as cross-contamination and resulting impacts to the endpoints of interest after application of the herbicide.

As noted previously, in nearly all cases there was a decrease in cover and shoot density from T_0 to T_{30} (Table 6) indicative of senescence of the plants. Cover decreased on the upper and lower elevations across the study plots 8.6 and 18.8 percent, respectively. Comparable values for shoot density were 12.9 and 16.1 percent. Visual inspection of the quadrats suggested an increase in dead (brown) leaves and broken seed (flowering) stems. Of concern is the extent of senescence that may occur in 2014 between the application of the herbicide and post-treatment monitoring 30 days later that may mask any short-term treatment effects. One approach to address this would be to conduct the post-treatment monitoring 365 days after application. While this would not allow for the detection of short-term impacts, it would identify long-term impacts that may be of greater concern. An alternative would be to conduct post-treatment monitoring at both time points: 30 and 365 days post treatment. If the sampling effort were reduced to 21-24 *vs* 45 quadrats, there would be little additional effort to conduct the monitoring at the two times post treatment. Taylor Shellfish Farms has indicated that the study plots will be managed according to the needs of the monitoring effort, and therefore would not impose additional constraints. We believe the latter option maximizes the information gathered by addressing short- and long-term impacts and the potential masking of effects by senescence of the plant by 30 days post treatment. It may be that monitoring 365 days post application would be better to assess any "net loss" of native eelgrass associated with the control of *Z. japonica* on commercial clam beds in Willapa Bay.

A decision on the importance of a distinction between direct toxic and indirect effects of the application of imazamox on native eelgrass is important. Direct toxic effects may result in a reduction in the selected endpoints 30 and/or 365 days following application resulting from the exposure of off-site eelgrass to the herbicide. Determination of direct toxic effects 365 days after application may be confounded by indirect effects on the environment on and off the study plots. Removal of *Z. japonica* on the herbicide-treated beds may alter water flows and/or depths along the study transects, particularly on the upper elevation transects. A reduction in water retention resulting from removal of the exotic eelgrass may result in a reduction in native eelgrass metrics not due to direct exposure of the latter to the herbicide. Therefore, there may be a "net loss" of off-site eelgrass, but not due to the direct exposure to the herbicide. "No net loss" needs to be clearly defined.

Supplementary Documentation

Supplementary documentation including GPS points for the corners of each study plot and the ends of each transect, digital files with all of the monitoring data and the photographs of

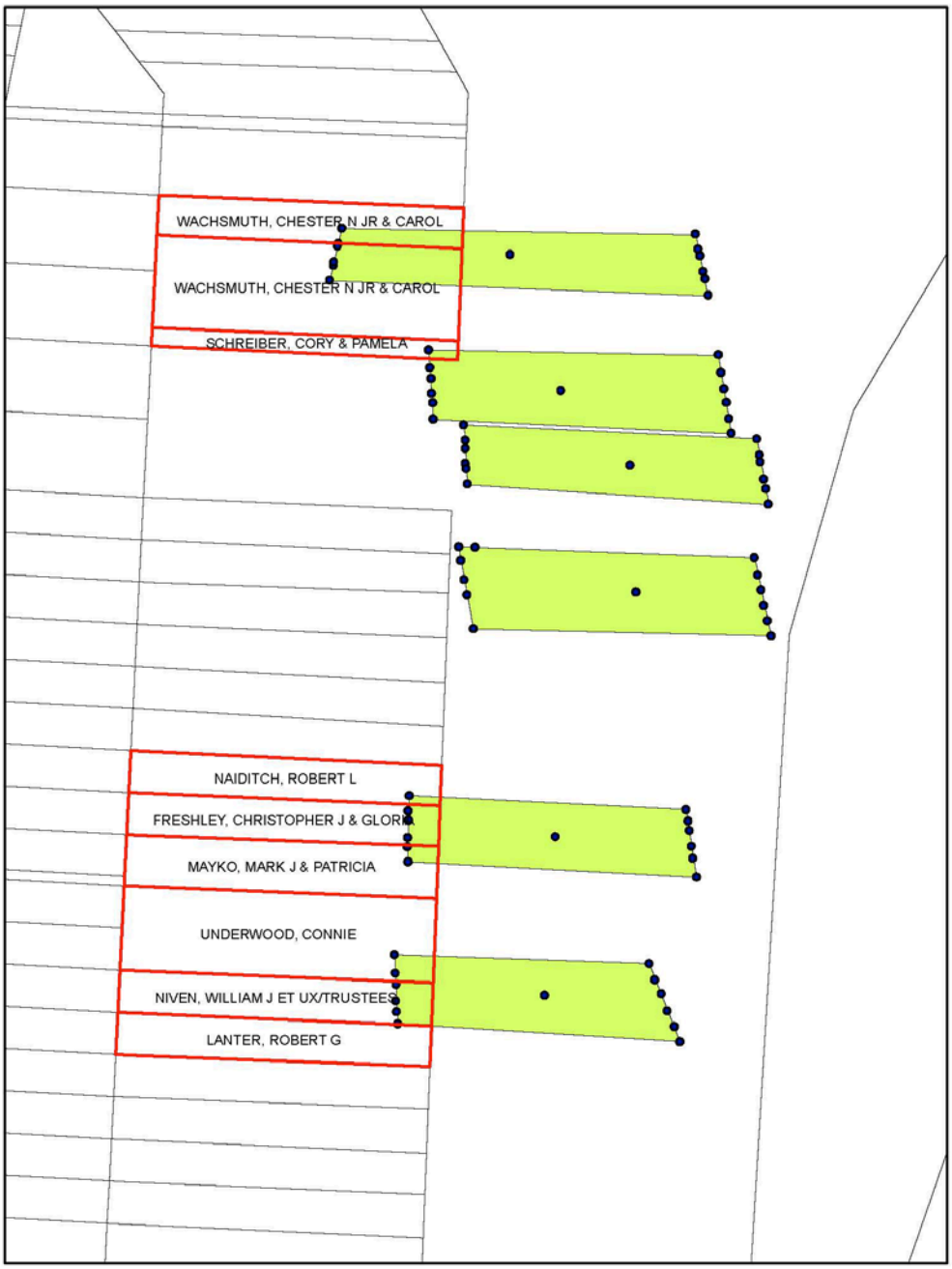

Figure 10. Ownership of study plots other than Taylor Shellfish Farms. The upper and lower plots are both to be treated with imazamox and have multiple ownership and are therefore the most problematic.

Table 8. Standard deviations (SD) associated with mean values for shoot density and cover for the top (high elevation) and bottom (low elevation) ends of the control and treatment clam beds (ca. 5 ac each) near Oysterville, WA at T_0 to T_{30}. Values are from 45 0.25 m^2 quadrats at the ends of each bed on 24-26 May 2013 (T_0) and 21 comparable quadrats on 25-26 June 2013 (T_{30}).

			Shoots SD T_0	Shoots SD T_{30}	Cover SD T_0	Cover SD T_{30}
Control	1	Top	10.4	12.5	5.2	5.6
Control	2	Top	9.0	13.0	6.2	5.5
Control	3	Top	9.2	11.5	3.6	5.0
Treatment	1	Top	13.7	17.2	5.6	4.1
Treatment	2	Top	8.6	9.1	6.3	5.4
Treatment	3	Top	10.7	15.1	8.9	10.0
Control	1	Bottom	8.1	12.2	5.0	5.7
Control	2	Bottom	10.5	11.1	3.7	6.1
Control	3	Bottom	7.7	7.8	6.1	6.4
Treatment	1	Bottom	7.8	11.2	6.9	5.8
Treatment	2	Bottom	11.8	13.6	5.0	2.6
Treatment	3	Bottom	8.3	9.2	4.4	6.1

the selected quadrats (n=108) taken during the T_0 sampling, and a copy of the sediment analysis report from ARI is provided in electronic format (CD) under separate cover.

Acknowledgments

Funding was provided by the Washington State Department of Ecology's Water Quality Program (Contract No. C1300168), and the Oyster Reserve Fund managed by the Washington State Department of Fish and Wildlife (Contract No. 13-1273), with in-kind support from Taylor Shellfish Farms and the Washington Cooperative Fish and Wildlife Research Unit (WCFWRU). C.E. Grue is employed by the U.S. Geological Survey (USGS) and the Unit is supported by the USGS, Washington State Departments of Ecology (WDOE), Fish and Wildlife (WDFW), and Natural Resources (WDNR), University of Washington (UW), and Washington State University (WSU). Field assistance was provided by Schuyler Pearman-Gillman (WACFWRU) and Jacob Moore (Willapa Grays Harbor Oyster Growers' Association). Consultations with Amy Yahnke (WACFWRU), and Dr. Kim Patten (WSU-Long Beach), as well as input from the growers, WDOE, WDFW, and WDNR were greatly appreciated. Thanks also to Alexandra Troiano for assistance with the frequency histograms. Reviews of earlier drafts by Nathan Lubliner, Jacob Moore, and Brian Sheldon were greatly appreciated.

References

Zar, J.H. 2010. Biostatisical Analysis, 5th Edition. Prentice Hall, Upper Saddle River, NJ. 944 pp.

Appendix 1. Criteria from the WDOE draft monitoring plan and meeting with stakeholders at study sites on 30 April 2013 used in the present evaluation.

Paired analysis

One-sided paired t-test *vs* time series analysis as the later 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.

Appendix 2. GPS locations for the corners of each of the study plots near Oysterville, WA used to evaluate the proposed monitoring design.

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. 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 4. Contact information for landowners whose property extends onto the study sites selected to be treated with imazamox in 2014 should the NPDES be approved.

Chester Wachsmuth
380 SE 9th Ave
Portland, OR 97202

Connie Underwood
13203 Silvercreek Dr
Tenino, WA 98589

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Watsonville, CA 95076

Robert Lanter
172 Mockingbird Rd
Burkeville, TX 75932
