Padilla Bay
National Estuarine Research Reserve

Technical Report No. 8

EFFECTS OF APPLICATION OF GLYPHOSATE ON THE EELGRASSES ZOSTERA MARINA AND ZOSTERA JAPONICA IN PADILLA BAY, WASHINGTON

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WASHINGTON STATE DEPARTMENT OF ECOLOGY

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ABSTRACT

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The herbicide, glyphosate (Rodeo® with X-77® spreader), was applied at five concentrations with a backpack sprayer to intertidal Zostera marina and Z. japonica and onto subtidal Z. marina whose leaves were floating on the water surface during low tide. Glyphosate was applied in July, 1992 during some of the lowest tides of the year to maximize the time of exposure before inundation of the eelgrasses by high tide. Glyphosate had no consistent effect on Z. marina nor Z. japonica in either microhabitat in the two months following application as measured by density of shoots, percent cover of live and dead leaves, above- and below-ground biomass or concentration of chlorophyll in the leaves. Nor was there any difference between treatment and control sites 12 months after application as measured by density and above ground biomass. It is suggested that the glyphosate did not have any measurable effect because of water retained on the leaf surface reducing absorption of the herbicide and because of the short time of exposure to the herbicide (three hours or less) before the flooding tide.

INTRODUCTION

The few studies on the effects of herbicides on seagrasses have been conducted both to determine effective means of killing eelgrass and acceptable concentrations to protect seagrasses. Eelgrass has been considered a nuisance plant by oyster growers (Taylor 1954, Thomas 1967) and swimmers (Bulthuis 1984). Thomas tested eight different herbicides on Zostera marina and recommended use of 200-400 pounds per acre (110 g m^{-2}) of the butoxyethanol ester of 2,4-dichlorophenoxyacetic acid (2,4-D) to kill eelgrasses (Thomas 1967, 1968; Thomas and Duffy 1968). Taylor (1954) recommended application of at least 200 quarts per acre (50 ml m^{-2}) of Benodor 3 ("a mixture of chlorinated benzenes") to control Z. marina in oyster growing areas. On the other hand, concern about the effects of runoff of herbicides into eelgrass habitat areas has been the impetus for studies on the minimum concentration of herbicide that will affect eelgrasses (Kemp et al. 1983). Correll and Wu (1982) reported inhibition of photosynthesis of Z. marina by 650 $\mu g\ l^{-1}$ of dissolved atrazine, but stimulation of photosynthesis at 75 $\mu g\ l^{-1}$. Mitchell (1987) reported a significant reduction in the growth of the seagrass Halodule wrightii in culture at a concentration of 30 ppm atrazine. Mayer and Elkins (1990) measured the concentration of herbicides in sediments and water in Padilla Bay in order to indicate whether herbicides were having any deleterious effect on eelgrasses in the bay. They reported only very low levels of dicamba and 2, 4-D and concluded that there were no ecologically significant levels of any of the fourteen pesticides studied in Padilla Bay.

We are not aware of any published studies on the effects of glyphosate on Zostera spp. or on any other seagrass. However, glyphosate effectively kills floating leaved freshwater plants. Barrett (1985) reported control of ten different species of freshwater floating weeds at doses of 1.2 - 5.4 kg ha^{-1} (1.4 - 6.0 pounds per acre), although some floating weeds were resistant to glyphosate. Lockhart et al. (1989)

reported a significantly reduced growth rate of the floating aquatic plant, Lemna minor, when fronds were sprayed with glyphosate but very little effect when similar concentrations were added to the water.

The eelgrasses Zostera marina and Z. japonica that grow near or intermingled with Spartina alterniflora in Washington State may be affected by glyphosate if the herbicide is used to control Spartina spp. While there are no published studies specifically testing the effect of glyphosate on Zostera spp., the previously mentioned studies on freshwater floating leaved plants indicate that glyphosate has the potential to affect Zostera spp. Zostera spp. may be susceptible when growing either intertidally and exposed to glyphosate spray or when growing in the shallow subtidal but with leaves floating on the surface during low tide. Lockhart et al. (1989) reported significantly reduced growth of the freshwater plant, Lemna minor, when the fronds were exposed to a surface spray of glyphosate for only six hours. Eelgrass with leaves that are floating on the surface similarly may be affected by sprayed glyphosate.

The primary objective of the present study was to determine the effects of glyphosate spray (Rodeo® with X-77® spreader) on intertidal Zostera marina and Z. japonica and shallow subtidal Z. marina. A second objective was to evaluate the relative sensitivity of Z. marina and Z. japonica by testing five different rates of application of this glyphosate spray.

METHODS

Study location. Padilla Bay is a shallow embayment located in Skagit County, Washington, north of Puget Sound. Extensive eelgrass meadows cover 3200 ha of Padilla Bay's 5000 ha (Bulthuis, 1991). The bay also has intertidal sand and mudflats dissected by dendritic channels that drain and distribute water to the flats during the unequal diurnal 4 m tides. Three sites were selected within the bay representing different eelgrass micro-habitats. Site one was located in a stand of intertidal Zostera japonica at the highest elevation of the three sites (Fig. 1). Site two was slightly lower in the intertidal and contained a mix of about 40% Z. marina and 60% Z. japonica. Site three was located in a shallow pool that was covered by subtidal Z. marina.

Twenty-one experimental plots of 1 m^2 were established at each of the intertidal sites (1 and 2). The plots were arranged in a grid pattern containing three columns and seven rows with two meters separating each experimental plot. The subtidal site (3) consisted of six experimental plots of 1 m x 2 m with 2 m separating adjacent plots.

Glyphosate Application. The herbicide glyphosate in the commercial aquatic formulation, Rodeo®, and a surfactant, Valent x-77®, were applied at both intertidal sites in five concentrations. The highest concentration was 4.44 qts/acre (17 l ha^{-1}) followed by 2.22 qts/acre (8.5 l ha^{-1}), 1.11 qts/acre (4.25 l ha^{-1}), 0.56 qts/acre (2.14 l ha^{-1}), and 0.28 qts/acre (1.07 l ha^{-1}). Control plots received an application of tap water. Spraying was done with a gas pressurized sprayer designed for research (KC-2L Koke Kap®) and 36 inch (0.91 m) boom equipped with two spray nozzles. The control plots were sprayed first to avoid accidental contamination of glyphosate in the spray equipment.

The Zostera japonica site (1) was sprayed on 2 July 1992 while sites 2 and 3 were sprayed on 1 July 1992. Herbicide application at the subtidal Z. marina site (3) was limited to only the highest glyphosate concentration (17 l ha^{-1}) and the control treatment. Tests were conducted on July 1 and 2 because the tide was predicted to be -3.2 ft (-0.98 m) on July 1, equal to the lowest tide of the year. Spraying was completed within 30 minutes of low tide at all three stations. The time interval between herbicide application and inundation by the incoming tide was 3 hours at the Zostera japonica site (1), 2.5 hours at the intertidal mixed Zostera site (2) and 2 hours at the subtidal site (3).

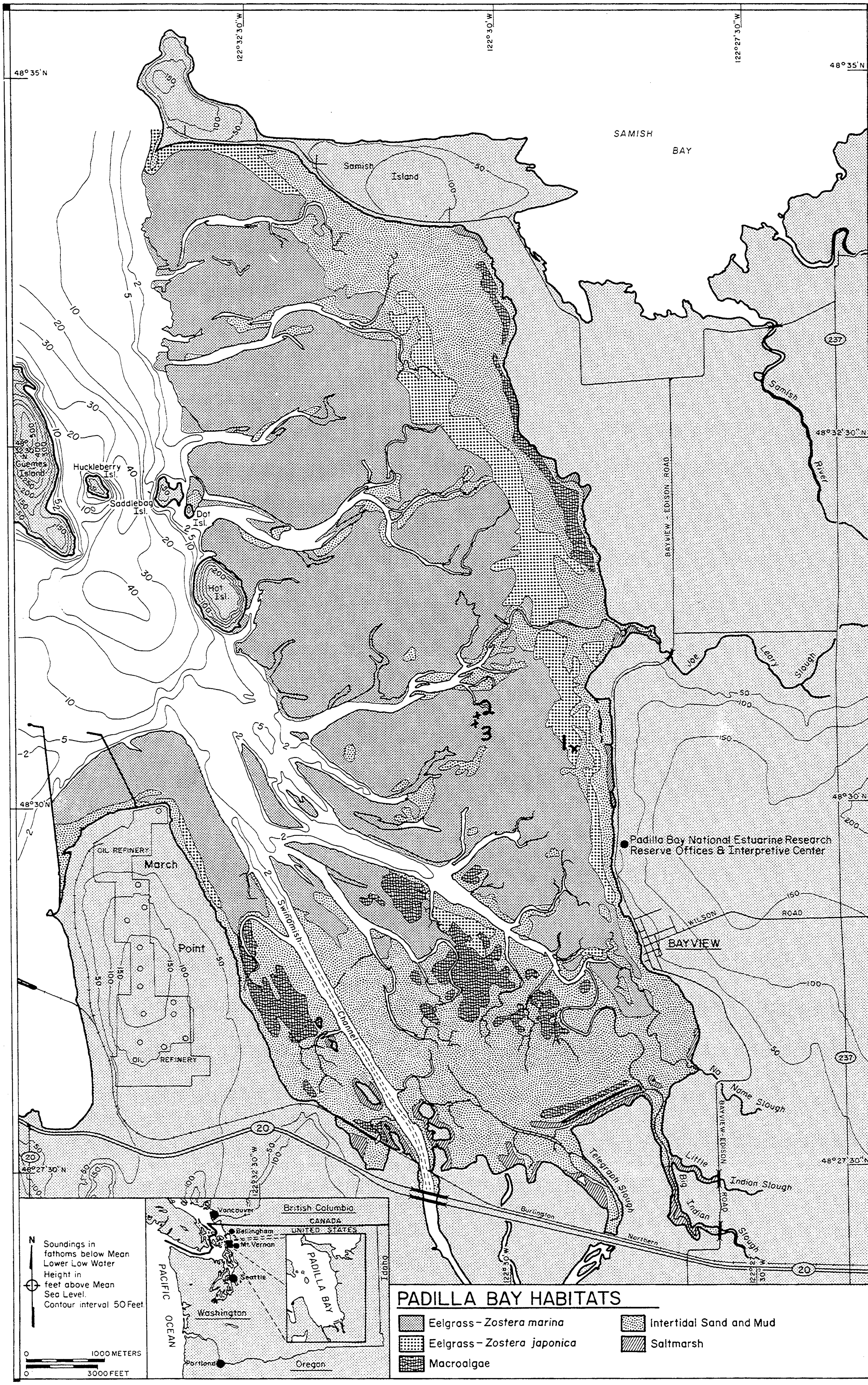

Figure 1. Location of experimental sites (1, 2 and 3) in Padilla Bay.

All treatments were conducted in triplicate with random allocation of plots within columns to meet the requirements of a randomized complete block experimental design.

Data Collection. Percent cover, shoot density, and leaf samples for chlorophyll determination were taken at each site prior to the herbicide application, 28 days after and 53-56 days after application. Biomass samples were collected prior to application and 53-56 days after application. Density and above-ground biomass also were measured one year after application, July 21 and July 23, 1993.

At the intertidal sites (1 and 2), above- and below-ground biomass was measured prior to application in three plots at each site (one in each column) randomly allocated for that purpose. Further measurements were not made in these six plots because of the disturbance caused by the pre-application sampling. After 53-56 days, above- and below-ground biomass was measured at the intertidal sites in plots that received the highest herbicide concentration ($17\ l\ ha^{-1}$) and in control plots. One year after application, above-ground biomass was measured in all plots at the intertidal sites.

At the subtidal site, disturbance to the plots was avoided by taking pre-application biomass samples from a site 25 m away rather than from within the site. Both sampling areas appeared to be part of the same relatively homogenous subtidal stand of Z. marina. Above-ground biomass was measured in all six subtidal sites 54 days after application.

Biomass sampling consisted of placing a 25 cm X 25 cm frame over a randomly selected section of the experimental plot. Above ground biomass within the frame was then cut at the sediment surface and placed into plastic bags. Below ground biomass within the frame was removed to a depth of 8 cm. All samples were transported from the field in a cooler and stored at $5^{o}C$ in the lab. Samples were washed and sorted within seven days of collection. Photosynthetic biomass was separated from non-photosynthetic biomass during the washing process with leaf color used as the primary criteria for this sorting. Dry weights were determined after drying to a constant weight at 105°C in a drying oven.

Shoot density was measured by placing a frame over a randomly selected section of each experimental plot and counting all shoots within the frame. Frame size was 10 cm X 10 cm at the Zostera japonica site (1), 15 cm x 15 cm at site 2 and 25 cm x 25 cm at the subtidal site (3). Frame size differed between sites because of the order of magnitude difference in density of eelgrass among sites.

Percent cover was measured prior to and 28 days after application with a 15 cm x 15 cm frame containing a grid pattern providing 16 points of intersection. Duplicate measurements were made in randomly selected sections of each plot and the species present immediately below each point of intersection recorded. A similar sized frame with a grid containing 25 points of intersection was used for duplicate measurements on the last sampling (53-56 days). [The number of points was increased in an attempt to reduce the coefficient of variation and increase the ability to detect any treatment effects. However, there were no statistical differences ($P < 0.05$, $\underline{t}$ test) between the coefficients of variation of the two grid types at all three sites.]

The concentration of chlorophyll was measured in two leaves per plot haphazardly selected from randomly identified sections within each experimental plot. Leaf samples consisted of the distal 15 cm of the plant's first leaf and were placed in a cooler for transport to the laboratory where they were frozen at -5°C until grinding. A 1 cm^2 section was removed from each leaf sample and ground in a tissue grinder with 10 ml of 90% acetone (Dennison 1990). This solution was held overnight in dark conditions at 5°C. The absorbance of the solution was determined with a diode spectrophotometer at 646 nm, 648 nm and 664 nm. Chlorophyll a and b content was calculated using the formulae of Jeffrey and Humphrey (1975).

Statistical Analysis. All data were tested for compliance with the assumptions of ANOVA. Two factor ANOVA, with replication, was used to test the response for each growth parameter during each time period; the factors being treatment (six concentrations) and blocks (three columns) (Sokal and Rohlf 1969). [Two factor ANOVA without replication was used to test the effects of treatment on density 28 days after application because only one count per plot could be collected at that time.] When the two factor ANOVA indicated significant treatment effects, the Bonferroni post hoc test was used to determine which treatments were significantly different from each other. (Systat 1992; Rice 1989)

RESULTS

Application of glyphosate. The glyphosate (Rodeo® and X-77® spreader) was applied with a backpack sprayer with a spray boom that treated an area slightly wider than 1 m. At site 1 (intertidal Zostera japonica), some water is retained on the sediment surface when the mudflats are exposed. At the time of application the upper layer of leaves were above this layer of water, although almost all leaves had a wetted surface layer of water. After application of the glyphosate the water level on the mud surface continued to go down, exposing more leaves, all of which continued to have wetted surfaces. The microtopography at site 2 (intertidal Z. marina) was similar to that at site 1. At site 3 (subtidal Z. marina) the plants had leaves about 1 m long and were standing in water that decreased from about 50 cm deep to 30 cm deep. The upper portions of the leaves were floating on the water surface and were exposed directly to the spray, while the lower portions of these plants were not exposed to the glyphosate spray. A slight current was discernable in the lower part of the water column at site 3 at all times. However, the surface film did not move, and the area within the treated plots had a visible scum from the glyphosate and spreader spray that remained in the plot area in contact with the leaves on the water surface for the 2 hours until return of the flooding tide.

Effects of glyphosate application. The percent cover, density, and chlorophyll content of the leaves of Zostera japonica (site 1) in control plots and in plots at all five levels of glyphosate treatment were similar 28 days and 53 days after treatment; and above and below ground biomass was similar 53 days after treatment (Table 1, Appendices A, B, C, and D). One year after application of glyphosate there were no differences in biomass of Z. japonica among treatments (Table 2). Two factor analysis of variance (ANOVA) indicated a significant ($P < 0.05$) treatment and treatment times block interaction for density of Z. japonica (Table 2). Because the treatment main effects was significant, a post hoc test, Bonferroni, was used to determine which treatment means for density were significantly different. Only the 0.28 qts/acre was higher than the 0.56 and 2.2 treatments. No other treatments were significantly different from each other. Application of glyphosate (Rodeo® with X-77® spreader) had no significant effect on Z. japonica in any of the characteristics measured in this study with exception of some anomalous density data one year after treatment.

The percent cover, density, chlorophyll content of leaves, and above ground biomass of Zostera marina in subtidal plots (site 3) also were not different from each other at all levels of glyphosate application (Table 3, Appendices A, B, C, and D).

The density, percent cover of green leaves, and concentration of chlorophyll in the leaves of Zostera marina at site 2 in control plots and in plots at all five levels of treatment were similar 28 days after treatment (Table 4, Appendices A, B, C, and D). Two way analysis of variance indicated a significant ($P < 0.05$) treatment, block and interaction effect on percent cover of dead Zostera leaves. The Bonferroni post hoc test for differences among treatments indicated that only the 4.4 qts/acre treatment had a higher percent cover of dead leaves (11%) than the control (3%).

Fifty-six days after treatment, the density, percent cover of green leaves, below ground biomass, and concentration of chlorophyll in the leaves at site 2 in control plots and in plots at all five levels treatment again were similar (Table 5). On the other hand,above ground biomass was significantly higher in controls than in the highest level of treatment (141 vs 89 g m^{-2}, Table 5). This difference in above ground biomass was due to differences in the amount of photosynthetic biomass (Appendix A-2). For percent cover of dead leaves, two way analysis of variance indicated a significant ($P < 0.05$) treatment effect. The Bonferroni post hoc test indicated that the 2.2 treatment was different from the 1.1 treatment, but that all other treatments were not different from each other. However, there was a highly significant treatment times block interaction ($P < 0.001$) which may have caused an apparent treatment effect (Table 5).

One year after treatment there was no significant difference in the density of Zostera marina and Zostera japonica at site 2, but there was a significant treatment effect on above ground biomass (Table 6). The highest level of treatment (4.4 qts/acre) and the control were not different from each other, but the three lowest levels of treatment had less biomass than the control plots. This comparison of above ground biomass is complicated by a significant block times treatment interaction (Table 6).

Table 1. Intertidal *Zostera japonica* site: mean ±standard error of the mean (n=number of plots) of biomass, density, percent cover and chlorophyll concentration of the leaves of *Z. japonica* before and 28 days and 53 days after application of glyphosate at the indicated rates of treatment. Two measurements were made within each plot for all characteristics and dates except for the initial density and percent cover when one measurement was made within each plot. Two factor analysis of variance indicated no significant (P>0.05) treatment effects for any of the characteristics that were measured.

Characteristic (units)	(n)	Treatment	Before	28 days after	53 days after
Biomass (g/m^2)					
photosynthetic	(3)	Initial	115 ±12.4		
	(3)	Control			62 ±9.5
	(3)	4.4 qts/acre			78 ±13.9
total above ground	(3)	Initial	129 ±16.5		
	(3)	Control			73 ±11.0
	(3)	4.4 qts/acre			90 ±14.8
rhizome	(3)	Initial	49 ±10.8		
	(3)	Control			65 ±6.5
	(3)	4.4 qts/acre			70 ±2.9
Density (no/m^2)	(18)	Initial	3622 ±214		
	(3)	Control		3870 ±1020	2270 ±259
	(3)	0.28 qts/acre		3400 ±666	2900 ±73
	(3)	0.56 qts/acre		2670 ±186	2630 ±224
	(3)	1.1 qts/acre		4070 ±291	1980 ±411
	(3)	2.2 qts/acre		5070 ±888	2970 ±613
	(3)	4.4 qts/acre		2470 ±338	2470 ±17
Cover (percent)					
live Zostera	(18)	Initial	96 ±1.5		
	(3)	Control		96 ±2.8	83 ±1.8
	(3)	0.28 qts/acre		98 ±1.0	89 ±1.8
	(3)	0.56 qts/acre		96 ±1.0	84 ±3.1
	(3)	1.1 qts/acre		95 ±1.0	81 ±4.4
	(3)	2.2 qts/acre		92 ±1.0	80 ±0.0
	(3)	4.4 qts/acre		98 ±1.0	80 ±6.4
dead Zostera	(3)	Control		1 ±1.0	2 ±1.2
	(3)	0.28 qts/acre		2 ±1.0	1 ±0.7
	(3)	0.56 qts/acre		1 ±1.0	2 ±1.2
	(3)	1.1 qts/acre		1 ±1.0	1 ±1.3
	(3)	2.2 qts/acre		3 ±1.8	2 ±2.0
	(3)	4.4 qts/acre		0 ±0.0	1 ±0.7
Leaf chlorophyll a (mg chl/dm^2 leaf area)	(3)	Initial	1.8 ±0.05		
	(3)	Control		2.2 ±0.01	3.3 ±0.15
	(3)	0.28 qts/acre		2.4 ±0.22	3.4 ±0.28
	(3)	0.56 qts/acre		2.2 ±0.48	4.1 ±0.35
	(3)	1.1 qts/acre		2.4 ±0.42	3.8 ±0.33
	(3)	2.2 qts/acre		2.4 ±0.29	3.2 ±0.40
	(3)	4.4 qts/acre		2.5 ±0.22	3.4 ±0.12

Table 2. Mean ±standard error of the mean (n=number of plots) of above ground biomass and density of *Zostera japonica* one year (23 July 93) after application of glyphosate at site 1. (Two measurements were made within each plot for each characteristic.) Results of two factor analysis of variance (ANOVA) are indicated by: ns = not significant; * = P<0.05; ** = P<0.01. The Bonferroni post hoc test for differences among treatment means of density indicated only that the 0.28 treatment was significantly different from the 0.56 and 2.2 treatments.

Treatment	(n) [df]	Biomass (g/m^2)		Density (no/m^2)	
Control	(3)	40	±13.0	2000	±441
0.28 qts/acre	(3)	49	± 2.3	2900	±180
0.56 qts/acre	(3)	42	±17.4	1400	±751
1.1 qts/acre	(3)	47	± 7.5	1700	±333
2.2 qts/acre	(3)	34	±14.0	1600	±433
4.4 qts/acre	(3)	46	±22.7	1950	±304
ANOVA					
Treatment	[5]	ns		*	
Block	[2]	ns		ns	
Treatment X Block	[10]	**		*	

Table 3. Subtidal *Zostera marina* site: Mean ±standard error of the mean (n=number of plots) of biomass, density, percent cover and chlorophyll concentration of the leaves of *Z. marina* before and 28 days and 56 days after application of glyphosate at the rate of 4.4 qts/acre. Two measurements were made within each plot for all characteristics and dates except for the initial density when one measurement was made within each plot. One way analysis of variance indicated no significant (P>0.05) treatment effects for any of the characteristics that were measured.

Characteristic (units)	(n)	Treatment	Before	28 days after	56 days after
Biomass (g/m^2)					
photosynthetic	(3)	Initial	120 ±30.9		
	(3)	Control			134 ±18
	(3)	4.4 qts/acre			138 ±17
total above ground	(3)	Initial	158 ±36.4		
	(3)	Control			343 ±85
	(3)	4.4 qts/acre			261 ±32
Density (no/m^2)	(18)	Initial	131 ±14		
	(3)	Control		187 ±5	184 ±37
	(3)	Treatment		275 ±24	243 ±23
Cover (percent)					
live Zostera	(3)	Control		79 ±7	75 ±11
	(3)	Treatment		82 ±6.3	81 ±7.4
dead Zostera	(3)	Control		8 ±3.8	11 ±4.1
	(3)	Treatment		9 ±4.7	14 ±2.3
Leaf chlorophyll a (mg chl/dm^2 leaf area)	(3)	Initial	1.9 ±0.16		
	(3)	Control		2.2 ±0.21	2.1 ±0.10
	(3)	Treatment		1.8 ±0.19	2.3 ±0.07

Table 4. Intertidal *Zostera marina* site (2): mean ±standard error of the mean (n=number of plots) of biomass, density, percent cover and chlorophyll concentration of the leaves of *Z. marina* and *Z. japonica* before and 28 days after application of glyphosate at the indicated rates of treatment. Two measurements were made within each plot for all characteristics and dates except for the initial density and percent cover when one measurement was made within each plot. Two factor analysis of variance indicated no significant (P>0.05) differences among treatments except for the percent cover of dead *Zostera* for which the treatment effect, block effect and the treatment times block interaction were all significant. The Bonferroni post hoc test for differences among treatments are indicated beside the means: treatments joined by the same line are not significantly different from each other.

Characteristic (units)	(n)	Treatment	Before	28 days after		
Density (no/m^2)	(18)	Initial	995 ±100			
	(3)	Control		1200 ±302		
	(3)	0.28 qts/acre		1330 ±289		
	(3)	0.56 qts/acre		1450 ±146		
	(3)	1.1 qts/acre		1530 ±466		
	(3)	2.2 qts/acre		1350 ±283		
	(3)	4.4 qts/acre		1510 ±771		
Cover (percent)						
live Zostera	(18)	Initial	81 ±3.9			
	(3)	Control		93 ±4.5		
	(3)	0.28 qts/acre		89 ±4.5		
	(3)	0.56 qts/acre		92 ±5.5		
	(3)	1.1 qts/acre		86 ±6.8		
	(3)	2.2 qts/acre		89 ±6.3		
	(3)	4.4 qts/acre		81 ±9.0		
dead Zostera	(3)	Control		3 ±3.1	|	
	(3)	0.28 qts/acre		4 ±1.0	|	|
	(3)	0.56 qts/acre		5 ±3.8	|	|
	(3)	1.1 qts/acre		5 ±2.8	|	|
	(3)	2.2 qts/acre		5 ±1.0	|	|
	(3)	4.4 qts/acre		11 ±6.8		|
Leaf chlorophyll a (mg chl/dm^2 leaf area)	(3)	Initial	1.1 ±0.10			
	(3)	Control		2.2 ±0.14		
	(3)	0.28 qts/acre		2.2 ±0.24		
	(3)	0.56 qts/acre		2.4 ±0.09		
	(3)	1.1 qts/acre		2.1 ±0.22		
	(3)	2.2 qts/acre		2.1 ±0.12		
	(3)	4.4 qts/acre		2.0 ±0.05		

Table 5. Mean ±standard error of the mean (n=number of plots, two measurements were made within each plot for each characteristic) of biomass, density, percent cover and chlorophyll *a* content of the leaves of *Zostera marina* and *Z. japonica* 56 days after application of glyphosate at site 2. Results of two factor analysis of variance (ANOVA) are indicated by: ns = not significant; * = $P<0.05$; ** = $P<0.01$: *** = $P<0.001$. The Bonferroni post hoc test for differences among treatment means indicated that only the 2.2 treatment was different ($P<0.05$) from the 1.1 treatment for cover of dead *Zostera*..

Treatment	(n) [df]	above ground biomass (g/m^2)	below ground biomass (g/m^2)	(n) [df]	density (no/m^2)	cover of live Zostera (percent)	cover of dead Zostera (percent)	leaf chl a (mg chl/dm^2 leaf area)
Control	(3)	141 ±8.5	91 ±16.1	(3)	1580 ±386	82 ±1.2	15 ±2.4	1.9 ±0.15
0.28 qts/acre				(3)	1830 ±71	84 ±6.1	15 ±6.7	2.0 ±0.05
0.56 qts/acre				(3)	1820 ±368	89 ±1.8	11 ±1.3	2.2 ±0.29
1.1 qts/acre				(3)	1880 ±160	80 ±6.4	19 ±2.5	2.4 ±0.17
2.2 qts/acre				(3)	1400 ±128	88 ±3.5	9 ±3.7	2.2 ±0.05
4.4 qts/acre	(3)	89 ±9.5	93 ±12.0	(3)	1940 ±9	87 ±2.7	12 ±2.0	2.1 ±0.07
ANOVA								
Treatment	[1]	*	ns	[5]	ns	ns	*	ns
Block	[2]	ns	ns	[2]	ns	ns	**	ns
Treatment X Block	[2]	ns	ns	[10]	ns	*	***	ns

Table 6. Mean ±standard error of the mean (n=number of plots) of above ground biomass and density of *Zostera marina* and *Z. japonica* one year (23 July 93) after application of glyphosate at site 2. (Two measurements were made within each plot for each characteristic.) Results of two factor analysis of variance (ANOVA) are indicated by: ns = not significant; *** = $P<0.001$. The results of the Bonferroni post hoc test for differences among treatment means for biomass are indicated beside the means: treatments joined by the same line are not significantly ($P<0.05$) different from each other.

Treatment	(n) [df]	above ground biomass (g/m^2)	density (no/m^2)
Control	(3)	178 ±22.0	1300 ±200
0.28 qts/acre	(3)	50 ± 8.2	1800 ±300
0.56 qts/acre	(3)	56 ± 5.4	1600 ±220
1.1 qts/acre	(3)	45 ± 2.3	1800 ±390
2.2 qts/acre	(3)	94 ±48.7	1300 ± 87
4.4 qts/acre	(3)	128 ±27.0	1700 ±190
ANOVA			
Treatment	[5]	***	ns
Block	[2]	ns	ns
Treatment X Block	[10]	***	ns

DISCUSSION

The only treatment versus control comparisons that were significantly different from each other were observed at site 2 where a mixture of Zostera marina and Z. japonica grew intertidally. The percent cover of dead leaves in the highest level of treatment was significantly higher than controls 1 month after treatment (Table 4), but lower than controls 2 months after treatment (Table 5) and the second highest level of treatment was significantly lower than controls 2 months after treatment. We infer from the conflicting results 1 month apart that the significant differences in cover of dead leaves do not reflect a treatment effect, but rather a heterogenous percent cover of dead leaves in the experimental area. This explanation is consistent with the significant treatment times block interaction observed in both cases in which treatment effects on percent cover of dead leaves is significant (Tables 4 and 5). Similarly, the significant difference in the above ground biomass one year after treatment at Site 2 may be due to non-random heterogeniety within the experimental area as indicated by the significant treatment times block interaction and the lowest biomass in the lowest levels of treatment (Table 6). The significantly decreased above ground biomass 2 months after treatment (Table 5) seems a clear indication of a treatment effect due to application of glyphosate. This result seems contradictory compared with all other parameters measured after two months (density, percent cover of green and dead leaves, concentration of chlorophyll a in the leaves). It is possible that the highest level of treatment (4.4 qts/acre) had some deleterious effect at this site, but we infer that this result is spurious when considering all of the other measurements that indicated no treatment effect.

The present study showed very little effect of application of glyphosate (Rodeo® plus X-77® spreader) on intertidal or subtidal Zostera marina or on Z. japonica. These results were not expected since many other aquatic plants are sensitive to glyphosate

(Barrett 1985, Lockhart et al. 1989). The apparent lack of an effect of glyphosate on Z. marina and Z. japonica in the present study may be due to ineffective treatment of the plots, to lack of absorption of the glyphosate into the eelgrass, or to a lack of sensitivity of eelgrass to glyphosate.

The treatment method appears to have been effective in delivering the designated volume of glyphosate to the plots. A specially designed backpack sprayer for research applications was used. Independent measurements of the volume of liquid delivered by each nozzle were close to the manufacturer's specifications. There may have been some variation in application rate because a constant rate of speed was required over such a small area. However, repeated "dry run" measurements indicated this variation was small and not enough to negate all treatment effects. Thus, the treatment method appears to have been effective in delivering the glyphosate to the treatment plots at or near the reported rate of application.

A second possible reason for the lack of treatment effect may be a lack of absorption of the glyphosate into the eelgrass. The microenvironment of the intertidal eelgrass during low tide exposure may be one factor that minimizes the amount of glyphosate that is absorbed by the eelgrass. Both Zostera marina and Z. japonica, are essentially submerged plants that have a wetted leaf surface even during normal low tide exposure. That layer of water may partially dilute the sprayed glyphosate and may act as a barrier or retardant to rapid uptake of the glyphosate. For example, Rodeo label directions specify that leaves of cordgrass should be dry when herbicide applications are made. Lockhart et al. (1989) sprayed glyphosate on the leaf surface of the floating aquatic plant, Lemna minor (duckweed), and added glyphosate to the water in which L. minor was growing. Spray on the leaf surface stopped growth of L. minor whereas glyphosate in the water had no effect on growth except at the highest concentration tested (Lockhart et al. 1989). L. minor is a floating plant whose leaves float above the water surface microlayer and whose upper leaf surface is exposed to the air. Glyphosate sprayed on L. minor would directly contact the leaf surface whereas glyphosate sprayed onto intertidal Zostera spp. would rest on the film of water that covers the leaves of Zostera spp. Thus, much of the glyphosate that is sprayed onto eelgrass may not be absorbed by the leaves of Zostera spp. into the plant in the typical intertidal microhabitat.

A second factor that may have prevented absorption of glyphosate into the Zostera spp. in the present study was the short time period between application of the glyphosate and the return of the flooding tide. Sites 1, 2 and 3 were all selected in places where the time of low tide exposure following application would be as long as possible and yet where relatively homogeneous stands of Zostera marina or Z. japonica were growing. The date of the treatment was selected because the predicted tide was the lowest tide of the year. Experimental plots were treated shortly before the time of low tide in order to allow sufficient water to flow off of the eelgrass leaves before spraying, and to maximize the time before return of the tide. However, the time of exposure was only 2 hours to 3 hours at the 3 sites. This short time of exposure may have minimized the amount of glyphosate absorbed by the eelgrass in the experimental plots. Thus, the short time of exposure may be the cause for the lack of treatment effect seen in this study.

A third possible reason for the apparent lack of effect of glyphosate may be a lack of sensitivity of Zostera spp. to glyphosate. Such a lack of sensitivity is possible because specific tests of the effectiveness of glyphosate on Zostera spp. have not been conducted. Barrett (1985) reported two species of floating weeds that appear to be resistant to glyphosate and Comes and Yong (1981; quoted in Barrett 1985) showed that both the absorption and translocation of glyphosate was poor in the submerged weeds which they tested. However, glyphosate is a broad spectrum herbicide and most of the plants that have been tested are susceptible to glyphosate. It seems unlikely that Zostera spp. are not susceptible to glyphosate and that the lack of effects seen in the present study are due to lack of susceptibility.

Other possible reasons for the apparent lack of effect of glyphosate in the present study are the concentration of glyphosate used and the time of application in relation to the growth cycle of eelgrass.

The highest concentration of glyphosate used in the present study (4.4 qts/acre) is above the highest rate of application (3.75 qts/acre) recommended for control of Spartina on the Rodeo label. It is possible that a higher concentration of glyphosate would have had a deleterious effect on Zostera spp. under the conditions tested in the present study. However, overspray or drifting glyphosate spray used to control Spartina is unlikely to occur on Zostera spp. at concentrations above 4 qts/acre when Rodeo is used under label conditions.

Glyphosate also could have greater effect on Zostera spp. at other times during their growth cycle. In the present study, both Zostera japonica and Z. marina had an extensive cover of above ground biomass at the time of application and Thom (1990) reported peak above ground biomass values in May, June, and August in Padilla Bay depending on tidal height and species. Barrett (1985) reported that glyphosate was ineffective on aquatic plants when applied either too early or too late in the growing season. Based on Thom's (1990) work in Padilla Bay and Barrett's general comments, July and August probably would be the most suitable months to "control" Zostera spp. in Padilla Bay. However, there may be other seasonal effects that make Zostera spp. more susceptible at other stages in their growth cycle.

Thus, there are several possible explanations for the apparent lack of effect of the broad spectrum herbicide, glyphosate (Rodeo® and X-77® spreader) on Zostera marina and Zostera japonica. We suggest that the most likely explanation is the combination of the short times of exposure before the flooding tide (three hours or less) and the thin film of water that normally wets the leaf surface of Zostera spp. even during low tide.

The apparent lack of deleterious effect of glyphosate even when sprayed directly onto Zostera spp. indicates that eelgrass beds are unlikely to suffer major damage from overspray or drifting spray under the environmental conditions and spray concentrations used in the present study. Under other environmental conditions and spray concentrations Zostera spp. may be highly susceptible. In particular, in these experiments, Zostera spp. were exposed to glyphosate for only 3 hours or less, and longer exposure times may be deleterious. Such generalizations do not negate the need for very careful application of glyphosate and prevention of drift onto areas of eelgrass. In addition to the gross effects that were tested in the present study, there is need for laboratory studies of the sublethal effects of glyphosate on eelgrass, e.g. on photosynthesis and respiration.

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APPENDICES

Appendix A. Biomass of Zostera marina and Z. japonica at experimental sites.

Appendix B. Percent cover of Zostera marina and Z. japonica at experimental sites.

Appendix C. Density of Zostera marina and Z. japonica at experimental sites.

Appendix D. Concentration of chlorophyll a in leaves of Zostera marina and Z. japonica at experimental sites.

Table A-1. Mean biomass in g dry wt/m^2 prior to application with glyphosate. SE = standard error of the mean, n=2 except where otherwise noted.

	Plot	Photosynthetic Biomass	SE	Non-Photosynthetic Biomass	SE	Total Leaf Standing Crop	SE	Rhizome	SE
Intertidal Z. marina site:									
	A-2	70.80	2.48	45.36	6.32	116.16	3.84	57.20	10.64
	B-4	68.16	1.28	127.68	58.16	195.28	56.88	72.72	13.68
	C-3	55.92	1.84	145.52	10.64	213.68	12.48	91.28	0.56
	Mean (n=3)	64.96	4.58	106.19	30.85	175.04	29.92	73.73	9.85
Intertidal Z. japonica site:									
	D-3	106.32	2.27	6.00	0.09	112.32	2.35	28.64	0.58
	E-1	98.64	0.77	13.28	0.11	111.92	0.88	52.00	1.29
	F-5	139.20	0.26	22.48	0.26	161.68	0.00	65.44	0.10
	Mean (n=3)	114.72	12.44	13.92	4.77	128.64	16.52	48.69	10.75
Subtidal Z. marina:									
	G-1	102.88	0.73	29.28	0.43	132.16	1.16		
	G-2	77.76	2.50	34.88	0.14	112.64	2.64		
	G-3	180.32	3.17	50.08	0.97	230.40	4.14		
	Mean (n=3)	120.32	30.86	38.08	6.21	158.40	36.44		

Table A-2. Mean biomass in g dry wt/m^2 fifty-three to fifty-six days after application with glyphosate applied at a rate of 4.44 qts/acre and control applications of tap water. SE = standard error of the mean, n=2 except where otherwise noted.

	Plot	Photosynthetic Biomass	SE	Non-Photosynthetic Biomass	SE	Total Leaf Standing Crop	SE	Rhizome	SE
Intertidal Z. marina site:									
Control;	A-4	116.96	2.88	33.28	12.96	150.24	15.84	97.92	22.08
	B-5	116.16	39.52	33.28	4.00	149.44	43.52	59.76	4.40
	C-1	105.60	30.56	18.80	1.68	124.40	28.88	113.84	18.00
	Mean (n=3)	112.91	3.66	28.45	4.83	141.36	8.48	90.51	16.05
Treatment;	A-1	52.20	7.80	39.44	8.40	91.64	0.60	115.52	21.60
	B-6	51.04	3.20	20.08	0.40	71.12	2.80	74.96	3.76
	C-5	88.56	9.36	14.96	8.24	103.52	0.65	87.60	22.64
	Mean (n=3)	63.93	12.32	24.83	7.45	88.76	9.46	92.69	11.98
Intertidal Z. japonica site:									
Control;	D-5	79.60	13.84	13.76	1.44	93.36	12.40	71.04	13.76
	E-3	59.60	22.00	11.20	3.36	70.80	25.36	51.84	10.88
	F-4	47.12	12.88	8.32	1.12	55.44	14.00	71.44	16.88
	Mean (n=3)	62.11	9.46	11.09	1.57	73.20	11.01	64.77	6.47
Treatment;	D-1	59.36	38.08	11.36	8.64	70.72	46.72	64.16	5.76
	E-4	70.24	7.84	9.28	1.12	79.52	6.72	71.92	16.08
	F-7	105.28	4.80	13.60	1.76	118.88	6.56	73.52	8.24
	Mean (n=3)	78.29	13.85	11.41	1.25	89.71	14.81	69.87	2.89
Subtidal Z. marina site:									
Control;	H-1	140.96	3.20	92.64	6.24	233.60	3.04		
	H-3	100.48	21.76	184.64	88.64	285.12	110.40		
	H-5	162.00	40.72	348.64	233.60	510.64	274.32		
	Mean (n=3)	134.48	18.05	208.64	74.87	343.12	85.07		
Treatment;	H-2	106.64	29.04	119.28	12.24	225.92	16.80		
	H-4	145.52	32.40	179.92	39.60	325.44	72.00		
	H-6	162.40	28.64	68.48	24.64	230.88	4.00		
	Mean (n=3)	138.19	16.51	122.56	32.21	260.75	32.38		

Table A-3. Mean (± s.e. of the mean) above ground biomass (g dry / m^2) of *Zostera marina* and *Z. japonica* one year after application of glyphosate at site 2 (n=2 measurements per plot; n=3 plots per treatment).

	Plot	above ground dry wt (g / m^2)	s.e.
Treatment - 4.44 qts/acre;	A-1	157	±36.9
	B-6	153	± 7.0
	C-5	74	±25.8
	Mean (n=3)	128	±27.2
Treatment-2.22 qts/acre;	A-7	60	±11.5
	B-1	32	± 4.7
	C-7	190	±19.6
	Mean (n=3)	94	±48.7
Treatment - 1.11 qts/acre;	A-3	46	± 2.8
	B-2	49	± 2.1
	C-2	41	± 6.5
	Mean (n=3)	45	± 2.4
Treatment - 0.56 qts/acre;	A-6	62	±10.6
	B-3	45	± 0.0
	C-4	60	±17.8
	Mean (n=3)	55	± 5.3
Treatment - 0.28 qts/acre;	A-5	34	± 8.5
	B-7	60	± 6.9
	C-6	57	± 6.4
	Mean (n=3)	50	± 8.2
Treatment - Control;	A-4	156	±26.2
	B-5	156	±30.6
	C-1	222	±42.2
	Mean (n=3)	178	±22.2

Table A-4. Mean (± s.e. of the mean) above ground biomass (g dry / m^2) of *Zostera japonica* one year after application of glyphosate at site 1 (n=2 measurements per plot; n=3 plots per treatment).

	Plot	above ground dry wt (g / m^2)	s.e.
Treatment - 4.44 qts/acre;	D-1	1	± 0.2
	E-4	71	± 5.6
	F-7	67	± 7.8
	Mean (n=3)	46	±22.7
Treatment-2.22 qts/acre;	D-7	42	±15.8
	E-2	7	± 6.9
	F-3	54	±10.9
	Mean (n=3)	34	±14.0
Treatment - 1.11 qts/acre;	D-2	51	±10.4
	E-6	57	±20.7
	F-2	32	±15.6
	Mean (n=3)	47	± 7.5
Treatment - 0.56 qts/acre;	D-4	68	± 2.0
	E-7	48	±21.1
	F-1	9	± 8.9
	Mean (n=3)	42	±17.4
Treatment - 0.28 qts/acre;	D-6	53	± 1.8
	E-5	45	±24.9
	F-6	48	±12.4
	Mean (n=3)	49	± 2.3
Treatment - Control;	D-5	62	±13.8
	E-3	17	±11.8
	F-4	40	±25.8
	Mean (n=3)	40	±13.0

Table B-1. Percent cover at site 2 (intertidal *Z. marina* site) prior to application of glyphosate (n=18).

Plot	% Z. marina	% Z. japonica	Total % Zostera	% Bare
A-1	50.00	43.75	93.75	6.25
A-3	68.75	0.00	68.75	31.25
A-4	62.50	12.50	75.00	25.00
A-5	31.25	50.00	81.25	18.75
A-6	18.75	18.75	37.50	50.00
A-7	56.25	43.75	100.00	0.00
B-1	0.00	62.50	62.50	37.50
B-2	75.00	6.25	81.25	18.75
B-3	75.00	0.00	75.00	25.00
B-5	75.00	12.50	87.50	12.50
B-6	12.50	43.75	56.25	43.75
B-7	62.50	18.75	81.25	18.75
C-1	93.75	6.25	100.00	0.00
C-2	6.25	87.50	93.75	6.25
C-4	75.00	12.50	87.50	12.50
C-5	25.00	68.75	93.75	6.25
C-6	0.00	93.75	93.75	6.25
C-7	68.75	25.00	93.75	6.25
Mean	47.57	33.68	81.25	18.06
SE	7.15	6.95	3.91	3.5

Table B-2. Mean (±SE, n=2 within each plot, n=3 plots per treatment) percent cover per square meter following application of glyphosate at site 2 (intertidal *Z. marina* site).

	Plot	% Zostera	SE	% Dead	SE	% Bare	SE
28 Days After Application							
Treatment - 4.44 qts/acre;	A-1	65.63	3.13	25.00	0.00	3.13	3.13
	B-6	81.25	12.50	6.25	0.00	9.38	9.38
	C-5	96.88	3.13	3.13	3.13	0.00	0.00
	Mean (n=3)	81.25	9.02	11.46	6.83	4.17	2.76
Treatment-2.22 qts/acre;	A-7	78.13	3.13	3.13	3.13	3.13	3.13
	B-1	87.50	0.00	6.25	0.00	6.25	0.00
	C-7	100.00	0.00	6.25	6.25	6.25	6.25
	Mean (n=3)	88.54	6.34	5.21	1.04	5.21	1.04
Treatment - 1.11 qts/acre;	A-3	100.00	0.00	0.00	0.00	0.00	0.00
	B-2	78.13	9.38	6.25	6.25	6.25	6.25
	C-2	81.25	6.25	9.38	3.13	9.38	3.13
	Mean (n=3)	86.46	6.83	5.21	2.76	5.21	2.76
Treatment - 0.56 qts/acre;	A-6	81.25	6.25	12.50	0.00	6.25	6.25
	B-3	93.75	0.00	3.13	3.13	3.13	3.13
	C-4	100.00	0.00	0.00	0.00	0.00	0.00
	Mean (n=3)	91.67	5.51	5.21	3.76	3.13	1.80
Treatment - 0.28 qts/acre;	A-5	81.25	12.50	6.25	0.00	12.50	12.50
	B-7	87.50	6.25	3.13	3.13	6.25	0.00
	C-6	96.88	3.13	3.13	3.13	0.00	0.00
	Mean (n=3)	88.54	4.54	4.17	1.04	6.25	3.61
Treatment - Control;	A-4	100.00	0.00	0.00	0.00	0.00	0.00
	B-5	84.38	3.13	9.38	3.13	6.25	0.00
	C-1	93.75	0.00	0.00	0.00	0.00	0.00
	Mean (n=3)	92.71	4.54	3.13	3.13	2.08	2.08
55 Days After Application							
Treatment - 4.44 qts/acre;	A-1	90.00	2.00	10.00	2.00	0.00	0.00
	B-6	90.00	2.00	10.00	2.00	0.00	0.00
	C-5	82.00	2.00	16.00	4.00	2.00	2.00
	Mean (n=3)	87.33	2.67	12.00	2.00	0.67	0.67
Treatment-2.22 qts/acre;	A-7	88.00	8.00	4.00	0.00	0.00	0.00
	B-1	94.00	2.00	6.00	0.00	0.00	0.00
	C-7	82.00	2.00	16.00	0.00	0.00	0.00
	Mean (n=3)	88.00	3.46	8.67	3.71	0.00	0.00
Treatment - 1.11 qts/acre;	A-3	90.00	2.00	10.00	2.00	0.00	0.00
	B-2	82.00	2.00	16.00	0.00	2.00	2.00
	C-2	68.00	4.00	32.00	4.00	0.00	0.00
	Mean (n=3)	80.00	6.43	19.33	2.45	0.67	0.67
Treatment - 0.56 qts/acre;	A-6	92.00	0.00	8.00	0.00	0.00	0.00
	B-3	86.00	6.00	12.00	4.00	2.00	2.00
	C-4	88.00	4.00	12.00	4.00	0.00	0.00
	Mean (n=3)	88.67	1.76	10.67	1.33	0.67	0.67
Treatment - 0.28 qts/acre;	A-5	72.00	0.00	28.00	0.00	0.00	0.00
	B-7	92.00	0.00	8.00	0.00	0.00	0.00
	C-6	88.00	0.00	8.00	4.00	2.00	2.00
	Mean (n=3)	84.00	6.11	14.67	6.67	0.67	0.67
Treatment - Control;	A-4	84.00	4.00	16.00	4.00	0.00	0.00
	B-5	80.00	0.00	10.00	6.00	2.00	2.00
	C-1	82.00	6.00	18.00	6.00	0.00	0.00
	Mean (n=3)	82.00	1.15	14.67	2.40	0.67	0.67

Table B-3. Percent cover at site 1 (intertidal Z. *japonica* site) prior to application of glyphosate (n=18).

Plot	% Z. japonica	% Bare
D-1	93.75	6.25
D-2	100.00	0.00
D-4	100.00	0.00
D-5	81.25	18.75
D-6	100.00	0.00
D-7	100.00	0.00
E-2	87.50	12.50
E-3	100.00	0.00
E-4	100.00	0.00
E-5	100.00	0.00
E-6	100.00	0.00
E-7	93.75	6.25
F-1	100.00	0.00
F-2	100.00	0.00
F-3	81.25	18.75
F-4	100.00	0.00
F-6	93.75	6.25
F-7	100.00	0.00
Mean	96.18	3.82
SE	1.53	1.53

Table B-4. Mean (±SE, n=2 within each plot, n=3 plots per treatment) percent cover per square meter following application of glyphosate at site 1 (intertidal *Z. japonica* site).

	Plot	Total % Z. japonica	SE	% Dead	SE	% Bare	SE
28 Days After Application							
Treatment - 4.44 qts/acre;	D-1	96.88	3.13	0.00	0.00	3.13	3.13
	E-4	96.88	3.13	0.00	0.00	3.13	3.13
	F-7	100.00	0.00	0.00	0.00	0.00	0.00
	Mean (n=3)	97.92	1.04	0.00	0.00	2.08	1.04
Treatment - 2.22 qts/acre;	D-7	90.63	9.38	6.25	6.25	3.13	3.13
	E-2	90.63	3.13	3.13	3.13	6.25	6.25
	F-3	93.75	0.00	0.00	0.00	6.25	0.00
	Mean (n=3)	91.67	1.04	3.13	1.80	5.21	1.04
Treatment - 1.11 qts/acre;	D-2	93.75	0.00	0.00	0.00	6.25	0.00
	E-6	93.75	6.25	3.13	3.13	3.13	3.13
	F-2	96.88	3.13	0.00	0.00	3.13	3.13
	Mean (n=3)	94.79	1.04	1.04	1.04	4.17	1.04
Treatment - 0.56 qts/acre;	D-4	93.75	0.00	3.13	3.13	3.13	3.13
	E-7	96.88	3.13	0.00	0.00	3.13	3.13
	F-1	96.88	3.13	0.00	0.00	3.13	3.13
	Mean (n=3)	95.83	1.04	1.04	1.04	3.13	0.00
Treatment - 0.28 qts/acre;	D-6	96.88	3.13	3.13	3.13	0.00	0.00
	E-5	100.00	0.00	0.00	0.00	0.00	0.00
	F-6	96.88	3.13	3.13	3.13	0.00	0.00
	Mean (n=3)	97.92	1.04	2.08	1.04	0.00	0.00
Treatment - Control;	D-5	100.00	0.00	0.00	0.00	0.00	0.00
	E-3	96.88	3.13	3.13	3.13	0.00	0.00
	F-4	90.63	3.13	0.00	0.00	9.38	3.13
	Mean (n=3)	95.83	2.76	1.04	1.04	3.13	3.13
53 Days After Application							
Treatment - 4.44 qts/acre;	D-1	82.00	10.00	0.00	0.00	12.00	4.00
	E-4	68.00	12.00	2.00	2.00	22.00	10.00
	F-7	90.00	6.00	0.00	0.00	0.00	0.00
	Mean (n=3)	80.00	6.43	0.67	0.67	11.33	6.36
Treatment - 2.22 qts/acre;	D-7	80.00	4.00	0.00	0.00	2.00	2.00
	E-2	80.00	0.00	0.00	0.00	10.00	6.00
	F-3	80.00	8.00	6.00	6.00	2.00	2.00
	Mean (n=3)	80.00	0.00	2.00	2.00	4.67	2.67
Treatment - 1.11 qts/acre;	D-2	90.00	2.00	0.00	0.00	8.00	4.00
	E-6	76.00	8.00	0.00	0.00	16.00	8.00
	F-2	78.00	6.00	4.00	0.00	6.00	2.00
	Mean (n=3)	81.33	4.37	1.33	1.33	10.00	3.06
Treatment - 0.56 qts/acre;	D-4	86.00	2.00	0.00	0.00	6.00	2.00
	E-7	88.00	4.00	4.00	4.00	0.00	0.00
	F-1	78.00	2.00	2.00	2.00	4.00	4.00
	Mean (n=3)	84.00	3.06	2.00	1.15	3.33	1.76
Treatment - 0.28 qts/acre;	D-6	92.00	8.00	0.00	0.00	0.00	0.00
	E-5	90.00	2.00	0.00	0.00	2.00	2.00
	F-6	86.00	6.00	2.00	2.00	0.00	0.00
	Mean (n=3)	89.33	1.76	0.67	0.67	0.67	0.67
Treatment - Control;	D-5	86.00	2.00	0.00	0.00	2.00	2.00
	E-3	84.00	4.00	4.00	0.00	2.00	2.00
	F-4	80.00	8.00	2.00	2.00	2.00	2.00
	Mean (n=3)	83.33	1.76	2.00	1.15	2.00	0.00

Table B-5. Mean (±SE, n=2 within each plot, n=3 plots per treatment) percent cover per square meter following application of glyphosate (treatment = 4.4 qts/acre) at site 3 (subtidal *Z. marina* site)

	Plot	% Z. marina	SE	% Dead	SE	% Bare	SE
28 Days After Application							
Treatment;	H-2	81.25	0.00	12.50	0.00	6.25	0.00
	H-4	93.75	6.25	0.00	0.00	6.25	6.25
	H-6	71.88	21.88	15.33	9.38	12.50	12.50
	Mean (n=3)	82.29	6.34	9.28	4.71	8.33	2.08
Control;	H-1	65.63	3.13	15.63	3.13	18.75	0.00
	H-3	84.38	3.13	3.13	3.13	12.50	0.00
	H-5	87.50	6.25	6.25	0.00	6.25	6.25
	Mean (n=3)	79.17	6.83	8.33	3.76	12.50	3.61
56 Days After Application							
Treatment;	H-2	66.00	6.00	18.00	10.00	16.00	4.00
	H-4	86.00	2.00	14.00	2.00	0.00	0.00
	H-6	90.00	6.00	10.00	6.00	0.00	0.00
	Mean (n=3)	80.67	7.42	14.00	2.31	5.33	5.33
Control;	H-1	54.00	2.00	18.00	2.00	28.00	0.00
	H-3	82.00	10.00	4.00	0.00	2.00	2.00
	H-5	90.00	6.00	10.00	6.00	0.00	0.00
	Mean (n=3)	75.33	10.91	10.67	4.06	10.00	9.02

Table C-1. Shoot density (no./m^2) at site 2 (intertidal *Zostera marina* site) prior to glyphosate application. Mean and standard error of the mean shown for the 18 plots.

Plot	Z. marina	Z. japonica	Flowering Plants	Total Zostera
A-1	177.76	622.16	0	799.92
A-3	355.52	133.32	0	488.84
A-4	355.52	44.44	0	399.96
A-5	311.08	177.76	44.44	533.28
A-6	266.64	1377.64	311.08	1955.36
A-7	44.44	844.36	44.44	933.24
B-1	44.44	488.84	399.96	933.24
B-2	177.76	355.52	88.88	622.16
B-3	177.76	488.84	399.96	1066.56
B-5	1111	0	177.76	1288.76
B-6	533.28	0	0	533.28
B-7	577.72	0	266.64	844.36
C-1	222.2	933.24	177.76	1333.2
C-2	177.76	933.24	399.96	1510.96
C-4	133.32	933.24	177.76	1244.32
C-5	177.76	1111	311.08	1599.84
C-6	44.44	799.92	44.44	888.8
C-7	222.2	711.04	0	933.24
Mean	283.92	553.03	158.01	994.96
SE	60.03	101.31	36.32	100.35

Table C-2. Shoot density (no./m^2) of Zostera marina and Z. japonica at site 2 (intertidal Z. marina site) one and two months following application of glyphosate. One month after treatment: n=1 per plot; two months after treatment: n=2 per plot; n=3 plots per treatment. Mean and standard error shown when 2 measurements were made in each plot.

	Plot	Z. marina	SE	Z. japonica	SE	Flowering Plants	SE	Total Zostera	SE
28 Days After Application									
Treatment - 4.44 qts/acre:	A-1	133		356		0		489	
	B-6	444		2355		222		3022	
	C-5	400		533		89		1022	
	Mean (n=3)	326	97	1081	639	104	65	1511	771
Treatment-2.22 qts/acre;	A-7	311		667		44		1022	
	B-1	133		844		133		1111	
	C-7	44		1778		89		1911	
	Mean (n=3)	163	78	1096	345	89	26	1348	283
Treatment - 1.11 qts/acre:	A-3	222		2133		222		2444	
	B-2	400		711		89		1200	
	C-2	311		622		0		933	
	Mean (n=3)	311	51	1155	490	104	65	1526	466
Treatment - 0.56 qts/acre:	A-6	267		933		178		1378	
	B-3	400		711		133		1244	
	C-4	89		1422		222		1733	
	Mean (n=3)	252	90	1022	210	178	26	1452	146
Treatment - 0.28 qts/acre:	A-5	267		755		0		1022	
	B-7	622		311		133		1067	
	C-6	44		1822		44		1911	
	Mean (n=3)	311	168	963	448	59	39	1333	289
Treatment - Control;	A-4	133		400		89		622	
	B-5	133		1200		311		1644	
	C-1	222		933		178		1333	
	Mean (n=3)	163	30	844	235	193	65	1200	302
55 Days After Application									
Treatment - 4.44 qts/acre:	A-1	378	67	1489	511	89	0	1955	578
	B-6	444	222	1378	889	111	67	1933	733
	C-5	378	22	1444	378	0	0	1933	467
	Mean (n=3)	400	22	1437	32	67	34	1941	9
Treatment-2.22 qts/acre;	A-7	622	0	667	0	111	22	1400	22
	B-1	178	89	1311	200	67	67	1622	111
	C-7	289	111	733	289	156	67	1178	333
	Mean (n=3)	363	134	904	205	111	26	1400	128
Treatment - 1.11 qts/acre:	A-3	333	67	1822	933	44	0	2200	867
	B-2	311	89	800	89	22	22	1755	422
	C-2	378	244	1111	933	200	200	1689	889
	Mean (n=3)	341	56	1244	302	89	56	1881	160
Treatment - 0.56 qts/acre:	A-6	178	44	1000	422	44	44	1222	333
	B-3	578	311	1089	689	67	67	1733	444
	C-4	267	133	1866	489	356	178	2489	533
	Mean (n=3)	341	121	1318	275	156	100	1815	368
Treatment - 0.28 qts/acre:	A-5	356	267	1533	1267	0	0	1889	1000
	B-7	400	44	1422	222	89	44	1911	222
	C-6	156	67	1467	622	67	22	1689	578
	Mean (n=3)	304	75	1474	32	52	27	1829	71
Treatment - Control;	A-4	578	267	244	244	0	0	822	511
	B-5	89	44	1600	44	133	44	1822	44
	C-1	222	44	1822	489	44	44	2089	400
	Mean (n=3)	296	146	1222	493	59	39	1578	386

Table C-3. Shoot density (no./m^2) at site 1 (intertidal *Zostera japonica* site) prior to glyphosate application. Mean and standard error of the mean shown for the 18 plots.

Plot	Z. japonica	Flowering Plants	Total Plants
D-1	1900	300	2200
D-2	1800	800	2600
D-4	1600	800	2400
D-5	2500	1000	3500
D-6	2300	900	3200
D-7	3100	1300	4400
E-2	2000	700	2700
E-3	2700	400	3100
E-4	3300	1200	4500
E-5	3200	900	4100
E-6	4000	1000	5000
E-7	3100	700	3800
F-1	2100	1400	3500
F-2	1600	800	2400
F-3	3800	400	4200
F-4	4100	700	4800
F-6	3400	1300	4700
F-7	3700	400	4100
Mean	2788.89	833.33	3622.22
SE	198.51	77.96	214.13

Table C-4. Shoot density (no./m^2) of *Zostera japonica* at site 1 (intertidal *Z. japonica* site) one month after application of glyphosate. (n=1 per plot; n=3 plots per treatment; mean and standard error of the mean shown for each treatment).

	Plot	Z. japonica	SE	Flowering Z. japonica	SE	Total Live Z. japonica	SE
28 Days After Application							
Treatment - 4.44 qts/acre;	D-1	1800		0		1800	
	E-4	2500		400		2900	
	F-7	1800		900		2700	
	Mean (n=3)	2033	233	433	260	2467	338
Treatment-2.22 qts/acre;	D-7	5200		900		6100	
	E-2	2400		900		3300	
	F-3	5000		800		5800	
	Mean (n=3)	4200	902	867	33	5067	888
Treatment - 1.11 qts/acre;	D-2	3600		1000		4600	
	E-6	3100		500		3600	
	F-2	3500		500		4000	
	Mean (n=3)	3400	153	667	167	4067	291
Treatment - 0.56 qts/acre;	D-4	2800		100		2900	
	E-7	2000		300		2300	
	F-1	1900		900		2800	
	Mean (n=3)	2233	285	433	240	2667	186
Treatment - 0.28 qts/acre;	D-6	2400		1400		3800	
	E-5	2000		100		2100	
	F-6	3300		1000		4300	
	Mean (n=3)	2567	384	833	384	3400	666
Treatment - Control;	D-5	5000		900		5900	
	E-3	1800		1200		3000	
	F-4	2600		100		2700	
	Mean (n=3)	3133	961	733	328	3867	1020

Table C-5. Shoot density (no./m^2) of *Zostera japonica* at site 1 (intertidal *Z. japonica* site) two months after application of glyphosate. (n=2 per plot; n=3 plots per treatment; mean and standard error of the mean shown for each plot and treatment).

	Plot	Z. japonica	SE	Flowering Z. japonica	SE	Total Live Z. japonica		Dead Z. japonica	SE
53 Days After Application									
Treatment - 4.44 qts/acre	D-1	2250	50	200	100	2450	150	50	50
	E-4	2100	200	400	200	2500	0	150	150
	F-7	2200	500	250	150	2450	650	100	100
	Mean (n=3)	2183	44	283	60	2467	17	100	29
Treatment-2.22 qts/acre;	D-7	3000	500	700	200	3700	700	0	0
	E-2	1350	1050	400	200	1750	1250	100	100
	F-3	3100	800	350	250	3450	1050	350	50
	Mean (n=3)	2483	567	483	109	2967	613	150	104
Treatment - 1.11 qts/acre	D-2	2550	550	250	150	2800	700	200	100
	E-6	1350	150	300	0	1650	150	150	50
	F-2	1400	400	100	0	1500	400	50	50
	Mean (n=3)	1767	392	217	60	1983	411	133	44
Treatment - 0.56 qts/acre	D-4	1900	200	300	200	2200	400	250	50
	E-7	2550	650	400	100	2950	550	0	0
	F-1	2350	150	400	100	2750	250	250	150
	Mean (n=3)	2267	192	367	33	2633	224	167	83
Treatment - 0.28 qts/acre	D-6	2650	150	300	100	2950	250	300	100
	E-5	2700	600	150	50	2850	550	750	250
	F-6	2650	550	50	50	2700	600	50	50
	Mean (n=3)	2667	17	167	73	2900	73	367	205
Treatment - Control;	D-5	1350	650	400	100	1750	550	200	100
	E-3	2100	300	400	200	2500	100	350	50
	F-4	2250	250	300	100	2550	350	200	100
	Mean (n=3)	1900	278	367	33	2267	259	250	50

Table C-6. Shoot density (no./m^2) of *Zostera marina* at site 3 (subtidal *Zostera marina* site) prior to the application of glyphosate. Mean and standard error of the mean shown for each plot (n=2) and for the 3 plots.

Plot	Z. marina	SE
G-1	104	8
G-2	136	40
G-3	152	24
Mean (n=3)	131	14

Table C-7. Shoot density (no./m^2) of *Zostera marina* at site 3 (subtidal *Zostera marina* site) one month and two months after application of glyphosate. Mean and standard error of the mean shown for each plot (n=2) and for each treatment. Treatment consisted of glyphosate applied at a rate of 4.4 qts/acre.

	Plot	Z. marina	SE
28 Days After Application			
Treatment;	H-2	264	40
	H-4	320	32
	H-6	240	16
	Mean (n=3)	275	24
Control;	H-1	176	48
	H-3	192	96
	H-5	192	0
	Mean (n=3)	187	5
56 Days After Application			
Treatment;	H-2	224	32
	H-4	216	24
	H-6	288	48
	Mean (n=3)	243	23
Control;	H-1	136	24
	H-3	256	16
	H-5	160	32
	Mean (n=3)	184	37

Table C-8. Mean (± s.e. of the mean) shoot density (no/m^2) of *Zostera marina* and *Z. japonica* one year after application of glyphosate at site 2 (n=2 measurements per plot; n=3 plots per treatment).

	Plot	Z. marina (no / m^2)	s.e.	Z. japonica (no / m^2)	s.e.	Total Zostera (no / m^2)	s.e.
Treatment - 4.44 qts/acre	A-1	248		1040		1288	± 24
	B-6	462		1416		1888	±320
	C-5	422		1378		1800	±289
	Mean (n=3)	377	± 66	1278	±120	1659	± 94
Treatment - 2.22 qts/acre	A-7	311		1022		1333	±222
	B-1	133		1267		1400	±333
	C-7	432		680		1112	±312
	Mean (n=3)	292	± 87	990	±170	1282	± 87
Treatment - 1.11 qts/acre	A-3	200		1778		1978	±689
	B-2	244		2111		2355	±578
	C-2	111		933		1044	±156
	Mean (n=3)	185	± 39	1607	±351	1792	±390
Treatment - 0.56 qts/acre	A-6	489		1578		2066	±289
	B-3	333		978		1311	±244
	C-4	267		1289		1555	± 89
	Mean (n=3)	363	± 66	1282	±173	1644	±222
Treatment - 0.28 qts/acre	A-5	200		1422		1622	± 67
	B-7	311		1155		1467	±311
	C-6	267		2155		2422	±467
	Mean (n=3)	259	± 32	1577	±299	1837	±296
Treatment - Control:	A-4	368		944		1312	± 26
	B-5	208		720		928	± 32
	C-1	490		1120		1600	±176
	Mean (n=3)	355	± 82	928	±116	1280	±195

Table C-9. Mean (± s.e. of the mean) shoot density (no/m^2) of *Zostera japonica* one year after application of glyphosate at site 1 (n=2 measurements per plot; n=3 plots per treatment).

	Plot	density (no / m^2)	s.e.
Treatment - 4.44 qts/acre	D-1	1450	±1150
	E-4	2500	±800
	F-7	1900	±500
	Mean (n=3)	1950	±304
Treatment-2.22 qts/acre;	D-7	1600	±300
	E-2	400	±300
	F-3	2300	±100
	Mean (n=3)	1433	±555
Treatment - 1.11 qts/acre	D-2	2100	±400
	E-6	1850	±350
	F-2	1000	±100
	Mean (n=3)	1650	±333
Treatment - 0.56 qts/acre	D-4	1450	±250
	E-7	2700	±500
	F-1	100	± 0
	Mean (n=3)	1417	±751
Treatment - 0.28 qts/acre	D-6	3100	±100
	E-5	2950	±250
	F-6	2500	± 0
	Mean (n=3)	2850	±180
Treatment - Control;	D-5	2800	±600
	E-3	1300	±400
	F-4	1800	±700
	Mean (n=3)	1967	±441

Table D-1. Mean chlorophyll a concentration prior to the application of glyphosate at each experimental site (n=3). Data are presented in mg chl/ dm^2.

	Block	chlorophyll concentration	SE
Intertidal Z. marina site;	A	1.00	0.25
	B	1.04	0.24
	C	1.31	0.26
	Mean	1.12	0.10
Subtidal Z. marina site:	Mean	1.94	0.16
Z. japonica site;	D	1.92	0.17
	E	1.78	0.42
	F	1.74	0.11
	Mean	1.81	0.05

Table D-2. Mean chlorophyll a concentration following the application of glyphosate at the intertidal Z. marina site (n=2 per plot; n=3 per treatment). Data are presented in mg chl/ dm ^2.

	Plot	chlorophyll concentration	SE
28 Days After Application			
Treatment - 4.44 qts/acre;	A-1	2.04	0.12
	B-6	1.97	0.36
	C-5	1.88	0.49
	Mean (n=3)	1.96	0.05
Treatment-2.22 qts/acre;	A-7	2.38	0.64
	B-1	2.05	0.14
	C-7	1.99	0.62
	Mean (n=3)	2.14	0.12
Treatment - 1.11 qts/acre;	A-3	1.68	0.34
	B-2	2.26	0.17
	C-2	2.41	0.02
	Mean (n=3)	2.12	0.22
Treatment - 0.56 qts/acre;	A-6	2.31	0.37
	B-3	2.55	0.06
	C-4	2.24	0.42
	Mean (n=3)	2.36	0.09
Treatment - 0.28 qts/acre;	A-5	1.72	0.37
	B-7	2.27	0.08
	C-6	2.53	0.50
	Mean (n=3)	2.17	0.24
Treatment - Control;	A-4	2.24	0.04
	B-5	2.00	0.41
	C-1	2.49	0.34
	Mean (n=3)	2.24	0.14
55 Days After Application			
Treatment - 4.44 qts/acre;	A-1	2.21	0.13
	B-6	2.14	0.41
	C-5	1.98	0.49
	Mean (n=3)	2.11	0.07
Treatment-2.22 qts/acre;	A-7	2.23	0.02
	B-1	2.21	0.07
	C-7	2.08	0.44
	Mean (n=3)	2.22	0.05
Treatment - 1.11 qts/acre;	A-3	2.65	0.41
	B-2	2.36	0.23
	C-2	2.07	0.09
	Mean (n=3)	2.36	0.17
Treatment - 0.56 qts/acre;	A-6	2.69	0.07
	B-3	1.67	0.26
	C-4	2.10	0.05
	Mean (n=3)	2.15	0.29
Treatment - 0.28 qts/acre;	A-5	2.09	0.10
	B-7	2.03	0.31
	C-6	1.92	0.56
	Mean (n=3)	2.01	0.05
Treatment - Control;	A-4	2.22	0.13
	B-5	1.81	0.14
	C-1	1.72	0.13
	Mean (n=3)	1.91	0.15

Table D-3. Mean chlorophyll a concentration following the application of glyphosate at the Z. japonica si (n=2 per plot; n=3 per treatment). Data are presented in mg chl / dm^2.

	Plot	chlorophyll concentration	SE
28 Days After Application			
Treatment - 4.44 qts/acre;	D-1	2.95	0.64
	E-4	2.27	0.16
	F-7	2.31	0.10
	Mean (n=3)	2.51	0.22
Treatment - 2.22 qts/acre;	D-7	1.91	0.04
	E-2	2.90	0.42
	F-3	2.49	0.03
	Mean (n=3)	2.43	0.29
Treatment - 1.11 qts/acre;	D-2	2.24	1.11
	E-6	3.15	0.26
	F-2	1.70	0.31
	Mean (n=3)	2.36	0.42
Treatment - 0.56 qts/acre;	D-4	3.15	1.37
	E-7	1.74	0.14
	F-1	1.68	0.72
	Mean (n=3)	2.19	0.48
Treatment - 0.28 qts/acre;	D-6	2.23	0.97
	E-5	2.85	0.05
	F-6	2.14	0.50
	Mean (n=3)	2.41	0.22
Treatment - Control;	D-5	2.18	0.45
	E-3	2.16	0.44
	F-4	2.28	0.33
	Mean (n=3)	2.21	0.01
53 Days After Application			
Treatment - 4.44 qts/acre;	D-1	3.45	0.00
	E-4	3.61	0.02
	F-7	3.20	0.41
	Mean (n=3)	3.42	0.12
Treatment - 2.22 qts/acre;	D-7	3.85	0.54
	E-2	2.50	0.62
	F-3	3.36	0.38
	Mean (n=3)	3.24	0.40
Treatment - 1.11 qts/acre;	D-2	4.32	0.06
	E-6	3.84	0.39
	F-2	3.19	0.26
	Mean (n=3)	3.78	0.33
Treatment - 0.56 qts/acre;	D-4	4.68	0.21
	E-7	4.28	0.86
	F-1	3.48	0.44
	Mean (n=3)	4.14	0.35
Treatment - 0.28 qts/acre;	D-6	3.85	0.19
	E-5	2.89	0.63
	F-6	3.43	0.37
	Mean (n=3)	3.39	0.28
Treatment - Control;	D-5	3.55	0.47
	E-3	3.41	0.54
	F-4	3.04	0.07
	Mean (n=3)	3.33	0.15

Table D-4. Mean chlorophyll a concentration following the application of glyphosate at the subtidal Z. marina site. Treatment plots were sprayed with glyphosate at a concentration of 4.44 qts/acre (n=2 per plot; n=3 per treatment). Data are presented in mg chl / dm^2.

	Plot	chlorophyll concentration	SE
28 Days After Application			
Treatment;	H-2	1.44	0.11
	H-4	2.09	0.45
	H-6	1.75	0.43
	Mean (n=3)	1.76	0.19
Control;	H-1	2.24	0.04
	H-3	1.84	0.30
	H-5	2.58	0.00
	Mean (n=3)	2.22	0.21
56 Days After Application			
Treatment;	H-2	2.43	0.22
	H-4	2.23	0.31
	H-6	2.22	0.01
	Mean (n=3)	2.33	0.07
Control;	H-1	1.94	0.04
	H-3	2.29	0.36
	H-5	2.09	0.09
	Mean (n=3)	2.10	0.10