Padilla Bay
National Estuarine Research Reserve

Technical Report No. 9

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

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October 1994

Publication No. 94-170

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Padilla Bay National Estuarine Research Reserve is managed by the Shorelands and Environmental Assistance Program, Washington State Department of Ecology, in cooperation with the Estuarine Reserves Division, National Oceanic and Atmospheric Administration (NOAA), U.S. Department of Commerce. The preparation of this document was financially aided through a grant to the Washington State Department of Ecology with funds obtained from NOAA/Office of Ocean and Coastal Resource Management, and appropriated for Section 306 or 315 of the Coastal Zone Management Act of 1972, as amended.

WASHINGTON STATE DEPARTMENT OF ECOLOGY

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Bibliographic citation: Bulthuis, D.A. and M.J. Hartman. 1994. Effects of application of glyphosate during summer on epiphytes of the eelgrasses, *Zostera marina* and *Zostera japonica* in Padilla Bay, Washington. Padilla Bay National Estuarine Research Reserve, Mount Vernon. 40 pp. Padilla Bay National Estuarine Research Reserve Technical Report No. 9.

This is a report of the Washington State Department of Ecology pursuant to National Oceanic and Atmospheric Administration Awards No. NA270R0359-01, No. NA370R0229, and No. NA 470R0335; and Interagency Agreement DNR 93-001 between the Washington Department of Natural Resources and Washington Department of Ecology. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA, Washington Department of Natural Resources, Washington Department of Ecology, or any of the subagencies of these departments. Copies of this report are available from the Padilla Bay National Estuarine Research Reserve.

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ABSTRACT

Bulthuis, D.A. and M.J. Hartman. 1994. Effects of application of glyphosate during summer on epiphytes of the eelgrasses Zostera marina and Zostera japonica in Padilla Bay, Washington. Washington Department of Ecology, Padilla Bay National Estuarine Research Reserve, Technical Report No. 9, Mount Vernon, Washington. 40 pp.

The herbicide glyphosate (Rodeo® with X-77® spreader) was applied at five concentrations with a backpack sprayer onto experimental plots at three sites: an intertidal Zostera marina site, an intertidal Z. japonica site and a subtidal Z. marina site at which the leaves were floating on the water surface at the time of application of glyphosate. 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 and epiphytes by the flooding tide. Application of glyphosate had no effect on biomass of epiphytes as measured by total chlorophyll content of the epiphyte community and total dry weight of epiphytes in the eight weeks following application at the Z. japonica site and at the subtidal Z. marina site At the intertidal Z. marina site, total chlorophyll and chlorophyll c of epiphytes in the highest treatment plots was about one-half the biomass in the control plots after two weeks, but the glyphosate treatment had no effect on total biomass of epiphytes during the rest of the eight week study period. There was not any increase in the chlorophyll breakdown product, phaeo-pigment at any of the sites. It is suggested that the glyphosate had little measurable effect because of water retained on the leaf surface reduced absorption of the herbicide and because of the short time of exposure to the herbicide (three hours or less) before flooding tide. The natural epiphyte community on Zostera marina fluctuated widely; total chlorophyll decreased by 1/4 during the eight week study period. The biomass of the natural

epiphyte community on Zostera japonica was much lower than the community on Z. marina throughout the eight week study period.

INTRODUCTION

Epiphytes are an important component of the eelgrass community, but may be vulnerable to application of herbicides, such as glyphosate, when used to control nuisance wetland plants in bays and estuaries. Algal epiphytes of seagrasses may account for more than 50% of the community primary productivity (Penhale 1977, Fry 1984, Mazzella and Alberte 1986, Thom 1990), add structural complexity to the leaves that increases density of epifauna (Hall and Bell 1988; Schneider and Mann 1991), and interact with their hosts by shading (Caine 1980, Bulthuis and Woelkerling 1983), contributing nutrients (Harlin 1973, Capone and Taylor 1977), or competing for nutrients and carbon dioxide (Sand-Jensen 1977; Orth and vanMontfrans 1984).

There are few reports on the effects of glyphosate on algae (Grossbard 1985), although deleterious effects on growth of algae would be expected since the primary mode of action of glyphosate is inhibition of the shikimic acid pathway which occurs in plants and micro-organisms (Cole 1985). In laboratory studies glyphosate reduced photosynthesis of the algae Scenedesmus sp. (Van Rensen 1974), Euglena gracilis (Richardson et al. 1979), and Chlorella sorokiniana (Christy et al. 1981) and benthic algae (Goldsborough and Brown 1988). Powell et al. (1991) reported inhibition of photosynthesis and of synthesis of chlorophyll a in the bluegreen algae Synechocystis sp. and Anabaena variabilis but only at relatively high concentrations (5 mM glyphosate). Hernando et al. (1989) reported that glyphosate decreased photosynthetic pigment content of Chlorella pyrenoidosa, inhibiting synthesis of chlorophyll and causing a decrease in carotenoids.

Few studies have attempted to measure the effects of glyphosate on natural populations of aquatic algae. Sullivan et al. (1981) did not attribute any of the changes in population density of diatoms in an aquatic environment of a coastal forest to

spraying by glyphosate. However, Goldsborough and Beck (1989) attributed absorption of glyphosate in pond micro-organisms to the periphyton.

The micro-environment of algal epiphytes on the surface of leaves of seagrasses may make them vulnerable to applications of glyphosate. Any drifting glyphosate spray will contact the epiphytes before contacting the leaves of eelgrass. In addition, the leaves of intertidal eelgrasses often float on the water surface during lower tidal heights. In this position the epiphytes on the leaves are located to receive the initial contact of aerial sprays of herbicides and to be bathed in the sea surface microlayer where herbicides may be concentrated several fold more than underlying bulk water when herbicides are applied above the water surface.

The overall goal of this study was to indicate the effects of realistic applications of the herbicide glyphosate (Rodeo® with X-77® spreader) on natural populations of epiphytes of eelgrass. The specific objectives were 1) to determine the effect of application of glyphosate on the concentration of chlorophyll in epiphytes of eelgrasses in Padilla Bay, Washington; 2) to determine the effect on the concentration of chlorophyll degradation products, phaeo-pigments; 3) to determine these effects on epiphytes at three sites: an intertidal Zostera marina site, a subtidal Z. marina site and an intertidal Z. japonica site; and 4) to indicate the sensitivity of epiphytes by testing these effects at five different rates of application of glyphosate.

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 has extensive intertidal sand and mudflats dissected by dentritic channels that drain and distribute water to the flats

during the mixed durinal 4 m tides. Three sites were selected within the bay representing different eelgrass micro-habitats where effects on eelgrasses were also studied (Bulthuis and Shaw 1993). Site 1 was located in a stand of intertidal Zostera japonica at the highest elevation of the three sites (Fig. 1). Site 2 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 (Blocks) 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}). Herbicide application at the subtidal Z. marina site (3) was limited to only the highest glyphosate concentration (4.44 qts/acre) and the control treatment. Control plots received an application of tap water. Application of glyphosate and spreader was accomplished with a backpack mounted gas pressurized sprayer designed for research (KC-2L Koke Kap®) and a 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.

Spraying at the Zostera japonica site (1) was conducted on 2 July 1992. Sites 2 and 3 were sprayed on 1 July 1992. July 1 and 2 were chosen for application 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

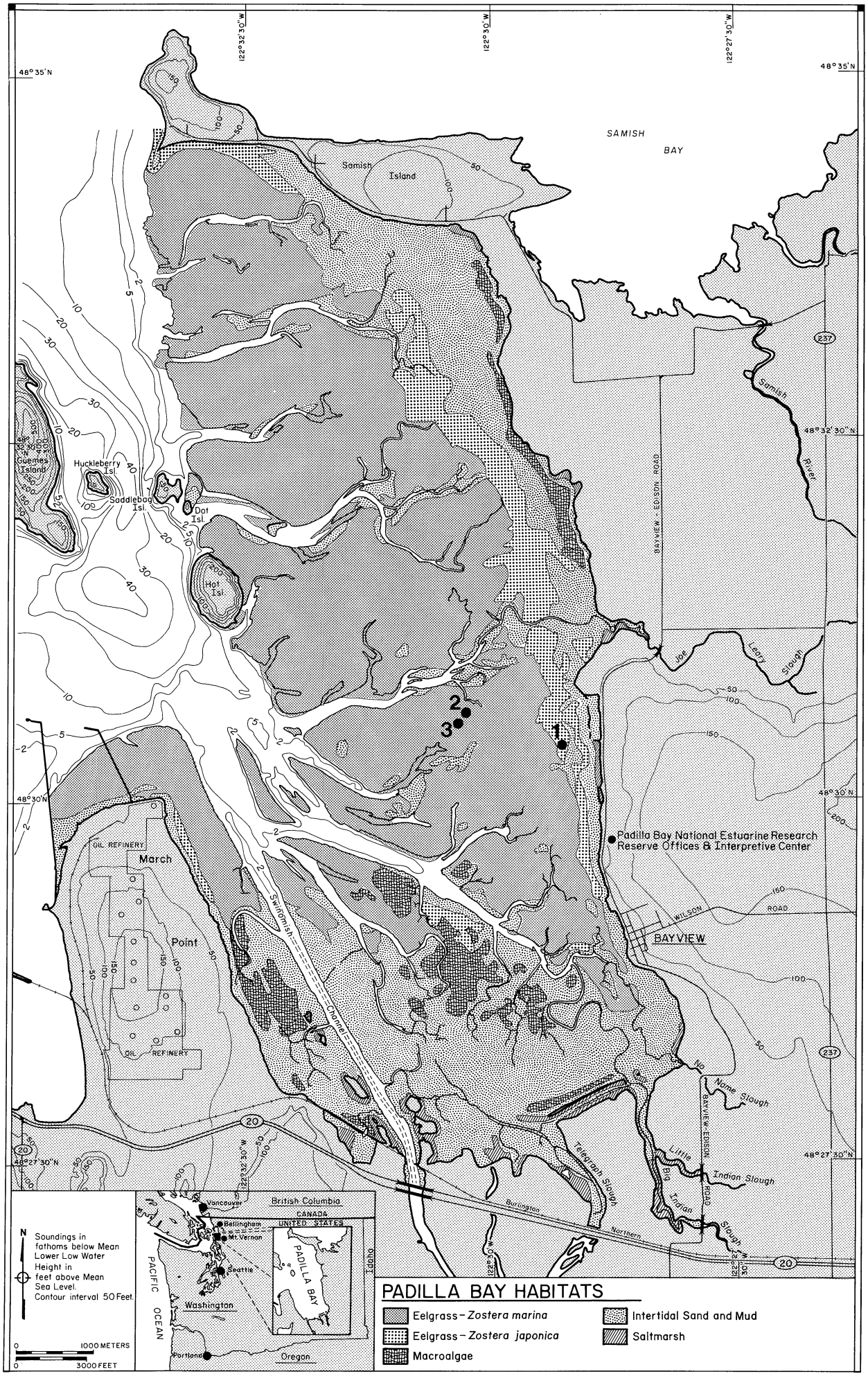

Figure 1. Location of experimental sites (1, 2, and 3) in Padilla Bay.

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).

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

Field sampling. Three eelgrass shoots within each plot were sampled for epiphytes. The third youngest leaf of each sample shoot (which leaf was fully grown, had a well developed epiphyte community, but did not have the tip missing nor the leaf decaying) was clipped and placed in a whirl pack, kept cool for transport to the laboratory and stored at 5°C until analysis. Samples for chlorophyll analysis were collected prior to treatment, one month after treatment and two months after treatment at all sites. In addition, samples were collected every two weeks at the intertidal Zostera marina site from the highest level of treatment and the control plots. Samples for dry weight determination were sampled prior to treatment and two months after treatment.

Chlorophyll analyses. Epiphytes were removed from the leaves on the same day they were collected in the field. The epiphytes were removed from the Zostera by scraping the leaves with a fingernail while wearing sterile gloves. The gloves were used to ensure no epiphytes would be lost by adhering to the researcher's hands. One drop of $MgCO_3$ (1 gram/100ml H_2O) was added to each filter to control acidity. Sea water was used to wash epiphytic material onto a Whatman GF/C filter and the algal suspension was allowed to filter through completely under 20 mm Hg pressure vacuum. Filters were folded together, wrapped in aluminum foil and stored in the freezer for later extraction. Leaves were later measured for surface area.

Extraction of chlorophylls was completed within the 30 day window as prescribed by Parsons et al. (1984). Filters were ground with a Wheaton overhead stirrer with 90% acetone for 5-10 minutes to completely break up the algal cells. The

mixture was transferred to a 15-ml centrifuge tube and 90% acetone was added as needed to fill to 15 ml. Tubes were stoppered and stored overnight in the refrigerator.

Within 24 hours of extraction, the contents of each tube were centrifuged for 10 minutes. The supernatant was pipetted into a 1-cm path length spectrophotometer cuvette and extinction of the sample was measured at the following wavelengths: 630, 646, 648, 664, and 750 nm. The readings at 646 and 648 were averaged to arrive at a reading for 647 nm. Each extinction was corrected for the turbidity blank by subtracting the 750 nm reading from the 630, 647, and 664 nm absorptions. The amount of pigment in the original sample was calculated using the equations below:

(Chla) Chlorophyll a = $11.85\ E_{664}-1.54E_{647}-0.08E_{630}$

(Chlb) Chlorophyll b = $21.03\ E_{647}-5.43E_{664}-2.66E_{630}$

(Chlc) Chlorophyll c = $24.52E_{630}-1.67E_{664}-7.60E_{647}$

where E stands for the absorbance at different wavelengths (after turbidity correction) and Chla, Chlb, and Chlc are the amounts of chlorophyll in µg/ml if a 1-cm light path cuvette is used; then:

$$\mu g\ \text{chlorophyll}/cm^2 = \frac{Cxv}{Vxl}$$

where v is the volume of acetone in ml, V is the surface area of the leaf in cm^2, l is the 1 cm path length of the cuvette and Chla, Chlb, and Chlc are the three chlorophylls which are substituted for C.

Phaeo-pigments were determined by measuring the extinction of the acetone extract of plant pigment before and after treatment with dilute acid. The change following acidification was used to measure the quantity of phaeo-pigments in the sample (Parsons et al. 1984). After the initial measurement of extinction of the extract, two drops of dilute hydrochloric acid (10% HCl) was added to the cuvette. Aluminum

foil was placed over the mouth of the cuvette and the sample was mixed by inverting the cuvette three times. The extinction of the extract was then remeasured. The 750 nm readings were subtracted from the other readings and the following equations (Parsons et al. 1984) were used to calculate the concentration of living chlorophyll a and phaeo-pigments in the sample:

$$\text{living Chlorophyll a (mg/m}^3\text{)} = \frac{26.7(665_o - 665_a)xv}{Vxl}$$

$$\text{phaeo-pigments (mg/m}^3\text{)} = \frac{26.7(1.17(665_a) - 665_o)xv}{Vxl}$$

where 665o is the extinction at 665 nm before acidification, 665a is the extinction at 665 nm after acidification, v is the volume of acetone extract (ml), V is the surface area of the leaf (cm^2) and l is the path length of the cuvette (cm).

Dry weight and ash weight analyses. Whatman glass fiber filters (934AH) were used for dry weight and ash-free dry weight measurements. Filters were washed by filtering 60 ml of distilled water through them and allowing them to dry out on the vacuum. Filters were dried to a constant weight in a 105° C oven. Epiphytes were scraped off as previously described and washed onto the filters using distilled water. Filters with samples were dried to a constant weight at 105° C. Samples were placed in a muffle furnace at 550° C for one hour, weighed, replaced in the muffle furnace for one hour, and reweighed. Sample weight after muffling was subtracted from dry weight to determine ash-free dry weight of each sample.

Statistical analyses. Two-way analysis of variance (ANOVA) with replication was used to test the response for each parameter during each time period; the factors being treatment (six concentrations at intertidal sites and two concentrations at the subtidal site) and blocks (three columns) (Sokal and Rohlf 1969). When the two-way

ANOVA indicated significant treatment or block effect, the post hoc Bonferroni test was used to determine which treatments were significantly different from each other (Rice 1989; Systat 1992).

RESULTS

The chlorophyll content of epiphytes on Zostera japonica was very low, < 0.1 μg chl per cm^2 of leaf area, at the initiation of the experiment on the 1st of July (Table 1). During the following month the biomass of epiphytes as measured by total chlorophyll increased more than tenfold, the majority of which was chlorophyll c, and then decreased the second month. Application of glyphosate had no effect on total chlorophyll, chlorophyll a, or chlorophyll c of epiphytes of Z. japonica, nor did the concentration of the degradation products of chlorophyll, phaeo-pigments, increase in treatment plots (Table 1, Appendix A).

Biomass of epiphytes of Zostera marina at both the intertidal (site 2) and subtidal (3) sites was high, > 6 μg chl/cm^2, at the beginning of the experiment, but decreased during the following eight weeks to concentrations around 1 μg chl/cm^2 (Tables 2-5, Fig. 2, Appendix A). Application of glyphosate, again, had no detectable effect on concentration of total chlorophyll, chlorophyll c, nor phaeophytin in epiphytes of Z. marina at the subtidal site (site 3, Table 5). However, at the intertidal Z. marina site (2), two weeks after application of glyphosate, total chlorophyll (a, b, and c) and chlorophyll c were significantly lower in plots with the highest level of treatment (4.4 qts/acre) compared to control plots (Tables 2 and 3). There was no comparable increase in phaeo-pigments (Table 4). Two weeks later (four weeks after treatment), there was no difference in total chlorophyll due to treatment by glyphosate, nor was

there a difference in the two subsequent sampling periods (Table 2). [The significant differences in chlorophyll c among plots on 28 July and 26 August (Table 3) were apparently not caused by the treatment because the lowest chlorophyll c per leaf area were scattered among the middle levels of treatment (Table 3)]. The biomass of epiphytes at an auxiliary sampling site (distant control) located 100 m from site 2 was similar to the biomass of epiphytes at site 2, showing that spread of the glyphosate over the whole site was not the cause for the decrease in biomass in the four weeks after application (Tables 2-4).

The dry weight and ash-free dry weight of epiphytes on Zostera japonica and Z. marina eight weeks after application of glyphosate was similar in control plots and in plots treated with glyphosate (Table 6, Appendix B). The dry weight of epiphytes paralleled the concentration of chlorophyll, increasing on Z. japonica during the eight week experimental period and decreasing on Z. marina (Table 6).

Table 1. Concentration of total chlorophyll *(a, b* and *c*), chlorophyll *c*, and phaeopigments in epiphytes of *Zostera japonica* at an intertidal site (site 1) at which the indicated concentration of glyphosate was applied on July 2, 1992. Units are µg chl/cm2 leaf area, mean ± standard error of the mean, n=3 plots, each plot mean based on 3 leaf samples per plot except for 1 July when plot means are based on 2 samples per plot. n.d.=no measurements taken. None of the control vs. 4.4 qts/acre comparisons are significantly (P>0.05) different from each other by two-way analysis of variance (ANOVA).

Treatment	1 July	30 July	28 August
Total chlorophyll			
Initial	<0.10 ±0.00		
Control		1.30 ±0.15	0.44 ±0.23
4.4 qts/acre		1.34 ±0.10	0.50 ±0.25
Chlorophyll c			
Initial	n.d.		
Control		0.90 ±0.09	0.10 ±0.06
4.4 qts/acre		0.93 ±0.11	0.11 ±0.06
Phaeophytin			
Initial	n.d.		
Control		0.03 ±0.01	0.04 ±0.04
4.4 qts/acre		0.03 ±0.03	0.03 ±0.02

Table 2. Concentration of total chlorophyll (*a, b* and *c*,) in epiphytes of *Zostera marina* at an intertidal site (site 2) at which the indicated concentration of glyphosate was applied on July 1,1992. The distant control was collected 100m east of the experimental site. Units are µg chl/cm2 leaf area, mean ± standard error of the mean, n=3 plots, each plot mean based on 3 leaf samples per plot except for 30 June when plot means were based on 2 samples per plot. Results of two-way analysis of variance (ANOVA) are indicated by: ns = not significant; * = P<0.05.

Treatment	30 June	14 July	28 July	11 August	26 August
Initial	6.39 ±0.90				
Control		4.20 ±0.35	1.01 ±0.16	2.25 ±0.12	0.67 ±0.12
0.28 qts/acre			0.87 ±0.14		0.94 ±0.16
0.56 qts/acre			0.56 ±0.05		0.85 ±0.11
1.1 qts/acre			0.79 ±0.18		0.60 ±0.12
2.2 qts/acre			0.73 ±0.17		0.59 ±0.02
4.4 qts/acre		1.74 ±0.80	1.03 ±0.17	1.84 ±0.04	0.74 ±0.14
Distant control			0.66 ±0.13		
ANOVA					
Treatment		*	ns	ns	ns
Block		ns	ns	ns	ns
Treatment X Block		ns	ns	ns	ns

Table 3. Concentration of chlorophyll *c*, in epiphytes of *Zostera marina* at an intertidal site (site 2) at which the indicated concentration of glyphosate was applied on July 1,1992. The distant control was collected 100m east of the experimental site. Units are µg chl/cm2 leaf area, mean±standard error of the mean, n=3 plots, each plot mean based on 3 leaf samples per plot except for 30 June when plot means were based on 2 samples per plot. Results of two-way 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 indicated for 28 July that the only significant difference was between the 0.56 qts/acre mean and the 4.4 qts/acre mean and that row B was different from row C; and for 26 August that none of the treatment means were different from each other.

Treatment	30 June	14 July	28 July	11 August	26 August
Initial	1.05 ±0.12				
Control		0.77 ±0.06	0.19 ±0.02	0.38 ±0.03	0.14 ±0.01
0.28 qts/acre			0.10 ±0.01		0.22 ±0.03
0.56 qts/acre			0.04 ±0.02		0.22 ±0.03
1.1 qts/acre			0.19 ±0.10		0.13 ±0.04
2.2 qts/acre			0.07 ±2.00		0.17 ±0.02
4.4 qts/acre		0.35 ±0.15	0.21 ±0.10	0.32 ±0.02	0.14 ±<0.01
Distant control			0.13 ±0.01		
ANOVA					
Treatment		*	**	ns	*
Block		ns	**	ns	ns
Treatment X Block		ns	ns	ns	ns

Table 4. Concentration of phaeopigments in epiphytes of *Zostera marina* at an intertidal site (site 2) at which the indicated concentration of glyphosate was applied on July 1,1992. The distant control was collected 100m east of the experimental site. Units are µg chl/cm2 leaf area, mean±standard error of the mean, n=3 plots, each plot mean based on 3 leaf samples per plot except for 30 June when plot means were based on 2 samples per plot. Results of two-way analysis of variance (ANOVA) are indicated by: ns = not significant; * = P<0.05. The Bonferroni post hoc test for differences among means indicated that no one of the rows (Blocks) on 26 August was significantly (P>0.05) different from the others.

Treatment	30 June	14 July	28 July	11 August	26 August
Initial	0.06 ±0.03				
Control		0.09 ±0.06	0.06 ±0.03	0.12 ±0.01	<0.01 ±<0.01
0.28 qts/acre			0.03 ±0.03		0.03 ±0.03
0.56 qts/acre			<0.01 ±<0.01		0.01 ±0.01
1.1 qts/acre			0.01 ±0.01		<0.01 ±<0.01
2.2 qts/acre			0.02 ±0.01		0.01 ±0.01
4.4 qts/acre		0.13 ±0.08	<0.01 ±<0.01	0.07 ±<0.01	0.03 ±0.03
Distant control			0.02 ±0.01		
ANOVA					
Treatment		ns	ns	ns	ns
Block		ns	ns	ns	*
Treatment X Block		ns	ns	ns	ns

Table 5. Concentration of total chlorophyll (*a, b* and *c*), chlorophyll *c*, and phaeopigments in epiphytes of *Zostera marina* at a subtidal site (site 3) at which the indicated concentration of glyphosate was applied on July 1,1992. Units are µg chl/cm2 leaf area, mean±standard error of the mean, n=3 plots, each plot mean based on 3 leaf samples per plot except for 1 July when plot means are based on 2 samples per plot. None of the control vs. 4.4 qts/acre comparisons are significantly (P>0.05) different from each other by two-way analysis of variance.

Treatment	1 July	29 July	27 August
Total chlorophyll			
Initial	6.57 ±1.92		
Control		1.03 ±0.21	1.00 ±0.03
4.4 qts/acre		1.14 ±0.22	0.85 ±0.17
Chlorophyll c			
Initial	1.23 ±0.39		
Control		0.19 ±0.03	0.16 ±0.01
4.4 qts/acre		0.21 ±0.03	0.13 ±0.03
Phaeophytin			
Initial	0.48 ±0.24		
Control		0.05 ±0.01	0.06 ±0.01
4.4 qts/acre		0.09 ±0.04	0.08 ±0.03

Table 6. Dry weight of epiphytes on leaves of Zostera marina (sites 2 and 3) and Zostera japonica (site 1) in Padilla Bay at which the indicated concentration of glyphosate was applied on June 30 or July 1, 1992. Units are µg/cm2 leaf area, mean±standard error of the mean, n=3 plots, each plot mean based on 2 leaf samples per plot. Initial measurements: June 30 and July 1, 1992; Final measurements: August 26-28, 1992. One-way analysis of variance (ANOVA) indicated that none of the control vs. 4.4 qts/acre means were significantly different from each other.

	Total dry weight	Ashfree dry weight
Zostera japonica		
Initial	0.18 ±0.06	0.18 ±0.03
Final		
Control	0.77 ±0.15	0.43 ±0.03
4.4 qts/acre	0.63 ±0.23	0.47 ±0.15
Zostera marina intertidal		
Initial	10.23 ±1.23	3.57 ±0.34
Final		
Control	0.50 ±0.10	0.20 ±0.06
4.4 qts/acre	0.47 ±0.12	0.20 ±0.06
Zostera marina subtidal		
Initial	8.47 ±2.13	2.90 ±0.69
Final		
Control	1.03 ±0.59	0.43 ±0.28
4.4 qts/acre	0.53 ±0.03	0.20 ±0.00

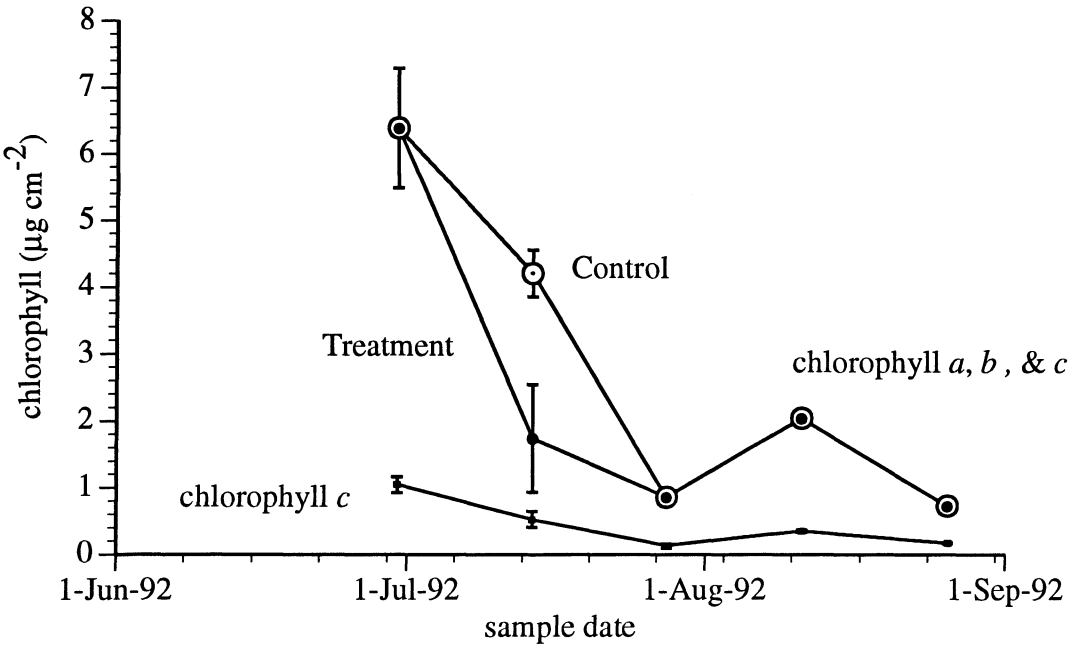

Figure 2. Concentration of chlorophylls *a*, *b* , & *c* and chlorophyll *c* in epiphytes of *Zostera marina* at site 2 from June 30 to August 28, 1992. Mean ± s. e. n=3 to 18 plots (depending on date, see Appendix A for complete data) with 3 leaves sampled per plot. On the 14th of July there was a significant diffence in the total chlorophyll (*a*, *b*, & *c*) between treatment and control plots. Therefore, chlorophyll *a*, *b*, & *c* are plotted separately for that date.

DISCUSSION

The application of glyphosate (Rodeo® and X-77® spreader) had little effect on the epiphytes of Zostera marina and Z. japonica in the present study. At one of the three sites, the biomass of epiphytes, as measured by total chlorophyll, decreased two weeks after application of glyphosate, but no further decreases in epiphyte biomass were observed. This lack of any appreciable effect may have been due to inappropriate measures of the epiphyte community, resistance (or non-vulnerability) of the epiphytes to glyphosate, or lack of absorption of the glyphosate by the epiphytes. These possibilities are discussed, in order, below.

The concentration of chlorophylls a, b, and c and the total dry weight and organic weight of epiphytes are gross measures of the total epiphyte community biomass. Changes in these parameters indicate gross changes to the epiphyte community. It is possible that application of glyphosate was causing some changes to the epiphyte community; yet, because of the diverse nature of the community (Heijs 1987, Borowitzka et al. 1990) and the short doubling times of most community members, decreases in one species of epiphytes may have been compensated by increases in another species. The results in this study indicate no overall effects on total biomass of the epiphyte community under the conditions of the treatment other than a short-term decrease in total chlorophyll in the first two weeks after treatment. There may have been other important effects at the species level. Such effects were not addressed in this study and are better addressed in laboratory studies of cultures.

A second explanation for the lack of an observed effect is that epiphytes may be resistant to the glyphosate and spreader. This seems unlikely because glyphosate's mode of action appears to influence photosynthesis (Cole 1985). Van Rensen (1974) reported decreased photosynthesis of the alga Scenedesmus sp.; Richardson et al. (1979) reported reduced chlorophyll production in Euglena when exposed to glyphosate;

and glyphosate caused cell destruction of the alga Chlorella sorokiniana (Christy et al. 1981). Glyphosate decreased cell density and photosynthetic pigment content of Chlorella pyreniodosa (Hernando et al. 1989). In the later study, glyphosate was shown to have two different effects on photosynthetic pigments: inhibition of chlorophyll synthesis and a decrease in carotenoids. Therefore, epiphytes are likely to be effected by glyphosate if the herbicide is absorbed into the cell.

A third explanation for the lack of an observed effect is lack of entry of the glyphosate at a high enough concentration into epiphyte cells. The epiphytes, at all three sites were positioned to intercept the glyphosate spray directly. In the case of epiphytes of the subtidal Zostera marina (site 3), the leaves with the epiphytes were floating on the water surface and the spray remained visibly stationary on the water surface within the treatment plots until the return of the tide. On the other hand, epiphytes on the opposite side of the leaf at this site would not have been exposed directly to the spray and would probably have been surrounded by a much lower concentration of glyphosate during the exposure period. The total time of exposure to the glyphosate before return of the flooding tide may have been too short for sufficient absorption of glyphosate into epiphytes. The date of the treatment was selected because the predicted tide was equal to the lowest tide of the year, and plots were treated shortly before the time of low tide in order to maximize the time before return of the tide. However, the time of exposure was only 2 to 3 hours at the three sites. This short time of exposure may have limited the amount of glyphosate that was absorbed by the epiphytes in the experimental plots. In addition to the short exposure time, another factor limiting the absorption of glyphosate into the epiphytes may be the film of water retained on the leaf surfaces of Z. marina and Zostera japonica. The epiphytes help retain such a film of water even during times of exposure of the eelgrass. Such a film may dilute the concentration of glyphosate and decrease the rate of absorption of glyphosate into the epiphytes.

Another noteworthy feature of the data on chlorophyll concentration and weight of epiphytes is the rapid changes in the total community size. At site 2, the concentration of chlorophyll a, b, and c decreased to less than 1/2 in two weeks and a further decrease to less than 1/6 of the initial concentration in 4 weeks. Such rapid fluctuations in biomass of epiphytes on seagrasses have been reported by other authors such as Thom in Padilla Bay (Thom 1990, Thom et al. 1991), Macauley et al. (1988) in the Gulf of Mexico, and Jacobs et al. (1983) on the Atlantic coast of France. Such rapid fluctuations in epiphyte community biomass combined with the spatial heterogeneity of the epiphytes may preclude detection of subtle effects of herbicides and limit field studies such as this one to an evaluation of overall gross effects.

The cause for the rapid decline in biomass of epiphytes is not known. However, grazers, such as snails, isopods, and amphipods can quickly reduce the biomass of epiphytes of seagrasses (Hootsmans and Vermaat 1985; Howard and Short 1986; Mazzella and Russo 1989). Thom et al. (1991), Caine (1991), and Shaw (1994) reported large fluctuations in the density of grazers such as the isopod Idotea resecata, the caprellid amphipod Caprella laeviuscula, the gastropod Lacuna variegata and the opisthobranchs Phyllaplysia taylori and Haminoea vesicula in Padilla Bay. Thom et al. (1991) reported increased densities of these grazers in Padilla Bay during summer and autumn. In laboratory studies Idotea resecata generally removed 100% of the epiphytes by the end of the experiments (Thom et al. 1991). Densities of Idotea resecata and Phyllaplysia taylori on leaves of eelgrass in Padilla Bay increased five- to ten-fold in a two week period during summer (Shaw 1994). Therefore, the rapid decline in standing crop of epiphytes on Zostera marina between 1 July and 28 July observed in the present study may have been caused by an increase in grazing by invertebrates.

In summary, the present study indicates that application of glyphosate did not have major deleterious effects on the epiphytes of eelgrasses when the application rate was around 2 quarts/acre and the time before immersion of the eelgrass was about 3

hours or less. Less obvious effects such as shifts in species composition may occur but were not measured. The total biomass of epiphytes on eelgrasses changed rapidly during the eight week summer study period.

ACKNOWLEDGEMENTS

We thank Steve Wirth for applying herbicide onto minute research plots; Sharon Riggs, Robin Cottrell, Jennifer Crain, Travis Shaw, and Brady Scott for assistance in the field and laboratory; Kurt Fresh, Washington State Department of Fisheries for advice and help in planning the study; Dr. Steve Sulkin and staff of the Shannon Point Marine Center, Western Washington University, for use of the laboratory and equipment for chlorophyll analyses; the Washington State Department of Natural Resources for a financial grant to conduct this study; Sharon Riggs for reviewing the manuscript; and Dr. Tom Mumford, DNR, for assistance in planning this research and encouragement throughout the study. The study was financially aided through an interagency agreement between the Washington State Department of Natural Resources and Ecology, and through grants to the Washington State Department of Ecology with funds obtained from NOAA Office of Ocean and Coastal Resources Management and appropriated for Sections 306 and 315 of the Coastal Zone Management Act of 1972, as amended.

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APPENDICES

Appendix A. Concentration of chlorophylls a b and c and phaeo-pigments in epiphytes on each sample leaf of Zostera marina and Zostera japonica at 3 treatment sites in Padilla Bay in June, July, and August, 1992.

Appendix B. Dry weight and ash-free dry weight of epiphytes on each sample leaf of Zostera marina and Zostera japonica at 3 treatment sites in Padilla Bay in June, July, and August, 1992.

Appendix A-1. Concentration of chlorophyll in epiphytes on leaves of Zostera japonica at site 1 on 1 July 1992. Units are µg chlorophyll per cm2 of leaf surface area. Sample number indicates row in which plot was located (A-H), plot number (1-7), and leaf number within plot (1-3). Detection limit was less than 0.10 µg chl/cm2 leaf area.

Sample	Chl a	Chl b	Chl c	Ca+Cb+Cc	living chl a	phaeophytin
D4#1	0.00	0.00	0.00	0.00	0.00	0.00
D4#2	0.00	0.00	0.00	0.00	0.00	0.00
F6#1	0.00	0.00	0.00	0.00	0.00	0.00
F6#2	0.00	0.00	0.00	0.00	0.00	0.00

Appendix A-2. Concentration of chlorophyll in epiphytes on leaves (#1-#3) of *Zostera japonica* at site 1 on 30 July 1992. Units are µg chlorophyll per cm2 of leaf surface area. Means are given for plots (n=3 leaves per plot), treatments (n=3 plots per treatment) and the mean for this sampling date (n=6 plots on this date). s.e.=standard error of the mean.

Sample	Chl a	Chl b	Chl c	Ca+Cb+Cc	living chl a	phaeophytin
D5#1	0.67	0.00	0.69	1.35	1.04	-0.68
D5#3	0.21	0.00	0.53	0.74	0.32	-0.20
E3#1	0.45	0.00	0.41	0.86	0.27	0.26
E4#1	0.28	0.00	0.61	0.89	0.32	-0.08
E4#2	0.38	0.00	0.43	0.81	0.39	-0.04
F4#1	0.26	0.00	0.43	0.69	0.20	0.08
D1#1	0.78	0.00	0.90	1.68	0.68	0.13
D1#2	0.22	0.00	1.07	1.29	0.14	0.14
D1#3	0.00	0.00	1.46	1.46	0.00	0.00
D5#2	0.90	0.00	1.60	2.51	1.55	-1.14
E3#2	0.04	0.00	1.09	1.13	0.11	-0.11
E3#3	0.38	0.00	0.70	1.09	0.38	-0.02
E4#3	0.48	0.00	1.27	1.75	0.60	-0.22
F4#2	0.41	0.00	1.73	2.14	0.57	-0.30
F4#3	0.25	0.00	0.95	1.20	0.03	0.35
F7#1	0.24	0.00	0.99	1.23	0.10	0.22
F7#3	1.12	0.00	1.23	2.34	1.29	-0.34
F7#2	0.13	0.00	0.43	0.56	0.17	-0.08
Control plots						
D5 mean	0.59	0.00	0.94	1.53	0.97	0.00
E3 mean	0.29	0.00	0.73	1.02	0.25	0.04
F4 mean	0.31	0.00	1.03	1.34	0.27	0.04
4.4 qts/acre						
D1 mean	0.33	0.00	1.14	1.48	0.27	0.09
E4 mean	0.38	0.00	0.77	1.15	0.43	0.00
F7 mean	0.50	0.00	0.88	1.38	0.52	0.00
Control mean	0.40	0.00	0.90	1.30	0.50	0.03
s.e.	±0.10	±0.00	±0.09	±0.15	±0.24	±0.01
Treatment mean	0.40	0.00	0.93	1.33	0.41	0.03
s.e.	±0.05	±0.00	±0.11	±0.10	±0.07	±0.03
30 July mean	0.40	0.00	0.92	1.32	0.45	0.03
s.e.	±0.05	±0.00	±0.06	±0.08	±0.11	±0.01

Appendix A-3. Concentration of chlorophyll in epiphytes of *Zostera japonica* at site 1 on 28 August 1992. Units are µg chlorophyll per cm2 of leaf surface area. Means are given for plots (n=3 leaves per plot), treatments (n=3 plots per treatment) and the mean for this sampling date (n=6 plots on this date). s.e.=standard error of the mean.

Sample	Chl a	Chl b	Chl c	Ca+Cb+Cc	living chl a	phaeophytin
D1-1	1.14	0.02	0.24	1.40	1.07	0.06
D1-2	0.31	0.07	0.16	0.54	0.31	0.00
D1-3	0.52	0.00	0.19	0.71	0.79	0.00
D5-1	0.53	0.02	0.14	0.69	0.67	0.00
D5-2	0.37	0.10	0.33	0.80	0.90	0.00
D5-3	0.72	0.00	0.13	0.84	0.47	0.37
E3-1	0.40	0.02	0.10	0.51	0.40	0.00
E3-2	0.48	0.00	0.04	0.52	0.47	0.00
E3-3	0.43	0.02	0.12	0.57	0.41	0.01
E4-1	0.47	0.03	0.17	0.67	0.82	0.00
E4-2	0.74	0.00	0.13	0.87	0.57	0.24
E4-3	0.13	0.00	0.10	0.23	0.30	0.00
F4-1	0.00	0.00	0.02	0.02	0.00	0.00
F4-2	0.00	0.00	0.00	0.00	0.00	0.00
F4-3	0.00	0.00	0.00	0.00	0.00	0.00
F7-1	0.00	0.00	0.01	0.01	0.00	0.00
F7-2	0.05	0.00	0.00	0.05	0.11	0.00
F7-3	0.02	0.00	0.00	0.02	0.05	0.00
Control						
D5 mean	0.54	0.04	0.20	0.78	0.68	0.12
E3 mean	0.43	0.01	0.09	0.54	0.43	0.00
F4 mean	0.00	0.00	0.01	0.01	0.00	0.00
Treatment						
D1 mean	0.66	0.03	0.20	0.89	0.72	0.02
E4 mean	0.45	0.01	0.13	0.59	0.56	0.08
F7 mean	0.02	0.00	0.00	0.03	0.05	0.00
Control mean	0.32	0.02	0.10	0.44	0.37	0.04
s.e.	±0.16	±0.01	±0.06	±0.23	±0.20	±0.04
Treatment mean	0.38	0.01	0.11	0.50	0.45	0.03
s.e.	±0.19	±0.01	±0.06	±0.25	±0.20	±0.02
28 Aug mean	0.35	0.02	0.10	0.47	0.41	0.04
s.e.	±0.11	±0.01	±0.04	±0.15	±0.13	±0.02

Appendix A-4. Concentration of chlorophyll in epiphytes on leaves of *Zostera marina* at site 2 on 30 June 1992. Units are µg chlorophyll per cm2 of leaf surface area. Means are also given for plots (n=2 leaves per plot) and the mean for this sampling date (n=3 plots on this date). s.e.=standard error of the mean.

Sample	Chl a	Chl b	Chl c	Ca+Cb+Cc	living chl a	phaeophytin
A5#1	2.66	0.01	0.57	3.24	2.56	0.01
A5#2	5.23	0.00	1.11	6.34	4.98	0.11
B6#1	8.18	0.00	1.43	9.61	7.79	0.16
B6#2	5.08	0.00	1.11	6.19	4.86	0.06
C5#1	4.30	0.00	0.92	5.22	4.29	-0.23
C5#2	6.62	0.00	1.12	7.75	6.32	0.10
A5 mean	3.94	0.00	0.84	4.79	3.77	0.06
B6 mean	6.63	0.00	1.27	7.90	6.32	0.11
C5 mean	5.46	0.00	1.02	6.48	5.30	0.00
30 Jun mean	5.34	0.00	1.05	6.39	5.13	0.06
s.e.	±0.78	±0.00	±0.12	±0.90	±0.74	±0.03

Appendix A-5. Concentration of chlorophyll in epiphytes on leaves of *Zostera marina* at site 2 on 14 July 1992. Units are µg chlorophyll per cm2 of leaf surface area. Means are given for plots (n=3 leaves per plot), treatments (n=3 plots per treatment) and the mean for this sampling date (n=6 plots on this date). s.e.=standard error of the mean.

Sample	Chl a	Chl b	Chl c	Ca+Cb+Cc	living chl a	phaeophytin
A1 #2	0.68	0.03	0.14	0.85	0.52	0.25
A1 #3	1.03	0.01	0.23	1.27	1.03	-0.06
A4 #1	2.95	-0.01	0.60	3.55	3.48	-1.07
A4 #3	1.40	0.00	0.30	1.70	1.35	0.00
B5 #1	2.61	-0.01	0.56	3.16	2.40	0.21
B5 #2	2.50	-0.01	0.58	3.08	2.40	0.03
B5 #3	3.60	0.03	0.80	4.43	3.26	0.36
B6 #1	0.61	0.00	0.18	0.79	0.00	1.00
B6 #2	0.44	0.00	0.08	0.52	0.42	0.00
B6 #3	0.85	0.00	0.25	1.10	0.91	-0.15
C1 #1	2.92	0.00	0.66	3.57	2.73	0.13
C1 #2	4.14	0.00	0.97	5.11	4.07	-0.13
C1 #3	3.42	0.00	0.80	4.22	3.16	0.23
C5 #1	4.72	0.00	1.09	5.80	4.37	0.32
C5 #2	2.70	0.00	0.63	3.34	2.58	0.05
C5 #3	0.68	0.00	0.20	0.88	0.70	-0.07
A1 #1	0.74	0.01	0.38	1.12	0.99	-0.46
A4 #2	7.38	-0.01	1.64	9.00	7.07	0.10
Control plots						
A4 mean	3.91	-0.01	0.85	4.75	3.97	0.00
B5 mean	2.90	0.00	0.65	3.56	2.69	0.20
C1 mean	3.49	0.00	0.81	4.30	3.32	0.08
Treatment plots						
A1 mean	0.82	0.02	0.25	1.08	0.85	0.00
B6 mean	0.63	0.00	0.17	0.80	0.44	0.28
C5 mean	2.70	0.00	0.64	3.34	2.55	0.10
Control mean	3.44	0.00	0.77	4.20	3.33	0.09
s.e.	±0.29	±0.00	±0.06	±0.35	±0.37	±0.06
Treatment mean	1.38	0.01	0.35	1.74	1.28	0.13
s.e.	±0.66	±0.01	±0.15	±0.80	±0.64	±0.08
14 July mean	2.41	0.00	0.56	2.97	2.30	0.11
s.e.	±0.56	±0.00	±0.12	±0.68	±0.57	±0.05

Appendix A-6. Concentration of chlorophyll in epiphytes on leaves of *Zostera marina* at site 2 on 28 July 1992. Units are µg chlorophyll per cm2 of leaf surface area. Means are given for plots (n=3 leaves per plot), treatments (n=3 plots per treatment) and the mean for this sampling date (n=18 plots on this date). s.e.=standard error of the mean.

Sample	Chl a	Chl b	Chl c	Ca+Cb+Cc	living chl a	phaeophytin
A5 #1	0.38	0.00	0.00	0.38	0.37	-0.01
A5 #2	1.89	0.00	0.27	2.16	1.79	0.05
A5 #3	0.85	0.00	0.07	0.91	0.66	0.26
A6#1	0.78	0.00	0.01	0.79	0.77	-0.04
A6 #2	0.39	0.00	0.00	0.39	0.40	-0.05
A6 #3	0.34	0.00	0.00	0.34	0.35	-0.04
A7 #1	0.22	0.00	0.03	0.25	0.12	0.11
A7 #2	0.67	0.00	0.03	0.71	0.67	-0.04
A7 #3	0.57	0.00	0.04	0.61	0.57	-0.04
B1 #1	0.85	0.00	0.09	0.93	0.82	-0.02
B1 #2	1.15	0.00	0.15	1.30	1.11	-0.01
B1 #3	0.87	0.00	0.07	0.94	0.87	-0.06
B3 #1	0.40	0.00	0.03	0.43	0.42	-0.06
B3 #2	0.80	0.00	0.10	0.90	0.79	-0.04
B3 #3	0.61	0.00	0.05	0.66	0.59	-0.01
B7 #1	0.51	0.00	0.05	0.57	0.51	-0.03
B7 #2	0.40	0.00	0.00	0.40	0.43	-0.09
B7 #3	1.00	0.00	0.15	1.15	0.96	0.00
C1 #2	0.56	0.00	0.10	0.66	0.54	0.01
C1 #3	1.32	0.00	0.22	1.54	1.16	0.20
C2 #1	0.49	0.00	0.01	0.50	0.47	-0.01
C4 #1	0.36	0.00	0.01	0.37	0.37	-0.05
C4 #2	0.25	0.00	0.01	0.26	0.30	-0.12
C5#1	0.95	0.00	0.13	1.09	0.87	0.07
C4 #3	0.76	0.00	0.10	0.87	0.74	-0.01
C6 #1	0.45	0.00	0.11	0.56	0.48	-0.08
C6 #2	0.80	0.00	0.11	0.92	0.81	-0.07
C6 #3	0.63	0.00	0.11	0.74	0.61	-0.02
C7 #1	0.45	0.00	0.04	0.48	0.37	0.10
C7 #2	0.49	0.00	0.04	0.53	0.44	0.04
C7 #3	0.72	0.00	0.10	0.82	0.70	-0.02
C1 #1	1.70	0.00	0.13	1.83	1.51	0.19
C2 #2	0.52	0.00	0.00	0.52	0.57	-0.17
C2 #3	0.33	0.00	0.03	0.35	0.42	-0.22
C5 #2	1.03	0.00	0.03	1.06	0.97	0.00
C5 #3	0.32	0.00	0.00	0.32	0.35	-0.09
A1 #1	1.12	0.01	0.24	1.37	1.13	-0.07
A1 #2	0.42	0.02	0.13	0.56	0.48	-0.13
A1 #3	0.72	0.02	0.18	0.92	0.71	-0.03
A3 #1	0.42	0.00	0.11	0.54	0.42	-0.03
A3 #3	0.65	0.03	0.21	0.88	0.64	-0.02
B5 #1	0.79	0.00	0.19	0.96	0.72	0.08
B5 #2	0.68	0.00	0.19	0.86	0.68	-0.04
B5 #3	0.83	0.00	0.22	1.05	0.78	0.04
B6 #2	0.57	0.00	0.09	0.60	0.47	0.12
A3 #2	1.04	0.00	0.30	1.29	0.89	0.18
A4 #2	0.72	0.00	0.23	0.80	0.60	0.14
A4 #3	0.66	0.00	0.21	0.82	0.78	-0.24
B2 #1	0.62	0.00	0.15	0.56	0.75	-0.29
A4 #1	0.87	0.00	0.25	1.05	0.72	0.20
B2 #2	1.09	0.00	0.28	1.22	0.93	0.18
B2 #3	1.16	0.00	0.60	1.74	1.20	-0.13
B6 #1	0.71	0.00	0.35	1.00	0.79	-0.18
B6 #3	2.16	0.02	0.74	2.92	2.10	-0.02

Appendix A-6. (continued).

Sample	Chl a	Chl b	Chl c	Ca+Cb+Cc	living chl a	phaeophytin
Control						
A4 mean	0.75	0.00	0.23	0.89	0.70	0.03
B5 mean	0.77	0.00	0.20	0.96	0.73	0.03
C1 mean	1.19	0.00	0.15	1.34	1.07	0.13
0.28 qts/acre						
A5 mean	1.04	0.00	0.11	1.15	0.94	0.10
B7 mean	0.64	0.00	0.07	0.71	0.63	0.00
C6 mean	0.63	0.00	0.11	0.74	0.63	0.00
0.56 qts/acre						
A6 mean	0.50	0.00	0.00	0.51	0.51	0.00
B3 mean	0.60	0.00	0.06	0.66	0.60	0.00
C4 mean	0.46	0.00	0.04	0.50	0.47	0.00
1.1 qts/acre						
A3 mean	0.70	0.01	0.21	0.90	0.65	0.04
B2 mean	0.95	0.00	0.35	1.17	0.96	0.00
C2 mean	0.45	0.00	0.01	0.46	0.49	0.00
2.2 qts/acre						
A7 mean	0.49	0.00	0.03	0.52	0.45	0.01
B1 mean	0.96	0.00	0.10	1.06	0.93	0.00
C7 mean	0.55	0.00	0.06	0.61	0.50	0.04
4.4 qts/acre						
A1 mean	0.75	0.02	0.18	0.95	0.77	0.00
B6 mean	1.15	0.01	0.39	1.51	1.12	0.00
C5 mean	0.77	0.00	0.06	0.82	0.73	0.00
Control mean	0.90	0.00	0.19	1.06	0.83	0.06
s.e.	±0.15	±0.00	±0.02	±0.14	±0.12	±0.03
.28 mean	0.77	0.00	0.10	0.87	0.74	0.03
s.e.	±0.14	±0.00	±0.01	±0.14	±0.10	±0.03
.56 mean	0.52	0.00	0.04	0.56	0.53	0.00
s.e.	±0.04	±0.00	±0.02	±0.05	±0.04	±0.00
1.1 mean	0.70	0.00	0.19	0.84	0.70	0.01
s.e.	±0.15	±0.00	±0.10	±0.21	±0.14	±0.01
2.2 mean	0.67	0.00	0.07	0.73	0.63	0.02
s.e.	±0.15	±0.00	±0.02	±0.17	±0.15	±0.01
4.4 mean	0.89	0.01	0.21	1.09	0.87	0.00
s.e.	±0.13	±0.00	±0.10	±0.21	±0.12	±0.00
28 July mean	0.74	0.00	0.13	0.86	0.72	0.02
s.e.	±0.05	±0.00	±0.03	±0.07	±0.05	±0.01

Appendix A-7. Concentration of chlorophyll in epiphytes on leaves of *Zostera marina* at site 2 on 11 August 1992. Units are µg chlorophyll per cm2 of leaf surface area. Means are given for plots (n=3 leaves per plot), treatments (n=3 plots per treatment) and the mean for this sampling date (n=6 plots on this date). s.e.=standard error of the mean.

Sample	Chl a	Chl b	Chl c	Ca+Cb+Cc	living chl a	phaeophytin
A1#1	1.33	0.04	0.27	1.64	1.24	0.08
A1#2	1.52	0.02	0.28	1.81	1.42	0.08
A1#3	1.62	0.03	0.31	1.96	1.54	0.04
A4#1	1.84	0.02	0.35	2.21	1.71	0.11
A4#2	1.88	0.02	0.34	2.24	1.75	0.12
A4#3	1.29	0.02	0.26	1.57	1.22	0.05
B5#1	1.30	0.02	0.26	1.58	1.23	0.04
B5#2	2.24	0.06	0.42	2.72	2.06	0.17
B5#3	2.41	0.04	0.49	2.95	2.25	0.14
B6#1	1.51	0.02	0.30	1.83	1.40	0.10
B6#2	1.76	0.02	0.41	2.18	1.63	0.12
B6#3	1.41	0.02	0.31	1.74	1.37	0.00
C1#1	2.72	0.00	0.60	3.31	2.43	0.31
C1#2	1.47	0.01	0.32	1.81	1.43	-0.02
C1#3	1.46	0.00	0.34	1.80	1.33	0.13
C5 #1	0.48	0.00	0.12	0.60	0.46	0.01
C5 #2	2.32	0.00	0.52	2.84	2.14	0.17
C5 #3	1.62	0.02	0.36	2.00	1.54	0.05
Control plots						
A4 mean	1.67	0.02	0.32	2.00	1.56	0.10
B5 mean	1.98	0.04	0.39	2.41	1.85	0.12
C1 mean	1.88	0.00	0.42	2.31	1.73	0.14
Treatment plots						
A1 mean	1.49	0.03	0.28	1.80	1.40	0.07
B6 mean	1.56	0.02	0.34	1.92	1.47	0.07
C5 mean	1.47	0.01	0.33	1.81	1.38	0.08
Control mean	1.85	0.02	0.38	2.24	1.71	0.12
s.e.	±0.09	±0.01	±0.03	±0.12	±0.08	±0.01
Treatment mean	1.51	0.02	0.32	1.84	1.42	0.07
s.e.	±0.03	±0.01	±0.02	±0.04	±0.03	±0.00
11 August mean	1.68	0.02	0.35	2.04	1.56	0.10
s.e.	±0.09	±0.01	±0.02	±0.11	±0.08	±0.01

Appendix A-8. Concentration of chlorophyll in epiphytes on leaves of *Zostera marina* at site 2 on 26 August 1992. Units are µg chlorophyll per cm2 of leaf surface area. Means are given for plots (n=3 leaves per plot), treatments (n=3 plots per treatment) and the mean for this sampling date (n=18 plots on this date). s.e.=standard error of the mean.

Sample	chl a	chl b	chl c	Ca+Cb+Cc	living chl a	phaeophytin
A1-1	0.53	0.03	0.06	0.62	0.54	0.00
A1-2	1.61	0.01	0.28	1.90	1.41	0.23
A1-3	0.46	0.00	0.08	0.54	0.46	0.00
A3-1	0.32	0.00	0.06	0.39	0.32	0.00
A3-2	0.34	0.00	0.07	0.42	0.36	0.00
A3-3	0.21	0.00	0.06	0.27	0.23	0.00
A4-1	0.71	0.00	0.14	0.86	0.70	0.00
A4-2	0.51	0.00	0.12	0.64	0.50	0.00
A4-3	0.72	0.00	0.16	0.89	0.70	0.00
B2-1	0.49	0.00	0.15	0.63	0.49	0.00
B2-2	0.59	0.00	0.14	0.72	0.58	0.00
B2-3	0.68	0.01	0.17	0.85	0.66	0.00
B5-1	0.64	0.00	0.15	0.78	0.61	0.00
B5-2	0.68	0.02	0.15	0.85	0.66	0.00
B5-3	0.55	0.00	0.15	0.70	0.53	0.01
B6-1	0.63	0.00	0.17	0.80	0.63	0.00
B6-2	0.41	0.01	0.13	0.55	0.40	0.00
B6-3	0.32	0.00	0.12	0.44	0.32	0.00
C1-1	0.28	0.00	0.09	0.37	0.28	0.00
C1-2	0.57	0.00	0.19	0.76	0.54	0.02
C1-3	0.11	0.00	0.08	0.18	0.13	0.00
C2-1	0.72	0.00	0.24	0.96	0.72	0.00
C2-2	0.67	0.00	0.20	0.87	0.65	0.00
C2-3	0.21	0.00	0.12	0.33	0.23	0.00
C5-1	0.55	0.00	0.14	0.69	0.54	0.00
C5-2	0.25	0.00	0.10	0.35	0.29	0.00
C5-3	0.59	0.00	0.17	0.76	0.58	0.00
A5-1	0.93	0.02	0.21	1.16	0.79	0.20
A5-2	1.49	0.00	0.40	1.89	1.43	0.02
A5-3	0.53	0.00	0.19	0.72	0.47	0.05
A6-1	1.08	0.04	0.26	1.39	1.00	0.09
A6-2	0.52	0.01	0.16	0.69	..5	0.00
A6-3	0.42	0.00	0.13	0.55	0.40	0.01
A7-1	0.41	0.00	0.14	0.55	0.39	0.00
A7-2	0.66	0.04	0.20	0.90	0.61	0.05
A7-3	0.26	0.00	0.12	0.38	0.26	0.00
B1-1	0.29	0.00	0.26	0.55	0.63	0.00
B1-2	0.41	0.00	0.25	0.66	0.46	0.00
B1-3	0.31	0.00	0.12	0.43	0.29	0.01
B3-1	0.42	0.00	0.17	0.59	0.42	0.00
B3-2	1.50	0.00	0.47	1.96	1.43	0.02
B3-3	0.38	0.00	0.17	0.55	0.39	0.00
B7-1	0.78	0.01	0.25	1.03	0.76	0.00
B7-2	0.37	0.00	0.17	0.55	0.37	0.00
B7-3	0.46	0.00	0.18	0.64	0.45	0.00
C4-1	0.37	0.00	0.16	0.53	0.37	0.00
C4-2	0.49	0.00	0.27	0.75	0.47	0.00
C4-3	0.47	0.00	0.16	0.63	0.46	0.00
C6-1	0.66	0.01	0.18	0.85	0.64	0.00
C6-2	0.85	0.00	0.22	1.08	0.81	0.02
C6-3	0.42	0.00	0.14	0.56	0.41	0.00
C7-1	0.58	0.00	0.17	0.76	0.57	0.00
C7-2	0.39	0.00	0.14	0.53	0.39	0.00
C7-3	0.38	0.00	0.16	0.54	0.38	0.00

Appendix A-8. (continued).

Sample	chl a	chl b	chl c	Ca+Cb+Cc	living chl a	phaeophytin
Control						
A4 mean	0.65	0.00	0.14	0.80	0.63	0.00
B5 mean	0.62	0.01	0.15	0.78	0.60	0.00
C1 mean	0.32	0.00	0.12	0.44	0.32	0.01
0.28 qts/acre						
A5 mean	0.98	0.01	0.27	1.26	0.90	0.09
B7 mean	0.54	0.00	0.20	0.74	0.53	0.00
C6 mean	0.64	0.00	0.18	0.83	0.62	0.01
0.56 qts/acre						
A6 mean	0.67	0.02	0.18	0.88	0.70	0.03
B3 mean	0.77	0.00	0.27	1.03	0.75	0.01
C4 mean	0.44	0.00	0.20	0.64	0.43	0.00
1.1 qts/acre						
A3 mean	0.29	0.00	0.06	0.36	0.30	0.00
B2 mean	0.59	0.00	0.15	0.73	0.58	0.00
C2 mean	0.53	0.00	0.19	0.72	0.53	0.00
2.2 qts/acre						
A7 mean	0.44	0.01	0.15	0.61	0.42	0.02
B1 mean	0.34	0.00	0.21	0.55	0.46	0.00
C7 mean	0.45	0.00	0.16	0.61	0.45	0.00
4.4 qts/acre						
A1 mean	0.87	0.01	0.14	1.02	0.80	0.08
B6 mean	0.45	0.00	0.14	0.60	0.45	0.00
C5 mean	0.46	0.00	0.14	0.60	0.47	0.00
Control mean	0.53	0.00	0.14	0.67	0.52	0.00
s.e.	±0.11	±0.00	±0.01	±0.12	±0.10	0.00
.28 mean	0.72	0.00	0.22	0.94	0.68	0.03
s.e.	±0.13	±0.00	±0.03	±0.16	±0.11	0.03
.56 mean	0.63	0.01	0.22	0.85	0.63	0.01
s.e.	±0.10	±0.01	±0.03	±0.12	±0.10	0.01
1.1 mean	0.47	0.00	0.13	0.60	0.47	0.00
s.e.	±0.09	±0.00	±0.04	±0.12	±0.08	0.00
2.2 mean	0.41	0.00	0.17	0.59	0.44	0.01
s.e.	±0.04	±0.00	±0.02	±0.02	±0.01	0.01
4.4 mean	0.59	0.01	0.14	0.74	0.57	0.03
s.e.	±0.14	±0.00	±0.00	±0.14	±0.11	0.03
26 August mean	0.56	0.00	0.17	0.73	0.55	0.01
s.e.	±0.04	±0.00	±0.01	±0.05	±0.04	0.01

Appendix A-9. Concentration of chlorophyll in epiphytes of *Zostera marina* at site 3 on 1 July 1992. Units are µg chlorophyll per cm2 of leaf surface area. Means are given for plots (n=2 leaves per plot) and the mean for this sampling date (n=3 plots on this date). s.e.=standard error of the mean.

Sample	Chl a	Chl b	Chl c	Ca+Cb+Cc	living chl a	phaeo-pigs
G1#3	11.27	0.27	2.85	14.38	9.93	1.67
G1#4	5.08	0.00	1.10	6.18	4.82	0.15
G3#3	5.71	0.00	1.35	7.06	5.26	0.44
G2#4	3.60	0.00	0.73	4.33	3.40	0.12
G2#3	2.76	0.00	0.60	3.36	2.65	0.02
G3#4	3.37	0.00	0.74	4.11	2.98	0.46
G1 mean	8.17	0.13	1.97	10.28	7.37	0.91
G2 mean	3.18	0.00	0.67	3.84	3.02	0.07
G3 mean	4.54	0.00	1.04	5.58	4.12	0.45
1 July mean	5.30	0.04	1.23	6.57	4.84	0.48
s.e.	±1.49	±0.04	±0.39	±1.92	±1.31	±0.24

Appendix A-10. Concentration of chlorophyll in epiphytes on leaves of *Zostera marina* at site 3 on 29 July 1992. Units are µg chlorophyll per cm2 of leaf surface area. Means are also given for plots (n=3 leaves per plot), and treatments (n=3 plots per treatment) and the mean for this sampling date (n=6 plots on this date). s.e.=standard error of the mean.

Sample	Chl a	Chl b	Chl c	Ca+Cb+Cc	living chl a	phaeophytin
H1 #1	0.28	0.00	0.09	0.37	0.26	0.01
H1 #2	0.54	0.01	0.13	0.67	0.49	0.04
H1 #3	0.76	0.03	0.16	0.96	0.70	0.06
H2 #1	0.80	0.01	0.17	0.98	0.75	0.03
H2 #2	0.53	0.00	0.14	0.67	0.50	0.02
H2 #3	0.53	0.01	0.14	0.68	0.49	0.05
H3 #1	0.81	0.01	0.18	1.01	0.76	0.04
H3 #2	0.77	0.00	0.18	0.95	0.73	0.03
H3 #3	0.95	0.00	0.21	1.17	0.90	0.04
H4 #1	0.96	0.03	0.21	1.19	0.89	0.05
H4 #2	0.91	0.00	0.25	1.17	0.83	0.08
H4 #3	0.79	0.00	0.18	0.97	0.73	0.06
H5 #1	0.91	0.00	0.23	1.14	0.85	0.06
H5 #2	1.00	0.01	0.22	1.22	0.96	0.01
H5 #3	1.47	0.02	0.28	1.77	1.34	0.13
H6 #1	2.62	0.04	0.53	3.19	2.33	0.35
H6 #2	0.82	0.00	0.18	1.01	0.74	0.09
H6 #3	0.34	0.00	0.09	0.43	0.28	0.07
Control plots						
H1 mean	0.52	0.01	0.13	0.67	0.49	0.04
H3 mean	0.85	0.01	0.19	1.04	0.80	0.04
H5 mean	1.13	0.01	0.24	1.38	1.05	0.06
Treatment plots						
H2 mean	0.62	0.01	0.15	0.78	0.58	0.03
H4 mean	0.89	0.01	0.21	1.11	0.82	0.07
H6 mean	1.26	0.01	0.27	1.54	1.12	0.17
Control mean	0.83	0.01	0.19	1.03	0.78	0.05
s.e.	±0.17	±0.00	±0.03	±0.21	±0.16	±0.01
Treatment mean	0.92	0.01	0.21	1.14	0.84	0.09
s.e.	±0.19	±0.00	±0.03	±0.22	±0.15	±0.04
29 July mean	0.88	0.01	0.20	1.09	0.81	0.07
s.e.	±0.12	±0.00	±0.02	±0.14	±0.10	±0.02

Appendix A-11. Concentration of chlorophyll in epiphytes on leaves of *Zostera marina* at site 3 on 27 August 1992. Units are μg chlorophyll per cm2 of leaf surface area. Means are also given for plots (n=3 leaves per plot), and treatments (n=3 plots per treatment) and the mean for this sampling date (n=6 plots on this date). s.e.=standard error of the mean.

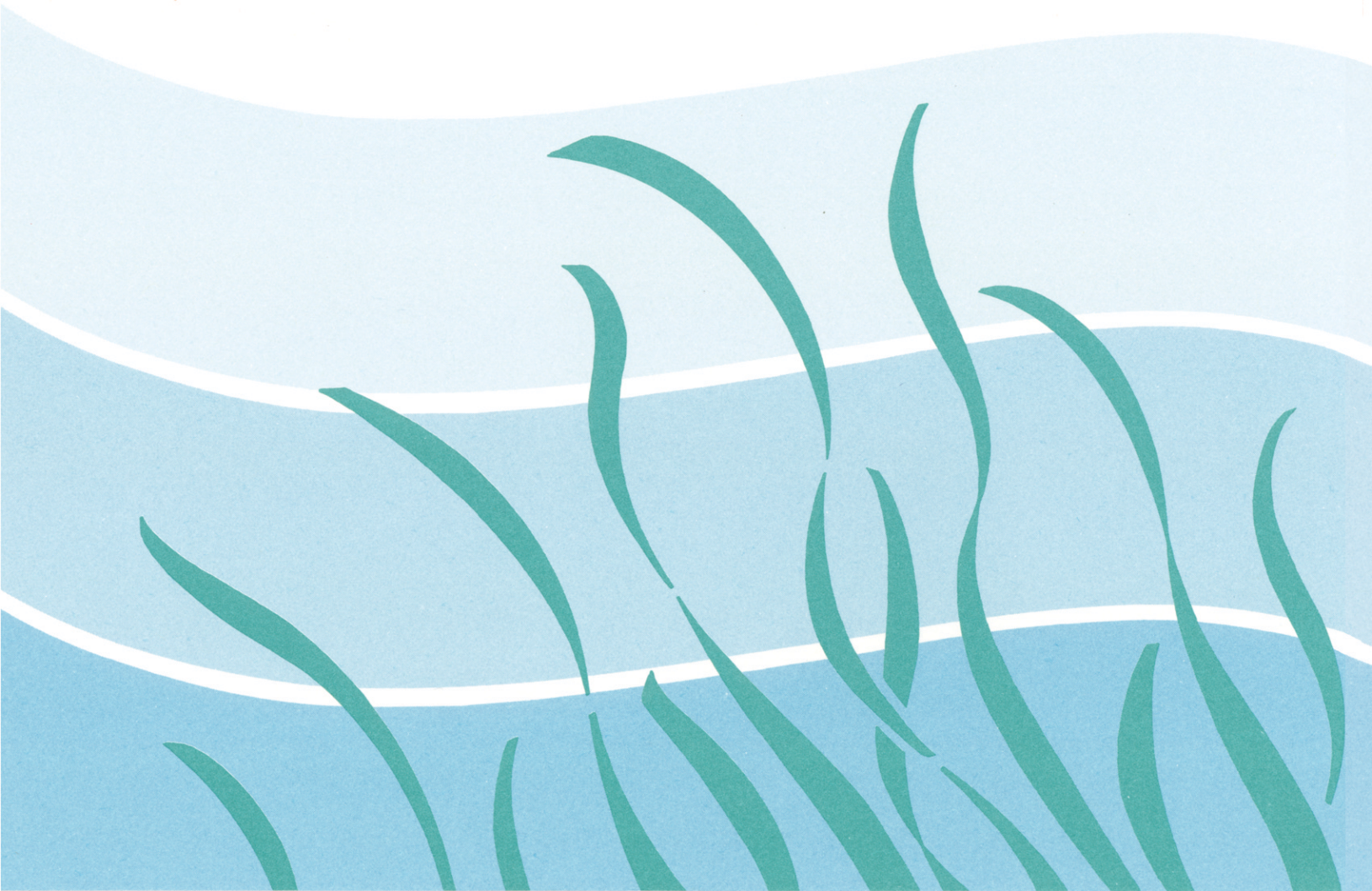
Sample	Chl a	Chl b	Chl c	Ca+Cb+Cc	living chl a	phaeophytin
H1-1	0.92	0.03	0.16	1.11	0.88	0.01
H1-2	0.84	0.01	0.16	1.01	0.77	0.08
H1-3	0.80	0.02	0.15	0.97	0.75	0.05
H2-1	1.33	0.03	0.24	1.60	1.24	0.08
H2-2	0.72	0.01	0.14	0.87	0.67	0.05
H2-3	0.93	0.00	0.17	1.11	0.86	0.06
H3-1	0.94	0.01	0.17	1.13	0.89	0.03
H3-2	0.50	0.00	0.09	0.60	0.47	0.03
H3-3	0.87	0.03	0.15	1.05	0.75	0.15
H4-1	0.54	0.00	0.10	0.64	0.52	0.01
H4-2	0.69	0.01	0.13	0.82	0.65	0.02
H4-3	0.58	0.00	0.12	0.70	0.54	0.05
H5-1	0.67	0.00	0.13	0.81	0.62	0.06
H5-2	0.61	0.00	0.12	0.73	0.54	0.08
H5-3	1.28	0.01	0.26	1.54	1.18	0.09
H6-1	0.94	0.00	0.15	1.09	0.73	0.31
H6-2	0.44	0.01	0.10	0.55	0.38	0.07
H6-3	0.24	0.00	0.04	0.28	0.22	0.02
Control plots						
H1 mean	0.86	0.02	0.16	1.03	0.80	0.05
H3 mean	0.77	0.02	0.14	0.93	0.70	0.07
H5 mean	0.85	0.00	0.17	1.03	0.78	0.08
Treatment plots						
H2 mean	0.99	0.01	0.19	1.19	0.92	0.06
H4 mean	0.60	0.00	0.12	0.72	0.57	0.03
H6 mean	0.54	0.00	0.10	0.64	0.44	0.14
Control mean	0.83	0.01	0.16	0.99	0.76	0.06
s.e.	±0.03	±0.00	±0.01	±0.03	±0.03	±0.01
Treatment mean	0.71	0.01	0.13	0.85	0.64	0.08
s.e.	±0.14	±0.00	±0.03	±0.17	±0.14	±0.03
27 Aug mean	0.77	0.01	0.14	0.92	0.70	0.07
s.e.	±0.07	±0.00	±0.01	±0.08	±0.07	±0.02

Appendix B-1. Dry weight and ash-free dry weight of epiphytes on leaves of *Zostera japonica* at site 1 in Padilla Bay at which glyphosate (treatment at 4.4 qts/acre) was applied on July 2, 1992. Units are µg/cm2 of leaf area. Means are given for plots (n=2 leaves per plot on 1 July and 1 leaf per plot on 28 August), treatments (n=3 plots per treatment), and the mean for each sampling date (n=2 plots on 1 July and 6 plots on 28 August). s.e. = standard error of the mean.

Sample	Date	dry weight	ash-free dry weight
D1-1	1-Jul-92	0.0002	0.0002
D1-2	1-Jul-92	0.0003	0.0002
E4-1	1-Jul-92	0.0000	0.0001
E4-2	1-Jul-92	0.0002	0.0002
D1-T	28-Aug-92	0.0011	0.0007
D5-C	28-Aug-92	0.0008	0.0004
E3-C	28-Aug-92	0.0005	0.0004
E4-T	28-Aug-92	0.0004	0.0005
F4-C	28-Aug-92	0.0010	0.0005
F7-T	28-Aug-92	0.0004	0.0002
1 July plots			
D1 mean		0.0003	0.0002
E4 mean		0.0001	0.0002
1 July mean		0.00018	0.00018
s.e.		±0.00008	±0.00002
28 Aug control mean		0.00077	0.00043
s.e.		±0.00015	±0.00003
28 Aug treatment mean		0.00063	0.00047
s.e.		±0.00023	±0.00015
28 August mean		0.00070	0.00045
s.e.		±0.00013	±0.00007

Appendix B-2. Dry weight and ash-free dry weight of epiphytes on leaves of *Zostera marina* at site 2 in Padilla Bay at which glyphosate (treatment at 4.4 qts/acre) was applied on 1 July, 1992. Units are µg/cm2 of leaf area. Means are given for plots (n=2 leaves per plot on 30 June and 1 leaf per plot on 26 August), treatments (n=3 plots per treatment), and the mean for each sampling date (n=3 plots on 30 June and 6 plots on 26 August). s.e. = standard error of the mean.

Sample	Date	dry weight	ash-free dry weight
A7-1	30-Jun-92	0.0111	0.0041
A7-2	30-Jun-92	0.0069	0.0025
B7-1	30-Jun-92	0.0072	0.0028
B7-2	30-Jun-92	0.0151	0.0048
C7-1	30-Jun-92	0.0102	0.0036
C7-2	30-Jun-92	0.0109	0.0036
A1	26-Aug-92	0.0007	0.0003
A4	26-Aug-92	0.0006	0.0002
B5	26-Aug-92	0.0006	0.0003
B6	26-Aug-92	0.0004	0.0002
C1	26-Aug-92	0.0003	0.0001
C5	26-Aug-92	0.0003	0.0001
30 June plots			
A7 mean		0.0090	0.0033
B7 mean		0.0112	0.0038
C7 mean		0.0106	0.0036
30 June mean		0.01023	0.00357
s.e.		±0.00064	±0.00015
26 Aug control mean		0.00050	0.00020
s.e.		±0.00010	±0.00006
26 Aug treatment mean		0.00047	0.00020
s.e.		±0.00012	±0.00006
26 August mean		0.00048	0.00020
s.e.		±0.00007	±0.00004

Appendix B-3. Dry weight and ash-free dry weight of epiphytes on leaves of *Zostera marina* at site 3 in Padilla Bay at which glyphosate (treatment at 4.4 qts/acre) was applied on 1 July, 1992. Units are µg/cm2 of leaf area. Means are given for plots (n=2 leaves per plot on 1 July and 1 leaf per plot on 27 August), treatments (n=3 plots per treatment), and the mean for each sampling date (n=3 plots on 1 July and 6 plots on 27 August). s.e. = standard error of the mean.

Sample	Date	dry weight	ash-free dry weight
G1-5	1-Jul-92	0.0047	0.0011
G1-6	1-Jul-92	0.0069	0.0015
G2-5	1-Jul-92	0.0080	0.0036
G2-6	1-Jul-92	0.0061	0.0026
G3-5	1-Jul-92	0.0189	0.0058
G3-6	1-Jul-92	0.0062	0.0028
H1	27-Aug-92	0.0006	0.0002
H2	27-Aug-92	0.0005	0.0002
H3	27-Aug-92	0.0003	0.0001
H4	27-Aug-92	0.0006	0.0002
H5	27-Aug-92	0.0022	0.0010
H6	27-Aug-92	0.0005	0.0002
1 July plots			
G1 mean		0.0058	0.0013
G2 mean		0.0071	0.0031
G3 mean		0.0126	0.0043
1 July mean		0.00847	0.00290
s.e.		±0.00207	±0.00087
27 Aug control mean		0.00103	0.00043
s.e.		±0.00059	±0.00028
27 Aug treatment mean		0.00053	0.00020
s.e.		±0.00003	±0.00000
27 August mean		0.00078	0.00032
s.e.		±0.00029	±0.00014