Padilla Bay
National Estuarine Research Reserve

Technical Report No. 4

ACUTE TOXICITY OF INTERTIDAL SEDIMENTS FROM PADILLA BAY, WASHINGTON, TO THE AMPHIPOD, *RHEPOXYINUS ABRONIUS*

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Travis Shaw

November 1992

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WASHINGTON STATE DEPARTMENT OF ECOLOGY

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ABSTRACT

Bulthuis, D.A. and T. Shaw 1992. Acute toxicity of intertidal sediments from Padilla Bay, Washington, to the amphipod, Rhepoxynius abronius. Washington Department of Ecology, Padilla Bay National Estuarine Research Reserve Technical Report No. 4, Mount Vernon, Washington. 37 pp.

The acute toxicity of intertidal sediments at nineteen sites in Padilla Bay, Washington was determined for the amphipod, Rhepoxynius abronius. Mean survival of R. abronius ranged from 14 to 92 percent at sixteen sites scattered throughout Padilla Bay and ranged from 7 to 40 percent at three sites located near a former landfill. It is concluded that Padilla Bay is unlikely to be a good reference area for noncontaminated sediments because of the toxicity of the sediments to R. abronius at sites throughout Padilla Bay. There was no consistent pattern of toxicity among the 16 scattered sites that would indicate a point source contamination. It is suggested that the sea-surface microlayer may be a mechanism for concentrating contaminants in intertidal sediments and that intertidal sediments throughout Puget Sound may have higher sediment toxicities than adjacent subtidal sediments.

INTRODUCTION

Padilla Bay, Washington, has few sources of contamination and is a potential reference area for studies on sediments in Puget Sound (PTI 1991). Potential reference areas that meet the performance standards may be used as reference sites for multiple Puget Sound sediment management programs (e.g. Puget Sound Dredged Disposal Analysis and Puget Sound Estuary Program). The interim performance standards for reference areas include standards for organic compounds, inorganics, total organic carbon, sulfides, bioassays and benthic macroinvertebrates (PTI 1991). The bioassays include amphipod mortality, bivalve larvae abnormality, and Microtox. Very little or no data on these parameters is available for sediments in Padilla Bay (Pastorok et al. 1989). The overall aim of the present study was to collect data on amphipod mortality in bioassays of intertidal sediments from Padilla Bay.

Only a single bioassay of amphipod mortality from Padilla Bay was reported by Pastorok et al. (1989) in their review of data on sediments from potential reference areas throughout Puget Sound. DeWitt (personal communication) reported 92% survival of the amphipod, Rhepoxynius abronius, for sediments from that intertidal site in Padilla Bay (Appendix A). Wiggins (1992) reported 100% mortality of R. abronius near the site of a former landfill in the southwest corner of Padilla Bay. Further information on the toxicity of sediments throughout Padilla Bay is not known.

The objectives of this study were to determine the toxicity of intertidal sediments throughout Padilla Bay to the amphipod, Rhepoxynius abronius, to compare toxicity of the sediments to particle size and concentration of organic carbon and to compare the toxicity of sediments in Padilla Bay with other estuarine sites in Puget Sound and the Pacific Northwest. These data could be compared to the performance standards to evaluate the suitability of Padilla Bay as a reference area.

METHODS

Study location and sampling methods. Padilla Bay is a 5000 hectare embayment located in Washington State, north of Puget Sound proper and east of the San Juan Islands and the channels linking the straits of Juan de Fuca and Georgia. The bay has extensive intertidal sand and mudflats dissected by dendritic channels that drain and distribute water to the flats during the mixed diurnal 4 m tides. Seagrasses cover extensive portions of the intertidal flats (Bulthuis 1991) and provide important refuge and food source (Thom 1990) for fish (Simenstad et al. 1988, Caine 1991), crabs (Dinnel et al. 1986), waterfowl (Reed et al. 1989, Jeffrey 1976) and other marine life.

At nineteen stations throughout Padilla Bay sediments were collected for bioassays of toxicity to Rhepoxynius abronius, Microtox, and analysis of total organic carbon and sediment grain size. The bay was divided into ten strata based on seagrass cover, proximity to the shore, shore type and north Padilla Bay vs south Padilla Bay for allocation of stations for sampling.

Four strata along the shore extended 10 m seaward from the higher high water mark. Six strata were delineated on the intertidal flats based on the mapping of seagrasses and sediment characteristics in Padilla Bay (Bulthuis 1991). All six strata in the intertidal and two of the shore strata had stations allocated within them (limited resources prevented sampling and analyses of stations in the two northern shore strata).

Two stations were randomly allocated from each strata. In addition, three stations were selected near the site of a former landfill where J. Wiggins (personal communication) had found that Rhepoxynius abronius survival was low in bioassays of the sediment. The approximate location for each site is shown in Fig. 1 and the latitude, longitude and strata listed in Table 1.

Sediments were sampled by 5 cm diameter cores taken by hand either by divers during high tide or by investigators who walked to the sites near shore during low tides on 22 August 1991 (test series 1), 4 September 1991 (test series 2), 18 and 19 September 1991 (test series 3) and 23 September 1991 (test series 4). Sediment samples were stored at 4^oC in the dark until initiation of the bioassays within 48 hours of collection for test series 1, 2, and 3 and within 12 days of collection for test series 4. The top 2 cm of each core were extruded into clean (detergent washed, acid washed, distilled water rinsed, methylene chloride rinsed) glass jars with Teflon lids, placed in a cooler with crushed ice for transport to the laboratory and stored at 4^oC until analyses or toxicity tests.

Amphipod bioassays. Sediment toxicity tests were conducted with the infaunal amphipod, Rhepoxynius abronius (Barnard), with the methods developed by Swartz and

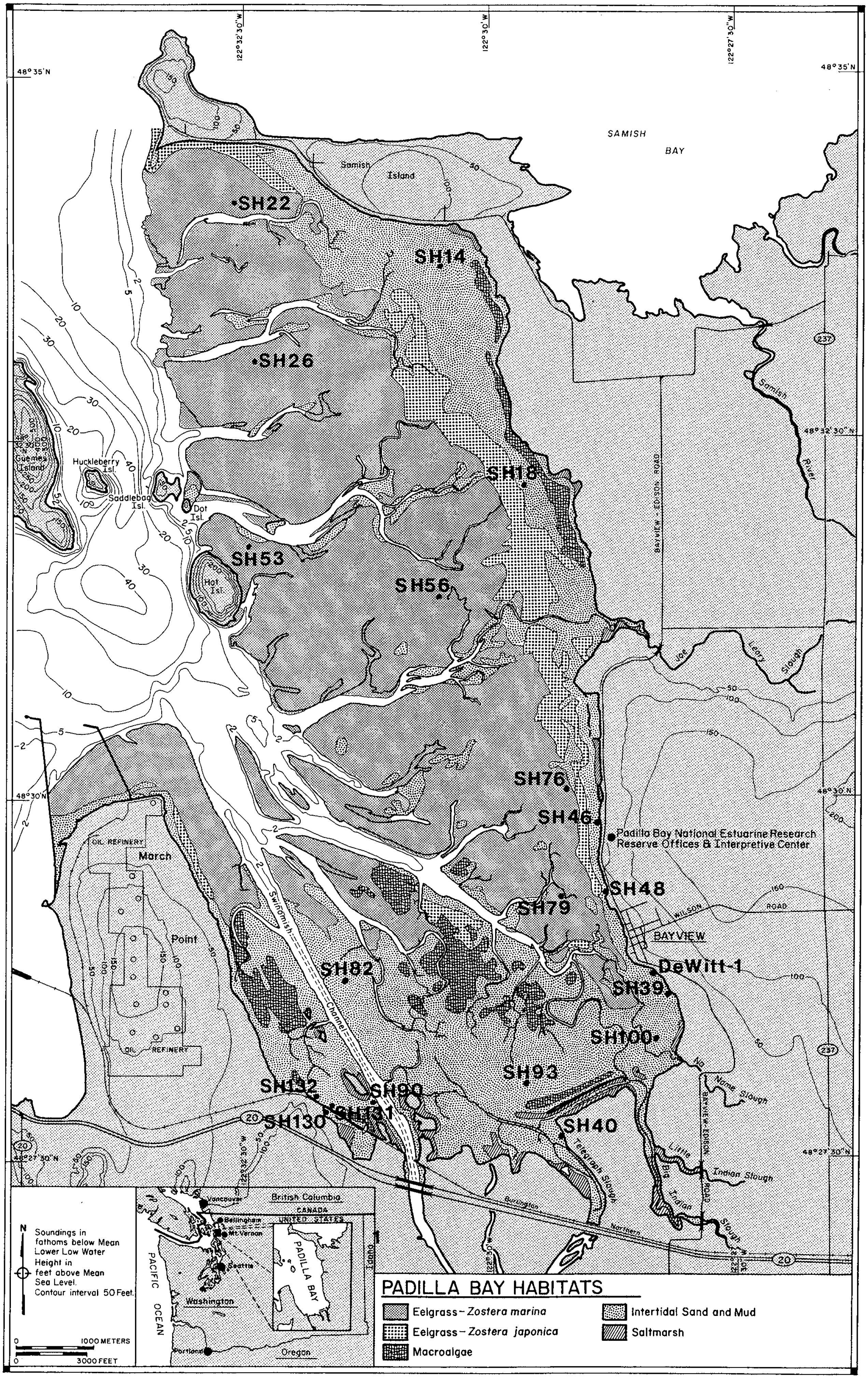

Figure 1. Location of sample sites for sediments in this study and in DeWitt's study.

Table 1. Location, sampling dates and characteristics of intertidal sites in Padilla Bay, Washington, from which sediments were sampled and tested for toxicity to Rhepoxynius abronius.

Site No.	Latitude	Longitude	Strata (habitat type)	Exposed or submerged at time of collection	Date Collected	Date bioassay initiated	Test Series
Intertidal flats - seagrasses							
SH 22	48° 34.10' N	122° 32.74' W	Zostera marina, north, sandy	submerged	18 Sep	20 Sep	3
SH 26	48° 33.06' N	122° 32.43' W	Zostera marina, north, sandy	submerged	4 Sep	6Sep	2
SH 53	48° 31.80' N	122° 32.45' W	Zostera marina, central, sandy	submerged	4 Sep	6 Sep	2
SH 56A	48° 31.41' N	122° 30.52' W	Zostera marina, central, sandy	submerged	19 Sep	20 Sep	3
SH 56B				submerged	19 Sep	1 Oct	4
SH 76	48° 30.06' N	122° 29.25' W	Zostera marina, silt & clay	exposed	22 Aug	23 Aug	1
SH 79	48° 29.32' N	122° 29.26' W	Zostera marina, silt & clay	exposed	4 Sep	6 Sep	2
Intertidal flats - bare							
SH 14	48° 33.66' N	122° 30.52' W	bare, north	exposed	22 Aug	23 Aug	1
SH 18	48° 32.18' N	122° 29.66' W	bare, north	submerged	18 Sep	20 Sep	3
SH 93A	48° 28.03' N	122° 27.76' W	bare, south, silt & clay	submerged	18 Sep	20 Sep	3
SH 93B				submerged	18 Sep	1 Oct	4
SH 100	48° 28.32' N	122° 28.35' W	bare, south, silt & clay	exposed	22 Aug	23 Aug	1
SH 82	48° 28.83' N	122° 31.58' W	bare, south, sand	exposed	22 Aug	23 Aug	1
SH 90	48° 27.90' N	122° 31.25' W	bare, south, sand	exposed	18 Sep	20 Sep	3
Shorelines							
SH 46	48° 29.35' N	122° 28.84' W	Bay View ridge, shore	exposed	22 Aug	23 Aug	1
SH 48	48o 29.35' N	122o 28.84' W	Bay View ridge, shore	exposed	4 Sep	6 Sep	2
SH 39	48° 28.65' N	122° 28.20' W	south dikes, shore	exposed	22 Aug	23 Aug	1
SH 40	48° 27.62' N	122° 29.30' W	south dikes, shore	exposed	4 Sep	6 Sep	2
Near landfill							
SH 130	48° 27.36' N	122° 31.70' W	bare, silt & clay	exposed	22 Aug	23 Aug	1
SH 131	48° 27.90' N	122° 31.62' W	bare, silt & clay	exposed	4 Sep	6 Sep	2
SH 132	48° 27.06' N	122° 31.82' W	bare, silt & clay	exposed	19 Sep	20 Sep	3

others (Swartz et al. 1979, 1985; Puget Sound Estuary Program 1986). Amphipods and control sediment for test series 1-3 were collected from West Beach, Whidbey Island, Washington, and for test series 4 from Yaquina Bay, Oregon. Adult amphipods were separated by sieving the sediment through a 1.0 mm screen and were maintained in the laboratory for 2 to 3 days before initiation of the toxicity tests. A two cm deep layer of test sediment was placed in each test chamber (1 liter glass beakers) and covered with filtered seawater from the Whidbey Island site (series 1-3) or Yaquina Bay (series 4). Interstitial water was adjusted up to 28 parts per thousand salinity when required. Temperature was maintained at 15°C ± 2°C in a water bath in test series 1-3 and at 13°C in test series 4. Continuous illumination was provided throughout the bioassays. Twenty R. abronius were placed in each test chamber. A few R. abronius did not bury themselves immediately into the sediment. Those not burrowing within 30 minutes were removed and replaced. The overlaying water was aerated throughout the tests. Temperature, dissolved oxygen and pH in the test chambers were measured regularly throughout the bioassays. Control sediments from the collection site were treated as for test sediments during each test series and positive controls (with cadmium chloride) were included during series 2 and 3. R. abronius were exposed to test sediments for 10 days. Emergence from the sediment and number dead at the sediment surface were recorded each day. After 10 days of exposure the test sediments were sieved through 1.0 mm screens and the number of survivors counted. Death was defined as no visible appendage movement or response to tactile stimulation. Missing amphipods were considered to be dead. Five replicates for each test sediment were conducted in test series 2-4, three to five replicates for each test sediment in series 1. Test series 1-3 were conducted at Padilla Bay National Estuarine Research Reserve by the authors, test series 4 was conducted in Newport, Oregon by Northwestern Aquatic Sciences.

Percent survival from each site was compared with the control for that test series by t-test.

Microtox. The inhibition of light emission by an extract of the sample sediments was measured by a modification of the methods described in Puget Sound Estuary Program (1986) by M. Stinson at the Manchester Laboratories, Washington Department of Ecology. Sediments were collected 18-24 September 1991 and stored at 4°C until testing. An extract was prepared by shaking 30g of sample sediment with 10 ml of Microtox Diluent in a 100 ml beaker for 24 hours at 4°C. The resultant supernatant was cleared by centrifugation before testing. Inhibition of light emission was measured by the method described in the Beckman Microtox System Operating Manual. Light emission was measured at 0, 5 and 15 minutes. The extraction procedure was begun on 1 October 1991 and testing conducted on 2 October 1991.

Sediment characteristics. Sediment samples from all sites were collected 22 August to 19 September 1991 by hand held corer, either by staff walking to the site during low tide or by divers during submergence of intertidal sediments during high tide. Interstitial salinity was measured with a refractometer. Particle size analysis was determined by the methods described by Buchanan (1984) and divided into 15 size classes from > 4.75 mm to < 0.9 μM. Total organic carbon (TOC) was determined by autoanalyzer and results expressed as mg C per kg of dry sediment. Particle size, percent water content and total organic carbon were determined by Sound Analytical Services in Tacoma, Washington.

RESULTS

The sediments at 19 sites in Padilla Bay ranged from 6% sand to 99% sand and total organic carbon 1.1 to 20 g C per kg dry sediment (Table 2). Sites closer to the shore and sediments in the southern part of the bay tended to have a higher percentage of fines (<0.062 mm) and a higher percentage of total organic carbon.

Rhepoxynius abronius survival was significantly lower ($p < 0.05$) than controls at 10 of the 16 scattered sites in Padilla Bay (Table 3). Sediments from five of the six sites with seagrass cover and three of the four sites near the shoreline decreased survival of the amphipod. The only seagrass covered site that did not affect survival of the amphipod was covered with a mixture of Zostera japonica and Z. marina, with Z. japonica the more abundant of the two. The remaining seagrass sites had a higher biomass of seagrasses, usually only Z. marina. Sediments from all three sites near the former landfill also decreased survival of the amphipod. Avoidance of the sediments by R. abronius generally was higher in the sediments that caused an increased mortality. The variation in rates of survival of R. abronius among sites was not correlated with either percent fines of the sediments ($r^2 = 0.147$, $p < 0.05$ that correlation is significant) nor with total organic carbon ($r^2 = 0.104$, $p < 0.05$ that correlation is significant) (Table 4, Fig. 2).

Sediments from two sites (SH56 and SH93) were tested both at Padilla Bay National Estuarine Research Reserve laboratories with Whidbey Island controls and by Northwestern Aquatic Sciences with Yaquina Bay controls. All four tests indicated

Table 2. Particle size, total organic carbon and water content of sediments at nineteen sites in Padilla Bay and one control site, West Beach, Whidbey Island at which sediments were collected for bioassay with the amphipod, R. abronius. Percent of dry weight for sand, silt and clay, percent of wet weight for water content.

Site No.	Sand %	Silt %	Clay %	Total Organic Carbon g C Kg^{-1}	Water Content %
Intertidal flats - seagrasses					
SH 22	99	<1	1	1.1	27
SH 26	93	4	3	3.9	36
SH 53	91	5	4	6.5	31
SH 56	48	34	18	15	43
SH 76	64	28	8	4.3	37
SH 79	41	44	15	6.3	50
Intertidal flats - bare					
SH 14	90	6	4	3.4	30
SH 18	78	16	6	6.8	39
SH 93	31	54	15	7.1	46
SH 100	8	70	22	15	49
SH 82	97	1	2	3.3	28
SH 90	82	13	5	4	38
Shorelines					
SH 46	99	1	<1	4.7	21
SH 48	100	<1	<1	1.6	22
SH 39	16	74	10	11	48
SH 40	8	62	30	19	41
Near landfill					
SH 130	9	67	24	20	64
SH 131	6	67	27	17	55
SH 132	9	69	22	16	52
WB	79	13	8	5.2	43

Table 3. Survival (percent) of Rhepoxynius abronius exposed for ten days to sediments from nineteen intertidal sites in Padilla Bay, Washington (mean ± standard deviation, n=5). Test series 1, 2, and 3 conducted by Padilla Bay National Estuarine Research Reserve, Test series 4 by Northwest Aquatic Sciences, Newport, Oregon.

Site No.	Test Series	Survival[1] mean	s.d.
Intertidal flats - seagrasses			
SH 22	3	***70	5.0
SH 26	2	***57	8.0
SH 53	2	*76	12
SH 56A	3	82	11
SH 56B	4	92	6.5
SH 76	1	*58	17
SH 79	2	***28	14
Intertidal flats - bare			
SH 14	1	65	23
SH 18	3	83	17
SH 93A	3	75	18
SH 93B	4	92	6.5
SH 100	1	**45	23
SH 82	1	70	19
SH 90	3	**66	11
Shorelines			
SH 46	1	70	23
SH 48	2	***14	11
SH 39	1	*62	22
SH 40	2	*63	21
Near landfill			
SH 130	1	***7	13
SH 131	2	***30	14
SH 132	3	**40	23
Control 1	1	93	2.9
Control 2	2	94	8.2
Control 3	3	94	8.2
Control 4	4	100	0

[1]Survival significantly different from respective control are asterisked: *p < 0.05, ** p < 0.01, ** p < 0.001

Table 4. Comparison of mean survival of Rhepoxynius abronius, Microtox, percent fines and total organic carbon at nineteen sites in Padilla Bay, Washington.

Site No.	amphipod toxicity (mean % survival)	Microtox (decrease of luminescence)	silt & clay (% dry wt)	total organic carbon (g kg^{-1})
Intertidal flats - seagrasses				
SH 22	70	>100	1.0	1.1
SH 26	57	>100	7.0	3.9
SH 53	76	>100	9	6.5
SH 56A	82	>100	52	15
SH 56B	92			
SH 76	58	>100	36	4.3
SH 79	28	>100	59	6.3
Intertidal flats - bare				
SH 14	65	>100	10	3.4
SH 18	83	>100	22	6.8
SH 93A	75	>100	69	7.1
SH 93B	92			
SH 100	45	>100	92	15
SH 82	70	>100	3.0	3.3
SH 90	66		18	4.0
Shorelines				
SH 46	70	>100	1.0	4.7
SH 48	14	>100	<1.0	1.6
SH 39	62	>100	84	11
SH 40	63	>100	92	19
Near landfill				
SH 130	7		91	20
SH 131	30	>100	94	17
SH 132	40	>100	91	16

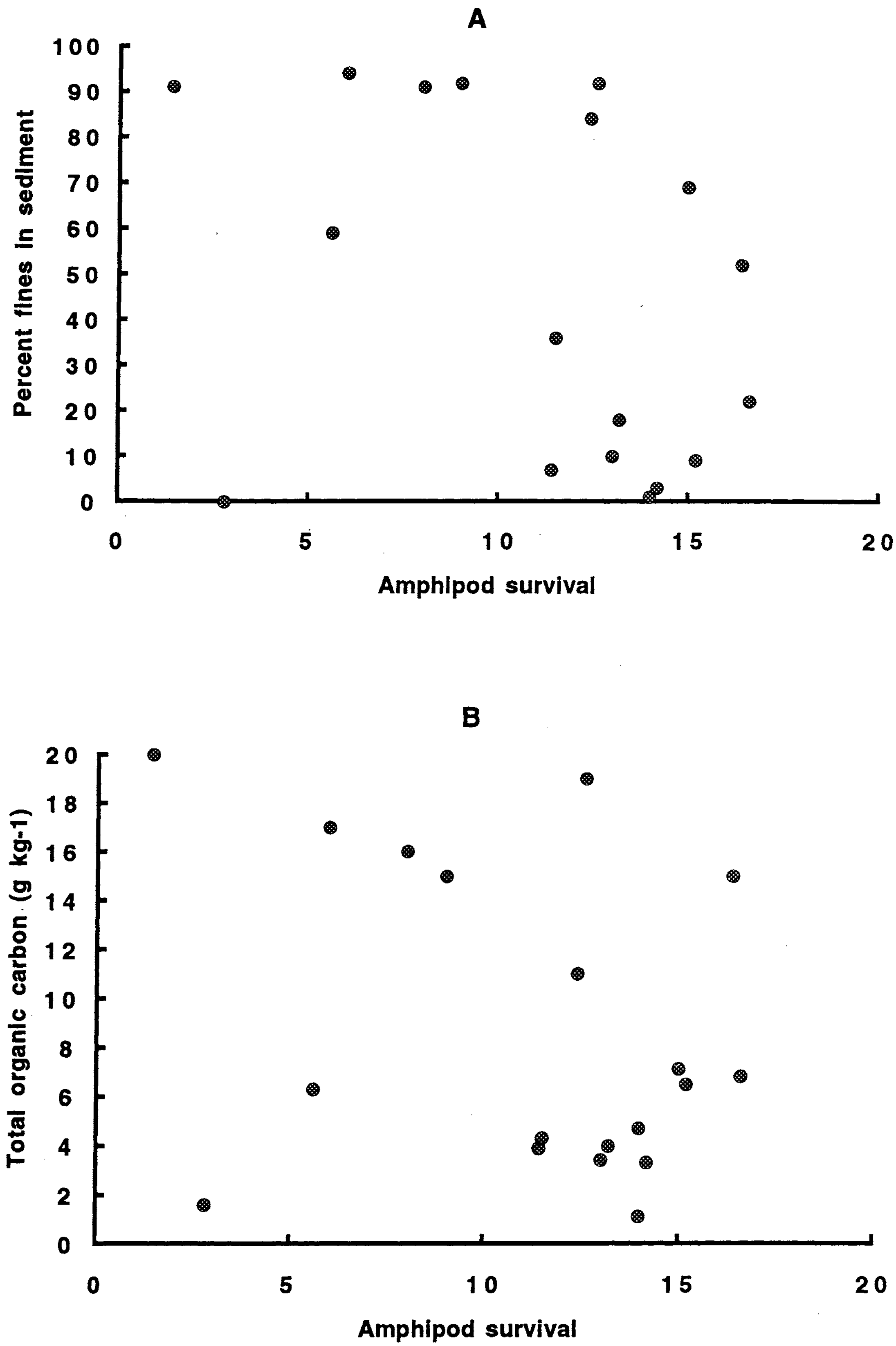

Figure 2. Survival of Rhepoxynius abronius as a function of percent fines(A) and total organic carbon(B) in the sediments at 19 sites in Padilla Bay. Points are mean (n=5) survival of 20 amphipods at each site.

somewhat lower survival than controls but the differences from controls were not significant at the 0.01 level of confidence. The survival of controls in the tests conducted by Northwestern Aquatic Sciences were higher than survival in tests conducted at Padilla Bay National Estuarine Research Reserve laboratories (Table 3).

The Photobacterium phosphoreum (Microtox) tests indicated no significant depression of light emission at any of the 19 sites in Padilla Bay (Table 4). Statistical analysis of the data generated by Microtox resulted in negative gammas in all but two of the samples and in the deionized water blank. The EL50 estimates for the reference toxicant were: 5 minutes: 1.9 mg l^{-1} phenol and 15 minutes: 2.6 mg L^{-1} phenol.

DISCUSSION

The rates of survival of Rhepoxynius abronius exposed to sediments from sixteen sites in the bay indicate widespread toxicity of sediments in Padilla Bay (Fig. 3). This apparent toxicity indicates that Padilla Bay would not be a suitable reference site for uncontaminated sediments.

The amphipod toxicity data for Padilla Bay can be compared with other bays in Puget Sound that are also considered potential reference sites and compared to the various performance standards that have been proposed for Puget Sound reference areas. Pastorok et al. 1989 proposed a reference area sample performance standard of 25% mortality for the amphipod bioassay. Twelve of the sixteen scattered sites as well as the three sites near the former landfill all exceeded this standard (Table 5). PTI (1991) proposed a performance standard of 30% or less mortality with a 95% confidence limit of 40%. Nine and five of the sixteen sites exceed these standards respectively (as well as the three sites near the landfill; Table 5). On the basis of these data most of Padilla Bay does not meet the standards to be a reference area for noncontaminated sediments.

Padilla Bay had several stations with a higher percent mortality than the maximums of ten other bays in Puget Sound considered as potential reference areas by Pastorok et al. (1989; Table 6). Much of the intertidal sediments in Padilla Bay appear to be more toxic to the amphipod, Rhepoxynius abronius, than the subtidal sediments of the potential reference areas. These comparisons also indicate that Padilla Bay would not be a good reference site for noncontaminated sediments.

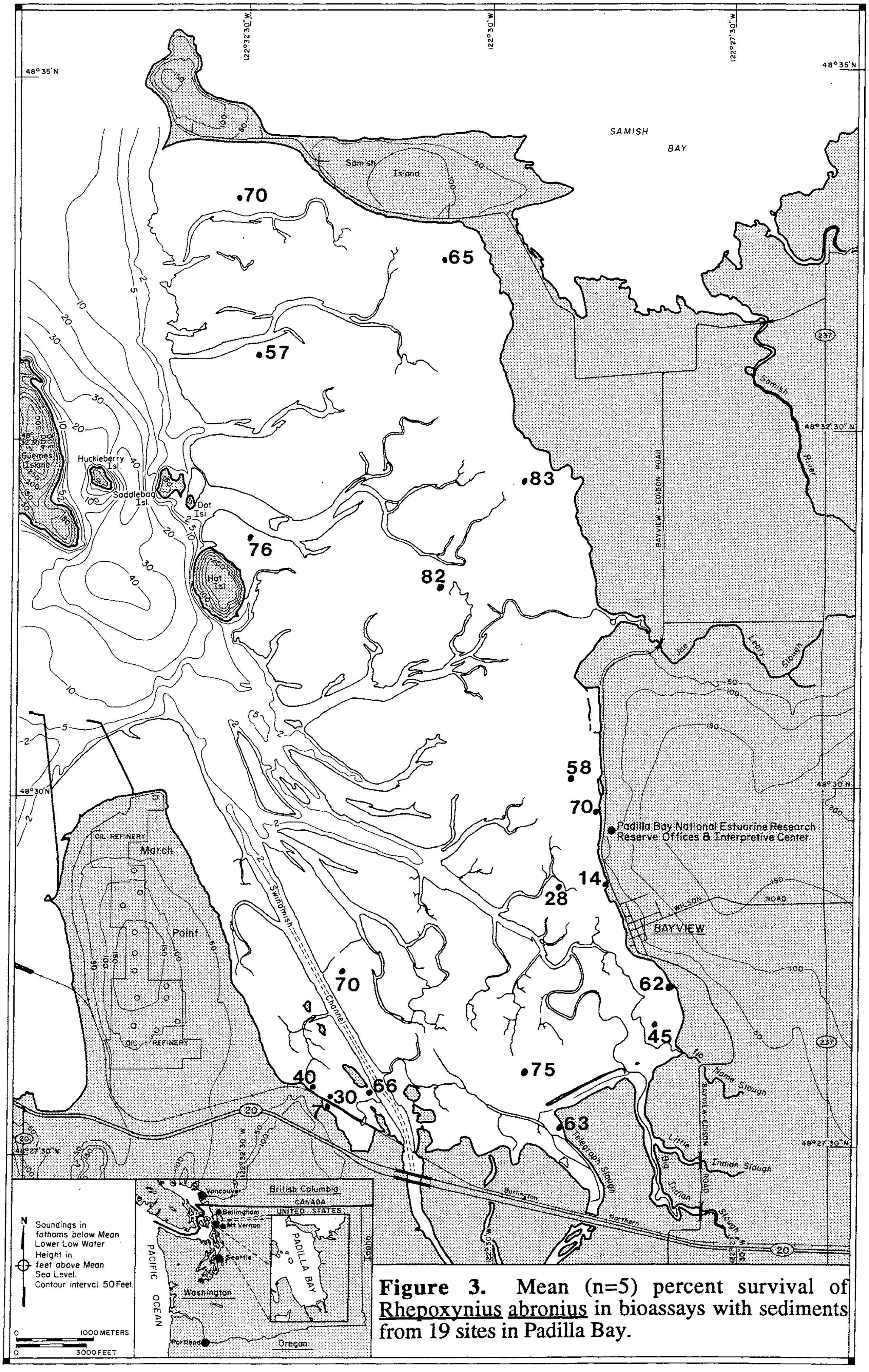

Figure 3. Mean (n=5) percent survival of Rhepoxynius abronius in bioassays with sediments from 19 sites in Padilla Bay.

Table 5. Comparison of mean percent mortality of Rhepoxynius abronius at 19 sites in Padilla Bay, Washington, with the quality control limits (10% or less) and reference area sample performance standard (25% or less) of the Puget Sound Estuary Program (PSEP) and the Proposed Performance standard (30% or less) or 95% confidence limit (40% or less) for PSEP by PTI (1991). Stations that exceed standard are asterisked.

Site No.	Mortality %	Exceeds Reference Area Sample Performance Standard	Exceeds Proposed Performance Standard	Exceeds 95% Upper Confidence Limit of Proposed Performance Standard
Intertidal flats - seagrasses				
SH 22	30	*		
SH 26	43	*	*	*
SH 53	24			
SH 56A	18			
SH 56B	8			
SH 76	42	*	*	*
SH 79	72	*	*	*
Intertidal flats - bare				
SH 14	35	*	*	
SH 18	17			
SH 93A	25			
SH 93B	8			
SH 100	55	*	*	*
SH 82	30	*		
SH 90	34	*	*	
Shorelines				
SH 46	30	*		
SH 48	86	*	*	*
SH 39	38	*	*	
SH 40	37	*	*	
Near landfill				
SH 130	93	*	*	*
SH 131	70	*	*	*
SH 132	60	*	*	*

Table 6. Comparison of mean amphipod mortality (%) at sixteen scattered stations in Padilla Bay with means of stations in eight other bays in Puget Sound considered as potential reference areas for sediment studies and for which data on amphipod mortality met the quality assurance review of Pastorok et al. (1989). Data extracted from Pastorok et al. (1989).

Location	Amphipod mortality (%)[1]		
	Minimum	Maximum	No. of Stations
Blakely Harbor	5	10	2
Carr Inlet	10	25	6
Case Inlet	30	50	54
Dabob Bay	10	35	10
Oak Harbor	5	20	9
Padilla Bay	**10**	**85**	**16**
Port Susan	10	40	8
Samish Bay	20	45	25
Sequim Bay	5	40	30

[1]Mean percent mortality for stations within each bay.

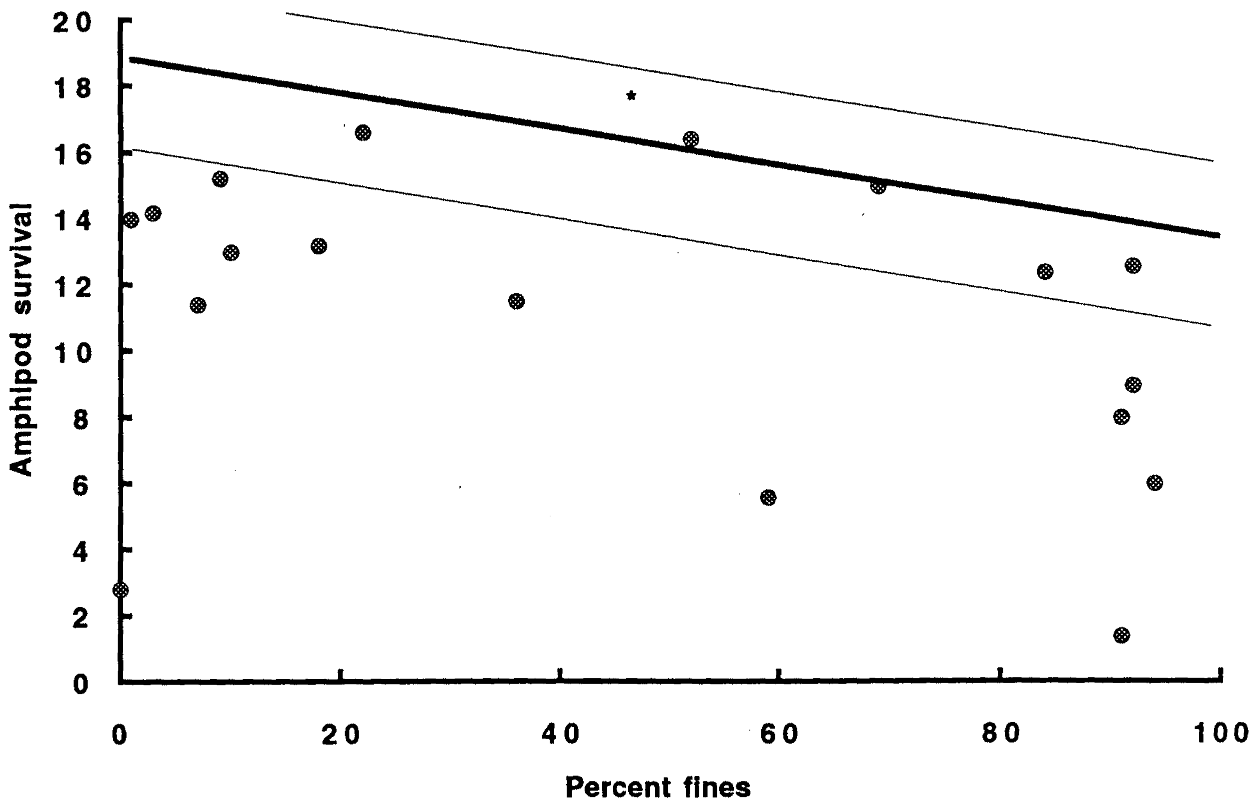

Figure 4. Survival of Rhepoxynius abronius as a function of percent fines of sediments from 19 sites in Padilla Bay (this study) and one site in Padilla Bay (DeWitt, personal communication - asterisk). The approximate regression line and 95% confidence limits for 78 urban sites from Puget Sound from a study by DeWitt et al. (1988) is also shown.

DeWitt et al. (1988) reported that sediment features such as sediment particle size and organic content could effect survival of R. abronius. They developed an equation to separate out the effects of fine particle size on R. abronius survival. Within the results for Padilla Bay, there was no indication of increasing toxicity with increasing percent of fines (Fig. 2). Survival of R. abronius was significantly lower in some coarse sediment and similar to controls in some fine sediment (Table 4). The results from Padilla Bay were compared to the regression line and 95% prediction limits of DeWitt et al. (1988) for 127 reference sites in Puget Sound. Using the criteria recommended by DeWitt et al. (1988) and Mearns et al. (1986), sediment contamination caused amphipod mortality in ten of the 16 scattered sites and all three sites near the former landfill (Fig. 4).

Variance of the survival of R. abronius was much greater in controls conducted at Padilla Bay National Estuarine Research Reserve (Table 3, controls 1-3) than for the test conducted by Northwestern Aquatic Sciences (Table 3, control 4). Two possible reasons for the difference are the somewhat higher range of temperature to which the test organisms were exposed (13°C - 17°C) at Padilla Bay National Estuarine Research Reserve than for the test conducted by Northwestern Aquatic Sciences (12.4°C - 15°C) (Appendix C) or the inexperience of Padilla Bay National Estuarine Research Reserve staff with handling R. abronius. The inexperience of the authors of the present study may have caused some unintended trauma to test organisms, or juveniles may have been included unintentionally with adults in the test chambers. In spite of the higher variance and the lower percent survival in control sediments, the quality control limits were not exceeded (Puget Sound Estuary Program 1986) and the data appear to be an accurate measure of the toxicity of Padilla Bay sediments.

The rates of survival of Rhepoxynius abronius exposed to sediments indicate that a low level toxicity is widely distributed in the intertidal sediments throughout Padilla Bay. This finding was unexpected because Padilla Bay has a generally non-urban, non-industrial watershed without any major outfalls. Potential sources of compounds that may contribute to the toxicity include the oil refineries at March Point, the City of Anacortes (including local industry), agricultural runoff from the Padilla Bay watersheds and atmospheric fallout. The pattern of sites showing some toxicity compared to those not showing toxicity (Fig. 4) is not consistent with a single point source of toxicity to the sediments from any of these sources. In particular sites SH53, SH56 and SH82 (Fig. 1) are close to any point source on March Point or from Guemes Channel (City of Anacortes) but survival at these sites was not significantly lower than controls. Instead, the widespread distribution of sites with sediments toxic to R. abronius, indicates either multiple sources or a low level diffuse source in which local patterns of sediment characteristics, circulation or deposition might cause a higher concentration at one site than at similar sites elsewhere.

One possible mechanism for transport of toxic compounds to the intertidal sediments is the sea surface microlayer. The sea surface microlayer usually contains organic compounds and metals at concentrations more than an order of magnitude higher than the water column beneath the surface microlayer (Hardy et al. 1987). As the mixed diurnal tides ebb, the sea surface microlayer contacts the sediments and various compounds may be deposited on the sediment surface. Such surface deposits in Padilla Bay had higher toxicity than surface deposits in Discovery Bay (Gardiner 1992). Repeated deposition with tides may increase the concentration in the sediments until the survival of R. abronius is affected. Such a mechanism also may increase toxicity of intertidal sediments in other parts of Puget Sound. There are no other reports on toxicity of intertidal sediments in Puget Sound to R. abronius that specify the sediments are intertidal except for a test site in Padilla Bay reported by DeWitt (1989; personal communication). However, if Padilla Bay, located some distance away from many potential sources of toxic compounds has a low level of toxicity in the sediments, then toxicity of intertidal sediments may be widespread in Puget Sound (Gardiner 1992).

Seagrasses are a possible natural source of toxic materials to the sediments. Water-soluble extracts from seagrasses (Zostera marina) inhibit growth of microbial algae and marine bacteria (Harrison 1982). Even more important for the present study, Harrison reported inhibition of grazing by the amphipod, Eogammarus confervicolus, by water soluble extracts of Zostera marina. It is possible that water soluble inhibitors (such as phenolic acids) may be present in the sediments where seagrass biomass is high and that these may increase the toxicity of sediments to Rhepoxynius. The significant toxicity found at five of the six seagrass covered sites is consistent with this hypothesis (Table 3). The one seagrass site that did not have significant toxicity (SH56) had only scattered Zostera marina plants and was mainly covered by a very short Zostera japonica. The hypothesis that natural compounds from seagrasses may be toxic to Rhepoxynius abronius requires further study.

The amphipod toxicity test has been developed for subtidal sediments. Its suitability as a test organism for intertidal sediments has not been documented and in this study the protocols for subtidal sediments were applied to intertidal sediments. The results, therefore, should be interpreted with caution because the test has not been proven as a reliable indicator of toxicity for intertidal sediments. There may be unique characteristics of intertidal sediments, either of physical compaction or chemical differences that influence their suitability for survival of Rhepoxynius abronius. R. abronius may not be a suitable organism for testing toxicity of intertidal sediments.

Leachate from the former landfill in the southwest corner of Padilla Bay appears to be introducing toxicants to the sediments. At all three sites around the landfill survival of R. abronius was 40% or less after 10 days of exposure. Mean survival at these three sites was 25.5% compared to 61.5% for the remaining 16 sites. This difference was significant at the 0.01 level of probability based on a one-way analysis of variance. The extent of the area affected by the leachate and the compound(s) responsible for the toxicity are not known. Further study of the extent of this toxicity has been conducted by Wiggins (1992).

Pastorok and Becker (1990) concluded that Photobacterium phosphoreum generally was one of the most sensitive bioassays in terms of ability to detect significant effects relative to reference sediments. This was not true in the case of the Padilla Bay sediments where negative gammas precluded calculation of an LC50 at all but two of the sites. None of the sediments were found to reduce the light emission by P. phosphoreum. These data indicate that the P. phosphoreum bioassay was less sensitive than the R. abronius assay in its ability to detect significant effects relative to reference sediments.

In conclusion, the sediment bioassays with Rhepoxynius abronius indicate that a low level of toxicity is widely distributed in the intertidal sediments in Padilla Bay. Sediments near the former landfill are highly toxic to R. abronius killing more than half of the test organisms in 10 days of exposure. Further study is required to more clearly delineate the areas of high toxicity in the intertidal sediments of Padilla Bay, the contaminant(s) in the sediment that is (are) causing the toxicity and the source(s) of the contaminant(s).

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APPENDICES

APPENDIX A.

Field notes, grain size of sediments and results of sediment toxicity tests conducted by Dr. Theodore H. DeWitt, Oregon State University, on sediments collected at Padilla Bay in July 1987.

Results of sediment toxicity tests and collection and location notes from notes sent by Dr. T. DeWitt to Dr. D. Bulthuis, April 1992. For location of site see Fig. 1, site DeWitt-1. These are further details for the Padilla Bay site of a study published in DeWitt et al. 1989.

Project B7GF - Intertidal Puget Sound Sediment Survey
Sediment Collecting Field Notes - Ted DeWitt

Collecting procedure: Approximately 1 liter of surficial sediment (top 2 cm) was collected at each site. Sediment obtained using 8 cm diameter glass corers. Sediment and water samples were kept on ice (periodically replenished) in large coolers. Surficial and interstitial salinities were measured by refractometer.

Padilla Bay 7/25/87 - 9:45 a.m.

Location: Large mudflat adjacent to small, private boat landing/pullout, about 1/2 mi. south of Padilla Bay State Park on Bayview Rd. Address of driveway/dirt road to landing is approximately 1147 Bayview (read from nearby mailbox). About 100 ft north of Bayview Mobile Court. Local and Mud: Very Broad mudflat (at least 1/4-1/2 mi wide) with exposed seagrasses (*Zostera*) and numerous epibenthic gastropods (unid.). Sandy mud collected about 20 m from shoreline. Interstitial salinity 29 ppt. Noticed that mud caked onto fingernail cuticles turned coppery-green about 1 hr after collection. High copper in mud?

Sediment grain size at site DeWitt-1:

Median 0 =	64.6 μm
Sand	51.7%
Silt	39.5%
Clay	8.8%

Toxicity tests:

Eohaustorius estuarius, 3 beakers, x = 19 survivors, 95%, 5.0 s.d.
Rhepoxynius abronius, 3 beakers, x = 18.3 survivors, 91.7%, 5.77 s.d.

APPENDIX B

Survival and daily emergence of Rhepoxynius abronius in each replicate of sediment toxicity bioassays

Table B-1. Daily emergence from the sediment (living and dead) and survival of Rhepoxynius abronius after ten days of exposure to test sediments in each replicate during test series 1-3 at Padilla Bay National Estuarine Research Reserve, and test series 4 at Northwestern Aquatic Sciences Laboratory. Twenty R. abronius added at Day 0 to each replicate.

Sample location and replicate no.	Daily and total emergence											Surviving *R. abronius*
	1	2	3	4	5	6	7	8	9	10	Total	
West Beach 1												
1	1	3	1	0	0	1	0	0	0	0	6	20
2	1	2	1	1	2	2	3	1	1	1	15	20
3	1	1	1	1	2	2	2	2	2	2	16	19
West Beach 2												
1	0	0	1	2	2	1	2	1	0	1	10	19
2	0	0	0	0	0	0	0	0	0	0	0	20
3	0	0	0	1	2	2	4	4	5	4	22	16
4	0	0	1	1	1	1	1	1	1	1	8	19
5	0	0	0	0	0	0	0	0	0	0	0	20
West Beach 3												
1	1	1	1	1	1	2	1	1	1	1	11	17
2	0	0	0	0	0	0	0	0	0	0	0	20
3	1	0	0	1	1	1	1	1	1	1	8	20
4	0	0	0	0	0	0	1	1	1	1	4	20
5	0	2	2	1	1	1	1	1	1	1	11	17
Yaquina Bay												
1	0	0	0	0	0	0	0	0	0	0	0	20
2	0	0	0	0	0	0	1	0	1	0	2	20
3	0	0	0	1	0	0	0	0	0	0	1	20
4	0	0	0	1	0	0	0	0	0	0	1	20
5	0	0	0	0	0	0	0	1	0	0	1	20
SH22												
1	1	3	2	0	1	1	1	0	0	0	9	13
2	0	0	0	0	0	0	0	0	0	0	0	14
3	1	1	0	0	0	1	0	0	0	0	3	13
4	2	2	3	2	2	0	0	0	1	1	13	15
5	0	0	0	1	1	1	1	0	0	0	4	15
SH26												
1	0	1	3	6	2	1	2	3	2	0	20	9
2	0	1	2	1	2	1	3	1	0	0	11	11
3	0	0	0	0	0	1	0	2	0	0	3	12
4	0	0	0	0	0	0	0	0	0	0	0	13
5	1	1	1	2	1	0	0	0	0	1	7	12

Sample location and replicate no.	Daily and total emergence											Surviving R. abronius
	1	2	3	4	5	6	7	8	9	10	Total	
SH14												
1	0	1	1	1	4	1	3	3	2	2	18	17
2	2	2	1	1	2	1	1	0	0	0	10	12
3	1	3	3	3	2	3	3	1	5	7	31	16
4	3	6	5	8	6	4	3	2	2	2	41	7
SH18												
1	0	0	1	1	1	0	0	0	0	0	3	16
2	1	0	0	0	0	0	0	0	0	0	1	18
3	2	2	0	0	0	0	0	1	1	1	7	18
4	0	0	0	1	1	0	2	1	1	0	6	20
5	0	0	0	1	2	1	0	0	0	1	5	11
SH53												
1	0	0	2	1	1	0	0	0	0	0	4	14
2	0	0	1	0	0	0	0	1	0	0	2	12
3	0	0	0	0	0	0	0	0	0	0	0	17
4	2	1	0	1	0	0	1	0	0	1	6	15
5	0	0	0	1	0	0	0	0	0	0	1	18
SH56A												
1	0	0	0	0	1	0	1	1	0	0	3	15
2	1	1	1	1	0	0	0	0	0	0	4	17
3	0	0	0	0	0	0	0	0	0	0	0	15
4	0	1	0	1	1	1	1	0	0	1	6	22
5	1	3	2	1	1	0	0	1	0	0	9	15
SH56B												
1	0	0	0	1	1	1	1	1	2	0	7	19
2	0	0	0	0	0	0	0	0	0	1	1	17
3	0	0	0	0	0	0	0	0	0	0	0	19
4	0	0	1	1	1	1	1	1	1	1	8	20
5	1	0	0	1	1	1	0	0	0	0	4	17
SH46												
1	4	5	4	6	6	6	4	0	1	2	38	8
2	2	1	1	1	1	1	1	0	0	0	8	14
3	2	4	2	4	3	1	1	1	1	1	20	19
4	0	1	1		2	1	0	0	0	1	6	15
SH48												
1	3	6	7	8	4	3	5	5	5	99	145	5
2	2	2	4	5	6	6	5	1	1	5	37	5
3	2	4	6	7	6	4	8	5	2	5	49	3
4	7	9	12	15	15	11	6	5	2	8	90	0
5	4	5	8	7	6	3	5	6	2	3	49	1

Sample location and replicate no.	Daily and total emergence											Surviving R. abronius
	1	2	3	4	5	6	7	8	9	10	Total	
SH76												
1	6	5	4	6	5	3	0	1	0	1	31	8
2	4	4	3	4	0	0	0	0	0	0	15	16
3	2	2	3	2	2	0	0	2	1	1	15	12
4	3	2	1	3	3	3	1	0	1	1	18	10
SH79												
1	0	0	0	2	2	1	0	1	0	0	6	8
2	0	0	0	0	1	0	0	0	1	1	3	1
3	0	0	0	1	0	0	0	0	1	0	2	7
4	0	0	0	1	0	1	0	0	2	0	4	6
5	0	0	1	0	0	0	1	0	1	1	4	6
SH39												
1	1	1	1	1	3	2	1	0	1	1	12	16
2	1	3	3	2	3	2	1	2	1	2	20	18
3	2	4	5	4	5	5	3	2	2	0	32	10
4	3	4	4	4	2	1	1	0	0	0	19	10
5	1	0	1	3	4	4	5	1	0	0	19	8
SH40												
1	6	4	1	0	3	5	3	1	0	4	27	15
2	0	2	6	2	3	1	1	2	0	1	18	16
3	2	4	1	5	6	3	4	4	0	4	33	7
4	4	6	3	6	5	4	3	6	3	3	43	16
5	1	4	5	8	6	6	7	6	3	4	50	9
SH93A												
1	0	0	0	2	1	3	2	1	1	0	10	9
2	0	1	1	1	1	0	0	0	1	1	6	14
3	0	0	1	1	1	0	1	2	2	1	9	17
4	0	0	0	1	0	2	0	0	0	1	4	17
5	0	0	0	1	2	2	2	1	3	1	12	18
SH93B												
1	0	1	1	3	2	3	1	2	2	1	16	17
2	0	0	0	0	0	0	1	0	0	0	1	20
3	0	0	1	0	0	0	0	0	0	0	1	17
4	2	0	0	1	1	1	0	0	0	1	6	19
5	0	0	0	1	0	0	0	1	1	0	3	19
SH100												
1	1	0	4	6	5	4	3	1	1	0	25	7
2	2	0	1	1	1	0	4	4	0	0	13	7
3	3	4	2	2	2	1	1	1	1	1	18	8
4	1	2	1	3	5	4	2	2	1	0	21	16
5	3	2	3	5	6	2	1	1	0	0	23	6

Sample location and replicate no.	Daily and total emergence											Surviving R. abronius
	1	2	3	4	5	6	7	8	9	10	Total	
SH82												
1	1	4	2	3	1	1	2	1	3	4	22	14
2	0	0	0	0	0	0	1	2	2	2	7	18
3	1	3	4	7	7	7	7	4	3	4	47	9
4	2	2	2	1	1	1	1	1	1	1	13	15
5	2	2	1	2	4	3	3	2	1	0	20	15
SH90												
1	5	4	4	4	3	4	6	4	5	9	48	12
2	0	5	3	4	4	3	5	5	5	9	43	11
3	4	3	3	5	3	4	5	4	5	8	44	13
4	1	2	0	1	0	1	4	6	5	10	30	10
5	1	1	4	2	7	8	5	10	12	10	60	17
SH130												
1	10	2	3	5	3	2	3	0	1	1	30	0
2	9	1	3	1	1	0	0	0	0	0	15	1
3	2	2	1	0	0	0	1	1	1	0	8	6
4	1	0	0	1	0	0	0	0	0	0	2	0
5	2	1	1	1	0	0	0	1	0	0	6	0
SH131												
1	0	1	4	0	0	0	0	0	1	2	8	4
2	0	1	1	0	2	1	1	2	2	1	11	9
3	1	1	1	1	0	0	0	0	0	0	4	4
4	0	0	1	0	0	0	1	1	0	2	5	9
5	1	2	2	2	2	2	1	0	0	0	12	4
SH132												
1	0	3	4	1	4	4	4	3	0	2	25	6
2	0	0	1	5	2	4	4	2	2	1	21	3
3	1	1	1	1	2	0	0	0	2	1	9	15
4	0	1	0	1	1	1	1	1	2	0	8	10
5	1	3	7	4	5	5	2	4	4	1	36	6

APPENDIX C

Water quality data for toxicity bioassays of sediments

Table C-1. Daily minimum and maximum temperatures (^{O}C) in water both of sediment toxicity bioassays Series 1, 2 and 3.

	Days										
	0	1	2	3	4	5	6	7	8	9	10
Test Series 1											
Minimum	15	15	15.5	14.2	15	14.8	14.2	14.5	16	14	14.2
Maximum	17	18	17.5	16.5	17.5	17	17	16	18	17.2	16
Test Series 2											
Minimum	15	14.5	13.5	13.5	13	14	14	14	14	14	16
Maximum	18	15.8	15.5	17	16	17	17	17	17	17	17
Test Series 3											
Minimum	14	13.5	13.5	14.8	14	13.5	15	14	14.5	13.3	14.5
Maximum	15.8	14.5	14.5	16	17	16	17	18	17	16.5	16.5

Table C-2. Initial and final salinities (parts per thousand) of interstitial water and overlying water in sediment toxicity bioassays Series 1 to 3 and final Eh in Series 2 (replicate number 1 for each treatment).

	Interstitial		Overlying water		
	Day 0	Day 10	Day 0	Day 10	Eh (mV) Day 10
Test Series 1					
SH 14	27	30	30		
SH 39	28	30	30		
SH 46	28	28	28		
SH 76	28	30	30		
SH 82	30	30	30		
SH 100	30	30	30		
SH 130	29	29	30		
Control 1	31	30	29	30	
Test Series 2					
SH 26	27	28		29	256
SH 40	27	29		30	257
SH 48	27	27		30	256
SH 53	27	27		30	259
SH 79	24 adjusted to 27	27		29	256
SH 131	24 adjusted to 26	27		30	256
Control 2	29	29		30	257
Test Series 3					
SH 18	28	30		30.5	
SH 22	28	30		30	
SH 56	28			32	
SH 90	26			31	
SH 93	28	31		31	
SH 132	28			30	
Control 3	32	30	30	30.5	

Table C-3. Minimum and maximum dissolved oxygen and pH in "water quality control" beakers that had no sediment or amphipods added in test series 1, 2 and 3

	Dissolved Oxygen (mg l-1)		pH	
	Minimum	maximum	Minimum	Maximum
Test Series 1	7.4	7.95	7.7	7.95
Test Series 2	7.2	7.8	7.87	8.07
Test Series 3	6.9	7.4	7.96	8.14

Table C-4. Temperature, salinity, dissolved oxygen and pH in test beakers (replicate 1) at beginning and end of Test Series 4.

	Day 0				Day 10			
Sample Identification	Temp (oC)	Sal (ppt)	DO (mg/L)	pH	Temp (oC)	Sal ppt)	DO mg/L)	pH
Control	15.0	28.0	8.6	7.9	12.4	30.0	8.6	8.1
SH 56B	15.8	28.0	8.5	7.9	12.7	29.0	8.6	8.2
SH 93B	15.0	28.0	8.5	7.9	12.5	28.0	8.6	8.2

APPENDIX D.

Survival of Rhepoxynius abronius
exposed to cadmium (positive controls).

Table D-1. Survival of Rhepoxynius abronius exposed for 96 hours to cadmium as cadmium chloride in Test Series 2. Twenty amphipods were added to each test beaker.

Cadmium concentration (mg l^{-1}	Surviving R. abronius (no) 24h	48h	96h	Percent survival after 96h
10	7	1	0	0
1	16	14	11	55
0.1	12	9	4	20
0.01	18	16	15	75
Control	17	15	13	65

Table D-2. Survival of Rhepoxynius abronius exposed for 96 hours to cadmium as cadmium chloride in Test Series 3. Twenty amphipods (presumably) were added to each test beaker.

Cadmium concentration (mg l^{-1})	Surviving R. abronius (no) 24h	48h	72h	96h	percent survival after 96h
10	12	4	0	1	5
1	19	15	5	3	15
0.1	20	17	12	9	45
0.01	21	19	16	10	50
Control	20	20	15	13	65

APPENDIX E

Grain size distribution of sediments for which toxicity bioassays were conducted.

Table E-1. Grain size distribution of sediments at 19 sites in Padilla Bay and at the control site (West Beach, Whidbey Island) from which sediments were collected for toxicity bioassay with the amphipod *Rhepoxynius abronius*. Percent of total dry weight in each size category.

Sample Location	Grain size (µm)															
	Gravel		Sand						Silt				Clay			
	>4750	4750-2000	2000-850	850-425	425-250	250-106	106-75	75-62.5	62.5-31.2	31.2-15.6	15.6-7.8	7.8-3.9	3.9-1.9	1.9-0.9	<0.9	Balance
SH22	0	0	0	1	21	76	1	0	0	0	0	0	0	0	1	0
SH26	0	0	1	10	23	54	4	1	1	1	1	1	0	1	2	0
SH14	0	0	0	1	10	68	8	3	3	1	2	0	1	1	2	0
SH18	0	0	1	6	21	34	12	4	9	3	2	2	1	1	3	1
SH53	0	0	1	12	29	44	4	1	2	2	1	0	1	0	2	1
SH56	0	0	1	1	3	18	16	9	15	9	6	4	4	4	6	4
SH46	20	2	1	3	40	32	1	0	0	1	0	0	0	0	0	0
SH48	14	5	1	1	19	59	1	0	0	0	0	0	0	0	0	0
SH76	0	1	1	13	18	18	8	5	16	6	4	2	2	1	4	1
SH79	0	0	0	1	3	13	14	10	23	11	6	4	3	3	7	2
SH39	0	0	0	0	1	4	6	5	39	24	8	3	2	1	5	2
SH40	0	0	2	2	1	1	1	1	11	22	18	11	8	6	16	0
SH93	0	0	0	1	1	6	11	12	34	11	6	3	2	3	5	5
SH100	0	0	0	1	2	1	2	2	24	25	13	8	5	5	12	0
SH82	0	0	0	3	26	62	5	1	0	1	0	0	0	0	2	0
SH90	0	0	0	3	13	52	10	4	8	2	2	1	1	1	3	0
SH130	0	0	1	1	1	3	2	1	7	27	21	12	6	4	12	2
SH131	0	0	0	0	1	2	1	2	12	25	18	12	7	6	13	1
SH132	1	0	0	0	1	3	2	2	16	27	17	9	5	4	10	3
W. Beach 3	0	0	1	0	4	53	15	6	7	3	2	1	2	1	3	2