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SEDIMENT BIOASSAYS IN VARIOUS B.C. COASTAL AREAS

Prepared for:

Environmental Protection Service 3rd Floor - Kapilano 100 Park Royal South West Vancouver, B.C. V7T 1A2

Prepared by:

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June 1984

Project 4711

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E.V.S. Consultants

Biological and Chemical Services for the Environment

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Our File: 4711

July 12, 1984

Mr. H. Nelson Environmental Protection Service 3rd Floor - Kapilano 100 Park Royal South West Vancouver, B.C. V7T 1A2

Dear Mr. Nelson:

Re: Sediment Bioassays in Various B.C. Coastal Waters

Please find enclosed twenty copies of our final report on the above project prepared following your review and comment.

The study has successfully accomplished its objectives including testing 12 sediment samples (9 were significantly toxic), comparing toxicity and sediment chemistry data (bioassay results reflect the actions of complex chemical mixtures), and comparing the results of data for other areas (a potentially serious sediment toxicity problem exists in the Vancouver Harbour area). On the basis of this and of previous studies conducted in the United States, we recommend that the EPS consider incorporating sediment bioassays into future ocean dumping and marine pollution assessments.

If you have any questions or concerns, please do not hesitate to call.

Sincerely,

E.V.S. CONSULTANTS LTD.

Peter M. Chapman, Ph.D.,

Vice-President

PMC:mkm

Attach.



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SUMMARY

Chapman, P.M. and C.T. Barlow. 1984. Sediment bioasays in various B.C. coastal areas. Report prepared for the Environmental Protection Service, West Vancouver, B.C. by E.V.S. Consultants Ltd., North Vancouver, B.C. 22 pp + appendix.

Sediment bioassays using the sensitive amphipod Rhepoxynius abronius were used to test the toxicity of composite sediment samples collected from 12 marine sites in B.C. Nine of the 12 sites were significantly (P = 0.01) toxic compared to controls. The three most toxic sites were located in the area of Vancouver Harbour: Vancouver Wharves, Neptune Terminals and the East Basin of False Creek. Bioassay results were compared with the results of chemical analysis of the sediments for metals (including nine Priority Pollutants) and other inorganic elements. The single composite sediment sample with the highest levels of Priority Pollutant metals (including Cd and Hg) had 0% amphipod survival, but low survivals were also recorded in samples with lower measured sediment contaminant levels. The results indicated that the sediment bioassay measures a biological response to combinations of chemicals, and toxicity was not related to present ODCA criteria for Hg and Cd. These data indicated a potentially serious sediment toxicity problem in the Vancouver Harbour area, which should be The ODCA criteria would require revision to be consistent with addressed. sediment toxicity tests such as the bioassays used herein.



ACKNOWLEDGEMENTS

E.V.S. Consultants thank the Scientific Authority, H. Nelson, and D. Brothers and R. Kussat of the Environmental Protection Service (EPS) for their assistance with this study. We especially thank Dr. M. Waldichuk for recommending that this study be done. All sediment collections, chemical analyses and drafting were conducted by the EPS.

E.V.S. Consultants acknowledge the principal investigators, P. Chapman and C. Barlow, and the assistance of the following members of E.V.S. staff: K. McKim, H. Hobson, and G. Vigers. The report was word processed by M. Mees.

This study was funded directly by EPS - Pacific Region. The contract was handled by DSS, Science Procurement - Pacific Region under DSS File No. KE603-3-1523.



1.0 INTRODUCTION

This report documents the results of a study commissioned by the Environmental Protection Service (EPS) to determine the acute toxicity of marine sediments collected from six different B.C. coastal areas. A total of 12 sediments (2 from each of 6 sites) were tested for toxicity using the infaunal amphipod Rhepoxynius abronius. Sediment bioassays, particularly with this amphipod, are assuming increasing importance in the United States for regulatory purposes. The present preliminary study represents the first broad-scale use of these techniques in Canada.

Testing involved 10-d exposure of the amphipod \underline{R} , abronius to test sediments, with determination of daily emergence patterns and final mortality using techniques developed by Swartz et al. (1979, 1981, 1982, in press). Test sediments were selectively analysed for chemical contaminants by the EPS and these results were compared with the toxicity data. The relevance of study results to similar studies in contaminated areas of the United States is discussed.

1.1 Objectives

The objectives of the present study were:

- 1. To determine the acute toxicity of 12 field-collected marine sediments to a sensitive infaunal amphipod.
- 2. To compare toxicity data with selected sediment chemistry data.
- 3. To compare results with similar data for other areas.



2.0 METHODS

2.1 Field Collections

Sediment collections were conducted by the EPS, using either a Ponar or Smith-McIntyre benthic grab. Four separate grab samples were collected from each of 12 stations (6 sites; Figs. I - 6) on the following dates: March 28 - Vancouver Harbour, March 29 - False Creek, April 01 - Alberni Inlet, April 03 - Victoria Harbour, April 04 - Powell River, April 05 - Port Mellon.

Four separate grab samples were composited for each site, subsamples were removed by the EPS for chemical analysis, and the remaining sediment was sealed in clean labelled polyethylene bags. Samples were stored at 4° C in the dark prior to testing.

2.2 Sediment Chemistry Analyses

Chemical analyses were conducted by the EPS. Sediments were extracted using a combination of nitric and perchloric acids, and the extracts analysed for metals using Inductively Coupled Argon Plasma (ICAP). Standard reference sediments were analysed to determine analytical efficiency and precision.

2.3 Sediment Bioassays

The infaunal amphipod <u>Rhepoxynius</u> <u>abronius</u> was collected subtidally from West Bay on Whidbey Island (Washington State) using a bottom trawl. Amphipods were maintained and transported in clean coolers with ice, and were returned to the E.V.S. Consultants Laboratory within 6 h of collection.



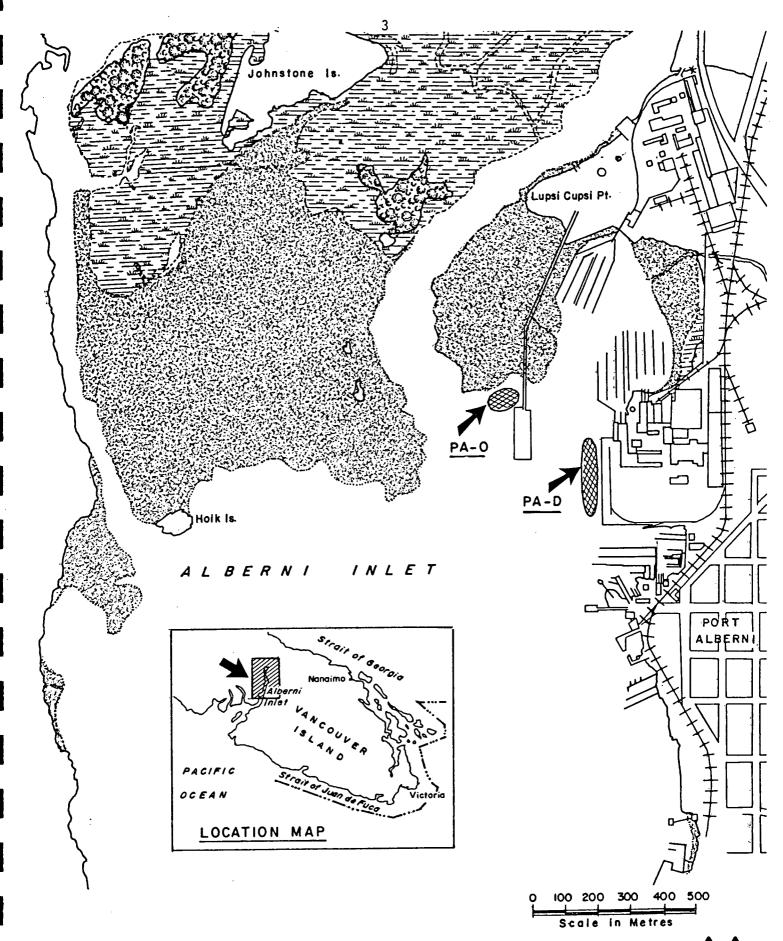


FIGURE 1 PORT ALBERNI SEDIMENT COLLECTION SITES FOR LETHER BIOASSAY TESTS - April, 1984

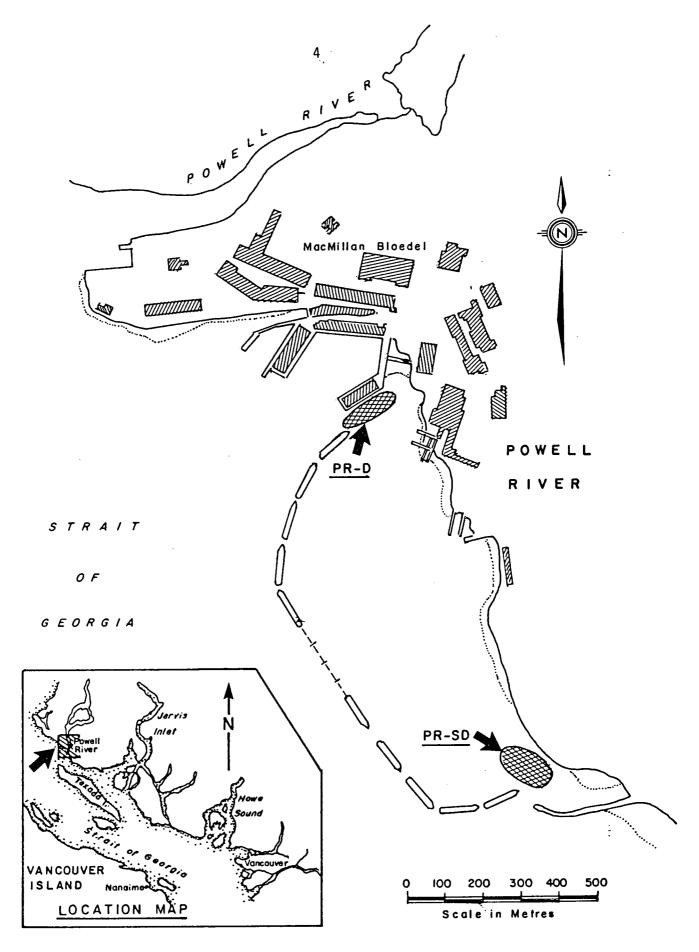


FIGURE 2 POWELL RIVER SEDIMENT COLLECTION SITES LETHALITY BIOASSAY TESTS - April, 1984



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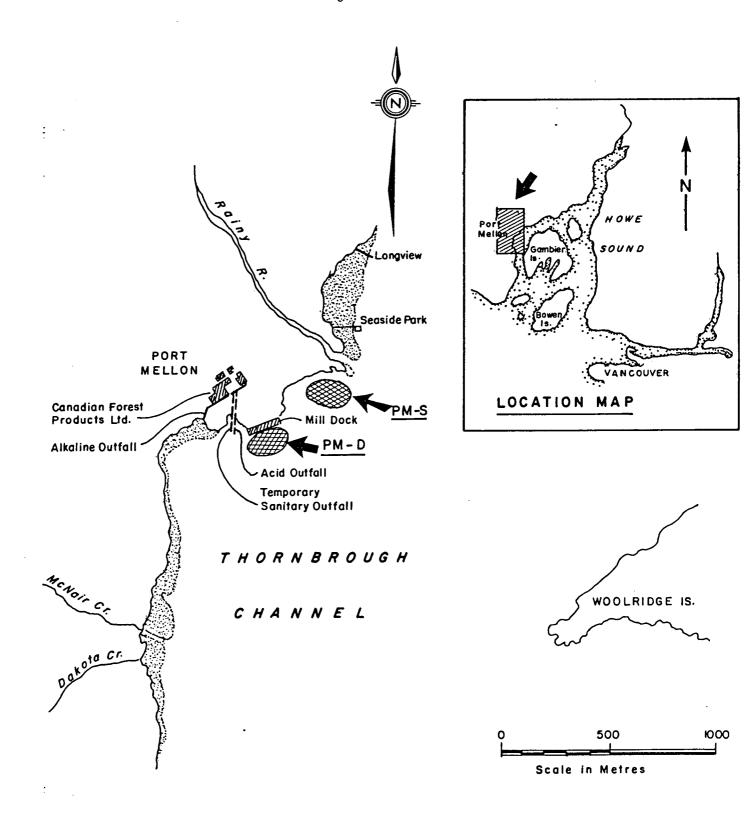
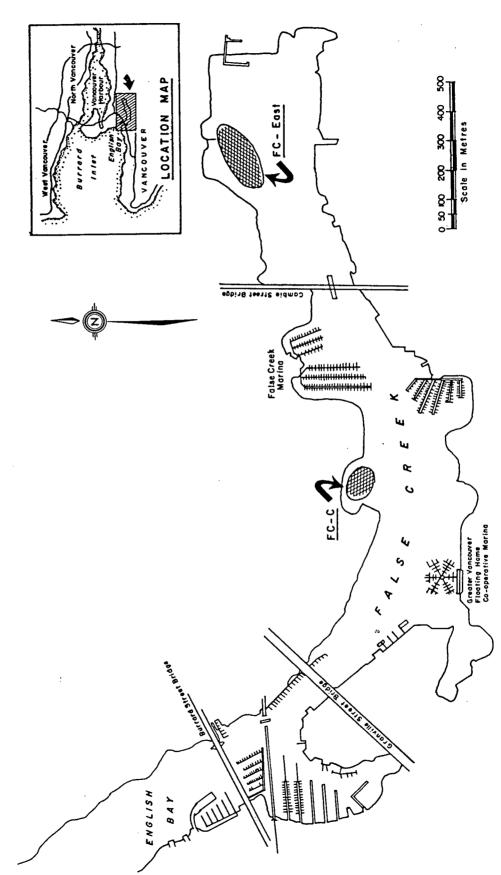
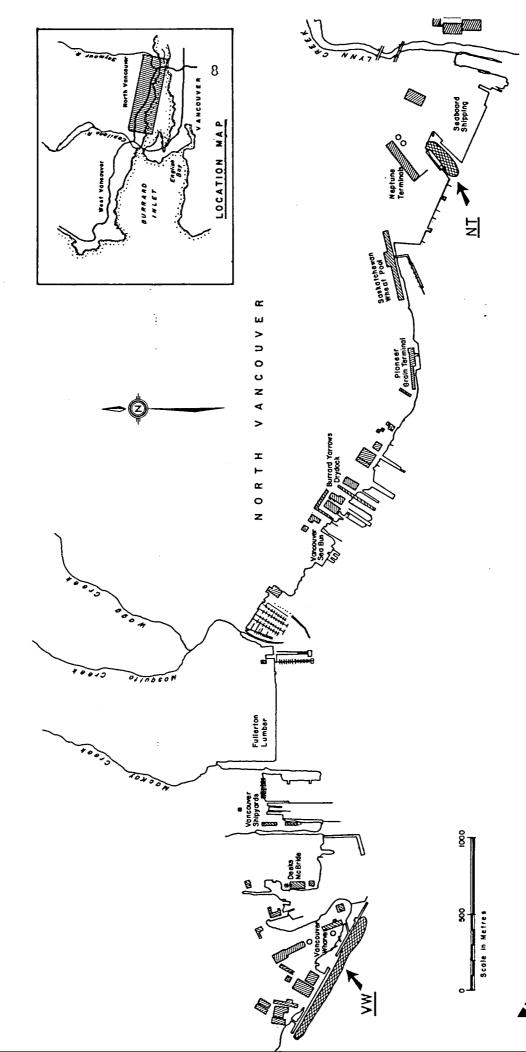


FIGURE 4 PORT MELLON SEDIMENT COLLECTION SITES FOR LETHALITY BIOASSAY TESTS - April, 1984





FALSE CREEK SEDIMENT COLLECTION SITES FOR LETHALITY BIOASSAY TESTS - April, 1984 FIGURE 5



FI 6 VANCOUVER

VANCOUVER HARBOUR SEDIMENT COLLECTION SITES FOR LETHALITY BIOASSAY TESTS - April, 1984

Following their arrival in the laboratory, amphipods were hand sorted from sediments and identifications were confirmed with a Wild M5 dissecting microscope. Damaged and dead individuals were discarded, the remaining amphipods being placed in clean polyethylene containers provided with clean natural substrate and seawater (28 ppt salinity). Cultures were acclimated for 48 h in a constant environmental room adjusted to $15 \pm 1^{\circ}$ C under continuous light. Cultures were aerated but not fed during acclimation.

Acute lethality of whole fresh (unfrozen) sediments was measured by the methodology of Swartz et al. (1982, in press), which involved a 10-d exposure to test sediments. A 2 cm layer of test sediment was placed in 1 L glass beakers, and covered with 800 mL of clean seawater (28 ppt salinity). Each beaker was seeded with 20 amphipods and aerated. Six replicates (20 amphipods each) were run per test sediment. Five beakers served to determine toxicity, while the sixth beaker served as a reference for daily measurement of water chemistry (pH, DO, salinity, temperature). Testing was conducted at 15 ± 1°C under constant light. Test containers were checked daily to establish early trends in mortality and sediment avoidance, and also to gently sink any amphipods which had left the sediment surface overnight and become trapped by surface tension at the air/water interface. A control sediment (from the amphipod collection site) was run concurrently with the test sediments.

Bioassay tests were terminated after 10 d when sediments were sieved (0.5 mm screen), and live and dead amphipods removed and counted. Amphipods were considered dead when there was no response to physical stimulation or microscopic examination revealed no evidence of pleopod or other movement. Missing amphipods were assumed to have died and decomposed prior to the termination of the bioassay



(Swartz et al., 1982, in press). Any significant differences in survivorship between test sediments was determined by analysis of variance. Differences in mean survival between test and control sediments was determined by Dunnett's procedure (Steel and Torrie, 1960). One-tailed Dunnett tables were used to determine if mean survival in each test series was significantly less than control values.

3.0 RESULTS

3.1 Sediment Chemistry

Results of the sediment chemistry analyses for Priority Pollutant metals are provided in Table 1. Results of analyses for other elements are provided in Table 2. Complete data sheets are provided in Appendix A.

All of the sediments tested contained cadmium concentrations exceeding the Ocean Dumping Control Act (ODCA) criteria of 0.6 µg/g. Two of the sediments also contained mercury concentrations exceeding the ODCA criteria of 0.75 µg/g.

3.2 Sediment Bioassays

Sediment bioassay results are provided in Table 3. Complete data sheets are provided in Appendix B.

Statistical data analysis indicated that amphipod survivals in 9 of the 12 sediments tested were significantly (P = 0.01) lower than controls. Survivals in sediments from the Powell River dock (PR-D), Port Mellon scow area (PM-S) and False Creek Area C (FC-C) were not significantly different than controls.



SEDIMENT LEVELS OF PRIORITY POLLUTANT METALS^a TABLE 1

						Metals (µg,	hetals (µg/g dry weight)	q(i		
Area	Station	As Is	81	밁	ان	ઢા	ΞĮ	ଣ	<u>Z</u>	뒴
Port Alberni	PA-U PA-0	18. 18.	*0.5 L 0.2	1.2	63.9 *64.2	91.4	36.2 44.	56.2 53.5	480. 1,995.	0.54
Powell River	PR-S PR-D	L 8. 10.	L 0.2 L 0.2	3.0	12.4 27.5	93.6 103.9	7.5	81.	190.5 376.2	0.54
Victoria Harbour	0-H H-H -H	17.	0.3	0.8	45.8 57.8	77.6	21.5	167.5 272.	158.5 264.2	0.71
Port Mellon	PM-S PM-D	L 8. 9.5	0.4 L 0.2	1.6	48.0 55.5	142. 130.	. 25. . 18.	64. 180.	197. 225.	0.62
False Creek	Α. Α.Ο.	12.5	*0.5	3.3	58.6 26.6	141. 56.5	30.2	227.8 224.	475.2 225.5	0.37
Vancouver Harbour	⊢ ¾ Z >	L 8. *95.3	0.3 L 0.2	1.1	29.8 38.8	*199. L 0.8	238. *992.8	59. *28,675.	145.	0.21 *2.47

a. Priority pollutant metals not measured: Sb, Se, Ag, Tl.

b. L = less than
* = highest concentration(s) measured
= Cd or Hg levels exceeding ODCA criteria



TABLE 2
SEDIMENT LEVELS OF NON-PRIORITY
POLLUTANT METALS AND

INORGANIC ELEMENTS

Element	Concentration Range (цд/д dry wt.)	Station with Highest Concentration
Ba	13.4 - 148.	FC-E
Со	4.5 - 86.	VW
Mn	114.0 - 504.	PA-O
Мо	3.9 - 239.	VW
Р	510.0 - 12,800.	NT
Sn	$L 0.2^{a} - 31.$	FC-E
Sr	27.3 - 238.	FC-C
Ti	318.0 - 1,950.	PA-D
V	41.0 - 131.	PA - D
ΑI	5,120.0 - 27,300.	PA-D
Fe	12,000.0 - 76,400.	VW
Si	130.0 - 1,760.	FC-E
Ca	100.0 - 101,000.	PM-D

4,890.0 - 12,400.

2,790.0 - 15,400.

PA-D

PA-O

a. L = less than

Mg

Na



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TABLE 3
SEDIMENT BIOASSAY RESULTS

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			Survival		Avo	Avoidance ^C
Area	Station	Mean No. Survivors	Standard Deviation	P=b	Mean	Standard Deviation
Port Alberni	Dock (PA-D) Outfall (PA-O)	13.2	1.5	0.01	2.6	2.1
Powell River	Stillwater Div. (PR-S) Dock (PR-D)	12.4	-6	0.01	3.2	3.3
Victoria Harbour	Outer (VH-O) Inner (VH-I)	12.2	h.4 4.4	0.0	1.7	1.8
Port Mellon	Scow Area (PM-S) Dock (PM-D)	18.2	1.3	n.s. 0.01	1.2	1.9
False Creek	East Basin (FC-E) Area C (FC-C)	2.6	2.3	0.01	2.5	2.0
Vancouver Harbour	Neptune Terminals (NT) Vancouver Wharves (VW)	00	00	0.01	6.1	4.0
Whidbey Island	(Control)	18.0	1.2	ŀ	1.2	-:

a. Five replicates; seeded with 20 amphipods per replicate.

b. Level of significance; n.s. = not significant.

c. Number of amphipods out of the sediment per replicate per day.

Data for avoidance (amphipods emerging from the sediments, Table 3) showed a good correspondence with the survival data. For instance, the two stations with no amphipod survivals (NT and VW) showed the highest avoidance response. Two of the three stations with survivals similar to controls showed low avoidance responses, also similar to controls. However, two exceptions to this correspondence were noted: station PR-D showed no significantly lower survival than controls but there was a relatively high avoidance response to the sediments; and, station VH-O showed significantly lower survival but a relatively low avoidance response.

4.0 DISCUSSION

4.1 Toxic Areas

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Although bioassays have been used extensively to document water column toxicity related to specific discharges, sediment bioassays involving laboratory exposures of test species to field-collected sediments have only recently been applied to the marine environment. Because sediments are a major repository for persistent aquatic contaminants, sediment bioassays provide data on the toxicity of contaminants which may be released to the water column through dredging or natural scouring processes, and which may be affecting marine fauna living in, on or near the bottom sediments.

The most sensitive species used in acute lethal sediment bioassay tests conducted to date is the phoxocephalid amphipod Rhepoxynius abronius (Chapman et al., 1982, in press a; Chapman and Fink, 1983, R. Swartz and J. Cummins, EPA, pers. comm.). This sensitivity is reaffirmed by field observations that this species and phoxocephalids as a group are not found in contaminated areas of Puget Sound, Washington (Swartz et al., 1982; Comiskey et al., in press; Chapman et al., in press b).



The results of the present study, using R. abronius as a test organism, indicate that nine areas caused significant amphipod mortality, and hence can be classified as acutely toxic. Three of these areas were characterized by less than 15% amphipod survival: Vancouver Wharves, Neptune Terminals and the East Basin of False creek. Sediments from two areas were characterized by 42-45% survival: the outfall area at Port Alberni and inner Victoria Habour. The remaining four areas were characterized by survivals of between 60 and 70%. Data on avoidance generally supported this characterization. Sediments from the Powell River dock area, which were not actually acutely toxic, induced a high level of avoidance suggestive of sublethal effects.

The above results are based on composite samples and do not serve to characterize the extent of toxicity in each area tested. Such characterization, including delineation of the depth and aereal extent of toxicity, is required to accurately delineate areas of concern. However, the results of the present study do serve to prioritize areas of concern as follows:

- 1. Vancouver Wharves, Neptune Terminals;
- 2. East Basin of False Creek,
- 3. Outfall area in Port Alberni, inner Victoria Harbour;
- 4. Port Alberni dock area, Powell River Stillwater Division, outer Victoria Harbour, Port Mellon dock area.

4.2 Comparisons Between Sediment Chemistry and Toxicity

Previous attempts to correlate sediment bioassay results with sediment contaminant concentrations have been largely inconclusive. Tsai et al. (1979) determined 48 h LC50 values for the mummichog (Fundulus



heteroclitus), spot (<u>Liostomus xanthus</u>) and a mollusc (<u>Mya arenaria</u>) exposed to constantly mixed Baltimore Harbor sediment concentrations of between 79 and 0.63 g/L. There was no correlation between bioassay results and measured concentrations of Pb, Cr, Zn, Cd, Hg, Ni, Cu, Mn, As and PCBs in the sediment. However, bioassay results did show a significant correlation with species diversity indices for the tested sites. Tsai et al. (1979) concluded that no single chemical contaminant accounted for sediment toxicity.

Similar results have been obtained for the New York Bight. Wurster (1982) tested eight sediment samples from the Hudson-Raritan estuary using a two species phytoplankton bioassay. He found no correlation between bioassay results and PCB and PAH concentrations, but did note some correspondence with levels of total heavy metals in sediments.

Tietjen and Lee (in press) used free-living nematodes as bioassay organisms to determine the relative toxicity of eight sediment samples from the New York Bight representing a gradient of stations from lightly to heavily impacted by pollution. Sediment contaminants measured were PCBs, PAHs, Cd, Cr, Cu, Hg, Pb and Zn. The natural daily increase in number of nematode generations was used as a measure of sediment quality. Population growth was found to be less than half when PCB concentrations were greater than 270 ppb, and when PAH concentrations were greater than 8700 ppb. Highest levels of sediment contamination for particular compounds were (in ppb): PCBs, 1560; PAHs, 15200; Cd, 32; Hg, 23.

Swartz et al. (1982) have sediment bioassay data for Commencement Bay and Waterways, Puget Sound. Sediment chemistry data relevant to these bioassays is reviewed by Schultz et al. (in prep.). Swartz (pers. comm.) is presently preparing a report on benthic community data



relevant to both the bioassays and the chemistry and will be attempting to correlate all three data sets using multivariate analyses. However, the results of these analyses are not expected to be available until mid-1984 (Swartz, pers. comm.).

Attempts by other Puget Sound investigators (i.e. Quinlan et al., in press; Chapman et al., in press b) to correlate sediment bioassay results with particular individual or combined sediment chemical contaminants have been unsuccessful. However, broad-scale comparisons of sediment bioassay results with sediment chemistry and fish histopathology data have shown correspondences (Quinlan et al., in press), while Chapman et al. (in press b) have shown that areas characterized as toxic in sediment bioassays are also characterized by changes in benthic infaunal composition and high overall sediment chemistry levels.

The results of the above analyses indicate that sediment bioassays provide data representative of <u>in situ</u> toxic conditions, but that the measured toxicity cannot be ascribed to any particular chemical compound or groups of compounds. As noted by Swartz et al. (1984), "the sediment bioassay is a method of pollution assessment whose validity is not dependent on correlation with sediment chemistry."

In the present study, only the results of testing at Vancouver Wharves (0% survival) were intuitively predictable based on the fact that this sediment had the highest levels of 6 of 9 Priority Pollutants measured, including cadmium (18.7 μ g/g) and mercury (2.47 μ g/g). In contrast, Neptune Terminals sediments, which also had 0% survival in the bioassays, had the second lowest levels of cadmium (1.1 μ g/g) and the lowest levels of mercury (0.21 μ g/g). Extremely high levels of phosphorous were, however, noted in the Neptune Terminal sediments, which may be significant.



The metals cadmium and mercury are regulated under the ODCA. When sediment levels exceed 0.75 μ g/g mercury and/or 0.6 μ g/g cadmium, more extensive testing may be required and/or site specific information may be considered during the evaluation for ocean disposal. In the present study, all sediments exceeded the cadmium criteria, but not all sediments exceeding the criteria showed toxicity. The range of cadmium concentrations noted for toxic responses was 1.1 - 18.7 μ g/g, and overlapped with the range for non-toxic responses, which was 1.0-3.0 μ g/g. Only two sediments exceeded the mercury criteria, and both showed toxic responses. The range of mercury concentrations noted for toxic responses was 0.21 to 2.47 μ g/g, and overlapped with the range for non-toxic responses, which was 0.37-0.59 μ g/g.

Previous sediment bioassays in the East Basin of False Creek showed toxicity at the following sediment contaminant levels (in µg/g dry weight): Cd, 4.3; Pb, 206; Hg, 0.44 (R. Waters, B.C. Place, unpub. data). No toxicity was noted at the following sediment contaminant levels: Cd, 0.87; Pb, 39.2; Hg, 0.18 (R. Waters, unpub. data).

Based on the present results and those of previous studies, it is apparent that sediment bioassay toxicity responses are not related to concentrations of individual compounds such as the metals Cd and Hg. Rather, the response is to overall sediment contamination which includes organic compounds in addition to synergistic/antagnostic reactions between chemicals. As such, the toxicity response provides data of more direct relevance to environmental effects of ocean dumping than do chemical analyses alone.



4.3 Comparisons with Other Areas

Comparisons between the results of sediment bioassay tests for different areas are commonly undertaken on the basis of number of stations showing toxicity and the overall degree of toxicity. However, comparisons here are made on the basis of single composite samples, and only for the most toxic areas.

The fact that two stations in Vancouver Harbour had 0% survival and one station in False Creek had less than 15% survival is of concern. It is relatively rare to obtain such low survival values in replicated amphipod bioassays. For instance, in recent studies at Commencement Bay in Puget Sound, designated by the EPA as among the top ten priority toxic waste dumpsites in the United States requiring remedial action, a total of 50 stations were tested for toxicity using the amphipod bioassay. Of these, only one station showed 0% survival with the two next most toxic stations showing survivals of 5 and 23% (Chapman, unpub. data).

It is therefore recommended that the three most toxic sites in the Vancouver Harbour area be given priority for additional studies. Specifically, toxicity tests should be undertaken at each site to determine the areal extent of the toxic response.

5.0 CONCLUSIONS

The following major conclusions can be derived from the results of this study, related to the study objectives.



1. Sediment bioassay tests served to prioritize the following tested areas on the basis of decreasing acute lethality (toxic = significantly lower survival than in controls):

Toxic

- I. Vancouver Wharves, Neptune Terminals;
- 2. East Basin of False Creek;
- 3. Outfall area in Port Alberni, inner Victoria Harbour;
- 4. Port Alberni dock area, Powell River Stillwater Division, outer Victoria Harbour, Port Mellon dock area;

Non-Toxic

- 5. Powell River dock, Port Mellon scow area, False Creek Area C.
- 2. Sediment bioassays indicated that the highest toxicity occurred at the site (Vancouver Wharves) with the highest concentrations of Priority Pollutant metals. However, toxicity was not related to concentrations of particular chemicals, and there was not relationship between the ODCA criteria for mercury and cadmium, and sediment toxicity.
- 3. The three most toxic sites, located in the Vancouver Harbour area, may be indicative of serious contamination problems; the toxicity data from this general area suggested a comparable level of sediment toxicity as the U.S. marine Superfund site in Commencement Bay.

6.0 RECOMMENDATIONS

The following major recommendations result from the present study.

 Sediment biaossays with the amphipod <u>Rhepoxynius abronius</u> provide information on sediment toxicity, and should be used in future assessments by the EPS, to supplement chemical analyses.



Following the recommendations of Chapman and Long (1983) and consistent with present techniques in the United States, it is further recommended that sublethal and genotoxic bioassay tests also be used, to provide complete information on sediment toxicity.

- 2. Additional sediment bioassay testing should be conducted on a grid of samples collected from at least the three most toxic areas: Vancouver Wharves, Neptune Terminals, and the East Basin of False Creek. Samples for testing should also be taken at different depths in the sediment, where possible, to determine the extent of toxic sediment deposition. Sampling design should endeavour to identify whether the cause(s) of contamination are historical, point source (storm, sewer, effluent discharge) or non-point source discharges.
- 3. The present ODCA criteria for Ocean Dumping are based on chemical analyses, most notably for Cd and Hg. These criteria should be reviewed and amended to reflect toxicity criteria.

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$\label{eq:appendix} \mbox{APPENDIX A} $$ \mbox{SEDIMENT CHEMISTRY DATA SHEETS}^{\mbox{$\bf q$}}$

Station	Lab. Identification (8404960 -)
PM-D PM-S PA-D PA-O FC-E FC-C NT VW PR-S PR-D VH-O VH-I	01, 02 03, 04 05, 06 07, 08 09, 10 11, 12 13, 14 15, 16 17, 18 19, 20 21, 22 23, 24
Reference Material	
MESS-I BCSS-I	25, 26, 27 28, 29, 30

a. Two replicates, plus random split samples analysed.



840496006

365613 840496006	- 6/6n	(8.	5.6	4.	1.1	17.3	63.	91.	418.	25. 1	36.	741.		7.	61.8	1680.	123.	476.	24700.	38900.	1220.	1 ଓଡ଼ଜ୍ଜ.	11940.	1ଜବେଡ.	;		-1	
840496902	5/6n	(8.	50.3	٠. •	1.4	17.5	63.7	91.3	488.	24.4	37.	734.	48.	12.	60.6	1,820.	126.	481.	୧୯୨ଉଡ.	38900.	1040	989 0 .	1 ଅଷ୍ଟର	9≥60.		1	m,	
848496888	576n	(8.	58.9	ű.	1.1	14.6	66.	92.5	436.	26.	36.	751.	67.	12.	63.8	1950.	131.	486.	27300.	4 ଫରବର ଦ	1040.	10300.	12400.	9550.			Œ	
84 0 496004	6/5n	(8.	75.4	4.	1.6	€	47.6	135.	476.	23.8	24.	1030.	61.	18.	148.	1570.	.88	194.	20300.	୧୨୭୭୭.	1250.	43600.	11200.	9410.				
840496003	6/8n	(8,	75.	ო.	1.7	18.	49.1	148.	475.	21.1	26.	931.	68.		148.	1630.	91,	200.	20900.	29500.	1460.	43000.	11300.	9350.			. -4	
84849688	ō/ōn	(8.	57.8	oi .	m m	6.5	ທີ່	134.	398.	22.6	18.	926.	174.	. 6	189.	1120.	ທີ່	ก ณ ณ	16000.	19400.	1440.	101000.	8180.	10200.			-	
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840496023		14.	146.	4.	1.3	11.7	58.1	104.	315.	7.7	45.	1150.	297.	14.	71.2	1330.	92.	272.	22200.	31000.	130.	7840.	9940.	7120.			
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840496022	SEDIMENT UG/G	. 687	1:1	!	
840496021	SEDIMENT UG/G	42.	1 1 1 1 1 1		0.153 0.157 0.157
840496020	SEDIMENT UG/G	. 471	B 1:1		MESS-1
	. Compared to the contract of	Q	REPLICATES DILUTION FACTOR		

APPENDIX B

SEDIMENT BIOASSAY DATA SHEETS



E.V.S. CONSULTANTS

AMPHIPOD BIOASSAYS - COMMENCEMENT BAY BIOASSAY DATA AND DAY 10 WATER CHEMISTRY

SAMPLE	VH-I			
			-	

			NUH							-			NUMBER		WATER	CHEHI	STRY AT	10 D
NO.	REP.	0	FRO	2	E D I	MEN 4	15	AT 6	DAY 7	5 0	_	10	ALIVE AT 10 DAYS	FAILING TO REBURROW	TEMP	SAL (ppt)	0.0. (mg/L)	рН
1	А		6		1	1	1	Γ			1	1	15		15.6	27	8.0	8.5
2	B -	Г	1	5	3	Γ				1	1	1	6		15.2	27	8.0	8.5
3	С		7	6	7	5	7	3	Π			Γ	6		15.1	27	8.0	8.5
4	D		5	3	3	2	Γ	2	1	Π	3	2	4	,	15.0	28	7.8	8.7
5	Ε		5	6	5	2	1	4	1	1	2	1	10		15.3	27	8.2	8.5

* all replicates of DAY 1 samples exposed to 14:10 light/dark photoperiod- all following days were continuous light exposure.

SAHPLE VH-0

AT DAYS 0-10 6 7 8 9 1 1 2 3	ALIVE FAILII AT 10 TO 10 DAYS REBURN	TEMP SAL	0.0.) (mg/L)	рН 8.6
1 1 2 3	5 5			8.6
				_ , _
2 1 1	2 13	15.4 26	*7.3	8.4
1 2	14	15.2 27	8.0	8.7
1 3 2 1	14	15.3 26	8.2	8.7
1 3	2 15	15.4 26	8.1	8.6
	1 2	1 2 14 1 3 2 1 14	1 2 14 15.2 27 1 3 2 1 14 15.3 26	1 2 14 15.2 27 8.0 1 3 2 1 14 15.3 26 8.2

* aeration stopped overnight

SAMPLE PR-D

					0F								NUMBER		WATER	CHEHI	TRY AT	10 D
LAB NO.	REP.		FRO	H Ş	EDI	HEN	TS	AT	DAY	S 0	-10		ALIVE AT 10	FAILING	TEMP	SAL	0.0.	рН
		0	1	2	3	4	5	6	7	8	9	10	DAYS	REBURROW	(°C)	(ppt)	(mg/L)	
13	A		9	2	4	3	3	3	5	2_	2	4	14		15.4	26	7.9	8.6
14	В		4	3	3	2			1		1	1	18		15.0	26	8.0	8.6
15	С		10	7	5	Γ	2	1	2	3	3	1	18		15.2	26	7.9	8.5
16	D		6	4	2	1	2	2	2	1	1		19		15.3	26	8.0	8.5
17	Ε		9	Г	4	Π	2	Π	1		1	2	17		15.1	26	8.2	8.5



	:		NUH	BER	OF	AH	PHI	POD	S E	MER	GED	,	NUMBER		WATER	CHEHI	STRY AT	10 D
LAB NO.	ş REP.		FRO	H S	EDI	HEN	TS	AT	DAY	S 0	-10	<u>.</u>	ALIVE AT 10	FAILING TO	TEMP	SAL	0.0.	рН
	ľ !	0	1	2	3	4	5	6	7	8	9	10	DAYS	REBURROW	(°C)	(ppt)	(mg/L)	
19	A		4	3	2	2	3	2	1	2	1	2	13		15.3	26	8.1	8.6
20	В	-	8	5	2	2	4	5	3	2	3		11		15.3	27 .	8.0	8.6
21	c	\vdash	12	ī	┰	2	1				Г	1	14		15.1	26	8.1	8.6
22	0	┢	11	5	2	4	5	4	4	4	2	4	12		15.2	26	8.2	8.6
23	Ε	\vdash	5	2	2	1	2	4	4	4	2	2	12		15.3	26	8.0	8.6

SAMPLE PM-D

	,		NUH	BER	OF	AH	PHI	POD	S E	MER	GED		NUMBER		WATER	CHEHI	STRY AT	10 D
LAB NO.	REP.		FRO	H S	EDI	HEN	TS	AT	DAY	'S 0	-10		ALIVE AT 10	FAILING	TEMP	SAL	D.O.	pН
110.	}	0	1	2	3	4	5	6	7	8	9	10	DAYS	REBURROW	(°C)	(ppt)	(mg/L)	
25	A		8	4	1		Γ		2	2	3	1	10		15.2	26	7.8	8.8
26	В			3	1	1	1	l	1	2	3	5_	16		15.3	27	0.8	8.8
27	С		1	2	2	1	2	3	2	3		2	18		15.2	26	8.0	8.8
28	0		5	Γ	Π	2		1		1	2_	2	14		15.4	26	7.9	8.8
29	E		9	3	1	2	l	2	2	3	5	3	12	1 .	15.4	26	7.6	8.7

SAMPLE PM-S

			NUM	BER	OF	AH	PHI	POD	SΕ	MER	GED		NUMBER		WATER	CHEMI	STRY AT	10 D
LAB NO.	REP.		FRO	H Ş	EDI	HEN	TS	AT_	DAY	S 0	-10		ALIVE AT 10	FAILING TO	TEMP	SAL	D.O.	рΉ
		0	1	2	3	4	5	6	7	8	9	10	DAYS	REBURROW	(°C)	(ppt)	(mg/L)	
31	A		4	2	Г		1			Г	Γ		19		15.2	26	8.4	8.7
32	В		1	2		2	1	1	1	1	1	1	17		15.3	26	8.1	8.7
33	С	-	3	一	\vdash	1	2	1	2	1	1	1	20		15.4	26	8.0	8.6
34	D	-		 		2	1	1	1	1	1	\dagger	17		15.4	26	8.1	8.7
35	F	\vdash	4	2	-	2	1	4	3	4	1	1	18		15.2	26	8.3	8.7

SAMPLE FC-EAST

		NUH	BER	0.F	AH	PHI	POD	SΕ	MER	GED		NUMBER		WATER	CHEHI	STRY AT	10 D
REP.		FRO	H S	EDI	HEN	TS	AT	DAY	S 0	-10		ALIVE AT 10	FAILING TO	TEMP	SAL	0.0.	рН
	0	\Box	2	3	4	5	6	7	8	9	10	DAYS	REBURROW	(96)	(ppt)	(mg/L)	
			_	3	5	3	2	3	4	3	4	1		15.1	27	8.1	8.7
	-	10	1	<u> </u>	ļ.	2	2	1	1	1	1	6		15.1	27	7.8	8.7
В	<u> </u>	1	<u> </u>	┖	ļ_	13	1:-	 -	1	1	17	1	 	15.2	26	7.8	8.7
c_		5	3	2	3	2	11	4_	14	4	<u>Ľ</u>	-			ļ		8.6
D	\Box	4		T		2	1	4	1		1	1	J.:	15.0	21		
F	┼	5	4	2	2	1	1	1	2	3	T	1		15.3	26	7.2	8.6
֡	A B	REP. O	REP. FRO. 0 1 A	REP. FROM S 0 1 2 A	REP. FROH SEDI 0 1 2 3 A 3 3 B 10 4 2 C 5 3 2 D 4 9	REP. FROM SEDIMENT OF THE NAME	REP. FROM SEDIMENTS 0 1 2 3 4 5 A 3 5 3 B 10 4 2 2 3 C 5 3 2 3 2 D 4 2 2	REP. FROM SEDIMENTS AT 0 1 2 3 4 5 6 A 3 5 3 2 B 10 4 2 2 3 2 C 5 3 2 3 2 1 D 4 0 2 2	REP. FROM SEDIMENTS AT DAY O 1 2 3 4 5 6 7 A 3 5 3 2 3 B 10 4 2 2 3 2 4 C 5 3 2 3 2 1 4 D 4 0 2 4	REP. FROM SEDIMENTS AT DAYS O 0 1 2 3 4 5 6 7 8 A 3 5 3 2 3 4 C 5 3 2 3 2 1 4 4 D 4 0 2 2 4	REP. FROM SEDIMENTS AT DAYS 0-10 0 1 2 3 4 5 6 7 8 9 A 3 5 3 2 3 4 3 B 10 4 2 2 3 2 4 4 1 C 5 3 2 3 2 1 4 4 4 D 4 0 2 2 4	0 1 2 3 4 5 6 7 8 9 10 A 0 3 5 3 2 3 4 3 4 B 10 4 2 2 3 2 4 4 1 1 C 5 3 2 3 2 1 4 4 4 7 D 4 0 2 4 0<	REP. FROM SEDIMENTS AT DAYS 0-10 O 1 2 3 4 5 6 7 8 9 10 DAYS A 3 5 3 2 3 4 3 4 1 1 6 C 5 3 2 3 2 1 4 4 7 4 D 4 0 2 4 1 1	REP. FROM SEDIMENTS AT DAYS 0-10 0 1 2 3 4 5 6 7 8 9 10 DAYS A 3 5 3 2 3 4 3 4 1 B 10 4 2 2 3 2 4 4 1 1 6 C 5 3 2 3 2 1 4 4 4 7 4 D 4 0 2 4 1 1 1	REP. FROM SEDIMENTS AT DAYS 0-10 ALIVE AT 10 DAYS REBURROW (°C) A	REP. FROM SEDIMENTS AT DAYS 0-10 A	REP. FROM SEDIMENTS AT DAYS 0-10 A



SAMPLE	FC-C	
JAMIELE .		

			אטא	BER	OF	AM	PHI	POD	S E	MER	GED		NUMBER		WATER	CHEHI	STRY AT	10 D
LAB NO.	REP.		FRO	H Ş	EDI	HEN	TS .	AT_	DAY	s 0	-10		ALIVE AT 10	FAILING TO	TEMP	SAL	0.0.	рΗ
		0	1	2	3	4	5	6	7	8	9	10	DAYS	REBURROW	(°C)	(ppt)	(mg/L)	
43	A								1	Г		1	18		15.1	26	8.1	8.4
44	В								2		Г	1	17		15.0	27	8.2	8.4
45	С				1	\Box			Π	1	Γ	Π	15		15.3	26	8.1	8.4
46	D		1		1	2	2	1	1	2	1		19		15.3	26	8.0	8.4
47	E	T	4		1							\vdash	18	1	15.4	26	8.0	8.4

SAMPLE PA-0

			HUH										NUMBER	NUMBER	WATER	CHEHI	STRY AT	10 D
LAB NO.	REP.		FRO	H -S	EDI	HEN	TS	AT	DAY	'S 0	-10		ALIVE AT 10	FAILING TO	TEMP	SAL	0.0.	рH
,,,,		0	1	2	3	4	5	6	7	8	9	10	DAYS	REBURROW	(°C)	(ppt)	(mg/L)	
49	Α		9 .	1	2	1	2		1				14		15.3	26	0.8	8.7
50	8		5	2	3	3	4	1	3	2	1	6	8		15.4	26	8.0	8.7
51	C		14	5	4	2	6	6	9	4	Б	3	7		15,5	26	7.5	8.5
52	0		6	5	4	4	3	4	3	1	1	1	9		15.3	26	8.0	8.7
53	Ε		16	13	8	5	7	11	7	7	4	3	7		15.1	26	7.9	8.7

SAMPLE PA-D

					OF								NUMBER		WATER	CHEHIS	TRY AT	10 D
LAB NO.	REP.		FRO	H Ş	EDI	HEN	TS	AT	DAY	s 0	-10		ALIVE AT 10	FAILING TO	TEMP	SAL	D.O.	рΗ
		0	1	2	3	4	5	6	7	8	9	10	DAYS	REBURROW	(°C)	(ppt)	(mg/L)	
55	A		5	5	4	4	3	4	2	3	5	8	14		15.1	26	8.0	8.6
56	В		7	1	1	3	1		1		2	3	13		15.1	26	8.1	8.5
57	C	_	\vdash	3	3	5	1	1	1	5	3	2	11		15.1	27	8.0	8.6
58	D	-	8	1		3	1	4	2	2	1	3	13		15.3	27	8.1	8.5
59	Ε		7	1	T	1	1			4	3	3	15		15.5	26	8.0	8.6

SAMPLE NT

			אטא	BER	OF	AM	PHI	POD	SE	MER	GED		NUMBER		WATER	CHEHI	TRY AT	10 (
LAB NO.	REP.		FRO	H S	EDI	HEN	TS	AT	DAY	s 0	-10		ALIVE AT 10	FAILING TO	TEMP	SAL	0.0.	рН
no.		0	1	2	3	4	5	6	7	8	9	10	DAYS	REBURROW	(°C)	(ppt)	(mg/L)	
61	A		11	6	4		11	10	10	12	10	11	0		15.0	26	8.0	8.3
62	В		15	7	5	├ 	9	7	7	7	2	5	Ó		15.3	26	8.2	8.2
	-	-	12	-	1	8	3	3	5	3		1	0		15.3	26	8.2	8.3
63	D	┼	14	3	1,	6	6	5	-		6	5	0	·	15.2	26	8.1	8.5
64	1	 	+	 -	 '	1	1-	╀——	13	-	├	+	0	 	15.2	26	8.2	8.3
65	E	1	14	9	11	4_	7	4	2	5	2	2	U	<u> </u>	13.2	120	0.2	٣.



			NUH	BER	OF	AH	PHII	200	S EI	HER	GED		NUMBER		WATER	CHEHI	STRY AT	10 D
LAB NO.	REP.		FRO	H S	EDI	HEN	TS	AT	DAY	s 0	-10		ALIVE AT 10	FAILING TO	TEMP	SAL	0.0.	рН
NU.		0	1	2	3	4	5	6	7	8	9	10	DAYS	REBURROW	(°C)	(ppt)	(mg/L)	
67	A		13	15	15	8	13	8	9	5	6	4	0		15.3	26	8.1	7.2
68	В		12	_	10	_	8	9	5	4	4	5	0		15.3	26	8.2	7.7
69	C	-	14		<u> </u>	_	10	7	7	5	7	3	0		15.4	26	*6.9	7.2
70	0		6	8	1	1	4	3	8	5	Γ	2	0		15.2	26	8.0	7.7
71	Ε	T	8	13	8	6	10	2	10	8	Ī	5	0		15.3	26	8.2	7.4

* aeration stopped overnight

SAMPLE	CONTROL	

							PHI						NUMBER		WATER	CHEHI	STRY AT	10 0
LAB .NO.	REP.	-	FRO	H Ş	EDI	HEN	TS		DAY		_	,	ALIVE AT 10 DAYS	FAILING TO REBURROW	TEMP	SAL (ppt)	D.O. (mg/L)	рН
		0	1	2	3	4	13	6	1	8	13	10	UAIS	KEBUKKU	1, 3,			
73	A				1	2	2_	1.	1	1_	1	2	18		15.1	26	8.3	8.3
74	В				2		П	1	2	2	2	2	18		15.0	26	8.3	8.3
75	С		2	3	3	3	3	3	3	3	3	3	17		15.0	26	8.4	8.2
76	D			<u> </u>		1	2	1	2	2	1	1	17		15.0	26	8.4	8.2
77	Ε			\vdash	\vdash								20		14.4	26	8.4	8.3

SAMPLE _____

		- 1	KUK	BER	OF	АН	PHI	POD	S E	MER	GED		NUMBER		WATER	CHEMI	STRY AT	10 D
LAB NO.	REP.		FRO	H Ş	EDI	HEN	TS	AT	DAY:	s 0	-10		ALIVE AT 10	FAILING TO	TEMP	SAL	0.0.	рН
NO		0	1	2	3	4	5	6	7	8	9	10	DAYS	REBURROW	(°C)	(ppt)	(mg/L)	
	Α									_				ļ				
	8								<u> </u>				<u></u>					
	С											<u> </u>						
	0														ļ	ļ		
	Ε			П									<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	

SAMPLE _____

			NUM	BER	0F	AH	PHI	POD	SΕ	MER	GED		NUMBER		WATER	CHEHI	STRY AT	10 D
LAB	REP.		FRO	H Ş	EDI	HEN	TS .	AT_	DAY	\$ 0	-10)	ALIVE AT 10	FAILING	TEMP	SAL	D.O.	рН
ΝΟ.		0	1	2	3	4	5	6	7	8	9	10	DAYS	REBURROW	(°C)	(ppt)	(mg/L)	
	Α										_	<u> </u>		ļ				
	В										_	_		ļ	 	ļ		
	С			L				L	L	_	<u> </u>	↓_		ļ	<u> </u>	ļ		
	0						_		ļ_	_	<u> </u>	1_		<u> </u>	 			
	Ε					<u>L</u>		<u>L</u>		Ļ	_	丄	<u> </u>	<u>.l</u>	<u> </u>	<u> </u>	<u> </u>	L





AMPHIPOD BIOASSAYS

					HC HC
•			(+00/ \Line 100	DISSOLVED OXYGEN (Mg/L)	١
		TEMPERATURE (°C)	SALINI I LPP'S	+	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
- A8	SAMPLE			_	
3	-	013 2 3 4 5 6 7 8 9 10	10 0 1 2 3 4 5 6 7 0 0 1	,	6. 10. 16. 18.1 8.3 8.4 8.1 8.1 8.4
5		+	1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1	185125 180 81 61 61 80 80 82 83 4.9	
	1-11	13.0 16.415.6 16.0 148 15.6 14.5 16.3 15.6 15.4		\perp	8 2 B 4 B 4 B 3 B 5 B 5 B 5 B 5 B 5 B 1
9	- 4			8.4 1.1 8.0 8.0 8.0 6.4 5.4 0.7 0.4	- 1
!	0.17	13.0 16.2 15.4 15.7 14.5 15.1 14.0 16.1 11.55 15.1	2 2 2 2 2 2 2 2 2 2 2 2	1	1 121 123 12 1 23 5 1 8 3 1 8 1 5 1 5 5 5
7	2 2 2		12 12 12 12 12 12 12 12 12 12	124 17, 1 80 8.0 8.0 18.0 18.0 18.7 18.3 18.1	
٩	400	15 2 15 3 15 3 15 3 15 3 15 3 15 3 15 3	87 87 87 87 97 92 92	1	1 0. 6. 12. 12. 12. 12. 12. 12. 12. 12. 12. 12
20	2.5			19,515 50 50 6.0 8.0 8.0 8 1 8 1 51	_
		13.6 1.5 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1.	26 26 26 26 28 28 28 38 38		
74	PR-50			1.5 1.8 10.8 10.0 18.0 18.4 18.4 18.4 18.4 18.4	
		25125115 112 5115 5115 5	30 m 20 12 15 31 21 21 92 92 92		L
30	D-M-0	13.010.113.6 6.0 11.0 10.0 10.0 11	١	C 2 1 2 C 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	
	6	4.5.4 15.4 15.4 14.5 115.3 14.3 14.0 15.5 15.4	27 17 11 11 21 32 37 97 42		10.10.10.10.10.10.10.10.10.10.10.10.10.1
36	PM-S		2/1 /2 2/2 22 22 24	36 7.6 8.0 8.0 8.0 8.1 8.0 8.4 8.4 8.4 S.	
41.	EC - CAST	13.0/18/15/2/15.6/14.8/15.3/14 4/5.9/15/6/15:3	20 20 20 20 20 20 20 20 20 20 20 20 20 2		
_	١				
•	4	TEMPERANCE ADJUSTMENT	Keduited.		
	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1				

DAILY WATER CHEMISTRY MONITORING

E.V.S. CONSULTANTS



E.V.S. CONSULTANTS AMPHIPOD BIOASSAYS

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MONITORING	
CHEMISTRY A	
WATER (
DAILY	

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13.0 16.4154150 14.5153 14.3 16.0 15.515.4 130/165/154 15.6/13.4/15.4/16.2/155/6.4 131/61/154/0.3/25/155/143/16.0/155/94 4.

27 27 23

13.418.0 15.5 16.0 15.4 15.0 11.1 16.0 15,1 18.3

CONTROL

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13. 18. 15. 6. 13. 6. 13. 6. 14. 6. 6. 6. 6. 6. 6. 6. 134 16.4135 15.9 15.4 13 41.3 16.1 15.6 15.1

PA-D

7 3

5.1 29 8.3 646.3 6.3 6.4 6.3 6.3 6.1

51 82 62 46. 16.3 6.3 6.1 6. 24 3 5.7

8.2 8.2 8.6 8. 8.3 8.4 8.4 6.3 8.1 8.4 3.2 8.1 8.4 8.4 6.3 8.3 8.4 8.5 8.4 8.8

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SAMPLE 1.0.

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アニー PA-0

SALINITY (ppt)

DISSOLVED OXYGEN (mg/L)

64 7.8 8.18.1 80 8.0 8.4 8.7 8.1 82 5. 12. 12. 16. 15. 0 8. 0 16. 0 18. 4 10. 0

45 7.7 80 80 80 0 10 1 8 2 81 82 8.3 7.5 5.0 8.0 8.0 8.1 8.1 8.1 8.1