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Sediment Bioassays with Oyster Larvae

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The oyster (Crassostrea gigas) larvae bioassay technique is described in Standard Methods (APHA 1980) as a rapid and reliable indicator of environmental quality. During the first 48 h of embryonic development, fertilized oyster eggs normally develop into free-swimming, fully shelled veliger larvae. Failure of the eggs to survive or the proportion of larvae developing in an abnormal manner, is used as an indicator of polluted conditions.

Numerous authors have used this technique to evaluate environmental contaminant effects (e.g. WOELKE 1967, 1972; BOURNE et al. 1981; COGLIANESE 1982). However, tests with naturally-occurring sediments are rare, sediment testing methodology is not standardized, and the results to date have been highly variable. In the present paper we present a simple methodology for undertaking sediment bioassays with oyster larvae, and present data from a recent study to prove the utility of this method.

MATERIALS AND METHODS

Test sediments were collected from a total of 22 stations in Puget Sound, Washington, generally from areas with high levels of chemical contamination and which showed evidence of toxicity in previous sublethal and mutagenic response tests (CHAPMAN et al. 1983a). Station location maps are provided by CHAPMAN et al. (1982, 1983a).

Adult Pacific oysters were thermally conditioned then spawned following procedures described in Standards Methods (APHA 1980). Fertilized eggs were washed through a Nytex screen (0.25 mm) to remove excess gonadal tissue, and were then suspended in 2.5 L of filtered, UV-treated seawater (20°C, 25 ppt salinity). When microscopic examination revealed the formation of polar bodies, egg density was determined from triplicate counts of the number of eggs in a 1 ml sample of a 1:99 dilution of homogeneous egg suspension.

Sediment bioassays were conducted by adding 15 g (wet weight) of each sediment to clean, acid-rinsed one litre Nalgene polyethylene bottles.

The volume in each bottle was then brought up to 750 ml with filtered, UV-treated seawater to make a final concentration in all test containers of 20 g (wet weight) of sediment per litre of seawater. Two controls were prepared and run concurrently. One control contained the same concentration of clean sediment, the other contained clean seawater. All containers were run in duplicate (22 sediments + 2 controls x 2 = 48 containers).

The sediments were resuspended by sealing the bottles and rotating them all together at 10 rpm for 3 h, following which period each container was innoculated with some 28,000 developing oyster embryos to given an approximate concentration of 35/ml. The suspended sediments were then allowed to settle. The innoculated cultures were covered with paper towelling and air-incubated for 48 h at 20 $\pm$ 1°C.

After 48 h, the contents of each container were carefully poured through a Nytex mesh screen (0.042 mm) without disturbing the settled sediment, thereby retaining and concentrating the surviving oyster larvae (larvae caught in the sediments were invariably dead). The concentrated larvae were then washed into a 100 ml graduated cylinder, quantitatively transferred to screw-cap glass vials with an automatic pipette, and preserved with 3% neutral formalin. Preserved samples (equal in volume to that containing 300-400 larvae in controls) were placed in Sedgewick-Rafter cells and examined at 100X magnification.

Normal and abnormal larvae were enumerated to determine percent survival and percent abnormalities. All larvae that failed to transform to the fully shelled, hinged, "D" shaped veliger were considered abnormal.

Salinity, dissolved oxygen and pH were initially adjusted in each container to 25 ppt, 8.0 mg/l and 8.0, respectively. These parameters were measured for each container at the termination of the bioassay.

RESULTS AND DISCUSSION

Bioassay results are summarized in Table I. Salinity, pH and dissolved oxygen values remained at acceptable levels in all cultures at termination. Detailed data are provided in CHAPMAN et al. (1983a).

Control cultures showed extremely low percentages of abnormal larvae (1.1-1.6%) well below the 3% abnormality rate suggested by WOELKE (1972) as acceptable for oyster larvae bioassay controls. Sediment samples gave dramatic differences in responses ranging from extremely toxic to non-toxic. Station 52 was the most toxic; no live larvae were

Table 1 Oyster Larvae Bioassay Data for Puget Sound Sediments

Geographic Location	Station[a]	Replicate	Total Larvae	Normal Larvae Total	Normal Larvae Percent	Abnormal Larvae Total	Abnormal Larvae Percent	Mean Values: Number of Larvae	Mean Values: Percent Abnormal	Mean Values: Percent Relative Survival[b]
ELLIOTT BAY										
47°37.9',122°24'	2	A	172	162	94	10	6	286	3	78
		B	400	391	98	9	2			
47°37.2',122°21.8'	4	A	51	14	27	37	73	107	30	30
		B	164	136	83	28	17			
47°36.1',122°20.5'	12	A	376	368	98	8	2	350	2	96
		B	324	315	97	9	3			
47°35.2',122°22.3'	15	A	408	403	99	5	1	322	6	88
		B	236	200	85	36	15			
47°34.2',122°25.1'	17	A	350	340	97	10	3	351	2	96
		B	352	345	98	7	2			
DUWAMISH WATERWAY										
47°34.5',122°21.5'	21	A	7	3	43	4	57	9	78	2.5
		B	11	1	9	10	91			
47°34.7',122°20.6'	26	A	23	8	35	15	65	20	50	6
		B	17	12	71	5	29			
47°34',122°20.8'	29	A	86	12	14	74	86	47	86	13
		B	8	1	12	7	88			
47°31.8',122°18.8'	37	A	32	20	62	12	38	26	31	7
		B	20	16	80	4	20			
COMMENCEMENT BAY AND WATERWAYS										
47°16.9',122°24.3'	42	A	13	1	8	12	92	22	91	6
		B	32	3	9	29	91			
47°16.4',122°22.7'	47	A	32	7	22	25	78	46	42	13
		B	60	35	77	14	23			
47°16.3',122°22.5'	49	A	64	44	69	20	31	84	24	23
		B	104	83	80	21	20			
47°15.9',122°21.8'	52	A	0	0	-	0	-	0	-	0
		B	0	0	-	0	-			

Table I (continued)

Geographic Location	Station[a]	Replicate	Total Larvae	Normal Larvae Total	Normal Larvae Percent	Abnormal Larvae Total	Abnormal Larvae Percent	Mean Values: Number of Larvae	Mean Values: Percent Abnormal	Mean Values: Percent Relative Survival[b]
47°16',122°23.5'	57	A	110	67	70	33	30	88	32	24
		B	67	45	67	22	33			
47°15.4',122°22.8'	61	A	81	56	69	25	31	166	22	46
		B	252	204	81	48	19			
47°16.2',122°25'	63	A	97	82	85	15	15	105	15	29
		B	112	96	86	16	14			
47°15.3',122°26.2'	67	A	351	333	95	18	5	272	4	75
		B	194	189	97	5	3			
47°15.1',122°25.9'	70	A	8	0	0	8	100	8	94	2
		B	9	1	11	8	89			
47°16.7',122°27.5'	71	A	162	153	94	9	6	200	13	55
		B	238	194	82	44	18			
SINCLAIR INLET										
47°33.1',122°38.4'	82	A	79	38	48	41	52	78	45	22
		B	78	49	63	29	37			
47°33.3',122°37.7'	84	A	97	80	82	17	18	74	18	20
		B	50	40	80	10	20			
PORT MADISON										
47°43.4',122°31.3'	91	A	173	165	95	8	5	167	4	46
		B	161	156	97	5	3			
	Sediment Control	A	497	488	98	9	2	487	2	134
		B	477	470	99	7	1			
	Seawater Control	A	355	351	99	4	1	364	1	100
		B	374	370	99	4	1			
				Combined Sediment and Seawater Controls				426 (n=4;S.D.= 72; $\bar{X} \pm 2$S.D.= 282-570)		

a. Station numbers from Chapman et al. (1982, 1983a).
b. In terms of the seawater control which, following standard (Cummins, 1973, 1974; A.P.H.A., 1980) procedures, is assigned a survival value of 100%.

found after 48 h. The percentage of abnormal larvae exhibited for Stations 4, 21, 26, 19, 37, 42, 47, 49, 57, 61, 70 and 82 exceeded the single sample water quality criterion of 20% larval abnormality proposed by WOELKE (1972). The following additional stations exceeded WOELKE's (1972) proposed multiple sample quality criterion of 5% larval abnormality: Stations 15, 63, 71 and 84. Stations 2, 12, 17, 67 and 91 all had less than 5% larval abnormalities.

The survival values generally agreed with the data on abnormalities. For the 12 stations with greater than 20% larval abnormalities, mean relative survival was low (range 2-46%). Of the four stations with between 5 and 20% larval abnormalities, only Station 15 had a mean survival rate greater than 55% (88%) which, coupled with a low (6%) rate of larval abnormalities, indicated low toxicity at this station. The five stations with less than 5% abnormalities had generally good survival rates (75-90%) with the exception of Station 91 which had a mean relative survival rate of 46%, indicating high acute lethality at this station.

The data thus indicated that 13 of the 22 stations tested were highly toxic to oyster larvae, 5 stations were moderately toxic, and 4 were non-toxic. The development of larval abnormalities in combination with mortality served as an indication of chemical toxicity in tested sediments.

Previous uses of oyster larvae bioassays with sediment samples have involved variable sediment concentrations (e.g. 0.5-10 g/L wet weight; SCHINK et al. 1974, CUMMINS et al. 1976) and different methodologies including rotating developing eggs together with test sediments (CARDWELL et al. 1977). As a result, it is difficult to compare studies or even, in some cases, to separate the effects of physical abrasion from actual toxicity. The methodology described here uses higher sediment concentrations than most other studies without problems from physical abrasion, has been shown to work, and provides comparable data to other toxicity tests.

Sediments from many of these 22 stations have been tested for toxicity using sensitive amphipod bioassays (SWARTZ et al. 1982, OTT et al. 1983). Sediments from all of these stations have been tested for sublethal and genotoxic effects (CHAPMAN et al. 1982). Subsamples from the actual sediments tested with oyster larvae have also been used in reproductive impairment tests including effects on polychaete life-cycles, surf smelt development stages, and fish cells (CHAPMAN et al. 1983a). Synthesis of this data indicates that the oyster larvae bioassays provided comparable toxicity data to other more expensive and specialized tests (CHAPMAN et al. 1983b). Thus, we recommend the use of the oyster larvae bioassay as described herein for future sediment bioassays.

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