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Effects of Puget Sound Sediments and Their Elutriates on the Life Cycle of *Capitella capitata*

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The marine polychaete worm Capitella capitata has been used in a variety of studies examining the environmental effects of toxicants (i.e. Reish, 1978, 1980; Reish and Carr, 1978) and can be cultured through a full generation within a few weeks (APHA, 1980). Previous life-cycle studies with C. capitata have concentrated on laboratory tests with specific chemicals (Reish, 1980) rather than the complex chemical mixtures found in contaminated marine sediments.

In the present study, we examined the effects of contaminated marine sediments from Puget Sound, Washington, on a complete life-cycle of C. capitata raised from the trochophore larvae stage with exposure to both sediment elutriates and whole sediments. We examined survival at all life-cycle stages, abnormalities, growth rate, and time from trochophore larvae to reproduction. The results of this study provide information on the toxic effects of the tested sediments and also provide comparative data regarding sediment bioassays conducted with whole sediments and with elutriates prepared from those sediments.

MATERIALS AND METHODS

Sediment samples were collected from 22 stations in Puget Sound using a 0.1 m^2 van Veen grab modified with top screens and rubber flaps to minimize surface sediment disturbance. Station location maps are provided by Chapman et al. (1982, 1983a). Five grabs were collected and pooled at each station. All sediment samples were frozen within 24 h of collection, stored with dry ice during transport, and kept frozen until tested.

Trochophore larvae of the marine polychaete Capitella capitata were purchased as isogenic strains from Dr. D. Reish (California State University, Long Beach). The larvae were received with 24 h of hatching and were previously acclimated to the test conditions (20°C, 35 ppt salinity). Testing was initiated within 4 h of receipt.

Sediment elutriates were prepared by placing 10 g (wet weight) of frozen sediment in a 1 litre acid-cleaned glass jar, and adding 500 ml of clean seawater (35 ppt salinity) to make a final concentration of 20 g (wet weight) of sediment per litre of seawater. The jars were shaken vigorously and the suspended sediments allowed to settle for 3 h. Fifty ml of elutriate were then withdrawn by pipette from the upper water layer and added directly to acid-washed 20 x 100 mm glass petri dishes. Whole sediment tests were

conducted by adding a 2 cm layer of sediment to the bottom of each petri dish following elutriate water addition. The use of these small petri dishes allowed for periodic observation of the worms with minimal disturbance.

Both elutriate and whole sediment testing was completed without replication. A total of four controls (2 seawater controls, 2 controls with clean sediment) were used in elutriate tests, and one control (clean sediment) was used in the whole sediment tests which were run concurrently.

Twenty free-swimming trochophore larvae were randomly pipetted into each dish along with a food suspension consisting of finely ground Tetramin and Enteromorpha in seawater. The dishes were then covered and kept at 20 ± 1°C under a 12 h light/dark regime in a controlled environment room.

Elutriate cultures were fed twice weekly ad libidum. The amount of food added was determined by the amount remaining from the previous feeding and was increased as the worms grew. Feeding schedules for sediment cultures followed those of the elutriate containers.

Elutriate solutions were changed in the cultures on a regular basis and replaced with fresh elutriate made with recently thawed sediment. The first change was made at 5 d after initiation when most of the larvae had settled; changes were then made at 3 and 4 d intervals for the duration of testing. Solutions were not changed in the sediment cultures.

Estimates of mortality were made 2 d after initiation of testing and prior to every elutriate change by placing each culture dish on a numbered 1 cm square grid and examining the bottom systematically with a dissection microscope. Direct observations included estimates of larvae and/or juveniles present, observations of any abnormalities and, during early growth stages, measurements of the lengths of individual worms. During late growth stages, examination of the dishes concentrated on egg production and laying. When mature trochophore larvae were noted in the female mucus tube, the tube was gently dissected under magnification for counting of eggs and larvae, and observation of abnormalities. Observations in the whole sediment dishes were made after termination of the experiment when worms were sorted from the sediments under a dissecting microscope.

Elutriate tests were terminated after 50 d. Sediment tests were terminated after 35 d. Percent survival, mean length, and the number of females bearing eggs were determined in these tests. Statistical comparisons of the data were made by means of a t-test.

RESULTS AND DISCUSSION

The results of the sediment elutriate tests are provided in Table 1, and those for whole sediments in Table 2. Evidence of sediment toxicity observed in both tests is provided in Table 3. A detailed account of experimental observations is provided by Chapman et al. (1983a).

Previous studies attempting to determine contaminant-related effects on Capitella capitata in Puget Sound, have examined natural populations for gross and microscopic lesions with negative results (Malins et al., 1980). In contrast, data from the present study indicated that sediments from 15 of the 22 stations tested were toxic to C. capitata during some stage of its life-cycle. No toxicity was attributable to Station 91 from Port Madison, the reference area.

Two types of larval mortality were distinguished during sediment elutriate testing: death before and death during metamorphosis. All stations including controls showed trochophore mortality, however only Stations 4, 17, 37 and 47 had significantly higher mortalities than controls.

Previous observations that C. capitata larvae are more sensitive than adults (Reish, 1980) are confirmed by the present test results. Significant mortalities only occurred prior to completion of metamorphosis. Induction of abnormal larval metamorphosis, resulting in death, was noted for seven sediment elutriate samples, and has not been previously reported.

Growth of juvenile and adult worms was monitored twice during elutriate tests. Prior to sexual maturation (up to Day 16), only one test sediment (Station 57) had a significantly different growth rate than controls; the rate was almost twice as high in this sediment. During the period of active egg development in naturing female worms (Day 29), length measurements indicated no significant differences between worms from control and test sediments.

Mature females with developing internal egg masses were observed in all sediment elutriates. However, due to the growth of bacteria in test cultures and associated worm mortalities, a complete comparison of timing to egg laying could not be made.

Mean lengths were measured once for the sediment tests, at termination. Worms exposed to sediments from eight stations had mean lengths significantly less than controls indicating slower growth: Stations 26, 37, 42, 49, 52, 67, 70, and 82. Of the whole sediment samples from which more than one worm was recovered at termination, only Stations 52, 70 and 82 did not contain females bearing eggs.

The growth of C. capitata in whole sediments was roughly half that observed in the sediment elutriate samples (mean = 0.16 $\pm$ 0.04 mm/d for sediments; 0.31 $\pm$ 0.05 mm/d for elutriates). Survival of C. capitata was generally lower in whole sediments (range 0 - 80%) than in elutriates (range 40 - 90%). Females recovered from the whole sediment samples were sexually mature at a much smaller size than females exposed to sediment elutriates. The reason(s) for these differences are unknown.

The present study results indicated that tested sediments can cause death, affect growth and reproduction. All of these effects have been previously demonstrated in laboratory tests with individual chemical contaminants such as metals (Reish, 1980) but have not been previously demonstrated for field-collected sediments. A previously reported contaminant effect, induction of

Table 1 Sediment Elutriate Toxicity to Capitella capitata

Geographic Location	Station[a]	Larval Mortality: Numbers During Metamorphosis	Larval Mortality: Total Numbers (%)	Growth and Development: Growth Rate (mm/d) to Day 16	Growth and Development: Growth Rate (mm/d) to Day 29	Growth and Development: Days to First Observed Eggs Produced	Overall Survival to Day 26: Total	Overall Survival to Day 26: Percent
ELLIOTT BAY								
47°37.9',122°24'	2	0	3 (15)	0.32	0.31	19	16	80
47°37.2',122°21.8'	4	0	10* (50)	0.28	0.27	26	10	50
47°36.1',122°20.5'	12	0	4 (20)	0.28	0.27	23	15	75
47°35.2',122°22.3'	15	0	9 (45)	0.32	0.27	26	11	55
47°34.2',122°25.1'	17	2	10* (50)	0.29	0.35	26	8	40
DUWAMISH WATERWAY								
47°34.5',122°21.5'	21	1	4 (20)	0.29	0.33	23	15	75
47°34.7',122°20.6'	26	4	5 (25)	0.33	0.29	19	15	75
47°34',122°20.8'	29	0	4 (20)	0.34	0.33	19	15	75
47°31.8',122°18.8'	37	0	11* (55)	0.17	0.25	29	8	40
COMMENCEMENT BAY AND WATERWAYS								
47°16.9',122°24.3'	42[b]	2	5 (26)[b]	0.34	0.37	26	13	68[b]
47°16.4',122°22.7'	47	2	10* (50)	0.25	0.28	26	10	50
47°16.3',122°22.5	49	0	6 (30)	0.36	0.32	26	14	70
47°15.9',122°21.8'	52	0	9 (45)	0.25	0.26	34	11	55
47°16',122°23.5'	57[b]	0	4 (21)[b]	0.39*	0.33	19	15	79[b]
47°15.4',122°22.8'	61	0	6 (30)	0.31		19	14	70
47°16.2',122°25'	63	0	2 (10)	0.34	0.29	23	18	90
47°15.3',122°26.2'	67	0	4 (20)	0.28	0.33	19	12	60
47°15.1',122°25.9'	70	2	6 (30)	0.35	0.40	23	13	65

Geographic Location	Station[a]	Larval Mortality: Numbers During Metamorphosis	Larval Mortality: Total Numbers (%)	Growth and Development: Growth Rate (mm/d) to Day 16	Growth and Development: Growth Rate (mm/d) to Day 29	Growth and Development: Days to First Observed Eggs Produced	Overall Survival to Day 26: Total	Overall Survival to Day 26: Percent
47°16.7',122°27.5'	71	0	8 (40)	0.28	0.33	19	12	60
SINCLAIR INLET								
47°33.1',122°38.4'	82	0	6 (30)	0.29	0.33	19	14	70
47°33.3',122°37.7'	84	2	6 (30)	0.32		19	13	65
PORT MADISON								
47°43.4',122°31.3'	91	0	2 (10)	0.33	0.32	19	17	85
	Sediment Control A	0	4 (20)	0.23	0.34	29	14	70
	Sediment Control B	0	3 (15)	0.26	0.23	34	16	80
	Seawater Control A	0	5 (25)	0.17	0.26	26	14	70
	Seawater Control B	0	6 (30)	0.25	0.30	29	13	65
	Combined Sediment and Seawater Control							
	Mean	0	4.5 (22.5)	0.23	0.28	29.5	14	71
	S.D.	0	(±1.3) (±6.5)	(±0.04)	(±0.05)	(±3.3)	(±1)	(±6)

a. Station numbers from Chapman et al. (1982, 1983a).

b. n=19 not 20; one trochophore lost during elutriate change.

* Significantly different (P=0.05) from controls.

N.D. = no data.

TABLE 2 Whole Sediment Toxicity to *Capitella capitata*

Station	Survival[a] Total	%	Mean Length (mm) ± S.D.	Number of Females with Eggs
2	9	45	8.3 ± 3.1	2
4	12	60	6.1 ± 1.1	2
12	11	55	5.6 ± 2.0	3
15	10	50	6.5 ± 2.0	3
17	11	55	8.1 ± 1.4	3
21	1[b]	5	-	-
26	10	50	5.1 ± 2.2*	2
29	15	75	6.0 ± 2.3	2
37	11	55	5.4 ± 1.3	2
42	14	70	5.6 ± 1.5*	2
47	6	30	6.3 ± 1.8	2
49	9	45	4.1 ± 1.6*	1
52	5	25	3.8 ± 1.6*	0
57	7	35	5.6 ± 2.1	1
61	12	60	5.5 ± 2.3	1
63	0	0	-	-
67	11	55	4.1 ± 1.8*	1
70	7	35	4.3 ± 1.1	0
71	11	55	7.1 ± 2.3	2
82	12	60	4.0 ± 0.7*	0
84	12	60	7.3 ± 2.3	3
91	11	55	6.6 ± 1.6	3
Control	16	80	7.1 ± 2.1	5

a. Numbers found alive on day 35; 20 trochophores originally added to each test chamber.
b. Two live fragments (an anterior and posterior end) were found; the anterior portion subsequently survived.
* Significantly different (P=0.05) from controls.

Table 3 Summary of Deleterious Effects Observed in Sediment and Elutriate Tests with Capitella capitata

Station	Elutriates			Sediments	
	Sig. Mortality	Abnormal Larvae	No Eggs Produced After 35 d	Survival[a] < 40%	Sig. Slower Growth[b]
2					
4	x				
12					
15					
17	x	x			
21		x	-c	x	-c
26		x			x
29					
37	x				x
42		x			x
47	x	x		x	
49					x
52			x	x	x
57				x	
61					
63			-c	x	-c
67					x
70		x	x	x	x
71					
82			x		x
84		x			
91					

a. Survival half or less that of controls (80%).
b. Mean lengths compared to controls.
c. Survival was 5% or less.

abnormal trochophore larvae during incubation, was rare in the present study, which may be due to the fact that significant numbers of abnormal trochophores only appear in the second generation following continuous exposure to specific toxicants (Reish et al., 1974). The present study was terminated after production of the first generation of trochophores, hence the induction of abnormal larvae in later generations remains a possibility.

Despite high initial mortalities in some samples, C. capitata was able to complete its life-cycle in all sediments. This result is somewhat surprising as the sediments used for testing were selected based on previous evidence of toxicity, are among the most chemically contaminated in Puget Sound (Chapman et al., 1983b), and subsamples of these sediments have been shown to be extremely toxic to oyster larvae (Chapman and Morgan, 1983).

The ability of C. capitata to survive and reproduce in contaminated sediments may be partly due to this species' opportunistic nature, which includes the ability to increase populations rapidly despite high larval mortalities (Grassle and Grassle, 1976). It is also possible that C. capitata living in contaminated areas of Puget Sound have developed an increased tolerance to toxicants present in these areas. For instance, Lee and Singer (1980) have shown that C. capitata exposed to petroleum and its components developed increased resistance to toxic effects through the third generation by the induction of increased activity in a mixed-function oxidase (MFO) system capable of detoxification.

The present study provides data on specific areas of Puget Sound showing toxicity, and also provides a comparison of elutriate and whole sediment toxicity tests (Table 3). Fourteen stations showed comparable results in both tests, giving a 64% level of agreement. Two stations showed toxicity only in elutriate tests (9%) and six stations showed toxicity only in sediment tests (27%). Thus although the elutriate and sediment test results were roughly comparable, additional information was obtained by using both tests in preference to one or the other. This conclusion has implications for future sediment bioassays (for example, related to ocean dumping): the utility of elutriate testing is confirmed, but the use of both whole sediment (solid-phase) and elutriate tests is suggested for specific studies where detailed information on toxicity is required.

The methodology used in the present study provided useful data for determining the relative toxicity and types of effects in tested sediments, and the results fit well with data from tests with other species (Chapman et al., 1983a,b). Life-cycle tests as described herein are both simple and easy to conduct requiring little glassware, a minimum of manpower and equipment, yet they provide extremely sophisticated information regarding toxicity. As such, these tests should be considered for implementation by researchers involved in marine pollution monitoring and assessment using the comprehensive approach described by Chapman and Long (1983).

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