SEDIMENT BIOASSAYS USING Bathyporeia sarsi AND Bathyporeia pilosa

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Introduction

It has been well established that many toxic chemical contaminants enter natural water resources and accumulate to high levels in bottom sediments. A variety of marine sediment bioassays have been developed to test the toxicity of these sediments on benthos locations. These range from short term acute tests involving the effects of individual contaminants on single species to complex long term determinations of the effects of chemical mixtures on the entire benthic ecosystem (Lamberson and Swartz , 1988). This report describes a short term acute sediment bioassay involving the infaunal amphipod Bathyporeia sarsi and Bathyporeia pilosa in two different tests. These two species are quite similar but from a practical point of view, B. pilosa are much easier to collect than B. sarsi , therefore a comparison of sensitivity between the two species is made with a contaminated sediment gradient in the first test, while the second test determines the effects of various sediments from different locations on B. pilosa .

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Bathyporeia species

Bathyporeia, a genus from the amphipod family Haustoriidae, can be characterized by the habit of burrowing into the soil of the sea floor (Watkin, 1939). These marine animals are commonly found in the North Sea off the Dutch coast. The species of quantitative importance in the intertidal areas of the Delta region are Bathyporeia pilosa and Bathyporeia sarsi (Vader ,1965). These two species differ anatomically mostly in the shape of their frontal antenna of the head, B.pilosa has a more rounded shape to the antenna while B.sarsi has a more squarish shape to the antenna. The swimming and burrowing habits of both species of the genus Bathyporeia have been shown to be alike (Watkin , 1939). As a result of swimming-like movements currents of water are

drawn in between the appendages and thus over the gills. Burrowing into the sand which is the natural home of the Bathyporeia is the second type of movement characteristic of this genus. It is known that the animals bury in the sand and feed by eating from the surface of sand particles and absorbing the nutrients while defecating indigestible material. Phoxocephalid amphipods are known to be sensitive to sediment contamination particularily since such animals are greatly dependent on the quality of interstitial water and sediment particulates (Swartz ,1984).

Animal Collection

In the first test both B.pilosa and B.sarsi were collected on May 20, 1989 on the small beach south of Ouwerkerk (the Eastern Scheldt). B.sarsi was found most abundantly in the upper 5 cm of the sediment layer close to the low water mark, in water of approx. 20 cm depth at low tide. In the higher littoral zone, B.pilosa was found among the upper 5 cm of the shallow sediment just below the high water mark. In order to obtain the animals , the sediment was sieved over a 3 mm sieve to separate the animals from shells and than over a 1 mm sieve to eliminate sand and organic debris.
For the second test , B.pilosa was collected on June 9, 1989 in Ouwerkerk. The top 5 cm layer of sediment from the high littoral zone was collected and sieved with a 1 mm pore size to recover the animals.

Sediment Processing

To obtain a contaminated sediment gradient for test 1 , clean and contaminated sediment were mixed in various proportions. A series was made with 0%, 10%, 25%, 50% ,100% wetweight percentages of contaminated sediment, the

counterpart being the clean sediment (reference sediment). In the following table the percentages of contaminated and ref. sediments are presented :

0% contaminated sediment / 100% ref. sediment

10% contaminated sediment/ 90% ref. sediment

25% contaminated sediment/ 75% ref. sediment

50% contaminated sediment/ 50% ref. sediment

100% contaminated sediment/ 0% ref. sediment

In test 2, no sediment processing was necessary, sediments from different locations were used in the various test jars.

Bioassay set-up

Test 1

Thirty 1L mason jars were filled with approx. 200 ml of the mixed (contaminated/ref.) sediment; 6 jars of each mixture, 3 replicates for each species. Raw (unfiltered) sea water was filled to the top of the mason jars and 1 day before the animals were introduced the sediment was allowed to settle while the incoming seawater flow was also set-up. After the sediment had settled, 19 animals were introduced in each jar. Unfortunately however, the screens of the mason jars became plugged with excess algae and some animals were lost thru the overflow of water. Hence this caused a variability of probably 2-5 animals in some jars. After this was discovered a system was set-up to have the sea water flow filtered. During the ten day exposure time, the test jars were examined daily. Temperature, oxygen saturation and the condition of the animals were measured: floating and swimming animals, emergent from the sediment or on the surface of the sedi-

ment and the number of surviving animals. Water temperature remained approx. 16°C during the test, oxygen saturation varied from 70% to 150%. After 10 days the exposure jars were emptied and the animals were recovered from the sediments by using a 1 mm sieve. The living Bathyporeia animals were collected and again allowed to rebury in clean sediment. After one hour the number of reburied animals was counted. The unburrowed animals were checked for viability; the living animals which failed to rebury giving an indication of sublethal stress.

Test 2

Thirty 1L mason jars were filled with ±200 ml of sediment from 10 different localities plus 1 reference sediment. Each location was tested in duplicate or triplicate (depending on the amount of sediment available from each location). A flow through system of filtered seawater was set-up and the test jars were all filled with seawater. After one day of allowing the sediment to settle, 20 B.pilosa were introduced to each test jar. Water temperature remained constant at approx. 17°C and oxygen saturation was approx. 80%. After the animals burrowed, air bubbling pipets were installed. During the 10 day exposure time, the test jars were daily examined and measured for temperature, oxygen saturation and the condition of the animals. The same criteria as in bioassay 1 was used to measure the condition of the animals except once the dead animals were noticed, they were removed. This was done to ensure an accurate measurement of the viable animals. After the 10 day test , the animals were recovered and counted but a reburial test was not performed.

Results

Test 1

In the following table the processed results are presented on the response of

B.pilosa and B.sarsi to the different contamination levels. The percentages are the mean of the three replicates.

Table 1	% mortality B.pilosa	% failure to rebury	%mortality B.sarsi	% failure to rebury
0 % contamination	8.9	3.2	46.4	1.6
10% contamination	24.7	3.2	39.4	15.7
25% contamination	38.9	24.2	71.9	8.7
50% contamination	40.5	27.8	85.4	8.7
100%contamination	48.5	34.7	80.1	12.1

Statistical analysis is not applied to this data. Although a clear trend is present, the control group (0% contamination) is quite high and thus statistically unacceptable.

The number of swimming amphipods is indicated in the following table: (numbers are total amphipods per contamination gradient)

Table 2 — No. of swimming animals per day of test

B.pilosa	day 3	day 4	day 5	day 6	day 9
0 %cont.	15	5	6	9	5
10 %cont.	10	8	3	3	4
25 %cont.	8	7	3	3	0
50 %cont.	10	6	8	4	1
100%cont.	21	10	8	9	1

Table 2 continued

B.sarsi	day3	day4	day5	day6	day9
0 %cont.	5	0	1	0	1
10 %cont.	3	3	3	2	5
25 %cont.	1	1	0	2	0
50 %cont.	1	2	1	5	1
100%cont.	8	6	4	6	0

Test 2

In Table 3, the effects of different sediments on B.pilosa are shown. The numbers presented are the mean values of replicate trials.

Table 3

Sediment	No. of Swimming(s) and Emerging(e) Animals per day					
Sample	day3	day4	day5	day6	day7	day 10
1	11s,1e	8s,-	6s,-	4s,1e	4s,1e	3s,3e
2	4s,-	4s,1e	3s,-	3s,1e	4s,1e	2s,2e
3	8s,1e	8s,2e	6s,2e	6s,3e	6s,3e	5s,4e
4	-	-	-	-	-	-
5	7s,-	5s,-	4s,1e	1s,-	3s,1e	3s,1e
8	-	-	-	-	-	-
9	9s,1e	7s,-	5s,1e	4s,1e	5s,1e	2s,1e
10	8s,-	3s,-	1s,1e	3s,2e	3s,1e	3s,4e
12	-	-	-	-	-	-
13	8s,-	3s,1e	3s,-	3s,-	2s,-	2s,-
blank	-	-	-	-	-	-

Table 4 describes the percent mortality and the number of surviving B.pilosa in different sediments. The numbers presented are the mean of replicate series. A statistical analysis with standard deviation and t-test is also included.

Table 4

Sample	% Mortality	Number of Surviving	Standard Deviation	t-test	p-values
1	18.4	16.3	1.16	4.47	0.005<p<=0.01
2	5.0	19.0	1.41	0.775	0.1<p<=0.375
3	16.5	16.7	2.89	4.765	0.05<p<=0.10
4	2.5	19.5	0.71	0.293	p>0.40
5	11.7	17.7	2.08	1.604	0.05<p<=0.10
8	5.0	19.0	1.73	0.632	0.10<p<=0.375
9	10.0	18.0	1.41	1.936	0.05<p<=0.10
10	21.7	15.7	0.58	8.485	p<=0.0005
12	1.7	19.7	2.58	0.0	p>0.40
13	10.0	18.0	1.73	1.581	0.05<p<=0.10
blank	1.7	19.7	0.58	-	-

Figures 1 and 2 illustrate the locations where the various sediments were collected.

Discussion

The results from test 1 indicate that B.sarsi are more sensitive to toxic sediment than B.pilosa. Although statistically, the mortality for the control group (0% contamination) was more than 10%, hence generally considered

unacceptable, a definite pattern is present.

Table 2 indicates another strong trend : B.pilosa appear to spend more time swimming above toxic sediment than B.sarsi and this could be a possible reason why B.pilosa are more resistant to sediment toxicity. If they spend more time swimming they are likely to spend less time burrowed in the sediment , hence have less contact with the toxic contaminants and possibly be less sensitive. However, on the other hand, it is also possible that B. pilosa swim more because they are quite sensitive to toxic sediments. It is difficult to conclude from this experiment which explanation is more probable but we can conclude that B.pilosa do swim more and have a lower mortality rate than B.sarsi. The amphipods were also measured for reburial ability which is supposed to indicate sublethal stress due to toxicity of sediment. However, the data obtained from the reburial test show very little pattern and appear quite random. Yet it is possible to note that B.pilosa spend more time swimming than burrowing in toxic sediment and also have a higher failure to rebury rate than B.sarsi which seem to burrow more and also rebury more in toxic sediment.

Test 2 indicates that the sediments from different locations are not very contaminated since most of the B.pilosa survived the test. However measuring the emergence of the amphipods may indicate some toxicity since few animals emerge from clean sediment under bioassay conditions (Swartz et.al.,1985). A slight trend can be noted from table 3 : B.pilosa appear to swim more in the beginning of the assay while the number of emerging animals increases towards the end of the assay. It also appears from table 4 that sample 10 is the most toxic while sample 12 is the cleanest (% mortality same as control group). However, statistically only samples 1 and 10 have mortality rates which are significantly different from the control. Samples 1 and 10 are located most inland, where the river Rhine is heavily polluted. Thus it is probable that

the mortality could be due to the toxicity of the sediment. A chemical analysis would be necessary to verify this.

The method of removing the dead animals daily in order to estimate the mortality rate was not accurate in this experiment. It is difficult to visibly recognize dead animals, especially once covered in sediment and daily enumeration of dead animals was not successful since at the end of the test, the number of live animals was not the same as that predicted by the daily observations. Thus the best method is to actually sieve the sediment and recover the live Bathyporeia after the 10 day exposure time.

Conclusion

Thus to summarize, data from test 1 indicates that B.pilosa spend more time swimming, have a greater number of animals which fail to rebury, and are " more resistant" to toxic sediment than B.sarsi. Therefore for future research B.sarsi which are quite sensitive to toxic sediment would be useful for bioassay monitoring systems. The reburial test did not give more information on sublethal stress in test 1, hence it was not included in test 2. The data from test 2 is useful since it does indicate some mortality probably due to toxicity. Also tne emergence and swimming behaviors of the Bathyporeia do indicate a small degree of toxic effects in some of the sediments. However, further work including a complete chemical analysis is required before our speculations can be confirmed.

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Figure 1: Locations of sediment samples 1, 2, and 3.

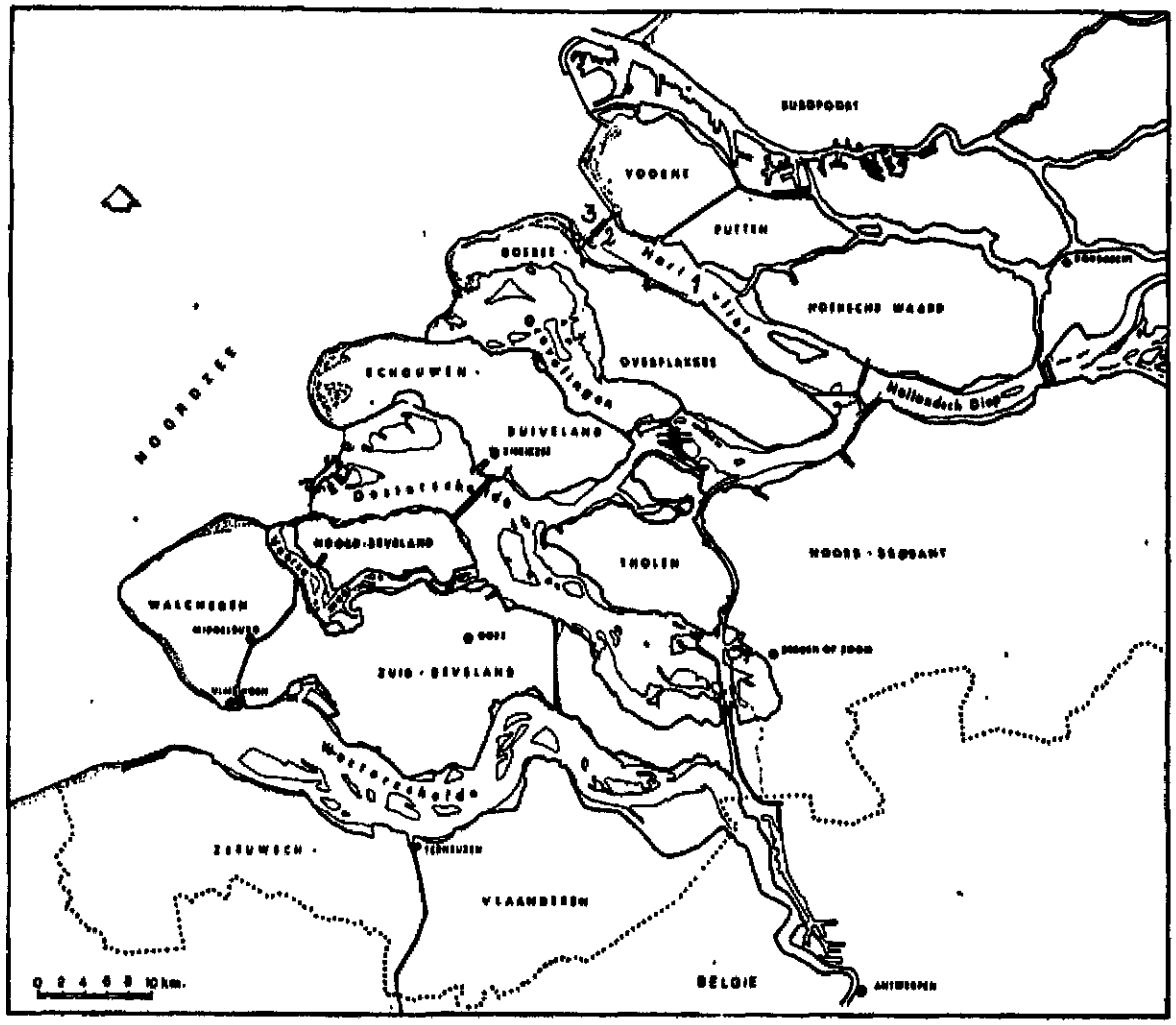

Figure 2: Locations of sediment samples 4, 5, 6, 7, 8, 9, 10, 11, 12, and 13.

Not all samples listed were available for testing.

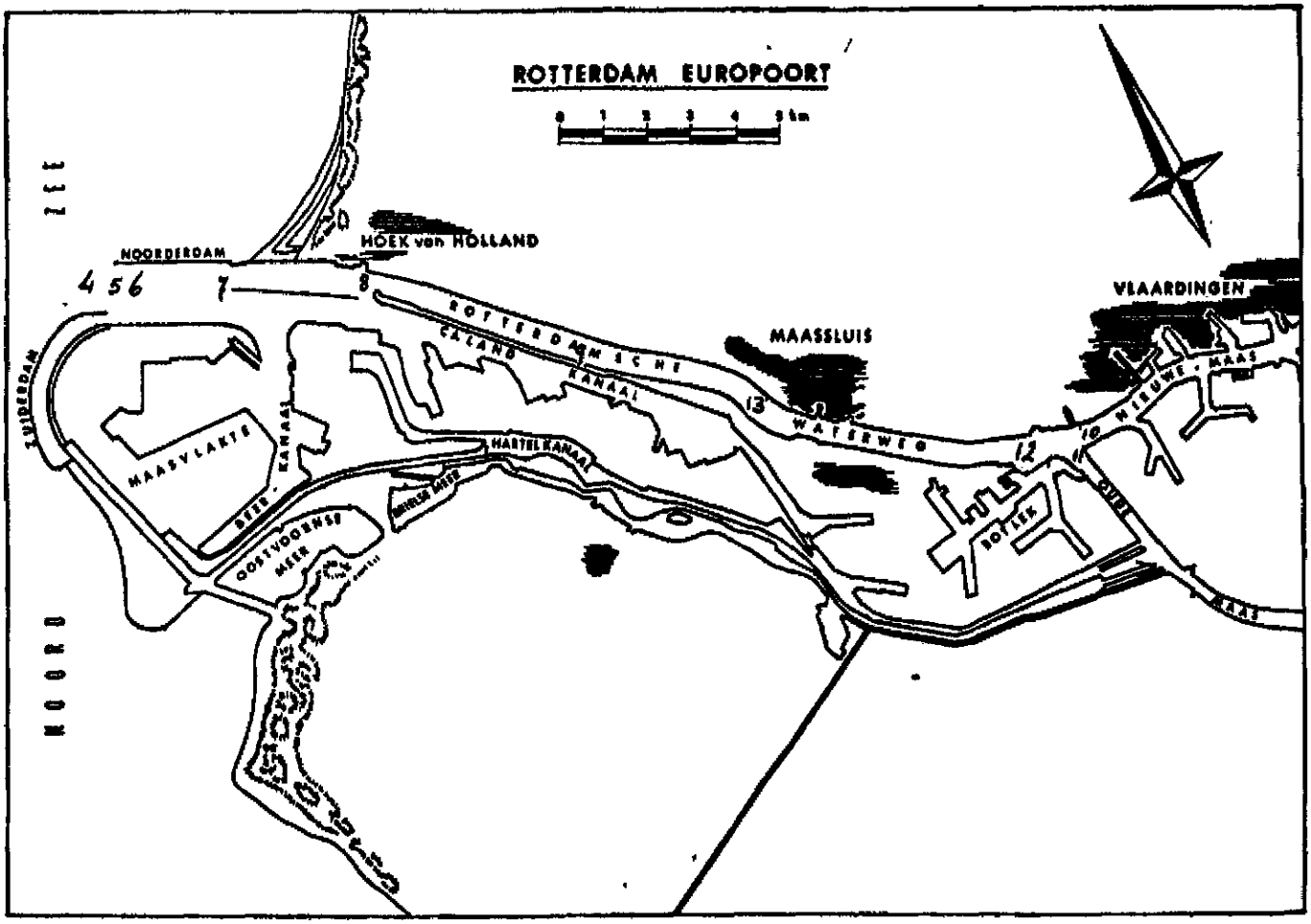

FIGURE 3: SPECIES COMPARISON