POSFORD HASKONING ON BEHALF OF THE TYNE AND WEAR PASSENGER TRANSPORT AUTHORITY

TOXICITY TESTING OF RIVER TYNE SEDIMENT SAMPLES USING THE AMPHIPOD *COROPHIUM VOLUTATOR*

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SUMMARY

A new tunnel crossing for the River Tyne (the New Tyne Crossing) between Jarrow and East Howdon is being sponsored by the Tyne and Wear Passenger Transport Authority (TWPTA). However, before the New Tyne Crossing can be approved it is necessary to carry out an Environmental Impact Assessment (EIA) of the proposals. The assessment will explore the benefits and possible problems brought about by the scheme both during the construction and when it is completed and operational. Once the environmental impacts have been noted any work needed to reduce them can be considered. Measures might include changes to the scheme, additional works, or works to protect the people, wildlife or objects affected.

The installation of the immersed tunnel will require dredging across the River Tyne. After the immersed tunnel has been installed, the remaining trench will be filled and disposal of excess dredged material will be required. The rivers sediments may contain various contaminants and during dredging sediments will be disturbed which may raise water quality issues. Therefore, as part of the environmental impact assessment, Posford Haskoning approached WRc-NSF to carry out toxicity testing on sediment samples taken across the river at the site of the crossing. The samples were to be taken from the surface down to a depth of 3.0 m and testing would be carried out using the estuarine/marine amphipod *Corophium volutator*. The Environment Agency requested that testing was to be carried out in manner which would allow comparisons with the results of a monitoring exercise carried out along the River Tyne in September 1997.

A series of 35 samples were collected by Allied Exploration and Geotechnics (AEG) Limited in the period 15 – 17 August 2001 and were tested with the 10 day *Corophium volutator* lethality test (PARCOM 1994). The test satisfied all the test validity criteria with the mean mortality level of the amphipods in the control sediment being 9.2% (Standard deviation 6.6%) and below the 20% mortality threshold level.

The mortality levels in the test sediments were typically less than 20%, with the exception of four samples BH 501 J5, BH 501 J6, BH 502 J6 and BH 503 J2. The samples which showed greater than a mean mortality level of 20% also showed greater variability between replicates than other samples. In the case of samples BH 502 J6 and BH 503 J2 this was due to one replicate showing high mortalities compared to the other two replicates.

Analysis of variance of the surface sediment sample data (J1 samples) indicated that no locations had statistically significantly higher levels of *C.volutator* mortality than that measured in the control sediment. The level of toxicity measured in the surface samples (J1's) was also consistent the result of 30% mortality (± 10%) recorded for the Howdon sediment samples taken during the September 1997 survey of the River Tyne.

Analysis of all the test sediments showed that there were no statistically significant differences in *C.volutator* mortality based on location or depth of the sample. On this basis the samples collected from the Howdon basin can generally be regarded as not being acutely toxic. However, although these samples showed a low level of toxicity to *Corophium volutator* this does not mean that they do not contain contaminants. Rather it shows that the levels of contaminants present and/or their bioavailability was not sufficient to cause mortality in the test species. It is possible that the level of contamination present would be sufficient to cause more subtle effects on parameters such as growth and reproduction of aquatic organisms.

1. INTRODUCTION

A new tunnel crossing for the River Tyne (the New Tyne Crossing) between Jarrow and East Howdon is being sponsored by the Tyne and Wear Passenger Transport Authority (TWPTA). The new tunnel is being planned to be of immersed tube construction where it crosses the River Tyne. This is relatively new technique pioneered in Holland which involves the building of prefabricated concrete or steel sections which are then lowered into a trench in the river. On the river banks the tunnel will be constructed using more conventional methods.

Before the New Tyne Crossing can be approved it is necessary to carry out an Environmental Impact Assessment (EIA) of the proposals. The assessment will explore the benefits and possible problems brought about by the scheme both during the construction and when it is completed and operational. Once the environmental impacts have been noted any work needed to reduce them can be considered. Measures might include changes to the scheme, additional works, or works to protect the people, wildlife or objects affected.

The installation of the immersed tunnel will require dredging across the River Tyne. After the immersed tunnel has been installed, the remaining trench will be filled and disposal of excess dredged material will be required. The rivers sediments may contain various contaminants and during dredging sediments will be disturbed which may raise water quality issues. Therefore, as part of the environmental impact assessment, Posford Haskoning approached WRc-NSF to carry out toxicity testing on sediment samples taken across the river at the site of the crossing. The samples were to be taken from the surface down to a depth of 3.0 m and testing would be carried out using the estuarine/marine amphipod *Corophium volutator*. The Environment Agency requested that testing was to be carried out in manner which would allow comparisons with the results of a monitoring exercise carried out along the River Tyne in September 1997.

Corophium volutator was chosen as the test organism as benthic organisms generally provide a more realistic indication of the bioavailability and hence toxicity of contaminants present in the sediment than tests on elutriates using water column dwelling organisms. Furthermore from a practical standpoint these organisms can be easily obtained from clean locations for use in sediment toxicity tests and a standardised test procedure has been developed (PARCOM 1994). This species can also be used on a range of sediment types from very fine sediments to very coarse sediments.

2. MATERIALS AND METHODS

2.1 Provision of samples

A series of 35 samples were collected by Allied Exploration and Geotechnics (AEG) Limited in the period 15 – 17 August 2001. The locations of the sampling sites is given in Table 2.1.

Table 2.1 Locations at which samples were collected

Code	Information on the sampling points		
	Location	Easting	Northing
BH 501	South bank of river	433047.4	565867.5
BH 502	South bank of river	433151.4	565868.7
BH 503	North bank of river	433051.4	566056.6
BH 504	North bank of river	433096.4	566049.0
BH 505	Navigation channel	433098.4	565948.2

The samples which were contained in 2.5 litre sealable plastic containers were transported to WRc-NSF by courier in insulated boxes containing cool packs and all samples were received by the 21 August 2001. Any sediment samples which were not dispatched by AEG at the end of a working day were held in a cold store until they were transported.

On receipt at WRc-NSF the samples were held in a cold store (4-6 °C) until they had all been received. The samples were then sieved through a 500 μm sieve to remove indigenous organisms and larger particles. The sieved material was retained in the containers in the cold store until testing was started.

The sediments were treated with caution as the full hazard of the test medium was not known and care was taken particularly when sieving sediments. Latex gloves, safety spectacles and long-sleeve laboratory coats were worn at all times when handling the samples.

The codes for the sample locations are given in Table 2.2 along with the codes for the depth at which samples were taken.

Table 2.2 Sample codes for the sediment samples

Codes for sediment samples taken at different locations and depths					
Depth (m)	Location1	Location 2	Location 3	Location 4	Location 5
0 - Bed level	BH 501 J1	BH 502 J1	BH 503 J1	BH 504 J1	BH 505 J1
0.5	BH 501 J2	BH 502 J2	BH 503 J2	BH 504 J2	BH 505 J2
1.0	BH 501 J3	BH 502 J3	BH 503 J3	BH 504 J3	BH 505 J3
1.5	BH 501 J4	BH 502 J4	BH 503 J4	BH 504 J4	BH 505 J4
2.0	BH 501 J5	BH 502 J5	BH 503 J5	BH 504 J5	BH 505 J5
2.5	BH 501 J6	BH 502 J6	BH 503 J6	BH 504 J6	BH 505 J6
3.0	BH 501 J7	BH 502 J7	BH 503 J7	BH 504 J7	BH 505 J7

2.2 Test procedure (following PARCOM 1994)

2.2.1 Source of test organisms:

The *Corophium volutator* used for the test were collected from Burnham-on-Crouch and maintained in the WRc-NSF laboratory until the start of the test.

Corophium were sieved from their native sediment on the day prior to initiating the test. This allowed any damaged animals to die ensuring that only healthy animals were used in the test. Approximately 4400 *Corophium* were sieved from sediment collected from Burnham-on-Crouch. The length of the test organisms used in the test was between 5 and 8 mm and this was estimated by comparing the test organisms with a scale marked on a piece of graph paper. The test organisms within the specified size range were placed into clean sea water and kept in tanks at 15 ± 2 °C until testing started the next day.

2.2.2 Preparation of the test vessels

The test vessels were 1 litre borosilicate glass beakers, filled with 300 g of test sediment and 850 ml of overlying seawater. Each sediment sample was tested with three replicates while six replicates of a negative control sediment (reference sediment) were prepared using the sediment from the species collection site. The sediment was placed in the test vessels on the day prior to the start of the test and left to settle overnight in the test facility.

On day 0 of the test the salinity, temperature, pH and dissolved oxygen of the overlying water in each vessel was recorded at and adjusted accordingly if they were outside the acceptable range for the test, namely:

Salinity:	30 – 38 ‰
Temperature:	13 – 17 °C
pH:	7.5 – 8.5
Dissolved oxygen:	>85%

The test vessels were then aerated for 0.5 hour prior to animal addition. Each of the beakers was gently aerated throughout the test exposure period via Plenhum chambers and small plastic pipette tips.

2.2.3 Addition of the test organisms

Test organisms were added randomly to the test vessels in groups of 5 held in small volumes of seawater in 20ml plastic vessels. Random number tables were used to assign the organisms to vessels and this approach was adopted to overcome any systematic bias. This process was continued until there were 20 organisms in each of the six replicates of the control sediment and 15 organisms in each of the three replicates of the test sediments.

2.3 Monitoring

The test vessels were visually inspected for mortalities on a daily basis and any dead *Corophium* were carefully removed from the test vessels and discarded. This information was recorded on the test data sheet.

The air supply to the beakers was also checked visually each day and if any of the pipette tips became blocked they were replaced. Finally, the water level in the vessels was checked daily since it may fall over the duration of the test due to evaporation and thereby increase the salinity of the overlying water. Therefore, the initial water level was marked on the outside of each test beaker using a black permanent marker and the water level was topped up to this mark with distilled water if required.

2.4 Termination of the test

On day 10 of the test measurements of the salinity, temperature, pH and dissolved oxygen of the overlying water in each test vessels were made. The content of the test vessels were then sieved through a 500 µm sieve and all living *Corophium* were counted. Since dead animals may decompose or be consumed during the test any animals which were apparently 'missing' (based on comparison of the number added initially and the number of living and dead animals recovered) were counted as dead. This information was recorded on the test data sheet.

2.5 Test validity

The test was considered valid if the survival of amphipods in the control sediment was greater than 80% (that is mortality in the control sediment was less than 20%)(PARCOM 1994).

2.6 Data interpretation:

The mean percentage mortality data for each sample was used to assess the spatial variability in toxicity both within sites (that is with depth) and also between sites. The information from this study was also compared with information from the previous testing exercise to assess temporal variability. A toxic response in a sample has been assumed if mortality in the test sediment was statistically different from the negative control sediment after the use of Analysis of Variance (ANOVA).

3. RESULTS AND DISCUSSION

3.1 Test validity

The mean mortality level of the amphipods in the control sediment was 9.2% (Standard deviation 6.6%) and since this is below the 20% mortality threshold level for the test (PARCOM 1994) the resultant data can be considered valid. Table 3.1 summarises the water quality data for the overlying water of the test vessels during the test and shows that salinity, temperature, pH and dissolved oxygen levels satisfied the test validity (see Section 2.2.2) .

Table 3.1 Summary of the water quality data for the overlying water in test vessels

Sample	Ranges of water quality parameters in the test for the sample replicates			
	Salinity (‰)	Temperature (°C)	pH	Dissolved oxygen (%ASV)
Control	30-37	13.3-13.9	7.84-8.27	92-99
BH 501 J1	31-37	13.2-14.0	7.94-8.30	94-99
BH 501 J2	30-37	13.2-13.8	8.00-8.36	94-99
BH 501 J3	31-36	13.3-13.8	8.00-8.33	95-99
BH 501 J4	31-38	13.3-13.7	7.99-8.35	93-99
BH 501 J5	31-38	13.0-13.7	8.04-8.37	95-100
BH 501 J6	30-38	13.0-13.6	8.05-8.40	95-99
BH 501 J7	32-38	13.1-13.8	8.03-8.41	95-99
BH 502 J1	31-38	13.1-13.8	8.04-8.42	96-100
BH 502 J2	31-37	13.1-13.8	8.04-8.43	96-100
BH 502 J3	31-37	13.1-13.6	7.96-8.42	95-99
BH 502 J4	30-37	13.1-13.7	8.00-8.42	95-99
BH 502 J5	31-38	13.1-13.7	8.03-8.42	95-99
BH 502 J6	31-37	13.0-13.7	8.08-8.43	96-99
BH 502 J7	31-38	13.0-13.5	8.05-8.44	96-99
BH 503 J1	30-36	13.1-13.6	8.03-8.46	95-99
BH 503 J2	31-37	13.1-13.4	8.06-8.46	96-100
BH 503 J3	31-37	13.1-13.4	8.04-8.45	96-99
BH 503 J4	30-37	13.0-13.4	8.05-8.46	96-99
BH 503 J5	30-37	13.0-13.4	8.07-8.46	96-99
BH 503 J6	31-38	13.0-13.4	8.09-8.43	96-99
BH 503 J7	32-38	13.0-13.3	8.02-8.41	96-99
BH 504 J1	32-36	13.4-13.8	8.09-8.39	96-100
BH 504 J2	31-36	13.5-13.8	8.07-8.37	95-100
BH 504 J3	32-37	13.5-13.6	8.05-8.35	96-99
BH 504 J4	31-37	13.6-13.8	8.05-8.34	93-99
BH 504 J5	30-35	13.5-13.8	8.08-8.30	96-99
BH 504 J6	31-36	13.4-13.8	8.06-8.31	95-99
BH 504 J7	30-35	13.6-13.9	8.04-8.30	96-99
BH 505 J1	30-35	13.3-13.9	7.98-8.30	96-100
BH 505 J2	31-35	13.5-13.9	8.01-8.28	95-99
BH 505 J3	30-36	13.4-13.9	8.06-8.26	96-99
BH 505 J4	31-36	13.5-14.0	8.02-8.27	96-99
BH 505 J5	30-36	13.7-14.0	8.06-8.25	96-99
BH 505 J6	30-36	13.6-14.1	8.06-8.26	96-99
BH 505 J7	31-37	13.0-14.2	8.08-8.27	96-99

3.2 Mortality in sediment samples

The mortality levels in the test sediments were typically less than 20%, with the exception of four samples BH 501 J5, BH 501 J6, BH 502 J6 and BH 503 J2 (Figures 3.1 and 3.2). The samples which showed a mean mortality level of greater than 20% also showed greater variability between replicates than other samples (Figure 3.2). In the case of samples BH 502 J6 and BH 503 J2 this was due to one replicate showing high mortalities compared to the other two replicates. The raw data for the test is given in Appendix A.

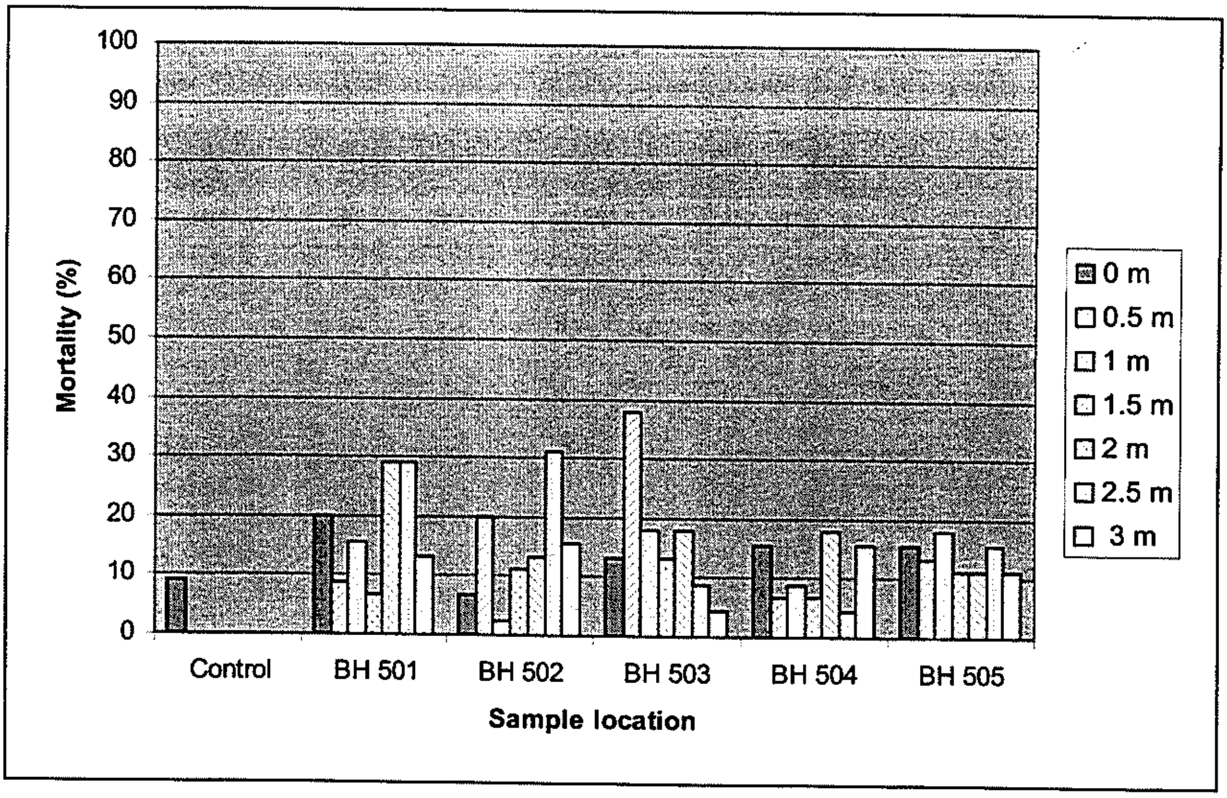

Figure 3.1 Mean *C.volutator* mortality data in the different sediment samples from the various locations and depths

Analysis of variance of the surface sediment sample data (J1 samples) indicated that no locations had statistically significantly higher levels of *C.volutator* mortality than that measured in the control sediment (F = 1.45, P[1] = 0.26). Analysis of all the test sediments showed that there were no statistically significant differences in *C.volutator* mortality based on location (F = 1.44, P = 0.23) or depth of the sample (F = 1.64, P = 0.15).

[1] Statistically significant results are evident when the probability (P) value is less than 0.05

3.3 Comparison of the current data with previous information

The level of toxicity measured in the surface samples (J1's) in this study was consistent the result of 30% mortality ($\pm$ 10%) recorded for a Howdon surface sediment sample taken during the September 1997 survey of the River Tyne.

The data are also consistent with a mortality level of 10% measured downstream of Howdon (54° 59.29' N 1° 28.28' W) as part of the 1995 National Marine Monitoring Programme (CEFAS 1998) which monitors the status of United Kingdom estuaries and coastal waters.

3.4 Interpretation of the data

In the control sediment the level of mortality was 9.2% and it is important to recognise that some level of mortality will generally result from exposure of organisms to even 'clean' sediments. CEFAS (1997) stated that experiences of testing sediments collected from around offshore oil platforms suggested that mortalities in excess of 20% can generally regarded as an adverse effect of biologically available chemical contamination. Mortality levels lower than 20% can be regarded as effectively background levels and should not be ascribed to the effects of chemical contamination. As the level of contamination increases the hazard presented by the sediments becomes increasingly unacceptable and a greater cause for concern. In the National Marine Monitoring Programme estuarine and coastal sediments which result in less than 39% mortality in the *C.volutator* lethality test are considered good. Sediment samples showing 40-99% mortality are considered to reflect substantial deterioration whilst samples showing 100% mortality are considered to be very poor (CEFAS 1998).

On this basis the samples collected from the Howdon basin can generally be regarded as not being acutely toxic. However, although these samples showed a low level of toxicity to *Corophium volutator* this does not mean that they do not contain contaminants. Rather it shows that the levels of contaminants present and/or their bioavailability was not sufficient to cause mortality in the test species. In terms of the results of whole organism sediment toxicity tests such as the 10 day *C.volutator* lethality test it is the bioavailable fraction of the total contaminant concentration present that is responsible for any effects observed.

Finally, although no acute effects were evident in the samples, it has to be recognised that is the level of contamination present may be sufficient to cause more subtle effects on parameters such as growth and reproduction of aquatic organisms.

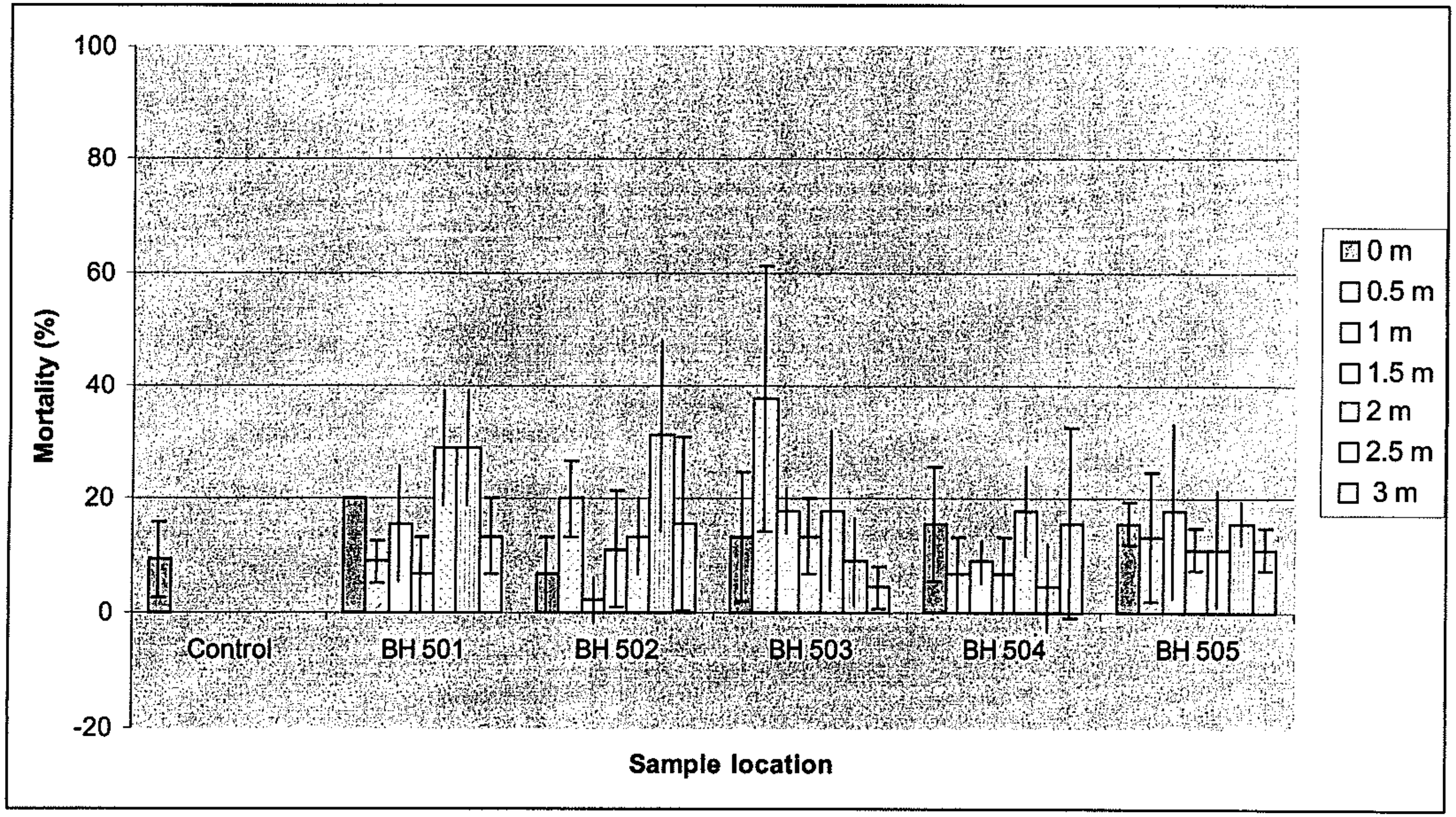

Figure 3.2 **Mean *C.volutator* mortality data (with standard deviations) in the different sediment samples from the various locations and depths**

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CEFAS (1997) Final Report of the Sediment Bioassay Task Team of the Marine Pollution Monitoring Management Group. Science Series Aquatic Environment Monitoring Report No. 48. The Centre for Environment, Fisheries and Aquaculture Science, Lowestoft.

CEFAS (1998) Monitoring and surveillance of non-radioactive contaminants in the aquatic environment and activities regulating the disposal of wastes at sea, 1995 and 1996. Science Series Aquatic Environment Monitoring Report No. 51. The Centre for Environment, Fisheries and Aquaculture Science, Lowestoft.

PARCOM (1994) A Sediment Bioassay using an Amphipod *Corophium sp.* Part A. Annex to the Summary Record of the eighteenth meeting of the Working Group on Oil Pollution, The Hague, 8-11 February 1994, Paris Commission GOP 18/13/1-E.

APPENDIX A RAW DATA FROM THE *COROPHIUM VOLUTATOR* LETHALITY TEST

River Tyne Sediment analysis - 10 day *Corophium volutator* toxicity test

Location	Sample code	Replicate	No. animals alive	No. found dead	No. missing	% Survival	Mean	SD
Burnham on Crouch	Control	1	16	0	4	80		
		2	18	0	2	90		
		3	18	0	2	90		
		4	19	0	1	95		
		5	20	0	0	100		
		6	18	0	2	90	90.8	6.6
South bank	BH 501 J1	1	12	1	2	80.0		
		2	12	0	3	80.0		
		3	12	1	2	80.0	80.0	0.0
	BH 501 J2	1	13	0	2	86.7		
		2	14	0	1	93.3		
		3	14	1	0	93.3	91.1	3.8
	BH 501 J3	1	13	0	2	86.7		
		2	11	0	4	73.3		
		3	14	0	1	93.3	84.4	10.2
	BH 501 J4	1	14	0	1	93.3		
		2	13	0	2	86.7		
		3	15	0	0	100.0	93.3	6.7
	BH 501 J5	1	12	0	3	80.0		
		2	9	0	6	60.0		
		3	11	0	4	73.3	71.1	10.2
	BH 501 J6	1	12	1	2	80.0		
		2	11	0	4	73.3		
		3	9	0	6	60.0	71.1	10.2
	BH 501 J7	1	13	1	1	86.7		
		2	12	0	3	80.0		
		3	14	0	1	93.3	86.7	6.7
South bank	BH 502 J1	1	13	0	3	86.7		
		2	15	0	0	100.0		
		3	14	1	0	93.3	93.3	6.7
	BH 502 J2	1	11	0	4	73.3		
		2	12	0	3	80.0		
		3	13	0	2	86.7	80.0	6.7
	BH 502 J3	1	15	0	0	100.0		
		2	14	0	1	93.3		
		3	15	0	0	100.0	97.8	3.8
	BH 502 J4	1	13	1	1	86.7		
		2	15	0	0	100.0		
		3	12	0	3	80.0	88.9	10.2
	BH 502 J5	1	13	1	1	86.7		
		2	12	0	3	80.0		
		3	14	0	1	93.3	86.7	6.7
	BH 502 J6	1	13	0	2	86.7		
		2	10	1	4	66.7		
		3	8	2	5	53.3	68.9	16.8
	BH 502 J7	1	10	1	4	66.7		
		2	14	0	1	93.3		
		3	14	1	0	93.3	84.4	15.4

Location	Sample code	Replicate	No. animals alive	No. found dead	No. missing	% Survival	Mean	SD
North bank	BH 503 J1	1	12	0	3	80.0		
		2	15	0	0	100.0		
		3	12	0	3	80.0	86.7	11.5
	BH 503 J2	1	9	1	5	60.0		
		2	6	0	9	40.0		
		3	13	0	2	86.7	62.2	23.4
	BH 503 J3	1	13	0	2	86.7		
		2	12	1	2	80.0		
		3	12	0	3	80.0	82.2	3.8
	BH 503 J4	1	12	0	3	80.0		
		2	14	0	1	93.3		
		3	13	0	2	86.7	86.7	6.7
	BH 503 J5	1	14	0	1	93.3		
		2	13	0	2	86.7		
		3	10	0	5	66.7	82.2	13.9
	BH 503 J6	1	13	0	2	86.7		
		2	15	0	0	100.0		
		3	13	0	2	86.7	91.1	7.7
	BH 503 J7	1	14	0	1	93.3		
		2	15	0	0	100.0		
		3	14	0	0	93.3	95.6	3.8
North bank	BH 504 J1	1	14	0	1	93.3		
		2	11	0	4	73.3		
		3	13	0	2	86.7	84.4	10.2
	BH 504 J2	1	14	0	1	93.3		
		2	13	0	2	86.7		
		3	15	0	0	100.0	93.3	6.7
	BH 504 J3	1	13	0	2	86.7		
		2	14	0	1	93.3		
		3	14	0	1	93.3	91.1	3.8
	BH 504 J4	1	13	1	1	86.7		
		2	14	0	1	93.3		
		3	15	0	0	100.0	93.3	6.7
	BH 504 J5	1	13	0	2	86.7		
		2	13	0	2	86.7		
		3	11	0	4	73.3	82.2	7.7
	BH 504 J6	1	13	0	2	86.7		
		2	15	0	0	100.0		
		3	15	0	0	100.0	95.6	7.7
	BH 504 J7	1	13	0	2	86.7		
		2	10	0	5	66.7		
		3	15	0	0	100.0	84.4	16.8

Location	Sample code	Replicate	No. animals alive	No. found dead	No. missing	% Survival	Mean	SD
Navigation channel	BH 505 J1	1	13	0	0	86.7		
		2	13	0	2	86.7		
		3	12	0	3	80.0	84.4	3.8
	BH 505 J2	1	11	0	4	73.3		
		2	14	0	1	93.3		
		3	14	2	0	93.3	86.7	11.5
	BH 505 J3	1	11	0	4	73.3		
		2	11	1	3	73.3		
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		3	14	0	1	93.3	88.9	3.8
	BH 505 J5	1	15	0	0	100.0		
		2	12	0	3	80.0		
		3	13	0	2	86.7	88.9	10.2
	BH 505 J6	1	13	0	2	86.7		
		2	12	0	3	80.0		
		3	13	0	2	86.7	84.4	3.8
	BH 504 J7	1	13	0	2	86.7		
		2	13	0	2	86.7		
		3	14	0	1	93.3	88.9	3.8