**RESULTS OF THE OYSTER (*Crassostrea gigas*)
EMBRYO-LARVAL BIOASSAY ON SAMPLES
TAKEN DURING THE WINTER PHASE
OF NATIONAL MARINE MONITORING
PROGRAMME 1997**

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## SUMMARY

This report documents the results of the oyster embryo bioassay on samples taken during the winter sampling period, 1997, of the National Marine Monitoring Programme from estuarine and offshore sites around the UK. Water quality was assessed using the 24-hour oyster (*Crassostrea gigas*) embryo-larval development test, following the ICES TIMES No.11 protocol. Results are presented for 44 sites. Of the 44 sites tested, the oyster embryo bioassay indicated that 4 were of poor quality (Percent Net Response (PNR) >25%).

## 1.0    INTRODUCTION

The Environment Agency (herein referred to as the Agency) Direct Toxicity Assessment (DTA) Newcastle Laboratory was contracted to perform the oyster embryo-larval bioassay on samples taken during the 1997 National Marine Monitoring Programme. A coordinated programme of water sampling is carried out by each of the Agency's regions, during winter and summer, at estuarine and offshore sites designated by the Marine Pollution Monitoring Management Group (MPMMG). The oyster (*Crassostrea gigas*) embryo-larval (OEL) development test was then performed on the samples to assess their quality.

## 2.0    MATERIALS AND METHODS

### 2.1    Sampling

Water samples were collected in duplicate by Agency personnel in acid washed glass bottles and sealed with PTFE lined lids. Samples were transported to the laboratory in suitable cool-boxes and were stored at 4°C prior to and after transportation.

### 2.2    Sample Preparation

Wherever possible samples were tested on the day of receipt and within 24 hours after sampling. On arrival the samples were logged and the water quality parameters recorded. Samples with an initial salinity of less than 27‰ were adjusted to between 32-34‰ by the addition of analytical grade sea salt (Sigma Sea salts, S-0131). Samples with a salinity greater than 27‰ were tested without adjustment. The resultant salinities of each sample and the amount of salts added were recorded. After salinity adjustment the pH and dissolved oxygen (DO) of each sample were measured.

### 2.3    Test Organism

Tests were performed using the Pacific oyster *Crassostrea gigas*. These were supplied on the day of the test from Guernsey Sea Farms, Channel Islands. The conditioned oysters are supplied sexed and yielded a high percentage of normal embryos upon fertilisation in reference sea water.

### 2.4    Reference Sea Water

The sea water used throughout the test was artificially made from Sigma Sea Salts, S-0131 in distilled water to a salinity of 32-34‰.

## 2.5 Test Method

Samples were tested according to the ICES TIMES NO. 11 protocol - Techniques In Marine Environmental Sciences (TIMES), Biological effects of contaminants: Oyster (*Crassostrea gigas*) embryo bioassay, International Council for the Exploration of the Sea (ICES 1991). Tests were performed in 50 ml glass bottles, four replicates of each sample were tested and eight control replicates were used for each batch of samples.

## 2.6 Reference Test

A reference test, using zinc (as zinc sulphate), was run with each batch of samples tested. Four replicates of 0.1 and 0.32mg Zn $l^{-1}$ were used enabling the concentration resulting in a Percent Net Response (PNR) of 50% to be calculated. This information was then recorded on a Shewart control chart to determine if there were differences in the sensitivity between batches of oysters.

## 2.7 ᐯ Calculation of Results

From the numbers of normal and abnormal larvae counted, the percentage abnormality was calculated. From these values the Percentage Net Response (PNR) was calculated. This adjusts the percentage abnormality of the sample taking account of abnormality in the control:

$$PNR = \frac{\% \text{ test abnormality} - \% \text{ control abnormality}}{100 - \% \text{ control abnormality}} \times 100$$

## 3.0 RESULTS

## 3.1 Sample Test Results

Table 1 gives details of the samples tested and the resultant PNR value. A total of 44 samples was tested on 8 different test occasions including one test which was repeated due to failure of development in the control.

During the winter phase of 1997 there were four sites resulting in reduced water quality i.e. with PNR values greater than 25%. These were Tees Bamletts Bight, Thames Woolwich, Cardigan Bay Intermediate and Mersey Monk's Hall. Of these Cardigan Bay Intermediate had a PNR value of 29.4 indicating a slight impairment of water quality, whereas the other three sites had PNRs ranging from 65.8 to 100 indicating poor water quality.

## 3.2     Quality Control

Table 2 lists the control and reference toxicant data for each of the test occasions. Control development remained well below the recommended level of 40% abnormality on all testing occasions, the highest abnormality recorded being 19.1%. This table also details the results for the zinc reference tests performed with each test. The results were subsequently plotted on a Shewart control chart (Fig. 1) and provide an indication of the degree of variability in sensitivity between the batches of oysters used. On less than half of the occasions was it possible to calculate the zinc concentration producing a PNR of 50 ($PNR_{50}[Zn]$). Of these occasions all $PNR_{50}[Zn]$ values fell within the warning limits. This did not compromise the results as all control values fell within the acceptable limits.

## 4.0     DISCUSSION

There were four sites resulting in reduced water quality during the winter phase of 1997. Of these Cardigan Bay had an unusually high PNR of 29.4. This would not normally be expected at this site and it is possible that contamination of the sample occurred. Tees Bamletts Bight had a PNR of 65.8 and has also shown poor water quality in winter sampling phases for the previous two years. Monks Hall, Mersey resulted in a PNR of 100 consistent with the poor water quality that has been demonstrated at this site for the last four years. Thames Woolwich had a PNR value of 92.8 indicating poor water quality, as in the 1995 winter sampling phase.

## REFERENCES

ICES (1991) Biological effects of contaminants: Oyster (*Crassostrea gigas*) embryo bioassay. Techniques In Marine Environmental Sciences No.11. International Council for the Exploration of the Sea.

WRc (1994) Ecotoxicology Methods Manual. R&D Note 322.

Table 1 Summary of sample details and PNR values

| EA Region | NMP Site No. | Site Description | Date Sampled | Date Tested | Initial Salinity |
|---|---|---|---|---|---|
| North East | - | Tees - Bamletts Bight | 5-6-97 | 6-6-97 | 24.6 |
| North East | - | Tees - No 23 Buoy | 5-6-97 | 6-6-97 | 31.4 |
| North East | - | Tees - Phillips Approach | 5-6-97 | 6-6-97 | 32.9 |
| North East | 255 | Wear - South Hylton | 4-2-97 | 6-2-97 | 11.2 |
| North East | 265 | Wear - Queen Alexandra Bridge | 4-2-97 | 6-2-97 | 18.7 |
| North East | 275 | Wear - Sandy Point | 4-2-97 | 6-2-97 | 21.6 |
| North East | 235 | Tyne - Lloyds Hailing | 20-1-97 | 22-1-97 | 27.3 |
| North East | 225 | Tyne- Hebburn | 20-1-97 | 22-1-97 | 22.5 |
| North East | 215 | Scotswood Bridge | 20-1-97 | 22-1-97 | 6.6 |
| Anglian | - | W39 | 12-3-97 | 18-3-97 | 32.2 |
| Anglian | - | W42 | 12-3-97 | 18-3-97 | 33.7 |
| Anglian | 336 | Humber 7728 | 25-3-97 | 3-4-97 | 32.3 |
| Anglian | 356 | Humber Site III | 26-3-97 | 3-4-97 | 11.0 |

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| Salinity Adjustment | | Corrected Salinity | pH | DO % ASV | PNR Value |
|---|---|---|---|---|---|
| Y/N | Salt (g) | | | | |
| Y | 3.6 | 34.8 | 7.68 | 86 | **65.8** |
| N | - | - | 7.64 | 90 | **-2.5** |
| N | - | - | 7.77 | 79 | **-5.0** |
| Y | 6.72 | 33.8 | 7.84 | 86 | **1.4** |
| Y | 4.75 | 34.6 | 7.67 | 81 | **5.7** |
| Y | 3.93 | 33.3 | 7.83 | 83 | **2.1** |
| Y | 2.6 | 34.1 | 7.54 | 85 | **-12.9** |
| Y | 3.7 | 34.7 | 7.75 | 95 | **-14.2** |
| Y | 7.5 | 34.2 | 7.12 | 110 | **-4.3** |
| N | - | - | 7.68 | 80 | 1.3 |
| N | - | - | 7.77 | 93 | 0.7 |
| N | - | - | 7.94 | 110 | -5.4 |
| Y | 5.2 | 31.3 | 7.90 | 106 | -4.0 |

| EA Region | NMP Site No. | Site Description | Date Sampled | Date Tested | Initial Salinity |
|---|---|---|---|---|---|
| Anglian | 366 | Humber 7702 | 26-3-97 | 3-4-97 | 17.9 |
| Anglian | 375 | NSTF 16, Jonus OSP2 | 25-3-97 | 3-4-97 | 34.6 |
| Anglian | 405 | Ouse - Stowbridge | 23-3-97 | 3-4-97 | 2.3 |
| Anglian | 415 | Ouse - Freebridge | 23-3-97 | 3-4-97 | 29.0 |
| Anglian | 425 | Ouse - The Point | 23-3-97 | 3-4-97 | 18.6 |
| Thames | 435 | Thames - Woolwich | 18-6-97 | 20-6-97 | 6.6 |
| Thames | 445 | Thames - West Thurrock | 18-6-97 | 20-6-97 | 16.4 |
| Thames | 455 | Thames - Mucking | 17-6-97 | 20-6-97 | 27.2 |
| Thames | 465 | Thames - Warp | 17-6-97 | 20-6-97 | 34.3 |
| Southern | 495 | Selsey Bill | 27-2-97 | 4-3-97 | 33.9 |
| Southern | 505 | Southampton Water-Dock Head | 27-2-97 | 4-3-97 | 30.8 |
| Southern | 515 | Southampton Water-EBrambles Buoy | 27-2-97 | 4-3-97 | 32.5 |
| Southern | 525 | Calshot | 27-2-97 | 4-3-97 | 32.7 |
| South West | 545 | Tamar - Halton Quay | 17-2-97 | 19-2-97 | 2.3 |

| Salinity Adjustment | | Corrected Salinity | pH | DO % ASV | PNR Value |
|---|---|---|---|---|---|
| Y/N | Salt (g) | | | | |
| Y | 3.82 | 33.6 | 7.96 | 108 | -5.2 |
| N | - | - | 7.85 | 107 | -0.7 |
| Y | 6.94 | 31.3 | 7.99 | 92 | 5.9 |
| Y | 1.6 | 34.6 | 7.84 | 100 | 2.0 |
| Y | 3.68 | 33.6 | 7.96 | 108 | 0.9 |
| Y | 8.5 | 34.9 | 7.74 | 70.5 | 92.8 |
| Y | 5.65 | 33.9 | 7.51 | 61.3 | -1.5 |
| Y | 2.5 | 34.4 | 7.80 | 79.3 | -1.2 |
| N | - | - | 8.09 | 101 | 7.1 |
| N | - | - | 8.02 | 102 | -0.2 |
| N | - | - | 7.91 | 100 | -0.1 |
| N | - | - | 8.00 | 102 | -0.2 |
| N | - | - | 8.01 | 101 | -1.0 |
| Y | 8.75 | 32.7 | 8.19 | 115 | -1.7 |

| EA Region | NMP Site No. | Site Description | Date Sampled | Date Tested | Initial Salinity |
|---|---|---|---|---|---|
| South West | 565 | Tamar - Hamoaze | 17-2-97 | 19-2-97 | 19.1 |
| South West | 555 | Warren Point | 17-2-97 | 19-2-97 | 17.9 |
| Welsh | 615 | Severn- Nash Point | 1-4-97 | 3-4-97 | 31.5 |
| Welsh | 625 | Severn- Old Severn Rail Bridge | 1-4-97 | 3-4-97 | 15.5 |
| Welsh | 635 | Severn - No.1 Beacon | 1-4-97 | 3-4-97 | 20.5 |
| Welsh | 645 | Severn - English & Welsh Grounds | 1-4-97 | 3-4-97 | 25.3 |
| Welsh | 655 | Cardigan Bay Intermediate | 9-1-97 | 15-1-97 | 33.2 |
| Welsh | 675 | Dee - Shotton | 13-3-97 | 18-3-97 | 27.9 |
| Welsh | 685 | Dee - Flint | 13-3-97 | 18-3-97 | 29.6 |
| North West | 695 | Dee - No2 Buoy | 13-3-97 | 18-3-97 | 32.4 |
| North West | 705 | Burbo Bight | 2-4-97 | 3-4-97 | 31.6 |
| North West | 725 | Mersey - Monks Hall | - | 15-1-97 | 1.0 |
| North West | 735 | Mersey - Runcorn Old Quay | - | 15-1-97 | 21.7 |

| Salinity Adjustment | | Corrected Salinity | pH | DO % ASV | PNR Value |
|---|---|---|---|---|---|
| Y/N | Salt (g) | | | | |
| Y | 9.57 | 34.1 | 8.09 | 112 | -0.1 |
| Y | 4.95 | 33.9 | 8.12 | 110 | -4.1 |
| N | - | - | 7.96 | 98 | -5.3 |
| Y | 4.3 | 32.9 | 7.98 | 108 | -1.7 |
| Y | 3.3 | 34.2 | 7.92 | 107 | -4.8 |
| Y | 2.34 | 34.7 | 7.96 | 104 | -4.7 |
| N | - | - | 8.00 | 100 | 29.4 |
| Y | 2.01 | 34.9 | 7.83 | 107 | -0.1 |
| Y | 1.60 | 35.0 | 7.85 | 106 | 1.1 |
| N | - | - | 7.90 | 114 | -0.1 |
| N | - | - | 7.96 | 110 | -3.4 |
| Y | 9.86 | 34.5 | 7.55 | 88 | 100.0 |
| Y | 3.82 | 34.1 | 7.51 | 91 | -0.4 |

| EA Region | NMP Site No. | Site Description | Date Sampled | Date Tested | Initial Salinity |
|-----------|--------------|------------------|--------------|-------------|------------------|
| North West | 745 | Mersey - Bromborough E1 ·Buoy | - | 15-1-97 | 29.2 |
| North West | 755 | Mersey - Seacombe Ferry | - | 15-1-97 | 30.2 |
| North West | 765 | Mersey - C1 Buoy | 2-4-97 | 3-4-97 | 31.1 |
| North West | 785 | Off Wyre | 2-4-97 | 3-4-97 | 31.6 |

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| Salinity Adjustment | | Corrected Salinity | pH | DO % ASV | PNR Value |
|---|---|---|---|---|---|
| Y/N | Salt (g) | | | | |
| Y | 1.50 | 34.7 | 7.70 | 104 | -1.7 |
| N | - | - | 7.76 | 108 | 12.9 |
| N | - | - | 7.95 | 109 | -0.1 |
| N | - | - | 7.96 | 110 | -4.7 |

Table 2 - Control and reference toxicant summary data - Mean percent abnormality (MPA), PNR value and $PNR_{50}$ - [Zn] value

| Test Date | Control MPA | 0.1 mg Zn $l^{-1}$ | | 0.32 mg Zn $l^{-1}$ | | $PNR_{50}$- [Zn] |
|---|---|---|---|---|---|---|
| | | MPA | PNR | MPA | PNR | |
| 15-1-97 | 13.8 | 93.4 | 92.4 | 100 | 100 | * |
| 6-2-97 | 8.3 | 28.7 | 22.2 | 99.7 | 99.7 | 0.15 |
| 22-1-97 | 19.1 | 51.1 | 39.5 | 100 | 100 | 0.12 |
| 19-2-97 | 10.7 | 95.2 | 94.6 | 100 | 100 | * |
| 4-3-97 | 1.4 | 21.3 | 20.2 | 99.6 | 99.6 | 0.16 |
| 18-3-97 | 1.1 | 12.4 | 11.4 | 100 | 100 | 0.17 |
| 3-4-97 | 6.5 | 100 | 100 | 100 | 100 | * |
| 6-6-97 | 6.0 | 91.2 | 90.7 | * | * | * |
| 20-6-97 | 2.1 | 99.1 | 99.1 | * | * | * |

* - unable to calculate $PNR_{50}$ - [Zn] value

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Figure 1. Shewart control chart for results of zinc reference toxicant