Comparison of the relative toxicities of UK offshore, solid-phase, seawater extracts using marine bioassay techniques

S. J. Brooks, W. J. Reynolds, P. H. Roberts and J. E. Thain
Centre for Environment, Fisheries & Aquaculture Science, Burnham on Crouch
www.cefas.co.uk

Introduction

A suite of bioassays have been applied to seawater samples in order to demonstrate their suitability in offshore seawater quality assessment. Contaminant concentrations within offshore samples are unlikely to be of sufficient magnitude in order to determine effect concentration values in acute biological effects assays. This work program has attempted to assess the biological responses to offshore seawater samples by concentrating large volumes of offshore seawater through extraction techniques. This has enabled comparisons of the relative toxicities of water bodies to be made.

The aim of the work was to assess the combined suitability of extraction techniques (C8 and ENV+ solid phase extraction procedures) and bioassays (Oyster embryo, *Tisbe* and *Skeletonema*) as a useful tool of offshore seawater quality assessment.

Method

Collection

Seawater samples were collected from selected stations aboard RV *Cefas Endeavour* as part of the UK wide annual monitoring programme (2004-2006). A 50 L seawater sample was collected at each station by submerging a stainless steel water sampler to a depth of 1m.

Extraction

The seawater sample was immediately placed into a 50 L pressure vessel and extracted by C8 and ENV+ columns. The columns are a generic screen for non-polar compounds and polar compounds respectively and have been found in previous studies to remove the contaminants that are often responsible for the observed toxicity of waters. The columns were eluted in the laboratory by solvent extraction techniques and then taken back up with seawater to give a range of test concentrations of 1, 3.2, 10, 32, 100 and 320x the seawater extractable contaminants.

Bioassays

The dilution series described above was bioassayed using the oyster embryo, *Tisbe battagliai* (copepod) and *Skeletonema costatum* (microalgae).

Oyster Embryo Bioassay (OEB)

Oysters were manually stripped of gametes. Gamete quality was assessed microscopically and the sexes of individuals identified. Gametes were combined in an egg to sperm volume ratio of 1:100, and incubated at 16°C for fertilisation and development to 16-32 cell stage to be achieved. Embryo suspensions were added to each test treatment to achieve a final density of approximately 50 embryos/ml. The embryos were incubated in the dark at 24 ± 2°C for 24 ± 2h. The number of embryos that had successfully developed into normal D-shaped larvae after this time were counted microscopically.

Figure 1: Crassostrea gigas

Marine Algae Bioassay (*Skeletonema*)

The *S. costatum* assay was conducted in 96-well microplates. Each sample was run as a dilution series in 2 microplates with a control of clean seawater. Each plate was incubated in continuous light (approx. 2000 Lux) at 21 ± 2°C whilst being shaken at 100 rpm on an orbital shaker. Algal growth was measured by fluorescence (excitation 430 nm, emission 670 nm) at 0 h, 24 h, 48 h and 72 h.

Figure 2: Tisbe battagliai

Tisbe battagliai Bioassay

Tests were conducted in 12 cell well plates. Each well contained 5 mls of test solution, 4 replicates were used for each concentration. At the start of the test 5 T. battagliai were placed in each well. The test was carried out at 20 ± 2°C. The number of surviving copepods in each treatment after 48 ± 2 h was determined.

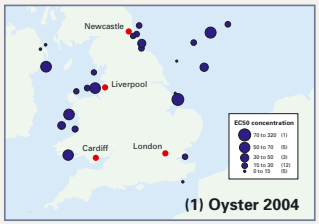
Figure 3: Skeletonema costatum

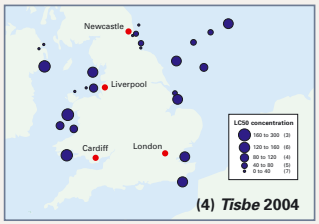
For all bioassays, a positive control ($ZnSO_4$) was run concurrently with each batch of samples, this was to check that the sensitivity of each batch did not vary significantly. Effect concentrations were determined from the analysis of raw data using the Toxcalc statistical package (Tidepool Scientific, USA).

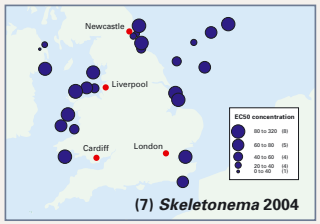
Results and discussion

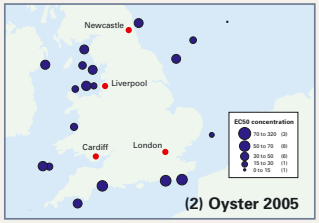
The position of the data points on each map indicate the location at which the seawater samples were taken, whilst the size indicates the EC50/ LC50 value range. The larger the diameter of the data point the larger the EC50/ LC50 concentration and lower the toxicity.

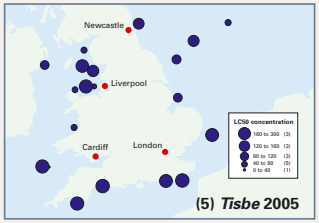
- For all seawater samples, non-concentrated seawater (i.e. 1x concentrate) did not cause any deleterious effects on oyster, *Skeletonema* or *Tisbe* development.

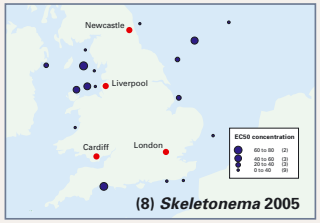


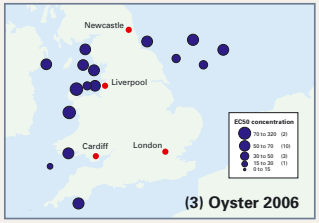


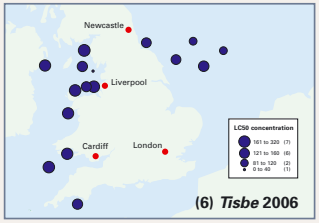


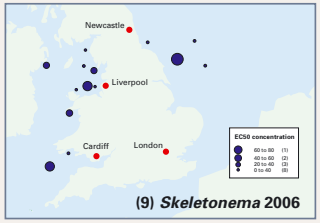












Maps 1-3: The EC50 concentrations of the Oyster embryo larvae (Crassostrea gigas) following 24 h exposure to extracted offshore seawater samples. EC50 values – times the original seawater concentration.

Maps 4-6: The LC50 concentrations of Tisbe battagliai (copepod) following 48 h exposure to extracted offshore seawater samples. LC50 values – times the original seawater concentration.

Maps 7-9: The EC50 concentrations of Skeletonema costatum (microalgae) following 72 h exposure to extracted offshore seawater samples. Effects on growth inhibition. EC50 values – times the original seawater concentration.

Summary of data for Oyster embryo

- Oyster 2004: Lowest EC50 values (highest toxicity) were found in samples from Belfast Lough (0-15x concentrate). Relatively low EC50s were found at the Mouth of the Tees and Tyne as well as two sites towards the Dogger Bank (North Sea). In general, offshore sites had a higher EC50 of >30x concentrate.
- Oyster 2005 & 2006: Overall, high EC50 concentrations at all sites (>30x concentrate).

Summary of data for *Tisbe battagliai*

- *Tisbe* 2004: Lowest LC50 values (highest toxicity) were found in samples from Belfast Lough (0-15x concentrate). Relatively low LC50s were found at the Mouth of the Tees and Tyne. Many offshore sites had a high LC50 concentration (>80x concentrate).
- *Tisbe* 2005 & 2006: Higher LC50s than previous year. Offshore sites generally low LC50s (i.e. >120x concentrate).

Summary of data for *Skeletonema costatum*

- *Skeletonema* 2004: Highest toxicity Belfast Lough and Tyne stations. EC50 concentrations at most offshore sites were high >60x concentrate.
- *Skeletonema* 2005: in contrast to Oyster and *Tisbe,* high toxicity at most of the sites with 9 of the 17 stations with an EC50 of ≤ 20x concentrate.
- *Skeletonema* 2006: Also high toxicity at all stations, 8 of 14 stations with an EC50 ≤ 20x concentrate.

Conclusions

- Good comparison between EC/LC50 values of oyster embryos and *Tisbe,* with both bioassays finding higher toxicity values in coastal waters and reduced toxicity offshore.
- In contrast, the toxicity results of the *Skeletonema* bioassay did not compare well with the oyster embryo or *Tisbe.* The reasons for these differences are unclear but the difference between animal and plant toxicity mechanisms may provide some explanation, with certain contaminants more toxic to one group than another.
- Overall, seawater quality was good at most stations sampled, particularly those stations located further offshore. Relatively poorer seawater quality was found at coastal water stations adjacent to industrialised centres.
- There are clear differences in response year on year and these could be due to a number of factors e.g. algal blooms/periods of calm or rough weather and water mixing.
- The assessment of water quality using solid phase seawater extracts with bioassay techniques has provided a tool for evaluating relative water quality that would otherwise not be possible using bioassays on water alone.

Acknowledgements

This study was part of a work program funded by the Department for Environment, Food and Rural Affairs (Defra).

steven.brooks@cefas.co.uk