Biomarker responses in the Blue mussel, *Mytilus edulis*, an integrated approach to biological effects measurements

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Introduction

Biological effects techniques and chemical residues in tissues have been measured in *M. edulis* with the aim in providing an integrated assessment of environmental status. The biological techniques used include whole organism responses (scope for growth), tissue responses (histopathology) and subcellular responses (lysosomal stability, comet assay and MXR). Application of these biomarker responses to selected native coastal water mussel populations in the UK has been carried out.

The main objective was to apply mussel biological effects methodology to a biological monitoring program. To integrate these biological effects methods with chemical analysis in order to provide an overall assessment of mussel health, which can be used to determine environmental status.

Method

Sample collection

Mussels were collected from five sites in the British Isles (Figure 1). All mussels were collected on a falling tide within 1 m above the water line. Where possible the same mussels were used for several biomarker assays.

Figure 1: Location of the five native mussel populations used.

Tissue chemistry: Whole mussel homogenates prepared from 50 mussels were used to determine metal, organotin and organic (PAHs & CBs) concentrations.

Histopathology: Excised samples were fixed in Davidson's seawater fixative for 24 h before transferred to 70% IMS. Samples were embedded in paraffin wax and thin sections were taken and stained with haematoxylin and eosin. Sections were evaluated for numerous health index parameters.

Scope for growth: carried out in accordance with the manual 'Practical Procedures for the measurement of scope for growth' for marine mussels (Widdows and Staff, 1997).

MXR assay: Sections of gill tissue (approx 4 x 4 mm) were exposed to 0.5 µM calcein AM solution. The gills were incubated in the dark for 20 min. After this time the gills were briefly rinsed in filtered seawater. The fluorescence of the gill sections following exposure to calcein was measured using image analysis software. (Method: A. Kohler pers. com.).

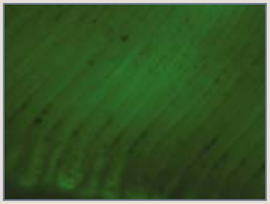

Figure 2: Example of a fluorescing mussel gill segment using fluorescence microscopy and digital image analysis.

Lysosomal stability: Measures the ability of the haemocyte lysosomes to retain the neutral red solution. The test was terminated and the time recorded when greater than 50% of the haemocytes leaked neutral red dye out of the lysosome into the cytosol.

Comet assay: The technique requires individual cells from the target tissues to be isolated and embedded into agarose gel. These embedded cells are then lysed and exposed to high pH (alkaline unwinding) in order to relax the nuclear material. The cells are then micro-electrophoresed and stained with a florescent stain to reveal any broken fragments of the DNA, which have migrated towards the anode. The comet like appearance of these cells gives rise to the assays name and the size and staining intensity of the "comet tail" provides a quantitative measure of DNA damage.

Results and discussion

Note: For practical reasons it was not possible to deploy all techniques at all sites.

Speciation

Table 1: Mussel speciation determined from PCR and gel electrophoresis (n = 20).

Site	Clyde	Southampton	Thames	Tees	Lunderston
M. edulis	95%	33.33%	100%	95%	100%
M. galloprovincialis	0%	33.33%	0%	0%	0%
Hybrid	5%	33.33%	0%	5%	0%

Scope for growth

Table 2: Scope for growth measurements of mussels collected from 4 sample sites. (mean ± SE, n=16).

	Clearance rate (l/h/g)		Respiration (µmol/h/g)		Scope for Growth (J/h/g)	
Site	mean	SE	mean	SE	mean	SE
Thames	3.09	0.17	17.13 †	1.76	6.07	1.05
Clyde	2.16	0.07	8.93	0.72	5.61	0.48
Tees	2.18	0.13	10.94	0.70	4.78	0.64
Southampton	3.89*	0.52	-	-	-	-

* Significant difference in clearance rate from Tees and Clyde mussels, $p<0.05$ (ANOVA, Tukey). † Significant difference in respiration from Tees and Clyde mussels, $p<0.05$ (ANOVA, Tukey).

Multi Xenobiotic Resistance (MXR) assay

Mussels exposed to elevated contaminant concentrations will produce MXR proteins (detoxifying enzymes) in order to reduce the intracellular contaminant concentrations. Therefore, low fluorescence indicates high MXR protein expression as a result of pre-exposure to environmental contaminants.

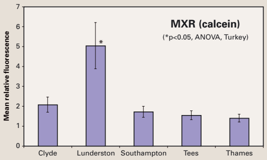

Figure 3: The relative fluorescence of mussel gill sections following exposure to 0.5 µM calcein only solution.

Lysosomal stability:

The results of the lysosomal stability test were inconclusive with no significant differences between the mean retention times of the five mussel populations (ANOVA, $p>0.05$). The mean retention times for the five populations ranged from approx 72 - 86 min.

COMET assay

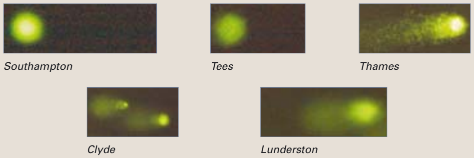

*Figure 4: Mean tail moment of mussel haemocytes taken from the 5 mussel populations (mean ± SE, n=10). * significant difference from mean tail moment for Southampton and Tees mussels (p<0.05 ANOVA). Pictures above represent an example of the actual tail moment of haemocytes from the 5 mussel populations.*

Tissue chemistry

Table 3: Summary of main chemistry data.

CBs	Low or undetected at all sites - Highest in Clyde ∑28CBs = 0.033 µg/kg ww.
PAHs	Highest in Tees ∑25 PAHs 822.7 µg/kg ww. Low at all other sites.
Organotins	Only detected in Southampton mussels at low concentrations (0.045 mg/kg).
Metals	Low and almost identical at all sites.

Histopathology

A total of 33 health index parameters were recorded from each mussel from each of the sampling sites. Parameter groups were: reproductive markers, non-specific inflammatory lesions, infectious diseases and pathology.

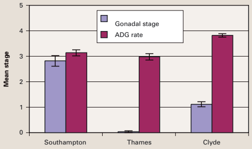

Figure 5: Reproductive markers.

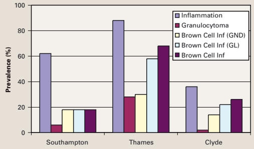

Figure 6: Inflammatory lesions.

Reproductive markers: The gonadal status of the Thames mussels was significantly lower than Southampton and Clyde and was considered to be abnormal due to the significant similarity of ADG rate between Southampton Water and the Thames (Figure 5).

Inflammatory lesions: Thames mussels exhibited the highest prevalence (Figure 6).

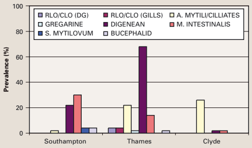

Figure 7: Infectious diseases.

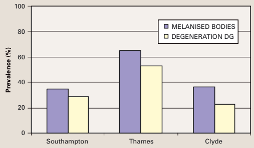

Figure 8: Digestive gland pathology.

Infectious Diseases: Thames mussels exhibited the widest range of infectious diseases (Figure 7).

Digestive gland pathology: Thames mussels showed the highest prevalence of melanised bodies and digestive gland degeneration (Figure 8).

Summary

Table 4:

	Thames	Clyde	Lunderston	Southampton	Tees
Species	100% edulis	95% edulis 5% hybrid	100% edulis	33% edulis 33% galloprovincialis	95% edulis 5% hybrid
Histopathology ADG rate Lesions DG Pathology Kidney melanised aggregates	 Abnormal Highest prevalence Highest prevalence Highest prevalence				
Scope for growth Clearance rate Respiration SFG	 Sig. higher (healthier)			 Sig. higher (healthier)	
MXR	Lowest fluorescence - high contam expo		Sig. higher fluorescence - low contam expo		
Lysosomal stability	No significant difference between populations				
COMET	Significantly higher DNA damage			No DNA damge	
Chemistry Organics (PAHs, CBs) Metals and organotins	 No marked differences between populations (low or undetected concentrations) No marked differences between populations (low or undetected concentrations)				

Conclusions

Overall, the mussels in poorest health were those collected from the Thames, which showed the highest prevalence of inflammatory lesions, digestive gland pathology and kidney melanised aggregates as well as abnormalities in ADG rate, signifying adverse reproductive effects. This coincided with the lowest fluorescence reading in the MXR assay (indicating increased contaminant exposure) and significantly higher DNA damage from the COMET assay.

The mussel chemistry data was either low or undetected in all mussel populations measured. Consequently, contaminant concentrations were unable to explain the differences in biomarker response.

This study has shown how biological effects techniques can be applied to determine the overall health status of a particular water body. In this study the biological response methods proved to be more effective in determining the differences in health status between the mussel populations compared to analytical chemistry methods. This suggests that contaminants other than those measured may have caused the poor mussel health, and emphasises the need to measure chemistry and biological effects at the same time.

Acknowledgements

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References

Widdows, J., & Staff, F., 1997. Practical procedures for the measurement of scope for growth. Produced for European ring-test by authors: Plymouth Marine Laboratory, Prospect Place, West Hoe, Plymouth, PL1 3DH, UK.

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