The effects of air exposure on the digestive diverticula of mussel: Recommendations for sampling procedures

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Introduction

Mussels have been utilised as a sentinel species for assessing the biological effects of aquatic contaminants. This trend is likely to increase in the future primarily due to their widespread distribution, sessile nature and an ability to exhibit a range of biological and chemical responses that can be measured. For these reasons they lend themselves well to integrated monitoring programmes for environmental assessments.

Mussel histopathology has been recognised by the International Council for Exploration of the Seas (ICES) Working Group on Biological Effects of Contaminants (WGBEC) as a promising technique for inclusion under the Oslo Paris (OSPAR) commission Mussel Integrated Approach (Thain *et al.*, 2008). Designation as a "prioritised core technique" will result in the need for quality assurance protocols with respect to diagnosis and sampling techniques when incorporating mussel histopathology. The digestive diverticula has long been a target organ of interest in mussels and exhibits morphological changes in relation to contaminant exposure in field and laboratory studies. These changes present themselves as a reduction in the cell height of the digestive tubule epithelium and have previously been measured using morphometric techniques (Lowe *et al.*, 1981; Vega *et al.*, 1989; Cajaraville *et al.*, 1992). Similar changes have previously been observed in mussels that have undergone starvation and air exposure. Furthermore, it is well established that digestive tubules have four distinct phases which are affected by the tidal cycle and subsequent air exposure (Langton, 1975). The 'disintegrating' phase and 'reconstituting' phase both exhibit reduced epithelial height albeit not to the same extent as those tubules severely affected by contaminants.

We have investigated the potential for a confounding factor (air exposure) to effect morphological measurement and therefore the consequent interpretation of contaminant related changes in the digestive diverticula of mussels from two UK estuaries. Standardised sampling protocols were utilised to ensure quality assurance whilst attempting to replicate conditions often encountered in monitoring programmes that incorporate mussels.

Materials and Methods

- Mussels were sampled from below the waterline at low tide on the River Tamar and Exe estuaries in Devon, UK
- For both sites cross-sections from ten mussels were dissected along a standardised plane and immediately preserved (t=0)
- The sampling was repeated following 24 and 48 hours storage, representing typical sampling and transport conditions (t=24 and t=48)
- Following Formalin Fixed Paraffin Embedding (FFPE) histology, morphometric parameters were measured
- These included Specific Diverticula Area (SDA), Specific Luminal area (SLA) and Specific Epithelial Area (SEA) were made for 25 tubules per mussel, and used to calculate the ratios SEA:SDA and SLA:SEA as indicators of stress

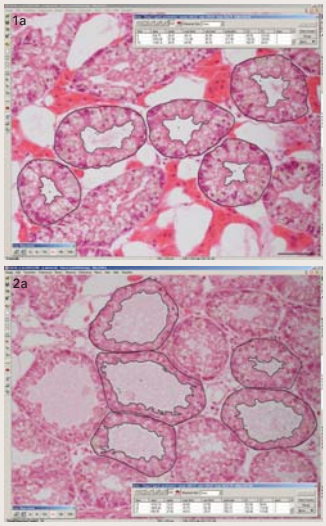


Figure 1: Representative image analysis micrographs of digestive tubules from (a) Exe t=0 and (b) Exe t=24.

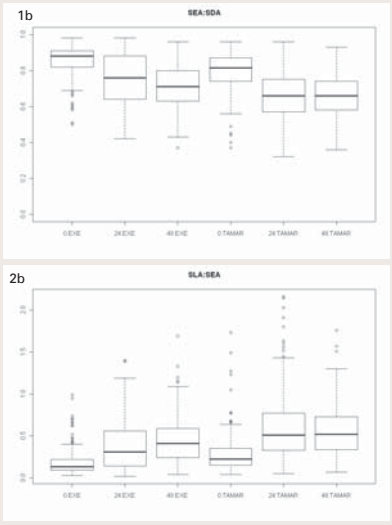


Figure 2: Box and whisker plots for the ratio (a) SEA:SDA and (b) SLA:SEA.

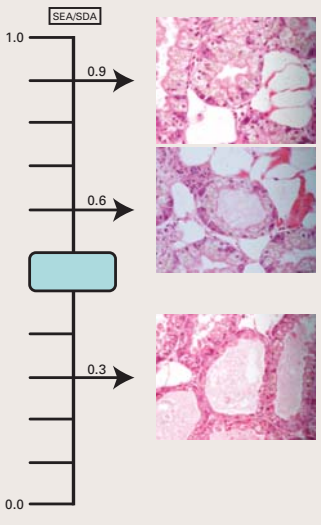
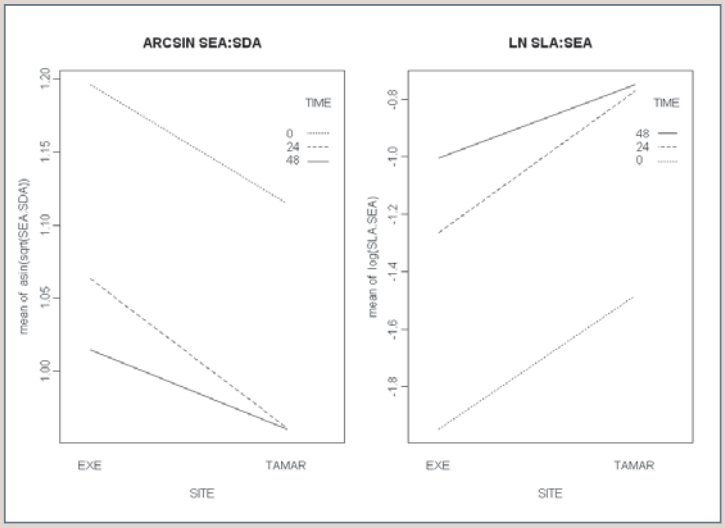


Figure 3: Representative scale showing micrographs of typical SEA:SDA values for an individual tubule.



Two-way ANOVA table: Response: arcsin(√SEA:SDA)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
TIME	2	8.2238	4.1119	204.3587	< 2e-16	***
SITE	1	2.3448	2.3448	116.5346	< 2e-16	***
TIME:SITE	2	0.1409	0.0705	3.5018	0.03039	*
Residuals	1494	30.0609	0.0201			

Two-way ANOVA table: Response: log(SLA:SEA)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
TIME	2	201.37	100.68	200.0845	< 2e-16	***
SITE	1	60.79	60.79	120.7974	< 2e-16	***
TIME:SITE	2	4.18	2.09	4.1557	0.01586	*
Residuals	1494	751.78	0.50			

Conclusion

We investigated the potential for air exposure to adversely affect the ratios SEA:SDA and SLA:SEA in mussels from the River Exe and Tamar as indicators of stress. We conclude:

- There is a significant difference between the three time points measured, with respect to air exposure and the mean ratios of SEA:SDA and SLA:SDA
- The greatest changes were observed between the time periods t= 0 and t= 24
- This was demonstrated at the River Exe and the River Tamar
- SEA:SDA and SLA:SEA in mussels from the River Tamar was lower and higher than the River Exe respectively. This could indicate the presence of other potential environmental factors having a compounding effect

Summary

These results illustrate the importance of standardised sampling methodology when sampling mussels for measurement of biological effects in marine monitoring programmes. We recommend that the duration of air exposure should be kept consistent across all sampling events in biological effects monitoring programmes utilising mussel histopathology, specifically relating to the digestive diverticula. Adopting quality assurance principals with respect to sampling and transit to the analytical laboratory will help to limit any sampling artefact that may be related to consequent air exposure.

Acknowledgments

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