THE USE OF NORMAL PHASE HPLC IN EFFECT DIRECTED IDENTIFICATION STUDIES USING THE MUSSEL *MYTILUS EDULIS*

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Introduction

During the 1970's and 80's scientists at Plymouth Marine Laboratory (PML) developed and applied a marine coastal environmental health monitoring procedure based on the physiological condition of mussels.

The monitoring procedure developed is termed Scope for Growth (SFG). SFG is a measurement of the energy balance of mussels determined from the rate of food uptake and its assimilation efficiency. This energy balance is strongly indicative of the health of the mussel, since a high positive energy balance is only achieved when a wide range of biochemical processes within the organism are functioning correctly.

A key element of this work was the simultaneous measurement of body burdens of selected chemical contaminants. These chemical measurements provided a means to link observed poor mussel health to causative agents

At many sites the levels of the limited number of contaminants analysed were insufficient to account for the severity of the biological impact. The failure to identify the cause(s) of major deleterious biological effects represents a serious challenge to water quality management

There is therefore a need to develop a practical protocol which can be used to augment SFG in monitoring programmes where toxic effects are unexplained through chemical monitoring inventories

Toxicity-Identification Evaluation (TIE) like procedures are now frequently applied to sediment and water samples taken from the environment.

These procedures essentially involve extraction of the toxic agents from the matrix of interest, fractionation of the extracts using physicochemical techniques to separate the individual toxic agents or groups of related toxic agents, testing and quantifying the toxicity of these fractions and identifying, where possible, the toxic chemical agents by means of appropriate analytical techniques

There are very few published studies of the successful application of such procedures to the tissues of organisms. Organisms are a highly complex matrix of organic and inorganic chemicals, from which pollutant chemicals are not readily resolved. Indeed, some naturally occurring biochemical agents when released from their compartmentalised form within the organism, can themselves be toxic

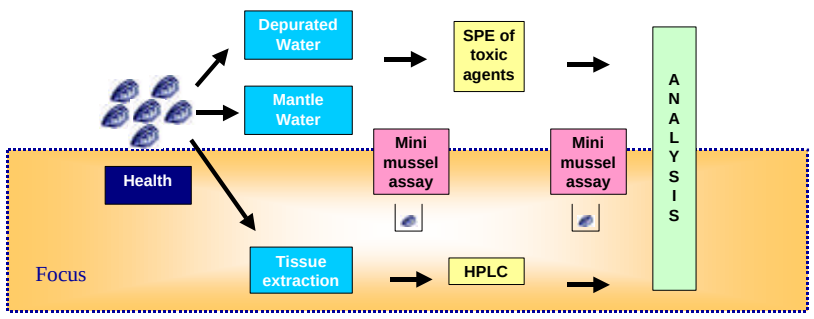
TIE type studies on the tissues of organisms represent a considerable technical challenge

Objectives

Do the tissues of field populations of mussels exhibiting reduced SFG contain toxic pollutants that are causing this reduction?

Can these toxic pollutants be identified?

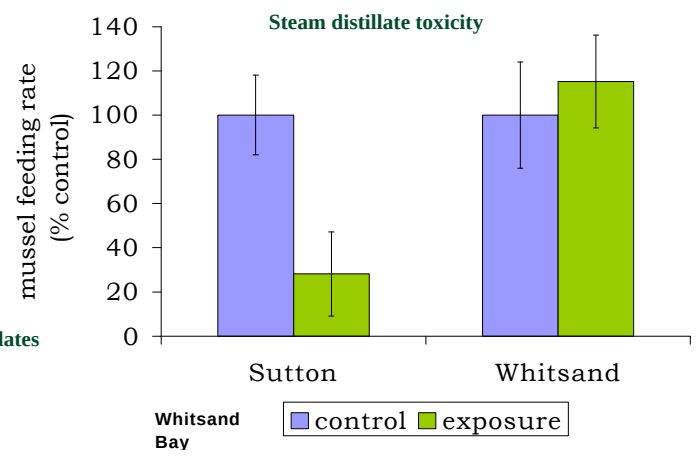
Overall Procedure



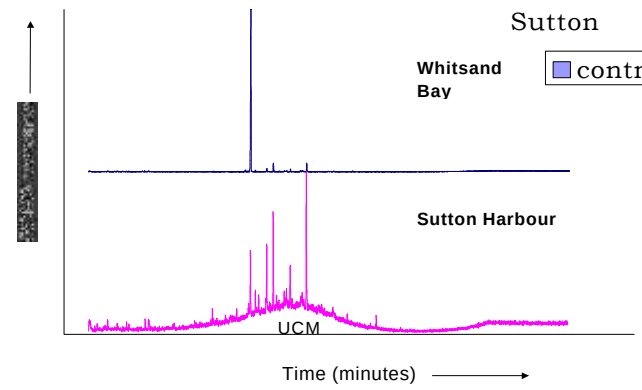
To ensure maximum speed, sensitivity and direct relevance to field monitoring programmes using SFG we further developed our existing sub-lethal effect toxicity assay based on the determination of feeding rate of small juvenile mussels. Feeding rate is the key pollution sensitive parameter used in SFG determination of adult mussels

Example of toxicity of pollutants in mussels from a clean site (Whitsand) and a polluted site (Sutton)

- the steam distillates from a clean site (Whitsand Bay) do not cause a decrease in mussel feeding rate
- the steam distillates from Sutton Harbour (polluted site) decrease mussel feeding rate by 70%



Gas chromatograms of distillates



- the distillates from the clean site (Whitsand Bay) exhibit only naturally occuring compounds
- the distillates from the polluted site (Sutton Harbour) exhibit a large UCM characteristic of oil pollution

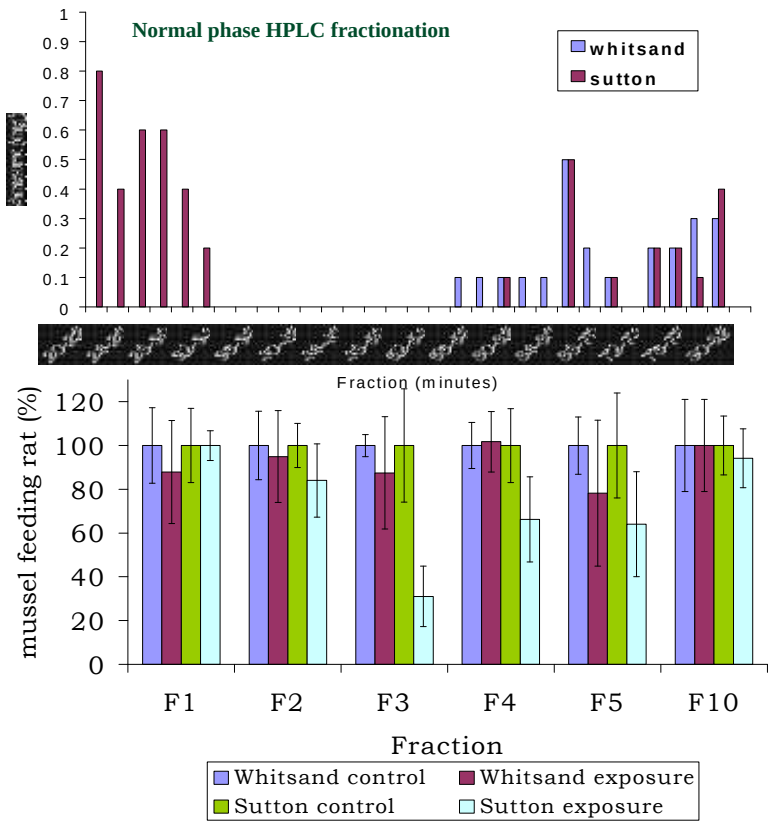
Toxic pollutants in the tissues of mussels

Pollutants were extracted from the tissues of polluted mussels by steam distillation and extracts shown to be toxic in the juvenile mussel feeding rate assay

Questions are sometimes raised as to whether reduced SFG in field populations of mussels is due to observed tissue residues of toxic pollutants or whether other factors are involved

This tissue extraction/toxicity test procedure provides unequivocal demonstration that tissues of mussels from polluted sites contain agents toxic to mussels, irrespective of whether these agents can be identified

Normal phase HPLC fractionation



Toxicity of HPLC fractions

Normal Phase HPLC fractionation

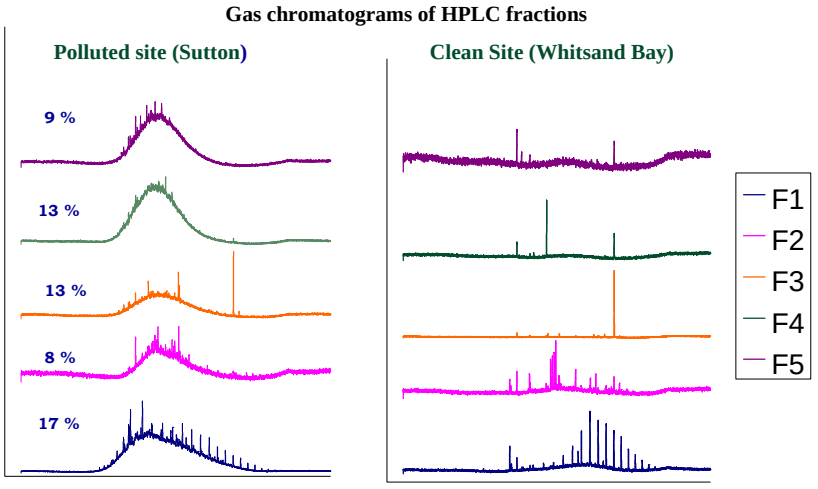
The steam distillates were further fractionated (2 min cuts from 20 – 80 min) using normal phase HPLC (Dionex GP40 gradient pump [Dionex Corp., USA] with 3 x Hypersil Hyperprep HSAPS-2 [ThermoHypersil-Keystone] 250 mm x 10 mm x 8 µm columns in series with a guard column; flow rate 2 ml min^{-1}; 0 – 40 min [100 % hexane], 40 – 45 min [gradient to 100 % DCM], 65 – 70 min [gradient to 100 % hexane], with a Dionex AD20 UV absorbance detector at 254/280 nm).

Despite separation of the extracts into 30 fractions each toxic fraction contained complex mixture of chemicals unresolvable by high resolution gas chromatography

The toxic agents in extracts of mussels from a polluted harbour occurred largely in 3 fractions

The toxic agents were structurally complex aromatic hydrocarbons derived mainly from the use of mineral oils particularly fuel oil such as diesel

Gas chromatograms of HPLC fractions



Summary

- Scope for Growth monitoring data highlights locations of greatest concern
- Toxicants are accumulated in animal tissue - complex matrix
- Whole animal sub-lethal bioassay
- Direct link between toxicity test and field
- Steam distillate method provides rapid assessment of sites
- Normal phase HPLC allows fine fractionation of accumulated chemicals
- Normal phase HPLC fractions show different toxicity
- Even using fine fractionation complex mixtures of compounds are found
- Toxicity cannot be assigned to one compound
- Importance of mixture toxicity
- Polar chemicals may be masked by natural polar compounds at other sites

Acknowledgements: We wish to thank DEFRA for supporting this research. Grant CDEP 84/5/267