MUSSELS AS A TOOL IN IMPACT ASSESSMENT

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ABSTRACT

The mussel *Mytilus edulis* L. has a substantial filtering capacity and is therefore capable of concentrating large amounts of toxic compounds, both dissolved in water, or adsorbed to particles. Many hydrophobic xenobiotics are not excreted so easily and will therefore be present in the mussels for long time. I describe some methods used in Sweden, technique of sampling, toxicity tests, such as the mussel embryo bioassays, energy budget tests (scope for growth) and behaviour tests.

INTRODUCTION

All over the world biologists are working with applied research connected to water pollutants. Lately, there has been an increasing interest among authorities and scientists to use biological indicators, not only because it is often a more direct way to get an answer about the state of impact on fauna and flora, but also that many methods could be used without the need of expensive, sophisticated laboratory equipment or chemical analyses.

During recent years, international organizations like IOC and ICES have focused on bottom sediments as these normally reflect what has happened in the water column during a certain time, compared to a water sample which just will show the instantaneous condition at the time of sampling. Furthermore, the sediment is an important part of the aquatic environment as a habitat for sessile fauna and flora which serve as a food source for benthic and pelagic life. Sediment samples can be taken back to the laboratory where a battery of different toxicity bioassays can be used. The results from these will give an answer of the degree of bioavailable pollutants at each site.

Early in 1978, the first meeting about the possibility to use mussels as an indicator of pollution was held in Barcelona, Spain. At that time the "mussel watch" programme was introduced, implying that the level of contamination is measured in mussels along coastal areas to support environmental monitoring or regulation programmes. Such "mussel" programmes operate now in many countries around the world, including Asia (Goldberg 1986). In the course of time, new methods have also been developed for further investigations of mussels both in the laboratory and in the field. Effect parameters, which span studies from the cellular level to the organism level, are used. Search for new „biomarkers" to serve as early warning systems for pollutant impact, is continuously in progress. For example, defence mechanisms in the organism can be used as markers for early detection of environmental stress (Hansen *et al.* 1991; Herbert & Zahn 1989). It is then possible to study various cellular and subcellular parameters which could reflect the state of defence mechanisms such as changes of plasma membranes, mitochondria, lysosomes, *etc.* (Sanders *et al.* 1991) or enzymatic activities (Coppage & Braidech 1976; Bocquene *et al.* 1990).

The use of mussels as test organisms has many advantages. *Mytilus edulis* can filter 7-10 l sea water h^{-1}. The bivalve is capable of concentrating large amounts of toxic compounds, both dissolved and adsorbed to particles. Many hydrophobic xenobiotics are not excreted so easily and will therefore be present in the mussels for a long time. As a consequence of this, molluscs cultivated in polluted waters may be a health risk for consumers.

This paper describes examples of some methods used in Sweden, technique of sampling, toxicity test methods (mussel embryo bioassays), energy budget tests (scope for growth) and behaviour tests.

REVIEW OF METHODS AND RESULTS

Sediment sampling

Sediment is taken by grab samplers which allow collection of an undisturbed part of the bottom sediment. The excess water is carefully removed, the upper two

cm scraped off, stored in glass vials, and immediately brought back to the laboratory.
Because the pore water (interstitial water) is in close contact with the sediment particles, and also quite isolated from the water column, an equilibrium partitioning is established between pollutants adsorbed on particles and those dissolved in water. The pollutants dissolved in water are the most bioavailable part, the use of pore water for testing will therefore be highly relevant.
Pore water is obtained by centrifugation. The water may then be used directly for testing, or may be stored frozen for later use. By applying stepwise procedures (Mount & Anderson-Carnahan 1988 a,b,c) the pore water may be treated in different ways in order to isolate specific groups of pollutants. For example, by adding chelators as EDTA it is possible to bind metals to non-bioavailable chelates. Thus, testing before and after such addition will give a guidance to whether present metals were responsible for the toxicity obtained. After extraction of the pore water with a solvent, for example hexane, it is also possible to separate most of the organic pollutants. Toxic remains in the water phase will then indicate the presence of inorganic pollutants such as metals.
When toxicity of pore water is low, there is a possibility to use solvent extraction to separate a greater part of the sediment-bound pollutants. This may be done by extracting a sediment sample by, *e.g.*, acetone in a Soxhlet apparatus. The extracts can then be used for a variety of biological tests which permit the finding of a dose-response relationship. By an extraction procedure, the main part of organic hydrophobic pollutants are transferred from the sediment to the solvent, involving also the ones which in a real field situation are firmly adsorbed to particles and then less bioavailable. In consequence, results from extract tests are hard to interpret and are therefore most suited for identification and ranking of toxic sediments.

Mussel embryo bioassay

When man changes the environment beyond the natural limits for populations, then production of gametes, reproductive behaviour or development of the young life stages may be altered and lead to a decline of a population, or even to its disappearance. The study of early developmental stages may therefore be of high priority. Larval stages have also shown to often constitute the most sensitive links in the life cycle of an organism.
Changes of environmental conditions during the period when most of the specializations occur, may have great influence on these processes. The result may appear as abnormalities, delayed growth, or mortality of the embryos. The mussel embryo bioassay may be used for pore water or solvent extracts. The following is a description of the method used at the Kristineberg Marine Research station (KMF):

Pore water is tested after adjustment of pH, and a coarse filtering to remove particles which otherwise may disturb fertilization and early development of the embryos. Spawning is induced in adult mussels by rigorous shaking and exposure to an increased water temperature. Eggs (50 ml^{-1}) are then transferred to two parallel sets of glass jars (1.2 ml), one droplet of sperm is added, and fertilization occurs. Three hours from start, the development is interrupted in one set of jars by adding a droplet of 5 % formaldehyde. The frequency of fertilization can now be measured by counting the embryos and the unfertilized eggs. In the other set of jars, development is interrupted after 72 hrs and the number of veliger larvae as well as abnormal larvae forms are counted (Fig. 1).
An example of results from a mussel larvae bioassay with sediment from Skagerrak, a part of the North Sea, is presented in Fig. 2. As seen some hot spots could be identified which then may be correlated with sediment chemical data.

Scope for growth (SFG)

This method is an effort to evaluate the energy budget in individual mussels by using parameters like oxygen consumption, excretion, food efficiency and filtering capacity.
SFG was developed in Great Britain (Widdows 1985). It is used in several monitoring programs in Europe as well as in the United States and Sweden (Magnusson *et al.* 1988). In two ICES/IOC workshops (Oslo and Bermuda) where several different toxicity tests were compared, the scope for growth method showed to discriminate very well with the pollution gradient chosen (IOC 1987). Recently this method has been recommended by ICES (1994) for use in biological monitoring. Also in laboratory experiments with single chemicals the method has worked well (Granmo *et al.* 1989). The SFG index is derived from the expression:

$P = SFG = A - (R+U)$ J $h^{-1} * g^{-1}$ dry weight mussel tissue

where:
A = energy absorbed from food
R = energy lost through respiration
U = energy lost through nitrogen excretion
P = energy stored in the organism as somatic tissue and gamete production

The practical procedure of the SFG method is described by Widdows & Salkeld (1992) The general principle can be illustrated as in Fig. 3. The method requires some rather expensive equipment: a particle counter and an oxygen meter. The mussels must be measured as quickly as possible after they have been transferred to the laboratory, so it is an advantage to have two or more sets of equipment in order to save time.
A series of field experiments were performed outside an industrial plant on the west coast of Sweden. An example of results is given in Fig. 4. During the experiment an accidental release of hydrocarbon waste from one of the industrial plants could later be detected as an uptake in the mussels as shown in Fig. 5. The measured bioaccumulation data gives a good example of the possibilities to study pollution by using biological indicators, like mussels.

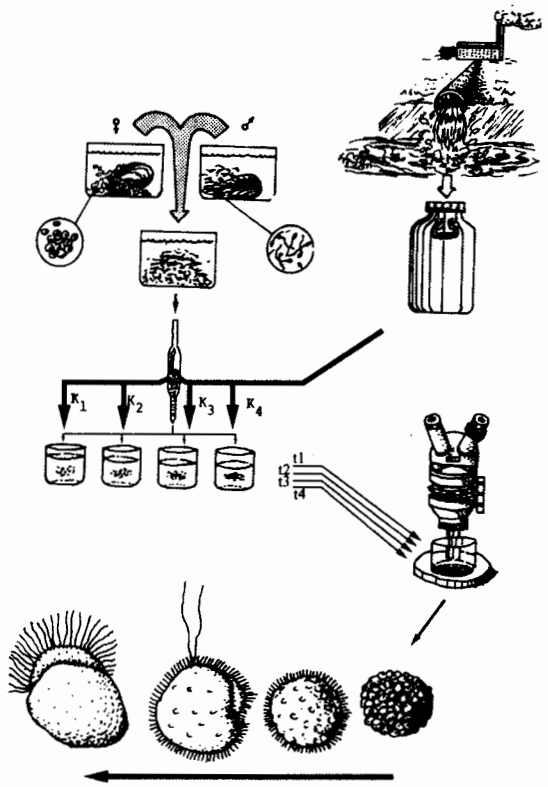


Figure 1. General principle for mussel embryo bioassay.

Burrowing behaviour
This test has been developed in our laboratory for benthic deposit feeding mussels, *Abra* sp., *Macoma* sp. *etc.*, but can be used also with other burrowing invertebrates. The method has shown to be very sensitive for certain pollutants. It is very simple to perform and can be used as a first screening method for detection of contaminated sediments. For this test, newly sampled whole sediment has to be used. The mussels are exposed to the sediment for at least 3-5 days. After that, they are transferred to the surface layer of a „standard sediment" from a less polluted area. The time needed for burrowing is continuously recorded during a couple of hours, and the results from different sediment samples can then be compared. Records of burrowing can be done by direct visual observation, or with a videocamera.
An illustration from a test in a pollution gradient outside a bleached-pulp mill (Fig. 6) indicates that the burrowing behaviour could be a sensitive tool for the study of organochlorine pollution (Granmo *et al.* 1991).

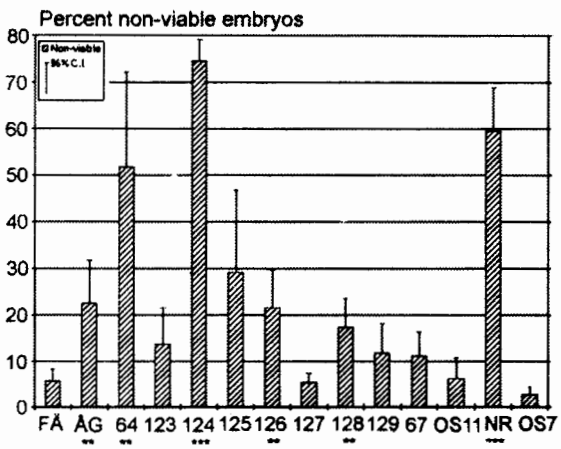
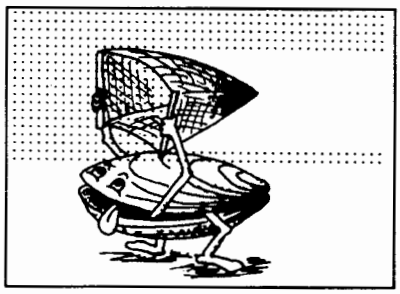


Figure 2. Percent of nonviable embryos (72 h) from fertilized eggs of *Mytilus edulis* incubated in pore water from sediments sampled at different stations in Skagerrak. Vertical bars indicate 95 % confidence limits. Nonparametric statistical test for significance (Mann-Whitney) between reference (Fä) and other stations indicated by stars.

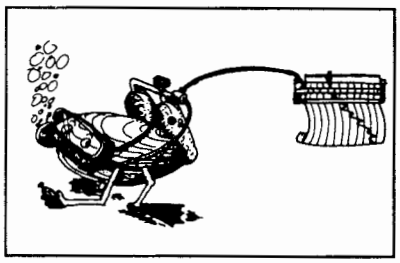
P = SFG = A-(R + U) in J/(h'g) dry weight mussel tissue

Where:

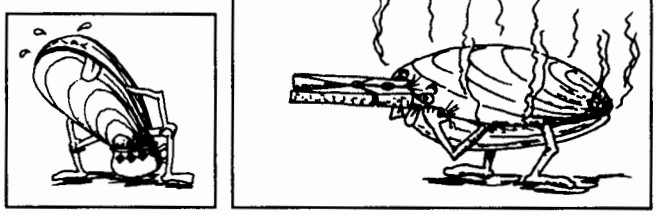
A = egergy absorbed from food



R = energy lost through respiration



U = energy lost through excretion



P = energy stored in the organism as somatic tissue and gamete production

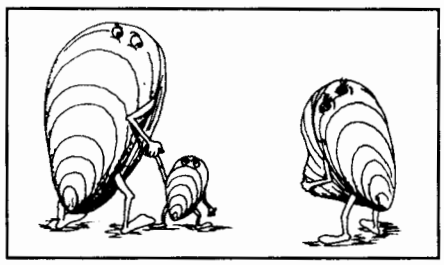


Figure 3. General principles of the measurements of Scope For Growth. Illustrations by Matz Berggren.

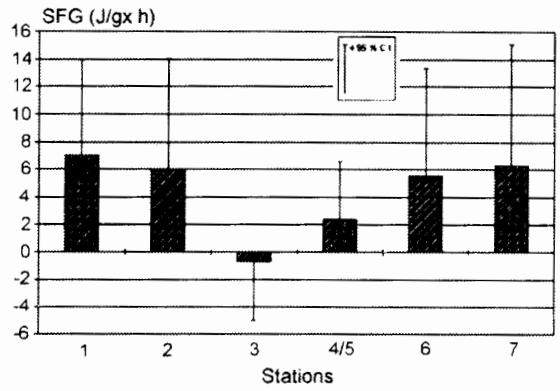


Figure 4. Scope for growth indexes for caged mussels (*Mytilus edulis* L.) in receiving waters from a petrochemical plant. Vertical bars indicate 95% confidence limits.

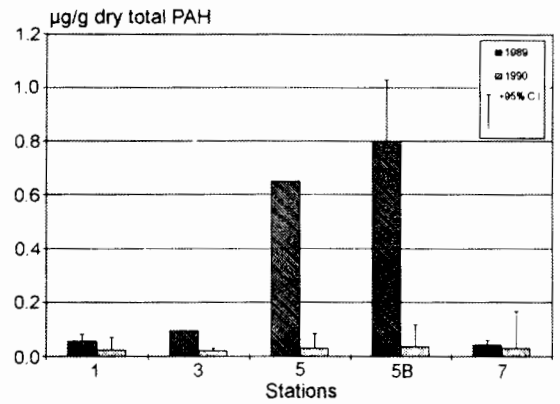


Figure 5. Bioaccumulation of PAH in caged mussels following an accidental waste from a petrochemical plant. Vertical bars indicate 95% confidence limits.

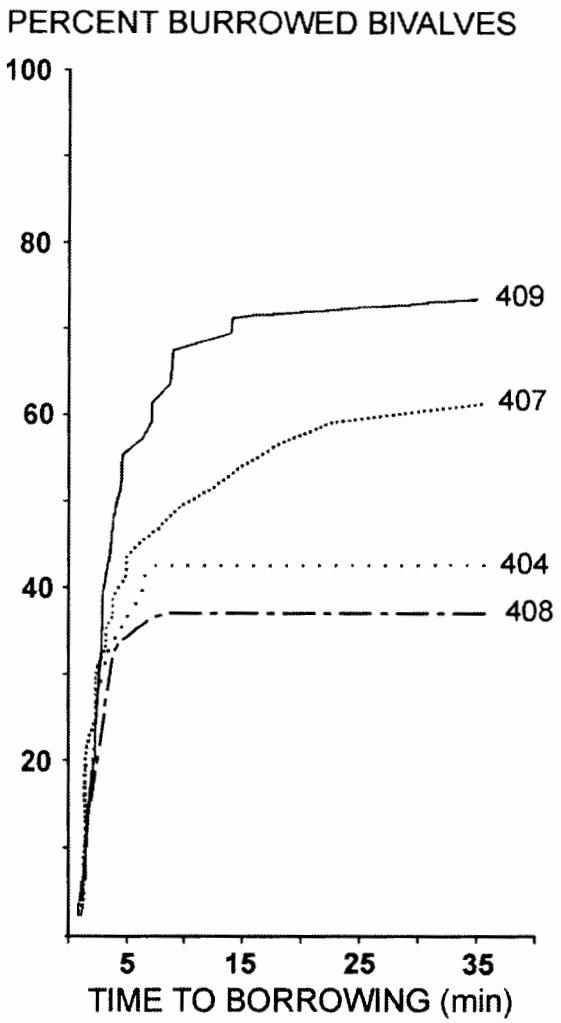


Figure 6. An example of burrowing activity of *Abra nitida* when exposed to contaminated sediments in a bleached-pulp mill pollution gradient. From Granmo *et al.* (1991).

CONCLUSIONS

By using biological methods it is often possible to get a good indication of pollution impact. The use of such methods are often quite simple and cost-effective and provide an integration of all contaminants present, known and unknown. This biology-led monitoring strategy may therefore be used as a first step in investigations of an area before the more costly chemically based programmes are initiated.

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