USE OF CAGED MUSSELS TO DETECT ENVIRONMENTAL EFFECTS OF POLLUTANTS

Å. Granmo
Göteborg University, Kristineberg Marine Research Station, SE-450 34 Fiskebäckskil, Sweden

ABSTRACT

In the marine environment, ecologically important organisms such as sessile invertebrates are often at great risk as they are unable to avoid changed environmental conditions. However, our knowledge about effects of pollutants in this group is restricted to a few widely used species. Among these, bivalves are of major ecological importance. In the present paper, different established methods but also new promising methods are described where caged mussels are used to assess bioavailability and associated bioeffects of contaminants. Suggestions are given for investigations directed towards general as well as more specific impacts of pollution. The described methods cover effects from the cellular up to the population level of organization.

INTRODUCTION

The global environment has been influenced by man much more than by any other organism. During the last 150 years, industrial development together with the great increase in human population has accelerated this process. Today, we are concerned about the global impact on both water and atmosphere. The measures necessary to stop this development are very complex and depend on several factors of political, economic, social and scientific origin.

During many years technical and chemical methods were the dominating scientific tools for the detection of water pollution. However, this presupposes the presence of chemically trained personnel and access to a well equipped chemical laboratory, which generally may not be found elsewhere than in industrialized countries.

During the last years the interest has increased to use more biologically directed methods which have many advantages as they are more focused on the living resources in the sea and could therefore reflect the actual state of water quality more directly. Once a problematic area has been identified, a more specific investigation where also chemical analyses are included can be carried out.

After identification of water quality the next step will be to make improvements for a better environment. Factors such as legislation, economic interests or national or local political attitudes may here be crucial barriers. However, these problems may be overcome by other tools and will not be dealt with here. Generally, it has in many cases been shown that if presented results are based on a solid and high quality scientific basis the possibilities for positive actions are good.

There are many advantages of using *in-situ* bioassays, *i.e.* to transfer organisms to different sites of interest, as the results give direct information on marine environmental quality that would be impossible to obtain through chemical monitoring alone or laboratory assays.

In the present paper some simple methods are described which have shown promise for further use and which can be used even at laboratories not especially instrumentally equipped.

General advice for caging and transplanting molluscs

Sessile species have often been shown to be very sensitive to different kinds of pollutants and will therefore constitute a suitable tool for impact studies in most water bodies. The transplant method with molluscs combines laboratory testing under controlled conditions with the realism of field exposure (Green *et al.* 1985) and also time integration. Bivalves and gastropods have hard shells and are generally well suited for caging and transplant studies. By caging it is possible to select gradients, moving animals for detoxification studies or studying dose-response relationships over different environmentally realistic conditions.

Many practical aspects have to be taken into account. For example, in the open sea you need to keep the animals so that they are not stressed or hurt by strong mechanical movements *e. g.* by waves or currents. One way of doing this is to use compartmentalized cages, i.e. trays or mesh bags with one mussel per cell (Salazar and Salazar (1995), (Fig. 1). In that way each individual could be studied and the bivalves do not clump, which might affect filtration, water pumping and accumulation of pollutants for the animals in the center of a clump.

Use of juvenile bivalves is recommended as they are normally more sensitive and have a higher growth rate. When collecting mussels for transplant studies it is favourable to use as small a range of length as possible (preferably within 5 mm). By using an analysis of variance (ANOVA) before start of exposure it is also possible to ensure a homogenuous material in the different cages. This will automatically give better and more solid results. The number of specimens for use depends on the kind of measurements to be done, but normally between 50 – 100 test animals per site should be used. These bivalves should be taken from a supposedly clean area with no known pollutants.

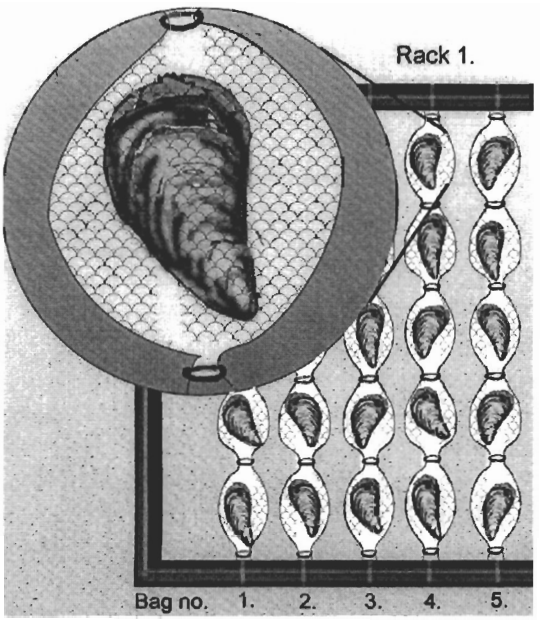

Fig.1. Rigs for caging bivalves according to a method by Salazar *et al.* (1996).

The exposure period may last 60 – 90 days which would be enough for bioaccumulation of major priority pollutants as also for different cellular or physiological processes to be activated within the organism. During such a long time, fouling on the cages may arise and the cages have to be cleaned now and then to avoid worse life conditions for the test animals.

METHODS

There are many different possibilities to study the caged animals and a battery of methods is recommended.

At the cellular level

Lysosome stability

Cellular changes are used as indicators of toxic impact in testing new chemical products in laboratory rats and have also been used in environmental investigations. During the last ten years, molluscan blood cells have been investigated. It is known that they play a role in the immune system

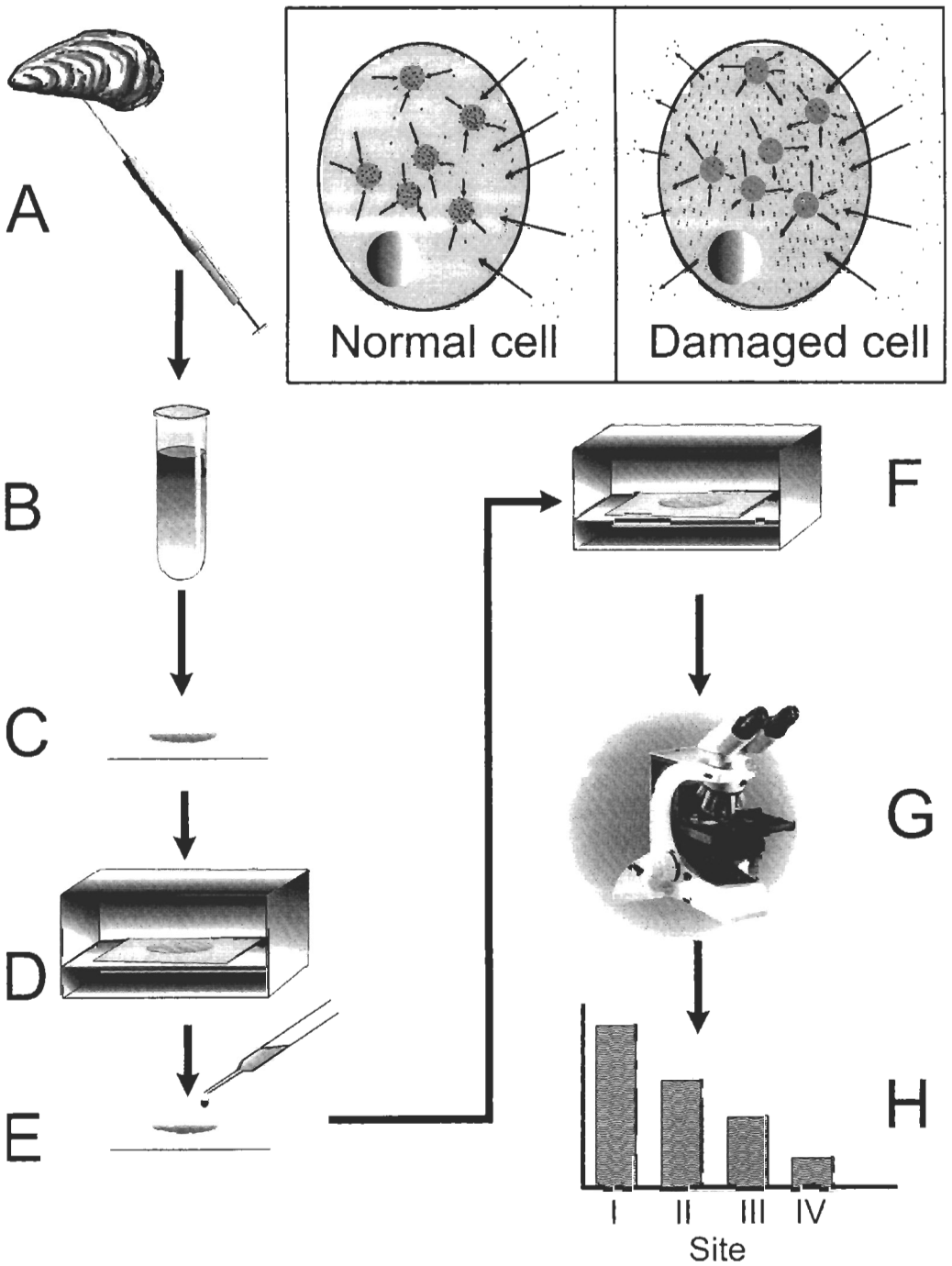

Fig. 2.Measurement of lysosomal stability according to Lowe *et al.* (1995).
A: haemolymph sampling; B: dilution in physical saline; C: transfer to a slide; D: store in a humidity chamber; G: read under microscope; H: result presentation.

response as the blood cell lysosomes release enzymes (acid hydrolases) which are able to degrade circulating pathogens (Lowe *et al.* 1995). However, this release of acid hydrolases must happen in a controlled manner otherwise general damage of the cell will occur. The lysosomes have a high ability to accumulate a diverse range of toxic metals and organic chemicals (Moore 1990). This may result in a higher risk of cell injury. Now, it has been found that the capacity of the cell to take up the dye neutral red could be used as an indicator of cell damage, as it was found that healthy cells could accumulate and keep the dye much better than damaged cells (Borenfreund & Puerner 1985). The method implies that after exposure in the field the bivalves are incubated in neutral red solution. The amount of dye is then extracted from the animals and quantified by a spectrophotometer. By a modification of this method, Lowe & Pipe (1994) and Lowe *et al.* (1995) used a microscope to measure neutral red uptake in living cells. In a study taken place in the Venice lagoon, Italy the method showed a high sensitivity and the results correlated well with the body burden of organochlorines, mercury and PCB (Lowe *et al.* 1995). Some practical details for the measurements are given in Fig. 2 and in Tab. 1.

Phagocytosis

In all animals there are cells which have the capacity to ingest particles, and in single celled animals it is the means of food ingestion. This function also serves as a defence mechanism of invertebrates.

Phagocytosis involves different sequences as: foreign particle identification, uptake, destruction and disposal. The hemocytes in the blood are responsible for the elimination of invading microorganisms and foreign particles.

A simple method for the determination of phagocytic activity using mussel blood cells (hemocytes) has been described by Hansen *et al.* (1991) (Fig. 3).

Hemolymph is taken from the blood sinus in the adductor muscle. Yeast cells are added and after 30 min. incubation at 20°C and addition of lectin, an agent for agglutinating the hemocytes, a phagocytosis index, i.e. the number of phagocyted yeast cells per 100 hemocytes can be determined by counting under the microscope.

Hansen and coworkers also mention the possibility to add bioluminiscent bacteria (*Photobacterium phosphoreum*) normally used for the Microtox®- test system.The hemocytes of the mussels will engulf the bacteria and the decrease of the bioluminiscence measured by a Microtox® spectrophotometer set up is directly related to the phagocytic activity.

Table 1. General practical advice for measurement of lysosome stability in molluscs.

- The techniques can be applied to a broad range of bivalve and gastropod molluscs as well as teleost fish.
- Sampling of >10 animals of the same juvenile size class.
- Avoid sampling during the spawning season
- Take animals from the sublittoral region
- Handle carefully when transported back to laboratory. Use tissue paper soaked in water to keep humidity.
- If transported more than 3-4 h. use ice pack in the transport box.
- Use a binocular light microscope xlO, 25 and x 40. If possible use a green filter 580 nm.
- For the performance of the test follow detailed descriptions given by Lowe *et al.*(1995).

Individual level

Measurement of growth

Growth is a parameter which is often used in field investigations. It is often used as a measure of effects because it provides an integration of many biological processes (Bayne *et al.* 1985). Advice of practical handling of this has been given in literature e. g. by Salazar (1992). Generally these measurements should be done together with recording of survival. Poor growth or high mortality at the control sites (> 20%) indicate bad condition among the test animals and then the test should be repeated. Measurements of growth may be repeated during considerable time at different conditions to get optimal information.

The most common ways to measure growth are shell length, height and weight measurements. Length measurements should be made to the nearest 0.1 mm and weight to the nearest 0.01 gram. Weight measurements are generally more accurate than length measurements and dry weight better than wet weight. However, length measurement is a non-destructive method which allows multiple measurements during the exposure time. Before weighing the water in the mantle cavity has to be removed. In order not to stress the animals, they should not be measured more often than every second or third week.

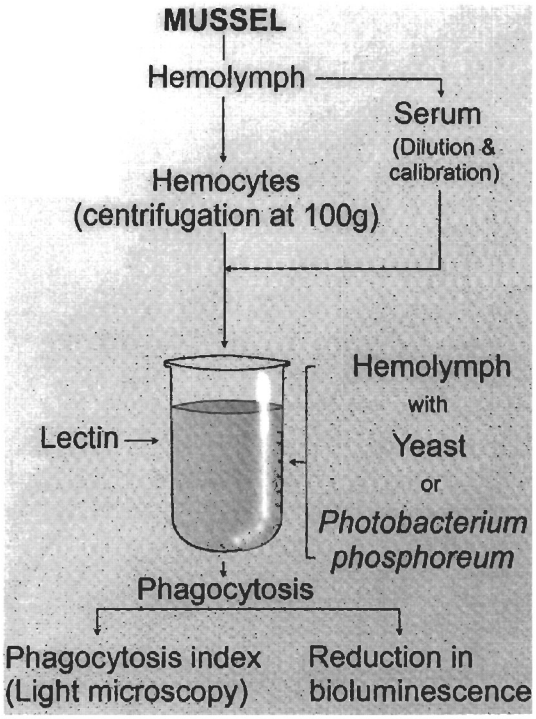

Fig.3. Principle for the phagocytosis test with bivalves. Redrawn after Hansen *et al.* (1991).

Another method is to measure the soft tissue weight at the end of the exposure. This was used successfully by Salazar and Salazar (in press) and showed to provide information more useful than length and total weight measurements.

Growth data should be statistically analyzed by an ANOVA procedure. Individual bivalves in each cage are then treated as replicates to increase the statistical power.

Finally, another conventional method much used is to measure the condition index. The index of body condition (CI) has been formulated by Fischer (1988) as: CI = 100% x (soft tissue dry weight) : (soft tissue dry weight + shell weight).

When using growth as an indicator of toxic effects in the field, one has to be aware of that growth is also affected by other factors such as nutrient supply, temperature and salinity.

Biaccumulation

The great capability of bivalves to accumulate pollutants has been used all over the world. There are many reasons for this phenomenon *e. g.* a great pumping and filtering capacity (a common mussel may filter 5 – 10 l/h). Furthermore, most bivalve species have less developed physiological detoxification systems, *i.e.* a lower capacity to convert the pollutants into intermediate products which can be more easily excreted. As bivalves constitute an essential link in

the marine food web as a food source for predatory vertebrates or invertebrates they are of great importance to study for the understanding of the impact of pollution on the whole ecosystem. Furthermore, as there is a large number of literature data from all over the world there are many possibilities to compare results. Most investigations have used common mussels or oysters, but in Asia also for example green-lipped mussels (*Perna viridis*) have been used as bioindicators of pollution (Tanabe and Tatsukawa 1987). Chemical analyses of tissues of bivalves give important complementary data to other measurements but due to high costs it is often performed on a restricted number of samples which in turn gives a low statistical power. Nevertheless, if possible, such measurements are recommended.

Anoxia tolerance

Along many coastal areas there are strong tides which regularly cause periods of dryness to the littoral fauna. Bivalves have the capability to close their valves during these periods. By extending the time period

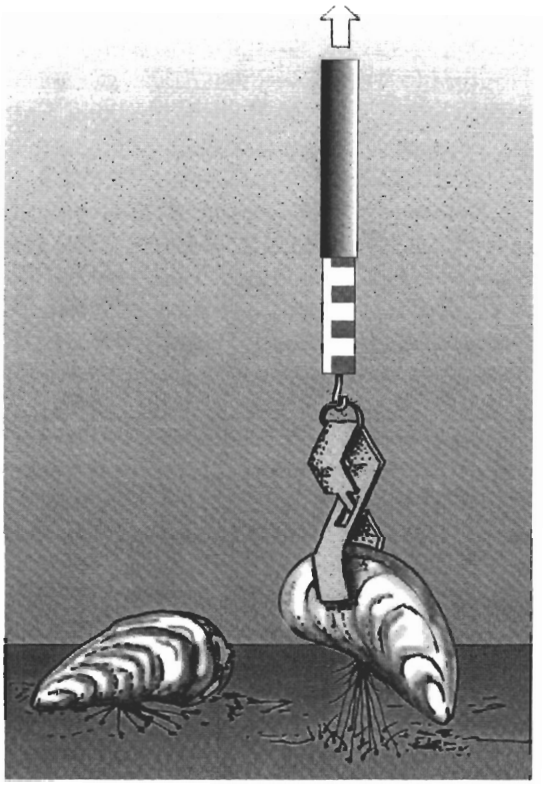

Fig. 4.Method for measuring byssal thread formation and strength in bivalves

Table 2. Different monitoring methods with caged bivalves.

Method	Pollutants	References
Reproduction	Metals, oil, organochlorines, PAH, surfactants	Ekelund *et al.* 1983; Granmo 1995; Thain 1991
Growth	Metals, organochlorines, oil, paper and pulp wastes	Fischer 1988; Salazar 1992; Salazar & Salazar 1995; Salazar *et al.* 1996
SFG	Metals, oil, PAH, organochlorines	Ekelund *et al.* 1983; Granmo 1995; Widdows & Salkeld 1992
Byssal thread formation	Metals, oil, surfactants	Ekelund *et al.* 1983; Roberts 1975
Lysosome stability	Metals, oil, PAH, PCB, pesticides	Borenfreund & Puerner 1985; Lowe *et al.* 1995; Lowe & Pipe 1994; Lowe *et al.* 1995; Moore 1990
Phagocytosis	Metals, oil, pesticides	Hansen *et al.* 1991
Bioaccumulation	Many metals and organic pollutants	Salazar & Salazar 1995; Salazar *et al.* 1996; Tanabe & Tatsukawa 1987
Anoxia tolerance	Metals, oil, PCB, general pollution	Veldhuizen-Tsoerkan *et al.* 1991; Zandee *et al.* 1986

of dryness it is possible to study the ability of the animals to survive exposure to air. This is interpreted as a decreased ability of the bivalve populations to cope with natural stress. The method is extremely simple to perform (Zandee *et al.* 1986) and implies measuring the actual time for survival in dryness in a constant moisture. It has been used on bivalves exposed in the field in the Netherlands (Veldhuizen-Tsoerkan *et al.* 1991) where significant differences were found after 2-5 months exposure between polluted and unpolluted sites.

Byssal thread formation and strength
Different sessile species of mussels develop byssus threads in order be safely attached to a suitable substratum. Byssal threads are formed by means of glands producing a secretion which rapidly hardens to strings when released into the water. Any change in this formation has a drastic influence on the possibility of attachment and survival of the mussels. It has been shown that this formation could be a sensitive indicator to study impact of pollution (Roberts, 1975, Ekelund and Granmo, 1983). The mussels could be taken from different sites and brought to tanks in the laboratory. After 1-2 days the number of specimens with threads are counted. By using a spring balance equipped with a clip it is also possible to measure the strength necessary to loosen the attached mussels from their bedding, an even more sensitive parameter (Fig.4).

Reproduction
Early life stages normally represent the most sensitive part of the life cycle of an animal. Therefore reproduction tests have been much used. A method for mussels and oysters has been described by for example Granmo (1995) and Thain (1991).

Population level

Energy budget
The scope for growth index (SFG) is a value which shows how much energy from food is left for growth and reproduction after basic metabolic requirements have been covered. This index has shown to be a sensitive parameter to study and several investigations are described in the literature and a detailed description of the method has been given earlier (Widdows and Salkeld 1992; Granmo 1995).

A summary of the different described methods and their application is given in Table 2. When planning a monitoring programme for water quality assessment such methods may provide a first valuable step in the identification process.

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