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# The valve movement response of mussels: a tool in biological monitoring

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## Abstract

Biological sensors are becoming more important to monitor the quality of the aquatic environment. In this paper the valve movement response of freshwater (*Dreissena polymorpha*) and marine (*Mytilus edulis*) mussels is presented as a tool in monitoring studies. Examples of various methods for data storage and data treatment are presented, elucidating easier operation and lower detection limits. Several applications are mentioned, including an early warning system based on this valve movement response of mussels.

## Introduction

Monitoring of the quality status of natural waters has traditionally been carried out with physico-chemical techniques. For many years now, attempts have been made to include the biological response of organisms in monitoring systems for the detection of pollution in the aquatic environment (Cairns, 1979; Bayne *et al.*, 1985; Gruber & Diamond, 1988). Bivalves agree very well with the requirements that should be met when selecting a suitable monitoring organism. They are sedentary, abundant and available throughout the year. Furthermore the organisms should have a manageable size and be hardy enough to be handled in the laboratory. (Phillips, 1977, 1980).

The concentration of pollutants in molluscs can serve as an indicator for the level of pollution. Mussels (e.g. the blue mussel, *Mytilus edulis*) have been widely adopted in chemical monitoring and surveillance programmes (Goldberg *et al.*, 1978; NAS, 1980; Widdows *et al.*, 1981; de Kock, 1986). Their ability to accumulate toxicants to a level representative for the integrated environmental conditions, makes them suitable for the characterization of specific ecosystems. In the passive form of chemical biomonitoring local populations are sampled for chemical analysis, whereas the active version involves the exposure of organisms translocated from reference sites. The disadvantage of these bioaccumulation studies is, that the equilibrium concentration is usually obtained only after several weeks of exposure. This makes them unsuitable as an early warning system.

In contrast to the former method, physiological and behavioural changes or reactions are usually fast and thus potentially suitable for a fast response in continuous biological monitoring.

Several biological monitoring systems, using fish, have been developed in recent decades, which are based on the change in rheotaxis, respiration, gill activity or electrical field alterations (Juhnke & Besch, 1971; Poels, 1977;

Gruber & Diamond, 1988; Geller, 1984). The change in activity of *Daphnia sp* (Knie, 1982), the metabolic luminescent activity of bacteria in test systems like 'Microtox' (Beckman) and physiological effects of bivalves (Slooff *et al.*, 1983) have also been applied.

Following the criteria for optimal functioning of organisms in a biological warning system, bivalves offer many possibilities with respect to continuous and automatic detection, reliable and fast response, handling and data interpretation, (Koeman *et al.*, 1979; Cairns, 1979).

If we limit ourselves to the studies of the bivalve molluscs, physiological parameters in particular have been used to detect environmental changes, caused not only by pollutants, but also due to natural variation (Widdows, 1973; Davenport, 1979; Akberali & Trueman, 1985), e.g. the heart rhythm of *Scrobicularia plana* (Akberali & Black, 1980), reproduction and larval development, respiration, heart rhythm, pumping or filtration rate, shell growth and valve movement of *Mytilus edulis* (Abel, 1976; Manley & Davenport, 1979; Sabourin & Tullis, 1981; Manley, 1983; Slooff *et al.*, 1983; Manley *et al.*, 1984; Bayne *et al.*, 1985), the activity of *Anodonta cygnea* (Barnes, 1955; Salanki & Lukacsovics, 1967; Salanki & Varanka, 1976) and the burrowing activity of *Venerupis decussata* (Stephenson & Taylor, 1975). The valve movement of both freshwater and marine mussels has attracted much attention. The method is based on the fact that most mussels have their shells open for respiration and feeding most of the time. It has been shown that they close their shells under stress for an extended period of time. This valve movement detection method was used to study both natural changes in the environment and the effect of pollutants, e.g. the effects of temperature (Hiscock, 1950), light (Bennett, 1954; Ameyaw-Akumfi & Naylor, 1987), tidal movements, salinity (Davenport, 1979, 1981; Akberali & Davenport, 1982), food quantity and quality (Higgins, 1980) and a series of toxicants like trace metals (Salanki & Varanka, 1976; Davenport, 1977; Manley & Davenport, 1979; Kramer *et al.*, 1989), pesticides (Salanki & Varanka, 1978) and other trace organics (Sabourin & Tullis, 1981; Slooff *et al.*, 1983).

Since Marceau (1909) first used sooted glass and a mechanical system to record the valve movement of mussels, several comparable systems have been described (Salanki & Balla, 1964). Other investigators used strain gauges for the detection of the valve movement (Djangmah *et al.*, 1979; Higgins, 1980). Schuring & Geense (1972) were the first to use electromagnetic induction to measure the valve displacement. This system was improved into a High Frequency (HF) electromagnetic induction system, EMIS (Noppert, 1987; Jenner *et al.*, 1989). In this paper we will discuss the response of the zebra mussel and the blue mussel to several toxicants, the various possibilities of data collection and data interpretation as recorded with this new system. Examples will illustrate the various approaches that have been followed.

## Materials and methods

### *Monitoring apparatus*

The electromagnetic induction system basically contains a high frequency oscillator, two tiny coils and an amplifier, with a power supply. One coil acts as transmitter of a magnetic field generated by a HF oscillating current (500 kHz). The other coil will, depending on the distance from the transmitting coil, intercept part of this magnetic field and a current will be induced which is proportional to the distance between the shell halves (Schuring & Geense, 1972; Jenner *et al.*, 1989).

The received signal (continuous scanning) is amplified and corrected for maximum opening of the valves. The minimum span between the transmitting and receiving coils is set to about 20 mm, by appropriate placement of the coils on the shells. Each amplifying circuit (one for each mussel) is trimmed for optimal sensitivity by zero setting when the valves are closed and maximum amplification when fully open. The resolution of the device is dependent on the recording method used; an 8 bit A/D converter results in a resolution of better than 0.02 mm.

The coils are manufactured by winding copper

wire (0.08 mm diameter; 155 windings) around a ferrite core (3 mm × 1.5 mm diameter). The coils are each fixed to a thin poly-urethane coaxial cable (ca. 2 m, 1 mm diameter) (Capable BV, Breda, NL) and encased in plexiglass. The dimensions of the encapsulated coils is approximately 14 × 9 × 5 mm ($l \times w \times h$). The coils are glued to opposite shell valves of the mussel with a non-toxic two-component acrylic resin (Unifast, GC Dental Industrial Corp.). More technical details and electronic schemes are given in Jenner *et al.* (1989).

Our design using coaxial cables is not restricted to mussels with a byssus, fixed to a solid substrate. The method can well be applied to free-living, infaunal mussels, and allows sufficient movement at the sediment surface.

To prevent the mussel from moving through the tank, which may cause interference between the different signals, the mussels are usually glued to perspex plates attached to the tank. So far we did not observe any negative effects of the EMIS system, either on free moving or on (artificially) attached test mussels. A dampproof plastic box contains the necessary electronic parts for the signal generation and reception of 5 mussels. Output is directed either to a chart recorder or, after A/D conversion of the signal, to a computer. Measurements were generally carried out with 10 mussels simultaneously. A schematic diagram is presented in Fig. 1.

The (prototype) early warning system consisted of a waterproof PVC housing containing a dedicated microcomputer for evaluation of the mussel response (see later) and a battery operated power supply. Eight mussels were glued to the outside of the container, the total system was submerged in the river. An output line was connected to a printer on shore (De Zwart & Slooff, 1987).

*Experimental set-up*

The experiments were performed with two different bivalve species: the freshwater zebra mussel, *Dreissena polymorpha* (3–3.5 cm) and the marine blue mussel, *Mytilus edulis* (3.5–4 cm). Three types of experiments were performed, two in a laboratory system:

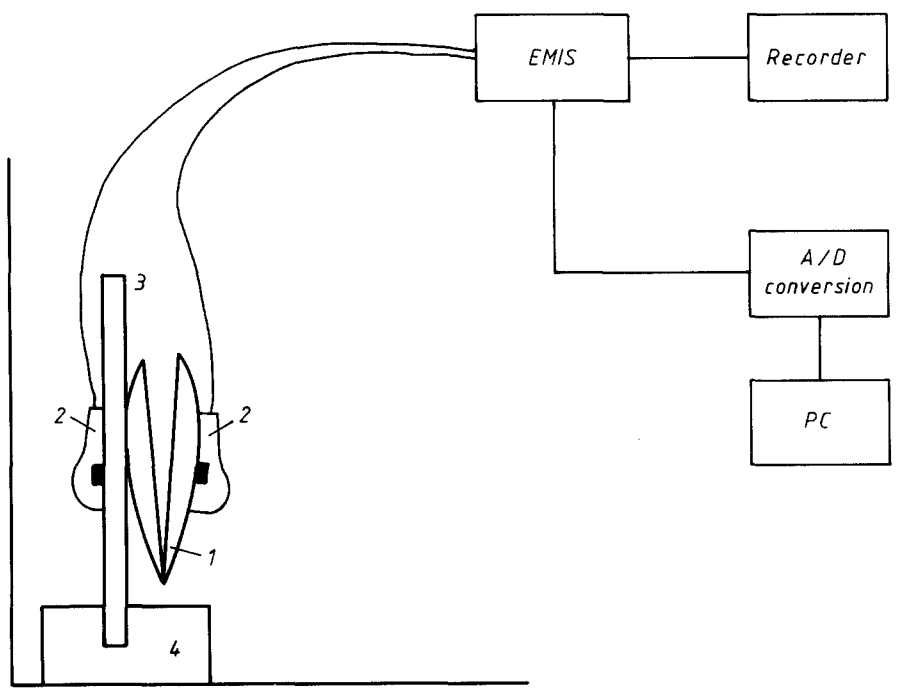


*Fig. 1.* Schematic presentation of the electromagnetic induction system (EMIS) based valve movement detection system. 1: Mussel; 2: coils; 3: perspex substrate; 4: support.

- continuous, single toxicant exposure of *Dreissena*;
- single toxicant exposure of *Mytilus*, with additions of separate spikes; and one in the natural environment:
- the use of *Dreissena* in an early warning system.

In the continuous toxicant exposure experiment ten organisms (*Dreissena polymorpha*) were kept in flow-through tanks ($2 \times 0.25 \times 0.25$ m, $l \times w \times h$), using river Rhine water at a flow rate of 1 l/min. The temperature was 15-18 °C. The water passed over a series of partitions (lamella separator) which resulted in a major reduction of the silt content; however, algae remained available. The experiments lasted 24 hours. Toxic chemicals (trace metals or hypochlorite) were added continuously using a peristaltic pump (Microperpex, LKB) from newly prepared stock solutions. Addition of Tributyltinoxide (TBTO) from a stock solution is hampered because of its adsorption to container walls and tubing. Therefore, inflow water was passed over different sizes (for different concentrations) of TBTO-impregnated, neoprene rubber sheets (NoFoul, Goodrich). This proved to be an efficient continuous TBTO source.

Duplicate groups of five *Mytilus edulis* were kept in Dutch coastal seawater, in static systems (16 l) for about 20 hours. The behaviour of the mussels was compared before and after addition of the toxicants (trace metals, hypochlorite, oil or TBTO), which were performed in one spike, using a timer and a peristaltic pump (Gilson). To minimize external effects upon the experiments (light, vibration, etc.) the additions were carried out during the night.

Actual pollutant concentrations were checked by chemical analysis, using standard methods for trace metal analysis (atomic absorption spectrometry or anodic stripping voltammetry). TBTO was analysed by AAS after extraction in hexane. Concentrations of hypochlorite were based on amperometric measurement of the total residual oxidants.

For the early warning system experiment zebra mussels, collected in a lake near Stockholm, were acclimated to water of the Göta Alv river for a period of three days. The early warning system was tested in the period August 23–28, 1985 in the plume of the regulation tunnel entering the Göta Alv river at the Trollhätan Olidenhalan power station (Sweden) (De Zwart & Slooff, 1987).

## Results and discussion

### *Valve movement response to pollutants*

A characteristic response of *Dreissena* to constant levels of chlorine (added as hypochlorite) is presented in Fig. 2. It shows a distinct increase of the time closed, with intermittent periods of activity. The length of these active periods decreases with the chlorine concentration until virtually no activity is recorded at a concentration level of 500 µg/l. Slooff *et al.* (1983) gave detection limits for several organic compounds of the valve movement response of *Dreissena*. The average of three measurements ranged from 105 mg/l for chloroform to 0.11 mg/l for γ-Hexachlorocyclohexane.

In the real time recording of the valve movement response of *Mytilus* in Fig. 3 an example of a mussel in the blank seawater is given. It will be clear that most of the time the mussel stays open and closes only for short periods. The time between these short enclosure events may vary substantially between mussels. Addition of 37.5 µg/l copper (as copper sulphate) results in a closure response within minutes, while at an addition of 20 µg/l Cu the closure response is delayed and is less clearly visible. This is confirmed by earlier work of e.g. Manley & Davenport (1979). A more detailed study on the effect of various copper species is given by Kramer *et al.* (1989). Addition of lower copper concentrations are less easy to detect, as the mussels instead of closing completely, only reduce the maximum 'open' reading. Application of a computer programme for data interpretation (see later) results in a detection limit of about 1–10 µg/l copper. Other estimated sensitivities are given in Table 1.

Interestingly both *Mytilus* and *Dreissena* have

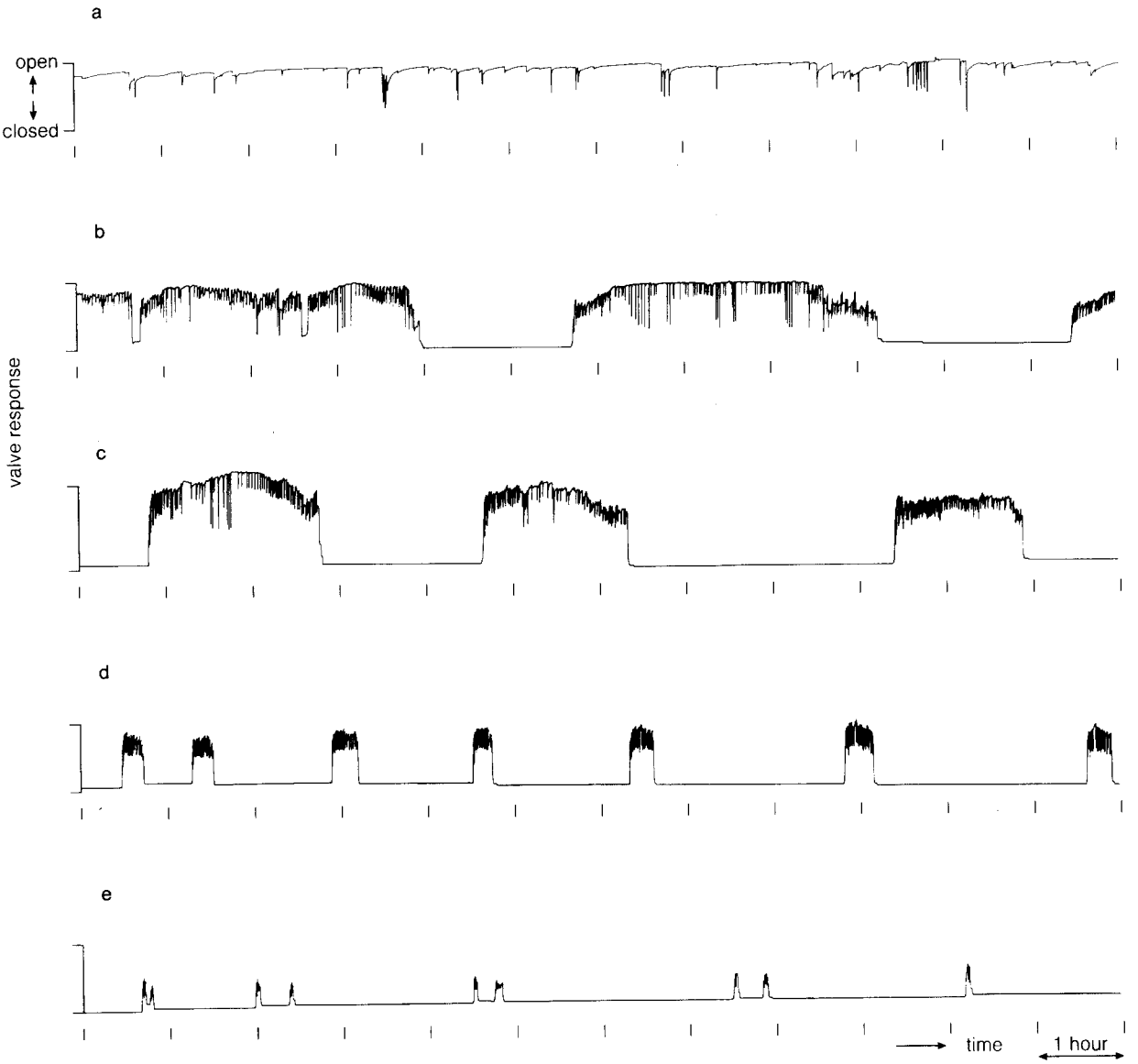


*Fig. 2.* Real time recording of the valve movement of *Dreissena polymorpha* under a continuous stress of chlorine. Chlorine additions (in μg/l): A:blank; B: 37; C: 55; D: 180; E: 550.

comparable low detection limits for the typical antifouling agents copper, hypochlorite and TBTO.

*Data collection*

The collection of the data and the kind of the information stored will be dependent on the objective of the experiment or the monitoring purpose, the storage device and the method of data treatment. Considering the kinetics of the valve movement, it is reasonable for short-term experiments to store and interpret the data in (pseudo) real-time. However, if monitoring will take weeks or even months, data storage and certainly data treatment becomes a serious problem. Therefore we distinguish:

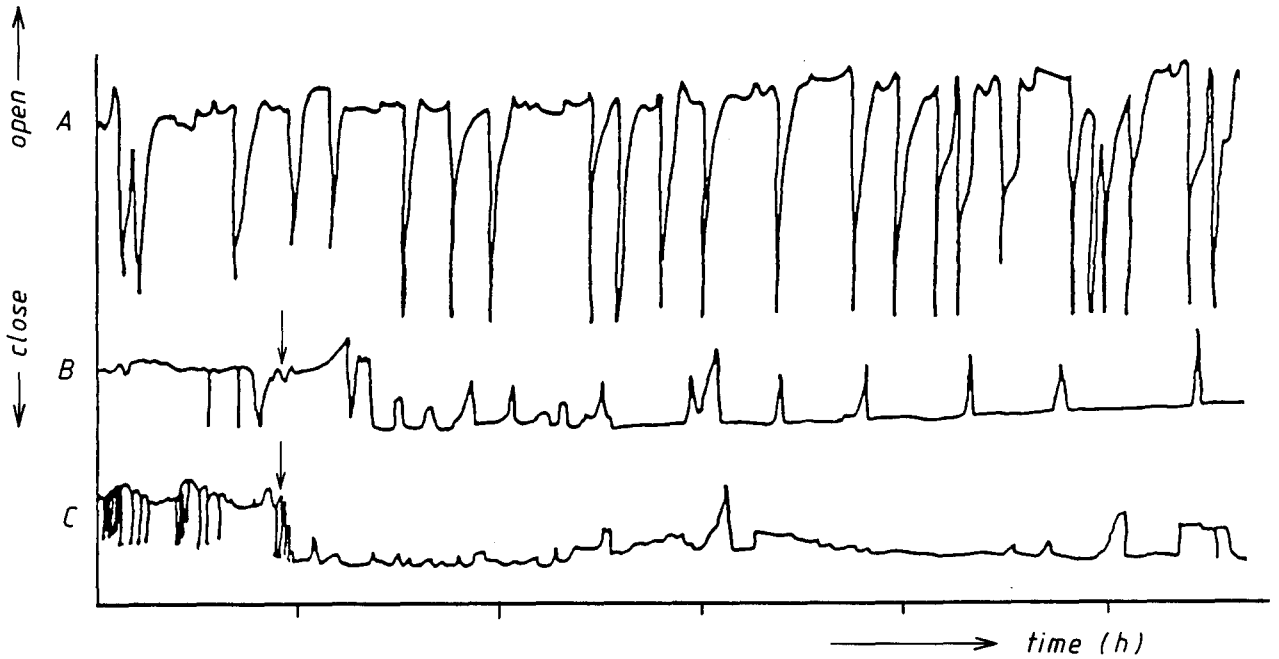


*Fig. 3.* Real time recording of the valve movement response of *Mytilus edulis* to the additions of one spike of 20 µg/l (B) and 37.5 µg/l (C) copper (see arrows), and in a blank situation.

– *Real-time data collection*
In real-time collection a continuous recording is made, either on a chart recorder or on magnetic tape. The graphs in Fig. 2 are made this way. On chart recorders the number of pens is usually limited, reducing the number of mussels that can be followed simultaneously.

*Table 1.* Estimated detection ranges for several toxicants as detected by the valve closure response of *Mytilus edulis* (M) and *Dreissena polymorpha* (D) using the EMIS.

| Compound | Estimated detections limit (µg/l) | Organisms |
|---|---|---|
| Copper | <10 | MD |
| Cadmium | <100 | MD |
| Selenium | <100 | D |
| Zinc | <500 | MD |
| Lead | <500 | MD |
| TBTO | <10 | MD |
| Chlorine | <10 | MD |
| Dispersed crude oil | <6000 | M |

* A positive response was recorded when seven or more mussels out of ten reacted (a closing response or a change in activity, while validity was checked by analysis of variance).

– *Pseudo real-time data collection*
Application of an A/D conversion (datalogger), offers the possibility to sample the analogue signal at frequent intervals. Five second intervals results in a picture (Fig. 4a) that can hardly be distinguished from a real-time graph. Increase of the sampling intervals tends to reduce the sharp peaks recorded, but it takes relatively long (4 min intervals), before the information stored becomes difficult to interpret (Fig. 4b–e).

– *Activity registration*
It is commonly observed that mussels do not change their valve opening position for some time. To reduce the stored data volume, in this case data are collected only when changes in the signal are observed. Therefore, after an evaluation procedure, only the change from a constant value to a more positive (or a negative) value will be recorded vs time. As no values are recorded when there is no change in the signal, no 'real' time

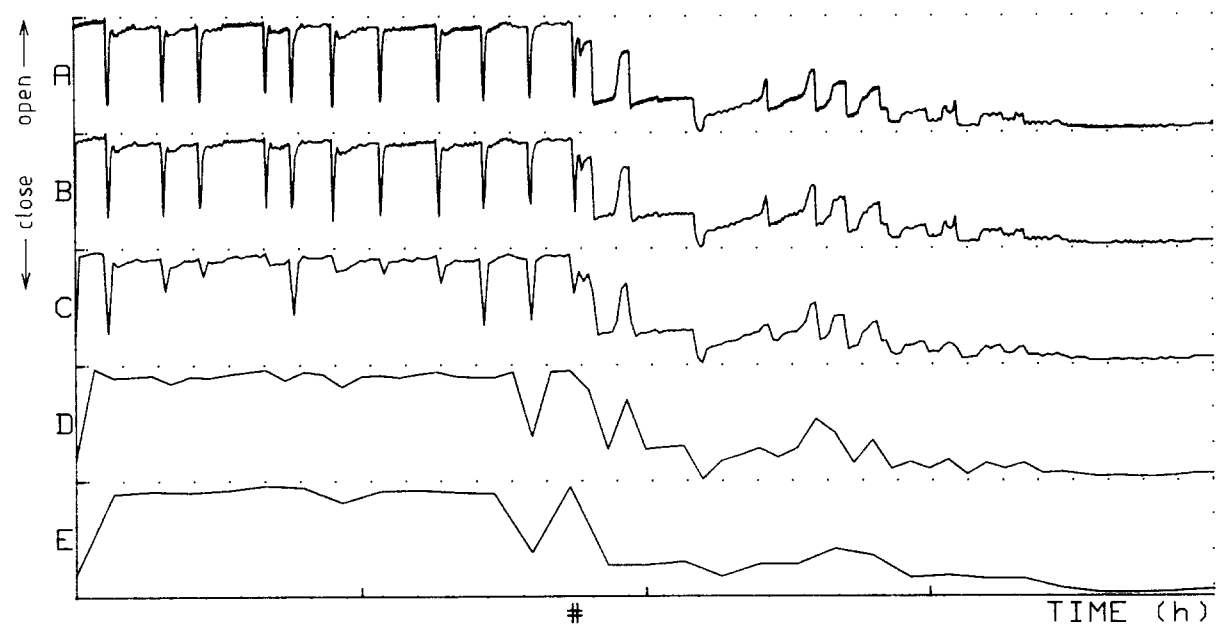


*Fig. 4.* Pseudo real-time recording of the valve movement response of *Mytilus edulis* as a function of sampling intervals: every 5 s (A), 20 s (B), 60 s (C), 4 min (D) and 8 min (E). A spike of 50 µg/l copper was added at '#'.

graph can be (re)constructed from the stored data. This method results, for the mussels described, in a data reduction of about 97%, as compared with the 5 s sampling interval.

– *Dedicated Early Warning system*

Based on the fact that it is very unusual that several mussels are closed for a prolonged period, it is possible to construct a dedicated Early Warning system that only reacts if a preset alarm criterion has been attained. This means that if no criterion is reached almost no data have to be stored. A prototype of the early warning system was tried in the Göta River. The alarm criterion which was preset at: 'if 6 out of 8 mussels are closed for more than 5 min' made the system not sensitive enough (De Zwart & Slooff, 1987). However, from the printout of the individual mussel behaviour, information on the quality of the aquatic system could be obtained. In Fig. 5 an example is given, where the vertical axis represents the percentage of time that the eight mussels are closed for more than 5 min. In the beginning of the test some of the mussels were still responding to handling. After this first 8 hours there was no closure for any extended time. Suddenly a closure response was recorded, indicating a discharge into the river, as was also found by other biomonitor systems applied (De Zwart & Slooff, 1987). The chemical nature of the cause of the stress was not determined. After half a day the stress disappeared.

*Data treatment*

One must distinguish between visual interpretation of chart recorder paper and the data treatment of digitized data. Visual inspection is virtually impossible if large data-sets are involved. For drastic changes in the environmental conditions within short periods of time a strip chart recorder is still very useful.

Once data are stored digitally it is obvious that data treatment should be performed by computer. We have developed three methods to interpret valve movement response data:

– *Average valve reading*

In this case the data-set is scanned for minimal and maximal reading of each mussel recorded,

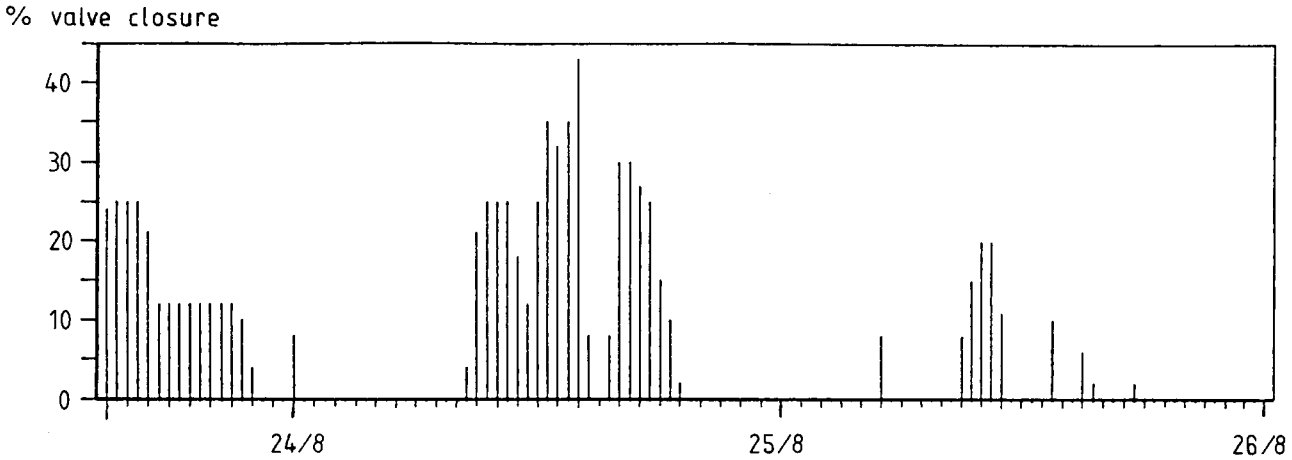


*Fig. 5.* Average percentage of time that *Dreissena* valves in the Early Warning system were closed for longer than 5 min during 30 min intervals (after: De Zwart & Slooff, 1987).

and set to 0 and 100% respectively. All data are thus normalized and mussels can be compared easily. Only the average reading per hour (or half hour) is recorded. Following a given mussel in time, a decrease in average value will indicate the detection of an effect. In Table 2 an example, resembling data also presented in Fig. 4, is given. About the first 2 hours represent the blank period, then copper is added to the system. A closing response can be clearly observed. It is interesting to observe that the average reading method is rather insensitive to lower sampling frequencies (Table 2). Some deviation from the 5 s sampling interval averages is visible only at relatively large sampling intervals. Two-way analysis of variance may be used to determine the confidence level or reliability of any observed effect.

– *Activity interpretation*

If the activity of the mussels as valve closure response parameter has been stored, it is easy to calculate the activity per unit of time (usually per h). The activity interpretation as a function of chlorine concentration that was calculated for the data set presented in Fig. 2 is given in Table 3. The ratio of the time closed/open clearly indicates that under natural conditions the mussels are closed for only a limited time (a maximum of 0.5-1 h per day was observed for *Dreissena*).

The activity per unit of time during the 'open' periods shows a marked increase in activity under stress conditions. At the lower additions of chlorine the mussels tend to be more open. Inclusion of the 'closed' periods in the calculations, makes the results less clear.

*Table 2.* Calculated average valve reading (per half hour) as function of the sampling frequency of the analogous signal, as graphically presented in Fig. 4. After 2 h (#) 50 µg/l copper was added.

| Average reading intervals: | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| Sampling intervals (s): | 5 | 87 | 87 | 86 | 68 | 24 | 26 | 10 | 4 | 9 |
| | 60 | 87 | 89 | 87 | 66 | 23 | 25 | 10 | 4 | 9 |
| | 180 | 89 | 91 | 90 | 59 | 22 | 26 | 10 | 4 | 9 |
| | 300 | 90 | 90 | 89 | 61 | 26 | 26 | 11 | 4 | 9 |
| | 600 | 87 | 93 | 91 | 49 | 26 | 24 | 8 | 4 | 10 |
| | 1200 | 84 | 93 | 90 | 61 | 22 | 26 | 15 | 5 | 10 |

*Table 3.* Calculated activity interpretation of the response of *Dreissena polymorpha* under continuous stress of several concentrations of chlorine (the data are graphically presented in Fig. 2).

| Chlorine conc. (µg/l): | 0 | 37 | 55 | 180 | 550 |
|---|---|---|---|---|---|
| Ratio t(closed)/t(open) | 0.06 | 0.5 | 1.3 | 5 | 18 |
| Activity (per half h) | | | | | |
| open period: | 4 | 47 | 47 | 97 | 62 |
| total period: | 4 | 31 | 20 | 16 | 3 |
| Subsequent hourly activity (per h) | | | | | |
| hour 1 | 4 | 72 | 28 | – | – |
| hour 2 | 8 | 80 | 93 | – | – |
| hour 3 | 2 | 110 | 74 | – | – |
| hour 4 | 16 | 102 | 0 | – | – |
| hour 5 | 10 | 0 | 40 | – | – |

*Mytilus* showed much less changes in activity during our experiments. If plotted against time, a closure response will be indicated by a drastic decrease in hourly activity (e.g, the activity drop after 4 hours of exposure to chlorine), showing the possibilities of this method for monitoring functions.

– *Alarm criterion*

If mussels are to be used in a sentinel function of the environmental condition, the above-mentioned data treatments can be used to build an early warning system. It seems more appropriate to construct a dedicated system, however. If the only task of the system is to exhibit an alarm function there is no need for complicated data treatment. This is not only too time consuming (a delay in the alarm) but also requires substantial computer back-up. By looking only for the present alarm criterion, e.g. 'if 6 out of 8 mussels are closed for more than 4 minutes', the alarm function can be triggered within this short period.

## Applications

A number of applications for the use of the valve closure response can be developed, especially for environments where steep gradients in the toxicological conditions are to be expected.

Some, mainly biological problems have to be sorted out, like the effect of adaptation, seasonal variation, reproduction, etc. Also a better idea of combined toxicity and the detection limits of various other chemicals should be tested. Since both freshwater and marine mussels seem to react comparably, the proposed bio-sensor can be applied anywhere in the aquatic environment. Possible applications include:

- effluent monitoring (discharge pipes);
- general water quality monitoring (rivers, coastal environments);
- monitoring of water inlets (drinking water, aquaculture);
- early warning system (alarm function, triggering a water sampler for chemical proof);
- toxicity testing;
- physiological and behavioural studies.

## Conclusion

The described method has several advantages over the other methods:

- the electronic interface facilitates automated data collection and data interpretation;
- since transmitting and receiving coils attached to the mussel are quite small, and the connecting wires are thin and supple, burrowing bivalves are free to move to some extent;
- the small size and rigidity of the system allows its use both under laboratory and (semi) field conditions, the latter being essential for the application in an Early Warning system.

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