Standardisation, Quality Assurance and Data Evaluation of Online Biological Alarm Systems

3. MOSSELMONITOR® (DeltaConsult, Kapelle, NL)

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KWR Watercycle Research Institute

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Colophon

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Part 3. MOSSELMONITOR® (DeltaConsult, Kapelle, NL)

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This report has been distributed to BTO participants and is public. This report will be distributed to members of the Deutsche Expertenkreis Biomonitoring.

Preface

In 2004, the Biological Alarming Steering Committee published a method standardisation report on the bbe Daphnia Toximeter. This report was drafted in response to a need for more insight into the use and possibilities of the standardisation of online biomonitors and to increase the reliability and comparability of the monitor data collected (Wagenvoort *et al*., 2005). The second report in this series dealt with the implementation, validation, standardisation and quality assurance of the bbe Algae Toximeter and was published in November 2006 (Penders *et al*., 2006). This third report deals with the MOSSELMONITOR® (Delta Consult, Kapelle, the Netherlands) and contains information on the standardisation, reliability, implementation, quality assurance and data evaluation. All three reports are to help Dutch and German users in the implementation and operation of their biomonitors.

This document describes the possibilities of standardisation, quality assurance and data-evaluation for the MOSSELMONITOR®. The second chapter deals with the theoretical background, the construction and the operation principles of the MOSSELMONITOR®. This is followed by more details concerning the quality control and the evaluation of the measurement results. The last part comprises a conclusion and suggestions for improvement with regard to the manufacturer.

The work of the Biological Alarming Steering Committee is based on the contributions and experience of members of the Biological Alarming study group. The Steering Committee's activities are partly financed by the BTO.

Summary

The MOSSELMONITOR® (DeltaConsult, Kapelle, NL) is a biomonitor with 8 mussels (bivalves), suitable for monitoring the quality of various types of surface and sea water. In general, the Zebra Mussel (*Dreissena polymorpha*) and the Blue Mussel (*Mytilus edulis*), both sedentary filter-feeding species, are used to monitor freshwater or marine water quality respectively. However, other species (suspension-feeders) can be used as well. In the Netherlands, four MOSSELMONITOR® systems are presently in use for monitoring the quality of surface water systems. Three of these are used for intake control monitoring of water used as a source for the production of drinking water. However, the MOSSELMONITOR® is well-known as an application in the control of biofouling in e.g. water cooling systems of power plants. This application is not discussed in this document.

In order to improve the *online* biomonitors' operationality in general and the MOSSELMONITOR® in particular, a study was performed on the critical factors that influence the measurement results of this monitor. When the MOSSELMONITOR® is installed in a field situation, it is important to consider the influence of factors, such as vibration, light, temperature, water supply, salinity, suspended matter and food conditions. Some of these factors are induced by man, and are relatively easy to detect. A well-considered configuration and installation of the MOSSELMONITOR® at the measurement location can eliminate the negative effects of many of these factors and thus increase the reliability of the results.

Regular checking of the MOSSELMONITOR® with regard to the opening of the valves and possible "gaping" alarms is necessary to keep the MOSSELMONITOR® operational over a long period of time. Maintenance of the MOSSELMONITOR®, mainly consisting of cleaning and removal of sediment and biofouling, contributes considerably to the functionality of the monitor. This report provides an example of maintenance planning and performance on the MOSSELMONITOR®.

When evaluating the results of the measurements, it is especially important to verify the alarm reports correctly so a technical alarm can de distinguished from a true quality alarm without any unnecessary measures being taken. Natural phenomena which might influence the result of the MOSSELMONITOR® also need to be taken into consideration. Under normal environmental conditions, mussels are submersed and their shells are open to allow for feeding and respiration. Closure of the shells for longer periods is to be considered escape behaviour. If one bivalve is closed for a prolonged time, e.g. for 5 minutes, it is not considered unusual; if several mussels do this simultaneously, e.g. 5 out of 8 mussels, it is deemed highly unusual and the reason for an alarm. The "close" alarm is seen to be the most reliable and useful alarm parameter of the MOSSELMONITOR®. In a recent study, a clear relationship between MOSSELMONITOR® alarms and pollutants present in the surface water has been established.

Contents

1 Introduction

1.1 The MOSSELMONITOR®

Water quality management is an expensive and time-consuming business. In order to monitor water quality, samples must be taken regularly and analysed chemically. On the basis of these results, a decision is then taken as to whether there is an alarming level of contamination. Very often, the result of the analysis indicates that contamination thresholds have not been exceeded.
The MOSSELMONITOR® is an early warning system based on the valve movement of mussels, capable of noting contamination and triggering an alarm without external control and without an extensive analysis system. The system was developed in order to monitor the water continuously (Figure 1.1). The early warning system is based on the behaviour of mussels and can be used in freshwater as well as in marine ecosystems. In clean water, mussels move their shells according to a characteristic pattern. They remain open most of the time, and only close for short periods. A mussel in contaminated water behaves differently.

Figure 1.1 The MOSSELMONITOR® (left: *in situ* (submersible) and right flow-through instrument)

Depending on the type and level of contamination, mussels show a movement pattern which can differ greatly from the normal pattern. This includes a more rapid opening and closing of the shells, keeping the shell closed for a long period, opening the shell less far, and in the worst case if a period of (high) contamination continues for too long, the death of the mussel (gaping).
By entering the data of the movements of the shell into a microprocessor, and by carrying out calculations on this data, it is possible to ascertain the behaviour of the mussel. Currently, a memory is used to store the data for evaluation of trends in shell movement behaviour.
By fitting a miniature coil to each shell, the distance between the shells can be measured. A high-frequency current is passed through the first coil, such that a magnetic field is created. This magnetic field induces a current in the other coil. The strength of the induced signal is dependent on the distance between the two coils, and is therefore indicative of the shell opening.
Tests have shown that mussels react differently to different substances, in particular heavy metals, chlorinated organic compounds and anti fouling compounds (TBT). Just like all other biological sensors (including fish, daphnia and bacteria), they are not equally sensitive to all substances. Detection levels have been determined for many substances (the list is continuously being extended, see Annex I). Using the evaluation of the movement pattern, it is possible to monitor water quality, both in fresh and in marine water. The in situ MOSSELMONITOR® has been designed to operate on a stand-alone basis, using built-in, rechargeable batteries. The housing for the electronics is a practical in situ field unit, which can be used in many locations. There is also a flow-through system, which is commonly used in field stations.

1.2 Reading guide

This document is a guideline for the online monitoring of surface water quality using of the MOSSELMONITOR® and will be regularly updated by the Biological Alarming Steering Committee. Chapter 2 describes the working principle of the monitor. The influence of the preliminary treatment of

the sample water is described in Chapter 3, and is in Chapter 4 followed by the installation and the parameters setting for the automatic alarm evaluation. Regular maintenance comprising the collection of the test organism, the maintenance of the MOSSELMONITOR® and the checking of the technical operation will be described in more detail in Chapter 5. Chapter 6 describes quality assurance and quality control. Chapter 7 deals with the evaluation of measurement data. Chapter 8 comprises conclusions and recommendations to improve the usability of the MOSSELMONITOR®.

2 Working Mechanism of the MOSSELMONITOR

2.1 Biological information

2.1.1 Bivalves used in the MOSSELMONITOR are molluscs with two shells

Snails, slugs, oysters, clams, octopuses and squids are all molluscs. In all, the phylum Mollusca has more than 50,000 known species. Most molluscs are marine, though some inhabit fresh water, and there are snails and slugs that live on land. Molluscs are soft-bodied animals (Latin: molluscus, "soft"), but most are protected by a hard shell made of calcium carbonate.

Despite their apparent differences, all molluscs have a similar body plan. The body has three main parts: a muscular foot usually used for movement, a visceral mass containing most of the internal organs, and a mantle, a heavy fold of tissue that drapes over the visceral mass and may secrete a shell. Many molluscs feed by using a strap-like rasping organ, called a radula, to scrape up food. Most molluscs have separate sexes, with gonads (ovaries or testes) located in the visceral mass. The body plan of molluscs has evolved in various ways in the different classes of the phylum. Mussels, oysters and clams belong to the class of the bivalves (Bivalvia).

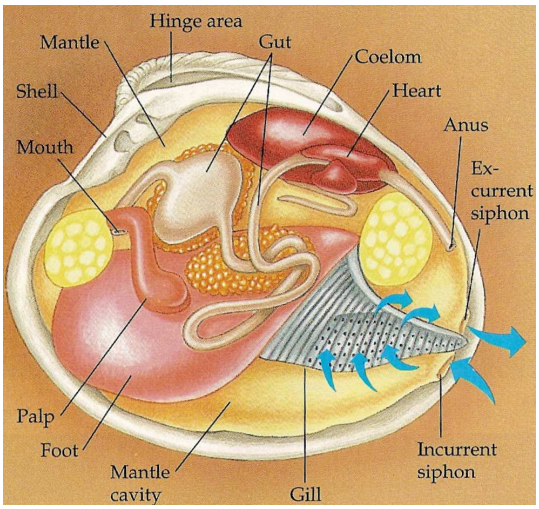


Figure 2.1 Anatomy of a clam (Campbell, 1993)
The left half of the bivalve shell has been removed. Food particles suspended in water entering through the incurrent siphon are collected by the gills and passed to the mouth via elongated flaps, called palps.

Bivalves have two shells divided into two halves. The two shells are hinged at the mid-dorsal line, and powerful adductor muscles draw the two halves tightly together to protect the soft-bodied animal. When the shell is open, the bivalve may extend its hatchet-shaped foot for digging or anchoring. The mantle cavity of a bivalve contains gills that are used for feeding as well as gas exchange (Figure 2.1). Bivalves have no distinct head, and the radula has been lost. Most bivalves are suspension-feeders. They trap fine food particles in a mucus that coats the gills, and then use cilia to convey the

particles to the mouth. Water flows into the mantle cavity through an incurrent siphon, passes over the gills, and then exits the mantle cavity through an excurrent siphon (Figure 2.2). Being suspension-feeders, most bivalves lead rather sedentary lives. Clams can pull themselves into the sand or mud, using the muscular foot for an anchor. Sessile mussels, such as e.g. Dreissena polymorpha and Mytilus edulis, secrete strong threads (byssus, Figure 2.3) that secure them to rocks, docks, boats, and the shells of other animals. In the group of bivalves there are also deposit-feeders of bivalves that can feed on plankton and benthic organisms. The MOSSELMONITOR® is used to determine water quality, so only suspension-feeders are used.

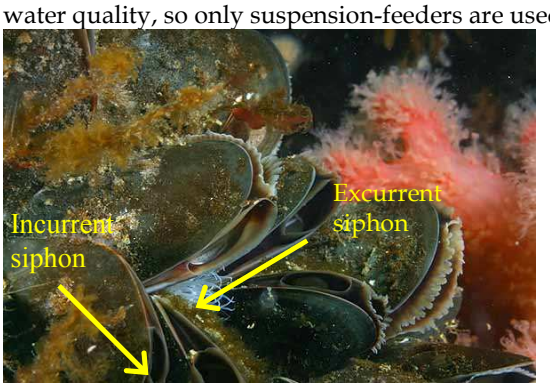


Figure 2.2
Mytilus edulis and its incurrent and excurrent siphons.

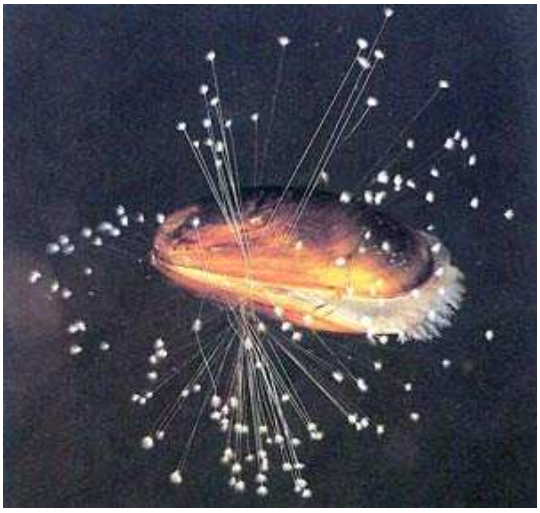


Figure 2.3
Mytilus edulis fixed on the substrate by byssus threads.

(http://seaotter.com/marine/research/mytilus/edulis/pics/edulis.jpg(www.uni-duesseldorf.de).

2.1.2 Life cycle of mussels

All bivalves have a similar life cycle. Most molluscs have separate sexes, with gonads (ovaries or testes) located in the visceral mass. In summertime, after releasing the eggs or sperm, fertilisation takes place in the water. Afterwards, the fertilised egg develops into larvae. This stage is the main dispersal mechanism of bivalves. In a few weeks time, this larva develops a shell, starts to settle on a suitable substrate, and becomes a sessile (benthic) organism for several years, till its death (Figure 2.4).

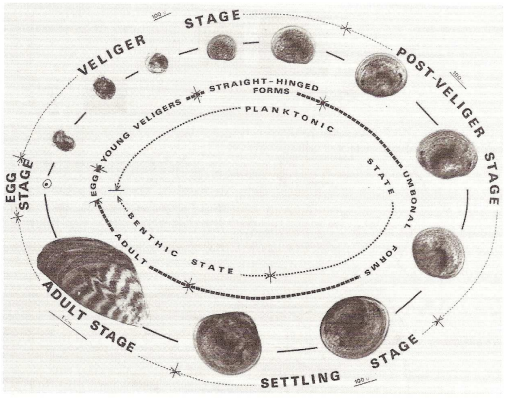


Figure 2.4 Life cycle of the Zebra mussel, *Dreissena polymorpha* (Claudi & Mackie, 1993).

2.1.3 Bivalves are the sensor

The sensor in the MOSSELMONITOR® is the bivalve (mussels, oysters or clams) itself. The bivalve shows a variation in its normal valve movement behaviour due to changes in its environment, e.g. caused by pollution or turbidity of the water. This valve movement is measured by the MOSSELMONITOR® and used for detecting abnormalities. By closing the shells (escape behaviour) the bivalves physically exclude the outside environment, and provided that the conditions do not become too bad, many species can survive for days. When the valve movement of several bivalves is assessed and shows a significant deviation from normal behaviour, the MOSSELMONITOR® automatically generates an alarm. For the results in this report only mussels were used, but other bivalves such as oysters and clams can be used as well.

Under normal environmental conditions, mussels are submersed and their shells are open to allow for feeding and respiration. The typical behaviour may vary from species to species, but almost all species tested close their shells occasionally for a short period, e.g. to defecate (Kramer & Foekema, 1999). Four typical examples of "normal" behaviour are shown for *Dreissena polymorpha* (Figure 2.5a, b), and for *Mytilus edulis* (Figure 2.5c, d). The data were normalised to fully closed (set at 0 %) to fully open (set at 100 %), thus allowing similar scaling and easy comparison. The average valve opening of about 70 – 95 % is evident, as are the short periods of closure. The difference between the two graphs per species is the difference between the individual behaviour of the two example mussels: one is more active ("nervous") than the other, a quite normal situation when comparing individuals.

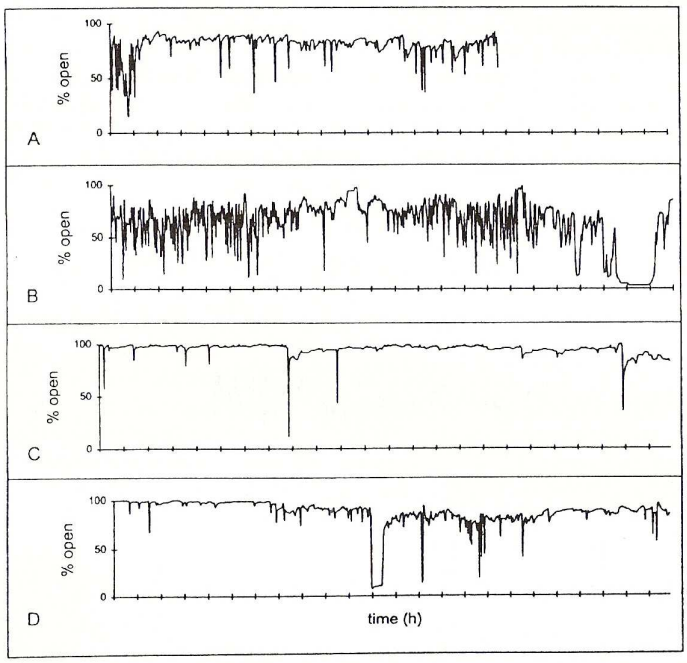


Figure 2.5 Typical registration of the valve movement of individual bivalves in natural (sea)water (Kramer & Foekema, 1999).
A and B: the fresh water Zebra Mussel (*Dreissena polymorpha*)
C and D: the Blue Mussel (*Mytilus edulis*)
From each species a relaxed (A and C) and a more nervous example (B and D) is displayed. Time scales are kept the same; data are normalized to closed (0 %) and fully open (100 %).

Closure of the shells for longer periods is to be considered as escape behaviour. If one bivalve is closed for a prolonged time, e.g. for 5 minutes, it is not considered as unusual; if several mussels do this simultaneously, e.g. 5 out of 8 mussels, it is deemed highly unusual and the reason for an alarm. However, some species, such as *Dreissena polymorpha* and *Unio pictorum*, have shown occasionally prolonged closure times (maximum 2 to 4 hours), obviously as part of their natural behaviour. However, even in these cases, the simultaneous closure of (nearly) all bivalves remains highly unusual, and is the reason for an alarm (see Box 1, Chapter 7).

The MOSSELMONITOR® determines and evaluates the average behaviour of the valve movement of 8 bivalves. Typical behaviour as a result of different types and levels of contamination are:
- closure of the shells for a prolonged period;
- a reduction in the average value of opening (normally 70 - 80%);
- an increased activity, e.g. the mussel opens and closes more frequently than under normal behaviour patterns;
- excessive opening, no further movement; this occurs when the mussel is no longer alive (so called "gaping").

Being closed for prolonged periods does not harm species such as the Zebra or Blue Mussel, as they can change from an aerobic to an anaerobic metabolism (Zandee *et al.*, 1986; Eertman & de Zwaan, 1994). Unlike other species used in online biological alarm systems, such as water fleas and fish, these bivalves have a distinct advantage in water that may tend to become temporarily hypoxic or anoxic. The closure of the shells continues until the mussels find the water conditions acceptable. Opening of the shells is

a guarantee that the water quality is no longer unacceptable for the bivalves. However, the moment of opening may be later (up to several hours) than the actual improvement of the water quality.

2.1.4 Suitable species

Both freshwater and marine mussels are commonly used in the MOSSELMONITOR®, mainly the Zebra Mussel (*Dreissena polymorpha*), *Unio* sp. and *Anadonta* sp. in freshwater ecosystems and the Common or Blue Mussel (*Mytilus edulis*) or oysters (*Ostrea* sp. or *Crassostrea* sp.) in marine ecosystems. However, other bivalves, including clams and oysters, have also been used successfully. The MOSSELMONITOR® is designed to measure water quality. For this purpose only suspension-feeder can be used, deposit-feeders are not suitable.

There is a slight preference for sedentary species that are attached by byssus threads to hard substrate (Section 2.1.1 and Figure 2.3), but there is no proof that the normally free-moving benthic species are negatively affected by a fixed mounting in the MOSSELMONITOR®. As the MOSSELMONITOR® is used to provide information on the quality of water, filter-feeding species are preferred (e.g. *Dreissena polymorpha* and *Mytilus edulis*).

2.2 Technical information

The MOSSELMONITOR® is available as an *in situ* (submersible) instrument and a flow-through instrument (Figure 2.6a, b). The *in situ* version can be submersed in the water to be tested, e.g. mounted on a buoy. This version requires no constant water flow, no housing and no pumping system. The flow-through instrument requires a constant water flow of at least 50 l/hr. To ensure rapid replacement of the water, a flow rate up to 150 l/h is recommended.
Many users have access to a constant water flow and in most cases the flow-through version is chosen. At several sites (e.g. Heel and Zuuk in the Netherlands), an *in situ* version was placed in an exposure container, making a kind of flow-through system, after problems occurred by placing the MOSSELMONITOR® in a canal or brook.

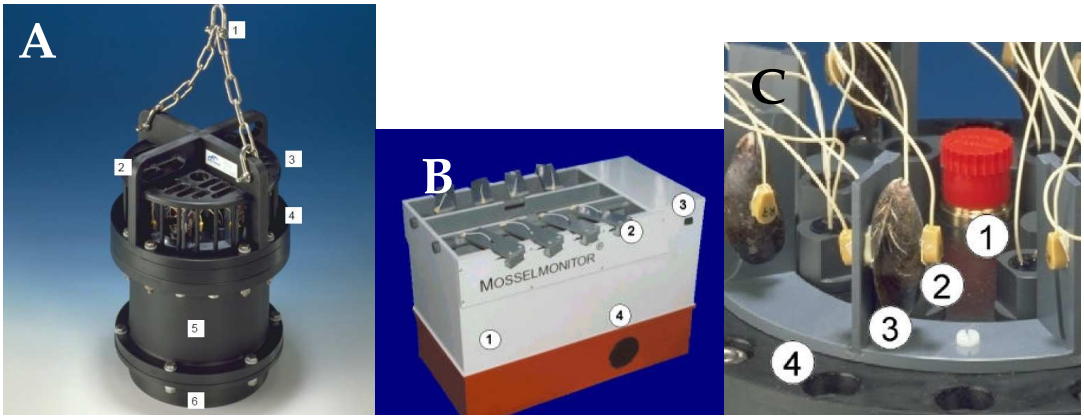


Figure 2.6 the MOSSELMONITOR®

A The *in situ* (submersible) instrument (1: mounting chain; 2: handle; 3: protective cover; 4: mussel compartment; 5: electronics housing; 6: ballast)
B The flow-through instrument (1: containment; 2: mussel holder; 3: power on/off signal; 4: wash out valve)
C Two sensors are fitted to every mussel (1: watertight connector/ sensor conduit; 2: sensor; 3: mussel; 4: mussel mounting ring)

2.2.1 Electronic information

Two sensors are fitted to every mussel, one on each shell half. A 250 kHz signal is sent to the first coil (Figures 2.6c; 2.7). This induces a current in the coil fitted on the opposite shell half, with a strength inversely proportional to the distance between the two coils. The strength of the current is a measure for the position of the shells. In order to avoid cross-over between the various measurement signals, the currents are transmitted individually, and are only read into the corresponding receiver (multiplexing). For the purposes of reading in the induced current, the signal is rectified and digitalised via an A/D converter. A microcomputer processes the data and ensures checking against the prescribed evaluation criteria. Through internal evaluation, the occurrence of an alarm situation can be ascertained and indicated via the (potential-free) alarm contact.

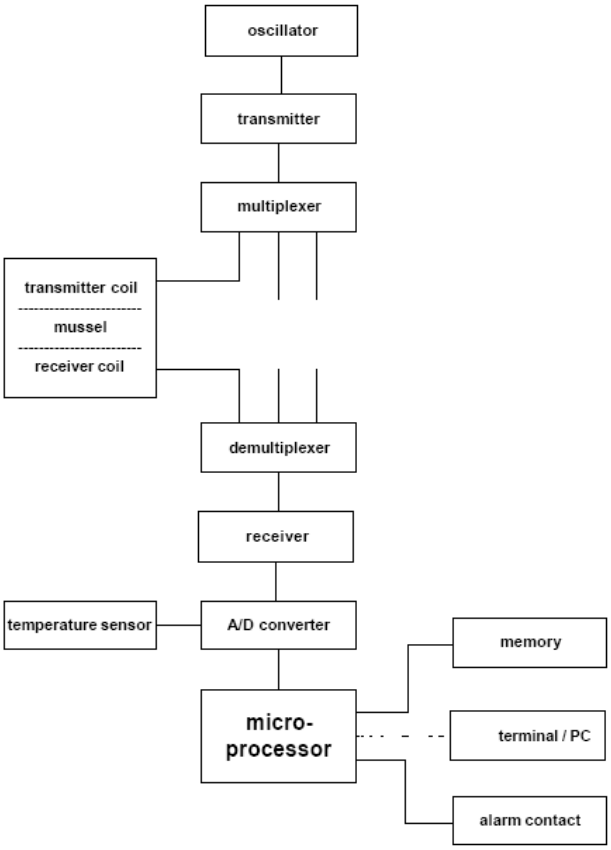


Figure 2.7 Schematic diagram of the MOSSELMONITOR®

Data, alarm reports and possible evaluation values can, if desired, be sent to the terminal port (RS422) to interface with a computer or terminal. Using an optional PC as a terminal, (in combination with the supplied RS422 - RS232 converter and analogue output, 4–20 mA) this data can be stored and processed.

2.2.2 Functional information

The shell position of each mussel is measured semi-continuously, at intervals adjustable between 10 and 600 seconds. The shell positions are translated internally by the processor to absolute values ranging between about 50 and 550 units (A/D conversion). Absolute values will vary from mussel to mussel as a result of their differing sizes and shapes, and are further dependent on the setting and adjustment of the electronics. They can be used for further evaluation of the measurements. However, when using the (measured) minimum and the maximum absolute values, obtained individually for each

of the mussels, the software can normalise the measured values. Minimum, or fully closed, is set at 0% open, maximum reading at 100% open. The current shell position can thus be expressed as a percentage of the shell opening. This largely facilitates comparison of the individual behaviour of the different mussels, despite size differences (of the individual mussels) and adjustment differences (in the electronics). These Figures are internally evaluated according to fixed criteria. Via the menu-driven program, the MOSSELMONITOR® can be tuned to a specific sensitivity/reliability. The user, enabling optimum use of the system (see later) can set many variables. Thus so-called alarm criteria can also be defined. If these threshold values are exceeded, an alarm is generated. This alarm closes a (potential-free) alarm contact on the cable reel (*in situ* version) or containment (flow-through version). The alarm can also be viewed on the PC screen and or transmitted to e.g. a control room. The system can be connected to a SCADA-system, an intranet, the internet and a PSTN/ISDN-modem connection.

In addition to the measured (normalised) opening values of each mussel, the date/time of the measurement, battery voltage and temperature are also recorded in each measuring cycle.

3 Prerequisites and Conditions for MOSSELMONITOR® Installation

Before installation of the MOSSELMONITOR® can be successfully completed, a number of prerequisites and conditions need to be taken into account. These are described in the following paragraphs.
The flow-through instrument (Figure 2.6b) requires a constant water supply of 150 $l.h^{-1}$, in order to allow for an early response, and a power supply (230 V). A constant water flow can be ensured using buffer stock with a certain height of the water column (Figure 3.1a). The height of the water column and diameter of the inflow pipe determine the flow of the water. A flow meter can be used to ensure a constant and regulated flow rate (Figure 3.1b). The flow to the buffer stock and the capacity of the pump should be much larger than the flow to the MOSSELMONITOR® and the surplus of water should overflow to the drain. Instead of a buffer stock, the water flow to the MOSSELMONITOR® is also regulated using a flow meter. The diameter of the drain of the MOSSELMONITOR® should be two sizes larger than the inflow, to avoid an overflow of the system.

Figure 3.1 Constant water flow to the MOSSELMONITOR®
A Using a buffer stock (location Heemskerk, photo: Kees Kramer)
B Using a regulator and flow meter (location Heel, photo: Paul Mulders).

The *in situ* version of the MOSSELMONITOR® (Figure 2.6a) can easily be located in the field but should be connected firmly. A solar panel can be used as a power supply. When the MOSSELMONITOR® is directly located in the field, it should be protected against environmental conditions (e.g. wear and tear of cables, and shackles) and vandalism.

3.1 Environmental conditions

The water quality at the investigated site should be within the range of the tolerance of the exposed bivalve, e.g. bivalves close their shells at low oxygen concentrations. Being closed for prolonged periods does not harm species such as the Zebra or Blue Mussel as they can change from an aerobic to an anaerobic metabolism (Zandee *et al.*, 1986;

Eertman & de Zwaan, 1994). An overview of the tolerance to salt of different species is given in Table 3.1.

Table 3.1 Tolerance to salt (chlorine) of commonly used species in the MOSSELMONITOR®.

species		tolerance to salinity (‰ or g.L^{-1} NaCl) lower limit	upper limit	references
Dreissena polymorpha	Zebra Mussel	0.0	1.2 (4.7)	Smit *et al.* (1993); Gittenberger *et al.* (2004)
Anodonta cygnea	Swan Mussel	0.0	1 - 2	Gittenberger *et al.* (2004)
Unio pictorum	Painters Mussel	0.0	3	Gittenberger *et al.* (2004)
Mytilopsis leucophaeta	Dark False Mussel	0.2	14.6	Gittenberger *et al.* (2004)
Mytilus edulis	Blue Mussel	16 (5)	> 35	Almada-Villela *et al.* (1982); Almada-Villela (1984)

In general, no specific preliminary treatment of the water is necessary before the mussels on the MOSSELMONITOR® can be exposed to the sample water. Some users have a coarse filter in order to remove large particles, e.g. leaves. In case of large amounts of suspended or particulate matter a fine sieve ($\geq$ 100µm) is recommended.
In case the MOSSELMONITOR® is placed directly in the sample water or uses untreated water, the user should be aware that the environmental conditions, e.g. temperature, turbidity and oxygen content may influence the behaviour of the mussels. Mussels respond to rapid changes in chemical and physical parameters. Possible influence of the different parameters should be monitored using a standard set of online sensors, such as temperature, oxygen, turbidity, conductivity and acidity. Of these, the temperature sensor is incorporated in the MOSSELMONITOR® and the data are recorded at each measurement. The values of these parameters should be available for data and alarm evaluations.

3.1.1 Temperature

All year round, water temperature should be within the tolerance of the exposed species. If the upper limits of the tolerance, in general 25 to 30 °C, are reached, the use of another species should be considered.
Temperature is of major influence in many biological processes, and also a positive correlation between temperature on the one hand and filtration rate and respiration rate on the other has been observed in mussels (Hinz & Scheil, 1972; Schulz & Baldes, 1982, both in: Kramer & Foekema, 1999). Valve movement activity (frequency) and absolute valve opening increase with temperature (Kramer & Foekema, 1999):

- the valve movement of mussels slows down when the temperature in lowered, but they still react to the sudden occurrence of a stress factor;
- the valve opening of mussels drops from the normal 80-90% towards about 65%, when the temperature was lowered with 10 °C in only a few hours; this indicates that the maximum (absolute) values recorded at e.g. 20 °C are not relevant at lower or higher temperatures – this has obvious implications for the setting of the various alarm criteria, and therefore a function is built into the MOSSELMONITOR® to update the span at regular intervals; only then can the criteria be correctly applied when there is a (seasonal) change in temperature.

In other types of biomonitors (such as algae and daphnia monitors), the sample water is artificially adjusted to a constant temperature before entering the system. However, this is impossible for the MOSSELMONITOR® since much larger amounts of water flow through the system than is the case for the algae and daphnia monitors. Therefore, any user should keep in mind that the activity of the MOSSELMONITOR® changes throughout the seasons, with the highest activity in summer and lowest activity in winter periods. Generally, this is not considered to be a problem, since many pollutants such as pesticides are also mainly used in summer periods, so the risk of water contamination is the highest during the period of the highest activity of the MOSSELMONITOR®.

3.1.2 Oxygen

The behaviour of mussels will be normal at oxygen concentrations of at least 4 mg.L^{-1}. At lower concentrations mussels can change their metabolism from aerobic to anaerobic and can survive low oxygen conditions for several days. During such a period the shells of the mussel are closed. Recently, it has been observed that mussels close their valves after a fast decrease in oxygen concentration from 10 to 8 mg.L^{-1} (Wagenvoort *et al.*, 2008) (Figure 3.2). The authors showed that this change in behaviour was caused by the respiration of algae, which were abundant at high concentration, during night time. After a sharp decrease of the oxygen concentration, it stayed constant for several hours. At these lower levels, the mussels re-open their valves and showed a normal behaviour.

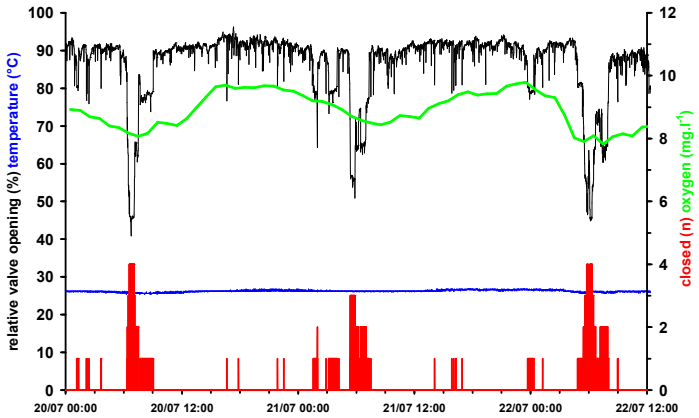


Figure 3.2 The effect of mussels a sharp decrease in oxygen concentration on the relative valve opening of *Dreissena polymorpha*. (Wagenvoort *et al.*, 2008).

—: oxygen concentration; —: relative valve opening; | : number of mussels closed

3.1.3 Salinity (or conductivity)

The selection of the test species depends primarily on the salinity of the water at the investigated site. In Europe, one of the species listed in Table 5.1 can be used, depending on the salinity. The origin of the test mussels is discussed in Section 4.2.

In Europe, freshwater systems use *Dreissena polymorpha, Unio pictorum* or *Anadonta cygnea* and marine systems apply *Mytilus* sp.. *Mytilopsis leucophaeta* is a species that can be used in brackish systems. However, it should be clear that there is no experience with the use of this species (personal comment Kees Kramer, 6th September 2007).

3.1.4 Suspended material

Seston, or suspended particulate matter, is composed of an inorganic and an organic fraction. The first fraction contains silt and clay particles, and the second consists of e.g. living, and dead plankton cells. In general, the Zebra Mussel and the Blue Mussel can handle a large range of suspended materials. In experiments, Kramer & Foekema (1999) showed that there was no obvious change in behaviour from very low (1 mg.L^{-1}) to very high loads (210 mg.L^{-1}) of suspended materials, or from algal biomass (chlorophyll content ranged between <2 to 75 µg.L^{-1}).

In one case, the Zebra Mussels showed a distinct behaviour towards food limitation, often leading to long periods of closure (as the non-food conditions in Figure 3.3), despite the relatively high seston content (average 20 mg.L^{-1}). It appeared that the nutritional value of the seston was rather low (average algal biomass < 3 µg.L^{-1} chlorophyll). This was due to the fact that the seston mainly consisted of sediment that was resuspended by ship movements and hence originated from deeper sediment layers (Kramer & Foekema, 1999).

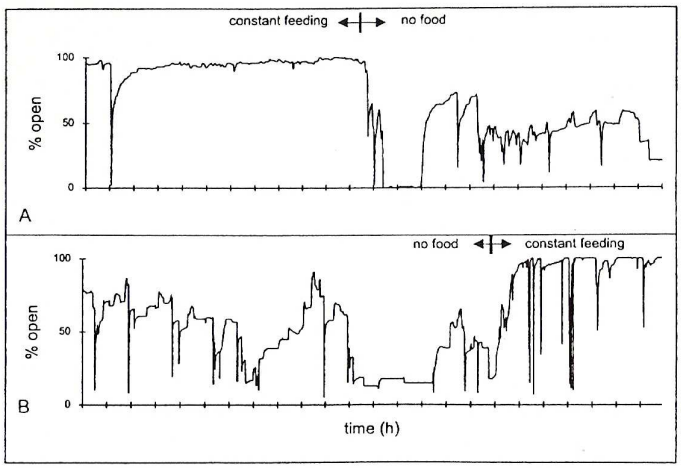


Figure 3.3 The effect of (interrupted) feeding with an algal suspension (*Pheaodactilum* spp.) in a laboratory exposure of the Blue Mussel (*Mytilus edulis*) (Kramer & Foekema, 1999).
A: effect of low food conditions by ending the dosing of algae
B: starvation and the effect of dosing of algae.
In between the two events, a typical "low food" pattern is recorded.

Ship movements may cause a strong, short-term (several minutes) increase in the turbidity (resuspension of sediments), resulting in a "Close" Alarm (Wagenvoort, 2006a, b) (Figure 3.4). In all data evaluations, the turbidity should be taken in account when "abnormal" deviations of the valve movement are recorded. Filtration of the sample water with a coarse mesh size (100 µm) can be applied to avoid too high loads of suspended material. In general, Zebra mussels feed on organic food particles up to a size of 40 µm, but also larger particles up to 750 µm can be ingested (Ten Winkel & Davids, 1982). Filtration, leaving the fraction smaller than 100 µm unaffected, does not influence the minimum amount of food required by the mussels. However, it is recommended to pay special attention to this and if necessary supply the mussels with additional food (Section 3.2.2).

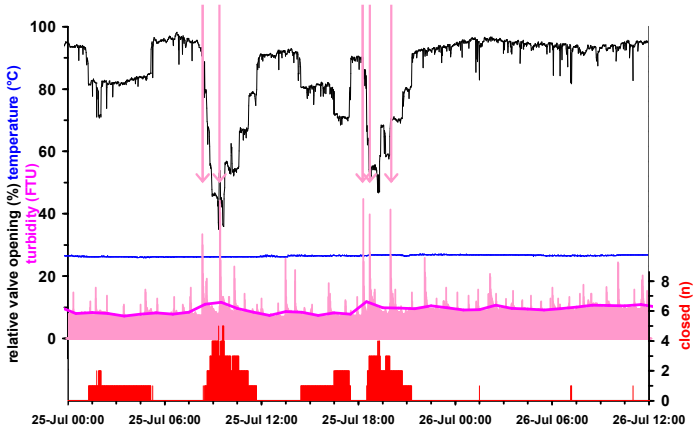


Figure 3.4 The effect of mussels of resuspension of sediment (registered as short distinguished peaks in turbidity) silt on the relative valve opening of *Dreissena polymorpha*. (Wagenvoort *et al*., 2008).
—: turbidity (hour average); | : turbidity (minute value); ↓: increased turbidity caused by a passing ship ; —: relative valve opening; | : number of mussels closed

Suspended material may deposit and can accumulate. This leads to a "smothering" of the mussels, which tends to close more often (Figure 3.5). In order to avoid false alarm readings during routine operation, the water level should be kept above the sensors. In turbid waters, the regular cleaning interval should be increased.

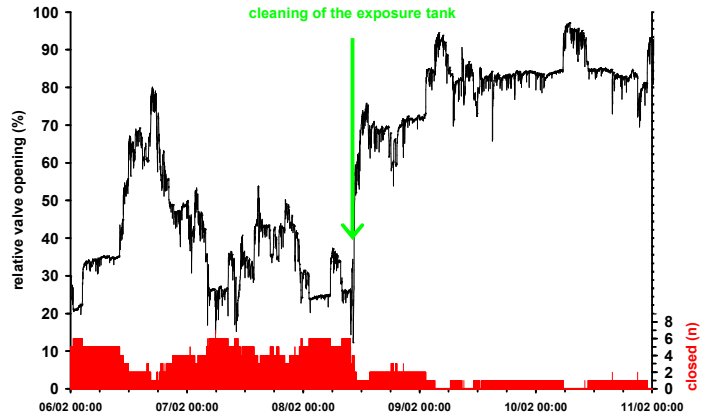


Figure 3.5 The effect of mussels smothered with silt on the relative valve opening of *Dreissena polymorpha*. After maintenance the behaviour returned to normal (Wagenvoort *et al.*, 2008).

—: relative valve opening; | : number of mussels closed

3.2 Preliminary treatment of sample water

3.2.1 Filtration

In the flow-through version it is recommended to use a coarse filter (mesh size 1 mm) in order to remove large particles, e.g. leaves. In case of large amounts of suspended or particulate matter a fine sieve (mesh size $\geq$ 100 µm) is recommended (Section 3.1.4).

3.2.2 Degassing

If water is pumped under pressure or during massive algal growth, degassing of the water in the flow-through instrument can occur. The formation of air or oxygen bubbles on the gills of the mussels can cause a closure of the mussel shells. Mussels experience these bubbles as being removed from the water and as a response they close. To prevent degassing of the water in the exposure container, the use of a degassing container (residence time 5 to 10 minutes) is recommended. The use of a buffer stock ensures degassing of the water before it flows to the MOSSELMONITOR® (Figure 3.1a).

3.2.3 Food supply

It is possible to use the MOSSELMONITOR® even in drinking water or water without any food (upstream parts of rivers and brooks without phytoplankton). In these cases, a preliminary treatment of the water is essential. In waters where no food is available, as e.g. in groundwater, drinking water, or water from storage under laboratory conditions, additional feeding may be required. For short-term laboratory tests, such as toxicological research, it is easier not to feed the mussels, as they show no effect within the first few days. For continuous monitoring this is not an option, and the addition of a suspension of algae has proven to be effective. Kramer & Foekema (1999) noticed that mussels immediately detected the lack of food, when the food supply was switched off (Figure 3.3).

Continuous feeding is recommended, while interrupted feeding interferes with the valve movement response and hampers alarm evaluation. However, in most surface waters,

the food supply is more or less continuous, and no problems should be expected. The Automated Food Device is available as an option on the MOSSELMONITOR®.

3.2.4 Dechlorination of drinking water

Bivalves, such as e.g. Zebra Mussel and Blue Mussel, are very sensitive to chlorine or other disinfectants. If chlorinated (drinking) water is being investigates, the water should be dechlorinated to prevent the interference of chlorine on the valve movement. For this purpose automatic dechlorination units (i.e. with thiosulfate) can be used.

3.2.5 Biofouling

Especially in summer the mussels in the MOSSELMONITOR® may be covered with fouling consisting of sponges (De Zwart, personal comment), or bryozoans (Wagenvoort, personal comment). This fouling may be so serious that the free access of the water to the mussels is blocked. In such situations the system should be cleaned.

3.3 Limitations to the bivalve size

The size of the bivalves that can be used in the MOSSELMONITOR® may vary considerably. Although a rather constant distance between the sensors is preferred, some variation can be allowed. However, there are two physical limitations to the size of the bivalve. The species may be too small so that one can not attach the sensors, or when this is possible, the movement of the sensor cables in running water would induce too much stress to the organisms. For this reason Kramer & Foekema (1999) failed, for example, to use Pea Mussels (*Pisidium* spp.) successfully. The minimum length required is about 1.5 cm. The maximum length in the *in situ* version of the instrument is limited to about 10 cm by the protective cover. Without the cover, or in the flow-through system, the maximum size is less restricted.

3.4 Interferences

3.4.1 Temperature

Bivalves react (closure) to drastic changes in temperature, as in the case of effluents, but for the monitoring of drinking, river or lake water, the large heat capacity of the medium prevented rapid changes. The MOSSELMONITOR® is equipped with a built-in temperature sensor and temperature measurements are recorded together with the valve movement data. In the flow-through version, a strong deviation in water temperature indicates a dramatic decrease in the flow through the system and the most likely result is a clogged outflow or a stopped pumping system (Section 5.2.1).

3.4.2 Magnetic fields

As part of the measurement, one sensor emits a small magnetic field, which induces an electronic current in the other coil. Kramer & Foekema (1999) never observed that this magnetic field affected the valve movement behaviour. However, external magnetic fields may interfere with the measurements. In a laboratory study, unexplained sudden shifts in the output signals were observed, which were caused by a large electrical oven. Even at a distance of about 6 m, the magnetic field generated by the coils in the oven affected the sensitive measurements.

3.4.3 Adaptation

In contrast to most other online biological alarm systems, such as systems with daphnia of fish, the organisms used in the MOSSELMONITOR® only need to be replaced after some months instead of days or week. This considerably cuts the cost of maintenance. As long as the bivalves do not show signs of malfunctioning, and they take up food and

oxygen from the ambient water, there is no reason to replace them. However, an exposure time of two months is recommended (Kramer & Foekema, 1999; Section 4.2).

3.4.4 Vibrations

Many biological alarm systems are sensitive to disturbances by vibration. This is also the case for the mussels in the MOSSELMONITOR®. A sudden vibration (e.g. caused by the slamming of a door) causes a direct closure of the shells, usually of all mussels. However, when the vibration is only a short pulse, the bivalves re-open almost immediately, and in most cases no alarm is generated since the closure does not last sufficiently long (Figure 3.6). Kramer & Foekema (1999) saw many data files where the entry of staff into the test facility caused these kind of "spikes" in the data. These spikes can be recognised relative easily and in general the alarm parameter "Decreased average" will be activated. For the flow-through version, it is recommended to place the MOSSELMONITOR® on a firm support, in order to avoid the influence of vibration.

Continuous vibration is something different. The bivalves normally adapt to the vibration, and continue their normal behaviour. However, if the vibration is present during a shorter period, the mussels close. During the development of a set-up for a verification test, vibration due to the action of a membrane pump caused closure of several mussels. The use of a peristaltic pump solved this problem and no mussels closed at the time the pump was started (Wagenvoort & Engels, 2007).
In conclusion, vibration is not considered to be a problem in the operation of the MOSSELMONITOR®, provided abrupt disturbances are avoided.

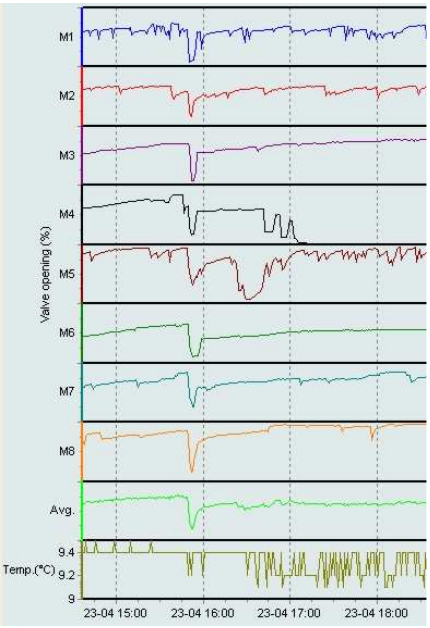


Figure 3.6 The effect of a short disturbance (e.g. vibration or exposure to air) at 15:50 on 23rd April on the relative valve opening of mussels (*Dreissena polymorpha*) (data: K. Kramer).

3.4.5 Light

Mussels are sensitive to light. A sudden change in light level, e.g. when the light is switched on, or when a shadow of a person (or e.g. a boat for the *in situ* version) falls on the bivalves, a closure reaction may occur. In general, all mussels react with a sharp closure peak, which can be recognized relative easily and the alarm parameter

"decreased average" may then be activated. For the flow-through version, it is recommended to place a dark housing over the MOSSELMONITOR®, in order to avoid the influence of light.
For the *in situ* version the light absorption effect of the water column is usually sufficient to minimize the effects of light.

4 Installation of the MOSSELMONITOR®

4.1 The MOSSELMONITOR® in the Netherlands

In the Netherlands, four MOSSELMONITOR® instruments are currently in use for water quality monitoring. They have been located along the main rivers, the Rhine and the Meuse, and in smaller water systems. For an overview of locations, see Table 4.1.

Table 4.1 Locations of the MOSSELMONITOR® in the Netherlands.

Location	Water	Company/institution
Heel	River Meuse	Water company Limburg, Ltd. (WML)
Nieuwegein	River Rhine	Amsterdam Waterworks (Waternet)/ Het Waterlaboratorium (not in use)
Zuuk	Klaarbeek brook	Vitens
Heemskerk	Assumburg Nature Reserve /Oud-Haerlem	Hoogheemraadschap Hollands Noorderkwartier

4.2 Origin of mussels

In the MOSSELMONITOR® sedentary (attached to hard substrate by byssus threads), filter-feeding species are preferred. Furthermore, it is highly preferable to use local species than to import new populations or – even worse - new (alien) species. Local species are best adapted to the environmental conditions, provided of course that these specimens are **not** already adapted to polluted waters. Mussels should be retrieved from "natural" ecosystems. The systems should be clean, without pollution and produce enough mussels (100 per year) to supply users in the future. Organisms collected from less polluted (pristine) sites are more sensitive than those from polluted waters (Kramer & Foekema, 1999). In any case, it is best to regularly determine the sensitivity of the mussels and pollution in the mussels from the selected site before they are used.

The selection of the test species depends firstly on the salinity of the water at the investigated site. In Europe one of the three species listed in Table 3.1 can be used, depending on the salinity of the water investigated.

To retrieve the mussels form nature, the individual mussels should be carefully removed from the substrate. In case of a species with byssus threads (e.g. *Dreissena polymorpha* or *Mytilus edulis*), it is best to loosen the mussels from the substrate by cutting the byssus threads with a pair of scissors or scalpel. Picking up the mussels by hand may cause the mussels to be damaged, causing unnecessary mortality in the stored batch of mussels. After retrieving the mussels, it is essential to store them in clean, unpolluted surface water for future use. Best practice is to place them in a kind of net, with large sized holes, in order to ensure maximum possible exchange of the water (exchange of oxygen and food). Here they can be kept for several months.

Breeding of mussels is also very possible but has several disadvantages:
- it requires (specially) skilled personnel;
- smaller species have to be cultured for several years before they can be used;
- it is expensive.

4.3 Gluing the mussels

The best results have been achieved using rapid hardening glues, with good filling characteristics. The glue must be non-toxic and may not affect the mussels. For this purpose, the use of the dental glue UNIFAST®, produced by the G-C Dental Industrial Corp. (supplied with the MOSSELMONITOR®) is recommended.
The glue requires the mixing of two components, but the setting time is short, depending on the temperature. It is recommended to prepare an amount of glue that is sufficient for 1-2 mussels only, and repeat this until all gluing has been completed. Glue each mussel on the flat side of the mussel holder so that in case of a closed mussel the sensors are situated approximately 20 mm apart. During the installation of the mussels the option for installation of mussels should be selected in the menu of the MOSSELMONITOR®. This provides continuously measured values on screen.
The mussels should be cleaned with a tissue to remove any biofouling and to ensure optimal attachment. The mussels are glued on a dry MOSSELMONITOR® and as a result of the exposure to air the mussels are closed. The mussels should be glued in such a way that MOSSELMONITOR® shows a reading of 100 to 200, so that the opened mussels are and stay in the range of magnetic field after some growth has occurred. A value of "550" is assumed for an open mussel (for instance a sensor distance of 25 mm). Deviations of +"50" will have no negative effects on the results. Due to different electromagnetic transparencies of water and air, the measured values in water are approx. 50 absolute units higher than in air. Mussels do not have a uniform thickness and the tapered form may be used as an advantage. For a large mussel the sensors should be attached closer to the front edge ensuring a sensor distance of approximately 20 mm at closed position. Ideally the sensors should be positioned in line, in order to guarantee the correct (electrical) functioning of the coils. When using small mussels, i.e. with a thickness smaller than 20 mm, more glue should be used in order to fill the space between mussel and mussel holder and obtain a signal of 100 for closed mussels. In case of very thin mussels the support may be thickened by gluing an additional plastic (5 mm PVC) plate to the support before positioning the mussel.

4.4 Parameter settings

The functioning of the MOSSELMONITOR® for measurement, communication, and evaluation is contained in the MOSSELMONITOR® stored on EPROMS inside the monitoring system. This software is loaded by the manufacturer and cannot be changed (or deleted) by the user. Because of this set-up, the monitor functions even when no personal computer is connected. Only for the presentation of the results, for example by using PRESENT^{IT}2, and/or data storage does a personal computer need to be attached to the system.
The moment the communication cable (*in situ* model) is attached to the MOSSELMONITOR® or the button of the flow-through system is switched on, the MOSSELMONITOR® software starts automatically. The program uses default values stored in the MOSSELMONITOR® software. For initial use they are settings made by the manufacturer, otherwise the parameters entered on the last occasion by means of a personal computer are used. It is possible to alter the parameter settings for evaluations so that the MOSSELMONITOR® operates according to the user's specifications. For this, the user needs to have direct access to the MOSSELMONITOR® by using PRESENT^{IT}2 or Windows HyperTerminal program. During the alteration of the parameters of the software, via the main menu, no measurements are made and consequently no data are stored.
The different relevant parameters will be explained in the following subsections. More detailed information can be found in the manual of the MOSSELMONITOR®. This information is essential when the user wants to optimise its application. In general, the MOSSELMONITOR® becomes more sensitive if an alarm is generated after a response of

a lower number of mussels and if this response lasts shorter than the standard settings (Box 1, Chapter 7). Unfortunately, in this case, the alarms also become less reliable and the occurrence of false alarms increases. The data of the measurements of the MOSSELMONITOR® can be evaluated relatively easily with standard software, e.g. MS Excel. Annex 2 lists the values of the standard parameter settings of the MOSSELMONITOR®.

4.4.1 Selection of mussels to be measured

In general, the MOSSELMONITOR® operates with eight mussels. If a mussel shows abnormal behaviour, it is possible to indicate which mussel(s) should be excluded from the calculations and alarm evaluation.

4.4.2 Measuring interval

The measuring interval (unit: second; range: 10 to 600 s, or 10 seconds to 10 minutes) determines the period after which the valve positions are measured, the values are stored and t processed in the calculations. An interval of 60 is recommended and gives relatively small data files but enough detailed information.

4.4.3 Evaluation delay

After new mussels have been glued onto the MOSSELMONITOR®, the mussels are heavily disturbed, and the chance of false alarm is strongly increased. During the acclimatisation period of the mussels, a time delay can be entered ("Evaluation delay", unit: hour; range: 0 to 24 h) to prevent any false alarms occurring in the first few hours after installation. This delay ensures that no calculations are carried out on the measured valve positions. Maximum and minimum levels are still calculated, but no alarm is generated. After new mussels have been glued on, the standard delay is 12 hours.

4.4.4 Adjusting span

The span (unit: day; range: 1 to 50 days) is the area within which the valve position of the mussel alters (from fully closed, 0%, to fully opened valves, 100%). The minimum and maximum valve positions are determined for each individual mussel. All intermediate valve positions are related to this minimum and maximum. The valve positions are expressed as a percentage of this 100% span (0% and 100% respectively match the minimum and maximum **absolute** values measured in the current time). The first time span during which a minimum and maximum are calculated is the period during which the evaluation delay takes place. The second time span is the following full 24-hour period. The measured valve positions are expressed as percentages, using the minimum and maximum recordings from the first time span. Maximum values are automatically increased and minimum values are automatically decreased if the measured valve position exceeds respectively is lower than those measured during Time Span l. In other words, after the second time span, minimum and maximum valve positions from Time Span 1 and Time Span 2 are taken into account. The 24-hour period following evaluation delay is a fixed period, and entirely independent of the period entered by the user. This guarantees a reliable minimum and maximum value.
This update is necessary because the (absolute) minimum and maximum positions of the mussels can alter as a result of environmental influences, for example as a result of growth and decreasing water temperature in the winter period.

4.4.5 Suspend evaluation

Under certain circumstances you might wish to suspend the evaluation of the valve positions and adjustment of spans and variables. E.g.: In case the MOSSELMONITOR® is temporarily out of the water (*in situ* model) for inspection and cleaning, or removing the deposited sediment from the flow-through model by evacuating the water. The sensors give a different reading in water and in air. Consequently, false alarms can be generated.

Before maintenance the "evaluation delay" should be activated in order to avoid false alarms. To prevent a restart (reset) of the system, the evaluation can be suspended and no alarms occur (except battery alarm).

4.4.6 Alarm evaluation

There is a balance between the sensitivity and the reliability of the system. Very sensitive settings may result in a relatively high number of alarms, but many may be considered "false". In contrast, low sensitivity results in alarms that are highly reliable: if under such conditions an alarm occurs, the user may be quite sure of the alarm. For example, 3 mussels closed for 2 min is a situation that can occur even in non-polluted situations, but when 7 mussels are closed for 5 min this means a definite alarm (the chance that such a situation occurs under natural conditions is statistically very low). The relation between the possibility of random closure and the number of mussels is illustrated in Box 1 (Chapter 7).

4.4.6.1 Closed evaluation

Using the "closed" evaluation, it is possible to detect whether a relatively high number of mussels remain closed for an unusually long time, e.g. as a result of occurring pollution. If the user wishes to evaluate the data for this alarm, the following parameters must be set:

- Percentage considered closed (unit: percentage %; range: 1 to 99 %);
  Mussels in the "closed" position are not always entirely closed. First of all, a percentage of the maximum open position must be entered, at which the mussel is considered to be "closed". If the mussel closes further than this set percentage, a timer is initiated. If the mussel re-opens to a level equal to or higher than the set percentage, the timer is reset. The higher the "closed" percentage, the more sensitive the alarm system, but also the chance of the occurrence of false positive alarms increases.
- Time closed in minutes (unit: minute; range: 1 to 30 minutes);
  A minimum time must be entered, during which the number of mussels must remain closed before the alarm threshold is reached. The shorter the minimum "closed" time, the more sensitive the system.
- Number of mussels closed (unit: number; range: 1 to 8 mussels);
  A sufficient number of mussels must remain closed for a sufficient period of time, for the alarm threshold to be reached. The smaller the number of mussels, the more sensitive the alarm system becomes. This parameter has the largest influence on the sensitivity of the MOSSELMONITOR®.

4.4.6.2 Decreased average valve position evaluation

A reduction in mussel activity is also shown by a decrease in average valve position. The physiological effect is that the mussels stop pumping and the siphons are withdrawn. The shells move closer together. This too can be the result of pollution in the water. Generally, this alarm signal is more sensitive than a "closed" alarm. The interval should be 15 minutes to obtain a fast response, but the number of mussels involved has the largest influence on the sensitivity of this alarm parameter.

4.4.6.3 Activity evaluation

With certain types of pollution, the mussels show an increase in the open-close-open frequency. Activity is interpreted as a minimally detectable change in valve position (in one direction) between two subsequent measurements. Several subsequent changes in valve position from the mussel in the same direction count as one activity. A change in direction of the valves can thus produce one activity. The use of the activity alarm under field conditions has not yet been demonstrated (personal comment Kees Kramer, 24th July 2007).

4.4.6.4 Gaping evaluation

When a mussel dies, the two shell halves of the mussel are open fully, since the closing muscle disintegrates, and is no longer tensed. This position is known as "gaping". A gaping mussel is noted if the maximum open position compared with a previous time block shows an increase in percentage greater than, or equal to that of the entered percentage. In the standard setting, the span is measured every day and as soon as the percentage of one mussel reaches 200%, this alarm is active.
The same affect happens when a coil and a mussel become detached and the alarm is activated (Figure 5.1).

4.4.7 Data acquisition

The values are stored in the data buffer of the MOSSELMONITOR® or are sent to the computer attached to the MOSSELMONITOR® after each measurement. There are different options of data stored, type of alarms and "watchdog" results sent to the terminal or computer. The different parameters that are recorded are:

- measured values to terminal (type of valve opening: absolute or percentage or both);
- results of evaluated values to terminal;
- all alarms to terminal;
- low battery voltage detection;
- alarm contact;
- OK message from the MOSSELMONITOR® ("watchdog").

This last parameter result periodically releases an OK message as a sign that the system is still operational.

5 Maintenance of Appliances and Monitoring the Mosselmonitor®

5.1 Maintenance of the MOSSELMONITOR®

Maintenance can be divided into a number of activities with different frequencies. Table 5.1 gives an overview of the different maintenance activities.
It is important to keep an accurate record of the maintenance that has been carried out. The evaluation of the measurement data forms an essential source of information for tracing false-positive signals. An example of a maintenance plan is included in Annex 3.

Table 5.1 Overview of the maintenance of the MOSSELMONITOR® (flow-through version), and the corresponding frequency.

Frequency Activity	Explanation	Sect.
Daily (from a distance by way of the modem connection or internet with the monitor)		
- Evaluate the measurements for deviating behaviour and alarms		5.2.1 7 5.2.1
- Check for loose coils or mussels		
Weekly maintenance		
- Clean hoses and exposure container (residue).	Sedimentation of suspended material deposits in the box of the MOSSELMONITOR® (flow-through system). The amount and rate of this sedimentation depends on the turbidity of the water. If necessary more often.	3.2.1.2
- Visual inspection for loose coils or mussels		5.1.1
- Check for biofouling		3.2.1.3
- Check for abnormalities	E.g. Degassing in the exposure container	
Maintenance at least every 2 months		
- Visual inspection new mussels		6.1
- Replace mussels		4.2 and 4.3
- Check the response of the coils and standard distance block		5.3 and 6.2
- Check the temperature sensor		5.2.1
Maintenance by manufacturer, when necessary		
- Check electronics		5.3
- Replacement of the sensors		5.3
- Calibration of temperature sensor		5.2.1

5.1.1 Checking mussels and sensors

The measurements are only reliable when the coils are attached to the mussels. If a coil becomes loose, the valve opening is no longer measured accurately and in general a very constant (often wide) value of the valve opening (absolute and %) is recorded and sometimes a "gaping" alarm is activated. If a "gaping" alarm is triggered, the value "#100" is recorded as measurement for that individual mussel. The typical pattern of measurements of mussels after coils have been unfastened is shown and explained in Figure 5.1. The coils can be re-glued to the mussels, and measurements can be continued. In special situations, e.g. in case of long travelling time, the untied mussels can be excluded from the data evaluation.

As described above, the occurrence of a coil no longer attached to a mussel can been seen quite easily by a short evaluation of the measurements. Of course, it is also relatively

easy to perform a visual inspection of the attachment of the coils to the mussels during additional and weekly services. This prevents the occurrence of completely detached coils.

5.2 Evaluating the measured parameters

5.2.1 Temperature of the sample water

In the MOSSELMONITOR® the temperature of the sample water is also used to detect technical malfunctions. The temperature of the sample water is relatively constant, due to the great heat capacity of water. When using a flow-through system, a relatively sharp increase or decrease in temperature (depending on the season) up to the temperature of the air temperature very likely indicates a malfunction of the system. The most likely outcome is that the water supply is reduced or clogged, or the pumping system has stopped.

Because temperature is an important parameter to determine the operation of the MOSSELMONITOR®, the sensor on the MOSSELMONITOR® should be checked regularly by using a calibrated reference thermometer. In case of deviations the sensor should be calibrated or replaced by the manufacturer.

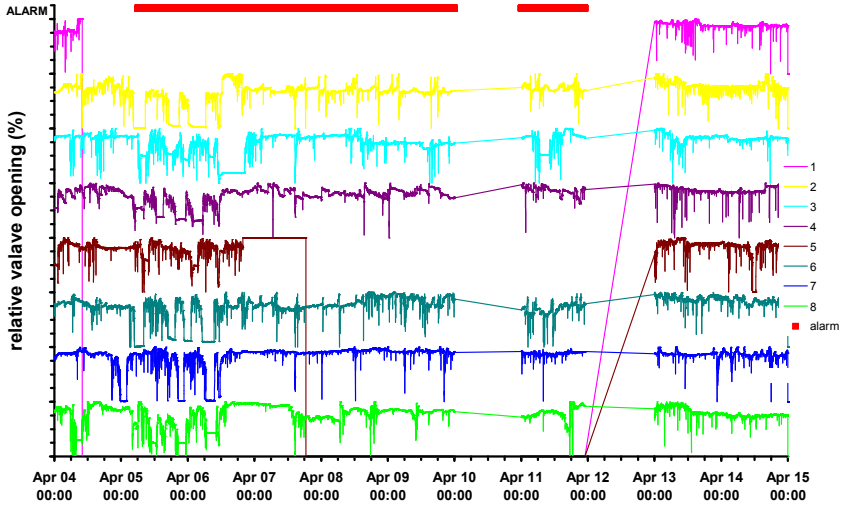


Figure 5.1 Typical pattern of coils becoming loose from a mussel.

Mussel 1 and 5 caused a "gaping" alarm due to a coil that was no longer attached to the mussel. The characteristic behaviour of this technical failure is illustrated: normal behaviour is shown, and after several maximum values (100 %) a "gaping" alarm is activated. On 13th April the mussels show normal behaviour again after they have been re-glued to the coil.

5.2.2 Valve movements

Under normal environmental conditions, mussels are submersed and their shells are open to allow for feeding and respiration. The average valve opening of about 70 – 95 % is evident, as are the short periods of closure (Figure 2.5). The behaviour of individual mussels can show a variation in activity, but this is quite common, and should be marked as normal valve behaviour.

In case food resources are limited, a typical kind of pattern of the valve movement becomes apparent, the so-called "low food' pattern (Figures 3.2 and 3.3).

For reproduction, the mussels have to release their eggs or sperm. In general, this takes place in spring and often after a sharp increase in temperature above 12 to 14°C (Ketelaars & Wagenvoort, 1995). During this period, called spawning, sensitivity is affected and the valve movement alters. The combination of abnormal behaviour and an increase in temperature in spring causing spawning can be confirmed by a microscopic analysis of the sample water for mussel larvae (Figure 2.4).

5.3 Response of the coils

Measurement of the valve opening is done using coils which record the strength of the magnetic field generated by the other coils. The only possible failure of this robust system could be broken wires. Drift of the measurement does not occur. In order to ensure proper operation, it is recommended to record the response of the pairs of coils at the time the mussels are replaced (small value (reading) for closed mussels and an increased reading for filtrating (open) mussels). Measurements of the distance of calibration blocks, with a specific distance, give essential information on the functioning of the sensors. If there is no response or abnormal readings are observed, the coils should be replaced and the system checked by the manufacturer.

6 Quality Assurance

6.1 Macroscopic and microscopic check of the mussels (parasites)

Before the mussels are glued to the MOSSELMONITOR®, a visual inspection of the tissues of several mussels for parasites (trematodes) is recommended, since infested mussels have a lower biomass and contain higher concentrations of heavy metals than non-infested mussels (Davids & Kraak, 1993). How this influences the sensitivity of the infected mussels is still unknown.

After opening several mussels, by cutting the muscles (posterior adductor muscle, and posterior byssal refractor muscle, Figure 6.1) with a scalpel, the tissue of these mussels can be inspected. For the inspection of small parasites the use of a dissection microscope (magnification at least 40 times) is recommended. If a large fraction of the mussels (more than 10%) contains parasites (Figure 6.2) or other abnormalities are observer, it is best to collect another batch of mussels.

Davids & Kraak (1993) provide more information on trematode parasites.

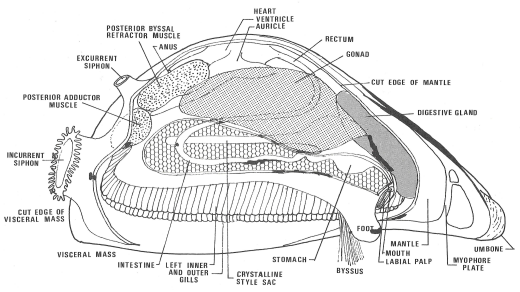


Figure 6.1

Diagram of location of major internal organs of adult Zebra Mussels.

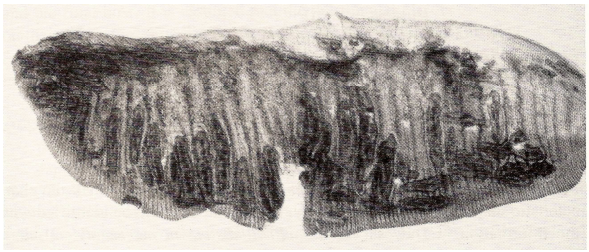


Figure 6.2

Gill of Dreissena polymorpha infested with Sporocysts of the trematode *Phyllodistomum folium* (Davids & Kraak, 1993)

6.2 Pollution in mussels

It is expected that organisms collected from less polluted (pristine) sites are more sensitive than those from polluted waters (Kramer & Foekema, 1999). It is best to regularly determine the sensitivity of the mussels (Section 5.3) and the pollution in the mussels (by chemical analysis) from the selected site before they are used. If pollution in mussels is to be expected or is measured, this should be monitored by examining the mussel (tissue) at regular intervals.

6.3 Function of the sensors in the MOSSELMONITOR®

Measurements of the distance of calibration blocks, with a specific distance, give essential information on the functions of the sensors. It is best to record this information at the time the mussels are replaced.

6.4 Sensitivity of the mussels

6.4.1 Anoxic survival time: a simple test for sensitivity to stress

Blue Mussels (and Zebra Mussels) are subject to unfavorable environmental conditions, such as fluctuations in salinity, temperature, food and oxygen availability. Sustained valve closure, accompanied by oxygen depletion, is a response to a stress-generating

condition. *M. edulis* can tolerate hypoxic or anoxic for days or even weeks due to the evolved biochemical strategy: a strong reduction energy demand and activity of highly efficient pathways of anaerobic energy metabolism. The survival time of the Blue Mussel under stress (closed shells) depends on the temperature (Figure 6.3a), season (glycogen content, Figure 6.3b) and pollution (Veldhuizen-Tsoerkan *et al.*, 1991; Smaal *et al.*, 1991; Eertman *et al.*, 1992a). Of course, the anoxic survival time differs between different species of the Bivalvia.

The parameter "Anoxic survival time" can be used as a simple standard test for the sensitivity of mussels to stress (Eertman *et al.*, 1992b). The combination of the anoxic survival time test and glycogen content in mussels is useful to screen the sensitivity of a batch of mussels before they are used on the MOSSELMONITOR®. The use of these tests makes the comparison with other datasets of mussels on the MOSSELMONITOR® possible. The data of the sensitivity measurement varies in time, but can be tested against dynamic guidelines and displayed in control charts. If the mussels originate from a different location than used before, these tests provide essential information on the sensitivity of the mussels used. If the measured sensitivity is not sufficient, another batch of mussels originating from an alternative site should be used.

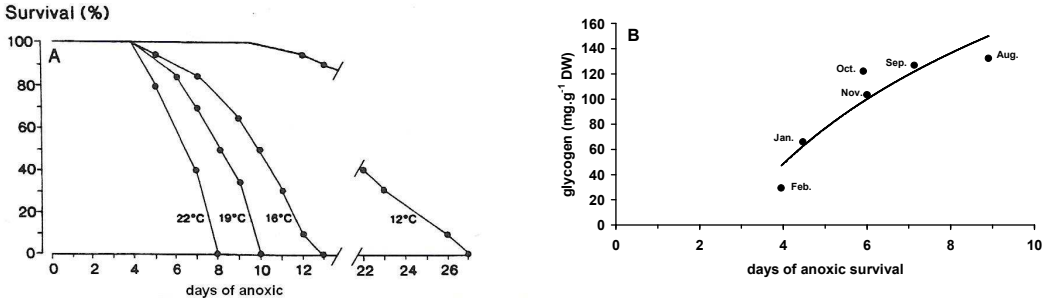


Figure 6.3 Anoxic survival time of the Blue Mussel, Mytilus edulis

A Influence of the temperature on the days of anoxic (Veldhuizen-Tsoerkan *et al.*, 1991)

B Influence of season or glycogen at a standard temperature of 18 °C (Eertman *et al.*, 1992a)

6.4.2 Control sample

With the use of a control samples, the operationality of the whole system configuration of the MOSSELMONITOR® can be determined periodically. Recently a standardised test is developed and is implemented at the monitoring station situated at the abstraction point of WML (Wagenvoort & Engels, 2007). In this test, the response to copper (a solution of $CuSO_4$ in tap water added through a static mixer to the flow of the sample water with the use of an additional pump) at a concentration of 20 to 40 $\mu g.L^{-1}$ or sodium chloride at a concentration of 3 ‰ is measured. The responses, type of alarm, duration of the alarm, number of responding mussels, and so on can then be recorded, tested against guidelines, and displayed in control charts.

The main response of the mussels to the control sample will be the closure of the shells. This means that the mussels will not accumulate the copper in their tissues and the sensitivity will not be altered. It is recommended to perform such a test at least once a month, or when there is a doubt about the recorded measurements or the sensitivity of the mussels.

6.5 Verification test

In order to test the response of mussels on the MOSSELMONITOR® to specific chemicals or changes, so-called verification tests can be performed. In these tests the set-up is comparable to the test of the control sample. In these experiments it is essential to record all relevant information, such as:

- screening of the sample water with the use of online sensors (temperature, oxygen, turbidity, acidity, and conductivity) for abnormalities;
- chemical screening of the sample water for pollutions;
- record of the sensitivity of the mussels used (Section 5.3);
- other relevant information.

7 Evaluation of Valve Movements

7.1 Parameter settings

The data evaluation of the MOSSELMONITOR® depends on the settings of the different parameters. Usually, the default setting of the manufacturer is adequate for most systems (see Annex 2). Moreover, each user has the possibility to define his own set of parameters. However, before the user compiles his own set of parameters, it is important to provide insight into which changes are implemented and the rationale behind them. The implemented changes can be tested by means of validation experiments (Section 6.4). In general, the MOSSELMONITOR® becomes more sensitive if an alarm is generated after a response of a lower number of mussels (BOX 1), at a shorter response time (see Section 3.5), or by shortening the time of the threshold of the alarm condition (per mussel). Unfortunately, the alarms become less reliable when the sensitivity is increased and the occurrence of false positive alarms increases.
Clearly, in the case of an alarm, it should be clear which settings have been used to evaluate the data and induce the alarm. Reports of measurement data and generated alarms must indicate which set of parameter settings was used at the time of the alarm. It is recommended to initially implement the same settings to all measuring systems in the same river system, and to deviate from these agreed settings only when there is clear evidence of settings generating too many false-negative/-positive alarms.

7.2 Alarm signals and the evaluation of measurement data

In principal, every generated alarm is regarded as a true alarm, even if the duration is very brief. In order to validate the alarm, it is important to evaluate whether there has been a failure in the monitoring system or e.g. in the monitoring station (such as a pump failure), or regular occurring events which did not influence the quality of the water prior to the measurements (such as a regular increase in turbidity caused by shipping-traffic). For data evaluation it is essential to register all maintenance, alarms, and other remarkable phenomena. In Annex 4, there is an example of such a registration worksheet for WML at Heel.

7.2.1 Gaping alarm

A "gaping alarm" is generated after the relative valve opening of a mussel during a relatively short period dramatically increases, e.g. more than 100% on top of the last maximum valve opening. This may happen after a mussel has died and the closing muscle no longer functions (Figure 7.1). It may also be an indication of a sensor (or mussel) being disconnected from the support (after incorrect gluing during installation).

The "gaping alarm" is also activated in case of a loose coil or the breakdown of a coil (Figure 5.1 and Section 4.4.1). In this case, the alarm is caused by a technical failure and the coil should be reattached as soon as possible. After a gaping alarm the measurements of the mussel should be excluded, the mussel, or in case of a broken sensor, the sensor should be replaced and finally the system reset.

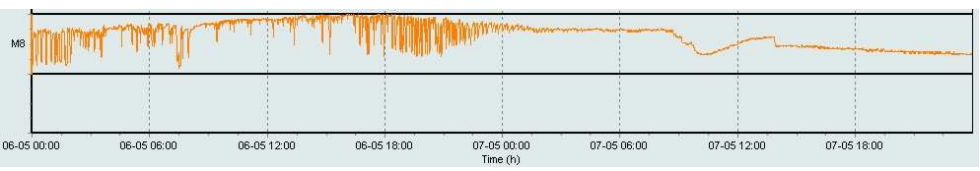


Figure 7.1 Mussel 8 died on the 7th May and the relative valve opening no longer showed variation.

After several days the relative valve opening will increase to a gaping situation (alarm) after the closing muscle is disintegrated (this situation is not shown here).

7.2.2 Decreased average alarm

A reduction in mussel activity is shown by a decrease in average valve position. As a result of water pollution, a possible physiological effect may be that the mussel stops pumping and the siphons are withdrawn. As a result the shells move closer together. In theory, this alarm signal is more sensitive than a "close alarm". Therefore, this alarm parameter seems to be more sensitive for false-positive alarms than a "close alarm".
The period in which the alarm can be generated is short and the alarm parameter is more sensitive for short disturbances, such as e.g. maintenance, turbidity peaks, light (Section 3.4.5), and vibrations (Section 3.4.4) (Kramer & De Maat, 2007 in prep.). However, "decreased average" alarms caused by maintenance can easily be avoided by switching the MOSSELMONITOR® off during maintenance (Section 4.4.5).

During maintenance the container of the flow-through MOSSELMONITOR® is drained, and the container and mussels are rinsed. During these steps the mussels are exposed to air, close their shells, and if the MOSSELMONITOR® has not been switched off, this can generate a "decreased average alarm" unless the parameter "suspend evaluation" is activated (Section 4.4.5; Figure 7.2a), sometimes followed by a "close alarm". If maintenance is recorded properly, these alarms can be marked as caused by maintenance (Annex 4).

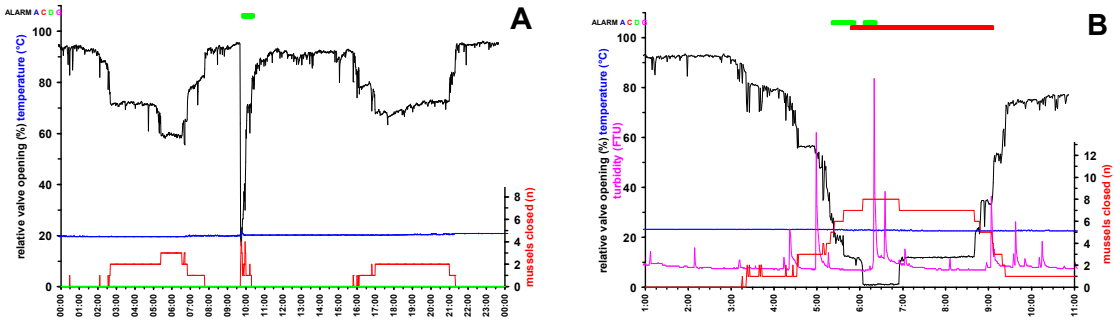


Figure 7.2

A Maintenance on 4th June 2007 09:40 until 10:00, caused a "decreased average alarm".

B Increased turbidity in the River Meuse caused the closure of the mussels on the MOSSELMONITOR® of WML at Heel generating a "decreased average alarm" followed by a "close alarm" on 21st June 2007 05:20 until 9:00.

In rivers or canals with intensive shipping traffic, strong, short-term (several minutes) increases in turbidity (amount of suspended material) may occur. Sometimes these short-term disturbances cause the closure of mussels and a "decreased average alarm", which is sometimes followed by a "close alarm" (Wagenvoort, 2006a,b). These events can be recognised easily when the turbidity of the sample water is recorded and displayed together with the valve opening of the mussels of the MOSSELMONITOR® (Figure 7.2b). Combining both signals the alarm can be classified as caused by turbidity and shall be eliminated.

BOX 1 Reliability and sensitivity of the "close" alarm evaluation

Closure of the valves for longer periods is considered to be escape behaviour. If one bivalve is closed for a prolonged time, e.g. for 5 minutes, it is not considered unusual; if several mussels do this simultaneously, e.g. 5 out of 8 mussels, it is deemed highly unusual and the reason for an alarm. However, some species, such as *Dreissena polymorpha* and *Unio pictorum*, have shown occasionally prolonged closure times (maximum 2 to 4 hours), obviously as part of their natural behaviour.

However, even in these cases, the simultaneous closure of (nearly) all bivalves remains highly unusual, and is the reason for an alarm. The probability of a certain configuration of the "close" alarm evaluation is explained in this box.

In this case, the assumption has been made that all mussels close during a relative long period of 120 minutes (2 hours) per day. To simplify this, this closure period occurs in one interval. In the data evaluation of the "close" alarm at least 5 mussels should be closed simultaneously for at least 10 minutes.

In our example, the chance that a mussel closes its valves and that they remain shut for at least an half an hour is 1 hours and 50 minutes out of 24 hours, or (1+5/6)/24. So if we have five (=n) mussels, the probability that all of these are closed can be calculated by:

$$p = \left(\frac{1\frac{5}{6}}{24}\right)^{n} = \left(\frac{1\frac{5}{6}}{24}\right)^{5} = 2.60 \cdot 10^{-6}$$

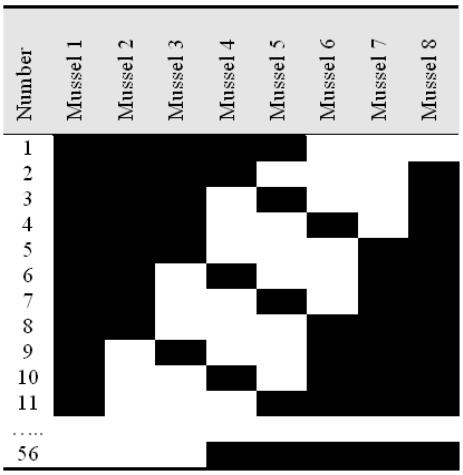
However, in the MOSSELMONITOR® there are 8 (=N) mussels, so 3 (=N-n) other mussels should be open. The chance that a mussel is open can be calculated as: 1-(1+5/6)/24 per mussel. The probability that mussels 1, 2, 3, 4 and 5 are closed and mussels 6, 7, and 8 are open can be calculated as follows:

$$p = \left(\frac{1\frac{5}{6}}{24}\right)^{n} \cdot \left(1 - \frac{1\frac{5}{6}}{24}\right)^{(N-n)} = \left(\frac{1\frac{5}{6}}{24}\right)^{5} \cdot \left(1 - \frac{1\frac{5}{6}}{24}\right)^{3} = 2.049 \cdot 10^{-6}$$

The probability should not be calculated for only one defined combination (mussels 1, 2, 3, 4 and 5 are closed and mussels 6, 7, and 8 are open), because other combinations are also possible (Table Box .1).

BOX 1 continued

Table Box 1 Illustration of the combinations that 5 mussels are closed (■) and 3 mussels are open (□).



In total, 56 combinations are possible. The number of combinations can be calculated as follows:

$$\text{combinations} = \frac{N!}{n!\cdot(N-n)!} = \frac{8!}{5!\cdot(8-5)!} = \frac{40320}{120\cdot 6} = 56$$

The probability that 5 mussels are closed and 3 mussels are open is:

$$p = \left(\frac{1\frac{5}{6}}{24}\right)^{n} \cdot \left(1-\frac{1\frac{5}{6}}{24}\right)^{(N-n)} \cdot \text{combinations} = \left(\frac{1\frac{5}{6}}{24}\right)^{5} \cdot \left(1-\frac{1\frac{5}{6}}{24}\right)^{3} \cdot 56 = 0.000115$$ or 0.12‰

The assumption was made that at least 5 mussels should be closed, so the probability that 6, 7 or all mussels are closed should be added. In Table Box 2 this is calculated and the probability is only 0.12 ‰ or less than once every 22 years.

Table Box 2 The probability of simultaneously closing of different numbers of mussels caused by their natural behaviour

number of mussels closed	number of combinations	probability (for combination in defined order)	probability (for combination in random order)	probability (for at least that number)	p	remarks
1	8	0.0438	0.3504	0.4704	>0.05	not significant, very likely
2	28	0.0036	0.1014	0.1201	>0.05	not significant, very well possible
3	56	0.0003	0.0168	0.0186	<0.05	weak significant, happens appr. 7 times a year
4	70	2.48E-05	0.0017	0.0019	<0.005	strong significant, unlikely, less than once a year
5	56	2.05E-06	0.0001	0.0001	<0.001	very strong significant, very unlikely
6	28	1.69E-07	4.75E-06	4.86E-06	<0.001	very strong significant, very unlikely
7	8	1.40E-08	1.12E-07	1.13E-07	<0.001	very strong significant, very unlikely
8	1	1.16E-09	1.16E-09	1.16E-09	<0.001	very strong significant, very unlikely

7.2.3 Close alarm

As we have seen, the simultaneous closure of several mussels for a prolonged time will generate a "close alarm". This indicates that the mussels experience the water quality as unfavourable. Afterwards, an alarm evaluation, as to whether it is a true or false alarm, should be preformed. The alarm evaluation procedure follows several steps:

- exclude false positive alarms generated by known disturbances (e.g. due to maintenance, turbidity peaks, light, vibrations, lack of food, or spawning);
- evaluate the data of the online sensors of turbidity, oxygen, temperature, acidity, and conductivity for causating factors (e.g. heavy rainfall);
- analyse water sampled at the time the measurements prior to the alarm started to deviate from normal by means of advanced chemical techniques;
- record the observations and causating factors in order to gain more inside information on observed changes (e.g. spills of pollutions).

By closing the shells the bivalves physically exclude the outside environment, and can thus survive for days during unfavourable conditions. However, on the one hand prolonged closure of the valves influences the mussel's sensitivity and can cause false positive alarms. On the other hand, at low concentration, before the mussels close their shells accumulation of pollution takes place. It is recommended to renew the mussels at least every two months or if the mussels have been closed for a long time. This is defined as the (relative) duration of the total of all "close alarms" (Annex 4):

- in one week the sum of the "close alarms" is more than 15% of the time (15% is approximately 1 day or 24 hours);

Or

- when the sum of all "close alarms" recorded by the actual batch of mussels on the MOSSELMONITOR® has a duration of more than 100 hours.

7.2.4 Activity alarm

In experiments performed under laboratory conditions Kramer & Foekema (1999) observed for several species (*Mytilus* spp. And *Dreissena polymorpha*) that the activity (open-close-open movement frequency) can increase dramatically when the species are exposed to toxic substances (organic solvents, free chlorine, and others); for evaluation the present frequency is compared with information collected 2 hours previously. The use of the activity alarm under field conditions has not yet been proven (personal comment Kees Kramer, 24th July 2007).

7.2.5 Start and features of an alarm signal

After an alarm is generated, the data should be analysed. The first step in the alarm evaluation is to exclude possible false-positive alarms. In data evaluation the number of mussels that have reacted (decreased/closed valves), the start of the decreased (closed) valves and the duration of the observed response is analysed. Depending on the parameter settings, the observed closure of the valves may be recorded some time before the alarm is generated. This is a result of the evaluation time that is set. For illustration, an alarm evaluation is performed in Figure 7.3 and Table 7.1.

As the characteristics are described, the causative factor is examined. For this, the readings of the online sensors are evaluated and in order to verify the alarm it is recommended to perform a chemical screening for organic micropollutants of a sample taken at the beginning of the alarm. In the example, several chemical pollutants were present at the time the alarm started (Table 7.1). These pollutants have also been found before in samples taken at the beginning of an alarm (Wagenvoort *et al.*, 2008).

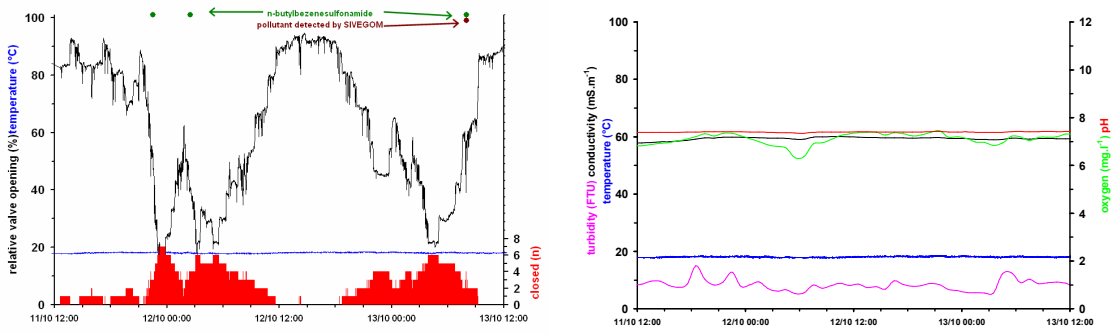


Figure 7.3 The average valve movement of Dreissena polymorpha exposed to water from Lateraal Canal

Data of online sensors and the presence of observed pollutants (samples with (○) SAMOS and (○) SIVEGOM, detected respectively ●, ●) (Wagenvoort *et al.*, 2008).

Table 7.1 Number, period and duration of the closure of Dreissena polymorpha exposed to water from Lateraal Canal (Wagenvoort et al., 2008). Data evaluation of "close" alarms from 11th to 13th October 2007

	5 mussels			6 mussels			7 mussels			8 mussels			remarks
date	start	end	Dura-tion	start	end	Dura-tion	start	end	Dura-tion	start	end	Dura-tion	
Oct 11	22:44			22:58			23:14	23:49	00:35				n-butylbenzenesulfonamide (± 2 mg.L^{-1}) and
Oct 12		0:50	02:06		0:24	01:26							N-(2-cyclohexyl-1-methylethyl)-N-methylacetamide
Oct 12	3:01	6:18	03:17	3:08	3:34	00:26							
Oct 12				4:55	5:33	00:38							Idem
Oct 13	3:25	6:46	03:21	3:54	5:00	01:06							very strong increase of turbidity
													in the regular sample (of 8 o'clock) n-but benzenesulfonamide was detected (2,1 µg.L^{-1})

7.2.6 End of an alarm

The end of an alarm can be determined in two different ways:

- *By evaluating the valve movements*

Being closed for prolonged periods does not harm species such as the Zebra or Blue Mussel as they can change from an aerobic to an anaerobic metabolism (Zandee *et al.*, 1986; Eertman & de Zwaan, 1994). Unlike other species used in online biological alarm systems, such as water fleas and fish, the bivalves have a distinct advantage in water that may tend to become temporarily hypoxic or anoxic. The closure of the shells will continue until the mussels find the water conditions acceptable. Opening of the shells is a guarantee that the water quality is no longer unacceptable for the bivalves. However, the moment of opening will be later (up to several hours) than the actual improvement of the water quality.

- *By monitoring the cause*

In case a causative factor, e.g. a pollutant, turbidity or oxygen content, is established, it is possible to monitor the presence or the undesirable concentration of this causative factor. As soon as the respective parameter returns to normal conditions, the alarm will be withdrawn.

8 Conclusions and Recommendations

To determine the toxicity of fresh or marine water for respectively Zebra or Blue Mussel the MOSSELMONITOR® is a useful monitor. The system is placed in a water system or connected to the water flow of untreated or course filtered (provided that sufficient food particles are available) surface water.
The quality assurance of the MOSSELMONITOR®:

- Test organisms (mussels) can be retrieved from nature, provided that the selected site is a pristine (unpolluted) one. Before the mussels are used relative simple quality controls for infections and sensitivity are strongly recommended. If mussels of unknown sites are used, the presence of accumulated pollutants (heavy metals, organic micropollutants) should be determined.
- In practice, it is possible to standardise the installation of the MOSSELMONITOR®. In this document the essential and prior conditions are listed and discussed. These conditions should be taken into account when installing the MOSSELMONITOR®.
- Maintenance is standardised and should only be adjusted to site-specific conditions.
- The functioning of the MOSSELMONITOR® (the whole configuration) can be confirmed by a control sample. This method is recently developed by Wagenvoort & Engels (2007). This test can also be used in verification tests. In these verification tests, the effects of chemicals can also be examined under standardised experimental conditions.
- In case of deviant behaviour, a secure system (quality control and scheme of maintenance) will contribute to the acceptance of alarm signals.

Alarm evaluation:

- The alarm evaluation is closely related to the chosen parameter settings. In alarm reports it is essential to indicate which settings have been used to detect an alarm.
- For alarm verification, measurement results of the online sensors for temperature, oxygen content, acidity, conductivity and turbidity should be available. It is also recommended to use a sampling device for sampling the water before, during and after an alarm period.
- In an alarm signal the alarm should be specified, thereby stating the number of mussels that have closed their shells and the duration of this alarm. In this document an example is given.
- In many cases it is not easy to determine the end and duration of an alarm. It is recommended to determine the end of an alarm using one, but preferably more, of the following criteria:
    - evaluating the valve movement series (opening of the shells is a guarantee that the water quality is no longer unacceptable for the bivalves; however, the moment of opening will be later (up to several hours) than the actual improvement of the water quality.);
    - monitoring the cause (pollutant or factor).

The MOSSELMONITOR® has proven to be a successful instrument in the detection of pollutants. In a recent study, data evaluation of the MOSSELMONITOR® at the Lateraal Canal has shown that at the time an alarm is generated by the MOSSELMONITOR® pollutants were also present in the canal water (Wagenvoort *et al.*, 2008).

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I Detection limits of three bivalves in the MOSSELMONITOR® (Delta Consult, February 2006)

Component detection limit in $mg.L^{-1}$ (nominal) fresh water mussels *Dreissena polymorpha* and *Unio pictorum* and the marine mussel *Mytilus edulis* (www.mosselmonitor.nl)

	Fresh water		Marine
	Dreissena polymorpha	*Unio pictorum*	*Mytilus edulis*
Ammonia (Unionised)			0.59
Atrazine	0.5	0.5	
Bentazone	0.75	0.75	
Cadmium (CdCl2)	0.15		0.1
Cloroform	43.0		
Cloropyriphos	0.05	0.05	
Copper (CuSO4)	0.01	0.01	0.005
Cyanide (KCN)	0.4		
1.3 Dichlorobezene	1.4		
Dichlorometane			50.0
Formaline			10.0
Hexachlorbutadiene	0.15		
Y-hexachlorcyclohexane	0.06		
Hipochlorite (chlorine)	0.037		0.005
Lead	0.25		0.25
Lindane	0.11		
Oil (dispersed)			6.0
Pentachlorophenol	0.01	0.01	
Phenol	14.0		
Selenium (selenite)	0.1		
Tetrachloromethane			2.5
Toluene	6.0		
Tributyltinoxide (TBTO)	0.006		0.01
Trichlorethylene	8.0		
Xylene	16.0		
Zinc	0.5		0.5

II Parameter settings of the MOSSELMONITOR®

(Delta Consult, 2002)

09-07 12:50:44 Date/Time ok? (Y/N):	Y
Change password? (Y/N):	N
Measure all mussels? (Y/N):	Y
Interval in seconds (10-600):	60
Evaluation delay in hours (0-24):	12
Updating span in days (1-50):	1
Suspend evaluation? (Y/N):	N
'Closed' evaluation? (Y/N):	Y
Percentage considered closed (1-99):	20
Time closed in minutes (1-30):	40
Number of musselsclosed (1-8):	4
Must alarm activate alarm contact? (Y/N):	N
Reset computed values? (Y/N):	N
'Activity' evaluation? (Y/N):	Y
Interval in minutes (10-120):	30
Comparison with interval -N (1-6):	2
Min. span variation in percentage (1-50):	20
Min. increase ratio of act/mussel (1-50):	10
Number of mussels (1-8):	4
Must alarm activate alarm contact? (Y/N):	N
Reset computed values? (Y/N):	N
'Decreasing average' evaluation? (Y/N):	Y
Interval in minutes (15-120):	15
Comparison with interval -N (1-6):	4
Decrease in percentage (10-90):	20
Number of mussels (1-8):	4
Must alarm activate alarm contact? (Y/N):	N
Reset computed values? (Y/N):	N
'Gaping' evaluation? (Y/N):	Y
Increase in percentage (+100%) (1-200):	100
Comparison with interval -N (1-6):	2
Must alarm activate alarm contact? (Y/N):	N
Reset 'gaping' mussels? (Y/N):	N
Measured values to terminal? (Y/N):	Y
1. Absolute	
2. Percentage	
3. Both	
Your choice :	2
Evaluated values to terminal? (Y/N):	Y
All alarms to terminal? (Y/N):	Y
Low battery detection? (Y/N):	N
Must alarm activate alarm contact? (Y/N):	N
Alarmactive in seconds (30-3600):	600
Interval ok-message in hours (0-24):	8

III Example of a Maintenance Plan (WML, version 5, 27th June 2007)

Maintenance plan MOSSELMONITOR® - abstraction point: Lateraalkanaal

AqWa ecologisch advies

Version: 5 — AqWa-code: 2005F23
Date: June 27th 2007 — Page: 1 of 1

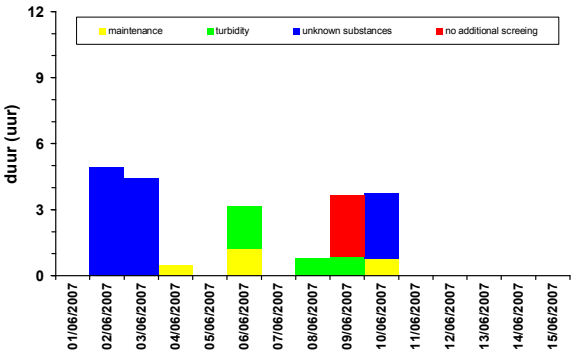
Year and Week _____

Date						Remarks
Time frame maintenance						
Operator						
Additional maintenance						
Clean the branch pipes	Y/N	Y/N	Y/N	Y/N	Y/N	
Remove the deposits on the mussels ("showering")	Y/N	Y/N	Y/N	Y/N	Y/N	
Clean exposure container	Y/N	Y/N	Y/N	Y/N	Y/N	
Weekly maintenance						
Clean the Rotameter (regulation of volume).			Y/N	Y/N		
Adjust the Rotameter at 500 L per hour			Y/N	Y/N	Y/N	
Are there loose coils/mussels?			Y/N	Y/N	Y/N	If YES, number:
Are there death mussels?			Y/N	Y/N	Y/N	If YES, number:
Are there mussels replaced?			Y/N	Y/N	Y/N	If YES, number:
Is there biofouling			Y/N	Y/N	Y/N	
Maintenance at least every 3 months						
Replace all mussels				Y/N		
Deviates the temperature sensor less than 0.5 °C				Y/N	Code reference thermometer:	Temperature reference: _____ °C Reading temperature sensor MOSSELMONITOR®: _____ °C; difference: _____ °C
Responds all coils?				Y/N	If NO, number:	

In case of an alarm or failure (add additional remarks):

IV Recording of Alarms, Maintenance, and Remarks of the MOSSELMONITOR® at WML

date	type alarm	start	end	duration (hh:m	duration (d)	remarks	reference	configuration	cause
Friday 01/06/2007					0.00			normal	
Saturday 02/06/2007	C	04:39	07:16	02:37	0.11	STOP abstraction unknown substances, ISAMDS RT [20.99 - max 2.9 µg L-1) en 2 SIVEGOM (RT 17.09, R(n)) 0.525 - 15 µg L-1; RT 23.38, R(n)) 0.798 - 178 µg L-1)	Aq'e-VML 2007N39		unknown substan
Saturday 02/06/2007	D	14:07	14:52	00:45	0.03		Aq'e-VML 2007N39		unknown substan
Saturday 02/06/2007	C	14:48	16:22	01:34	0.07		Aq'e-VML 2007N39		unknown substan
Sunday 03/06/2007	C	01:17	05:42	04:25	0.18		Aq'e-VML 2007N39		unknown substan
Monday 04/06/2007				00:00	0.00	START abstraction	Aq'e-VML 2007N39		
Monday 04/06/2007	D	09:52	10:22	00:30	0.02	maintenance	Aq'e-VML 2007N39		maintenance
Wednesday 06/06/2007				00:00	0.00	maintenance*	Aq'e-VML 2007N39		
Thursday 07/06/2007	D	02:07	02:22	00:15	0.01	STOP abstraction increased turbidity, no abstraction unknown chemical measured at Eijsden vall pass.	Aq'e-VML 2007N39		turbidity
Thursday 07/06/2007	C	02:11	03:52	01:41	0.07		Aq'e-VML 2007N39		turbidity
Thursday 07/06/2007	D	12:22	13:37	01:15	0.05	maintenance	Aq'e-VML 2007N39		maintenance
Friday 08/06/2007				00:00	0.00	START abstraction	Aq'e-VML 2007N39		
Saturday 09/06/2007	D	09:07	09:21	00:14	0.01	STOP abstraction	Aq'e-VML 2007N39		turbidity
Saturday 09/06/2007	C	09:43	10:18	00:35	0.02		Aq'e-VML 2007N39		turbidity
Sunday 10/06/2007	D	01:52	02:07	00:15	0.01		Aq'e-VML 2007N39		turbidity
Sunday 10/06/2007	C	02:37	05:25	02:48	0.12		Aq'e-VML 2007N39		no additional scre
Sunday 10/06/2007	C	16:37	17:12	00:35	0.02		Aq'e-VML 2007N39		turbidity
Monday 11/06/2007	C	02:54	06:40	03:46	0.21	unknown substances, ISAMDS [RT 20.99 - max 1.9 µ L-1) en 2 SIVEGOM (RT 17.09, R(n)) 0.525 - 1.9 µg L-1; RT 23.38, R(n)) 0.718 - 2.0 µg L-1]	Aq'e-VML 2007N39		unknown substan
Monday 11/06/2007	D	12:37	13:22	00:45	0.03	maintenance			maintenance



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