BTO 2005.015
10th February 2005

Standardisation, quality assurance and data evaluation of on-line biological alarm systems

1. *Daphnia*-toximeter (BBE-Moldaenke, Kiel, D)

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Colophon

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Standardisation, quality assurance and data evaluation of on-line biological alarm systems

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

Foreword

The first on-line biomonitors were developed in the eighties and implemented to monitor the water quality of the river Meuse and the Rhine, both of which were more heavily polluted at the time. However, the water quality of these rivers has improved substantially over the years. During the nineties a strong technological development took place, as well as a growing insight into the implementation of on-line biomonitors for water quality assurance. This made it possible to develop biomonitors that are able to detect toxic substances quickly and at the required lower detection level.

On-line biomonitors are currently being used to monitor the water quality of the surface water at seven locations in the Netherlands. Five of the locations are inflow points of water companies and two are RIZA monitoring stations. The aim of the inflow control of the different water companies is to signal pollution peaks at an early stage and to selectively abstract good quality water to avoid unnecessarily taxing subsequent purification or the purifying capacity of reservoirs.

Nowadays on-line biomonitors have reached a level of technical development that has made them extremely useful in the practical field. However there are still a number of aspects that need to be improved:

1. **A sound validation and standardisation of the monitors used, thereby demonstrating that they provide reproducible, reliable and good, comparable information on water quality.**
   Validation and standardisation are essential for a wide acceptance and implementation of on-line biomonitors. So far there has been too little focus on this so that measurement results within a catchment area are insufficiently comparable.
2. **Good quality assurance.**
   With regard to laboratory analyses there are sufficient guidelines to produce a good quality assurance of the analyses and their associated reports. There are ISO-, CEN- and other (inter)national guidelines for sampling, analysis methods, the laboratory and the reports. For on-line biomonitoring, where the sampling, analysis, data interpretation and processing are all found in one appliance, there are no clear quality guidelines yet.
3. **A heightened awareness of the consequences of on-line biomonitoring.**
   On-line monitoring requires an approach that is different to that of batch sampling, especially with regard to data management and reports. In a sampling programme with a frequency of one month it is clear which results must be reported. With a measurement result that is generated every minute or every ten minutes, the data must first be processed and translated into meaningful information before it can be reported to a third party. On the other hand, a certain degree of detailed information must remain available. In addition to this, on-line monitoring also requires on-line management and many organisations are not (yet) geared to this.

The Stuurgroep Biologisch Alarmering (SBA) (Biological Alarming Steering Committee), which is made up of the authors of this document, was established on the 8th April 2003 in order to fill in these knowledge gaps. During the course of the project Ad Jeuken was replaced by Ria Kamps (both RIZA representants). The SBA originated in the Bio Alarming Study Group, which organises the user's consultation for the biomonitors.

The aims of the SBA can be summarized as follows:
- Transforming data into information and a clear interpretation of this information;
- Preparing action plans in case of an alarm;
- Protocols for standardisation , quality assurance (and implementation and validation);
- Strategy for using Early Warning Systems (EWS).

At the first meeting it was decided to start with the standardisation of the *Daphnia*-toximeter. Generally speaking this biomonitor is fully developed and has achieved a high operational level. Moreover, this type of toximeter is currently being deployed in about four institutions in the Netherlands. The first step towards standardisation and validation of the *Daphnia*-toximeter is described in this document, which has been compiled by the members of the SBA. Its realisation included using the expertise of several German colleagues: Michael Lechelt (Hamburger Landerinstitut für Lebensmittel-sicherheit, Gesundheitsschutz und Umweltintersuchungen), Christine Werth (Landesanstalt für Umweltschutz Baden-Württemberg, Karlsruhe) and personnel of the company supplying the appliance, BBE-Moldaenke in Kiel. A parallel implementation report on the Algae Toximeter and the *Daphnia*-toximeter of the Keizersveer (EVIDES) monitoring station has also been drafted (Wagenvoort and De Hoogh, in prep.).
This report was submitted to the Bio Alarming Study Group for comments before the final version was printed.

The activities of the steering committee were partly financed by BTO research.

Contents

1 Introduction

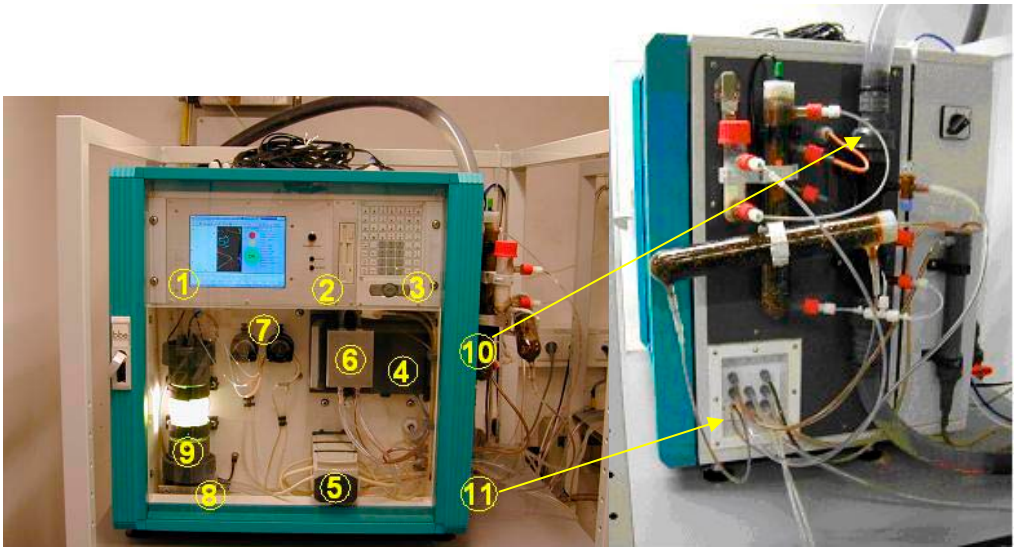
The Biological Alarming Steering Committee decided to commence with the validation of the *Daphnia*-toximeter. This biomonitor is technically more or less fully developed and has generally achieved a high operational level. This type of biomonitor is currently deployed at four locations in the Netherlands. This first step towards validating the *Daphnia*-toximeter could subsequently serve as a guideline for validating biomonitors that use other organisms as a sensor.

1.1 The *Daphnia*-toximeter

The BBE *Daphnia*-toximeter was developed with the aim of acquiring an on-line toxicity test with the *Daphnia magna* as a test organism. This organism is also used in acute and chronic toxicity tests (NEN, 1998; NEN, 2000). In the on-line test the swimming behaviour is recorded by means of digital image processing and continual evaluation.

In figure 1.1 we see how the *Daphnia*-toximeter looks like and which components it comprises. After filtration (10) the test water is transported by way of the supply (11) to the measuring chamber (4) with the aid of a pump (5). There the peltier-element (6) ensures that the measuring chamber is supplied with test water of a constant temperature. On the screen (1) we see the real-time measurement of the swimming movements of the *Daphnia* and the previous measurements that have been converted into several behavioural parameters (swimming speed, swimming height, etc.). Instructions can be given or data viewed by way of the keyboard (3). Nutrition for the *Daphnia* is pumped at intervals from the algae fermenter (9) to the measuring chamber (7). These algae are fed a nutrient solution that is supplied at regular intervals to the fermenter (pump 7). A peltier element (8) keeps the fermenter at a constant temperature.

A typical feature of the *Daphnia* is the vertical migration under the influence of light. The BBE *Daphnia*-toximeter makes use of this swimming behaviour. Under normal circumstances the swimming behaviour of the *Daphnia* is a relatively calm movement. Under the influence of toxic substances (depending on the type, concentration and reaction time) behaviour can change into a hypo- or hyper-activity. This means that the *Daphnia* either move much more slowly or much more quickly. An alarm signal is generated if the movement deviates from normal (previous) behaviour. In this way information on the water quality is obtained (long) before the *Daphnia* dies. The mortality rate of the *Daphnia* is also taken into account when evaluating the water quality.



a. b.

Figure 1.1 Components of the *Daphnia*-toximeter ; a. front view, b. side view. 1. LCD colour display; 2. disk drive; 3. keyboard; 4. measuring chamber (situated directly behind 6; in a single chamber system, the space behind 4 is empty); 5. test water pump; 6. peltier element (temperature regulation measuring chamber); 7. pumps for dosing nutrients and algae suspensions; 8. peltier element (temperature regulation fermenter); 9. algae fermenter for nutrient supply to *Daphnias*; 10. cross-flow filter; 11. water supply and drainage.

The software and the picture processing system continually record and analyse the tracks of the swimming. For this the position of every individual waterflea is determined every 40 milliseconds. By using the swimming tracks of the *Daphnia* a number of behavioural parameters are measured and recorded. These parameters are: average swimming speed, speed-class index, average height in the measuring chamber, average distance between the *Daphnia* and fractal dimension[1]. In addition to this, a number of other parameters of the *Daphnia* and of the technical operation of the system are recorded. These are: number of test organisms, average size, recognition rate and temperature of the measuring chamber, fermenter, sample water and pre-heater.

1.2 Reading guide

This document forms a guideline for carrying out the on-line monitoring of surface water quality with the aid of the *Daphnia*-toximeter (BBE, Moldaenke, Kiel, D) and will regularly be updated by the SBA.
Chapter 2 deals with the installation and the effect of the pre-treatment of the sample water. The regular maintenance, comprising the breeding of the test organisms, the maintenance of the *Daphnia*-toximeter and checking the technical functioning, is dealt with in chapter 3. Chapter 4 looks at quality assurance. The evaluation of the measurement data and the signalling of the alarm

[1] The fractal dimension is a way of indicating the curve of the swimming track of *Daphnia* in a number between 1 and 2. A fractal dimension with a value of 1 means a straight line; a value of 2 is a completely random movement (also see section 3.5.5.).

is fairly complex with regard to the *Daphnia*-toximeter . This is dealt with extensively in chapter 5. Chapter 6 deals with conclusions and recommendations for further improving the operation of the *Daphnia*-toximeter and data evaluation.

2 Installation of the *Daphnia*-toximeter

2.1 The BBE-*Daphnia*-toximeter in the Netherlands

In the Netherlands four BBE *Daphnia*-toximeters are currently in use. They have been placed along the Rhine and the river Meuse. For an overview of this see Table 2.1.

Table 2.1 Locations of the BBE Daphnia-toximeter *in the Netherlands.*

Location	On river	Company/institution
Lobith/Bimmen	Rhine	RIZA
Nieuwegein	Rhine	Amsterdam Waterworks/ Het Waterlaboratorium
Eijsden	Meuse	RIZA
Keizersveer	Meuse	Evides watercompany

As these monitors are not all fitted in the same way, details of the differences can be found in supplement 1. The supplement provides a guideline for the relevant parameters based on practical experience with the four monitors described and directions from BBE-Moldaenke.
Two companies (RIZA and the Amsterdam Waterworks) use a double chamber system and Evides uses a single chamber system. A double chamber system comprises two measuring chambers with a parallel link, and several water fleas in each chamber (more or less comparable to a simultaneous duplo measurement).

2.2 Preliminary treatment sample water

Before exposing the *Daphnia* to the sample water, the water has to undergo three treatments, namely: filtration, heating and re-cooling. The successive heating and re-cooling of the water generates degassing. These processes affect the potential toxicity of the sample water. The following subsections will deal with the effects of these processes.

2.2.1 Filtering the sample water

The standard *Daphnia*-toximeter has a cross-flow filter with a mesh size of 100 µm. Other mesh grades are also available. Fine particles (< 100 µm) therefore remain in the sample water. Consequently the mesh grade size of the cross-flow filter is selected so that the water fleas can ingest the remaining particles. *Daphnia magna* can ingest particles larger than 50 µm. (Burns, 1968). This means that in addition to the substances in solution, the *Daphnia*-toximeter is also sensitive to silt-related pollutants. In the *Daphnia*-toximeter the degassing pipe therefore contains iron wool which plays an important role in the degassing process (section 2.2.2).
In the measuring stations of RIZA and the Amsterdam Waterworks, a fine-mesh filter (Wafelin-filter, 0,2 µm) is used instead of the cross-flow filter. This membrane filter prevents particles of silt and organisms from passing through. The silt fraction therefore no longer plays a role in the *Daphnia*-

*toximeter*s used by these measuring stations so that only the toxicity of the water phase (only dissolved substances) is determined. In these systems the addition of feed to the *Daphnia* in the measuring chamber is of essential importance because feed is no longer present in the sample water. This is dealt with further in section 3.4.3.

2.2.2 *Degassing*

In the pre-heater the water is first heated up to a temperature that is higher than that of the water in the measuring chamber. As the solubility of gas decreases with an increase in water temperature and at a low pressure, the heated water degasses in the degassing pipe with a pressure free outflow. In the degassing pipe the substrate (glass wool or steel wool) facilitates the formation of air bubbles. Before the sample water enters the measuring chamber it is re-cooled to ensure that the measuring chamber remains free of air bubbles. Air bubbles would disrupt the image processing and could therefore affect the measurements. Moreover, it will also prevent air bubbles forming under the carapace of the water fleas, thus ensuring that the test organisms can move freely and independently.
A difference in temperature of at least 5°C is used in order to achieve full degassing. This means that the water is heated to a temperature of 25°C throughout the year. This heating of the water affects the number of dissolved volatile substances. The higher the temperature interval between the original test (surface) water and the water in the pre-heaters, the easier it is for volatile substances to show evasive behaviour. As these substances evaporate in the pre-treatment of the testwater in the *Daphnia*-toximeter, the possible effect on behavioural changes is weakened so that it is then (possibly) no longer observable. When monitoring for the production of drinking water, this does not generally form a big problem, as due to evaporation the influence of these substances on the system is only temporary. The role of volatile substances in the water phase in river ecosystems is limited, as due to the flow the water has a more intensive exchange with the atmosphere and the substances will quickly disappear from the water phase. Because these substances can easily become attached to particles of silt, it could be important for water managers to take substances like these into consideration. Pollution related to particles of silt does not affect the *Daphnia* in the toximeter if the water is filtered by means of a fine-mesh filter.

2.2.3 *Heating the sample water*

Daphnia are cold-blooded (poikilotherm) organisms, which means that temperature strongly affects the physiological processes. The rate of these processes doubles with every increase in temperature of about 10°C, also known as the Q_{10} (Peters, 1986). However, there is a maximum level reached in this relationship.
If we look at the swimming speed for example, this also appears to increase at a higher temperature (figure 2.1). In the measuring chamber a fixed temperature is maintained throughout the year (20°C). This means that under normal conditions the swimming movements will have an equal average value and therefore not be influenced by seasonal changes (outdoor temperature).

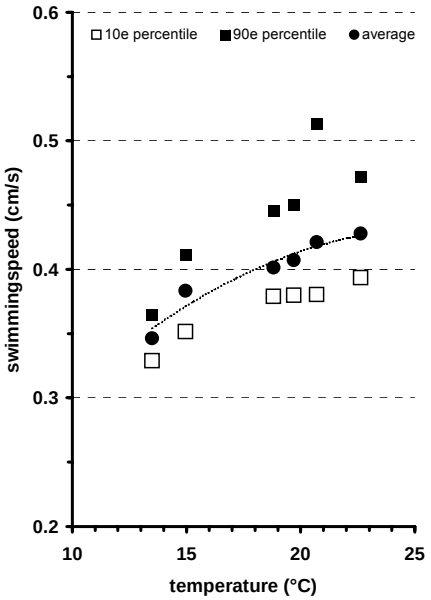
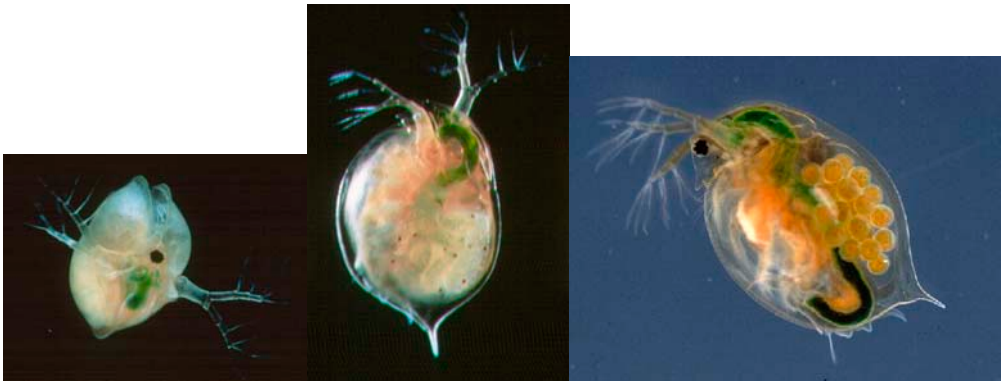


Figure 2.1
The relationship between water temperature and the swimming speed of *Daphnia magna* (size ± 1 mm) (Wagenvoort & De Hoogh, in prep.).

3 Breeding *Daphnia*, maintenance of appliances and monitoring the toximeter

3.1 General features of *Daphnia magna* (from: Flößner (2000), unless otherwise stated)

In the *Daphnia*-toximeter the *Daphnia magna* water flea is used as a test organism (figure 3.1). Some of the features and the ecology of *D. magna* are mentioned below.



a. b. c.

Figure 3.1
Photographs of the *Daphnia magna*.
View from above (a), a water flow for nutrients and oxygen is created through the opening in the shell (carapace), an intestine filled with (green) algae is visible;
Side-view (b), in the brood pouch, the clutch (the embryos' eyes) is visible;
Daphnia with eggs (c).

D. magna is a member of the Crustacean order, suborder Anomopoda, and feeds on small particles, bacteria and algae no larger than 50 µm (Burns, 1968). The size of the adult females is between 1.8 to 6.0 mm at the most, but on average the animals grow to a size of 3 to 4 mm.
The body is encased in a hard chitin shell, the carapace. In order to grow, the animals have to molt. The carapace, which has become too small, is shed and replaced by a new one.
D. magna lives in small to very large fresh water systems with a salinity of up to 8 g. per litre. They have a high tolerance for pH values: they are found in a pH greater than 5.5 but smaller than 10.8. *D. magna* lives in eutrophic systems which generally have few fish. The smaller juveniles may be prey for invertebrate predators such as preying water fleas, copepods and mysids. In the wild the large mature animals are usually positively selected by plankton-eating fish.
In section 2.2.3 is shown that temperature affects the speed of the physiological processes. Consequently, this is also true for the development of the *Daphnia magna*. At a temperature of 25°C the water flea reaches maturity within a week, at 20°C it takes 7.6 days (Geller, 1987).
For more information, see Flößner (2000) or Lampert (1987).

Under normal conditions the *D. magna* reproduces asexually (parthenogenetic) and the population (culture) is made up exclusively of females. If conditions are less favourable, males are produced, which are slightly smaller than the females (2.2 to 3.5 mm), have a lengthened and broadened primary antenna and have a very long appendage on the first pair of limbs (figure 3.2). After fertilization has taken place, the females produce resting eggs (or ephippium). These resting eggs are visible in the brood pouch as dark (black) dots. This resting egg makes it possible for the *Daphnia* to survive unfavourable environmental conditions.

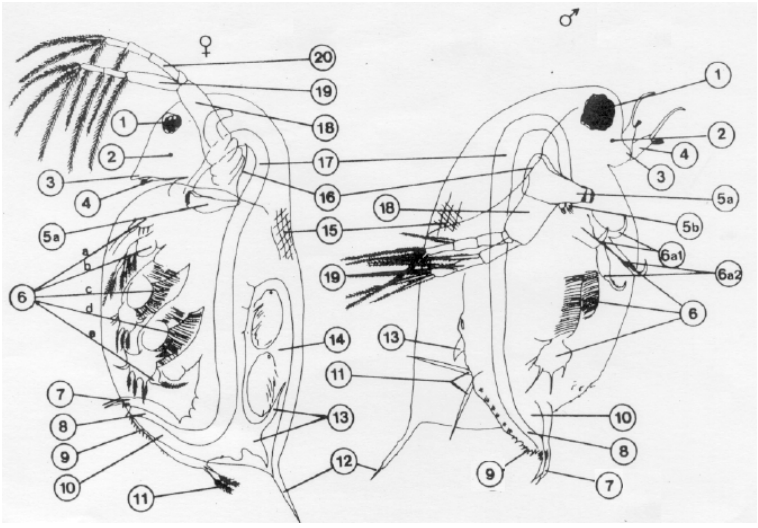


Figure 3.2
Anatomy of an Anomopoda, female and male (Notenboom-Ram, 1981, corrected).
1. compound eye; 2. nauplius eye or eye spot or ocellus; 3. crest or rostrum; 4. antennula (1st antenna) with olfactory setae and filtering combs; 5A. mandibula (mandible or upper jaw); 5B. maxillula (lower jaw); 6 (A-E). Pairs of limbs with the filtering combs on pair number 3 and 4; 6A-1. claw-like appendages on a pair of limbs, found in males only; 6A-2. bristles on a pair of limbs, found in males only; 7. claw (furca); 8. anus; 9. anal teeth; 10. post-abdomen (tail); 11. abdominal setae; 12. spina (shell-spine); 13. abdominal porcesses (spurs); 14. brood pouch; 15. shell (structure of the carapace consists of rigth and left valves); 16. fornix; 17. Intestine or stomage; 18. antenna (2nd antenna); 19. three-part inner limb with swimming bristles; and 20. four-part outer limb with swimming bristles.

3.2 Breeding *Daphnia magna*

This document contains a brief description of how to breed *Daphnia magna*. For a more detailed description please consult RIZA (1991) or WCB (2001). WCB (2001) includes a sensitivity test, a static LC_{50}-test with KCr_2O_7 (potassiumdichromate).

RIZA maintains a constant culture of *Daphnia magna* in Lelystad. Should there be any doubts about your own culture, for instance after sexual reproduction has taken place or when a culture has collapsed, new animals are available on request from RIZA.
When breeding, artificial water, the so-called Elendt medium (Appendix 2, WCB (2001)), may be used. This medium consists of ultrapure water (Nanopur) in which a large number of substances are dissolved. Filtered surface water may also be used instead of an artificial medium (mesh size smaller than 0.45 μm). The advantage of filtered surface water is that the culture has been bread in the same matrix as in which the exposure takes

place. Consequently, during the transfer no changes (or as few as possible) caused by a change in the matrix are registered. The absence of knowledge on the level of pollutants of the used filtered surface is a disadvantage. Consequently, one should always bear in mind that there is a certain amount of background pollution in the water, which could result in the animals adapting to the substances present.
Water fleas used in static tests should be cultivated in Elendt medium so as to exclude any possible influences of background pollution. WCB (2001) gives a detailed description of how to breed *Daphnia magna*; a short summary of this is given in the box below.

Green algae, *Chlorella vulgaris*, are used as food for the water fleas. These green algae are cultivated to a high density and fed in amounts of about 3000 µg chlorophyll/l or about 5.10^8 cells/ml of 0.1 to 0.2 mg C per *Daphnia* per day. The more nutrition there is the larger the clutche size is. If only small numbers of young are produced the amount of nutrition may be increased slightly. If there is too little nutrition, sexual reproduction takes place and resting eggs are observed in the cultures. One should try and avoid this at all times. Green algae may be kept in the refrigerator for a maximum of 3 weeks.

Method for breeding *Daphnia magna*

- Aerate the Elendt medium at a room temperature of 20°C for at least one hour through a narrow 1 ml glass pipette.
- Half-fill beakers (1000 or 2000 ml, glass) with this aerated water.
- Add the female animals (density ≈10 per liter) and place the beakers at a room temperature of 20 ± 2°C and a light system of 14 hours' light (10 to 50 µE/m^2 cm^2) and 10 hours' darkness.
  The beakers may be aerated with moistened and filtered air (50 to 100 ml air per hour) if necessary. Cover the beakers as much as possible (with glasses) to prevent evaporation.
- Feed the water fleas a few drops of algae concentrate at least three times a week.
- Check the individual beakers for resting eggs or (macroscopic) infections at least three times a week. If this proves to be the case, destroy all water fleas from the beaker in question.
- Change the medium at least once every fortnight.

3.3 Maintenance of the *Daphnia*-toximeter

Maintenance can be divided into a number of activities with different frequencies. In table 3.1 the activities have been grouped per frequency with a short explanation where necessary. A number of issues affect the evaluation of the measurement data and the alarms, and are discussed in more detail in section 3.4. The maintenance intervals mentioned in table 3.1 apply to river systems in which a substantial number of substances are generally suspended

in the water; it may be necessary to (temporarily) deviate from these intervals depending on local conditions. Using a Wafelin-filter (paragraph 2.2.1) for pre-filtering reduces the pollution of the system to a minimum and the formation of biofilm is substantially less, so that a weekly clean may suffice.

Table 3.1 Overview of the maintenance of the Daphnia-toximeter and the corresponding frequency.

Frequency Activity	Explanation	Sect.
Daily (from a distance by way of the modem connection with the monitor)		
- Check the functioning of a number of preconditions and conspicuous variations in temperature.	In addition to transgressing the fixed boundaries, the variation in the measurement signal may point to a technical defect (e.g. blockage).	3.4
- Check the noise (on the data) of the behavioural parameters	The variation of the indiviual data needs to be less than the average value of the signal itself.	3.4.6
Additional maintenance (at least twice a week)		
- Clean crossflow filter and replace filter wool.	More frequently when turbidity is high (>25 FTU).	3.3.1
- Replace test organisms.	Age of test organisms: 2 days. Juveniles appear most sensitive. Sensitivity decreases as the animals mature.	3.4.6
- Check nutrient stocks and supplement if necessary.		3.3.2
- Check algae cultures and replace if necessary	Visual inspection for flakes and concentration	3.3.3
Weekly maintenance		
- Clean measuring chamber. - Clean hoses (residue). - Clean degassing tube and tube with heating element.		
- Check temperature sensor in the measuring chamber	Use a reference thermometer	3.4.1
Monthly maintenance		
- Replace pump hoses - Replace extremely dirty PVC-hoses - Clean draining hoses		
- Measure pump flow	Check 3 to 5 days after replacement of pump hoses, once they have adjusted to the pump head.	3.3.4
- Microscopic check on algae culture for uni-algality and infections		3.3.3
Quarterly maintenance (in collaboration with BBE)		
- Replace all hoses. - Check pump bearings. - Clean fermenter and reconnect. - Replace fermenter lamp. - Clean cooling system.		

It is important to keep an accurate record of the maintenance that has been carried out. The evaluation of the measurement data will form an essential source of information for tracing false positive signals. An example of a maintenance plan is included in supplement 3.

3.3.1 *Cleaning the crossflow filter*

To guarantee a good filtration capacity of the crossflowfilter it is necessary to clean it in good time. Cleaning the crossflow filter comprises rinsing, ultra-sonic cleaning (15 minutes) and renewed rinsing with tap water.
The fibreglass wool is used to catch the smaller particles and it is replaced at every service.
The degree of pollution of both filters is closely connected to the amount of sediment in the test water. The following maintenance frequencies are necessary to prevent the filters from silting up.

- If the water is relatively clean (turbidity <<10 FTU, summer months), a weekly cleaning of the filters is sufficient.
- A turbidity of between 10 to 25 FTU, the filters need to be cleaned twice a week.
- A turbidity of between 25 and 75 FTU means that the filters need to be cleaned at least three times a week.
- If the turbidity is higher than 75 FTU, the filters need to be replaced every day or even more frequently. One should consider no longer keeping the monitors operational in conditions like this.

3.3.2 *Supply of nutrients*

The algae with which the water fleas are fed, also need to be fed in turn. The nutrient fed to the algae has a composition as described in table 3.2.

Tabel 3.2 Composition of the nutrient solution for algae.

Composition	**Concentration (mg/l)**
$CaCl_2.2\ H_2O$	11.6
$FeSO_4$	10.7
$CO(NH_2)_2$ (urea)	500
K_2HPO_4	240
KH_2PO_4	153.3
$MgSO_4.7\ H_2O$	200
Trace element solution 'woods hole'	10 ml/l (see BBE, 2003)

If algae from a flourishing algae culture (solid green) are pumped into the measuring chamber for 3 seconds every 600 seconds, the water fleas will have sufficient nutrition. By pumping nutrients into the algae fermenter for four seconds during the same time interval, the culture will continue to grow well and the fermenter will remain completely filled.

3.3.3 *Checking algae- and* **Daphnia-***culture*

During each service, the algae culture is visually checked for flakes and the intensity of the green colour. Flakes may be an indication of infections (for instance by mould) and if the intensity of the green colour diminishes, this means that the algae culture is thinning out or that the algae are dying. The nutritional value of the algae culture diminishes and this has a detrimental effect on the *Daphnia* in the measuring chamber.

The macro- and microscopic monitoring of the *Daphnia*-culture is a part of the quality assurance and will be explained in detail in section 4.1.

3.3.4 Measurement pump flow

Wear and tear of the hoses causes the pump flow to vary a little in time. Once the pump hoses have been renewed, the flow can vary considerably compared to before the replacement was made. The new hoses need a few days 'to adjust' to the shape of the pump and to discharge a constant pump flow. It is best to redetermine the pump flow between three to five days after the hoses have been replaced.

3.4 Checking parameters

In the *Daphnia*-toximeter the following parameters are evaluated in order to trace technical malfunctions:

1. temperature of the fermenter, pre-heater and test water;
2. detection rate (also referred to as recognition rate);
3. swimming height of the water fleas in the measuring chamber;
4. number of water fleas in the measuring chamber;
5. size of the water fleas;
6. signal-noise ratio.

Boundaries have been implemented for temperature and the detection rate. If this boundary is transgressed it generates a failure warning and transgressions of the toxicity index are not reported as a quality alarm. This automatic warning system only registers a limited number of malfunctions however, for which relatively strict boundaries sometimes apply. In real terms, a quick daily check can distinguish a number of supplementary checking parameters and malfunctions can be detected early. The monitoring parameters are explained in detail in the following subsections (also see supplement 4 for an overview).

3.4.1 Temperature

Malfunctions in the appliance caused by blockages in the cooling system for instance, will only result in a transgression of the boundary values after some time. Deviations in temperature within the set boundaries may be an early warning signal for a blockage. Malfunctioning may be prevented by checking the system for its proper functioning from a distance. A periodical check of the temperature sensors by way of a reference thermometer will ensure a good evaluation of the water quality. This is especially true for the sensor in the measuring chamber, as deviations in the temperature of the test water in the measuring chamber have direct consequences for the activity of the water fleas.

3.4.2 Detection rate

An important condition for a reliable measurements is that the camera, which records the behaviour of the water fleas, must distinguish the animals clearly from particles (e.g. dirt or air bubbles). A measure for this 'recognition rate' of the water fleas is the so-called detection rate. This is expressed in the percentage of measurement points, which the camera can clearly identify as the water flea. A measurement point is registered every 40 ms (= 25 times per second) during a measurement cycle of 1 minute. A maximum of 15,000 measuring points are distinguished per measurement cycle in a number of 10 *Daphnia* (60 * 25 * 10 = 15.000). A detection rate of 75% or more

is enough for a sufficiently reliable result. When the detection rate is lower, the fixed malfunction boundary for this parameter may be transgressed, after which a malfunction report is generated. This signal is not reported as a quality alarm, because it is a monitoring parameter, which does not contribute to the construction of the toxicity index. Deviations in the detection rate may be caused by dirt or air bubbles in the measuring chamber, or by the birth of a large number of water fleas.

3.4.3 Swimming height of the water fleas

Low concentrations of nutrients may lead to behavioural changes in water fleas and in extreme cases the lack of nutrition may even result in death. In order to avoid variations in the measurement result caused by changing nutrient conditions, it is important for the water fleas to get enough nutrition during their stay in the measuring chamber. Water fleas are able to ingest particles up to the size of about 50 µm as a source of nutrition. If these particles have been removed from the test water by pre-filtering, it is necessary to supplement the animals' nutrition with an algae suspension. If pre-filtering allows particles of up to 50 µm to pass through, it may still be necessary to supplement the animals' nutrition, especially in winter. In order to ensure that the water fleas get enough nutrition during their stay in the measuring chamber, they are provided with supplementary algae from the fermenter. The 'swimming height' parameter can be used to monitor whether the water fleas have indeed had enough nutrition. If nutrition is insufficient the water fleas are less active and will generally remain close to or on the bottom of the measuring chamber. In many instances they try to feed off the grid of the measurering chamber, where material often collects.
Besides the swimming height parameter, the visual inspection of the density of the culture in the fermenter may also provide information on adequate nutrition for the *Daphnia*. The fermenter contains a sufficient amount of algae if it is a deep green colour (more exact criteria have yet to be determined).

3.4.4 Number of water fleas

Toxic substances in the test water may cause mortality in water fleas; static toxicity tests are based on this. (NEN, 1998; NEN, 2000). Because the *Daphnia*-toximeter registers behavioural changes rather than mortality, it can detect toxic effects in a lower concentration, which makes it more sensitive than a static test.

The mortality of water fleas in the measuring chamber will generally be preceded by behavioural changes (particularly swimming speed, speed-class index and fractal dimension). If these behavioural changes are not detected, it is possible that the mortality has been caused by something other than a toxic substance. The mortality may have been caused by lack of nutrition. In this case, the position of the animals in the measuring chamber may be an indication (also see section 3.4.3). Another possibility is damage during the handling of the animals. In this case mortality will occur directly after the start up of a new measurement series.

The number of test organisms in the measuring chamber is very important for the reliability of the measurement values. If there are too many test organisms (for instance after hatching) this hampers good alarm detection as the identification of the separate animals by the image processing software will decrease dramatically and the detection rate drops. If there are too few test organisms, the problem is that behavioural changes of a single water flea will weigh too heavily on the average. With five test organisms, the contribution of the separate animals is 20% of the average; with eight organisms this is only 12.5%. Practically speaking, it is best to use between a minimum of 6 and a maximum of 10 water fleas. Consequently, 10 water fleas are deployed at the start of a new measurement series.

3.4.5 Size of the water fleas

During the period the water fleas are in the measuring chamber, the animals gradually increase in size. The size of the animals is determined at each measurement and represented as an average area (mm^2). Water fleas are crustaceans, who shed their shell (the carapace) in order to grow. At a temperature of 20 °C, the *Daphnia magna* molts approximately every 1½ to 2 days (figure 3.3). If there is little nutrition available, this period may be longer. This molting has a direct effect on the measurement values of the different parameters as there is a temporary behavioural deviation as each individual sheds its skin. The swimming speed generally decreases and the molting animals are positioned closer to the bottom. Because animals of similar ages are used (albeit from different female parents), a number of animals will molt during a short time interval. Consequently, during a period in which molting occurs, distances between the animals are short and the swimming height is low. In the measurement result this manifests itself as a sudden increase in the average size of the animals (figure 3.3). In cases like this, during the evaluation of a higher toxicity index it soon becomes clear that this is caused by molting (normal behavioural change).

3.4.6 Signal-noise ratio

Another phenomenon that needs to be taken into account is the variation or noise in the measurement values. In figure 3.3 we see the influence of the size (age) on the average swimming speed. As the animals grow, the variation of the separate measurement values often increases. This is caused by an increase in the range of the swimming speed: larger animals can swim faster than the smaller ones, but large animals can also swim slowly. As a result, the discrimination ability of the system (the alarm detection) decreases. When the water fleas are deployed, they are two days old. Generally, the noise (dispersion) increases rapidly if the animals have been present in the system for longer than 5 to 6 days and have an average size of about 6 mm^2. This increase in noise is avoided if the animals are replaced twice a week.

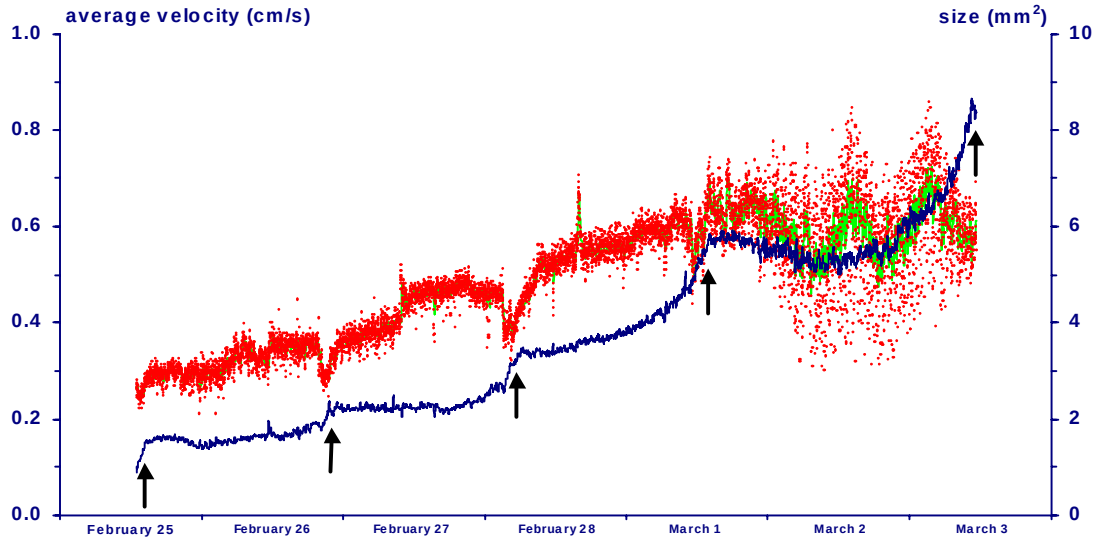


Figure 3.3 Increase in the average swimming speed caused by growth and a temporary decrease in swimming speed caused by simultaneous molting. ▪: individual measurement; —: average swimming speed; —: average size; ↑: molting events).

This is also beneficial for the sensitivity of the toximeter as young animals are in general more sensitive than adult water fleas. At a temperature of 20°C the water fleas reach maturity after about a week, after which they can lay their eggs in their brood pouch (Geller, 1987). This change in development influences the animals' sensitivity.

3.5 Behavioural parameters

Five behavioural parameters are evaluated and used to set up the toxicity index. If a parameter changes significantly, a number of points are awarded to the toxicity index. If this index exceeds a particular marginal value it generates an alarm signal.

The evaluated behavioural parameters are:

1. avarage swimming speed;
2. speed-class index;
3. average swimming height;
4. average distance;
5. fractal dimension.

3.5.1 Average swimming speed

The presence of toxic substances may cause the swimming speed to increase (fleeing action), or decrease (early physiological damage). Swimming speed is therefore an important factor in detecting the condition of the water fleas.

The temporal interval with regard to the previous measurement point and the distance of the vector are known for each individual measurement point of the track. These data are used to calculate the momentary swimming speed for each point and the consequent average speed for each measurement cycle

(60 seconds = 1500 measurement points per *Daphnia*, 1 measurement point per 40 ms). The average swimming speed has a unit of cm/s.

3.5.2 *Speed-class index*

In addition to the average swimming speed, there is a division into classes of the momentary swimming speeds on the separate measurement points. Through this division into classes, groups of (measurement values of) fast- and slow-swimming water fleas (compared to the average swimming speed) are distinguished. A large share of the smaller classes and the larger classes, i.e. a high speed-class index, could mean that some of the *Daphnia* are dying or are displaying a fleeing action. A small share indicates that the water fleas are displaying a more or less similar response. The parameter therefore indicates whether the swimming speed is more or less the same for all the animals.

The speed-class distribution is a histogram and cannot be represented as one individual parameter. In order to interpret the data from the speed-class distribution, a new parameter is calculated from these data, i.e. the speed-class index.

In order to gain insight into the development of this parameter, two histograms of the speed-class distributions at two different times are shown. In figure 3.4 the average swimming speed of the associated time interval is represented as a vertical line. A certain range around the average is then indicated as shaded areas. The range of these areas covers 80 to 120% of the average swimming speed. The speed-class index is the sum of all the percentages of the speed-classes that fall outside the range concerning the average swimming speed of the associated time interval. In the example, the speed-class index at moment 1 is 48% and at moment 2 it is 62%.

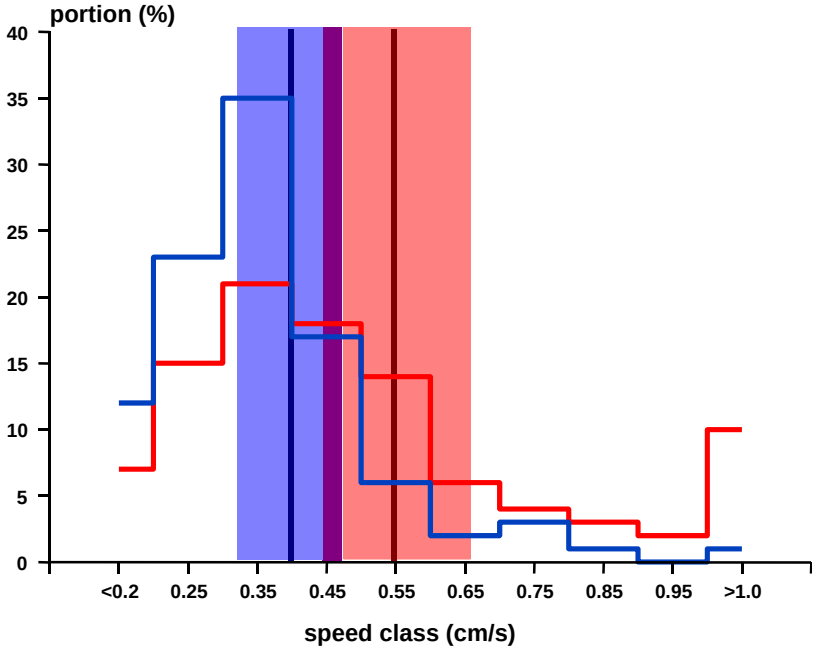


Figure 3.4 Histograms of the speed-class distribution at two moments. —: speed-classes at moment 1; ▮: average swimming speed at moment 1, ▪: range at moment 1; —: speed-classes at moment 2; ▮: average swimming speed at moment 2; ▪: range at moment 2;▪: concurring range at both moments.

3.5.3 Average swimming height

The average swimming height in the cuvette is calculated using the vertical components of the co-ordinates of the measurement points. Live *Daphnia* will always have altitude, whereas dead animals remain at the bottom of the measuring chamber. The average swimming height may be temporarily decreased due to a lack of nutrition (section 3.4.3) or simultaneous molting (section 3.4.5).

3.5.4 Average distance

The average distance between the *Daphnia*s is a measure for the behaviour in the group. The distance is calculated on the basis of the average distance between two measurement points at the same time interval. This can be used to distinguish changes in normal 'social' behaviour. Dead animals are positioned at the bottom of the measuring chamber and the distance between them will be small and constant. The distance between animals moving normally is between 1.5 and 3.5 cm.

3.5.5 Fractal dimension

If the *Daphnia* swimming movements are examined as a reaction to toxic substances, these movements appear hectic under exposure. They swim in small circles and start to spin as it were. In short, the shape of the track of the swimming water fleas changes.
The fractal dimension describes the curviness of geometric shapes. Fractal models were first used to describe coastlines, the circumference of leaves, coral reefs and animal movements. It is possible to represent the *Daphnia*'s swimming movements as a fractal dimension, in which case the following equation is used:

$$L(\delta) = K \cdot \delta^{1-D}$$

In which:

$L(\delta)$	: the entire length of all tracks with regard to the 'ruler' length (time interval, δ= 150 coherent measurement points, duration = 6 seconds);
K	: a constant;
D	: fractal dimension.

To get a better idea of the fractal dimension of various patterns, see figure 3.5. The value of the fractal dimension may vary between 1 and 2. In a straight line, the fractal dimension has a value of 1. Koch's snow flake, a typical fractal pattern, produces a fractal dimension of 1.26. The Brownse movement (random) has a fractal dimension of 1,5. When the water fleas start to spin and the tracks start to cover almost an entire area, the fractal dimension approaches a value of 2.

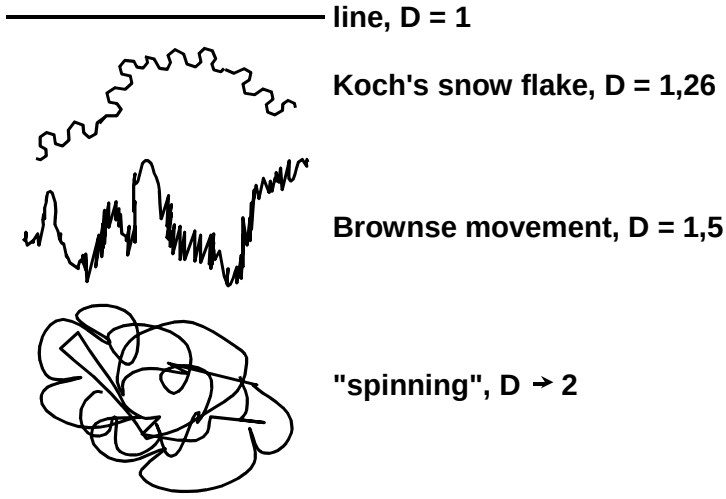


Figure 3.5 Fractal dimension of various patterns.

4 Quality Assurance

4.1 Macro and microscopic monitoring of the *Daphnia* culture

In the *Daphnia*-toximeter the same species, the *Daphnia magna*, and preferably the same clone, is always used in order to obtain a constant behavioural response. It is important that the culture is given sufficient nutrition in order to avoid the formation of resting eggs through sexual reproduction (section 3.2). This ensures the continued presence of the same clone. A number of cultures are used (beakers) which are visually inspected for the presence of conspicuous resting eggs at least 3 times a week. Should water fleas with resting eggs be found in a culture, it is no longer used. Should the clone used no longer be in stock, a new batch of the same clone can be ordered from RIZA Lelystad (section 3.2) and the behavioural response of this new batch should be validated (sections 4.2 and 4.3).

A periodical microscopic check in which abnormalities such as infections (of fungi for example) are registered, is also recommended.

4.2 Sensitivity of the *Daphnia magna*

The sensitivity of the test organisms can be periodically determined with the aid of a static test with potassiumdichromat. For more details see NEN-ISO 6341 (NEN, 1998) and NEN-ISO 10706 (NEN, 2000). It is important to realise that a check like this only determines lethality and therefore does not provide information on the sensitivity of water fleas in sublethal concentrations, in which only behavioural changes are determined. Moreover, this type of test only provides information on the response of the *Daphnias* to one substance only.

4.3 Control sample

A control sample must be measured periodically in order to determine the functioning of the whole system configuration. Preferably this should take place at least once a month. Every control measurement analyses the behavioural changes in the presence of the same substance in a similar concentration. By comparing the response with previous measurements one acquires information on the functioning of the total configuration of the system. These control measurements must include a recording of the toxicity index and which detectors are activated.

A variety of solutions of substances such as 5 g/l NaCl of 4 µg/l trichlorfon may be used as a control sample. During the control measurement the substances are added to the matrix (e.g. river water), so that only the response of the substance is measured (section 4.4). The advantage of a NaCl solution is that the exposure during the test can easily be registered by means of a conductivity meter. This saves supplementary analyses for verifying the final concentration of the control sample. Trichlorfon cannot be detected on-line and the final concentration in the measuring chamber will have to be determined in supplementary analyses. The advantage of this substance is that it reveals a real effect of a toxic class of substances rather than the purely physiological effect of an increase in the concentration of salt.

4.4 Validation test

When performing validation tests and determining the detection levels of the different substances, it is important to meet a number of prior conditions. One must ensure that side effects do not play a role. A number of control parameters and their pitfalls are mentioned in section 3.4.

Moreover, the matrix should not have any influence on the measurements and these must remain unchanged with the exception of the substance added. This means that a good system for carrying out these measurements must be devised. Preferably a validation test should be carried out in the same matrix as the sample water; Wagenvoort (2004) describes an experimental set up for carrying out a validation test with river water.

When using containers (storage vessels) of sample water, this water should constantly be mixed well and aerated. When carrying out the test the concentration of the substance must be adapted to the range of behavioural changes. In many cases research has shown that the EC_{50} and LC_{50} values are too high for detecting behavioural changes and it can occur that, if used in these concentrations in the dynamic test, no behavioural changes will be detected, only mortality (supplement 5). Once every test has been carried out the water fleas should be replaced as these test organisms could be influenced by exposure. This can also apply, even if no observable effects have taken place.

5 Evaluation of measurement results

5.1 Parameter settings

In the *Daphnia*-toximeter the detected behavioural changes are integrated in the toxicity index. If no (high concentrations of) toxic substances are present, the toxicity index is equal to zero. Only if the behaviour of the *Daphnia*s deviates significantly from the preceding values, there will be a visible increase in the toxicity index.
The value of the awarded points is reduced once again per detector and per parameter in a specific period of time. An image like this is highly suitable for use under normal monitoring conditions, in which the action of false positive alarms is prevented as much as possible. To generate an alarm signal (toxicity index greater than alarm value of 10), in many cases at least three detectors must have been activated in a limited time frame.

The principle of reducing the toxicity index within a certain time frame is necessary to prevent small changes in the water quality from developing over a longer period of time and reaching an alarm level. If, due to a small change in quality, there is an increase in the toxicity index by one or two points, followed by a certain period of no change, then these points are reduced once more after 2 or 4 hours for example. If this did not take place and these points remained as they were, an increasingly higher baseline would develop within a few days and this would finally result in a false alarm. In this case the alarm was caused by an accumulation of quality changes over time rather than by an acute change in quality within a period of a few hours.

With the evaluation and signalling of alarms it is important to know which settings have been used for the different parameters. In BBE (2001) it is indicated which parameters the system measures and how the Hinkley and gradient detectors work. Schwörer & Marten (2000) described the operation of the detectors in more detail. Three standard sets of settings are predefined by BBE, namely one for low, one for normal and one for high sensitivity. Under normal conditions the setting with normal sensitivity is sufficient for river systems. Moreover each user has the possibility to define his own set of parameters. In a set of parameters like this the sensitivity of the detectors to the different behavioural parameters can, amongst other things, be modified, and the contribution of the different behavioural parameters weighed differently. However, before the user compiles his own set of parameters it is important to provide insight into which changes are implemented and the reasons behind them. The setting changes implemented can be tested by means of validation experiments (section 4.4).
The three settings defined by BBE and those of the Dutch users can be found in supplement 6. Compiling a personal set of parameter settings is extremely complex and should be well-founded, after which this choice should be validated. In the compilation it is necessary to determine a wide range of variables (points per parameter, duration of the period in which the allocated points are reduced and the static boundaries of the detectors per parameter).

For example, a possible option is to allow the main parameters (swimming speed, speed-class index and fractal dimension) to weigh more heavily or to extend the period in which the allocated points are reduced.

Clearly, in the case of an alarm, it should be laid down which setting of the appliance has been used to evaluate the data and signal the alarm. In reporting measurement data and alarms it must be indicated which set of parameter settings has generated the alarm. It is highly advisable to implement the same settings on a national level (or at least with regard to all measuring systems on one and the same river).

5.2 Alarm signals and the evaluation of measurement data

In principal every transgression of the alarm boundary of the toxicity index is regarded as an alarm, even if the transgression is very brief. In order to determine, whether it is a real alarm signal, but also true during the evaluation of measurement data, it is important to know if there was any failure in the appliance or the measuring station (such as a pump defect) prior to the measurements. Moreover, control parameters should be used to determine whether the configuration was functioning well technically before

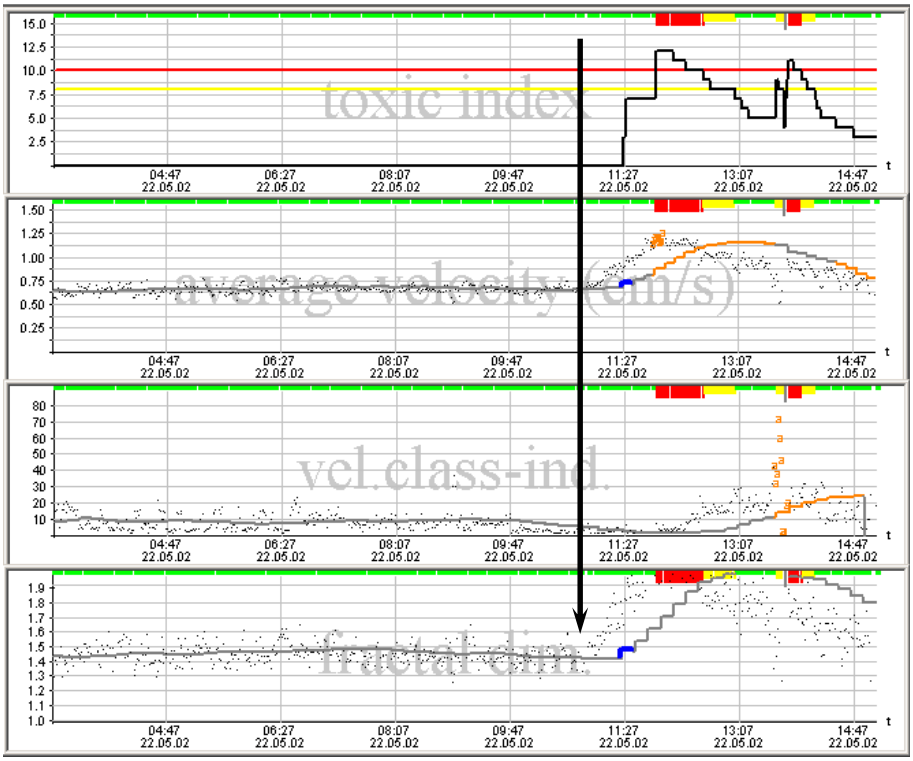


Figure 5.1 Overview of the toxicity index and behavioural parameters ('average velocity', 'vel.class-ind.', 'fractal dim.') in the validation test with diazinon on 22nd May 2002.
The exposure was started at 10:35 and ended at 12:05.
—: Hinkley detector used; —: gradient detector used (short period); ↓: start of alarm signal, fractal dimension is the first parameter to show a deviation with regard to the previous measurements.

the alarm signal (chapter 3). In *Daphnia*-toximeters with two measuring chambers there is a duplo measurement available and the measurement data of the two measuring chambers could contribute towards the exclusion of false positive alarms (section 3.4.5). Carrying out measurements with regard to a control sample could contribute to the evaluation of the technical functioning of the configuration (section 4.3).
In addition to alarm signals, a number of increases of the toxicity index in a longer time frame could be a reason to perform additional assessments of the measurements even if the toxicity index was lower than the alarm value. It could prevent the time frame in which the different detectors were activated from being too long to generate an alarm (due to the principle of reduction). If a clear link can be established between the different increases in the toxicity index, such as a shift in the level of one or more parameters (section 5.2.3), this could form a reason to evaluate these changes as an alarm signal.
In section 5.1 we see the evaluation of an alarm based on an experiment with an insecticide (diazinon, 8 µg/l). The following relevant parameters are determined in alarm signals: average speed, speed-class index and fractal dimension. These are discussed in detail in the following subsections.

5.2.1 Start of an alarm signal

A Hinkley or gradient detector is only activated if the measurement value shows a deviation over a longer period of time (up to several hours). This always causes an increase in the toxicity index and the transgression to occur at a later point in time than when the first deviations were perceptible; there is a certain response time. The point in time when the alarm starts is determined later on, at the point in time when the first parameter shows a deviation (in figure 5.1 the increase in the fractal dimension), and therefore not at the point in time when the toxicity index began to rise.
In addition to the time it takes to activate the detectors, the response time also depends on the 'dead' volume of the configuration (from the inflow point in the river to the measuring chamber), the parameter settings (weighing the detectors, the sensitivity of the integration interval of the measuring values) and the substance that causes the behavioural changes (concentration, operation mechanism and speed with which the organism can ingest the substance). In this way the (total) response time of the *Daphnia*-monitor at Keizersveer is at least between three quarters of an hour and an hour.

5.2.2 Features of the alarm

With an alarm there is always a strong increase in the toxicity index. The level of this value depends on the type and number of detectors that have been activated and the time frame in which this takes place. The altitude of the toxicity index itself does not provide us with much information. When there is an alarm signal it is important to indicate in which parameters a deviation has been detected. This information should be registered in an evaluation and an alarm signal. In addition to determining the deviations, it is important to determine whether there is a hypo (decreased) or hyper-activity (increased) and how the deviant activity progresses.

5.2.3 *Reference behaviour*

In order to determine how much the measurement values deviate from 'normal values' it is necessary to determine the values of the reference behaviour of the *Daphnia* on the relevant location (in the relevant matrix). A parameter such as swimming speed depends on the size of the water fleas. Although larger animals can swim faster than smaller animals, they can also swim slowly. Larger animals cover a greater speed range than smaller animals. This means that the size of the animals should be included in the evaluation.

Because the reference behaviour depends on the size of the animals, it is necessary to register this behaviour per size-class. The range of the swimming speed of *Daphnia* can be seen in figure 5.2.

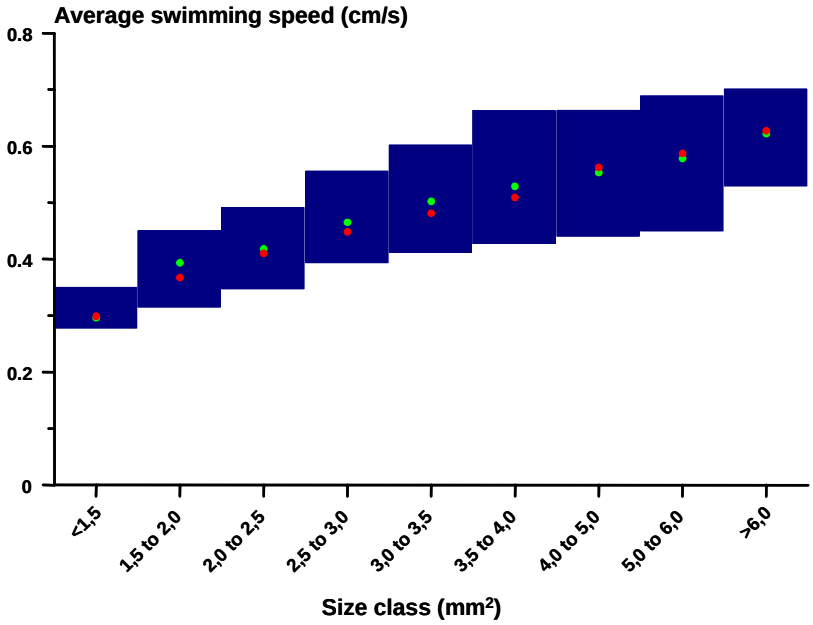


Figure 5.2 Swimming speed range of *Daphnia* in different size classes. ●: average; ●: median; ▮: range between 10^{e} and 90^{e} percentile.

The aging of the water fleas also causes changes with regard to the sensitivity of the test organisms. In general the youngest juveniles are more sensitive than older animals. Adults are (in many cases) the least sensitive stage. If a constant sensitivity of the configuration is desirable, the animals should be regularly replaced in order to limit the effect of aging.

5.2.4 *End of an alarm*

After an alarm signal has been generated and the start of the alarm has been determined, it is important to register the end (and therefore also the duration) of the alarm. As the toxicity index is reduced in time (see section 5.1) it will eventually drop back to the base line (zero). However this does not (automatically) mean that the water quality has also returned to normal conditions.

The measurement values are continually evaluated by using the previous values, and if the previous values have increased, the new measurement values will be compared with them. In this way, measurement values, which

lie outside the reference behaviour of the *Daphnia*, can nevertheless be evaluated as 'normal' because they do not deviate from the previous measurement values, which also lie outside the reference behaviour. The moment that the water quality improves and the behaviour of the *Daphnia* changes due to improved conditions, it is quite possible that this generates an alarm. Consequently, the toxicity index itself is not a good parameter for determining the end of an alarm.
Moreover, animals that were present in the appliance during the alarm can no longer be used as they could be affected or even damaged.

Because of all this, there is no simple way to determine the end of an alarm. In order to determine the end of an alarm it is necessary to meet one, or preferably, more of the criteria listed below:

- *Evaluating the measurement values of a new measurement series*
  It is necessary to evaluate the measurement values of the separate parameters of other water fleas that have been freshly placed in the measuring chamber at the start of the measurement series. In this evaluation it is important to change as few variables as possible in the culture and during exposure. Preferably, deviations should only be ascribed to the harmful substance itself. Consequently it is best to keep the cultures under the same conditions (e.g. 20 °C) and in the same matrix (filtered surface water) as the surface water where the toximeter is stationed. If deviations are no longer observed, the alarm can be stopped and the monitoring resumed. This method of evaluating an alarm is described by Wagenvoort *et al.* (2003).
- *Monitoring the cause*
  If it is possible to indicate a clear cause, for example by means of measurement data of physical continual measurement systems or chemical analyses, a measurement programme for this parameter can be started. As long as this measurement programme reveals deviations, there is no need to spend energy on evaluating whether the behaviour of the water fleas is still deviant. Monitoring can be resumed when this measurement programme shows that the causating factor has been removed and when it is determined that the water fleas no longer show deviant behaviour.

- *Static test*
  A possible solution is to carry out a static *Daphnia*-test (NEN, 1998). By using concentrated samples, it is possible to follow changes of the EC_{50} over time. This involves the use of columns (such as XAD-columns) (Penders & Hoogenboezem, 2001). On these columns most apolar organic substances are retained. After elution with an extraction solvent a small volume of the concentrated bonded substances is released. The extract can be used to make dilutions in Elendt medium, after which the sample can be tested by means of the static test. The concentration factor, which reflects an effect, provides us with information on the toxicity of an alarm sample with regard to an extract of an unsuspected sample. In this strategy it is important to know the EC_{50} of the surface water under normal conditions.

6 Conclusions and Recommendations

Breeding the *Daphnia* and the maintenance of the *Daphnia*-toximeter:

- The breeding of *Daphnia* and the maintenance of the *Daphnia*-toximeter can easily be standardised. A periodical monitoring of a number of essential variables produces a substantial increase in the up time, sensitivity and reliability aspects of the measurement system.

The validation and quality assurance of the *Daphnia*-toximeter:

- In practice it is very well possible to standardise the installation of the *Daphnia*-toximeter, but this has not been done yet. For example, each of the companies involved implemented a different sample filtration. The reasons for this are related to the features of the measuring station, the request of the client(s) and the requirements of the users.
- The functioning of the *Daphnia*-toximeter (the whole configuration) can be assured with a control sample. The monitoring of the *Daphnia*-culture and validation tests should be carried out periodically with the toximeter. In case of deviant behaviour a secure system will contribute towards 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 were used to detect the alarm. Moreover, it is extremely important to further optimise the parameter set including a behavioural evaluation based on size of the daphnids.
- In an alarm signal the alarm should be specified, thereby stating the detectors implemented and indicating the level of the different behavioural parameters.
- It is advisable to use the same settings and configuration on a national level (but in any case on all measurement systems on one and the same riverbasin).
- 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 measurement value of a new measurement series;
    - Monitoring the cause;
    - Carrying out a static *Daphnia*-test

7 Bibliography

BBE. 2001. Manual for the bbe *Daphnia* Toximeter. bbe Moldaenke, Kiel.

BBE, 2003. Herstellung der Nährungslösung V für Algen in Fermentern von BBE-Toximetern. BBE-Merkblatt D19, Version 2.0.2.6 D2, BBE, Kiel (D).

Burns, CW. 1968. The relationship between body size of filterfeeding Cladocera and the maximum size of particle ingested. *Limnology and Oceanography* **13**: 675-678.

Evides. 2004. Maintenance plan *Daphnia*-toximeter. Work list F 2016, version 3. Evides watercompany, Rotterdam (in Dutch).

Flößner, D. 2000. Die Haplopoda und Cladocera (ohne Bosminidae) Mitteleuropas. Backhuys Publishers, Leiden.

Geller, W. 1987. On estimating the age and the development time of *Daphnia* as a function of body size and temperature. *J. Plankton Res.* 9: 1185-1230.

Lampert, W. 1987. Feeding and nutrition in *Daphnia*. R.H. Peters and R. de Bernardi (Eds) *Memorie dell'Istituto Italiano di Idrobiologia*, 45: 143-192.

Maas-Diepenveen, JL. 1991. Preparation and composition of the Elendt-medium. Internal standard procedure, SOP AOCE/13. Riza, Lelystad (in Dutch).

NEN. 1998. Water – Determining the inhibition of the mobility of the *Daphnia magna* Straus (Cladocera) – Acute toxicity test. Norm NEN-EN-ISO 6341. NEN, Delft (in Dutch).

NEN. 2000. Water – determining the long-tern toxicity of substances *Daphnia magna* Straus (Cladocera). Norm NEN-ISO 10706. NEN, Delft (in Dutch).

Notenboom-Ram, E. 1981. Distribution and ecology of the Branchiopoda in The Netherlands. RIN-report 81/14. Rijksinstituut voor Natuurbeheer. Leersum (in Dutch).

Penders, EJM & Hoogenboezem, W. 2001. Biological tests, a suitable instrument for the quality control of surface water?. RIWA, Amsterdam.

Peters, RH. 1987. Metabolism in *Daphnia*. *Memorie dell'Istituto Italiano di Idrobiologia* **45**: 193-243.

RIZA. 1991. Manual for breeding the water flea *Daphnia magna*. Internal standard procedure, SOP; AOCE/02. RIZA, Lelystad (in Dutch).

Schwörer, R en M. Marten (2000). Mathematische Sprungerkennung (Hinkley-Detektor und Steigungsoperatoren) im Einsatz bei der Erkennung von Messwert-Niveausprüngen bei kontinuierlichen Biotestgeräten. Deutsche Gesellschaft für Limnologie e.V. Tagungsbericht 2000, Kaltenmeier Söhne, Krefeld, pag. 692-696.

Tomlin C. (ed.). 1995. The pesticide manual. Crop Protection Publications. The Bath Press, Bath.

Verschueren, K. (ed.). 1983. Handbook of environmental data on organic chemicals. Ban Nostrand Reinhold Company, New York.

Wagenvoort, AJ. 2004. Validation experiment – BBB-*Daphnia*-toximeter. Instruction. (AqWa, Goes (in Dutch).

Wagenvoort, AJ & de Hoogh, CJ. (in prep.). Biological monitoring of the water of the Meuse at Keizersveer, Implementation of the algae- and *Daphnia*-toximeter. Report). Evides, Rotterdam (in Dutch).

Wagenvoort, A, Pikaar-Schoonen, K, De Hoogh-Carpentier, C & Ketelaars, H. 2003. Algae and water fleas monitor the drinking water quality of the southwest Netherlands. *H_20* **22**: 21-24 (in Dutch).

WBB. 2003. Werk List – Functioning of the *Daphnia*-toximeter. Werk list F 2007, version 4. N.V. WBB, Werkendam (in Dutch).

WCB. 2001. Regulations WCB laboratory for breeding the water flea *Daphnia magna*. Regulation A-0071N. WRK, Nieuwegein (in Dutch).

I Overview of the BBE-*Daphnia*-toximeters at measuring stations in the Netherlands and the guideline proposed by the Biological Alarm Steering Committee (situation October 2003)

Subject		Location: Bimmen (BRD)	Nieuwegein	Eijsden	Keizersveer	Guideline	Comments
Owner		RIZA	Amsterdam Waterworks	RIZA	Evides		
Maintenance		RIZA	Water Laboratory	RIZA	Evides (and hire of Aqualab)		
River system		Rhine	Rhine/Lek	Meuse	Meuse/Amer		
Distance from the NL – B/D border (km)		0	?	0	247		
Inflow	flow (m^3/hour)	35 – 40	3	35 – 40	5 – 30		
	pressure (bar)	2	6	2	3		
	mesh size filter (µm)	1000	100	1000	800	$\leq$ 1000	Coarse particles are removed.
	duration to toximeter (min)	< 1	<5	< 1	< 6	$\leq$ 10	The 'dead' volume should be as small as possible.
Pre-filter	type	Wafelin filter	Wafelin filter	Wafelin filter	Crossflow-BBE		See sections 2.2.1 and 3.4.3
	counterpressure (bar)	0	2	0	0.3 – 0.5		
	use (l/hour)	15	15	15	1200		
	mesh size (µm)	0.2	0.2	0.2	100	50 – 100	
	production (l/hour)	15	15	15	> 4		

[Supplement I continued]

Subject		Location				Guideline	Comments
		Bimmen (BRD)	Nieuwegein	Eijsden	Keizersveer		
Pre-heater	type	Water bath containing litre bottle with glass beads	BBE	BBE	BBE		
	Flow rate volume (l/h)	6.3	5.6	6.3	3.7		
	temperature (°C)	25 to 30	25	25	25	maximum 25	
Glass wool filter and degassing through rise in temperature		Degassing without glass wool	Degassing with glass wool	Degassing without glass wool	Glass wool filter in extra tube	Degassing by heating and cooling down of water temperature	Reducing the water pressure can also be applied.
extra pump head on sample pump (W&M), degassing through decrease in pressure		no	no	no	yes (3.6 l/hour) ø 4.8 x 1.6mm Marprene		
Measurement chamber type		Helma (BBE)	Helma (BBE)	Helma (BBE)	Helma (BBE)	Helma (BBE)	Measurement cuvette content: 64 ml (2.1 x 4.5 x 6.8 cm)
	number of chambers	2 (parallel)	2 (parallel)	2 (parallel)	1		Measurement chamber content: 17 ml (1.0 x 3.1 x 5.5 cm)
	flow rate volume (l/h)	2 x 1.65	2 x 1.65	2 x 1.65	2.04	1.5 – 2.5	
	type hose	ø 4.8 x 1.6mm Marprene	ø 4.8 x 1.6mm Marprene	ø 4.8 x 1.6mm Marprene	ø 3.2 x 1.6mm Marprene		
	pump speed W&M (%)	60	60	60	60		
	temperature (°C)	20.0	20.0	20.0	20.0	20.0	
	outflow passive	yes	yes (4.74 l/hour)	yes	yes	yes	
	outflow active (l/h)	0.35	0.89	0.35	1.7	0.35 - 2	
	type hose	ø 1.6 x 1.6 mm Marprene	ø 1.6 x 1.6 mm Marprene	ø 1.6 x 1.6 mm Marprene	ø 1.6 x 1.6 mm Marprene		
	replacement degree (per h)	20	20	20	100	20 – 120	The replacement level should be sufficiently so that the accumulation of waste products can not have an effect. A replacement level that is too high causes a too high flow-rate. The flow-rate should be substantially lower (<5 cm/min) than the swimming speed.
	rate of flow (cm/min)	1.8	1.8	1.8	9	1.5 – 12	
	rate of flow (cm/s)	0.03	0.03	0.03	0.15	0.03 – 0.20	

[Supplement 1 continued]

Subject		Location				Guideline	Comments
		Bimmen (BRD)	Nieuwegein	Eijsden	Keizersveer		
Fermenter	type of algae	*Chlorella vulgaris*	*Chlorella vulgaris*	*Chlorella vulgaris*	*Chlorella vulgaris*	*Chlorella vulgaris*	If very fine filters are used there are no longer any nutrients in the (filtered) sample water. In this case it is very important to feed the test organisms extra algae; a flourishing culture is essential. Supplementary feeding is less critical if the water is not filtered.
	nutrient solution	type V BBE	own medium	type V BBE	type V BBE		
	temperature (°C)	24.0	24.0	24.0	24.0	24.0	
	type hose	ø 1.6 x 1.6 mm silicone	ø 1.6 x 1.6 mm silicone	ø 1.6 x 1.6 mm silicone	ø 1.6 x 1.6 mm silicone		
	pump speed Verder (%)	60	40	60	60		
	halogen lamp (% on)	100	100	100	100		
	dosage nutrients	3 s per 1000 s	50s per 10000s	3 s per 1000 s	4 s per 1000 s	$\geq$ 3 s per 1000 s	
	dosage nutrients algae	2 s per 1000 s	15s per 10000s	2 s per 1000 s	3 s per 1000 s	$\geq$ 2 s per 1000 s	
Daphnia	type	*D. magna*	*D. magna*	*D. magna*	*D. magna*	*D. magna*	See section 3.4.5
	minimum age (days)	2	2 tot 3	2	2	2	
	maximum age (days)	9	10	9	6 (noise!)	no strong increase in noise	
	number	10	10	10	10	10	
	size (mm^2)	undetermined	undetermined	undetermined	1.5 – 6		
	measurement series (n/week)	1	1	1	$\geq$ 2		

[Supplement 1 continued]

Subject		Location				Guideline	Comments
		Bimmen (BRD)	Nieuwegein	Eijsden	Keizersveer		
Quality assurance		Is implemented in	yes	no	yes	yes	See section 4.1
	breeding *Daphnia*	Düsseldorf	standard	standard	standard	standard	
	Clone and type		macro- and microscopic		macro- and microscopic	macro- and microscopic	
	Type of medium		Elendt	Elendt	Elendt and filtered water reservoirs	Elendt	
	control culture		LC_{50} dichromats				
	frequency		4 x per year		5 g NaCl/l		
	control sample				≤ 1x every 3	NaCl or trichorfon	
	frequency				weeks	≤ 1x every month	
Evaluation	used parameter-set	based on normal	self defined	based on normal	normal	normal	In case of alarm signals (to another station) it is important to know which parameter settings have been used. The standard setting for normal sensitivity is used in rivers in the Netherlands. A 'no alarm time'' (3x response time control sample ≈ 3,75 hours) is set up to prevent false positive signals from occurring. Depending on the use and features of the river, individual users can define and use an additional data set on site or apply an additional evaluation to the measurement values.
	no alarm time (min)	0	60	0	240	240	
	extra evaluation	no	no	no	yes		
	namely:				comparison with normal values per size-class		
					- swimming speed		
					- fractal dimension		
					- speed-class		
					- distance		
					- number		

II Composition of the Elendt medium (Maas-Diepenveen, 1991)

Trace elements

B	(H_3BO_4)	0.5000	mg/l
Fe	($FeSO_4.7H_2O$)	0.2000	mg/l
Mn	($MnCl_2.4H_2O$)	0.1000	mg/l
Li	(LiCl)	0.0500	mg/l
Rb	(RbCl)	0.0500	mg/l
Sr	($SrCl_2.6H_2.O$)	0.0500	mg/l
Mo	($Na_2MoO_4.2H_2O$)	0.0250	mg/l
Br	(NaBr)	0.0125	mg/l
Cu	($CuCl_2.2H_2O$)	0.0063	mg/l
Zn	($ZnCl_2$)	0.0063	mg/l
Co	($CoCl_2.6H_2O$)	0.0025	mg/l
J	(KJ)	0.0025	mg/l
Se	(Na_2SeO_3)	0.0010	mg/l
V	(NH_4VO_3)	0.0003	mg/l
$Na_2EDTA.2H_2O$		2.5000	mg/l

Macro-nutrients

$CaCl_2.2H_2O$	293.8	mg/l
$MgSO_4.7H_2O$	123.3	mg/l
$NaHCO_3$	64.8	mg/l
KCL	5.8	mg/l
$Na_2SiO_39H_2O$	10.0	mg/l
$NaNO_3$	0.27	mg/l
KH_2PO_4	0.14	mg/l
K_2HPO_4	0.18	mg/l

Vitamins

Thiamine	75.0	µg/l
Cyanobalamine	1.0	µg/l
Biotin	0.75	µg/l

The hardness of the medium is 250 mg/l $CaCO_3$ (on a basis of Ca and Mg).

III Example of a maintenance plan (Evides, 2004)

Maintenance plan *Daphnia*-toximeter

Version: 3
Datum: 28-oct-04

Evides-code: P 2016
Blad: 1 van 1

Year and week: _____

	1	2	3	Comments
Date				Day 1:
Carried out by:				
Extra maintenance				
Does the toximeter have any malfunctions	yes / no	yes / no	yes / no	
Replace glass wool in filter	yes / no	yes / no	yes / no	
Water fleas replaced	yes / no	yes / no	yes / no	
Replace nutrients	yes / no	yes / no	yes / no	
Weekly maintenance				
Replace RVS filter and clean dirty specimen	yes / no	yes / no	yes / no	
Clean (blow through with compressed air) narrow hoses	yes / no	yes / no	yes / no	
Measuring chamber cleaned	yes / no	yes / no	yes / no	Day 2:
Clean degassing tube and tube with heating element	yes / no	yes / no	yes / no	
Code reference thermometer				
Temperature sample water measuring chamber °C				
Monthly maintenance				
Clean (blow through with compressed air) drainage hoses	yes / no	yes / no	yes / no	
Monitor flow rate of pumped inflow and outflow water (note down the flow rate in the comments)	yes / no	yes / no	yes / no	
Fermenter unialgal	yes / no	yes / no	yes / no	
Quarterly maintenance				Day 3:
Replace all hoses	yes / no	yes / no	yes / no	
Check pump bearing of nutrient dosage and replace if necessary	yes / no	yes / no	yes / no	
Clean fermenter	yes / no	yes / no	yes / no	
Replace fermenter lamp	yes / no	yes / no	yes / no	
Clean cooling system (appliance and pipes)	yes / no	yes / no	yes / no	
Other				
Algae replaced in fermenter	yes / no	yes / no	yes / no	
Status (operational / non-operational)	O / NO	O / NO	O / NO	
System cleared (time)				
Control measurement carried out	yes / no	yes / no	yes / no	
Spike test carried out	yes / no	yes / no	yes / no	

IV Example of a plan for registering the functioning and evaluation of the water quality (WBB, 2003)

technical functioning over the last period: week number: _____

Day:				Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
Date:										
Date and time of check										
Initials:										
technical alarm on screen				YES / NO *	YES / NO *	YES / NO *	JA / NEE *	YES / NO *	YES / NO *	YES / NO *
Air bubble(s) in the cuvette				YES / NO *	YES / NO *	YES / NO *	JA / NEE *	YES / NO *	YES / NO *	YES / NO *
Particles in the cuvette				YES / NO *	YES / NO *	YES / NO *	JA / NEE *	YES / NO *	YES / NO *	YES / NO *
Are the parameters below outside the boundaries?				YES / NO *	YES / NO *	YES / NO *	JA / NEE *	YES / NO *	YES / NO *	YES / NO *
If so, fill in the whole column										
Parameter	unit	lower limit	upper limit							
number of test organisms	n	6	10							
size of test organisms	mm^2		6							
temperature fermenter	°C	23	25							
temperature pre-heater	°C	24	26							
temperature sample water	°C	19.5	20.5							
recognition-rate	%	30								
degree of tubidity (www.aqualarm.nl)	FTU									

Conclusion defect? YES / NO * **report PVO (ks/aw/hk)** to: _____ on: _____ **Description defect**: _____

[Supplement 4 continued]

Evaluation water quality

Day:		Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
toxicity index higher than 10		YES / NO *	YES / NO *	YES / NO *	YES / NO *	YES / NO *	YES / NO *	YES / NO *
size of test organisms (mm^2)								
Are the parameters below outside the reference values of normal behaviour? **If so, fill in the whole column**		YES / NO *	YES / NO *	YES / NO *	YES / NO *	YES / NO *	YES / NO *	YES / NO *
average size	[mm^2]							
average swimming speed	[cm/s]							
speed-class index	[%]							
fractal dimension	[-]							
distance	[cm]							

Conclusion: (possible alarm? YES / NO *

Report PVO (ks/aw/hk) to: _____

date: _____

Description Alarm: _____

Reference value of normal behaviour

Size-class (mm)	Average swimming speed (cm/s)		Speed-class index (%)		fractal dimension		distance (cm)	
	lower limit	upper limit	lower limit	upper limit	lower limit	upper limit	lower limit	upper limit
All classes			7	32			1.89	3.34
<1,5	0.28	0.32			1.22	1.35		
1,5 to 2,0	0.31	0.45			1.23	1.37		
2,0 to 2,5	0.35	0.49			1.25	1.40		
2,5 to 3,0	0.39	0.56			1.23	1.40		
3,0 to 3,5	0.41	0.60			1.22	1.41		
3,5 to 4,0	0.43	0.66			1.24	1.42		
4,0 to 5,0	0.44	0.66			1.26	1.43		
5,0 to 6,0	0.45	0.69			1.27	1.44		
>6,0	0.53	0.70			1.28	1.46		

V Detection levels for the *Daphnia*-toximeter for a number of different substances and the associated LC50 in a static *Daphnia*-test over a period of 48 hours

Substance	Group of substances	Detection level	LC_{50}	Source	Comments
3-Cyclohexyl-1,1-dimethylureum	Additive for hydraulic liquids; herbicide	< 30 µg/l		Evides, Kiwa, AqWa, 2004	
Chloroform		> 10 mg/l	353 mg/l [3]	Landesanstalt für Umweltschutz Karlsruhe 1999	
Cyclosarin (GF)	Neurotoxin	> 10µg/l		Wehrwissenschaftliches Institut für Schutztechnologie der Bundeswehr	
l-Cyhalothrin	Insecticide	> 500 µg/l	0.38 µg/l [1]	Umweltbehörde Hamburg 8/2000	Dynamic test carried out with a concentration of 500 µg/l .
Diazinon	Insecticide	> 8 µg/l	1.4 mg/l [1]	WBB, 2003	Dynamic test carried out with a concentration of 8 µg/l .
Endosulfan	Insecticide	> 100 µg/l	75 – 750 µg/l [1]	Landesanstalt für Umweltschutz Karlsruhe 1999	
Lindaan (γ-HCH)	Insecticide	> 30 µg/l	516 µg/l [2]	Landesanstalt für Umweltschutz Karlsruhe 1999	
Sodium chloride	Salt	> 3.5 g/l		RIZA, 2002	Control sample, with these concentrations test carried out by RIZA or WBB.
		> 5 g/l		WBB, 2003	
Pendimethaline	Herbicide	> 100 µg/l	46.8 mg/l [4]	Umweltbehörde Hamburg 8/2000	

Substance	Group of substances	Detection level	LC_{50}	Source	Comments
Propetamphos	Insecticide	> 100 µg/l	14.5 µg/l [1]	Landesanstalt für Umweltschutz Karlsruhe 1999	
Sarin (GB)	Neurotoxin	> 6.4 µg/l		Wehrwissenschaftliches Institut für Schutztechnologie der Bundeswehr	
Soman (GD)	Neurotoxin	> 6.4 µg/l		Wehrwissenschaftliches Institut für Schutztechnologie der Bundeswehr	
Tabun (GA)	Neurotoxin	> 35.5 µg/l		Wehrwissenschaftliches Institut für Schutztechnologie der Bundeswehr	
Terbuthyhlazine	Herbicide	> 250 µg/l	21.2 mg/l [1]	Umweltbehörde Hamburg 8/2000	
Trichlorfon	Insecticide	> 2 µg/l	0.96 µg/l [1]	Umweltbehörde Hamburg 8/2000	

[1]: Tomlin C. (ed.).1995. The pesticide manual. Crop Protection Publications. The Bath Press, Bath.
[2]: Verschueren, K. (ed.). 1983. Handbook of environmental data on organic chemicals. Ban Nostrand Reinhold Company, New York.
[3]: http://w3dibit.hsr.it/nfc/w3dibit/safety/schede/Chloroform_US.pdf
[4]: http://www.basf.de/file/1212011.pdf

VI Settings of the *Daphnia*-toximeter at different stations in the Netherlands and Germany (Excel work sheet)

	LOW	NORMAL	HIGH	WBB	RIZA Eijsden	RIZA Bimmen	WRK	Hamburg	Karlsruhe	
Software version				2.0.2.5.	2.2.0.7		2.2.0.8	2.2.09		
MFC42.DLL version				6.00.8665.0	6.00.8665.0			6.00.8447.0		
MSVCRT.DLL version				6.10.8637.0	6.10.8924.0			6.00.8337.0		
level for the yellow alarm	8	8	8	8	8	8	8	6	6	tox
level for the red alarm	10	10	10	10	10	10	12	10	10	tox
minimal level for number of objects	3	3	3	3	3	3	5	3	3	
rating number of objects	0	0	0	0	1	0	3	0	5	tox points
rating decrease of objects	0	0	0	0	1	0	3	0		tox points
rating minimal level for number of objects	5	5	5	5	5	5	8	5		tox points
rating alarm velocity	4	4	4	4	4	4	8	4		tox points
rating alarm height	2	2	2	2	2	2	6	4		tox points
rating alarm frc.dim.lin	3	3	4	3	4	4	3	4		tox points
rating alarm frc.dim.box	0	0	4	0	4	4	3	0		tox points
rating alarm distance	2	2	2	2	2	2	3	3		tox points
rating alarm vel.class index	4	5	5	5	5	5	4	3		tox points
rating slope alarm velocity	5	5	5	5	5	5	1	5		tox points
rating slope alarm height	3	3	4	3	4	4	1	3		tox points
rating slope alarm frc.dim.lin	3	3	3	3	3	3	0	4		tox points
rating slope alarm frc.dim.box	0	0	3	0	3	3	0	0		tox points
rating slope alarm distance	2	2	2	2	2	2	2	3		tox points
rating slope alarm vel.class index	5	4	5	4	5	5	3	4		tox points
follow-up time min. number of objects	30	30	30	30	30	30	180	500		min
follow-up time decrease per object	30	30	30	30	120	30	180	500		min
follow-up time alarm velocity	120	120	120	120	120	120	120	240		min
follow-up time alarm height	120	120	120	120	120	120	60	240		min
follow-up time alarm frc.dim.lin	120	120	120	120	120	120	120	240		min
follow-up time alarm frc.dim.box	120	120	120	120	120	120	120	240		min
follow-up time alarm distance	120	120	120	120	120	120	120	240		min
follow-up time alarm vel.class-index	120	120	120	120	120	120	120	240		min
follow-up time slope alarm velocity	150	150	150	150	150	150	30	300		min
follow-up time slope alarm height	150	150	150	150	150	150	60	200		min
follow-up time slope alarm frc.dim.lin	150	150	150	150	150	150	30	120		min
follow-up time slope alarm frc.dim.box	150	150	150	150	150	150	30	120		min
follow-up time slope alarm distance	150	150	150	150	150	150	30	120		min
follow-up time slope alarm vel.class-index	150	150	150	150	150	150	120	120		min
no-alarm time at measuring start	0	0	0	240	0	0	60	0		min
Mode for the combined toxic index	0	0	0	0	0	1	0	0		

		LOW	NORMAL	HIGH	WBB	RIZA Eijsden	RIZA Bimmen	WRK	Hamburg	Karlsruhe	
HINKLEY CHANNEL 0 (VELOCITY) (parameter set	hinkley)										
HINKLEY CHANNEL 0 (VELOCITY)	damping	1	1	1	1	1	1	1	1		
HINKLEY CHANNEL 0 (VELOCITY)	damping value	1	1	1	1	1	1	1	1		
HINKLEY CHANNEL 0 (VELOCITY)	Lg.SqrtN factor B	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002		
HINKLEY CHANNEL 0 (VELOCITY)	Lg.SqrtN factor A	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1		
HINKLEY CHANNEL 0 (VELOCITY)	fit points curve	27	27	27	27	27	27	27	28		data points
HINKLEY CHANNEL 0 (VELOCITY)	fit points SqrtN	51	51	51	51	51	51	51	51		data points
HINKLEY CHANNEL 0 (VELOCITY)	fit point's slope 1	20	20	24	24	24	24	24	20		data points
HINKLEY CHANNEL 0 (VELOCITY)	fit point's slope 2	51	51	51	51	51	51	51	51		data points
HINKLEY CHANNEL 0 (VELOCITY)	hinkley factor	0.8	0.6	0.6	0.6	0.6	0.6	0.6	1.2		
HINKLEY CHANNEL 0 (VELOCITY)	SqrtN factor	3	3	3	3	3	3	3	3		
HINKLEY CHANNEL 0 (VELOCITY)	log.SqrtN	1	1	1	1	1	1	1	1		
HINKLEY CHANNEL 0 (VELOCITY)	sigma coefficient	3	3	3	3	3	3	5	3		
HINKLEY CHANNEL 0 (VELOCITY)	jump height	1.3	1.3	1.3	1.3	1.2	1.2	1.1	1.4		
HINKLEY CHANNEL 0 (VELOCITY)	gradient limit 1	0.38	0.38	0.25	0.38	0.25	0.25	0.4	0.4		
HINKLEY CHANNEL 0 (VELOCITY)	gradient limit 1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5		
HINKLEY CHANNEL 0 (VELOCITY)	use Hinkley50	1	1	1	1	1	1	1	1		
HINKLEY CHANNEL 0 (VELOCITY)	alarm detection range hinkley alarm	12	12	12	12	12	12	12	12		data points
HINKLEY CHANNEL 0 (VELOCITY)	alarm detection range slope alarm	12	12	12	12	12	12	12	12		data points
HINKLEY CHANNEL 0 (VELOCITY)	dead time alarm	30	30	30	30	30	30	30	30		data points
HINKLEY CHANNEL 0 (VELOCITY)	Ausreißerbegrenzung	5	5	5	5	5	5	5	5		
HINKLEY CHANNEL 1 (HEIGHT) (parameter set	hinkley)										
HINKLEY CHANNEL 1 (HEIGHT)	damping	1	1	1	1	1	1	0	1		
HINKLEY CHANNEL 1 (HEIGHT)	damping value	1	1	1	1	1	1	1	1		
HINKLEY CHANNEL 1 (HEIGHT)	Lg.SqrtN factor B	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002		
HINKLEY CHANNEL 1 (HEIGHT)	Lg.SqrtN factor A	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1		
HINKLEY CHANNEL 1 (HEIGHT)	fit points curve	27	27	27	27	27	27	25	27		data points
HINKLEY CHANNEL 1 (HEIGHT)	fit points SqrtN	51	51	51	51	51	51	51	51		data points
HINKLEY CHANNEL 1 (HEIGHT)	fit point's slope 1	27	27	24	27	24	24	15	27		data points
HINKLEY CHANNEL 1 (HEIGHT)	fit point's slope 2	51	51	15	51	51	15	20	51		data points
HINKLEY CHANNEL 1 (HEIGHT)	hinkley factor	1	1	1	1	1	1	0.2	1		
HINKLEY CHANNEL 1 (HEIGHT)	SqrtN factor	3	3	3	3	3	3	3	3		
HINKLEY CHANNEL 1 (HEIGHT)	log.SqrtN	1	1	1	1	1	1	1	1		
HINKLEY CHANNEL 1 (HEIGHT)	sigma coefficient	3	3	3	3	3	3	2	3		
HINKLEY CHANNEL 1 (HEIGHT)	jump height	1.1	1.1	1.1	1.1	1.1	1.1	1.2	1.2		
HINKLEY CHANNEL 1 (HEIGHT)	gradient limit 1	0.75	0.7	0.5	0.7	0.36	0.36	0.2	0.55		
HINKLEY CHANNEL 1 (HEIGHT)	gradient limit 1	0.35	0.3	0.85	0.3	0.5	0.5	0.5	0.7		
HINKLEY CHANNEL 1 (HEIGHT)	use Hinkley50	1	1	1	1	1	1	1	0		
HINKLEY CHANNEL 1 (HEIGHT)	alarm detection range hinkley alarm	12	12	12	12	12	12	12	12		data points
HINKLEY CHANNEL 1 (HEIGHT)	alarm detection range slope alarm	12	12	12	12	12	12	12	12		data points
HINKLEY CHANNEL 1 (HEIGHT)	dead time alarm	30	30	30	30	30	30	30	30		data points
HINKLEY CHANNEL 1 (HEIGHT)	Ausreißerbegrenzung	5	5	5	5	5	5	5	5		

[Supplement 6 continued]

		LOW	NORMAL	HIGH	WBB	RIZA Eijsden	RIZA Bimmen	WRK	Hamburg	Karlsruhe	
HINKLEY CHANNEL 2 (FRC.DIMENSION) (paramet	hinkley)										
HINKLEY CHANNEL 2 (FRC.DIMENSION)	damping	1	1	1	1	1	1	1	1		
HINKLEY CHANNEL 2 (FRC.DIMENSION)	damping value	1	1	1	1	1	1	1	1		
HINKLEY CHANNEL 2 (FRC.DIMENSION)	Lg.SqrtN factor B	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002		
HINKLEY CHANNEL 2 (FRC.DIMENSION)	Lg.SqrtN factor A	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1		
HINKLEY CHANNEL 2 (FRC.DIMENSION)	fit points curve	27	27	27	27	27	27	27	27		data points
HINKLEY CHANNEL 2 (FRC.DIMENSION)	fit points SqrtN	51	51	51	51	51	51	51	51		data points
HINKLEY CHANNEL 2 (FRC.DIMENSION)	fit point's slope 1	24	24	24	24	24	24	24	20		data points
HINKLEY CHANNEL 2 (FRC.DIMENSION)	fit point's slope 2	51	51	51	51	51	51	51	51		data points
HINKLEY CHANNEL 2 (FRC.DIMENSION)	hinkley factor	0.8	0.8	0.8	0.8	0.8	0.8	0.6	1.1		
HINKLEY CHANNEL 2 (FRC.DIMENSION)	SqrtN factor	3	3	3	3	3	3	3	3		
HINKLEY CHANNEL 2 (FRC.DIMENSION)	log.SqrtN	1	1	1	1	1	1	1	1		
HINKLEY CHANNEL 2 (FRC.DIMENSION)	sigma coefficient	3	3	3	3	3	3	3	3		
HINKLEY CHANNEL 2 (FRC.DIMENSION)	jump height	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.2		
HINKLEY CHANNEL 2 (FRC.DIMENSION)	gradient limit 1	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.2		
HINKLEY CHANNEL 2 (FRC.DIMENSION)	gradient limit 1	0.25	0.2	0.2	0.2	0.2	0.2	0.2	0.2		
HINKLEY CHANNEL 2 (FRC.DIMENSION)	use Hinkley50	1	0	0	0	0	0	1	0		
HINKLEY CHANNEL 2 (FRC.DIMENSION)	alarm detection range hinkley alarm	12	12	12	12	12	12	12	12		data points
HINKLEY CHANNEL 2 (FRC.DIMENSION)	alarm detection range slope alarm	12	12	12	12	12	12	12	12		data points
HINKLEY CHANNEL 2 (FRC.DIMENSION)	dead time alarm	30	30	30	30	30	30	30	30		data points
HINKLEY CHANNEL 2 (FRC.DIMENSION)	Ausreißberbegrenzung	5	5	5	5	5	5	5	5		
HINKLEY CHANNEL 3 (FRC.DIM (BOX)) (parameter	hinkley)										
HINKLEY CHANNEL 3 (FRC.DIM (BOX))	damping	1	1	1	1	1	1	1	1		
HINKLEY CHANNEL 3 (FRC.DIM (BOX))	damping value	1	1	1	1	1	1	1	1		
HINKLEY CHANNEL 3 (FRC.DIM (BOX))	Lg.SqrtN factor B	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002		
HINKLEY CHANNEL 3 (FRC.DIM (BOX))	Lg.SqrtN factor A	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1		
HINKLEY CHANNEL 3 (FRC.DIM (BOX))	fit points curve	27	27	27	27	27	27	27	27		data points
HINKLEY CHANNEL 3 (FRC.DIM (BOX))	fit points SqrtN	51	51	51	51	51	51	51	51		data points
HINKLEY CHANNEL 3 (FRC.DIM (BOX))	fit point's slope 1	24	24	24	24	24	24	24	24		data points
HINKLEY CHANNEL 3 (FRC.DIM (BOX))	fit point's slope 2	51	51	51	51	51	51	51	51		data points
HINKLEY CHANNEL 3 (FRC.DIM (BOX))	hinkley factor	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6		
HINKLEY CHANNEL 3 (FRC.DIM (BOX))	SqrtN factor	3	3	3	3	3	3	3	3		
HINKLEY CHANNEL 3 (FRC.DIM (BOX))	log.SqrtN	1	1	1	1	1	1	1	1		
HINKLEY CHANNEL 3 (FRC.DIM (BOX))	sigma coefficient	3	3	3	3	3	3	3	3		
HINKLEY CHANNEL 3 (FRC.DIM (BOX))	jump height	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1		
HINKLEY CHANNEL 3 (FRC.DIM (BOX))	gradient limit 1	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36		
HINKLEY CHANNEL 3 (FRC.DIM (BOX))	gradient limit 1	0.25	0.2	0.2	0.2	0.2	0.2	0.2	0.2		
HINKLEY CHANNEL 3 (FRC.DIM (BOX))	use Hinkley50	0	0	0	0	0	0	1	0		
HINKLEY CHANNEL 3 (FRC.DIM (BOX))	alarm detection range hinkley alarm	12	12	12	12	12	12	12	12		data points
HINKLEY CHANNEL 3 (FRC.DIM (BOX))	alarm detection range slope alarm	12	12	12	12	12	12	12	12		data points
HINKLEY CHANNEL 3 (FRC.DIM (BOX))	dead time alarm	30	30	30	30	30	30	30	30		data points
HINKLEY CHANNEL 3 (FRC.DIM (BOX))	Ausreißberbegrenzung	5	5	5	5	5	5	5	5		

		LOW	NORMAL	HIGH	WBB	RIZA Eijsden	RIZA Bimmen	WRK	Hamburg	Karlsruhe	
HINKLEY CHANNEL 4 (DISTANCE) (parameter set	hinkley)										
HINKLEY CHANNEL 4 (DISTANCE)	damping	1	1	1	1	1	1	1	1		
HINKLEY CHANNEL 4 (DISTANCE)	damping value	1	1	1	1	1	1	1	1		
HINKLEY CHANNEL 4 (DISTANCE)	Lg.SqrtN factor B	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002		
HINKLEY CHANNEL 4 (DISTANCE)	Lg.SqrtN factor A	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1		
HINKLEY CHANNEL 4 (DISTANCE)	fit points curve	27	27	27	27	27	27	27	27		data points
HINKLEY CHANNEL 4 (DISTANCE)	fit points SqrtN	51	51	51	51	51	51	51	51		data points
HINKLEY CHANNEL 4 (DISTANCE)	fit point's slope 1	24	24	24	24	24	24	24	24		data points
HINKLEY CHANNEL 4 (DISTANCE)	fit point's slope 2	15	15	15	15	15	15	51	51		data points
HINKLEY CHANNEL 4 (DISTANCE)	hinkley factor	0.9	0.9	0.9	0.9	0.9	0.9	0.6	0.9		
HINKLEY CHANNEL 4 (DISTANCE)	SqrtN factor	3	3	3	3	3	3	3	3		
HINKLEY CHANNEL 4 (DISTANCE)	log.SqrtN	1	1	1	1	1	1	1	1		
HINKLEY CHANNEL 4 (DISTANCE)	sigma coefficient	3	3	3	3	3	3	3	3		
HINKLEY CHANNEL 4 (DISTANCE)	jump height	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.3		
HINKLEY CHANNEL 4 (DISTANCE)	gradient limit 1	0.36	0.36	0.5	0.36	0.3	0.3	0.36	0.36		
HINKLEY CHANNEL 4 (DISTANCE)	gradient limit 1	0.75	0.75	0.75	0.75	0.5	0.5	0.2	0.2		
HINKLEY CHANNEL 4 (DISTANCE)	use Hinkley50	1	1	1	1	1	1	1	0		
HINKLEY CHANNEL 4 (DISTANCE)	alarm detection range hinkley alarm	12	12	12	12	12	12	12	12		data points
HINKLEY CHANNEL 4 (DISTANCE)	alarm detection range slope alarm	12	12	12	12	12	12	12	12		data points
HINKLEY CHANNEL 4 (DISTANCE)	dead time alarm	30	30	30	30	30	30	30	30		data points
HINKLEY CHANNEL 4 (DISTANCE)	Ausreißberbegrenzung	5	5	5	5	5	5	5	5		
HINKLEY CHANNEL 5 (CLASSINDEX) (parameter se	hinkley)										
HINKLEY CHANNEL 5 (CLASSINDEX)	damping	1	1	1	1	1	1	1	1		
HINKLEY CHANNEL 5 (CLASSINDEX)	damping value	1	1	1	1	1	1	1	1		
HINKLEY CHANNEL 5 (CLASSINDEX)	Lg.SqrtN factor B	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002		
HINKLEY CHANNEL 5 (CLASSINDEX)	Lg.SqrtN factor A	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1		
HINKLEY CHANNEL 5 (CLASSINDEX)	fit points curve	27	27	27	27	27	27	27	20		data points
HINKLEY CHANNEL 5 (CLASSINDEX)	fit points SqrtN	51	51	51	51	51	51	51	51		data points
HINKLEY CHANNEL 5 (CLASSINDEX)	fit point's slope 1	24	24	24	24	24	24	24	24		data points
HINKLEY CHANNEL 5 (CLASSINDEX)	fit point's slope 2	51	51	51	51	51	51	51	51		data points
HINKLEY CHANNEL 5 (CLASSINDEX)	hinkley factor	0.7	0.7	0.7	0.7	0.6	0.6	0.6	1		
HINKLEY CHANNEL 5 (CLASSINDEX)	SqrtN factor	3	3	3	3	3	3	3	3		
HINKLEY CHANNEL 5 (CLASSINDEX)	log.SqrtN	1	1	1	1	1	1	1	1		
HINKLEY CHANNEL 5 (CLASSINDEX)	sigma coefficient	3	3	3	3	3	3	3	3		
HINKLEY CHANNEL 5 (CLASSINDEX)	jump height	1.3	1.3	1.3	1.3	1.1	1.1	1.1	1.2		
HINKLEY CHANNEL 5 (CLASSINDEX)	gradient limit 1	0.8	0.7	0.4	0.7	0.5	0.5	0.36	0.7		
HINKLEY CHANNEL 5 (CLASSINDEX)	gradient limit 1	0.52	0.52	0.52	0.52	0.52	0.52	0.2	0.5		
HINKLEY CHANNEL 5 (CLASSINDEX)	use Hinkley50	1	1	1	1	0	0	1	0		
HINKLEY CHANNEL 5 (CLASSINDEX)	alarm detection range hinkley alarm	12	12	12	12	12	12	12	12		data points
HINKLEY CHANNEL 5 (CLASSINDEX)	alarm detection range slope alarm	12	12	12	12	12	12	12	12		data points
HINKLEY CHANNEL 5 (CLASSINDEX)	dead time alarm	30	30	30	30	30	30	30	30		data points
HINKLEY CHANNEL 5 (CLASSINDEX)	Ausreißberbegrenzung	5	5	5	5	5	5	5	5		

[Supplement 6 continued]

		LOW	NORMAL	HIGH	WBB	RIZA Eijsden	RIZA Bimmen	WRK	Hamburg	Karlsruhe	
CLASS DATA (V) (parameter set	class)										
upper distance from fit value		80	80	80	80	80	80	40	60		%
lower distance from fit value		40	40	40	40	40	40	40	45		%
weight		1	1	1	1	1	1	1	1		
alarm level		10	10	10	10	10	10	10	30		%
upper limit classic mode		0.8	0.8	0.8	0.8	0.8	0.8	2	0.3		cm/s
lower limit classic mode		0.3	0.3	0.2	0.3	0.2	0.2	0.5	0.3		cm/s
mode for calculation vel.class index (0=fit,1=MinMax,2=classic)		0	0	0	0	2	2	0	0		
Mode add upper and lower classes		5	5	5	5	5	5	4	10		
Mode add upper and lower classes		5	5	5	5	5	5	2	10		
min limit		0	0	0	0	0	0	0	0		cm/s
max limit		1.5	1.5	1.5	1.5	1.5	1.5	2	1.5		cm/s
CLASS DATA (H) (parameter set											
min limit		0	0	0	0	0	0	0	0		cm
max limit		5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5		cm
CLASS DATA (W) (parameter set											
min limit		0	0	0	0	0	0	0	0		cm
max limit		3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5		cm
CHAMBER DIMENSIONS (parameter set											
width of the chamber		3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5		
height of the chamber		5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5		
AVERAGE INTERVALS (parameter set	average)										
average interval	velocity class	10	10	10	10	10	10	1	10	5	data points
average interval	frc.dim.lin	10	10	10	10	10	10	10	10	7	data points
average interval	frc.dim.box.	10	10	10	10	10	10	10	10	10	data points
average interval	number of active organisms	1	1	1	1	1	1	60	15	7	data points
average interval	recognition rate	30	30	30	30	30	30	30	30	15	data points
average interval	distance	10	10	10	10	10	10	10	10	5	data points
average interval	height	10	10	10	10	10	10	1	8	5	data points
average interval	size	10	10	10	10	10	10	20	30	20	data points
average interval	velocity class index	10	10	10	10	10	10	5	3	4	data points
average interval	velocity	8	8	8	8	8	8	20	8	10	data points
average interval	temperatures	20	20	20	20	20	20	10	20	4	data points
VIEW SETTINGS (parameter set	special)										
24 hour view	yes/enabled				no		no		no		
VIEW SETTINGS (parameter set	limits)										
slope and plausibility	yes/enabled				yes		yes				

	LOW	NORMAL	HIGH	WBB	RIZA Eijsden	RIZA Bimmen	WRK	Hamburg	Karlsruhe	
IMAGE PROCESSING										
Grey level					230	232	160	160	230	pixel
Grey level (static)					230	232	160	160		pixel
Grey level (dynamic)					0	0	0.35	0.37		
Tolerance point traces					3	3	3	3	3	datapoints
Maximum value of the distance					24.801	24.801	6.933	6.989	6.99	cm
percentage of the avg size to get the new min. Size					20	20	50	25		%
minimum size detection maximum					90	90	90	90	4	pixel
maximum size					10000	10000	10000	10000	300	pixel
fit point regression					10	10	10	19	10	datapoints
min. Dimension x of objects					2	2	2	1	2	pixel
min. Dimension y of objects					4	4	4	2	4	pixel
show numbers					no	no	no	no		
camera channel chamber 1:					1-	1-	1-	1-		
camera channel chamber 2:					1-	1-	1-	1-		
Background compensation (static)					70	70	70	0	70	%
Use background compensation					yes	yes	yes	yes	yes	
period background compensation					3	3	10	10	10	datapoints
NUMBER TRACKING										
Period of number tracking					1	1	5	5	5	interval
minimum occurence level					10	10	10	10	10	%
summation limit					95	95	95	85	95	%
count detecion mode					summation	summation	summation	HAD		
CONTROL F (parameter set: regulation)										
ki value:					20	20	5			%/gr. C/s
kp value:					50	50	50			%/gr.C
ks value:					0	0	0			%/gr.C
period:					120	120	120			s
required temperature value:					24	24	25			gr. C
delay:					0	0	0			min
I-check interval:					600	600	600			s
I-ratio check:					no	no	no			
maximum power:					100	100	100			%

	LOW	NORMAL	HIGH	WBB	RIZA Eijsden	RIZA Bimmen	WRK	Hamburg	Karlsruhe	
CONTROL P (parameter set: regulation)										
ki value:					0.4	0.4	0.1			%/gr. C/s
kp value:					5	5	5			%/gr.C
ks value:					0	0	0			%/gr.C
period:					20	20	40			s
required temperature value:					20	20	20			gr. C
delay:					0	0	0			min
I-check interval:					600	600	60			s
I-ratio check:					yes	no	no			
maximum power:					100	100	100			%
CONTROL P2 (parameter set: regulation)										
ki value:					0.4	0.4	0.4			%/gr. C/s
kp value:					5	5	5			%/gr.C
ks value:					0	0	0			%/gr.C
period:					20	20	40			s
required temperature value:					20	20	20			gr. C
delay:					0	0	0			min
I-check interval:					600	600	60			s
I-ratio check:					yes	no	no			
maximum power:					100	100	100			%
CONTROL V (parameter set: regulation)										
ki value:					0.4	0.3	1			%/gr. C/s
kp value:					0.2	0.2	0.5			%/gr.C
ks value:					0.5	0.4	0			%/gr.C
period:					20	20	40			s
required temperature value:					25	28	25			gr. C
delay:					0	0	0			min
I-check interval:					600	600	600			s
I-ratio check:					no	no	no			
maximum power:					100	100	100			%

[Supplement 6 continued]

	LOW	NORMAL	HIGH	WBB	RIZA Eijsden	RIZA Bimmen	WRK	Hamburg	Karlsruhe	
TEMPERATURES (parameter set: calib)										
fermenter temperature offset:					-12.5	-12.5	-12.5			gr. C
fermenter temperature slope:					19.8	20.6	20.7			gr. C/Volt
sample temperature offset:					-12.5	-12.5	-12.5			gr. C
sample temperature slope:					19.5	19.8	19.5			gr. C/Volt
sample 2 temperature offset:					-12.5	-12.5	-12.5			gr. C
sample 2 temperature slope:					20.3	20	20.3			gr. C/Volt
pre-heater temperature offset:					-12.5	-10.5	-12.5			gr. C
pre-heater temperature slope:					20.2	20.5	20.7			gr. C/Volt
external temperature offset:					-12.5	12.5	-12.5			gr. C
external temperature slope:					19.9	20	20.8			gr. C/Volt
offset additional data 1:					-12.5	-12.5	-12.5			gr. C
slope additional data 1:					20	20	20			gr. C/Volt
offset additional data 2:					-12.5	-12.5	-12.5			gr. C
slope additional data 2:					20	20	20			gr. C/Volt
offset additional data 3:					-12.5	-12.5	-12.5			gr. C
slope additional data 3:					20	20	20			gr. C/Volt
distance in ms between datapoints 1:					1	1	1			ms
distance in ms between datapoints 2:					1	1	1			ms
distance in ms between datapoints 3					1	1	1			ms
count of datapoints to determine the adw-value 1:					1-	1-	1-			
count of datapoints to determine the adw-value 2:					1-	1-	1-			
count of datapoints to determine the adw-value 3:					1-	1-	1-			
TEMPERATURES (parameter set: control)										
required temp sample <= pre-heater minus 2':					no	no	no			
controlled by external temperature?:					no	no	no			
external temperature interval:					60	60	1			min
control difference pre-heater to external temp.:					5	5	0			min
control difference sample to external temp.:					-2	-2	-2			min
DURATION/DOSAGE (parameter set: activation)										
sample pump rotation speed:					60	60	40	60		%
algae pump on for:					2	2	50	2		s
algae pump off for:					1000	1000	10000	1000		s
halogen light on for:					100	100	30	100		s
halogen light off for:					0	0	10	0		s
nutrition pump on for:					3	3	15	4		s
nutrition pump off for:					1000	1000	10000	400		s

	LOW	NORMAL	HIGH	WBB	RIZA Eijsden	RIZA Bimmen	WRK	Hamburg	Karlsruhe	
ACTIVATION (parameter set: activation)										
algae dosage activation:					yes	no	yes			
nutrition dosage activation:					yes	no	yes			
halogen light activation:					yes	no	yes			
fermenter control activation:					yes	no	yes			
pre-heater control activation:					yes	no	yes			
sample control activation1:					yes	yes	yes			
sample control activation2:					yes	yes	yes			
sample pump activation:					yes	yes	yes			
air pump activation:					yes	yes	yes			
cooling water pump activation:					yes	yes	yes			
sample check activation:					yes	no	no			
alarm relay activation:					no	no	no			
hardware alarm relay activation:					no	no	no			
hardware alarm activation in idle mode:					no	no	no			
length of maintenance period:					120	120	120	120		min
detection rate limit:					50	50	50	50		%
TEMPERATURES LIMITS (parameter set: activation)										
fermenter temperature limit:					35	35	30	35		gr. C
halogen light temperature limit:					35	35	27	35		gr. C
sample temperature limit:					50	50	30	50		gr. C
pre-heater temperature limit:					50	50	35	50		gr. C