MONITORING OF SURFACE WATER BY ULTRA-SENSITIVE DAPHNIA TOXIMETER

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Abstract

Static biotests, such as daphnia tests and algae tests, are well known in the checking of water quality. But although they are often called “acute“ tests they are not able to identify short term accidents or time-dependent variations of toxic substances. Biomonitoring systems were set up to serve as “early warning systems“. Biomonitors are 24 hour a day working systems, using organisms to indicate changes due to the release of toxic substances.

This paper deals with the measurement of changes in the behaviour of daphnia with the bbe Daphnia Toximeter. Speed, speed distribution, height, distance between organisms, number of daphnia and “fractal dimension“ are evaluated to account for the appearance of toxic substances. In this way even low concentrations which do not lead to the death of daphnia can be detected within minutes.

Results of the last two years of field experience and laboratory tests are given.

Keywords

Biomonitoring, early warning system, daphnia, toximeter, toxicity test, online test.

Introduction

Since the early 80’s great efforts have been made in the field of early warning systems. Biomonitors using daphnia, algae, mussels or fish are now used throughout Germany and Europe. They are intended to replace manual biotests (toxkits) in order to provide a continuous overview of water quality at permanently installed water control stations.

Biomonitors feature fast detection and continuous water sampling, which limits potential damage to ecosystems. Static tests are generally much too

slow for this. The static daphnia test, for example, prescribes a reaction time of 24 or 48 hours.
A further disadvantage is that it is difficult, and in most cases impossible, to take the sample at the moment of the actual occurrence. The result may be that at the moment of sampling the toxic release has passed the sampling point. Although the biosphere of the water may be severely damaged, a toxkit would fail to indicate this state. A repetition rate of one test every 10 to 30 minutes is required. This means that up to 50,000 tests per year have to be performed. The required rate precludes the use of “toxkits“ because of their enormous costs.
To minimise the costs, biomonitors have to work unmaintained for at least 7 days continuously, with permanent sensitivity. Sensitivity implies a stable working system over the whole week, low detection limits for toxic substances and fast reaction times in case of toxic events. Many systems do not meet all of these requirements in combination.
The bbe Daphnia Toximeter was developed, not only to detect mortality rates, but also to evaluate changes in the behaviour of daphnia. Camera observation and a computer aided evaluation system are used. Fast reactions can be detected over a wide range of toxic concentrations. Stability was tested and the results of sensitivity tests over a 2 year period are presented.

Materials and methods

Operation principles are demonstrated in figure 1. A cross-flow filter takes pre-filtered water (regular 100 µm filter width) out of a current flow. To keep the daphnia at a definite temperature a peltier cooling system is installed between the measuring chamber and filter system. Additionally, an algae solution can be added to keep the daphnia behaviour independent from the food concentration in the water sample. The daphnia chamber itself is illuminated with red light, not visible to daphnia, but excellent for observation with a camera-system. Under normal conditions e.g. operation without toxic events, the test runs continuously, without any maintenance, for about seven days.

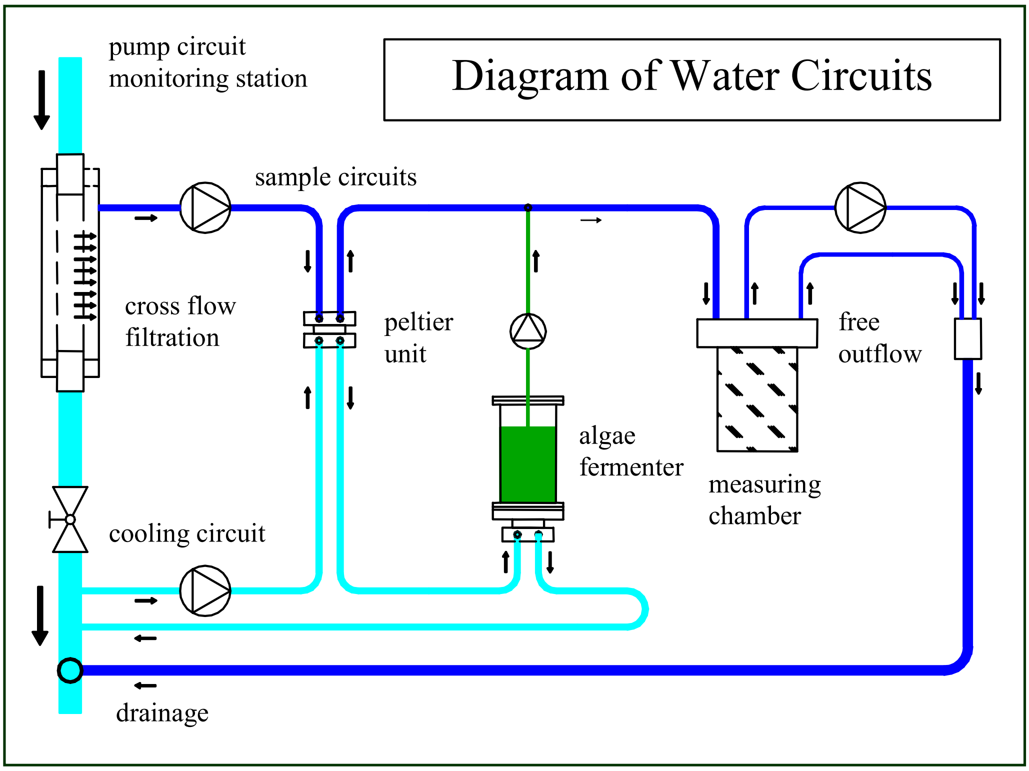


figure 1 Diagram of water circuit

To direct the daphnia, sunlight is simulated by a yellow LED at the top of the chamber. The camera in the back records the scattered light of the daphnia. The peristaltic pump provides the daphnia with 0,5-2 l/h of sample water. Within this range of flow rates the behaviour of swimming daphnia hardly varies.
The second circuit provides the peltier elements with water to ensure the efficiency of the water temperature control system. All of the materials are inert (mostly teflon) except for the tubes of the peistaltic pump.
The daphnia are fed by periodically adding to the algae (Chlorella vulgaris) to the sample stream from a continuously operating fermenter. This ensures that daphnia behaviour does not depend on occasional feeding[1]. On average, a biomass of Chlorella equivalent to 5 µg/l Chlorophyll, or more, should actually be available for the daphnia. The chemostatic working fermenter produces fully grown algae. The fermenter content is 0.5 Litres. About 0.2 l/day nutrient solutions are added.

25 black and white pictures/second are taken by a camera and all evaluations are made online every minute. The digital levels range between 0 for white and 255 for black background points. 756 * 581 points are taken into account by the camera system.

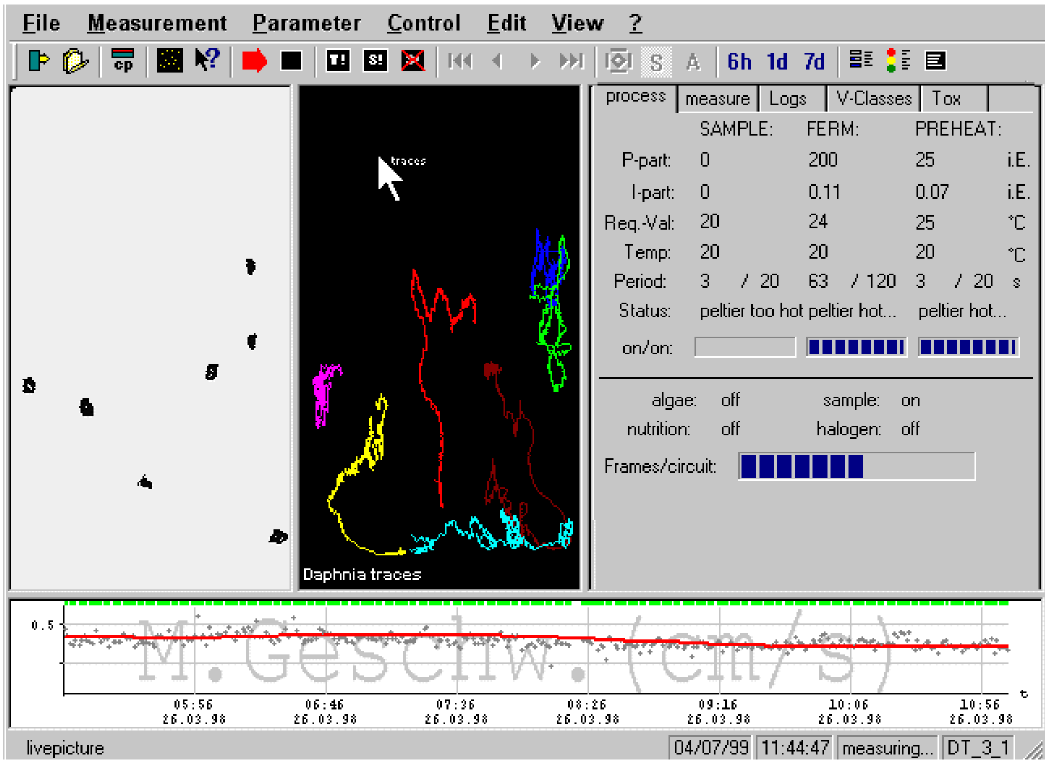


figure 2 Software Graphic Elements: moving daphnia on the left, described traces in the middle, temperature control parameter on the right

At the outset, and periodically thereafter, the background of non-moving objects is determined. Before further evaluation of a single picture begins the grey levels of the background points are subtracted from every point of the picture just taken. This procedure allows clear identification of daphnia. (figure 2, left side). Further, the centre of every single daphnia in every picture is counted, the movement of the centre points is calculated by vector analysis and traces can be determined (figure 2 centred picture).
In addition to the determination of moving daphnia, the determination of the traces allows the calculation of the following parameters:

- medium speed
- speed distribution, velocity class
- height
- distance between daphnia
- number of moving daphnia
- fractional dimension

Average Speed

Through the time information of the scanning intervals and the sum of the distance vector, the sum of the current speed is constantly available. Under observation of the actual chamber size, the speed of the physical size in cm/s is conveyed.

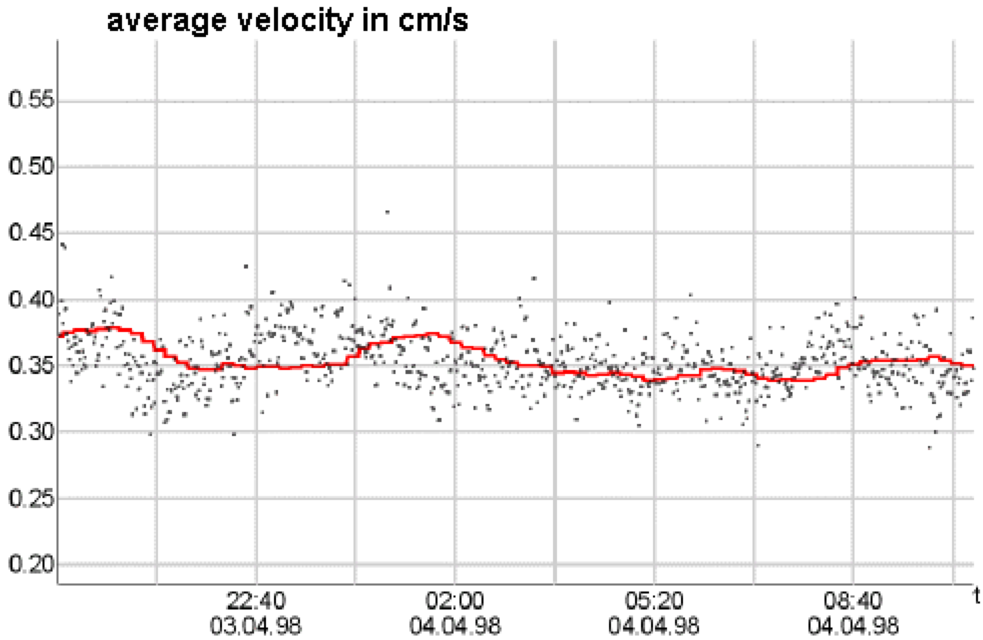


figure 3: Average speed

Figure 3 shows a normal course of the medium speed without any irregularities. The dots are the individual values in a period of one minute. The line consists of values standardised over a period of ten minutes. The possibilities of the dynamic recognition of jumps from this curve are evaluated by the Adaptive Hinkley Detector[2] (ADH).

Speed Classes

In addition to average speed, momentary speeds can be divided into classes. From this, the momentary values in the area (classes) are divided and, after an accumulation time, shown as percentage values.
The momentary values are only taken into account with the shortest track lengths and each value is standardised over this length. In the experiment it was shown that the speeds generally spread out around the normal value of 0.5 cm/s, and values above 0.8 cm/s are reached only rarely. For this reason the following classes are defined.
- slow daphnia: smaller than 0.2 cm/s
- daphnia classes in the region of 0.2 –1.0 cm/s in 0.1- steps and
- very fast daphnia likewise defects larger than 1.0 cm/s

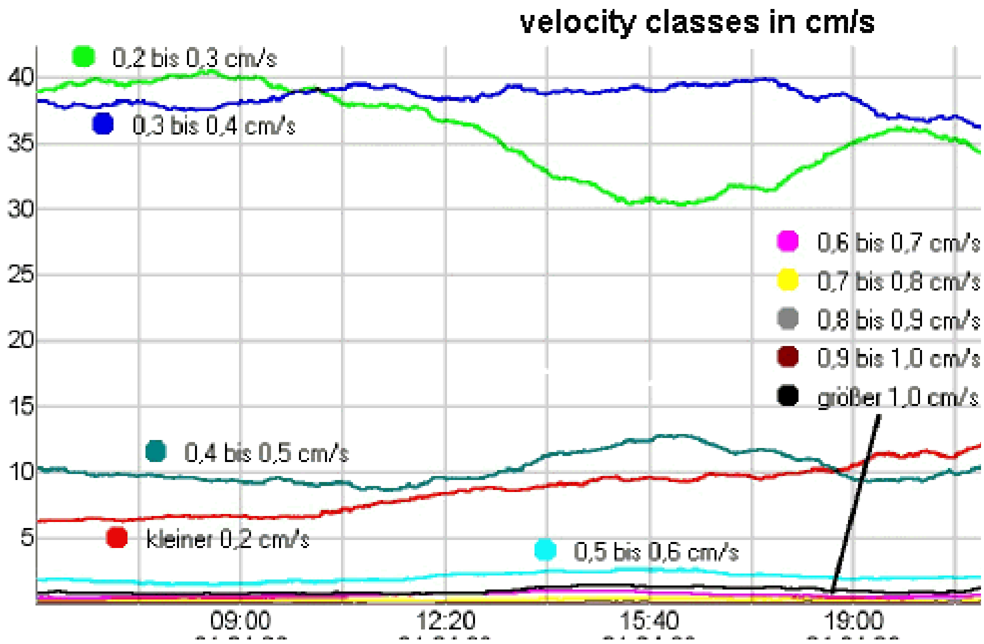


Figure 4: Speed Classes

Figure 4 shows the distribution of speeds during normal behaviour of the daphnia. Through classification groups of slow and fast daphnia, and defects are revealed. In data evaluation after an incident, the speed classes are an important means of ensuring accuracy in the assessment of changes in the behaviour of daphnia.

Speed Class-Index

This index is calculated from the information of the speed classes. In this process, the percentage of daphnia swimming faster than 0.2 cm/s (medium speed) and 0.2 cm/s slower than medium speed, are added together and displayed as a measurement. This, together with the mid-speed, is an important indicator for changes in the behaviour of the daphnia. For this reason dynamic recognition for this parameter is implemented.

Average altitude

The mid-level is calculated from the vertical co-ordination of the place information of the daphnia paths. A height profile of daphnia is shown in diagram 5. The line that has been traced connects the individual values to an average value. Dynamic jump recognition is also implemented here.

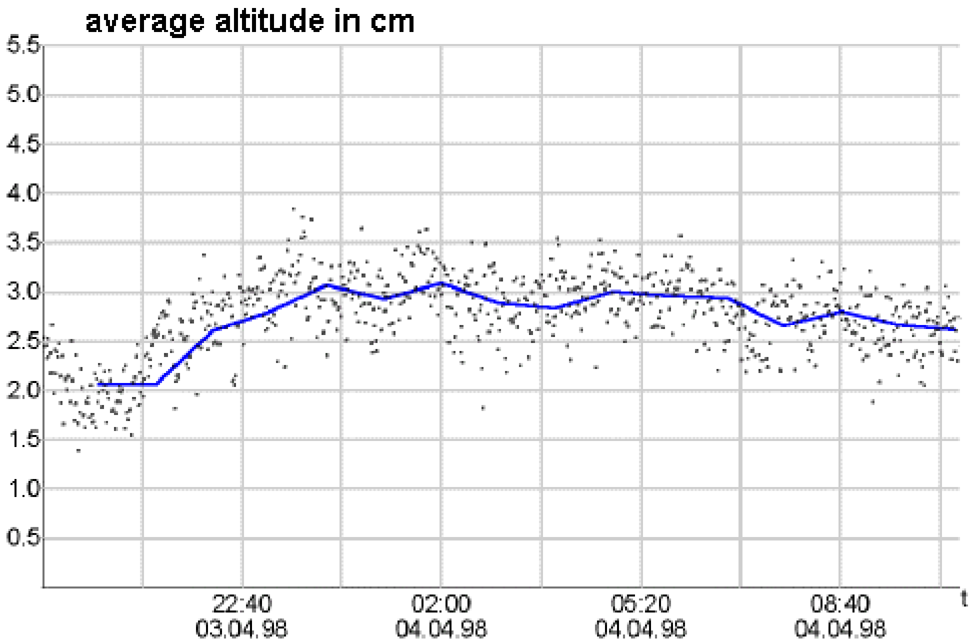


Figure 5: Average altitude

Fractal dimension

For detection of daphnia type behaviour (turning, circling) fractal dimension is determined.
Fractal models in nature describe the geometric forms of objects like coastlines, coral reefs, leaves and the movements of animals. In biology, methods from the research of Fractal have been used for many years in evaluation and classification.

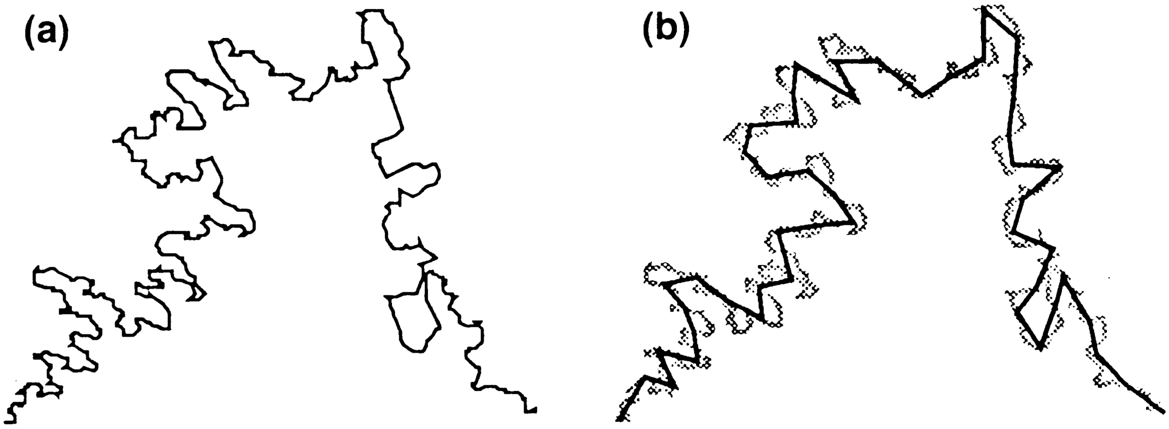


figure 6: Fractal Structures and their Measurement[3]

The most important discovery from fractal theory is that the length of a curve depends on the ruler length with which the curve is measured. This fact is illustrated in figure 6. The curve in diagram (a) has a complex and irregular nature. Diagram (b) shows that if a series of *N(d)* lines with a length of $l_i(\delta)$ are placed on top of one another in a fictitious 'pacing out' of the curve, at equal intervals δ, this occupies its starting and finishing point on the curve. Thus the Euclidean length $L(\delta)$ of all of these lines, with a measure δ is obtained[3,4].

(Formula 1)

$$L(\delta) = \sum_{i=1}^{i=N(\delta)} l_i(\delta)$$

If the number of lines N is increased and the ruler length δ decreased then the curve is more accurately measured, small fluctuations are reproduced more accurately and the length becomes greater. In the case of fractal shapes, the value of the exact length could only be determined by endless small scanning. It has been shown that these lengths in similar structures obey a simple potency law:

(Formula 2)

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

Therefore $L(\delta)$ is the length of the curve with a ruler length of δ, K is a proportionality constant and D is the fractal dimension.

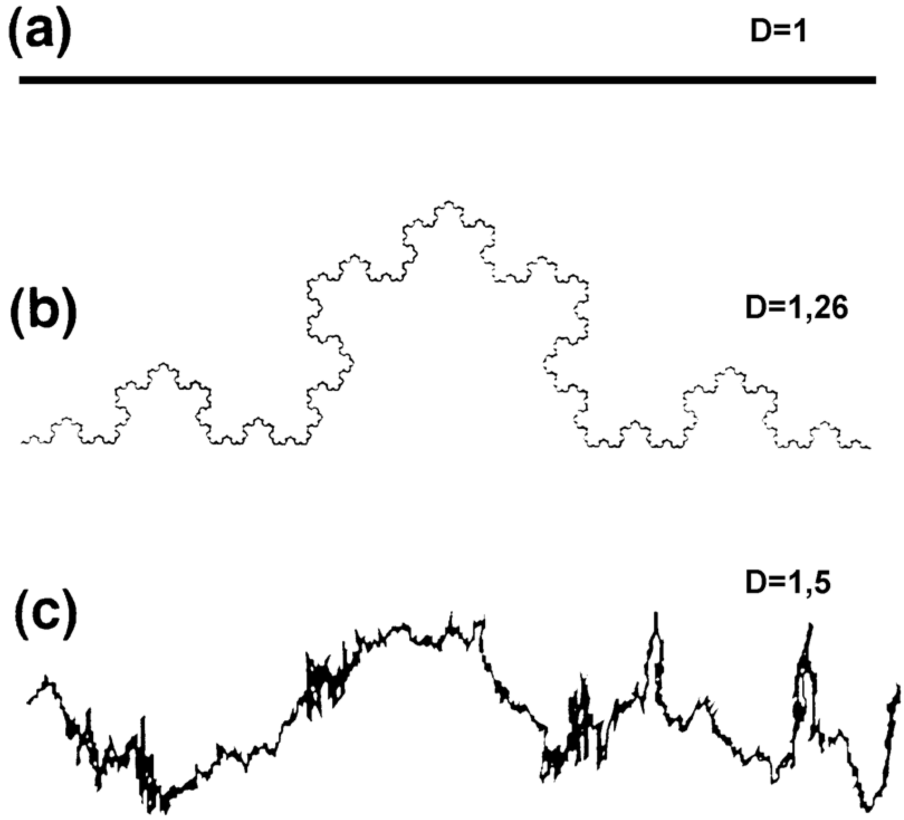
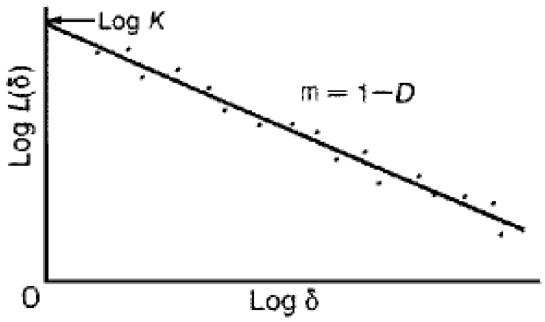


figure 7: Examples of fractal structures

The fractal dimension is thus a measurement for the 'curviness' of a path. In figure 7 three different curve formations are shown for orientation: A straight line (a) has the same length, therefore D=1.
The *Koch* snowflake (b) is a fractal shape and has a dimension of D=1.26, a Brown time series (c) has a dimension of D=1.5. An incidental movement of a part of the plane consequently has a dimension of D=2, since each point is equal, and so at some point the whole plane is affected.
With natural shapes the fractal dimension is not a genuine constant, unlike fractal shapes. However, when natural shapes show a self-similarity the fractal dimension can, in most cases, be determined by regression. These must, however, be individually examined. In particular, it can only lead to self-similar effects in parts of a ruler length.

- Calculation of the fractal dimensions over a linear regression[3].

  Here, the length $L(\delta)$ of the resulting polygons are determined with all complete factors of the entire length (with $\delta = n * \delta_{\min}$ and $n \geq 1$) and logarithmically applied in a diagram $\ln L(\delta)$ versus $\ln \delta$. Subsequently, a linear regression over all points of the curve is calculated.



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

figure8: Fractal dimension through linear regression. The quality of the regression is naturally determined by the similarity of the diagram with a straight line, as is the case in a fractal picture. The further this diagram deviates from a straight line the more difficult it becomes to calculate the gradient. There may also be one or more sections in which the gradient is constant.

- Calculation of the fractal dimension using the box counting method[5].

Using this method a grid of square boxes of side lengths e is placed over the curves whose fractal dimensions are to be determined and those segments $N(e)$ which contain one part of the curve are counted (counting)

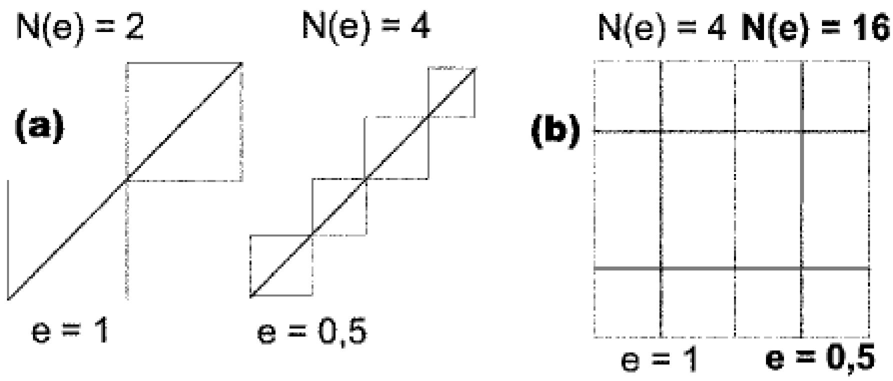


figure9: Box counting - Method

If this method is used to measure a straight line, then on halving e, twice the number of counted boxes results: $N(e)=\frac{k}{e}$, in which k is a constant. Should however, in an extreme case, every square be found, a square dependence arises:

(Formula 3)

$$N(e)=\frac{k}{e^2}.$$

(Formula 4)

Thus other shapes in the plane can be described by

$$N(e) = \frac{k}{e^D}$$

in which D lies in the area of 1<D<2 and which is the known fractal dimension.

In order to indicate a fractal dimension for the daphnia paths, only track lengths of a minimum length are used. Reduction to tracks ensures that direction is not altered to become unnatural through interference, as this would considerably influence calculation. By contrast to the previous parameters, the minimum length here equals 150 points.

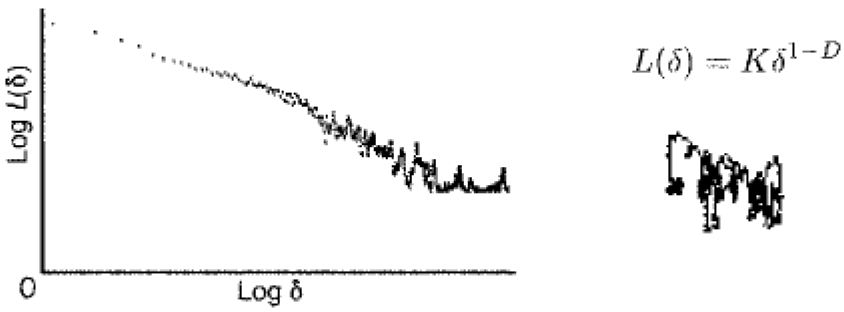


figure10: Fractal dimension: Actual Measuring Point

The above diagram shows a typical path with diagram. Here a regression shows a fractal dimension of D= 1.29. The box counting method, on the other hand, results in a fractal dimension of D=1.40, since it omits the non-linear part at the end of the curve and only uses the linear part for calculation. Both processes are implemented as independent parameters in the bbe Daphnia Toximeter.

<u>Number of animals capable of moving</u>

The number of animals capable of movement is a simple but decisive parameter for calculation. In the conception of the parameter so far the number of animals is not reduced. These are independent because of the average value. The advantage of this procedure is the constancy of, for example, the mid-speed in a change in the number of animals by new recognition or by dying off. Of course, when there are only a few daphnia the speed is somewhat affected by rushing, as the data basis is smaller. However, the data can be compared to that for a different number of animals. The number of animals capable of moving is, on recognition of lethal pollutants, a decisive factor. This corresponds to the criterion of the static daphnia test according to DIN or ISO, which evaluates the swimming performance of the daphnia under the influence of pollutants.

Average Distance

In assessment of group behaviour the average distance between each daphnia is determined.

Recognition rate

The recognition rate parameter exists because many of the parameters do not explicitly display the data basis, but the statistical reliability clearly depends on the number of used points. This parameter conveys the percentage of actual recognised path points compared with those possible in one measuring period.
The recognition rate is always less than 100% since path points are lost merely through crossing over. A value above 80% has been shown to be reliable.

Alarm Evaluation

The automatic alarm detection is one of the most important tasks of a biomonitor. For this purpose evaluation and recognition of a significant change in one or more of the parameters is a necessity. The bbe Daphnia Toximeter offers an integral parameter for this purpose: **the Toxic Index**.

The following events are evaluated and allocated weightings:

– a lower limit V_{min} for average velocity
– an upper limit V_{max} for average velocity
– a deviation in the number of Daphnia entered at the start of the program
– sudden changes in fractal dimension detected by a separate ADH
– sudden changes in fractal box counting detected by a separate ADH
– sudden changes in speed-class index detected by a ADH
– sudden changes in average height detected by a separate ADH
– sudden changes in average distance detected by a separate ADH

The toxic index of a measuring period is gathered from the current event weightings and compared with adjusted alarm limits, the weighting factors depend on the importance of the event.

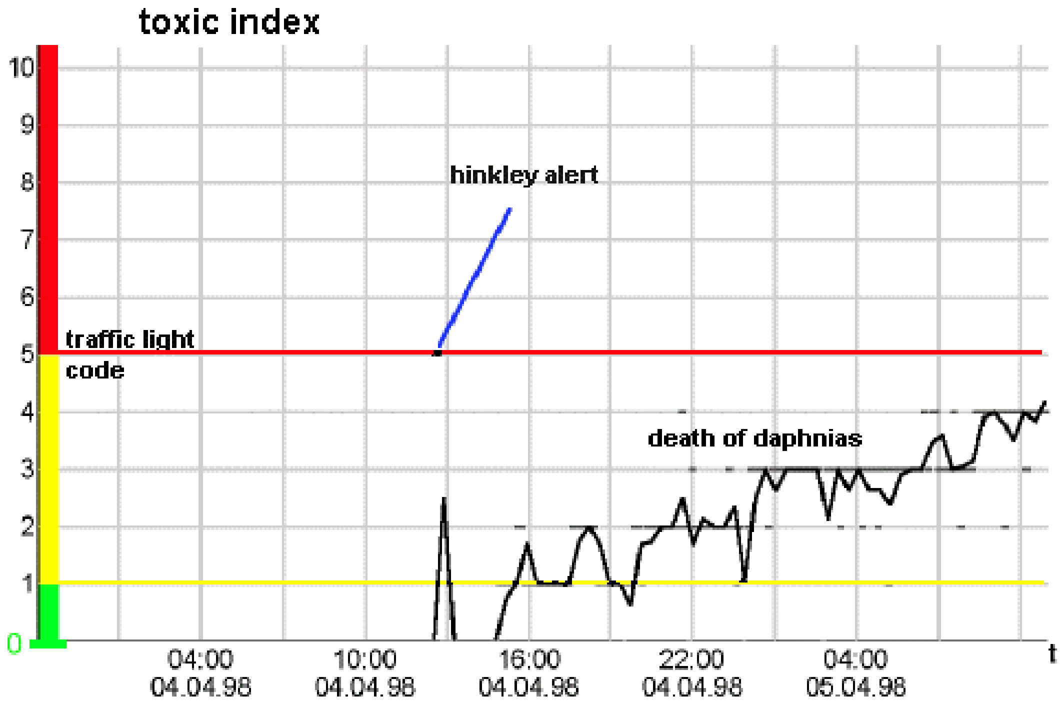


figure11: The Toxic Index and the Colour Coding

For basic evaluation the current alarm condition coded with the traffic light colours GREEN-AMBER-RED is printed at various situations.

- Speed limits

Here the minimum and maximum speed limits are displayed and on reaching these, provided with weighting factors.

- Number of Daphnia

The current number of the animals capable of movement is constantly compared with the amount at the start. A reduction is entered into the sum of the event weightings, along with a weighting factor & difference number.

- The Hinkley Channels

The Hinkley detector is deployed for recognition of sudden changes within the measuring parameter.

- average velocity
- fractal dimension
- fractal dimension box counting
- speed-class index
- average height
- distance

The Hinkley Detector

D.H.Hinkley[6] introduced the classic Hinkley detector. It was originally used to detect "switching" (sudden changes) in conductivity, while measuring cells. In doing this data was recorded by a so-called Patch-clamp experiment, in which "switching" could be shown to have "occurred". The following formulae describe the procedure of jump recognition (Schultze[7], Hansen[8])

(Formula 5)

$$g(t) = g(t-1) + z(t) - \frac{\mu_1 + \mu_0}{2},$$

$$g(t) = \begin{cases} g(t) \; if \; g(t) \geq 0 \\ 0 \; if \; g(t) < 0 \end{cases},$$

and alert, if $g(t) \geq \lambda$

Following each recording of a new measurement $z(t)$ at moment *t* the three formulae are worked out one after the other and subsequently the Hinkley sum $g(t)$ and the result of the (alarm) routine are determined. The following observations are valid for the algorithm:

1. $g(t)$ changes with every reading of a new measurement $z(t)$. In addition the value of $g(t)$ is taken into account at the previous moment and half of the jump height

   $$\text{half of the jump height} = \frac{\mu_1 + \mu_0}{2}$$

   with μ_0 = level before jump and μ_1 = level after jump.
2. $g(t)$ has 0 as its lower limit.
3. Should a significant jump occur, then even the reduction of half of the jump $(\mu_0 + \mu_1)/2$ can not prevent the increase of $g(t)$. This means that following the course of a certain series of measurements $g(t)$ the adjustable threshold value λ is reached. As a result, the alarm limit is exceeded and a Hinkley alarm is sounded.

The regular Hinkley detector requires that, e.g. because of optical analysis of the entire data, μ_0, μ_1, λ are known.

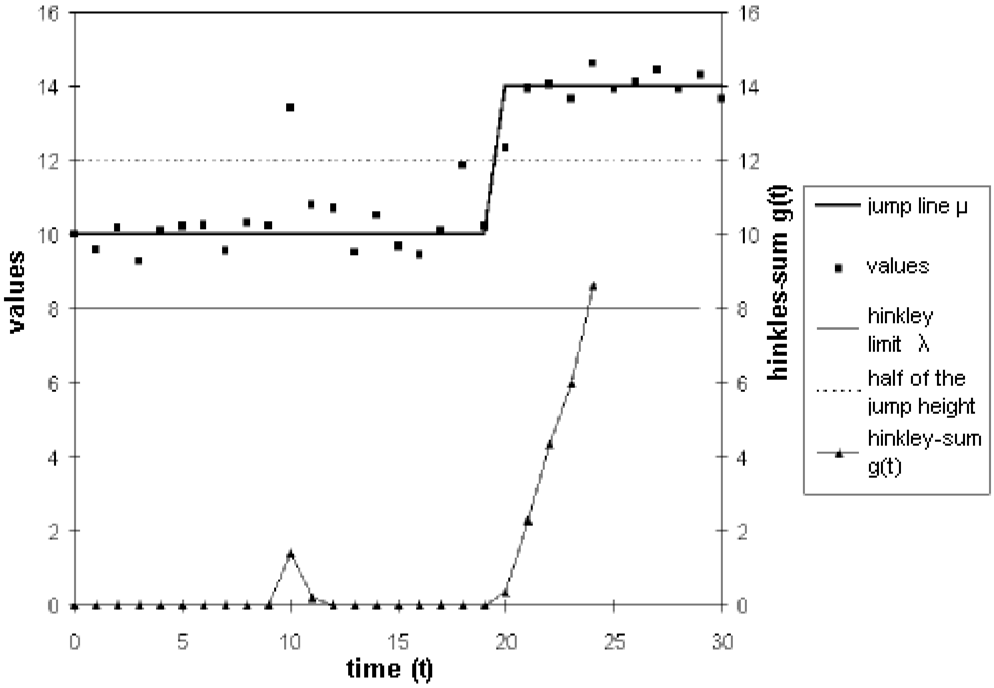


figure12: Recognising a jump using the Hinkley Detector

With the aid of figure12 the principle behind the Hinkley detector can be demonstrated by considering a real jump. The Hinkley sum $g(t)$ is, within the first 19 measuring points, greater than zero only once, namely at point "10" (t). At this point the measurement is larger than half of the jump height $(\mu_0 + \mu_1)/2 = 12$ and according to Formula 5, in which $g(t-1)$ at this moment is zero, therefore $g(t)$ is greater than zero. From point "20" (t) each measuring point is greater than half of the height of the jump. Each measurement then contributes to the Hinkley-sum $g(t)$, which is around half the height of a jump smaller than its actual sum. The measuring point at moment "23" lies slightly over the half jump limit. Its contribution to $g(t)$ is small. If this measuring point is smaller with regard to its sum than half the jump limit then the Hinkley-sum can (in the meantime) also become smaller. If the Hinkley-sum reaches the Hinkley-limit λ (here at point "24") then the jump is identified.

The form of the Hinkley detector described here is mostly of use in dynamic systems because in, for example, a drifting curve, neither the starting level μ_0 (then constant) nor the jump level μ_1, or the jump $(\mu_1 - \mu_0)$ is known. A solution to the problem of starting levels had been discovered by the introduction of the drift elimination. If the calculated drift of the measurement curve is subtracted, a curve results which staggers around the zero point. According to this $\mu_0 = 0$. It is understood from this that the absolute size of the measurement curve for the certain recognition of a jump $(\mu_1 - \mu_0)$ is not relevant in this curve.

The level of jump μ_1 must however be calculated. It should orientate itself to the noise σ of the previous value. Thus it shall be valid that $\mu_1 = f(\sigma, k, \text{jump height})$, with σ as standard deviation over the final measuring points yet to be calculated, with the proportionality constants k=20, and the co-efficient jump height whose optimum is yet to be ascertained.

(Formula 6)

$$\mu_1 = k * \sigma * \text{jump height}$$

μ_1 should prevent under increasing noise that the Hinkley-sum increases in proportion to the measurements. This is achieved by deducting $\mu_1 / 2$ from each procedure for summation (formula 5). The alarm limit under increasing σ is not reached, providing merely an increase in noise, but no significant jump, has occurred. The alarm limit $\lambda = f(\text{Hinkley factor}, k_\lambda)$ itself is a function of the proportionality constant $k_\lambda = 200$ and of the Hinkley-factor.

(Formula 7)

$$\lambda = k_\lambda * \text{Hinkley faktor}$$

By drawing up the functions μ_1 and λ it is guaranteed that the Hinkley detector works equally well for any type of curve. This means, in particular, that an increase in noise through changes in the system parameter, or merely through an increase of the average measurement under simultaneous constancy of the signal-noise relationship (relationship of the average impulse measurement to standard deviation) does not affect the quality of alarm detection.

The further development of the Hinkley detector (Formula 6) leads to an adaptive on the system working on noise relationships, which is called the *adaptive Hinkley detector* . Jump height and Hinkley-factor are adjustable values. The factors k and k_λ are fixed scale measurements (k = 20 and k_λ = 200).

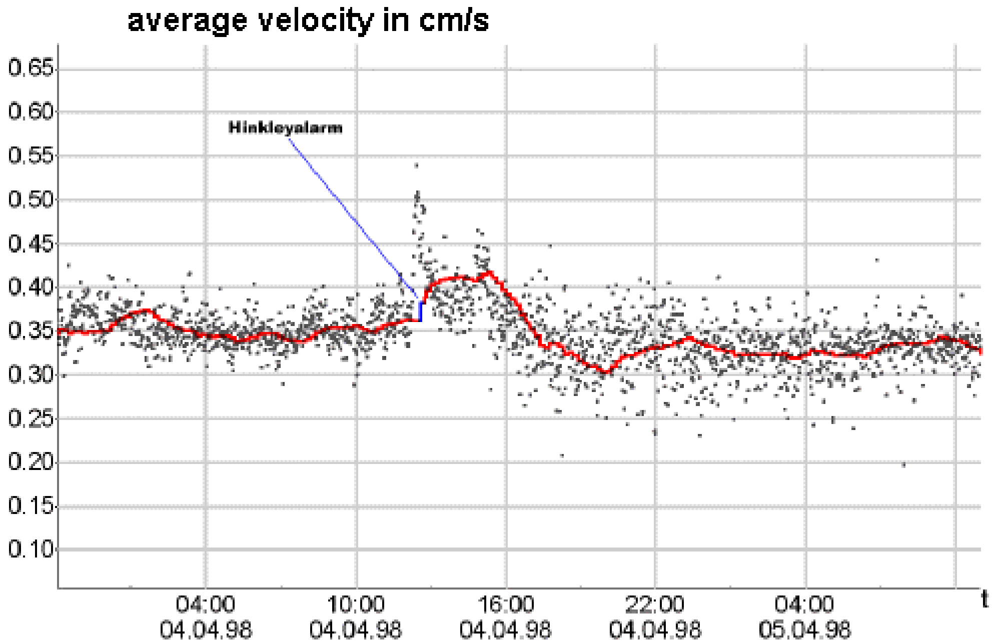


figure13: Hinkley Alarm at the Average Velocity

This algorithm is used in the bbe Daphnia Toximeter. The measurements are first of all standardised over ten minutes and subsequently evaluated. The evaluation contains a linear fit over, generally, 27 measuring points (270 minutes) After a drift elimination the adaptive Hinkley detector is employed and on reaching a threshold value, an alarm is sounded whereby the weighting factor of the corresponding Hinkley channel is activated. The adjustment of the values for the jump heights and the Hinkley factor is thus orientated to the optimal values already displayed, with a jump height of 1.1 and Hinkley factor of 0.6 [2,8].
The parameter “medium speed“ averages out the movement of all daphnia, while the “speed distribution“ classifies all movements in groups. The first group contains all movements of less than 0.1 cm/s, the next group indicates all movements between 0.1 and 0.2 cm/s and so on, until the group showing those of more than 1 cm/s. Changes in speed distribution in relation to the medium speed toward extreme classes are remarkable. The parameter “velocity class“ is used to add the percentage of extreme speed classes, such as slower then 0.2 cm/s and faster then 0.8 cm/s.
The parameters “height“ and “distance“ describe the medium height of all daphnia and the added distance between all daphnia. This makes it possible to take into account that in case of toxic influence, a different behaviour in the form of half the daphnia swimming to the top and half the Daphnia sinking to the bottom, can be recognised.

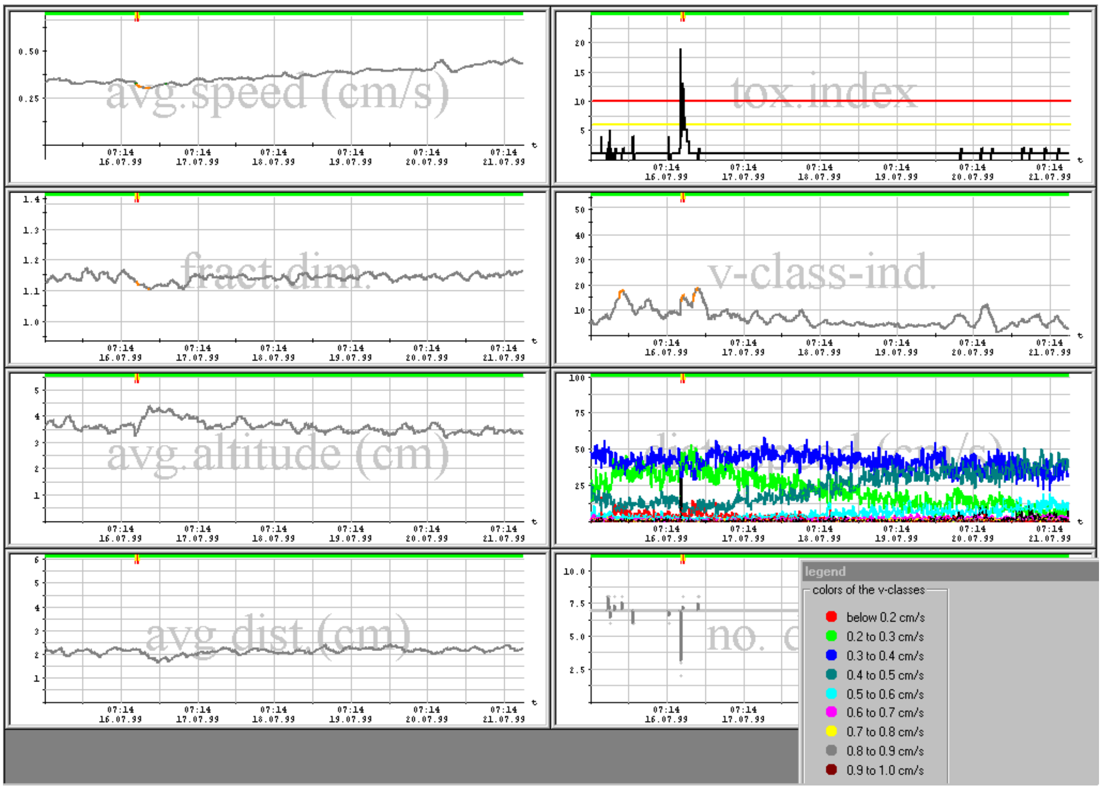


figure 14 shows data without any effect except knocking on the chamber lid with consequent alarm to that moment. On the left side *average speed, frac. dimension, height* and *distance* are displayed. On the right side *toxic index, speed class index, speed distribution and number of active daphnia* are displayed inclusively. The legend for explanation of the speed classes is also displayed.

It is obvious (figure 14) that data are drifting. Improved mathematical calculation is necessary. In the present case the best examples are the medium speed and the speed class distribution. Daphnia are growing and changing their speed, therefore after some days, the speed classes 0.4 to 0.5 cm/s is preferred, as the number of daphnia swimming in the speed class 0.2 to 0.3 cm/s is reduced.

Results and Discussion

Toxic events don't usually last for more than a few hours. Consequently tests have to be done with a limited incubation time. Data shown here (figure 15) derived from a two-hour treatment with the chemical compound Trichlorfon. The insecticide Trichlorfon is used in fish farms against parasites.

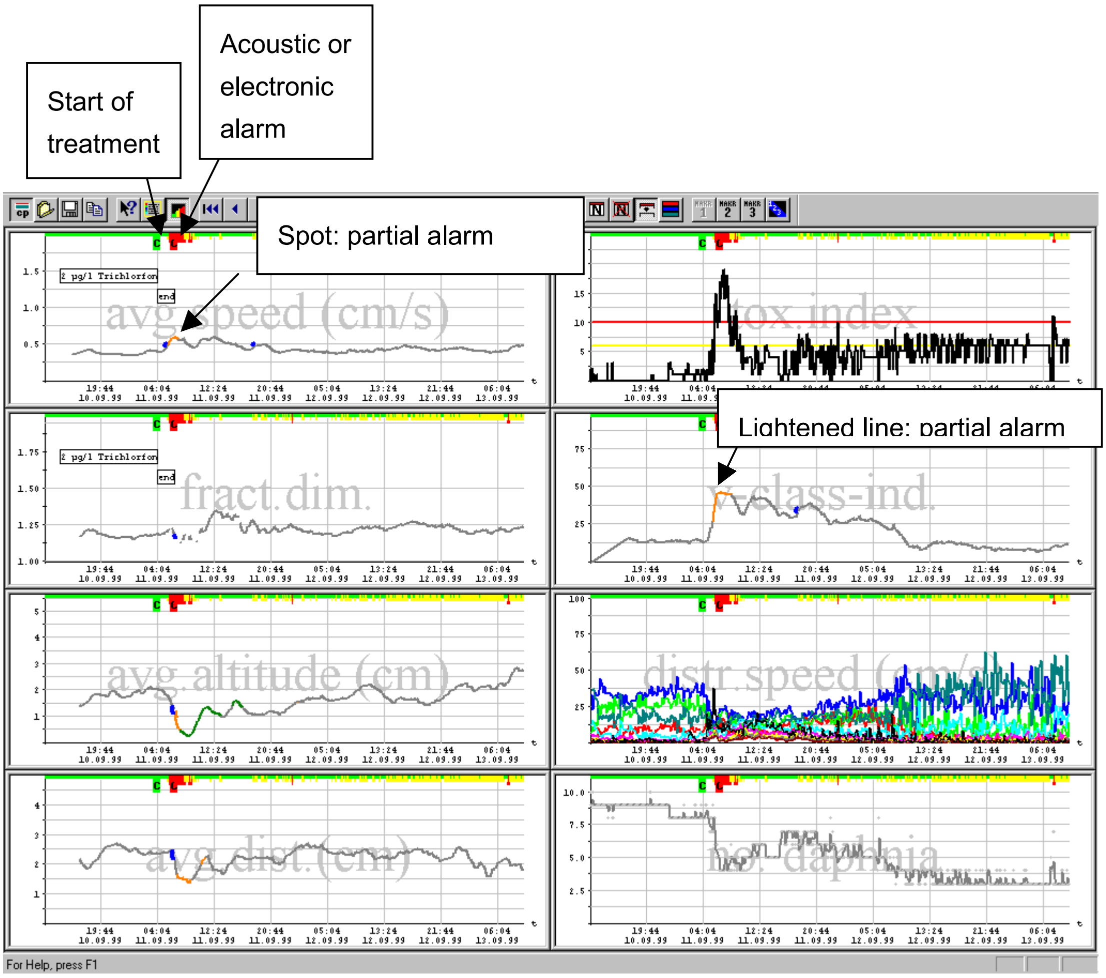


figure 15 Effect of 2 µg/l Trichlorfon

The dose used causes severe effects on daphnia behaviour. Very soon after treatment (indicated by a printed 'C' on the screen) daphnia swim faster (average speed), they are directed to the bottom (average altitude), they swim closer together (average distance), and the speed classes change to extreme classes (distribution speed, v-class-index). Some of the daphnia show no movements caused by the treatment. The amount of moving daphnia dropped from 9 to 4, but within the next 10 hours 3 daphnia recovered. After 48 hours three daphnia had survived. This results of a 48 hour test are easily obtained by a 30 min observation time course.

The Trichlorfon test has demonstrated the relationship with effects obtained from an 'acute' daphnia test (ISO). To make the daphnia test ultra-sensitive, however, the test-system has to prove that the user will get an alarm, even if the acute test does not show evidence for toxic substances. Figure 16 shows the effect of the herbicide Pendimethalin. The concentration used should not actually influence the daphnia. The ‘acute’ daphnia test shows no effect after 48 hours if daphnia are treated for 2 hours with 100 µg/l Pendimethalin. However, the effect on the daphnia´s behaviour is obviously demonstrated here. The parameters ‘average speed’, ‘fractional dimension’ and the evaluation of the speed distribution (‘v-class-index’) show significant deviation from the normal behaviour pattern. In contrast, average distance and height do not show clear changes and do not contribute to the ‘toxic index’.

figure 16 test of 100µg/l of the insecticide Pendimethalin. 8 of 11 daphnia survived the test after 48 h. The living daphnia return to behaviour similar to that shown before the test.

The examples show that of the bbe Daphnia Toximeter is considerably more sensitive than the so called ‘acute’ 48 h test. With the help of the described bbe Daphnia Toximeter, reliable control of water can be performed and fast response to toxic events is possible. Low maintenance cost (about 4 h per week) makes the bbe Daphnia Toximeter a favourable instrument.
If water is basically diluted it makes sense to use the dilution function of the instrument. The sensitivity is reduced but significant events of pollution can be detected very quickly. Extended algorithms can even help to estimate the distance between the place of event and the monitoring station[9].

Acknowledgement: We thank Dr. M. Marten from *State Agency for Environmental Protection of Baden-Würtemberg* for helpful support

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