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REAL-TIME BIOMONITORING OF WATER CONTAMINATION BY CYANIDE BASED ON ANALYSIS OF THE CONTINUOUS ELECTRIC SIGNAL EMITTED BY A TROPICAL FISH: *APTERONOTUS ALBIFRONS*

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Abstract—A new early warning system for monitoring the quality of water was developed using the information conveyed by the continuous electric organ discharges of the tropical fish *Apteronotus albifrons* (Gymnotiformes, family Apteronotidae). The principle is based on the time characterisation of the electric signal emitted by the fish and uses the fact that the frequency and the form of the signal vary as a function of the physico-chemical quality of the ambient water. Eight test fish were individually confined in a heat-proof test chamber in which a continuous water current, thermo-regulated at 27°C, was maintained. The electric signal sampled over 1 s periods were amplified. The computer processed the signals and their frequencies and determined the coordinates of the points where the temperatures were recorded. The results of the *A. albifrons* electrical activity were then visualised on a visual display unit. Subsequent mathematical processing helped to detect unusual electrical behaviour (crossing of lower and upper bounds). To illustrate the principle of this new early warning system, the electric response of the *A. albifrons* exposed to cyanide was tested and compared with the detection thresholds of other biological early warning systems. *A. albifrons* is able to detect a cyanide concentration of 34.6 $\mu g\,l^{-1}$ in less than half an hour. The recorded results suggest that this new biomonitor corresponds very well with the requirements of warning stations for surface waters subject to cyanide pollution. Copyright © 1996 Elsevier Science Ltd

Key words—water quality, water pollution, automated aquatic biomonitoring, early warning system, drinking water, toxicity, fish, electric fish, *Apteronotus*, hazard assessment

INTRODUCTION

It is now recognised that to assure good protection against the increasing risks of surface water pollution, there is a need to combine the survey of physico-chemical parameters with biological responses (Morgan *et al.*, 1981; Wallwork and Ellison, 1983; Botterweg *et al.*, 1989). If the exploitation of physico-chemical data in pollution monitoring programmes has become common practice, exploiting biological data is much more recent. Acute bioassay is perhaps the most classic biological approach to monitoring. This approach attempts to determine concentrations of a toxicant causing the death of 50% of the organisms within a predetermined period, usually 24, 48 or 96 h. Among the numerous shortcomings of this approach, the most important seems to be its difficult translation into a practical continuous on-line automated monitoring system. In addition, there is the need to use premortality symptoms as a means of recording rapid response to toxic stress. Research efforts have thus been turned to developing faster, more sensitive and more reliable biological warning systems.

Recent reviews of a large number of biological monitoring systems (van der Schalie, 1986; Kramer and Botterweg, 1991; Thomas, 1996) have identified a number of systems presenting promising perspectives. Among such systems are those based on the electric organ discharge (EOD) of the tropical fish, *Gnathonemus petersi* (Campbell *et al.*, 1990; Geller, 1984; Lewis *et al.*, 1990). Species of *Gnathonemus* (family Mormyridae) are freshwater Teleost. They generate electric pulse discharges. The monitor uses the EOD frequency fluctuations as an indication of the presence of pollutants in the surrounding water. However, the fact that *Gnathonemus* EOD frequency time distribution is irregular by nature, even in the absence of toxic substances, seriously weakens the attractiveness of the underlying principle. Unfortunately, the reason for this irregularity is not as yet fully known (Kemmer *et al.*, 1972; Mandriota *et al.*, 1965).

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For this reason, a new biological monitoring system has been developed in our laboratory with the goal of providing rapid and automated information about changes in the quality of ambient water. The system measures changes in the electrical behaviour of tropical knifefish, *Apteronotus albifrons* (family Apteronotidae) in order to provide early warning of water quality deterioration. This species has never been used in toxicological studies, even though it presents two major advantages: first it possesses electric organs which generate weak electric currents; and secondly the electric information it emits, in addition to being continuous, shows remarkable temporal stability.

The main objective of this study was to describe the new biological monitoring techniques. To accomplish this goal, detailed information about biological sensor and technical procedures are given. A second objective was to determine the sensitivity of this warning system to cyanide pollution.

MATERIALS AND METHODS

Biological sensors

The knifefish, *Apteronotus albifrons*, is a tropical electric Teleost found in the fresh waters of South America. It was identified from the key of Le Bail *et al.* (1984) and belongs to the Gymnotiformes order and to the Apteronotidae family. It is a solitary nocturnal predator. The *A. albifrons* is a popular domestic aquarium fish as it is easy to keep and raise. Two principal sources of supply exist: (i) from local importers exploiting fishing facilities and fishermen in Southern America and (ii) from Indonesia, a country whose fish farmers have mastered the artificial reproduction of this species. In its natural environment the *A. albifrons* feeds on insect larvae and on small fish, while in captivity it is either fed on frozen chironomidae larvae or worms, depending on its size.

The *A. albifrons* possesses a tubular electric organ of considerable length (Bennett, 1971). It continually emits weak amplitude electric discharges, a few volts in direct contact with the fish and a few millivolts on electrode sensors at a frequency close to 1000 Hz. This represents an extraordinarily high discharge rhythm for a biological oscillator and is common for neural electrogenerating organs (Florion, 1984). It emits two-phase EODs, starting with a positive phase alternating with a negative phase covering a large harmonic spectrum (Fig. 1). The *A. albifrons* two-phase EODs play neither offensive nor defensive roles. Just as with the *G. petersi*, they serve as a means of communication and perception of surroundings (Westby, 1984). As long as the external stimuli remain constant, the very high discharge frequency remains constant. Erskine *et al.* (1966) and Bullock (1969, 1970) estimated it at 0.01 to 0.1% under constant ambient water temperature conditions. In addition, the *Apteronotus* electric signal is not affected by a wide range of non-electric stimuli, e.g. touching or prodding with a stick, jarring of the tank, agitating the water, feeding, switching lights on and off or buzzing (Bullock, 1969). Temperature is the only parameter known to influence the frequency of the continuous discharge (Coates *et al.*, 1954). An ambient temperature increase (or decrease) of 1°C leads to an EOD frequency increase (or decrease) of around 40 Hz (Enger and Szabo, 1968). As a precautionary measure, the experimental unit was installed in an isolated room designed to reduce noise and visual stress on the test fish. We did not observe nycthemeral rhythm in the *A. albifrons* discharge frequency, as earlier experiments made by Larimer and McDonald (1968) did.

This species was selected due to its adaptability to laboratory conditions as well as for its electrical characteristics. The same group of juvenile fish from the Brazilian region of Manaus was used for the tests. They were obtained from local fish importers. The average weight

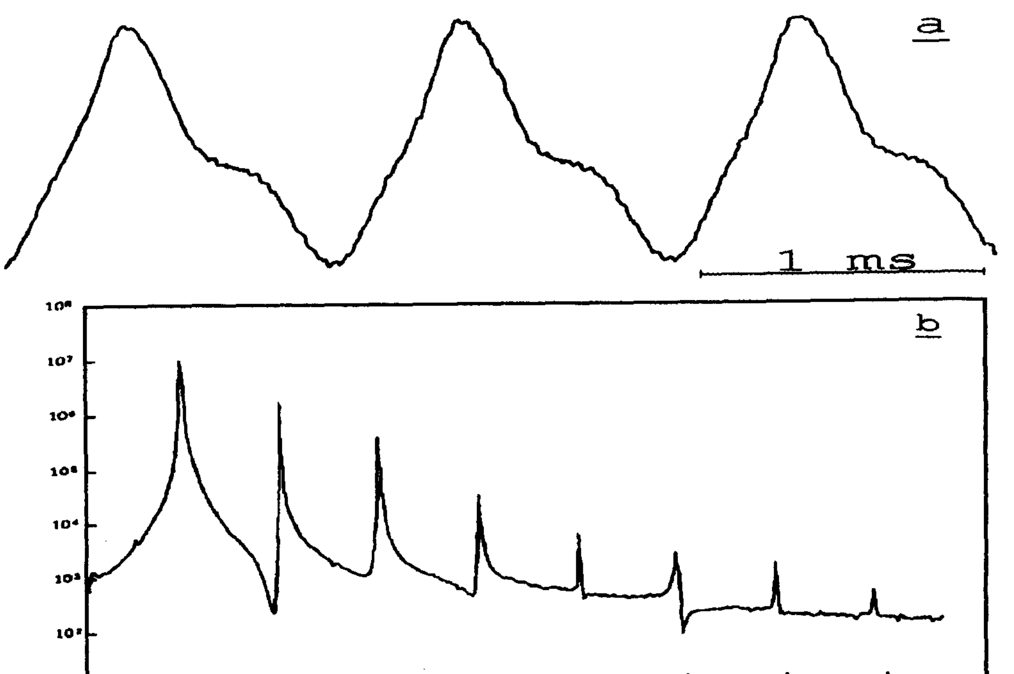


Fig. 1. *Apteronotus albifrons* EOD waveform after numerical analysis (a) and power spectra after fast Fourier transform (FFT) (b). *Y*-axis: amplitude (logarithmic scale); abscissa: EOD frequency (kHz). The fundamental and second to eighth harmonics are shown.

of the group used in the experiments was 29.4 g (9.7–57.2 g). Each fish was kept in the laboratory for at least a 4 week period of acclimatization prior to the experiments. Each fish was individually placed in a 60 l recirculating tank supplied with a continuous current of filtered water maintained at 27°C. Throughout the acclimatization, control and exposure periods, the fish were fed once a day with Tubifex worms. During the tests, no distinction was made regarding sex.

Monitoring apparatus

The hydraulic system was specifically designed to meet the thermal constraints imposed by the biology of the species and the temperature dependencies of its electric response. The system was also capable of functioning under the difficult conditions of an open circuit requiring flow control and renewal rates. In order to determine the sensitivity profile of the *A. albifrons* to different pollutants, the proposed biomonitor was first tested in a closed circuit. The aim was to allow better control of factors such as water temperature which, as is known, can modify the electric signal. Future research will focus on the problem of coping with on-site operational conditions when the technique is transferred *in situ*.

The experimental set-up comprised eight cylindro-conical test chambers. Each chamber had a 40 l cylindrical portion coupled to a 5 l conical portion. The eight replicate chambers were made heat-proof by a transparent double-casing. They were placed in a circle around a mixing tank with the same characteristics. A test substance injection device was mounted at the base of the mixing tank. Blending of the water and the toxic substance was ensured by rotating blades moving at a controllable speed. The surface mixture was fed into a 5 l cylindro-conical heating tank. The incoming liquid was sent to the base of the cylinder by a low heat capacity deflector situated at its centre. Six stainless steel immersion heaters were placed at maximum flow points to reduce the phenomenon of heat inertia.

A Pt-100 platinum probe leading from the heating tank communicated the water temperature to a thermoregulator. The latter sent a signal varying between 0 and 20 mA to a gradation device (a thyristor power unit) which converted it into proportional variation of the resistance power. Temperature-regulated water was then sent synchronously to the eight replicate chambers. The conical portion of each chamber was equipped with perforated plates to ensure uniform distribution of the liquid into the entire cylinder portion of the chamber. The latter portion contained a knifefish confined inside a well perforated PVC tube serving as a day-time shelter. The fish were therefore continually subjected to an ascending water current. The liquid was recycled back into the mixing tack by a system of surface collection. It took about 3 min to recycle the water through the hydraulic circuit. The proposed hydraulic system was designed for open circuit water quality biomonitoring, as well as for closed circuit toxicological studies.

Two stainless steel electrodes planted vertically in the median plane of each test chamber tapped the *A. albifrons* EODs. The quality of the information collected was superior when the test fish were located in the plane of the two electrodes. This position was chosen by preference through correct orientations of the test fish confining shelter. A third electrode was planted on the surface of the liquid in the centre of each test chamber and connected to the ground and to the zeros of the symmetrical power sources (+12 V, 0 V, −12 V; +5 V, 0 V, −5 V). With this arrangement, the periodic voltage variations tapped by the first two electrodes were kept small (of the order of millivolt). These variations were therefore amplified to raise their values to 2.5 V without waveform modification. The amplification was made in two stages. The first stage involved signal preamplification using the linear integrated circuit, TLC 272, the first level of which was a differential amplifier. The use of metal-coated resistances with exactly the same accuracy helped to achieve a fine symmetry between the output feedback to the inverter and non-inverter inputs. The resulting common mode rejection ratio was more than satisfactory to make the use of an RC filter redundant (the filter was initially meant to suppress the 50 Hz main-line parasitic noise which always accompanies the electric signal of the fish). As a result, any possibility of the RC circuit adversely affecting the characteristics of the signal spectrum was avoided. The second stage involved proper amplification, obtained with the second level of the TLC 272 circuit and two levels of the linear integrated circuit LM 1458. The signals were amplified by 1000-fold or more. A third integrated circuit, UA 741, finally carried out offset adjustment for the computer input.

Each of the eight replicate chambers was connected to separate preamplification and amplification units. This set-up appears expensive because of the electronics involved. However, it plays the crucial role of supplying the dispatcher in charge of sequential monitoring of the test chambers with a sufficiently powerful signal which protects against any kind of switching noise.

A computer system cyclically receives the resulting electrical data from a switching centre under its control for spectrum analysis. Simultaneously a monostable multivibrator transforms a copy of the electrical data for each knifefish into a positive rectangular impulse using a CMOS 4001 (with a quadruple NOR gate). This transformation facilitates counting of the exact number of zero crossing per unit time, from which the EOD frequency can be deduced. Fig. 2 gives a simplified scheme of this electronic circuit for recording and processing the *A. albifrons* EOD.

The water temperature of each test chamber was recorded at the same time as the *A. albifrons* EOD. This was done using an astable multivibrator, the frequency of which varies as a function of the product RC, where R denotes the resistance of the Pt-100 heat probe and C a fixed capacitance. The temperature measurement was thus transformed into a problem of frequency measurement and could therefore be treated like the EOD data.

The electric signal and temperature data thus assembled were sent to a computer station which carried out frequency and temperature counts and digitized the form of the EOD signal. The latter was carried out using an analog/digital converter, at a sampling frequency of 400 kHz and with 8-bit resolution. Figure 3 provides an overall flowchart for the computational steps.

Test procedures

General methods. All the tests were conducted at 27 ± 0.05°C under a 12 h photoperiod. Fish sensors were established individually in the monitoring chambers supplied with recirculating dechlorinated tap water from the municipal water supply system. Great care was taken to maintain the same physico-chemical characteristics for the dilution water in all tests (Table 1). The fish were allowed a 48-h period of acclimatization (Fig. 2) before data collection began. The indication for sufficient acclimatization for a given fish was provided by its remaining motionless all day long inside its shelter. The 48-h acclimatization period was followed by a phase of standardization. The electric signals of each fish were also sequentially recorded in terms of frequency and waveform. Each fish was used as its own standard to allow for individual variations. The phase of standardization termed "individual control period" (ICP) lasted at least 2 h. During the ICP, it should be noted that the two electrical characteristics (i.e. EOD frequency and waveform) remained very stable with respect to time.

Exposure protocol. The toxicant used in this study is potassium cyanide (KCN). This toxicant is considered as a major pollution risk in the River Moselle (France).

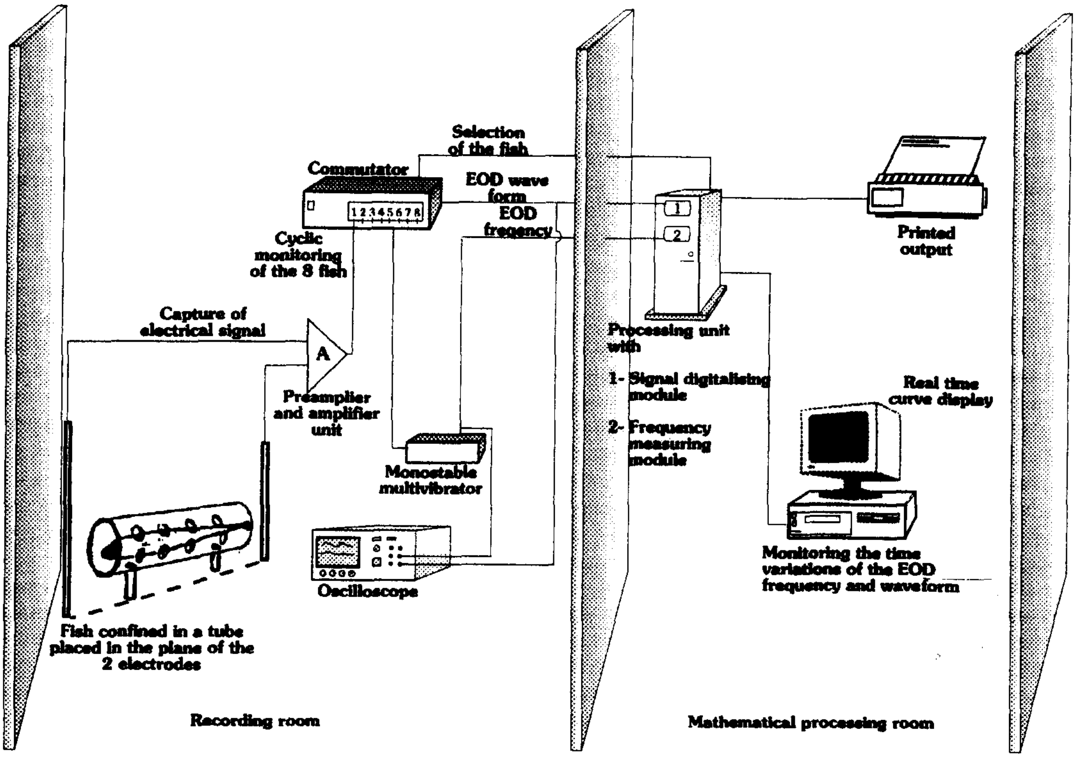


Fig. 2. Electronic circuit for recording and processing the electric organ discharge (frequency and waveform) of the eight electric knifefish, *A. albifrons*. To prevent any visual and noise stress, the recording data and the mathematical processing were realized in separate rooms.

The exposure solutions were prepared by dissolving KCN in tap water and were then directly introduced into the mixing tank from its base. This method best corresponds to accidental water pollution and the fish are thus exposed to a sudden change in water quality for a 2-h period. Potassium cyanide is readily soluble in water and requires no carriers. Eight fish were exposed to each concentration of the investigated pollutant. No fish was used more than once after being exposed to a pollutant. An intoxication period of 2 h maximum was chosen for these experiments to simulate the desired response time of the system, when used as a biological early warning system.

Cyanide analysis. At selected time intervals (15 min), three 50 ml water samples from three monitoring chambers, taken at random, were siphoned and immediately stabilized with NaOH up to a pH of 11. This step was necessary for subsequent analysis with the help of a specific probe using the ionometric method described by Rodier (1984). The results are presented in Table 2. The mean of each measured concentration varied by not more than $\pm 4\ \mu g\ l^{-1}$ from the scheduled nominal value.

Data processing and statistical analysis

The data collected were then displayed in real time on a computer screen and subjected to further mathematical treatment to detect abrupt slope changes in the evolution of the two parameters.

Exploiting the EOD frequency. To be able to exploit the rhythm information conveyed by the EOD signals, moving average frequencies were computed over 1-min period intervals for N consecutive periods. This operation corresponded to data smoothing. The cumulative slopes V_i were then computed with these values using the formula ($N = 15$):

$$V_i = \sum_{i=1}^{N} \frac{F_N - F_{N-i}}{T_N - T_{N-i}},$$

where F_N and F_{N-i} are the respective moving average frequencies computed as above at the dates T_N and T_{N-i}.

These transformations reduce the influence of small variations while accentuating that of larger variations. We next computed dynamic lower and upper bounds of the variations, denoted by LI and LS, respectively, according to the formulae:

$$\mathrm{LS} = \sum_{i=1}^{i=N'} \frac{V_i}{N'} + 2\sqrt{\frac{\sum_{i=1}^{i=N'}\left(X_i - \sum_{i=1}^{i=N'} \frac{V_i}{N'}\right)^2}{N'}}$$

$$\mathrm{LI} = \sum_{i=1}^{i=N'} \frac{V_i}{N'} - 2\sqrt{\frac{\sum_{i=1}^{i=N'}\left(X_i - \sum_{i=1}^{i=N'} \frac{V_i}{N'}\right)^2}{N'}},$$

where $N' = 120$.

The parameters LI and LS with the new value of V_i specifying the limits of dynamic warning were recomputed every minute. A point outside the interval [LI, LS] was consequently interpreted as showing unusual electrical behaviour.

Exploiting the EOD waveform. Fast Fourier transform mathematical treatment of the digitized data gave a spectral analysis from which the respective amplitude ratios of the second, third, fourth and fifth harmonics to that of the fundamental harmonic (H2, H3, H4 and H5, respectively) could be computed. Under normal conditions (during ICP), the four ratios belonged to the interval [LI′, LS′], where LI′ and LS′ designate the static lower and upper bounds, respectively. For this value, we calculated the mean of each ratio for $N' = 60$ min and then derived LI′ (or LS′) from the latter result by subtracting (or adding) twice the standard deviation. These limits were designated

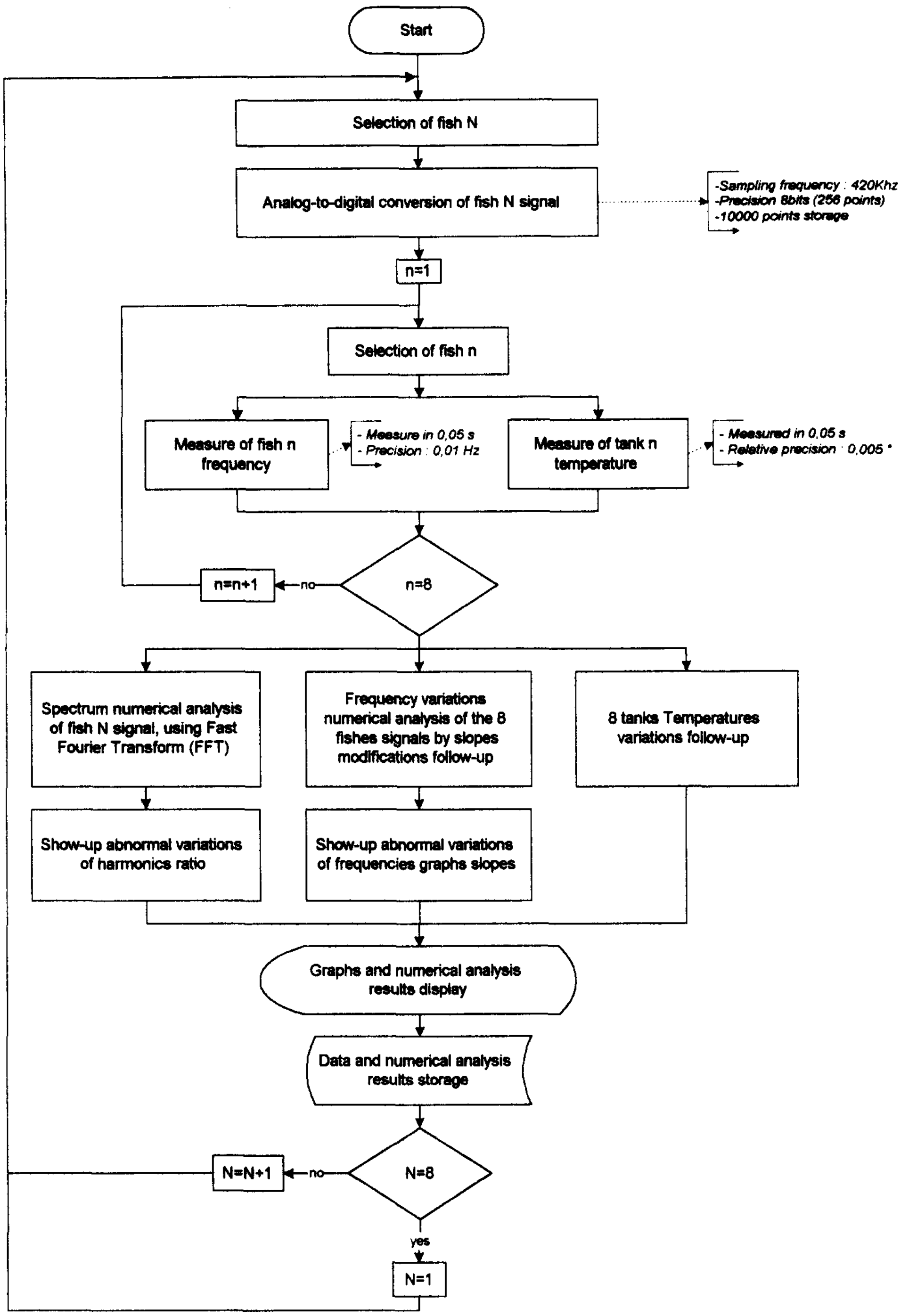


Fig. 3. Flowchart for computerized signal recording, processing, stocking and mathematical treatment.

static bounds because they were fixed over a period of an hour.

Alarm threshold. To further reinforce its reliability, the system resorted to a mathematical treatment which assigned the rank $Kx = 1$ to the first point lying outside [LI, LS]. If the next point was also external, it inherited the rank of its predecessor increased by 1. Subsequent successive points external to [LI, LS] also inherited the rank of their predecessor increased by 1. If, on the other hand, the next point was located inside [LI, LS], which amounts to boundary crossing, it inherited the rank of its predecessor decreased by 1. Subsequent successive points located inside [LI, LS] also inherited the rank of their predecessor minus 1. Moving from an inside point to an external point again triggered the rank decrement operation, and so on. Figure 4 gives a simplified illustration of the underlying procedure. A second parameter measures the deviation of a point from [LI, LS]. All points within [LI, LS] were assigned a zero deviation, while external points were assigned a deviation equal to the absolute value of its ordinate minus the closer of LI and LS. The degree of a point was then defined as the product of its rank and its cumulative deviation (i.e. the sum of the deviations of all points preceding it plus its own deviation). Following the same procedure, every point outside the static interval [LI′, LS′] was in turn assigned a rank and a degree.

Table 1. Chemical characteristics of the tap water used as dilution water for all experimental work

Characteristic	Unit	Mean*	Standard deviation
Dissolved oxygen	(mg l^{-1})	7.9	0.3
Conductivity	(μs cm^{-1})	275	13
Total hardness	(mg l^{-1} as $CaCO_3$)	110	11
Temperature	(°C)	27	0.05
pH	(–)	7.4	0.4

*Each value is the mean of nine measurements.

Table 2. Nominal and measured cyanide concentrations during experiments

Nominal concentrations (μg l^{-1})	Measured concentrations (μg l^{-1}) Mean*	Standard deviation
35	34.6	3.4
45	44.0	3.0
70	69.6	3.2

*Each value is the mean of 24 measurements (three water samples per 15 min, during the 2-h period of intoxication).

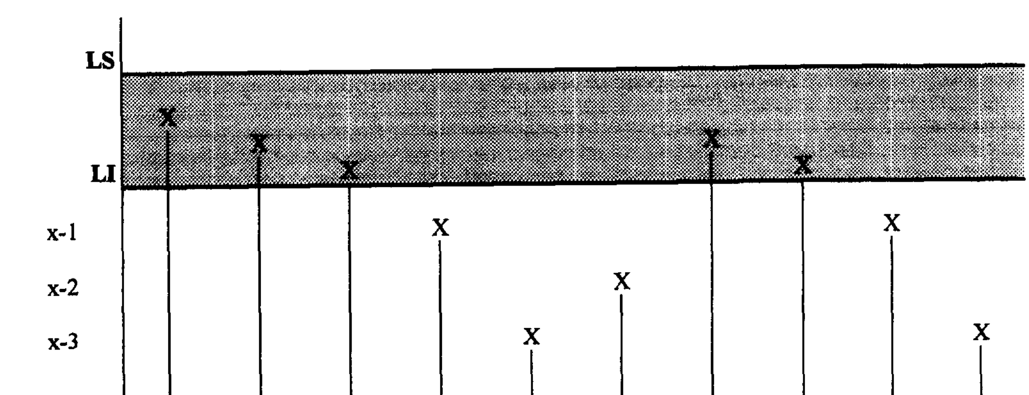
By definition, a toxicant was detected when 50% or more of the fish sensors had elicited a positive response. This was the case whenever the degree of the EOD frequency variation attained or exceeded 30 and 500, for LI and LS, respectively. A response was also considered positive whenever the degree of variation of the EOD waveform exceeded 2000, with static bounds LI′ and LS′. The preceding values which govern the warning system were determined empirically on the basis of several toxicological tests ($n = 10$) conducted according to the procedure described above and using a different group of eight fish each time. The pre- and post-intoxication values were recorded for each of the different signal parameters investigated (the EOD frequency; H2, H3, H4 and H5). These included deviation, rank and degree of each of the points involved. The question was then to determine, on the basis of the mathematical results, which values to adopt for the degree of variation of each parameter and for each context (crossing of lower and upper bounds. LI/LI′ and LS/LS′, in order) to reconcile the need for both system sensitivity and reliability. To achieve this, several mathematical filters were applied to the different series of data which were then computer analysed. The functioning of the system based on the values finally adopted (as given above) proved very satisfactory.

RESULTS

An adverse effect was shown in this study by a decrease in EOD frequency and an increase in EOD waveform when the fish were exposed to toxic conditions. It is noteworthy that changes in the locomotor behaviour of the *A. albifrons* were always preceded by changes in EOD characteristics.

EOD frequency. The temporal stability of the EOD frequency was affected by cyanide intoxication. For the three cyanide concentrations tested, between 62.5% and 75% of the fish population detected the toxic conditions (Table 3). The first responses appeared as early as a cyanide concentration of 34.6 μg l^{-1}. Attention is drawn to the rapidity of the EOD frequency response. Indeed, keeping to 50% detection as a warning threshold, it only took 27 min to detect the sub-lethal cyanide concentration of 34.6 μg l^{-1}. As the cyanide concentration increased, detection time decreased. More precisely, it was found that the electric fish based biomonitor took 17 and 6 min to detect cyanide concentrations of 44 and 69.6 μg l^{-1}, respectively. Figure 5a shows an example of the EOD frequency response developed by the *A. albifrons* before and during intoxication with 69.6 μg l^{-1} cyanide concentration. Two successive drops in the EOD frequency following the introduction of the pollutant alternated with a return to the pre-pollution frequency.

EOD waveform. The time variation of the amplitude ratios H2, H3, H4 and H5 remained relatively stable in the absence of the pollutant but was modified with the introduction of potassium cyanide (Fig. 5b). As could be expected, the



Rank = Kx	-	-	-	1	2	3	2	1	2	3
Deviation	-	-	-	1	3	2	0	0	1	3
Degree	-	-	-	1	8	18	12	6	14	30

Fig. 4. Computing the rank Kx, the deviation and the degree of EOD frequency data in relation to the interval defined by the dynamic lower (LI) and the upper (LS) bounds of the variation—a simplified version of the computational scheme. These bounds are the moving averages of the cumulative slopes $\pm$ twice the standard deviation (see text for more details).

Table 3. Detection potentials recorded with the EOD frequency and EOD waveform responses for *A. albifrons* subjected to cyanide intoxication

Mean measured concentrations of cyanide ($\mu g\ l^{-1}$)	% of fish detecting the toxic conditions using:		Response times (min) in 50% of fish within 2 h exposure using:	
	EOD frequency	EOD waveform	EOD frequency	EOD waveform
34.6	62.5	62.5	27	57
44.0	75.0	62.5	17	86
69.6	62.5	100.0	6	29

percentage of fish attaining the warning threshold rose with increasing cyanide concentration. This rose from 62.5% for the 34.6 $\mu g\ l^{-1}$ concentration, to 100% for the 69.6 $\mu g\ l^{-1}$ concentration (Table 3). It should be stressed that the EOD waveform pollution detection always lags behind EOD frequency based

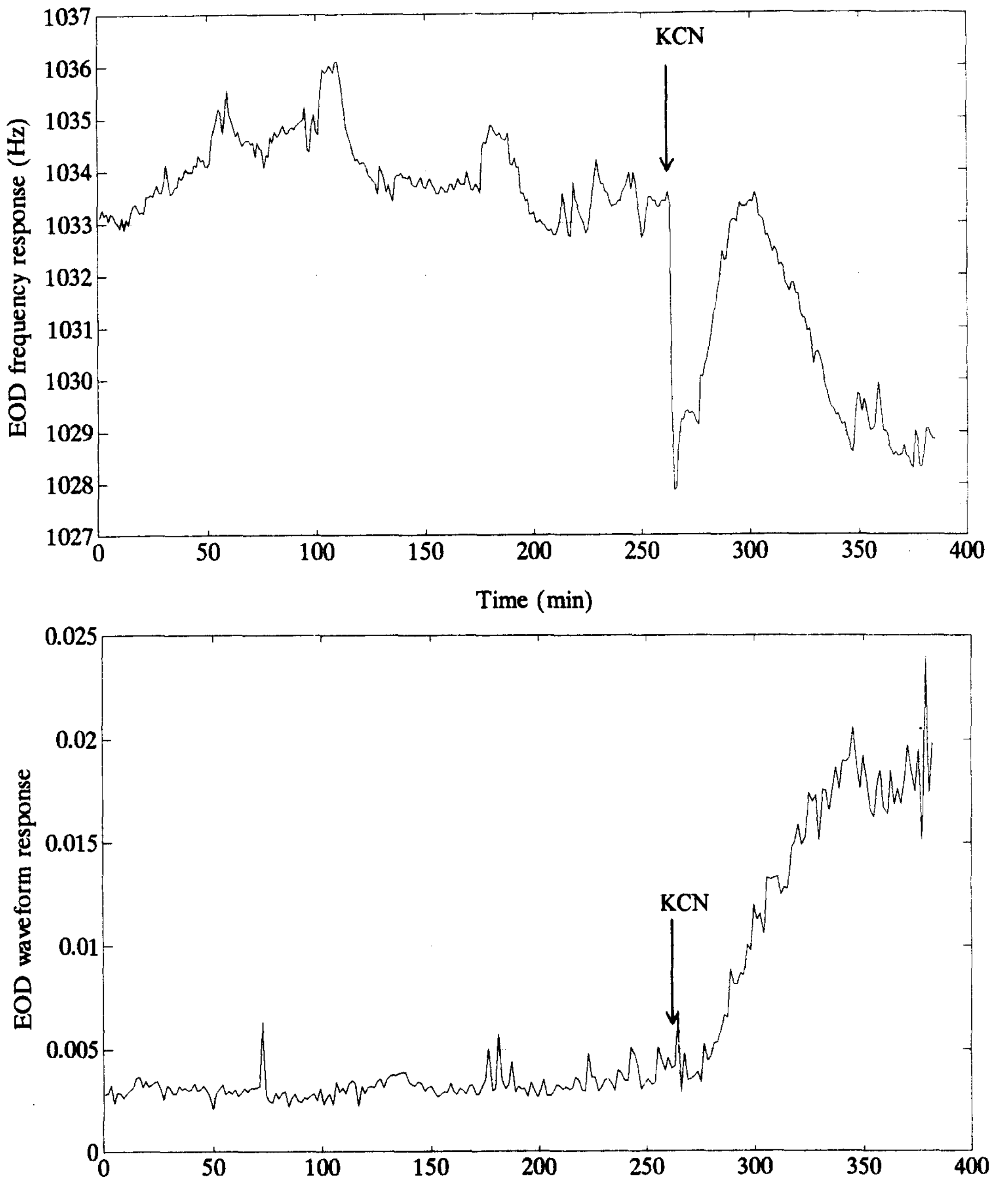


Fig. 5. EOD activity response of knifefish, *Apteronotus albifrons*, exposed to 69.6 $\mu g\ l^{-1}$ CN^-, within 2-h period of intoxication (the ICP is 0–260 min). (a) EOD frequency response; (b) EOD waveform response.

Table 4. Comparison of the *A. albifrons* cyanide pollution time response to that of other ichtyomonitors

Nominal concentration ($mg\,l^{-1}$)	Times for response (h)	Response monitored	Test species	Source
0.035	0.45	EOD frequency and waveform	*Apteronotus albifrons*	Present study
0.100	4.00	EOD frequency	*Gnathonemus petersii*	Geller (1984)
0.025	0.50	EOD frequency	*Gnathonemus tamandua* *Gnathonemus petersii*	Lewis *et al.* (1992)
0.100	0.35	Respiratory activity	*Micropterus salmoides*	Morgan (1979)
0.100	1.00	Locomotor behaviour	—	
0.400	0.50	Positive rheotaxis	*Oncorhynchus mykiss*	van Hoof (1980)

cyanide pollution detection. With the EOD waveform, the response times to cyanide pollution concentrations of 34.6, 44 and 69.6 $\mu g\,l^{-1}$ were 57, 86 and 29 min, respectively, after the onset of the pollution. Figure 5b illustrates the time variation of the EOD waveform response to 69.6 $\mu g\,l^{-1}$ cyanide pollution over time intervals of 0–260 min (before the cyanide pollution) and 260–380 min (during the cyanide pollution). As can be seen, the electric response was rapid and intense.

Table 3 shows the collected results from the exposure of *A. albifrons* to different measured concentrations of cyanide. It gives the proportions of the fish population showing a positive response within a 2-h exposure. The results illustrate the time delay for 50% of the fish to emit an EOD frequency and/or EOD waveform response(s) under the toxic conditions.

DISCUSSION

The present study gives a detailed description of a new biomonitor that exploits the *A. albifrons* electrical behaviour to obtain information on water quality changes. It was observed that this physiological end-point depended on the quality of the ambient aquatic medium. Under toxic conditions (cyanide pollution), the observed *Apteronotus* EOD frequency usually declined, while the waveform increased.

The ability of the *A. albifrons* to detect potassium cyanide pollution, as clearly demonstrated in this toxicological study, is particularly interesting. The inadequacy of periodic monitoring of the possible presence of cyanide due to its high toxicity and rapidity of action, and due to its transient persistence in the environment, is now recognized (Eisler, 1991). Comparison of the two parameters relating to *A. albifrons* electrical behaviour showed that with the EOD waveform, the positive responses were less prompt than with the EOD frequency. For this reason, the EOD waveform response can be envisaged as a validation for alarms triggered by the EOD frequency responses.

The early warning system developed in this study is able to detect a cyanide concentration as low as 34.6 $\mu g\,l^{-1}$ in less than half an hour. This value is below the current drinking water standard of 50 $\mu g\,l^{-1}$. It should be noted that two approaches are essential for a complete description of the *A. albifrons* EOD responses. The first deals with the weakest detectable concentrations and the second determines the necessary time to trigger the alarm. The values of both parameters are of particular relevance to water quality monitoring stations. Thus, in the particular case of cyanide intoxication, the noted rapidity of response and the levels of pollution (in terms of pollutant concentrations) detected were very satisfactory and corresponded very well to the requirements of warning stations. In this respect, Table 4 vividly highlights the remarkable cyanide detection performances of the electric fish compared to other biomonitors.

Unfortunately little is known about the toxicological mechanism which could explain the influence of toxic conditions on the recorded electric responses of the *A. albifrons*. It is however well known that cyanide induces a cytotoxic hypoxia and a lactate acidosis (Egekeze and Oehme, 1980; Eisler, 1991), and the combination of these two phenomena depresses the central nervous system, the most sensitive area of anoxia. As a consequence, the EOD command centre (pace-maker) can be affected. It is also possible that the action of the toxic substances modifies the endocrine activity of the hypothalamus. This could in turn affect the permeability of the electric cell membranes and therefore explain the variations of the levels of the EOD frequencies and waveforms under toxic conditions.

An important advantage of the proposed system resides in the use of the remarkably stable electric information generated directly by the fish. For this reason, *A. albifrons* can itself be likened to an electric generator. The electrical behaviour recording and processing are therefore easier. This very high temporal stability of *Apteronotus* electric activity is the major characteristic of the biomonitor developed in this study. This fact distinguishes it from Rausch's system (1980) based on the pulse discharges emitted by *G. petersi*. Another innovation in our system resides in the use of continuous waveform electric discharges, with the possibility of enhancing the system's reliability by exploiting the form of the signal. Finally, the *A. albifrons*, unlike the *G. petersii*, is a solitary species which adapts easily to test conditions.

It is very likely that fish locomotor behaviour also conveys information about the quality of the surrounding water. The movement of the fish is relatively easy to exploit by the biomonitor developed in this study. The fish movements do affect the recording quality of the signal emitted and the electric response for that matter. But in the case of cyanide pollution, we observed that changes in the movement of the *A. albifrons* were always preceded by changes in the EOD characteristics. Nevertheless, it is obvious that increasing the number of quality indicating parameters can reinforce the sensitivity and the reliability of the monitoring system and contribute to its overall robustness. Van der Schalie (1986) has in fact stressed the importance of relying on several parameters.

In conclusion, it appears necessary to continue the development of the biomonitor based on the real-time evaluation of the *Apteronotus* electric behaviour. Because this species has never been submitted to previous toxicological studies, a particular research effort must be made to determine the field of biomonitor validity for intermittent or continuous exposure protocols on a wider range of pollutants.

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