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MONITORING OF BEHAVIORAL PATTERNS OF AQUATIC ORGANISMS WITH AN IMPEDANCE CONVERSION TECHNIQUE

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An impedance converter, based on the tetrapole electrode system, was used to record the behavioral patterns of a wide range of aquatic organisms, such as *Daphnia magna*, *Gammarus pulex*, *Sialis lutaria*, *Leptophlebia vespertina*, *Baetis niger*, Simuliidae, *Dinocras cephalotes*, *Hydropsyche siltalai*, and tadpoles of *Rana temporaria*. The method proved to be sensitive for different kinds of behavior, e.g., ventilation, grazing, filter feeding, net spinning, and locomotion (swimming, creeping, and looping), which makes it a promising tool for continuous biomonitoring purposes.

INTRODUCTION

Responses of organisms to toxicants can be evaluated at various biological organization levels with increasing integration from biochemical analysis (such as changes in enzyme activities) to biocoenotical studies (such as changes in species diversity). Biochemical indicators are useful managers for water quality only if they can be related to changes at higher organization levels (Scherer 1992).

Behavioral responses are positioned at the whole organism level, between the biochemical and the ecological levels. Behavioral alterations rest on biochemical processes, but also reflect the fitness of the individual organism as well as potential effects on the population level, such as altered abundance of the species in the ecosystem. Behavioral responses appear to compare favourably with biochemical and physiological responses in terms of sensitivity and efficiency. In addition to their integrative nature and ecological relevance, behavioral responses are nondestructive, which makes continuous long-term monitoring possible (Scherer 1992).

Behavioral responses can be measured at different levels of integration and complexity, such as simple reflexes like rheotaxis or gill ventilation or more complex behaviors like locomotor behavior. The highest level of integration is represented by interspecific behavioral responses, such as competition or predation (Scherer 1992). In aquatic toxicology, behavioral endpoints have been applied for fish and crayfish for about 20 y (Atchison et al. 1987; Beitinger 1990). Behavioral toxicity tests for *Gammarus pulex* by use of the disruption of precopula have recently been described (Garmendia Tolosa and Axelsson 1993).

Behavioral aquatic toxicology is a comparatively young research area, partly due to the lack of suitable quantitative recording techniques. Infra-red lightbeam actographs have been successfully used in terrestrial environments, but are not suitable for aquatic organisms due to the high extinction rates of light in water. Lightbeam techniques have recently been used for the study of the activity of *Carcinus maenas* (Aagaard et al. 1991). Ultrasound beams have been used for behavioral studies by Huggins et al. (1973). Time-lapse photography and video-filming techniques have been mostly used in behavioral studies with fish and copepods (Atchison 1987; Vanderploeg and Pfaffen-höfer 1985). However, filming is labour intensive and the data analysis is time consuming. Recently the development of electrode chambers (Spoor 1971; Swain et al. 1977) made it possible to use the impedance conversion technique for recording behavioral responses of aquatic animals, such as marine fish (Wingard and Swansson 1992), chironomids and daphnids (Heinis et al. 1990; Heinis and Swain 1986), and copepods (Gill and Poulet 1986).

Biomonitoring systems for water quality surveillance often rely on physiological responses, such as luminescence of bacteria and algae or the heart rate of bivalvia. Behavioral responses have seldom been used for biomonitoring, with the exception of the activity of cladocerans, valve movements of mussels, or rheotaxis of fish (Borcherding 1992). As behavioral responses may be one of the first and most sensitive indicators of a chemical stressor, they offer great potential for biomonitoring purposes. Since aquatic insects are important links in the aquatic food web, easy to handle, and readily available, they should be included in biomonitoring procedures. The signals produced by the natural behavior of different aquatic organisms were observed and recorded to evaluate the impedance conversion technique for behavioral studies.

METHODS

Impedance measurements

The principle of the measurement of the impedance in an electrolyte solution is based on Ohms law. Two platinum electrodes in an electrolyte solution are connected to a generator, which produces a constant alternating current that is independent of the resistance of the electrodes. At low frequencies, the measurement of the dielectric properties of conducting materials is severely affected by electrode polarization (Schwan and Ferris 1968). Electrode polarization results in the voltage between the electrodes to be no longer a good approximation for the voltage across the sample (Schwan and Ferris 1968) due to "parasitic polarization impedances" and undesired voltages at the contact zone between the platinum electrodes and the electrolyte solution. This may affect the measurements of the impedance across the sample by distortion of the signals' shape and production of low-frequency noise. To avoid these disadvantages, a second, non-current-carrying pair of electrodes was introduced between the current-injecting electrodes to measure the voltage across the sample (tetrapole electrode technique). Thus, the two processes of current generation and impedance-dependent voltage registration occur at different electrode pairs (Fig. 1).

The behavioral monitoring system

The organism moves freely between two pairs of electrodes on each side wall of a test chamber which receives recirculating unfiltered streamwater from a water tank at a flow rate of 15 mL/min^{-1}. The organism's movements produce changes in the electrical field which can be measured as changes in the impedance of the system (Fig. 2). The sensitivity of the system for different kinds of movements depends on the intensity of the movements as well as on the size of the chamber. Two sizes of chambers made of plexiglas were used, dependent on the size of the species tested (10 x 3 x 2 cm^3 and 2 x 1 x 1 cm^3, Fig. 3). A nylon net (1 mm mesh size) was fastened on the bottom and on the sides of the chambers to prevent the organisms from directly contacting the electrodes. The large chamber was constructed with conical ends at the in- and outflow to minimize the formation of turbulence in the chamber. The level of background noise from the system without organisms was $\leq$ 20 mV.

The electrical signals from the chamber passed a 50 kHz bandpass filter to an amplitude demodulator. The demodulator unit is followed by a bandpass filter (1-20 Hz), where the signals are further smoothed

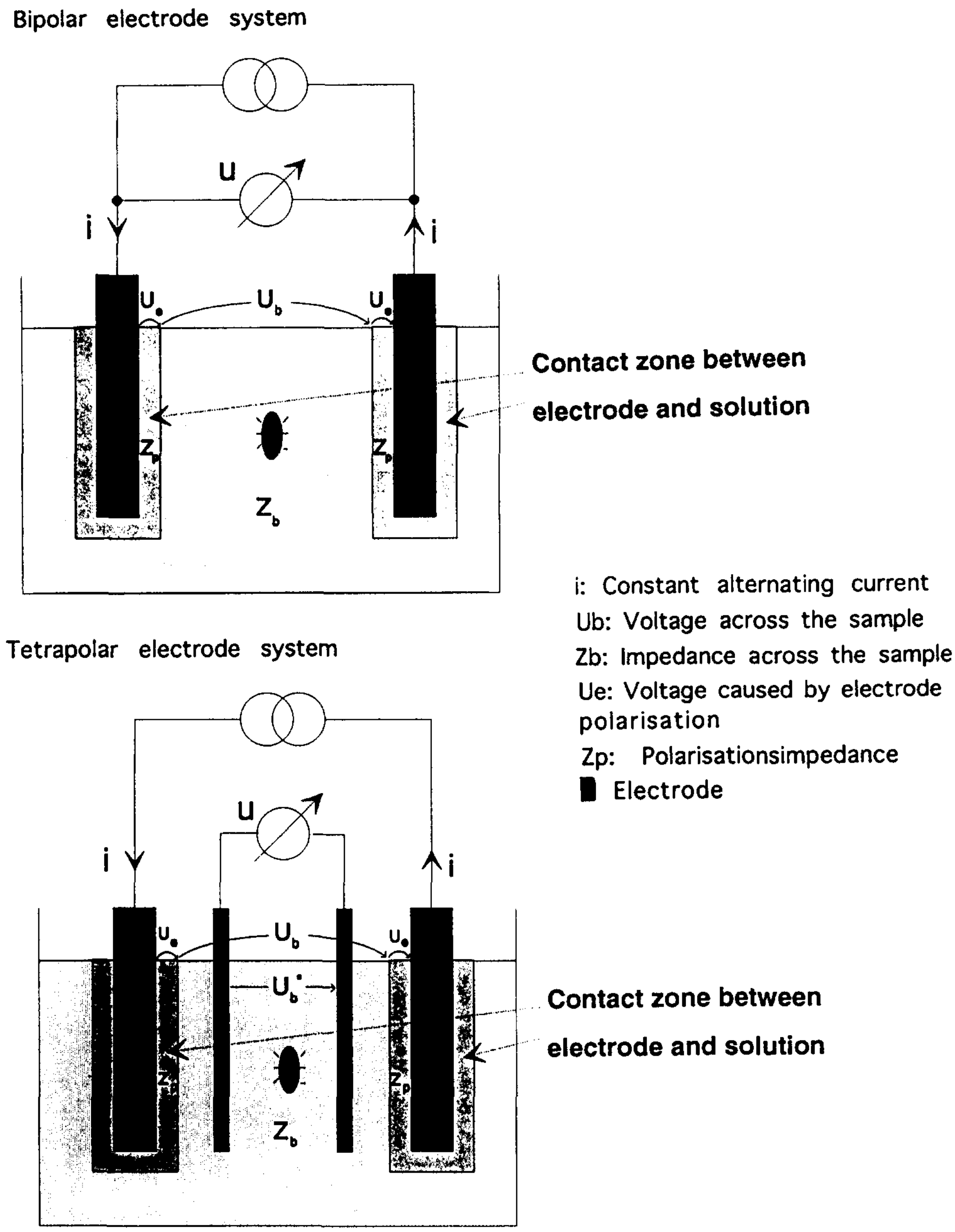


Fig. 1. Comparison of the bipolar and tetrapolar electrode system in impedance measurements.

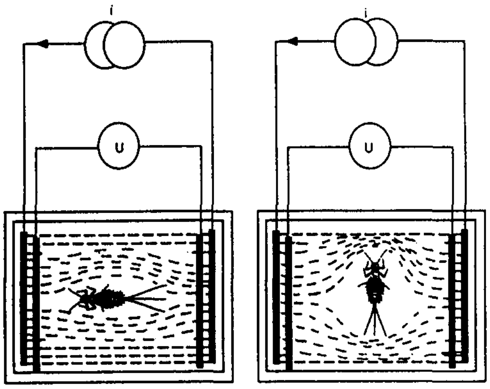


Fig. 2. Changes in the electrical field due to movements of organisms.

and noise is eliminated. A baseline adjust was included in the system to eliminate baseline drift of the signals (Fig. 4). The signals were calibrated by a separate pulse generator and amplified about 600 times. A voltage limiter allowed only signals ≤10 V to enter the Analog/Digital unit. The digital signals were processed in a Mac-LC3 computer (8, 80 MB). A powerful software (SuperScope) allowed for dense data registration (max 3600 points/s at one channel), simultaneous graphic display, and mathematical and statistical data processing.

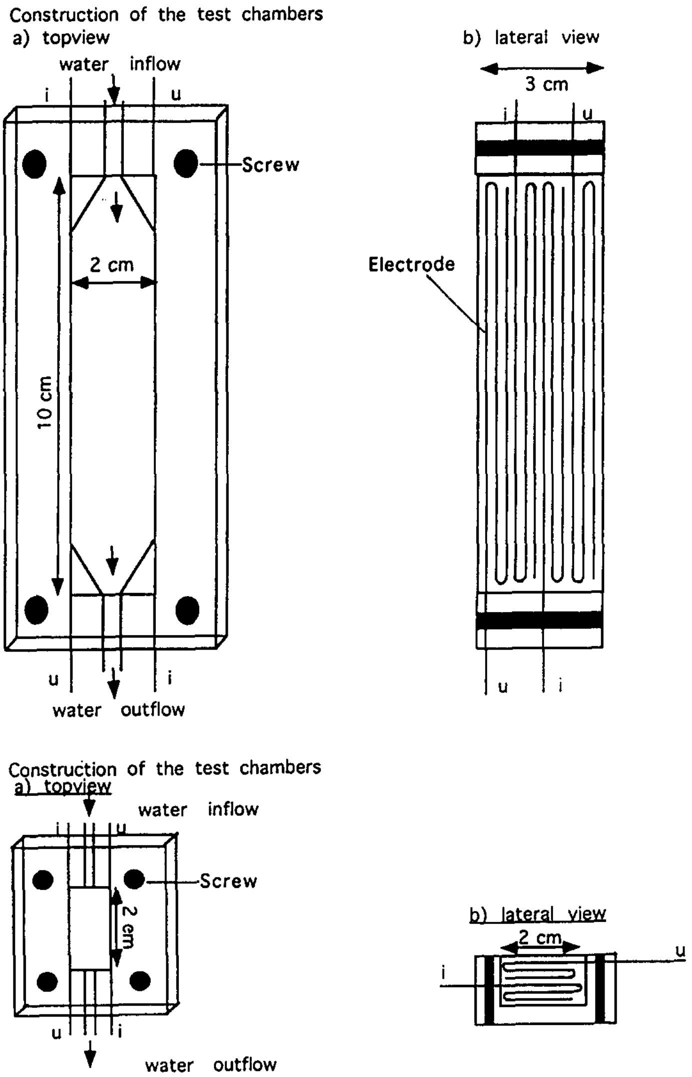


Fig. 3. Construction of the test chambers.

Test species

Several aquatic species were chosen according to the following criteria: 1) they should be common in freshwaters; 2) they should be frequently used in traditional toxicity tests (e.g., *Daphnia magna*, *Gammarus pulex*); 3) they should serve as indicators for different water quality levels (e.g., the plecopteran *Dinocras cephalotes* for oligosaprobe water, the crustacean *Gammarus pulex* for b-mesosaprobe water and the trichopteran *Hydropsyche siltalai* for b-a mesosaprobe water; and 4) they should represent different taxonomical groups, be of different sizes, and have different feeding habits.

With these criteria in mind, the following species were chosen to be tested in the impedance converter. Individuals were collected in pools and streams of different water quality in south Sweden (Table 1). The mayfly species, *Baetis niger* and *Leptophlebia vespertina*, were collected in Stream A, a small, episodically acidified, brownwater forest stream rich in organic matter. The crustacean *Gammarus pulex* and the plecopteran *Dinocras cephalotes* were collected in Stream B, a small, circumneutral, anthropogenically unaffected clearwater forest stream rich in grove detritus (allochthonous leaf packs) and aquatic mosses growing on the gravel substrate. The trichopteran *Hydropsyche siltalai* and the Simuliidae larvae were collected in Stream C, a small, circumneutral, eutrophic lake outflow. The megalopteran Sialis lutaria, the crustacean *Daphnia magna* and tadpoles of *Rana temporaria* were collected in a small abandoned marlpool with circumneutral pH.

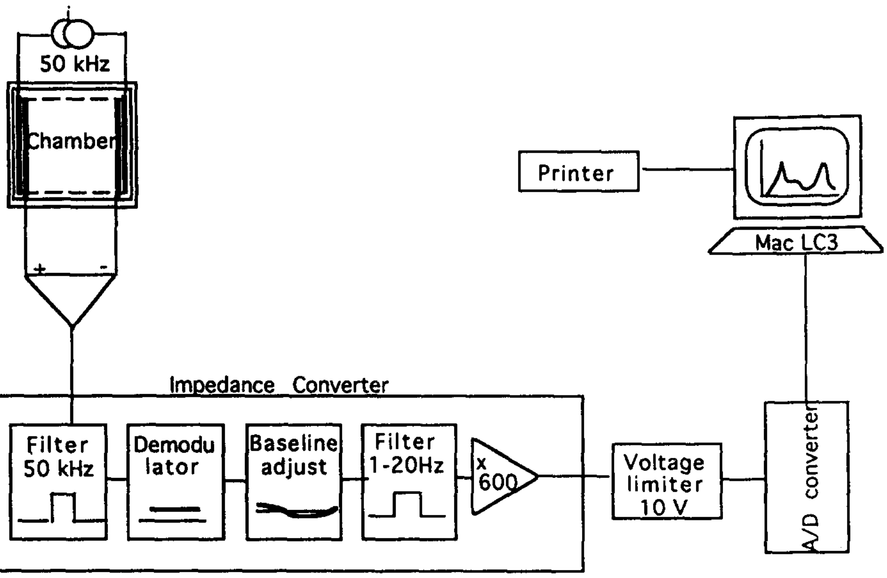


Fig. 4. Components of the behavioral monitoring system.

Table 1. Chemical parameters from the sample sites. All parameters in mg L^{-1}, except NO_3^- and CL^-, which are given in μM.

Stream	Al	Ca	Cd	Cu	Fe	Pb	P	S	Zn	NO_3^-	Cl^-
Stream A	0.44	9.41	0.00	0.02	5.80	0.00	0.09	6.05	0.03	43.2	273
Stream B	0.24	19.0	0.00	0.02	0.84	0.00	0.00	8.10	0.03	44.4	296
Stream C	0.00	61.8	0.00	0.07	0.23	0.00	0.26	10.42	0.14	346.0	540
Lake	0.00	52.8	0.00	0.00	0.15	0.00	0.00	2.04	0.00	3.4	419

Animal behavioral patterns

Locomotion: Locomotion comprehends all kinds of movements, such as swimming, crawling, and looping. Swimming and crawling signals are irregular with respect to their amplitudes and frequencies. Loopings of the larvae of Simuliidae include a series of different movements that allow the larvae to change their place without losing contact with the substrate. After searching for a suitable place with gyratory movements of the body while being attached to the substrate, the larvae investigate the substrate with their mouthparts. Afterwards the larvae fasten their thoracic prolegs to the substrate and produce a secretion with their mouthparts. Finally, the larvae move their abdomen from the old to the new place (Reidelbach and Kiel 1990). Drift behavior is characterized by passive floating in the water flow.

Resting: Resting or inactivity includes all small, not-interpretable signals that cannot be separated from background noise (≤ 20 mV).

Ventilation: Ventilation comprehends all kinds of regular, high frequency movements with the gills themselves or other body parts such as abdomen undulations in order to establish a constant water flow across the gills.

Feeding: Feeding includes behaviors like grazing, filtering, and hunting of predatory stoneflies. These behaviors have characteristic patterns for each species.

The different behaviors were observed for five specimens of each test species in such a way that the animals were observed under up to 40 x magnification and recorded simultaneously with the impedance converter. This procedure also revealed whether the electrical field affected the animals movements or not, after switching on the impedance converter.

Data collection

Behavioral patterns of five organisms from each test species were recorded for 5 min and preceded by an acclimation period of 20 min. During that period, the animals were supplied with unfiltered stream-water containing algae and detritus particles. Hunting of the predatory plecopteran *Dinocras caphalotes* was recorded in separate observations in which a mayfly was added to the test chamber. The trichopteran *Hydropsyche siltalai* was observed over a period of three days with a 5-min-long recording period every hour.

Data evaluation

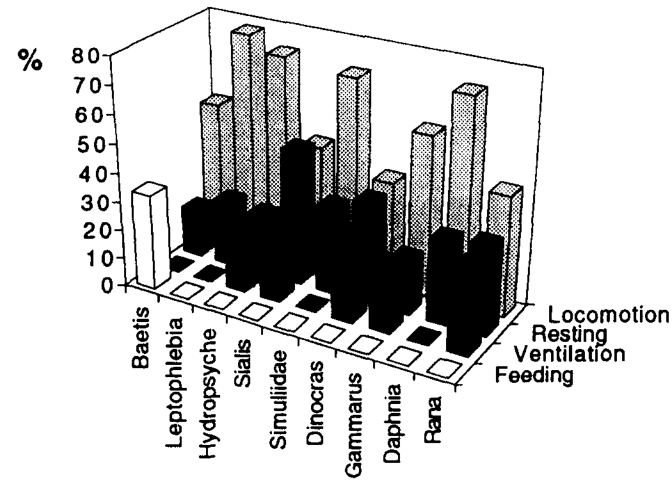
The electrical signals from different behaviors of each individual were analyzed as follows: The time spent on each type of behavior was calculated, and differences in the display of the behaviors were tested using one-factor ANOVA followed by Tukey HSD tests (Sokal and Rohlf 1987). For regular periodical signals, frequency analyses according to Fast Fourier Transformation were calculated. The different behaviors were described by frequencies and max-amplitudes. An absolute measure for overall activity was calculated from the integral over the whole behavioral record.

RESULTS

Baetis niger (Ephemeroptera) displayed three types of behavior, swimming being the most common behavior and significantly more often performed than grazing and resting (Fig. 5; $p < 0.002$). The swimming signal consisted of a typical pattern of peaks of up to 10 V followed by some lower peaks (> 2 V) (Fig. 6). The grazing signal consisted of movements with the head (1 V) followed by movements with the mouth parts (> 200 mV).

Leptophlebia vespertina (Ephemeroptera) is a poor swimmer. The larvae move mostly by creeping. Creeping phases were significantly longer than resting phases ($p < 0.0005$, Fig. 5). No feeding behavior was recorded, probably due to the fact that the nymphs were close to emergence, a period in the life cycle, in which they do not eat (Kjellberg 1972). Ventilation of the gills occurs only occasionally and was thus not recorded during the 5 min. measurements.

Hydropsyche siltalai (Trichoptera) displayed mostly locomotion (creeping and swimming), followed by few resting phases ($p < 0.03$) and even less ventilation, which is characterized by regular undulations of the abdomen from side to side ($p < 0.0005$). The signal pattern for locomotion consisted of a typical pattern of a whole body movement (≤ 10 V) followed



Percentage time spent on four types of behavior during 5 min. recording.

The values represent means of 5 organisms from each test species.

Fig. 5. Comparison of the behavioral pattern of different species.

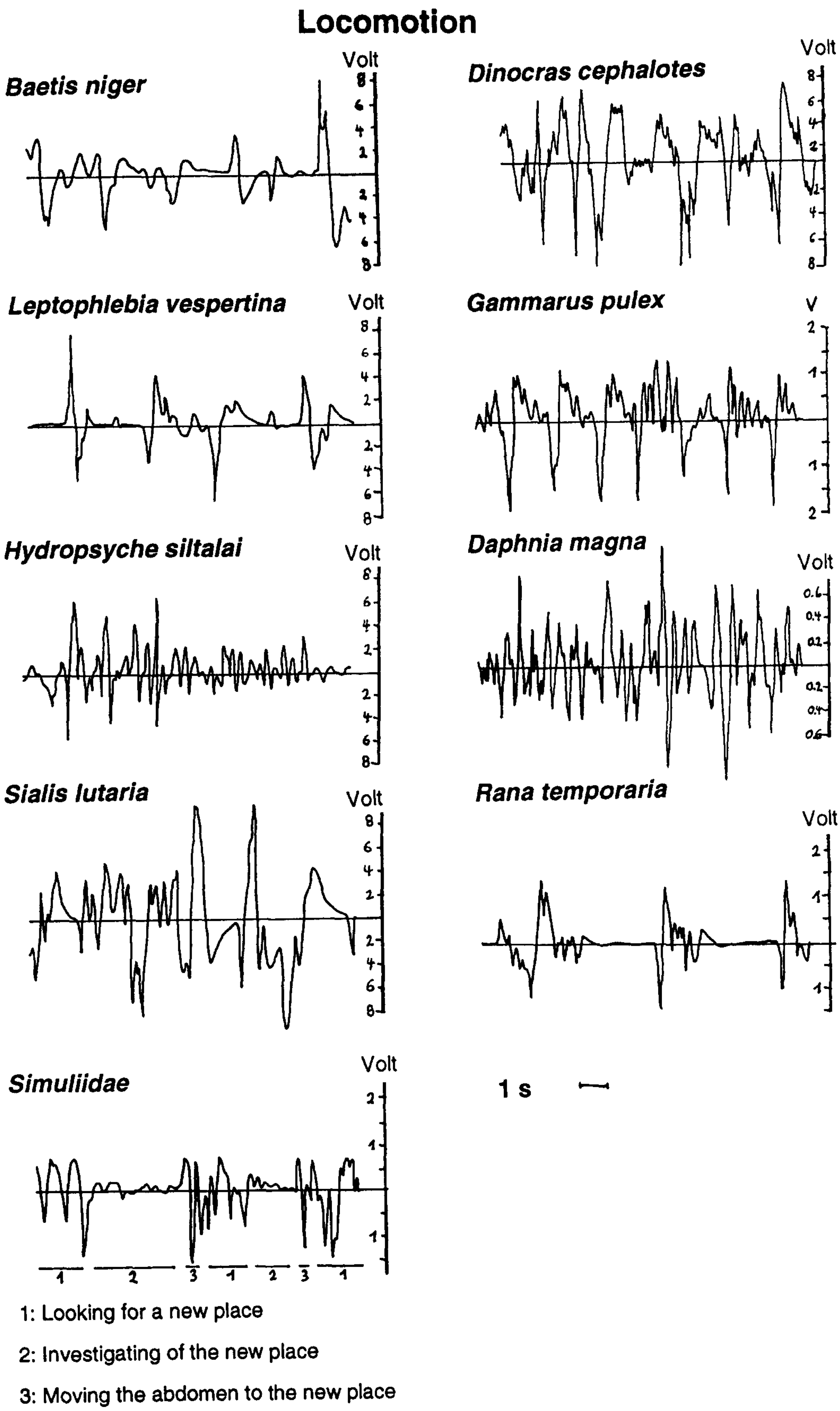


Fig. 6. Characteristic signals of different species for behaviors such as locomotion, ventilation, and grazing.

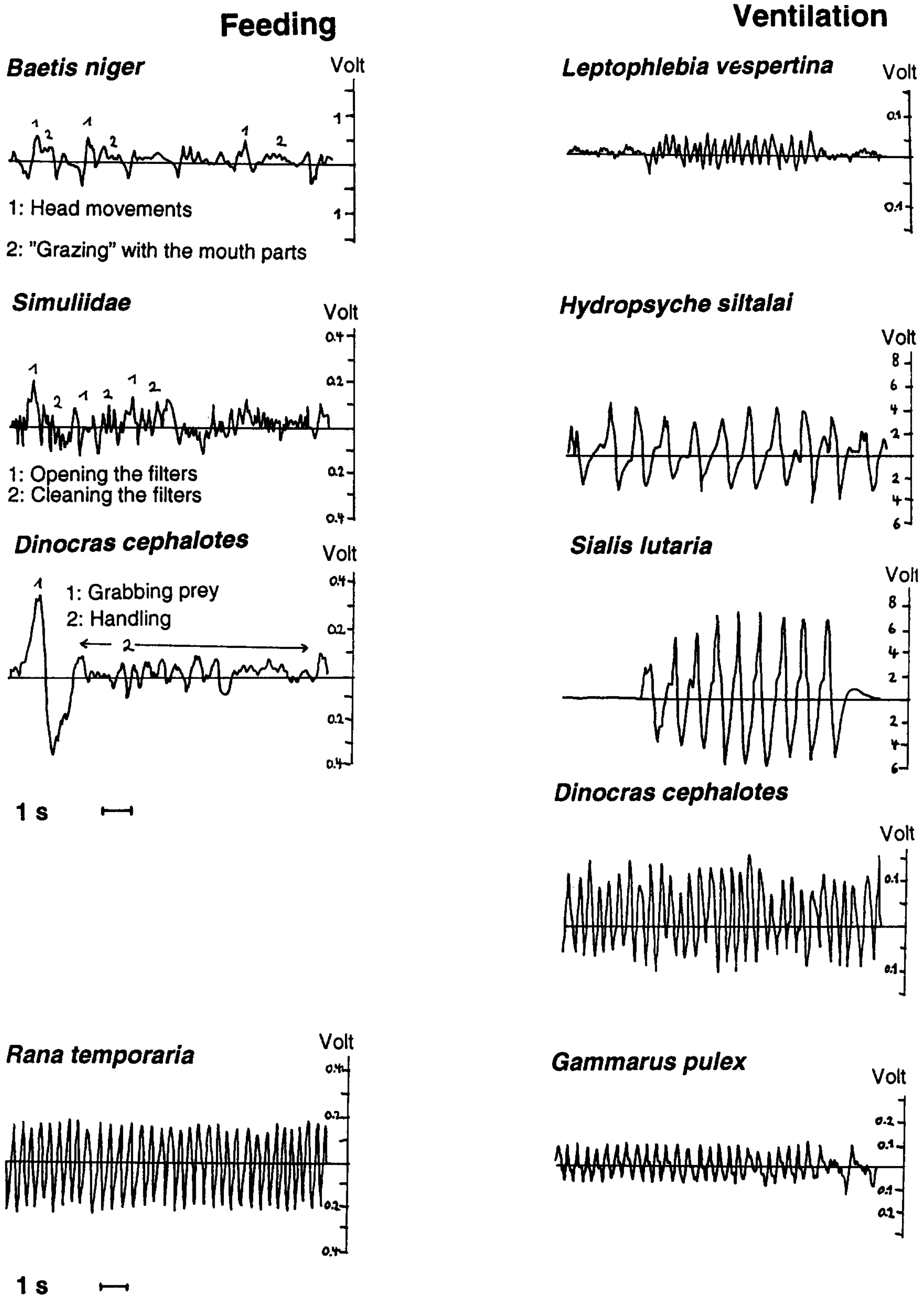


Fig. 6. Continued.

by leg movements (2 V) (Fig. 6). The time series revealed that after an acclimation period of about two hours, mainly characterized by locomotion, the larvae started to build a shelter and spin a net. This behavior alternated with phases of ventilation (Fig. 7). During the following days, the larvae always showed the same behavioral pattern consisting of alternating ventilation and resting phases. However, the length of the ventilation phases increased and the frequency decreased from 1.2 ± 0.2 Hz to 0.75 ± 0.1 Hz by the end of the observation period.

Sialis lutaria (Megaloptera) showed a similar behavioral pattern where the times spent on locomotion and resting were similar. No significant differences between the time spent on different types of behavior were found. *Sialis lutaria* was the most inactive species of those tested (Fig. 5). Ventilation occurred with similar frequencies as that of *H. siltalai* (1.4 ± 0.2 Hz).

Dinocras cephalotes (Plecoptera) showed three types of behavior, locomotion (creeping or swimming), ventilation consisting of push ups at 2.5 ± 0.5 Hz, and resting phases. No significant differences between the time spent on different types of behavior were found. *D. cephalotes* was significantly less active than *S. lutaria* ($p < 0.02$).

Hunting behavior and prey handling could be recorded with the impedance converter. After *D. cephalotes* encountered a mayfly larvae with the antennae, it grabbed the prey with the maxillae and started digestion (Fig. 6). Handling of the prey was interrupted by ventilation phases.

Simuliidae displayed different types of behavior, such as loopings, filter-feeding, drift, and resting. Most of the recording period was spent on locomotion such as loopings and drift ($p < 0.0005$). However, the overall activity of Simuliidae was significantly less than that of nonsessile invertebrates (p 0.001; Fig. 5). The typical series of movements for looping could be recorded precisely with the impedance converter (Fig. 6). The feeding behavior of the larvae consisted of an alternating opening of the filters (0.1 - 0.2 V) followed by movements of the mouth parts to clean the fitters (50 mV).

Gammarus pulex (Crustacea) showed three types of behavior such as swimming, resting, and ventilation. No significant differences between the time spent on the different behaviors were found (Fig. 5). The typical pattern of swimming was the periodical stretching of the body (< 10 V), followed by leg movements (1 V) (Fig. 6). Ventilation was performed by regular movements with the pleopodes at 3.6 ± 1.5 Hz).

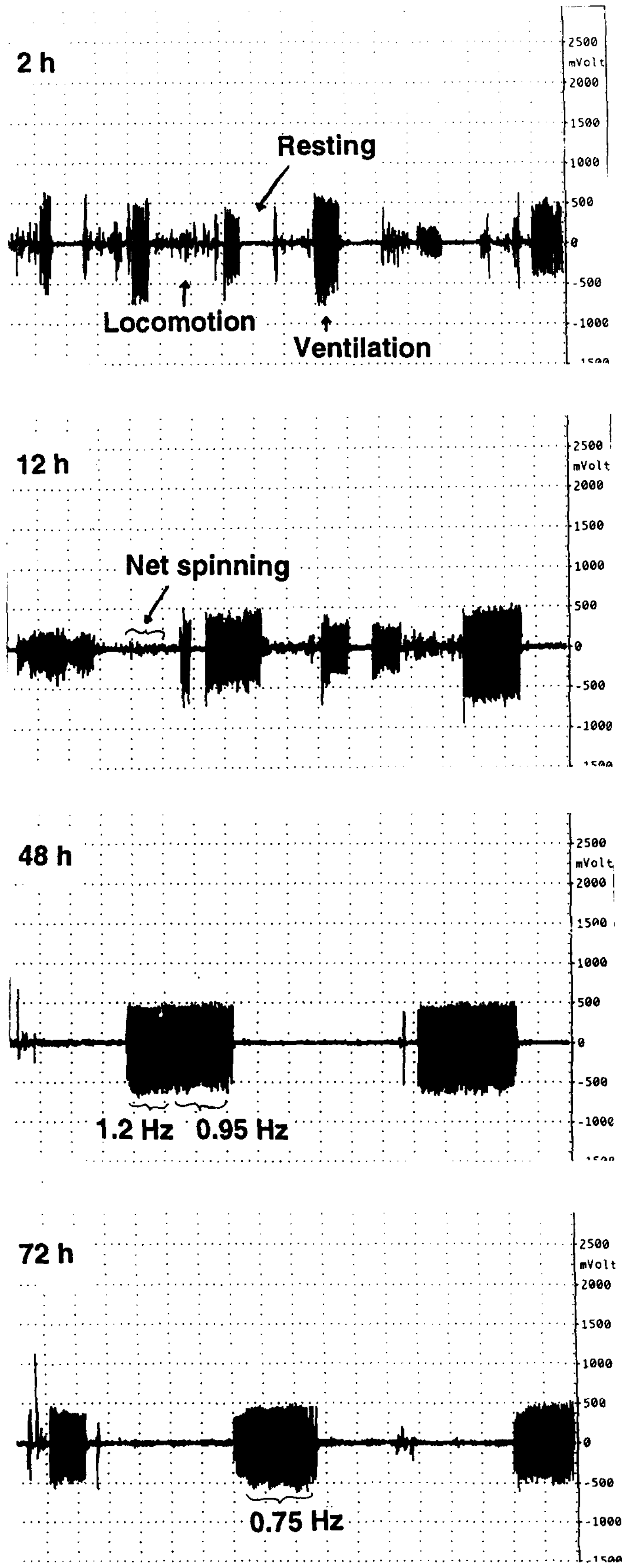


Fig. 7. The behavioral pattern of *H. siltalai* over a period of 72 h.

Daphnia magna (Crustacea) is a common cladoceran. The behavioral pattern consisted of fast jerky swimming movements with the antennae (200 mV) interspersed by few, short resting phases ($p < 0.0005$, Figs. 5 and 6). Ventilation movements of the legs within the carapax were superimposed by swimming and could not be registered at the same time.

Rana temporaria (Vertebrate, Amphibia) tadpoles displayed swimming and drinking in the test chamber. The swimming pattern was a typical series of an initial strong movement with the tail (> 1 V) followed by a few less intense movements (500 mV; Figs. 5 and 6). The swimming periods of *R. temporaria* were short and often interrupted by short resting phases which seemed to be used for new orientation. Ventilation occurred at a frequency of 2.1 ± 0.4 Hz.

DISCUSSION

Up to now, the impedance conversion method has been used to record respiratory movements of *D. magna* and chironomids (Heinis and Swain 1986), movements of the cephalic apendages of *Calanus helgolandius* (Gill and Poulet 1986), respiration of marine teleost fish (Wingard and Swanson 1992), and larvae of Odonata (Swain et al. 1977). Heinis et al. (1990) studied the activity pattern of the chironomid, *Glyptotendipes pollens*, which consisted of a typical sequence of net-spinning, pumping, elimination, and inactivity behaviors.

A detailed description of the impedance conversion method used in the above-mentioned studies has only been given in one case (Heinis and Swain 1986). As the construction of the impedance converter and its electronic parts severely affect the results of the measurements (sensitivity, background noise), a description of the apparatus used in the studies is necessary. In comparison to the method used by Heinis and Swain (1986), the present construction has the following advantage. The parasitic impedances are eliminated by use of the tetrapole electrode system. The system by Heinis and Swain (1986) uses the bipolar electrode system and a Wheatstone bridge circuit in combination with one chamber containing the insect and one chamber containing only water. This measurement device eliminates only coherent impedance changes in both chambers, which is a rare case. Noncoherent random impedances can, however, lead to background noise and baseline drift during the measurements.

Our impedance converter was appropriate for quantitative recordings of different kinds of behavior in aquatic species of different sizes. Locomotive behavior varied from creeping, swimming, and drift to looping. Regular movements of high frequency, like gill ventilation, abdomen undulations, and push-ups may serve to increase the waterflow over the respiratory tissues. Moreover, feeding behavior could be studied, such as filtration of Simuliidae, predation by *D. cephalotes* and grazing of Baetidae as well as the net-spinning behavior of *H. siltalai.*

These quantitative and sensitive recordings of different behaviors of aquatic species can be related to ecological parameters. For example, *G. pulex* was the most active species, followed by *B. niger*, *L. vespertina*, *H. siltalai*, and finally Simuliidae. These differences reflect differences in habitat choice and feeding habits. *Gammarus pulex* and *B. niger* are both good swimmers and occur frequently in the drift. *Gammarus pulex* also moves actively upstream to compensate for drift losses. *Sialis lutaria* and *L. vespertina* are living more closely to fine particulate sediment in slow-flowing habitats, the predator *S. lutaria* being of course more active than the detritus feeder *L. vespertina*. *Hydropsyche siltalai* is hemi-sessile, as it constructs a shelter with a drift-net to collect particles. Even less active are Simuliidae, as they are sessile filter feeders.

Not only activity but also ventilation behavior mirrors niche differentiation. Active animals with thin cuticles can rely on respiration through the skin, especially when they live in fast-flowing habitats, e.g., *B. niger*. Hemi-sessile animals, such as *H. siltalai* or those living in pool sites with only slow water flow, such as *S. lutaria*, may need ventilation to create a water flow across their gills. Crustaceans need ventilation as they cannot breath through their calcified carapace, which may explain the high ventilation frequencies of *G. pulex*. Large invertebrates, such as *D. cephalotes*, need additional ventilation to satisfy their oxygen demands, even if they live in fast-flowing habitats. As a consequence, *G. pulex* showed the highest ventilation frequencies, followed by *D. cephalotes*, *R. temporaria*, and *S. lutaria*, respectively.

Our tests showed that the impedance converter is an appropriate tool for behavioral and ecological studies, as it records behavioral patterns of aquatic species in a sensitive and quantitative way. The impedance converter can also record during the night, which is quite important as most aquatic invertebrates are active during the dark, e.g., drift and grazing of *Baetis niger* and hunting of *D. cephalotes*. Differences in specific behaviors between different species can be quantified (frequency, amplitude) and related to different adaptations of the species to their special habitats. Such knowledge is a useful tool in ecological studies, e.g., niche differentiation of species within a guild. Moreover, the impedance conversion method can be used for continuous behavioral biomonitoring by simultaneously recording changes in different types of behavior, such as feeding, swimming, predation, and ventilation as indicators for chemical stress. The more biological parameters are included in a biomonitoring system, the more sensitive it will be and the safety of the system as an early warning system will increase. Up to now, biomonitoring systems often only rely on only one or two physiological parameters, which may or may not be affected by a chemical. Impedance conversion also allows for simultaneous measurements of behavioral patterns of different species, e.g., from different trophic levels, which is necessary for a reliable biomonitoring system, as one species may or may not react to the chemical stress.

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