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Determination of *Chironomus javanus* Behaviour Using Multispecies Freshwater Biomonitor (MFB)

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Abstract: Study on *Chironomus riparius* behaviour in laboratory conditions was undertaken using Multispecies Freshwater Bioindicator (MFB). *Chironomus* larvae were collected from permanent culture tank and placed in MFB chamber. One individual *C. javanus* was placed in the chamber and the test repeated three times. Three different conditions were tested for *C. javanus* behaviour i.e., *C. javanus* free swimming in chamber, *C. javanus* placed in the modified case inserted in the chamber and *C. javanus* in its tube placed in the modified case. Locomotion, ventilation and inactivity were measured for chosen period. Result indicates that the signal produced in FFT and long-term modes did not much differ between each tests and one-way ANOVA confirmed that no significant difference was recorded ($p > 0.05$). However, *C. javanus* from third condition test (in tube and case) exhibited more constant and regular pattern in short-term signal (amplitude signal). The signal produced from third condition test exhibited more regular locomotion, ventilation and inactivity patterns. The one-way ANOVA test indicated that data from third condition test were significantly different from the first and second test condition.

Key words: Chironomid, behaviour signal, multispecies freshwater biomonitor, biomonitoring, biological indicator.

Introduction

Chironomids, also known as midges, are mosquito-like insects. They are widely distributed insects and can be found in all types of aquatic habitats especially in polluted areas (Pinder, 1986). Chironomids have received great concerns and various studies were undertaken on chironomids such as biology and taxonomy of chironomids, ecology of chironomids and also their responses to chemical and biological pollution especially in Europe, US and parts of Asia. Some established keys to selected species allow generic identification for larvae from Malaysia (Cranston, 2004) and from the Holartic region (Wiederholm, 1983). The development and establishment of this basic information on chironomids facilitate in determining the potential of chironomid as a biological indicator (Williams et al., 1992). Although chironomids are abundant in Malaysia, nevertheless, there are fewer studies on these organisms and that too at basic level. Systematic studies on chironomids have to be undertaken to determine population richness and species diversity towards environmental alterations.

These most widely distributed insects in freshwater can tolerate and grow in polluted waters including waste stabilization pond (Broza et al., 2000). Due to their feeding behaviour on debris in aquatic sediments, frequently chironomids are exposed to contaminants contained in the organic matter. According to Gerhardt et al. (1995), chironomids population information has widely been used to estimate the ecosystem quality especially organically polluted ecosystem. In addition, chironomids were also recognized as being capable to produce fast responses to the pollution. They produce responses through behaviour changes, ventilation rate or

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locomotion. Changes in organism behavior are among the fastest, easiest and sensitive indicators that can be used as a parameter for water pollution assessment (Gerhardt, 2000). The larvae of *Chironomus* spp. build and live in tubes composed of silk and assortment of substrate materials such as algae, detritus and sediments. Besides being associated with feeding and ventilation, tubes and tube-dwelling behaviour serve as anti-predation function (Hershey, 1987; Halpern et al., 2002). Recent studies indicate the potential of chironomids to react with pollutants and produce significant behaviour signals that can be extrapolated for biological monitoring purposes (Pascoe et al., 1989). This study was conducted to identify the best testing condition (inside the chamber) using chironomids as a biological indicator. Although from the same species, different individual could produce significant different behaviour in normal condition. Chironomids produce various activities such as swimming, looping, crawling and ventilation, which will be detected by the quadrapole as different patterns of signal. This could create problem in determining the most appropriate signal as regards the pollutants responses.

Materials and Methods

Chironomids were collected from natural habitat and cultured in the laboratory. Only third generation of culture were collected for taxonomic identification and being used for testing. Fourth instar chironomid larvae were sampled from fish pond in Bangi with a hand net. For species identification, the larvae were extracted using potassium hydroxide (KOH) 30% in digestor reactor Model DRB2000 and followed by neutralization with glacial acetic acid for 10 minutes. Morphological characteristics of chironomids were observed using microscope Model Olympus SZX9 and BX41 that were attached to a computer. Images were analyzed through Olympus Soft Imaging System software and identified to species level using Cranston (1995) and Wiederholm (1983) identification keys. Three species that were recognised are *Chironomus javanus*, *Chironomus kiiensis*, and *Chironomus bicoloris*. *Chironomus javanus* was selected for the study since it exhibits the most abundance.

For behavioural responses testing, the four instar *Chironomus javanus* was used. The larvae were kept in an aquarium in laboratory and sufficient oxygen was supplied using a water pump Model Aero-110. The multispecies freshwater biomonitor, MFB (eight measurement channels) connected to a computer was used to determine the behavioural responses of chironomid larvae (Figure 1). Quadrapole impedance conversion technique was applied. Movements of each tested larvae that was placed in a cylindrical test chamber alter the impedance across a pair of electrodes (opposite each other along the chamber walls). The other pair of non-current generating electrodes measured the changes in impedance.

The MFB has eight measurement chambers and each larval was placed in each chamber. The test was undertaken in normal condition (without a stressor) using dechlorinated water. The aim of the study is to identify the best test condition for stressor testing. Three different conditions were performed i.e., larval free swimming in

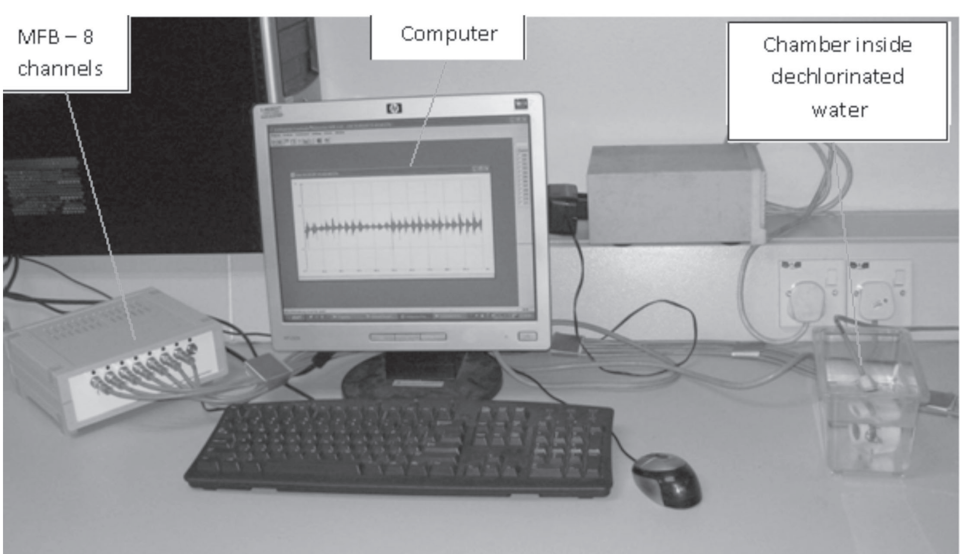

Figure 1: MFB connected to a computer (analysis unit).

the chamber; larval kept in their tube and located in the middle of the chamber, and larval kept in modified net (≤1 mm mesh size) and placed in the middle of the chamber.

Eight measurement chambers made of Plexiglas pipe (diametre: 2 cm, length: 5 cm, mesh width: 1 mm) were arranged in eight different aquariums filled with 800 mL dechlorinated water. Measurements were done at room temperature in laboratory and each chironomid larval was placed individually in different chambers for every measurement. The behavioural patterns of larval were recorded continuously for a period of two hours after 10 minutes of acclimation time (recording time: four minutes, interval time: six minutes). These patterns were plotted in electrical signal graphs (V) against time(s) that can be converted to frequency histogram (Hz) by Fast Fourier Transformation, FFT (Gerhardt, 2000). Data obtained were transported into excel numerical form and tested using univariate statistical method.

Results

Three species that were successfully identified are *Chironomus javanus*, *Chironomus kiiensis*, and *Chironomus bicoloris*. *C. javanus* was the most abundant species in the sampling area and was selected as the biosensor agent in this study.

From observation and with the help of the quadrapole conversion method, *C. javavanus* showed three types of behaviours, which are locomotion (swimming and looping), ventilation and inactivity (Figure 2). The noise level of the impedance converter was set for 20 mV. All signals below or the same with this level were considered as inactivity. Swimming was defined when chironomid wriggled with the whole body in open normal water (Gerhardt and Janssens de Bisthoven, 1995), within a range of 0.5–2 Hz and amplitude of about 200 mV. Looping was a highly multifrequent behaviour with amplitude ranging from 50 to 100 mV and kinematically distinguishable from swimming in open water. It usually occurs when a substrate is gripped by the prothoracic and abdominal pseudopods, and then attaching the head and moving the abdomen to a new place (Gerhardt and Svensson, 1994; Gerhardt and Janssens de Bisthoven, 1995).

Generally, larvae attached to a solid substrate at the bottom of the water perform ventilation. Larvae would also ventilate if it can hold on to a vertical surface by prothoracic or abdominal pseudopods (Brackenbury, 2000). This monofrequent undulations of the abdomen of chironomid produced frequencies of 2-3.5 Hz.

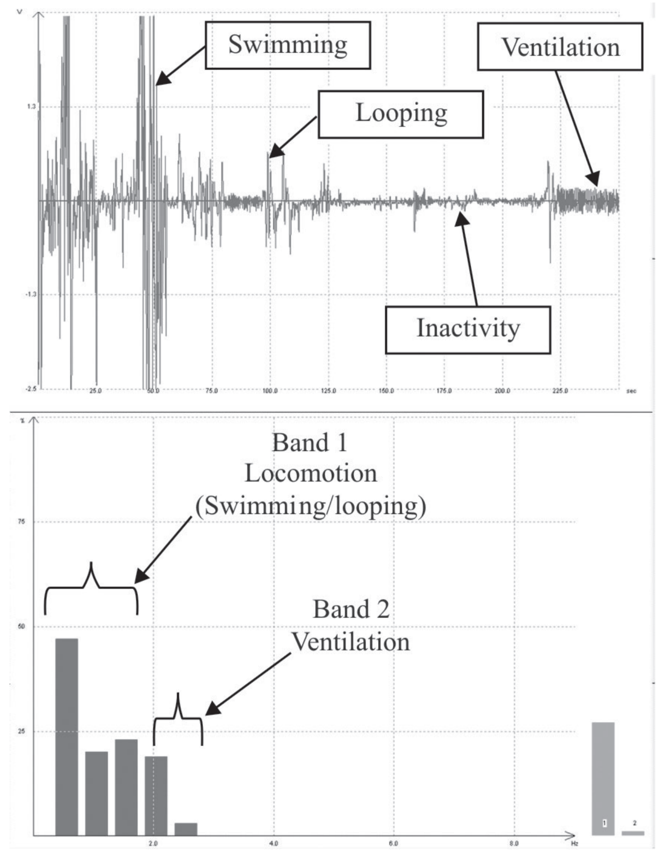

Figure 2: Characteristics of behavioural responses of *Chironomus javanus*.

Chironomids in Free Swimming Chamber

Chironomid from this chamber exhibits random movement and produced many variations in behavioural pattern (Figure 3). Swimming was the most common behaviour demonstrated by this larval. Chironomids are sessile organisms and need associates with substrate at most of the time. Since no substrate was provided in this chamber, the larval made the most variable movement compared to other two chambers. Swimming activity produce the largest amplitude followed by looping and ventilation. The FFT mode indicates that locomotion (low frequency) (band 1) has the highest percentage and ventilation produced was less than 20%.

Chironomids in Tube

Individual larval in this chamber demonstrates more consistent locomotion and ventilation in the amplitude mode compared to the first chamber (Figure 4). However, as regards the FFT histogram, individual placed in the modified net case exhibits approximately similar percentage (swimming and ventilation) as compared to the previous chamber.

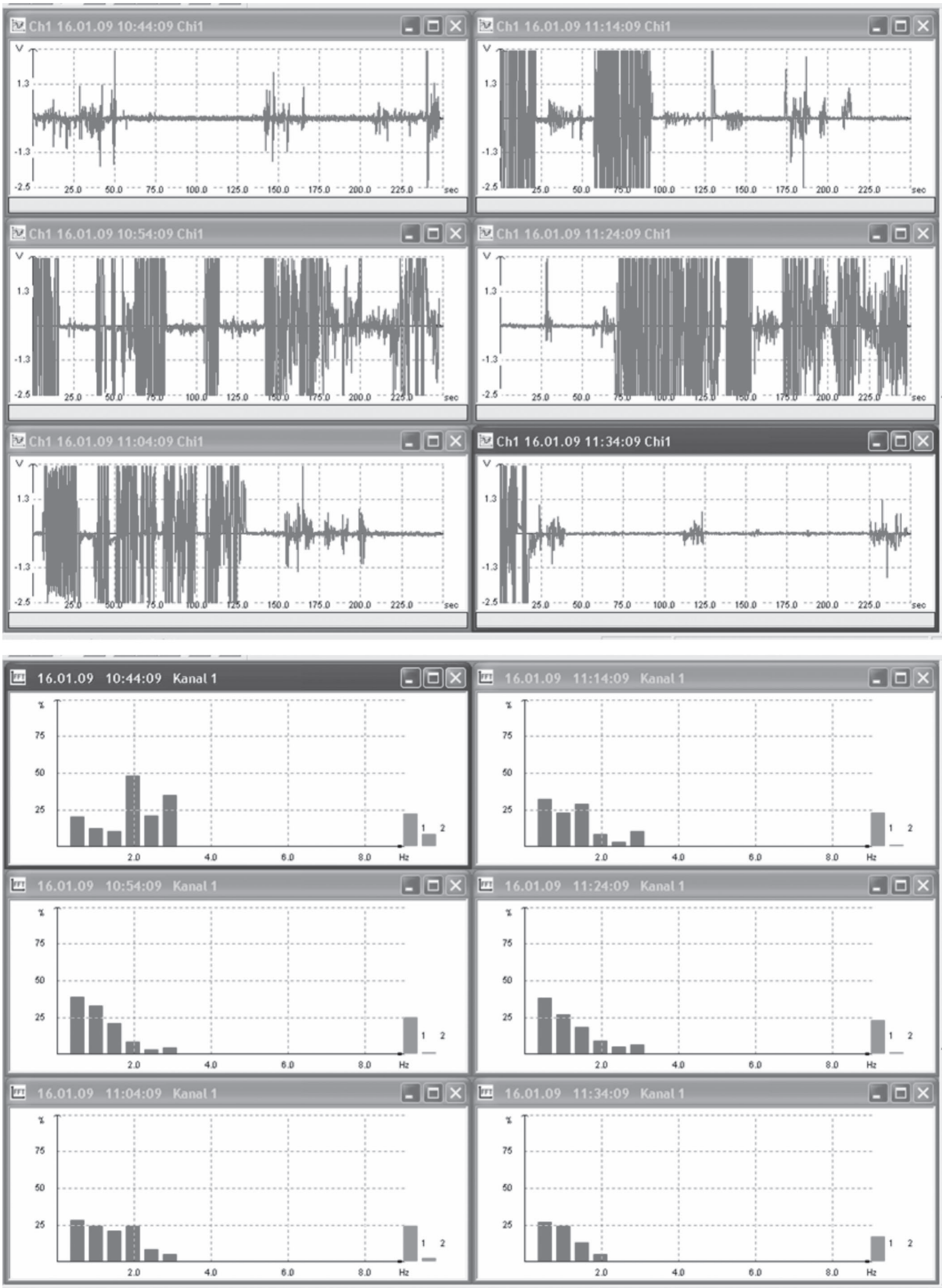

Figure 3: Examples of behavioural pattern of *Chironomus javanus* without tube: (above) signal graphs and (below) FFT histograms.

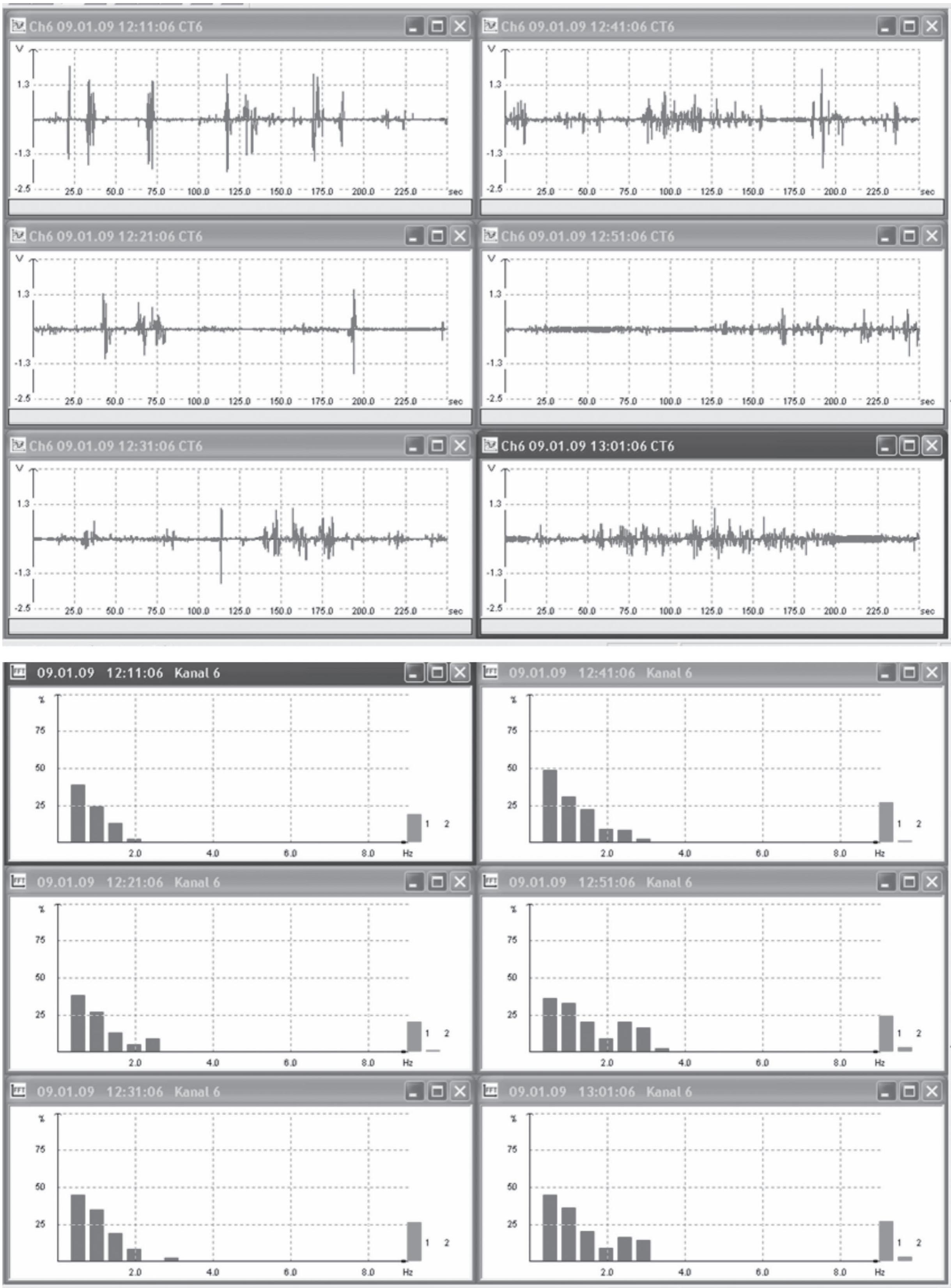

Figure 4: Examples of behavioural pattern of *Chironomus javanus* inside its tube: (above) signal graphs and (below) FFT histograms.

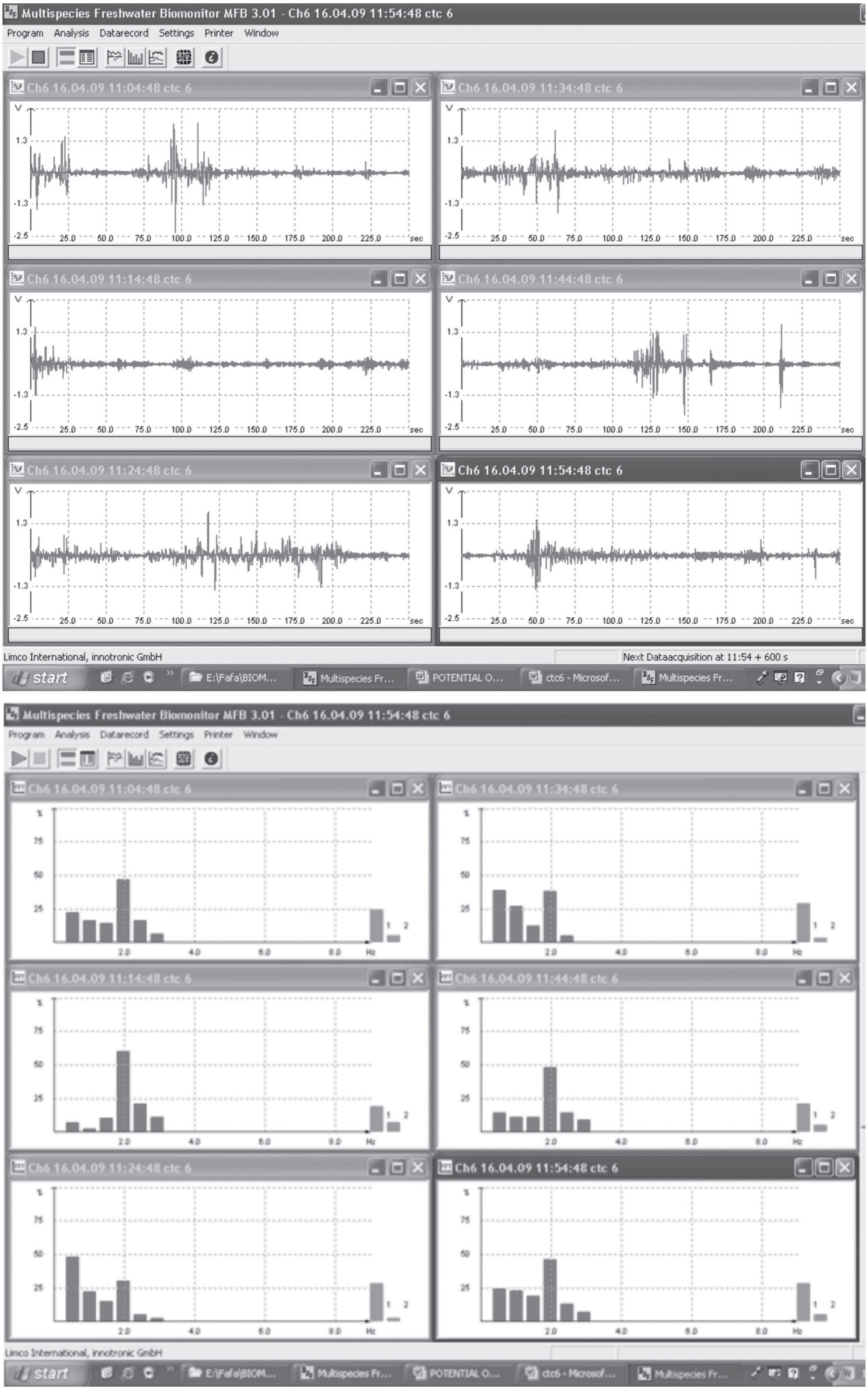

Figure 5: Examples of behavioural pattern of *Chironomus javanus* in tube and case: (above) signal graphs and (below) FFT histograms.

Chironomids in Tube and Case

The individual placed in tube in the modified net displays better amplitude compared to first chamber but almost similar with the second chamber (Figure 5). The whole behavioural pattern from the signal graph shows that this chamber produced less variation as compared to the first condition. Looping was defined as the most frequent behaviour compared to others.

Fewer variations in signal pattern were found in this condition. The FFT histograms also indicate that the frequencies produced by larvae movements were almost invariable by comparing the 10 minutes of recording time. The ventilation behaviour was found higher in this condition compared to other conditions. The minimum band for ventilation set was 2 Hz and was the highest in this chamber.

Discussion

Chironomus is one of the most diverse in tropical region and the most difficult in identification. *Chironomus javanus* was chosen as a biosensor agent because of their abundance and easy to be sampled. In addition, the sizes of 4th instar is quite large and suit the chamber used.

The behavioural responses showed three types of movements. The behaviours detected in this study were similar as reported by Gerhardt and Janssens de Bisthoven (1995). They use an impedance conversion method to show the behavioural pattern of chironomids. They characterized and compared the behavioural pattern of *Chironomus* gr. *thummi* from different polluted sites and deformed and non-deformed larvae. However, swimming frequencies (2.4 to 4.5 Hz) and ventilation frequencies (1.8 to 4.5 Hz) in their study was slightly different from ours probably due to the different sizes and species of larvae being used.

Brackenbury (2000) investigated the locomotory kinematics of *C. plumosus* and found three modes of motile activity, which were swimming, crawling and ventilation. The authors found that rhythmical ventilation behaviour (sinusoidal movement) has the same frequency as swimming behaviour (side-to-side flexure) provided it occurs in a plane that is parallel to the central plane of the sagittal suture. Swimming is an evolutionary development that resulted from pendular ventilation movements.

By visual observation, Cushman and McKamey (1981) indicated decreasing activity of *C. tentans* when exposed to acridine and quinoline. A decreased time spending spent on feeding and an increased time spent on ventilation in *C. riparius* upon exposure to Cd were found by Pascoe et al. (1989). As regards Cd exposure, increased ventilation and decreased activity were found in *Glyptotendipes pallens*, filter feeding chironomids sitting in their tubes (Heinis et al., 1990). The activity pattern of this species consisted of net spinning, pumping, elimination and inactivity behaviour.

High amplitudes in MFB test indicate locomotion activities such as swimming behaviour. However, in this study, in free swimming chamber, organisms frequently swim and tend to touch the electrodes of the chamber walls, that resulted in high amplitudes of the signal graphs. In these circumstances, the organisms may lie on the electrodes and the MFB still produce high amplitude; even the organism only did small movement or ventilate. This could produce false behavioural pattern of this organism. The presence of tubes in second condition test restricted the movements of chironomids from touching the electrodes and was good to prevent many variations of behavioural pattern in the signal graphs. Conversely, when chironomid being outside from its tube, it also held on to the electrodes of the MFB chamber. In order to avoid this problem, the net mass size used to build case must be sufficiently small to retain chironomids in the case to prevent chironomid from contact with those electrodes.

The third chamber (chironomids in chamber and tube) demonstrated the best signal in amplitude mode. The chironomids were left overnight in different beakers to allow them to build their tube. Chironomini is case maker and they build with any materials provided. In this study, chironomid samples were supplied with very fine sand and they built the tube overnight. There were numerous low amplitudes found because chironomid larvae were resting and only moved in their tubes within the cages. Even though the larval were found outside of the tube, it still did not touch the electrodes. Being outside a tube is considered when head, thorax and at least one abdominal segment are extended from a tube of chironomid (Hershey, 1987). Both data were tested for their normality using Kolmogorov-Smirnov test and band 1 data demonstrate the normal distribution, whereas band 2 data were not normal and been transformed using square root transformation. One-way ANOVA test was carried out to estimate their differences. Result indicates that only band 1 has a significant difference between tests ($p = 0.00$, $\alpha = 0.05$). The Tukey Post-Hoc test demonstrates that only third chamber (chironomid in tube and case) has a significant difference between other two tests. This explains that signal from third chamber (chironomid sample in tube and case) significantly differ from others ($p < 0.05$, $\alpha = 0.05$). As a result, signal produced by chironomids placed in tube and case are better and more constant. This will aid in determining variation of chironomids behaviour when chironomids are exposed to the stressor especially in amplitude mode.

Conclusion

C. javanus provided better behavioural pattern when tested in the tube placed in the case net compared to the naked plain chamber. Apart from that, Chironomini tribe is known as tube makers to facilitate feeding and respiration, as anti-predation shelter and also serves as chemical protector. This study concludes that by providing tube in the chamber, MFB produces better amplitude signal thus tube should be provided to chironomids in the next studies relating to the behavioural responses using MFB. The condition of chironomid sitting inside its tube, that was placed in a case, was the best condition to be chosen as a general signal pattern for behavioural biomonitoring test. This pattern can be used to compare between normal conditions (water without stressor) and with water that has been polluted.

Acknowledgement

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