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GILL VENTILATION RATES OF MAYFLY NYMPHS (EPHEMEROPTERA: HEPTAGENIIDAE) AS A BIOMONITORING TECHNIQUE

Richard A. MacKenzie[1] and Jerry L. Kaster[2]

ABSTRACT

Gill ventilation frequency (GVF) of the mayfly nymph *Stenacron interpunctatum* (Ephemeroptera: Heptageniidae) was studied to assess the applicability of a relatively simple, real time video methodology and to assess the potential of GVF rates for use in a chronic assay of sediment pore water. *Stenacron interpunctatum* nymphs were exposed to pore water samples taken along a transect from the mouth of the Fox River to Sturgeon Bay in the Green Bay area of Lake Michigan. This transect has previously been shown to exhibit several distinct gradients in sediment and water column conditions with distance from the Fox River. The highest GVF value of 6.68 ± 0.27 Hz was observed in pore water from the more polluted area near the Fox River. A lower GVF value of 5.44 ± 0.32 Hz was observed in pore water from the station near Sturgeon Bay and of 4.25 ± 0.27 Hz from the cleaner Lake Michigan station. GVF values exhibited a decreasing trend with relative distance from the mouth of the Fox River ($r^2 = 0.76$).

Invertebrates have been used in bioassays and as biomonitors over the past two centuries to determine water quality in aquatic and marine communities. These techniques include both traditional *in vitro* experiments examining mortality rates of invertebrates (e.g. LC_{50}s) (Anderson 1980, Maciorowski and Clarke 1980) and *in situ* taxonomic level biotic indices (Hawkes 1979, Hilsenhoff 1982). Although these procedures are widely accepted, certain problems may be overlooked when they are used.

Mortality tests generally determine the acute lethal dosages or effects of "biostressors", substances (e.g. organic pollutants, toxins) that cause biological organisms stress. The lethal dosage level is often represented by various endpoints such as immobility or cessation of vital activity since "death" is difficult to determine in invertebrates. Immobility, however, may occur at lower concentrations then death does, resulting in an overestimation of the effects the biostressors being studied are having on invertebrates. Furthermore, the exposure time in these tests is usually less than 100 hours. Any chronic lethal or sub-lethal effects are thus ignored as biostressors may affect invertebrates long after their exposure period (Maciorowski and Clarke 1980).

Biotic indices examine the community structure at a given point in time, determining to what degree the system is disturbed based on the presence or absence of pollution tolerant or intolerant organisms. This type of biomoni-

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toring also focuses on acute effects of biostressors and may ignore any chronic lethal or sub-lethal impacts starting to occur at the time of study. Furthermore, due to the contagious distribution of invertebrates in riverbeds, accurate sampling of organisms requires a number of samples, which may be impractical on a routine basis (Elliott 1973).

Although the presence of biostressors may not be immediately lethal, they could cause chronic lethal or sub-lethal stress in the organisms, which over a period of time, could impact the community. Stress can be measured through the use of simple, rapid quantitative bioassays that employ the combination of some physiological attribute or behavioral pattern with physical-chemical tests and result in the quantitative chronic measurement of biostressors. Physiological attributes previously used include protozoan ciliary movement (Bovee 1975), mussel byssal thread production (Roberts 1975), and reproduction in microcrustaceans (Sprague 1976). Behavioral patterns include stonefly ventilation "push-ups", helgrammite gill pulsation rates (Maki et al. 1973), burrowing ability of bivalves (Abel 1975), and filtration rates in blue mussels (Stirling 1975).

This study reports a simple, rapid bioassay designed to measure the gill ventilation frequency rates (GVF) of mayflies as an indication of stress that might result from the presence of contaminants (i.e., PCBs, Hg, Cu). The approach was to assess the applicability of a relatively simple real time video methodology for determining GVF, and to assess the potential of GVF rates for use in a chronic assay of sediment pore water.

Stenacron interpunctatum (Ephemeroptera: Heptageniidae) (Say), was chosen as the test organism for this study due to its wide distribution in lentic and lotic waters (Merritt and Cummins 1996) and its large, visible abdominal gills (Fig. 1). These mayflies are also considered to be a pollution (i.e., organic loading) tolerant organism in biotic indices (Hilsenhoff 1982, Hilsenhoff 1988), which suggests that, in the presence of a biostressor, the outcome might not be lethal, but could result in an increase of gill ventilation rates due to stress. If this were indeed the scenario, this increase in gill ventilation would result in an increase in energy expenditure, which could impact the organism's overall fitness with time.

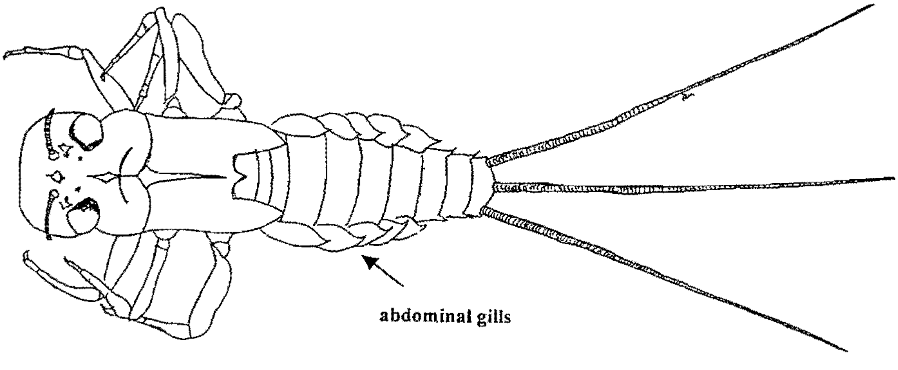

Figure 1. *Stenacron interpunctatum* mayfly nymph.

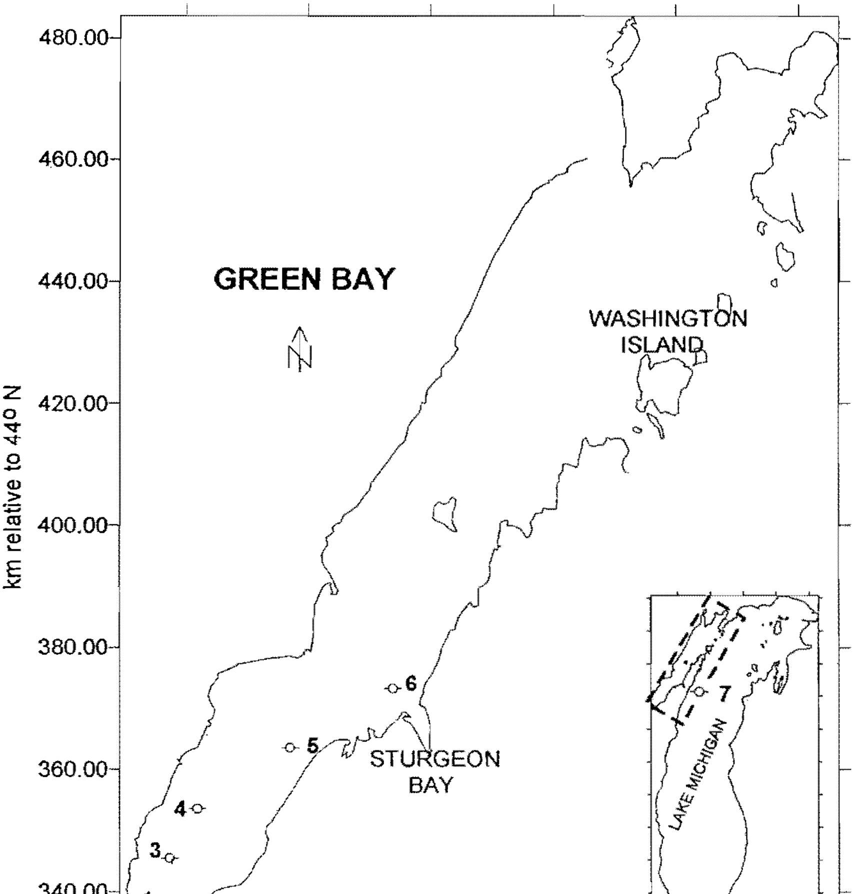

Figure 2. Green Bay map showing Green Bay and Lake Michigan sediment sampling stations from 1992 cruise.

MATERIALS AND METHODS

Research site. Green Bay, the largest freshwater estuary in the Laurentian lakes, is situated in the northwest corner of Lake Michigan (Fig. 2). The major fluvial system draining the Green Bay watershed is the Fox River, which flows into the bay's southern-most portion. Over the past 100 years, the Fox River has experienced an increase in agriculture, logging, and indus-

Table 1. Physical parameters of bottom water from Green Bay and Lake Michigan stations and relative distance from the mouth of the Fox River.

Station	Depth from surface (m)	Temp. °C	Dissolved oxygen (mg/L)	Distance from Fox R. mouth (km)
1	4	16.8	5.0	15.3
2	5	16.9	5.4	16.6
3	6.5	16.4	9.0	26.6
4	9	16.0	8.9	35.5
5	15	na	na	53.0
6	25	7.5	8.0	68.9
7	95	3.5	12.4	100.1

try (particularly paper mills) which has converted it into one of the most heavily developed and industrialized river valleys in the United States. As a result, the Fox River supplies nearly 70% of the total suspended sediment and nutrient load to Green Bay (Harris and Christie 1987). The consequence is a concentration of material in the southern portion of the bay leading to deterioration of water quality, contamination by PCB's and other wastes, and depletion of oxygen. These conditions, heavily influenced by the Fox River, have led to gradients in water column and sediment conditions as well as in biotic interactions. Several distinct gradients can be seen from the southern, hypereutrophic portion of the bay, to the northern, oligotrophic portion of the bay. These gradients include decreasing levels of pelagic primary productivity (Waples 1998), increasing concentrations of dissolved oxygen in the lower water column, increasing levels of water clarity (WDNR 1993), and decreasing concentrations of PCBs and other toxins found within the sediments (Sullivan and Delfino 1982, Hermanson et al. 1991, WDNR 1993, Manchester-Neesvig et al. 1996). We hypothesized that gill ventilation rates of mayfly nymphs exposed to Green Bay pore water would exhibit a similar gradient, with higher rates occurring in pore water samples collected closer to the mouth of the Fox River.

Pore water sampling and handling. Sediment samples were collected from stations on Green Bay (Table 1.) during a 5–day cruise during the summer of 1992 aboard the *RV Laurentian*. Single ponar grab samples were collected from stations along a transect from the mouth of the Fox River to Sturgeon Bay (Fig. 2). An additional sample was collected from a deep-water station in Lake Michigan and was used as a control (Table 1, Fig. 2). Bottom water temperature, depth, and dissolved oxygen were also measured at each station using a SBE 25–01 Sealogger CTD (Table 2).

Sediments were kept refrigerated at approximately 3°C until they were returned to the lab 10 days later and pore water was extracted using a small-volume, low pressure, diaphragm-type sediment squeezer (Robbins and Gustinis 1976). Approximately 20 mL of pore water was collected from each sample and kept at approximately 3°C until the analysis. It should be noted that, although dissolved oxygen concentrations in the pore water samples were never directly measured during the experiment, anaerobic water at 3°C reaches 80% oxygen saturation within 8 h of exposure to atmospheric oxygen based on Fick's first law (Hutchinson 1975, Stumm and Morgan 1996). Thus, it can be assumed that the pore water samples had sufficient time to equili-

Table 2. Mean gill ventilation frequencies (GVF)(1 SE) from observations (n = 5) of mayfly nymphs exposed to Green Bay and Lake Michigan pore water.

Station	GVF (Hz)
1	6.68 ± 0.27
2	5.77 ± 0.28
3	5.66 ± 0.32
4	5.64 ± 0.29
5	4.97 ± 0.32
6	5.44 ± 0.32
7	4.25 ± 0.27

brate with atmospheric levels of oxygen by the time the analysis took place seven days later.

Ephemeropteran sampling and handling. *S. interpunctatum* mayfly nymphs were collected in June of 1993 from the undersides of rocks in Genesee Creek, a second order stream located in Waukesha County, Wisconsin. The majority of the nymphs collected were 3rd and 4th instars, which were preferred due to their larger, abdominal gills (Fig. 1). The mean body length (head to tip of median caudal filament) of the nymphs was 1.5 ± 0.4 cm, with a range of 0.8 cm to 2.8 cm. Specimens were transported in coolers back to the laboratory, where they were acclimated in an artificial environment tank for 4 days. The tank consisted of laboratory-dechlorinated water continually flowing through an aquarium filled with rocks and sand from Genesee Creek. The water temperature of the holding tanks was kept constant at 15 °C and the velocity was similar to that of the creek's (0.5–0.7 m/s). A constant day/night photoperiod was maintained using an automated lighting system. Feeding occurred daily which consisted of ground up alfalfa pellets. Nymphs were kept here the entire duration of the experiment, which lasted a total of 8 days (4 days of acclimation plus 4 days of videotaping).

Exposure of heptageniid mayflies to pore water. Five replicate exposures were made for each of the seven pore water samples obtained from Green Bay and Lake Michigan, for a total of 35 exposures. A different nymph was used for each exposure, for a total of 35 nymphs. Nymphs were randomly selected from the environmental holding tank and allowed to acclimate for 5 min to the pore water inside a specially designed, temperature controlled containment chamber (Fig. 3). The acclimation period of 5 min was chosen since the majority of the mayfly nymphs appeared to "calm" down (e.g., relaxed gill ventilation, less movement) after this time. The containment chamber was constructed out of a 2 cm high, 3.5 cm internal diameter (ID) acrylic pipe attached to a 5 cm by 5 cm by 0.5 cm acrylic base. The chamber was then nested in a temperature control vessel that was a 4.5 cm high, 11.5 cm ID PVC dish in which chilled water was continually pumped through. The experimental temperature control vessel maintained the pore water temperature in the containment chamber at a steady 14.4 ± 0.5 °C, similar to that of the environmental holding tank.

After the acclimation period of approximately 5 min, each organism was videotaped for 2 min. The entire apparatus and test specimens were viewed using videomicroscopy, which consisted of a camcorder mounted above a dissecting microscope (Fig. 3), and gill ventilation frequency (GVF) was recorded. The data were later analyzed in slow motion to determine the ven-

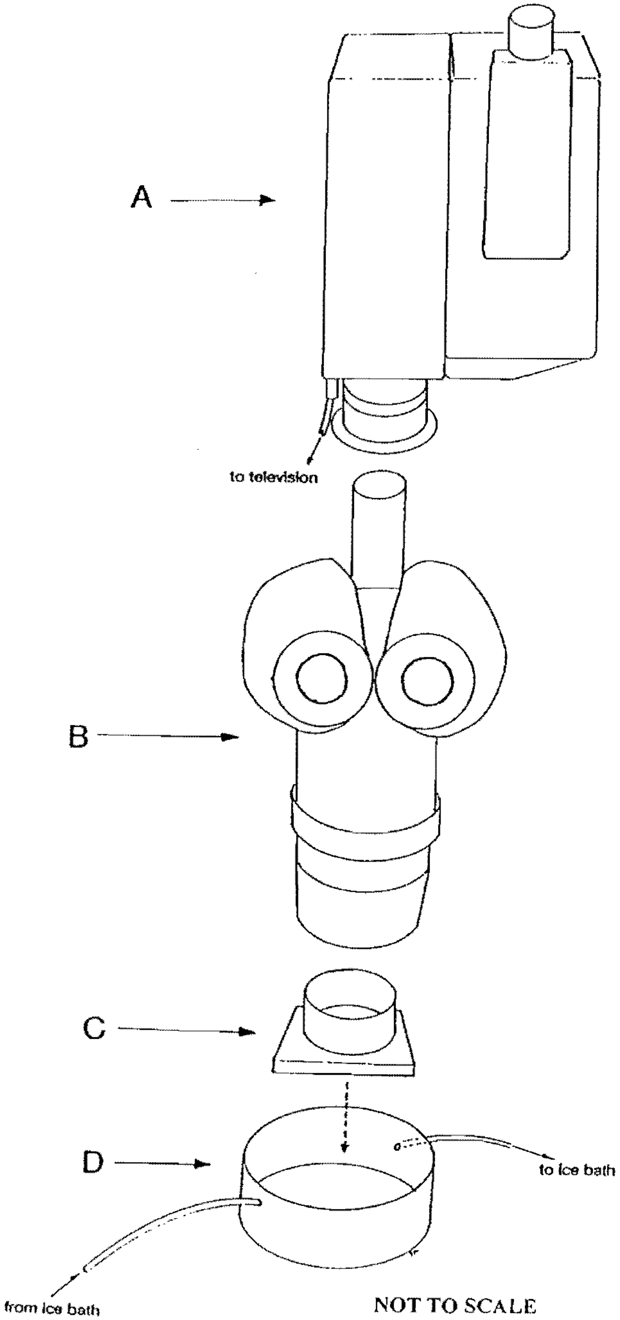

Figure 3. Videomicroscope apparatus: A. Camcorder, B. Dissecting microscope, C. Pore water containment chamber, D. Temperature control vessel.

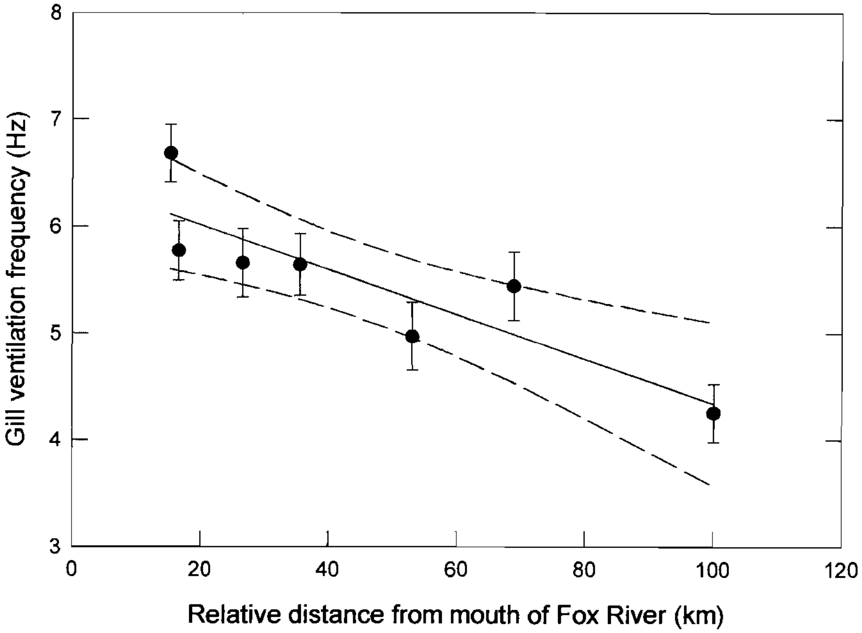

Figure 4. Linear regression ($r^2 = 0.76$) of mean gill ventilation frequencies for *Stenacron interpunctatum* mayfly nymphs ($n = 5$) exposed to pore water from Green Bay and Lake Michigan versus relative distance pore water was collected from the mouth of the Fox River. Bars represent ±1 SE of the mean and the dotted line represents a 95% confidence interval.

tilation rate and then extrapolated to real time (11.37 s of slow time = 1.0 s real time). Estimates of GVF for each nymph were made at four random two-minute intervals. Twenty observations (4 intervals by 5 replicates) were made for each pore water sample.

Statistical analysis. The mean GVF was determined for each station and reported as cycles per second (Hertz) (Table 3). A linear regression was then performed using Sigma Plot version 4, with relative distance of the stations from the mouth of the Fox River (Table 1) as the independent variable and mean GVF ($n = 7$) as the dependent variable (Fig. 4). The relative distance of station 7 in Lake Michigan was measured as the distance from the mouth of the Fox River to station 6 plus the distance from station 6 to station 7 through the Sturgeon Bay channel (Fig. 4).

RESULTS

Gill ventilation frequency rates of *S. interpunctatum* mayfly nymphs placed in sediment pore water collected from each of the seven stations in Green Bay and Lake Michigan exhibited a decreasing trend ($r^2 = 0.76$, $P < .001$) with distance from the mouth of the Fox River (Fig. 4). GVF rates in pore water collected from the Green Bay stations ranged from 5.44 ± 0.32 to 6.68 ± 0.27 Hz with the highest GVF observed from the station nearest the mouth of the Fox River in the southern portion of Green Bay.

The single sample from station 7 in Lake Michigan had a somewhat lower GVF than those from Green Bay. The 4.25 ± 0.27 GVF value obtained from mayflies exposed to Lake Michigan sediment pore water proved to be lower than values from Green Bay pore water, as was expected. The difference between the highest pore water GVF (at the mouth of the Fox River) and the lowest pore water GVF (from Lake Michigan) was 2.43 cycles per second (Fig. 4).

DISCUSSION

Exposure of heptageniid mayflies to pore water. Our results show a definite gradient of gill ventilation frequency in nymphs exposed to pore water collected from a system that is known to also exhibit several gradients. The exact stressor or variable responsible for this GVF gradient is unknown, but, assuming that oxygen levels in the containment chamber were maintained at a constant concentration, the pattern observed strongly suggested the presence of some "biostressor". Furthermore, the GVF gradient observed coupled nicely with gradients previously observed in Green Bay water column and sediment conditions from the nutrient rich, hypereutrophic regions of the southern bay to the oligo-trophic conditions of the northern bay. These gradients included an increase in bottom water dissolved oxygen concentrations with distance from the Fox River observed during the 1992 *RV Laurentian* sampling cruise (Table 1.).

The observed GVF suggests that a biostressor present in the system may have chronic lethal or sub-lethal affects on the organisms, which aren't detectable through the use of a biotic index. These affected organisms could suffer lowered fitness over time or they might overcome the stress by adapting to it. An example of the latter might be found in the positive correlation found between gill size and stressful environments (Pescador and Rasmussen 1994). Perhaps the increased gill size of organisms in pollution stressed environments could have resulted from an adaptation that has occurred over time.

A few assumptions were made with this technique and should be addressed. First, post-collection, pore water dissolved oxygen concentrations were measured in preliminarily exposures (9.7 mg/L). However, problems arose during videotaping due to the probe's interference with the videomicroscope apparatus. Continued use of the probe required assembling and disassembling the apparatus, which was time consuming. Since the goal of this experiment was to devise a rapid bioassay, the determination of dissolved oxygen was discontinued. Furthermore, the amount of time required for anaerobic pore water to equilibrate with atmospheric concentrations was determined for the volume extracted, the temperature the sample was kept at, and the surface area of the container using Fick's first law. By the time of the first exposure (7 days after pore water extraction), the samples were near, if not, saturated with oxygen. Second, laboratory experiments, like this one, tend to induce stress on the organisms when their ecological requirements aren't properly met. Several steps (e.g., flow, substrate, and oxygen) were taken to simulate the organisms' natural habitat. Nevertheless, it is still quite possible that stress was induced on our test organisms. Since each treatment was carried out exactly the same using several replicates (5) for each sample, it was highly unlikely that the observed trends reported here were an artifact, but represent a definite relationship between stress, gill ventilation rates, and the presence or absence of some biostressors.

Gill ventilation rate, a quick and easily determined metabolic sign of stress, proved to be a promising alternative to conventional bioassay and biomonitoring techniques. Although in this experiment it did not identify any particular biostressor present, it appeared to be useful in a system with many variables present that may have chronic lethal or sub-lethal effects on organisms. Further research may reveal that this simple, rapid approach may not only be useful in bioassays determining lethal concentrations or effects of biostressors, but it might also be a useful biomonitoring tool when coupled with traditional techniques.

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