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STUDY ON THE UTILITY OF CHIRONOMID DEFORMITIES IN THE ASSESSMENT OF WATER QUALITY

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ABSTRACT

A study was made of the utility of chironomid deformities in the assessment of water quality in the Yamaska River, Quebec.

Station 15, at Val Shefford, and stations 9 and 12 downstream from urban centres (Acton-Vale and Ste. Hyacinthe, respectively) are more environmentally stressed areas based on the frequency of chironomid deformities. The occurrence of higher frequencies of deformities below the urban centres (stations 9 and 12) lends credence to the use of deformities in the assessment of environment quality. However, the finding of deformed larvae in areas impacted upon by industrial effluents, municipal effluents and agricultural (pesticides) inputs as well as in control areas points to the lack of specificity in the response of deformities to environmental stressors. Also, with the low frequency of deformities found at most stations (i.e., <3%), in the present study, it is difficult to either rate areas according to environmental stress or to clearly differentiate between stressed areas and more natural clean areas.

At low frequencies of deformities, the use of deformities to assess water quality is probably not economically practical with respect to other bioassay methods, i.e., the cost and time involved in sampling, sorting, mounting, identifying, and scoring specimens for deformities would place costs at approximately $600 per station based on 200 specimens per station and $25/hour for labour for a <u>trained</u>

biologist. However, in such cases the use of deformities in combination with invertebrate community structure may be a more meaningful and economically practical (it costs little to score for deformities once all the other work of mounting and identifying the species is done) means of assessing environmental quality. That is, changes at the organism level, as indicated by deformities, might be more useful than changes at the community level for purposes of environmental monitoring because individual responses occur before community responses and, therefore, may provide an early warning of contaminant stress.

RÉSUMÉ

L'utilité de l'examen des difformités des chironomidés comme instrument d'évaluation de la qualité de l'eau de la rivière Yamaska au Québec, a été étudiée.

Selon la fréquence des difformités observées chez des chironomidés, la station 15 à Val Shefford et les stations 9 et 12, en aval de centres urbains (Acton-Vale et Saint-Hyacinthe, respectivement) sont situées dans des secteurs soumis à de plus grands stress du milieu qu'ailleurs. La fréquence supérieure des difformités en aval des centres urbains (stations 9 et 12) donne du poids à cette technique pour l'évaluation de la qualité du milieu. Cependant, le fait de trouver des larves difformes dans des secteurs exposés à des effluents industriels, des effluents municipaux et des apports d'origine agricole (des pesticides) ainsi que le fait d'en trouver dans des stations témoins révèlent le manque de spécificité de cette réaction à des stress environnementaux. En outre, compte tenu de la faible fréquence des difformités trouvées à la plupart des stations (c.-à-d. moins de 3 %), dans la présente étude, il est difficile de coter les facteurs selon des stress du milieu ou de faire nettement la distinction entre des secteurs soumis à des stress et des secteurs moins pollués.

Lorsque les difformités sont observées en faibles fréquences, le recours à ce moyen pour évaluer la qualité de l'eau n'est probablement pas économique si on le compare à d'autres méthodes de bioessais;

c'est-à-dire que les dépenses et le temps requis pour l'échantillonnage, le tri, les préparations, l'identification et le classement des spécimens par rapport aux difformités seraient tels qu'il en coûterait environ 600 $ par station à supposer qu'il y aurait 200 spécimens par station et qu'on aurait un biologiste formé à l'emploi au taux horaire de 25 $. Cependant, lorsque ce serait possible, le recours à l'examen des difformités en combinaison avec l'examen de la structure des communautés d'invertébrés pourrait constituer une méthode d'évaluation de la qualité du milieu qui soit à la fois significative et réalisable à bon coût (le classement par les difformités coûte peu une fois que les autres tâches de préparation des spécimens et d'identification des espèces sont faites). C'est-à-dire que des changements au niveau des organismes tels que révélés par des difformités peuvent se révéler plus utiles que des changements au niveau des communautés lorsqu'on exerce une surveillance du milieu car des réponses individuelles peuvent apparaître avant des réponses des communautés; ces tests pourraient servir de préalerte en cas de stress par des contaminants.

MANAGEMENT PERSPECTIVE

At low frequencies of deformities, the use of deformities to assess water quality is probably not economically practical with respect to other bioassay methods, i.e., the cost and time involved in sampling, sorting, mounting, identifying, and scoring specimens for deformities would place costs at approximately $600 per station based on 200 specimens per station and $25/hour for labour for a <u>trained</u> biologist. However, in such cases the use of deformities in combination with invertebrate community structure may be a more meaningful and economically practical (it costs little to score for deformities once all the other work of mounting and identifying the species is done) means of assessing environmental quality. That is, changes at the organism level, as indicated by deformities, might be more useful than changes at the community level for purposes of environmental monitoring because individual responses occur before community responses and, therefore, may provide an early warning of contaminant stress.

PERSPECTIVE-GESTION

Lorsque les difformités sont observées en faibles fréquences, le recours à ce moyen pour évaluer la qualité de l'eau n'est probablement pas économique si on le compare à d'autres méthodes de bioessais; c'est-à-dire que les dépenses et le temps requis pour l'échantillonnage, le tri, les préparations, l'identification et le classement des spécimens par rapport aux difformités seraient tels qu'il en coûterait environ 600 $ par station à supposer qu'il y aurait 200 spécimens par station et qu'on aurait un biologiste <u>formé</u> à l'emploi au taux horaire de 25 $. Cependant, lorsque ce serait possible, le recours à l'examen des difformités en combinaison avec l'examen de la structure des communautés d'invertébrés pourrait constituer une méthode d'évaluation de la qualité du milieu qui soit à la fois significative et réalisable à bon coût (le classement par les difformités coûte peu une fois que les autres tâches de préparation des spécimens et d'identification des espèces sont faites). C'est-à-dire que des changements au niveau des organismes tels que révélés par des difformités peuvent se révéler plus utiles que des changements au niveau des communautés lorsqu'on exerce une surveillance du milieu car des réponses individuelles peuvent apparaître avant des réponses des communautés; ces tests pourraient servir de préalerte en cas de stress par des contaminants.

INTRODUCTION

The presence of pesticides and other man-made contaminants in the aquatic environment has increased due to agricultural practices and industrial development. For example, the International Joint Commission (IJC) has reported the measured presence of more than 1,000 different industrially-produced chemicals in the Great Lakes ecosystem (Royal Society of Canada and National Research Council, 1985). About 300 toxic chemicals have been found in tissues of Great Lakes fish. Only a few toxicants are examined yearly, and there are probably many substances for which analytical techniques do not yet exist, or whose presence in the ecosystem is not suspected (Shear, 1981).

Sediments are a well-known sink for toxic substances. The evaluation of sediments has traditionally been by bulk chemical characterization (Munawar et al., 1985). However, the analytical chemical approach fails to address the issue of biological significance (Lee, 1980). Sediment-bound toxicity information, based on bioavailability instead of bulk chemical characterization, is badly needed for the management of both water and sediment quality (Munawar et al., 1984).

The effect of a pesticide or contaminant on the biological community has generally been assessed on the basis of acute lethality tests conducted in the laboratory. Although this provides useful information concerning the toxicity of a chemical, these tests have little relevance under field conditions. In comparison, field studies which measure changes in community structure or growth and reproduction of organisms in the field are more informative, but they are expensive and time-consuming and, therefore, are rarely carried out. Other assays are required to determine sublethal toxicant-induced stress, studies that are more sensitive and less expensive and time-consuming than monitoring changes in community structure.

Since chironomids spend most of their larval stages in surface sediments, they are exposed to toxicants present both in water and in sediments for prolonged periods through physical contact and ingestion. Their relatively sedentary foraging behaviour ensures that their home range is restricted to localized areas. This, together with a short life cycle, e.g., ten or more generations per year in the laboratory, makes chironomid larvae an ideal bioassay organism. In addition, they have been extensively used as test organisms in a variety of toxicity assessments, and a number of investigations have shown that chironomids may exhibit structural deformities in response to pollution (Hamilton and Saether, 1971; Hare and Carter, 1976; Koehn and Frank, 1980; Warwick, 1980a, b, 1985; Warwick _et al_., 1986).

Deformities include aberrations of mouthparts and antennae, heavy pigmentation of the head capsule and thickening of the head capsule and body wall. These deformities have been reported in _Chironomus_, _Microspectra_, _Procladius_, _Protanypus_, _Stictochironomus_, and an unidentified tanytarsine. Generally, it has not been possible to designate specific causative agents with certainty, but agricultural and industrial pollutants have been implicated. Cushman (1984) reported that coal liquid caused mentum deformities in Chironomus decorus, whereas Simpson (1980) found that chlorine and crude oil both caused stoneflies (Plecoptera) and caddisflies (Trichoptera) to have deformed trachael gills. In the Cushman (1984) study, the occurrence of deformities on the medial portion of the mentum was dose-dependent; however, occurrence of all deformities was independent of dosage. In the laboratory, a dose-dependent response in number of deformities was observed in Chironomus tentans exposed to a known genotoxicant, ethyl methane sulphonate (Hart et al., 1987) and C. decorus exposed to copper (Kosalwat and Knight, 1987).

The present study investigated the use of morphological deformities in benthic invertebrates, with emphasis on the Chironomidae, to assess the water quality of the Yamaska River, Quebec. In addition, cultured chironomid larvae were exposed to suspect sediments in the laboratory to determine whether the sediments induced deformities.

Study Site

The Yamaska River is located in southeastern Quebec between 45°15' and 46°05' latitude and 72°12' and 75°07' longitude about 70 km east of Montreal. The Yamaska River is a tributary to Lake St. Pierre, which is an enlargement of the St. Lawrence River, and drains an area of 5000 km^2. Tessier et al. (1980) reported a mean annual flow of 125 m^3/s at its confluence with the St. Lawrence River in 1975.

The source of the Yamaska River is in the Appalachian Mountains where Ordovician, Silurian and Devonian sedimentary and metamorphic (e.g., schists, sandstone and quartzite) rocks are prevalent, along with glacial and fluvioglacial deposits (at greater than 200 m altitude) and marine calcareous clays (at less than 200 m) (Tessier _et_ _al_., 1980). Lower reaches of the Yamaska River flow through the St. Lawrence lowlands where Ordovician and Cambrian sedimentary rocks (e.g., shales, calcareous shales and sandstone) are found under a thick layer (up to 70 m) of unconsolidated deposits of glacial tills and marine clays. The relief of the St. Lawrence lowlands is almost non-existent except for the Monteregian Hills. For example, Saint-Pie (Figure 1) is 40 m above sea level. In contrast, the relief of the Appalachians is varied and mountainous as is the Monteregian Hills, through which the Yamaska, Shefford and Brome Rivers flow, with a maximum elevation of 207 m for Mont Shefford. The average slope of the Yamaska River is 1.13 m/km (Desmeules and Gelinas, 1977).

According to Kloeppen's classification scheme, the climate of the basin is a boreal forest climate, humid with mild summers. Mean temperatures are above 5°C for five months of the year, with mean temperatures ranging from between 21°C in July to -11°C in January. Mean annual precipitation in the basin is 994 mm. Precipitation varies with relief. For example, the June, July and August monthly precipitation generally varies from about 80 mm in the area near the river mouth to 120 mm in the higher lands in the southeast (Quebec Ministere des Richesses Naturelles, 1979).

Agriculture is the primary land use in the Yamaska River basin. About 53% of the land area is in agriculture, 42% in forest, 2% in urban areas and 3% in other uses (Desmeules and Gelinas, 1977). Corn and hay/alfalfa are the principal crops grown in the basin. A breakdown of agricultural land use in the Yamaska River basin is presented by Metcalfe (1987a).

METHODS AND MATERIALS

Field Study

Samples were collected from various sites in the Yamaska River watershed representing areas impacted upon by agriculture (e.g., pesticides), industrial effluents, sewage effluents as well as from relatively natural areas (control stations). Samples were collected from June 9 to June 11 and from July 7 to July 9, 1988. In addition, site 15 was sampled in August (by K. Day and J. Metcalfe). Difficulties were experienced in collecting samples in June due to flooding conditions and samples were collected only from those sites (seven stations) that could be sampled safely. In the case of site 30, an alternate location farther upstream was sampled due to the high discharge conditions. In July, samples were collected during a period of low flow and 12 stations were sampled.

A D-shaped net was used to collect kick samples from riffle areas. In areas of slow flow sediment (silt) was sampled by stirring up the substrate by foot and passing the net through the suspended material or by dragging the net through surface sediments. Material collected in the net was placed in an enamel tray and examined for invertebrates. Usually the samples were partially sorted for invertebrates at the study sites with additional samples being collected, preserved in formalin and sorted later in the laboratory. An effort was made to collect at least 200 chironomid larvae and other more common taxa, such as Hydropsyche larvae and oligochaetes from each site. Sampling effort at each site varied and was inversely proportional to invertebrate populations at the site.

In the laboratory, more common taxa, particularly chironomids and to a lesser extent hydropsychid caddisflies and oligochaetes, were sorted by eye from the samples. An effort was made to sort 200 specimens of each of these taxonomic groups per sample site. Samples that initially yielded less than 200 chironomids were resorted under a dissecting microscope. This resulted in early instar chironomid larvae being included in the sample.

Chironomids were cleared in 8% KOH either by allowing the specimens to sit in cold KOH overnight or by gently heating the KOH for 30 to 60 minutes. Following clearing, larvae were placed in glacial acetic acid for 15 minutes to neutralize the KOH, placed in a 70% ethanol rinse for 15 minutes and then transferred to a final solution of 70% ethanol. Head capsules were mounted ventral side up (under a dissecting microscope) in either CMC-P, CMC-10 or Hoyers mountant media. Gentle pressure was placed on the glass cover slip to extrude the mandibles and to provide a clear view of the hypostomal teeth (although this often resulted in the mandibles obscuring the view of antennae). Identification of chironomids was to genera following Oliver and Roussel (1983). Mr. Bohdan Biljy (Freshwater Institute, Winnipeg, Manitoba) verified identifications and provided species identifications where possible.

Chironomid head capsules were examined for deformities under a compound microscope, generally at 400X magnification. Emphasis was placed on examining the hypostomal teeth for deformities, although the mandibles, premandibles and antennae (when clearly visible) were also examined for deformities as was the ligula of Tanypodinae. In the case of antennae, examination was only for gross deformities such as missing or extra segments, or major differences in size of segments between both antennae. No effort was made to examine antennae for more subtle deformities as described by Warwick (1985). Hypostoma and mandibles were described as deformed if they exhibited extra teeth, missing teeth including gaps, or were asymmetric or abnormal in shape. Care was taken to differentiate between deformities and abnormalities (e.g., missing or asymmetrical teeth) that may have resulted from breakage. Gaps were defined as deformities if their surface was smooth, as opposed to rough or jagged and obviously the result of breakage. Deformities were examined at 1,000X magnification for evidence of breakage (rough or jagged surface). In addition, a blind test was employed on a portion of the slides (165 specimens) to determine consistency of scoring between the author and a person familiar with chironomids (Mr. Alan Burt, Beak Consultants Limited).

Oligochaetes from sites which exhibited higher frequencies of chironomid deformities were identified and examined for setal deformities such as those described by Milbrink (1983) and Chapman and Brinkhurst (1988). Hydropsychid larvae were examined for deformed gills such as described by Simpson (1980). Since mayflies and stoneflies were present in only the control sites (Stations 1 and 35) in any number, and a quick examination of these specimens and those few specimens collected at other sites revealed no gill deformities such as those described by Simpson (1980), these taxa will not be discussed.

Sediments for laboratory sediment exposure studies were collected by dragging the net over the surface of the substrate. By this means only the surficial 2 or 3 cm of sediment was collected. Only three sites (Stations 15, 30 and 32) had sufficient fine sediments to allow collection and of these samples, those from Stations 30 and 32 were lost (while in storage in Granby). Hard substrate (e.g., cobble or bedrock) with little accumulation of fine material prevented collection of sediment at other sites.

Laboratory Studies

To determine whether contaminants in sediments induced deformities, Chironomus tentans* larvae were exposed to suspect sediment from Station 15, a site where deformed chironomids had been collected. Larvae were reared at 20°C in 1 L glass culture bowls containing 2 to 3 cm of sediment and dechlorinated tap water over a period of 24 days when pupae exuvia were first observed in culture bowls. Each culture bowl was continuously aerated and the larvae were fed ad libitum with a slurry of Cerophyll, a dried plant product, every three days. Water was gently siphoned from each bowl every week, almost to substrate level, and replaced, to prevent a build-up of metabolic waste products in the water.

The incidence of deformities in the presence of the suspect sediment was compared to that for larvae exposed to control substrates. Several authors (Batac-Catalan and White, 1982; Kosalwat and Knight, 1987) have utilized organic matter as a control substrate to prevent breakage of mouthparts (e.g., hypostomal teeth), whereas others (Hart et al., 1987) have used a mineral substrate. Warwick (1980a) suggested that a high degree of wearing of teeth and mandibles

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*The sediment exposure study was initiated in autumn. Therefore, it was not possible to obtain a fresh culture from field stock, since Chironomus tentans migrates to deeper waters and enters winter diapause in autumn; spring is the ideal time of year to start fresh cultures (Dr. B. Townsend, Freshwater Institute, Winnipeg, Manitoba, pers. comm.). Culture stock for the present study was obtained from an older culture maintained by Dr. Len Kalas (National Water Research Institute, Burlington, Ontario).

of larvae reared in a sand substrate may have obscured deformities in a toxicity study conducted by Hamilton and Saether (1971). Also, it is not clear whether the physical properties of the substrate influence deformities (e.g., gaps or asymmetrical teeth may be the result of breakage of mouthparts while ingesting mineral particles). For this reason, the influence of substrate, particularly particle size, on deformity frequency was investigated using a factorial experimental design with suspect sediment (Station 15, Yamaska River) and four control substrates: silt and clay (obtained from Dr. G. Wall, Dept. of Land Resource Sciences, University of Guelph), sandbox sand (commercially available from nurseries), canary grain sand (commercially available from pet stores), and organic matter (shredded paper towels). The influence of substrate on growth and survival was also investigated.

The mineral substrates were characterized as follows. Canary sand consisted primarily (74.2%) of particles in the size range from 0.71 to 1.0 mm. (The composition of the remaining particles was 10.5% in the size range from 1.0 to 1.7 mm, 13.4% from 0.425 to 0.71 mm, and 1.8% from 0.15 to 0.425 mm). In comparison, sandbox sand was primarily (61.9%) in the size range of 0.15 to 0.425 mm. (The composition of the remaining particles was 1.97% in the size range greater than 1.7 mm, 1.76% from 1.0 to 1.7 mm, 3.94% from 0.71 to 1.0 mm, 17.9% from 0.425 to 0.71 mm, 11.5% from 0.074 to 0.15 mm, and 1.06% less than 0.074 mm). The silt-clay substrate was 44.5% silt and 11.2% clay as determined by the pipette test. The remainder consisted of larger particles in the sand size range, which probably formed through coagulation of the silt/clay particles. The organic substrate consisted of paper towelling shredded in a blender, leached for three days in a 20% solution of HCl acid to remove juvenile hormone analogue (which inhibits larval development) and then rinsed in dechlorinated tap water.

Freshly hatched first instar larvae (125 specimens) were added to each replicate (3) per treatment. The larvae were collected from five egg masses daily over a four-day period as they hatched from the egg masses. The 125 larvae per culture bowl consisted of about 25 larvae from each egg mass.

This approach allowed for a more uniform group of larvae on initiation of the experiment. That is, the experimental design (five treatments x three replicates) required 1,875 larvae, a quantity which necessitated more than one egg mass, whereas delayed hatching within egg masses would have caused greater variability in size of larvae if fewer egg masses were employed. The variability in a single egg mass (i.e., larvae size and time to emergence) is as great as or greater than the variability among different egg masses (Dr. L. Kalas, pers. comm.).

Freshly hatched larvae were employed in the study since:

- eggs are protected by the chorion and are not as sensitive as larvae to toxicants (Kosalwat and Knight, 1987);

- it is difficult to use the same number of eggs per test to provide uniformity among replicates and treatments (although the number of eggs per sample could have been counted);
- if low survival occurred at termination of the study, it would not be possible to differentiate between mortality in the egg stage versus the larval stage; and
- to assess whether a particular substrate is preferable for use as a control substrate, measurement of growth and survival is essential (i.e., does substrate affect variability in size and generation time?).

To assess whether deformities induced by exposure to suspect sediments were transferable to offspring, a second set of larvae were exposed to Station 15 sediments (125 larvae per replicate, three replicates) and allowed to complete their life cycle to emerge as adults. Emerging males were collected each day for several days and mated with virgin females from the control cultures, in order to determine from their offspring whether heritable genetic damage had been induced. Since females can transmit cytoplasmic (non-genetic) effects in the egg cytoplasm to offspring, exposed females were not mated with control males to test for heritability.

To obtain virgin females, all adult specimens were removed from culture aquaria (covered with a fine screen) after which the aquaria were examined at intervals throughout the day collecting newly emerged adults at each visit until several females were collected from aquaria containing no males. These were paired with males from the exposed cultures. Egg masses resulting from these matings were placed in separate dishes of water until hatching occurred, then 125 larvae were counted and placed in separate culture bowls containing control sediment. These cultures were reared through to fourth instar before being sacrificed. Slide preparation and examination for deformities were as described previously. Statistical analysis was by ANOVA in combination with the Duncan multiple range test.

Because hypostomal teeth of larvae exposed to Station 15 sediment appeared to exhibit more wear than those from control substrates, the degree of wear was quantified. Teeth were classified as normal, which consisted of three subgroups: no wear (sharply pointed), slight wear and modest wear, or worn (Figure 4) and quantified per treatment.

RESULTS AND DISCUSSION

Field Study: Yamaska River

A total of 2,963 chironomids, collected from 12 stations representing areas impacted upon by agricultural, industrial or municipal pollution and relatively clean control areas within the Yamaska River system, were identified and their head capsules were examined for deformities. The seven stations sampled in June produced 543 chironomids, whereas 12 stations sampled in July yielded 2,386 chironomids.

Samples collected in June consisted primarily of Chironominae and Orthocladinae, which comprised 41.4 and 44.6% of the specimens, respectively. In July, 69% of the specimens were Chrionominae.

Hypostomal deformities were characterized as to their position of teeth on the hypostome (i.e., median teeth, 1st lateral or outer laterals) and nature of the deformity (i.e., extra teeth, gap or missing teeth, or asymmetry of teeth in size or shape). Ligulae were scored on the same basis. Tabulation of the data on the position and nature of deformities revealed no apparent trend (data not presented). Typical deformities observed in this study are illustrated in Figure 2.

Warwick (1985) proposed a classification system for scoring antennal deformities. His classification system requires considerable taxonomic expertise and is extremely time-consuming. More importantly, no evidence was presented to demonstrate that scores reflect a response in relation to levels of environmental contamination. However, in the event that severity, location or nature of deformities are dose-dependent, then a simple classification scheme could be developed based on the above scoring method. Presently, frequency of deformities is the best measurement of environmental stress and is the approach taken in the present study. A blind test for scoring of deformities in 165 specimens resulted in 96% agreement between scores.

The chironomid community structure and number of deformities observed at each station are presented in Tables 2 and 3 for samples collected in June and July, respectively. The frequency of deformities ranged from 0 to 10.5%, with a mean of 2.6 and 1.8% for June and

July, respectively. Based on the frequency of deformed chironomids per sample, Station 15, the Yamaska River at Val Shefford, was the most environmentally stressed site in June with 10.5% of the specimens being deformed. Deformities were found primarily in Chironomus (e.g., Figure 2b, c) of which 27.3% had deformities. A single Microtendipes larva collected at this site was also deformed (Table 2). In July, the incidence of deformities at Station 15 was low (0.95%), although 25% (one out of four specimens) of the Thienemannemyia larvae were deformed (Figure 2f). A higher incidence of deformities of 6.3% was observed in August. In addition, at Station 15, it was common to see yellow perch, Perca flavescens, with growths (presumably tumours) on their bodies, which also indicates a stressed environment. It is not clear why Station 15 is stressed. However, this station is located approximately 16 km downstream of Waterloo, and may be impacted upon by heavy metals from the metal plating industry located in Waterloo.

In July, Stations 9 and 12 were rated the most environmentally stressed sites based on frequency of deformities. Both these stations are located downstream of urban centres. Station 9 is approximately 1.5 km downstream of Acton Vale, and thus is impacted upon by textile mill and sewage effluents added to the river by the City. Station 12 is downstream of Ste. Hyacinthe, which contributes sewage effluent as well as effluents from textile mills and light industry to the river. In the present study, Station 12 is located upstream of the Ste. Hyacinthe sewage treatment plant outlet. (Dr. J. Maguire and Ms. J. Metcalfe sampled farther downstream below the STP outlet.) At

Station 12, several large concrete pipes (possibly stormwater drainage pipes) empty into the river upstream of the sampling station. At Station 9, 5.1% of the larvae were deformed (Table 3). Most of the deformities were seen in Chironomus larvae (9 of 172 specimens (5.2%) were deformed). Single deformed specimens were also found in Glyptotendipes (25% deformed) and Microtendipes (100%) (Table 3). In comparison, 5.23% of the larvae at Station 12 were deformed. Here, 20% of the Chironomus (2 of 10 specimens), 7% of the Polypedilum (7 of 100 specimens) and 5.7% of the Tvetinia discoloripes grp (6 of 106 specimens) were deformed (Table 3).

Initially, Station 32 was assessed to be the most environmentally stressed site in July with a deformity frequency of 9.46%. Twelve of 184 Stictochironomus larvae were found to have median teeth missing and, because the resulting gap was smooth, these were initially classified as deformities, although surrounding teeth exhibited evidence of wear. Further examination revealed specimens which had these two teeth worn to various degrees, i.e., some to the extent that they appeared as only one small (worn) tooth. Thus, it was concluded that these were not deformities, but broken and/or worn-off teeth, and the frequency of deformities for Station 32 was 4.95% in July.

If the toothed 'gap' has a concave depression (Figure 2b), then it most probably is a true deformity; otherwise, it should be considered breakage. However, in some cases, there can be a fine line in distinguishing between the concave deformity and the gap due to breakage (Mr. Bohdan Biljy, pers. comm.). This guideline would have saved some confusion in scoring deformities in this sample. Also, this illustrates the subjectivity in determining what is a deformity in certain instances.

Deformities have been associated with industrial wastes and/or agricultural pesticides. However, deformities are also observed in chironomids in non-polluted environments, but the incidence of deformities is low, i.e., generally much less than 1% of the population (Warwick, 1980a, b; Wiederholm, 1984). With increased pollution, the incidence and severity of deformities increases with high rates of deformities having been reported from more environmentally stressed areas. For example, Hare and Carter (1976) reported that 77% of the Chironomus (s.s.) ?cucini larvae collected from Parry Sound Harbour, Georgian Bay, Ontario, had deformed mouthparts, whereas only one specimen out of 73 larvae examined from outside the harbour was deformed. The sediments of Parry Sound Harbour are visibly contaminated with oil, cinders and sawmill leavings having received waste from lumber, smelting and explosives industries, as well as urban sewage over the past century. The harbour was also the site of a massive oil spill in 1950.

Similarly, a deformity frequency of 83% was reported for Chironomus specimens collected from the turning basin of Port Hope Harbour, Ontario, compared with only 14% for those specimens collected from the outer harbour (McKee et al., 1985; Warwick et al., 1986). The turning basin is heavily contaminated with U-238 decay chain radionuclides and several trace metals, whereas the outer harbour is relatively uncontaminated (Hart et al., 1986). Koehn and Frank (1980) reported frequencies of deformities ranging from 25.4 to 37.6% for Chironomus thummi at five stations in the Telkow Kanal, West Berlin, an area contaminated with high levels of cadmium, copper, lead and zinc. In the most polluted areas, 20% of the Chironomus riparus were also deformed (Frank, 1981). Boscor et al. (1974) reported the occurrence of large proportions of deformed Chironomus in Black River Bay, Lake Ontario, and implicated pollution by agricultural and industrial toxicants. In a study of Swedish lake sites, Wiederholm (1984) found that 5 to 25% of the chironomids (mainly Chironomus, Microspectra and Tanytarsus) at the strongly polluted sites were deformed, whereas less than 1% of the larvae from unpolluted sites, or unpolluted time periods in the fossil record, were deformed. Likewise, in a study of core sediments from the Bay of Quinte, Ontario, Warwick (1980b) found that frequency of deformed Chironomus and Procladius specimens clearly increased in the most recent sediments, i.e., from 0.09% in pre-European sediments to 1.06% at 4.5 cm (1951) and 1.99% in the 1972 population.

In the present study, the incidence of deformities was low, less than 2%, at most sites. No deformities were observed at two stations (Stations 6 and 31) in June and at three stations (Stations 1, 8 and 35) in July (Tables 2 and 3). These stations can be considered non-polluted. Stations 15 and 31 in July had frequency of deformities of less than 1% and, therefore, can also be considered non-polluted on this sampling occasion. Stations with frequency of deformities greater than 1% are considered polluted (Warwick, 1980a; Wiederholm, 1984), e.g., Stations 1, 5, 15 and 30 in June and Stations 5, 9, 12, 16, 30, 32 and 33 in July, respectively. The classification of Station 16 as non-polluted in June, but polluted in July, reflects the problems encountered when too small of a sample is collected (i.e., n = 2 in June; Table 2). Ideally, greater than 100 specimens and preferably 200 specimens should be examined per station, unless the environment is extremely stressed and the frequency of deformities is very high, e.g., greater than 25%.

Classification of Station 1, the Noire River upstream of Valcourt, as environmentally stressed in June (2.86% of the chironomids were deformed) was surprising since this site was chosen as a control station because of its relatively clean condition (Table 1). The presence of several mayfly and stonefly species at Station 1 is suggestive of clean water quality, although one of the most grossly deformed chironomid specimens (Figure 2h) was collected here. In this respect, a comparison of deformity frequencies with other water quality indicators, viz. community structure and diversity indices (Ms. J. Metcalfe, in progress) and with chemistry data (Dr. J. Maguire, in progress) would be informative, e.g., elevated levels of Al were recorded from this site (Appendix Table A1).

The presence of deformities in specimens collected from agricultural sites, Stations 30, 32 and 33 (Tables 2 and 3), suggests that agricultural pesticides are impacting upon the benthic fauna. In the present study, Station 30 was moved further upstream on the a la

Barbue (Figure 1) to a shallower area which was surrounded by corn fields. Corn fields were also located near Station 32. Station 33 on the Chibouet River at St. Hughes was similar to that sampled by others (Dr. J. Maguire and Ms. J. Metcalfe), and is impacted upon almost exclusively by agricultural inputs. It is not clear why a higher incidence of deformities of 4.95% occurred at Station 32 in July (Table 3).

In a study of atrazine export from these watersheds, Muir et al. (1978) found concentrations ranged from 0.01 to 26.9 µg/L and less than 0.02 to 1.34 µg/L for atrazine and N-deethylated atrazine residues, respectively. In this study, concentrations were highest in June and July during the herbicide spray season and when heavy rainfall was frequent. However, export was mainly during the spring runoff period and was related to the area of land planted in corn. In this respect, 15.6, 25.1 and 16.3% of the cultivated area of the St. Nazaire (Station 32), a la Barbue (Station 30) and Chibouet (Station 33) Rivers, respectively, was planted in corn (Muir et al., 1978). In the present study, deformities were also found at the other agricultural site, Station 31, on the Runnets River, but at background levels (i.e., less than 1%). In agreement with this, Muir et al. (1978) found the lowest levels of atrazine in Runnets River, which had 10.1% of its cultivated area in corn production.

Deformities were also observed at Station 5 (1.72% deformed; Tables 2 and 3, Figure 2e), downstream from Granby. This area is impacted upon by municipal sewage treatment plant effluents and by heavy metals from several metal processing plants in Granby, (e.g., Tessier et al., (1980) attributed elevated levels of copper (ca. 70 ppm), nickel (ca. 200 ppm) and zinc (ca. 700 ppm) at Ste-Alphonse, located further downstream from Granby).

The presence of deformities at frequencies greater than 1% (i.e., above background levels) at stations representing industrial effluents, municipal sewage effluents, agricultural runoff and control or natural conditions points to the widespread occurrence of deformities in the Yamaska River system and to the lack of specificity of deformities to particular environmental stresses. Deformities are, however, a good indicator of teratogenic effects within the environment.

It has been suggested that different types of chironomid deformities may be indicative of specific types of environmental stress, the frequency of deformities increasing with the severity of stress (Warwick, 1980a). Although there are no data to support the first conjecture that particular deformities are specific to certain types of contamination, higher frequencies of deformities downstream of urban centres (Stations 9 and 12) in the present study support the concept that the frequency of deformities may be a good indicator of the level of contamination (bioavailability of contaminants).

The low frequency of deformities observed in the present study of generally between 1 and 2%, and 5% for the urban areas, is comparable to the finding of others. For example, Wiederholm (1984) reported frequencies of deformities from 1.4 to 4.1% from areas that were characterized as slightly polluted to strongly polluted based on chemical data. In his study, other polluted-to-strongly-polluted sites had frequencies of 8.3 to 25%. Cushman (1984) reported that 2.7 to 4.6% of the Chironomus decorus larvae collected from experimental ponds to which a coal liquid had been added had deformities. Tennessen and Gottfried (1983) reported that 1.88 and 3.26% of the Tanypodinae collected from two man-made lakes had deformities, whereas 3.61% of those collected from strip-mine ponds were deformed, but did not investigate for causative agents. Warwick (1980a) found that 2.26% of the Chironomus spp. larvae collected from the western basin of Pasqua Lake had deformed mouthparts, whereas 3.13% of Paratendipes larvae in the eastern basins were deformed. The incidence of deformities was related to the trophic assessment for the basins, i.e., the western basin is eutrophic, whereas the eastern basin is strongly eutrophic. (Unusually high concentrations of heavy metals as well as the presence of organochlorine pesticides have been monitored in the Qu'Appelle River immediately above Pasqua Lake.) However, further analysis of Warwick's study revealed no difference in the frequency of deformities between polluted and relatively clean control areas (Dr. E. Ongley, pers. comm.). In a study of the Bay of Quinte, Ontario, Warwick (1980b) reported an incidence of deformities of 1.99%.

Difficulties arise in interpreting deformity levels around 1 to 2%. Are these levels indicative of pollution? In Wiederholm's (1984) study, two sites with frequencies in this range were affected by eutrophication, but were not particularly influenced by toxic substances. In contrast, an incidence of deformities of 2.26% in Pasqua Lake was attributed to contaminants (Warwick, 1980a). In the Yamaska River, I suspect frequencies of 1 to about 3% reflect areas that are slightly to moderately polluted (the 5% levels below urban centres are probably moderately polluted). However, this speculation requires confirmation with the data collected in the concurrent studies. In all probability, low frequencies of deformities indicate environmental stress due to pollution, but clearly should not be used alone as an indicator of environmental quality, i.e., deformity frequencies, unless very high, should only be used to provide additional information when analysis of the benthic fauna is carried out for the assessment of environmental quality.

A number of other occurrences of deformed chironomids have been reported, and these deformities were thought to be caused by industrial and/or agricultural pollution. For example, Brinkhurst _et_ _al_. (1968) and Hamilton and Saether (1971) reported that all of three specimens of _Chironomus_ spp. collected at stations in the western end of Lake Erie near the Maumee River were badly deformed. Examination of more than 1,700 larvae collected from other stations in the lake reveal no other deformed specimens. In a survey of the benthic macroinvertebrates of the central basin of Lake Erie, Krieger (1984) reported that one specimen of _Chironomus_ near the mouth of the Grand River at Fairport Harbor and two specimens of _Procladius_ near the mouths of the Black River at Lorain and the Cuyahoga River at Cleveland possessed deformed labial plates or ligulae (i.e., deformity incidences of less than 0.5%). Deformed Chironomus selanarius larvae were found in areas influenced by pulp and paper mill discharges in Thunder Bay, Lake Superior (Crowther and Luoma, 1984). Other deformed larvae (Procladius, Protanypus, Chironomus and Stictochironomus) have been collected from two lakes in the Okanagan Valley of British Columbia near known sources of pollution (Hamilton and Saether, 1971).

From the above, it is evident that chironomid deformities are associated with contamination by industrial wastes and/or agricultural pesticides. However, in most cases, the linkage between deformities and contaminants is based primarily on circumstantial evidence. Findings in the present study demonstrate that the frequency of deformities increases with environmental stress, i.e., higher frequencies of deformities downstream of urban centres and at Station 15. High frequencies (greater than 20%) of deformities have been reported from contaminated areas (discussed above). However, low frequencies of deformities may be expected in areas of contamination because of selected mortality of deformed specimens. Also, in more contaminated sites, only the more tolerant species persist, i.e., species more prone to exhibit deformities may be eliminated from the fauna. For example, Hamilton and Saether (1971) found deformed specimens generally near known sources of pollution, but samples taken nearer the source characteristically contained no specimens of the species. Likewise, Wiederholm (1984) reported that higher incidences of deformities at two strongly polluted sites in his study were based on limited material due to the scarcity of chironomids present under such conditions. The observation of an inverse relationship between frequency of deformed antennae and exposure concentration of DDE (Warwick, 1985) also supports the hypothesis of selective mortality of deformed specimens.

Deformities have also been reported in other invertebrate species, e.g., Plecoptera (Donald, 1980), Oligochaetes (Milbrink, 1980; Chapman and Brinkhurst, 1984) and Trichoptera (Petersen and Petersen, 1983). Simpson (1980) observed that the filamentous gills of Hydropsychidae larvae downstream of the effluent outfall from a plating mill were reduced to stubs, whereas upstream of the outfall larvae had normal gill filaments. Similar observations were made for the tracheal gills of the stonefly _Phasganophora capitata_. In the present study, hydropsychid larvae were common at several locations and, therefore, were examined for deformed gill filaments (Table 4). Of 1,992 specimens examined, three specimens from Station 15 in June exhibited a slight reduction in the degree of branching in their gill filaments. However, it was not clear as to whether this was due to natural variation in the population or not. Therefore, these specimens were considered normal. Other taxa, such as mayflies and stoneflies, were not examined for deformities because of their absence at most stations.

Setal deformities have been reported in oligochaetes collected from contaminated areas (Milbrink, 1980). Recently, Chapman and Brinkhurst (1988) demonstrated that the morphology of chaetae varied in response to different levels of pH, salinity, water hardness and mercury. Furthermore, they produced Tubifex tubifex with malformed pectinate chaetae in the presence of both mercury and salinity stress that were similar to those deformities described for worms in mercury-contaminated areas by Milbrink (1983).

In the present study, 283 Tubifex tubifex specimens from Station 5 and 100 specimens from Station 16 were examined for setal deformities such as those described by Millbrink (1980) and Chapman and Brinkhurst (1988). No setal deformities were found. Furthermore, no setal deformities have been observed in thousands of worms examined from a variety of polluted sites (Mr. Dennis Farara, Beak Consultants Limited, pers. comm.).

Several substances alone or together may produce deformed chironomid larvae. Heavy metals, oil, chlorine and pesticides cause deformities, but no substance has been identified as being more causative than others. Wiederholm (1984) reported high frequencies of deformities at several sites which had very high concentrations of heavy metals, e.g., mercury at 50 to 250X background concentration at two sites, lead at 15X background at another site, and chromium at 80X background at a fourth site. However, high incidences of deformities also occurred at three of his other sites where metal concentrations were not particularly high, but two of these were also contaminated with oil.

A dose-dependent response in chironomid deformities has been demonstrated in laboratory studies for larvae exposed to copper (Kosalwat and Knight, 1987) and to ethyl methanesulphonate (Hart _et_ _al_., 1987) and in the field for larvae exposed to oil (Cushman, 1984). Milbrink (1983) found a highly significant correlation between the incidence of setal deformities in oligochaetes and mercury deposits and speculated that synergistic effects between contaminants (e.g., Hg, Zn and Cd) may have been responsible for the deformities. Hamilton and Saether (1971) found that larvae exposed to 10 µg/L concentrations of DDE developed thickened body walls. No deformed mouthparts were observed in larvae exposed to either aldrin, DDE, PCBs at 10 µg/L or nitrilotriacetic acid or a mixture of 2,4-D and 2,4,5-T at 1 and 10 mg/L. This may partially be because of the high mortality in their studies. Also, Warwick (1980a) found that the sandy substrate used in their study caused a high degree of wearing of teeth and mandibles of larvae, and hence may have obscured the effects of chemicals tested (see discussion below). Deformities are not a common phenomenon (Saether, 1970; Mr. B. Biljy, Freshwater Institute, Winnipeg) and appear to be caused by environmental stress. Different levels of pH, salinity and water hardness can change the morphology of oligochaete chaetae (Chapman and Brinkhurst, 1987). However, it is unlikely that these stresses influence deformities in the heavily sclerotized mouthparts of chironomids, otherwise deformities would have been observed much more frequently in ecological studies.

Sediment Exposure Study

The incidence of deformities in C. tentans larvae reared in control substrates was higher than those specimens reared in suspect sediments from Station 15 (Table 5), although the difference was not significant with the exception of the clay/silt substrate ($p < 0.05$). Thus, suspect sediments from Station 15 did not induce deformities (Figure 3) in the culture larvae. However, the hypostomal teeth of specimens reared in the Station 15 sediment exhibited much greater ($p < 0.05$) wear (Figure 4d and e) than those larvae exposed to control substrates (Table 5). This raises the question as to whether contaminants in this sediment may be interfering with chitin deposition resulting in greater wear of the hypostomal teeth of these larvae. Also, it is noteworthy that the incidence of deformities in the field was much lower in July than in June (Table 3). This suggests that fresh sediments deposited at this site in July may not have been as contaminated as sediments deposited earlier in the year.

Inbreeding of stock culture may result in increased expression of recessive characteristics, including morphological abnormalities with a heritable genetic basis, and an increase in deformity incidence between 4th and 6th generation cultures has been observed (Hart et al., 1987). Since older cultures of Chironomus tentans were used in the present study, deformities (presumably due to inbreeding) were seen in each treatment (Table 5). These deformities were mainly confined to the median tooth, and consisted of uni-, bi- or tri-indentation (Figures 3c, d and e) of the median tooth to various degrees forming additional lobes or teeth. For example, a single indentation or cleft of the median tooth was the most common deformity observed. More extreme development of this cleft resulted in the formation of two lobes or teeth, or a forked median tooth (Figure 3b). Other deformities included gaps between teeth, often creating asymmetrical teeth, missing teeth, extra teeth, and deformed mandibles. Hart _et_ _al_. (1987) also observed a cleft median tooth and fusion of first lateral teeth in cultures. They reported that the symmetry of these deformities was consistent with an inherited genetic basis since somatic damage is much less likely to be symmetrical.

Single indentations (Figure 3c) in the median teeth were more common ($p < 0.05$) in specimens reared in the clay-silt substrate than the shredded paper or Station 15 substrates (Table 5). It is not clear why this should be the case. However, there was no difference in the incidence of single indentation deformities in specimens reared in the other control substrates, viz. shredded paper, sandbox sand or canary sand. No difference was seen in the number of larvae with a forked median tooth (Figure 3b) or the median tooth with multiple indentations (Figure 3e) with treatment.

Broken teeth were more numerous ($p < 0.05$) for larvae reared in the clay/silt substrate than the other substrates. The number of larvae exhibiting broken teeth was less than 10% of the sample in all replicates. Why broken teeth and incidence of deformities are higher for larvae reared in a clay/silt substrate than the other substrates is not known. However, there was no difference in the number of larvae with worn teeth in the various control substrates. Tooth wear was lowest in the canary sand with more ($p < 0.05$) larvae having sharply pointed teeth, exhibiting no wear, in this substrate than the other substrates (Table 5, Figure 4a).

Biomass per specimen and survival was higher for larvae reared in Yamaska River sediment from Station 15 than the other substrates, although the difference was not significant (Table 6). An exception is survival of larvae exposed to suspect sediment from Station 15, which was greater ($p < 0.05$) than that for larvae reared in the canary grain substrate. Better survival and growth (biomass) on the Station 15 substrate is probably a reflection of the higher organic matter content of the substrate compared to control substrates, with the exception of shredded paper.

Within the control substrates, high survival ($p > 0.05$, Table 6) of larvae reared in the paper towelling, together with their faster development (i.e., in larger size groups or pupating, Figure 5), indicates that shredded paper is a better control substrate.

Townsend _et_ _al_. (1981) found that late fourth instar larvae weigh about 22 mg for male larvae and 37.7 mg for female larvae. Values for biomass presented in Table 6 do not differentiate between sexes and are much lower than their values. In our study, a wide range of developmental stage was observed at the time of culture termination (Figure 5) and values in Table 6 reflect the inclusion of these early instar larvae. Hart _et_ _al_. (1987) attributed the finding of a large range of developmental stages in cultures to crowding effects. They also noted that mean biomass value per larvae was low under such conditions.

Thus, sediment from Station 15 did not induce deformities in larvae reared in this sediment, but the hypostomal teeth of several of these larvae exhibited more wear than those in control groups. There was no difference in biomass or survival of chironomid larvae reared in control substrates, with the exception of the coarser canary sand. The latter is not highly recommended for use as a control substrate because of lower survival and biomass ($p > 0.05$, Table 6) of larvae reared in this substrate than the other substrates. However, a distinct advantage of this substrate was that the hypostomal teeth of larvae reared in canary sand exhibited less wear ($p < 0.05$) than those for larvae reared in the other substrates (Table 5).

The choice of a control substrate appears to be governed both by whether it is necessary to match the physical characteristics of the suspect (test) substrate (e.g., particle size) and by convenience. Shredded paper required the most preparation time, but larvae were easiest to recover from the substrate at termination of the experiment. Also, larvae matured faster in this substrate than the other control substrates (Figure 5). The clay/silt substrate was easiest to use overall, but its practicability would depend on availability. The sandbox sand and canary sand are both readily available commercially and require little preparation, but moderately more effort is required to remove all of the larvae from these substrates.

Attempts to produce a F_1 generation by mating males exposed to Station 15 sediments with non-exposed (control) females have thus far proved unsuccessful. One mating resulting in the production of an egg mass, but no larvae hatched after four days after which the egg mass became infected with a fungus. This problem also has occurred with control stock. Another difficulty has been timing of emergence. Peak emergence of males from treatment sediments occurred at a time when few females were emerging in the control cultures. Egg masses had been added to culture aquaria several times a week to provide a continuous supply of emerging adults as described by Batac-Catalan and White (1982), but this did not produce the steady supply of adults as anticipated. Also, five other attempted matings did not result in egg masses being produced. Batac-Catalan and White (1982) recommend the replacement of cultures with fresh stock after about the seventh generation in order to maintain a viable culture, i.e., difficulties in successful production of a F_1 generation may be partially the result of working with an older culture. If future attempts are successful, the wear of teeth in the F_1 generation reared in a control substrate will be compared to the parent generation.

CONCLUSIONS

Station 15, at Val Shefford, and stations 9 and 12 downstream from urban centres (Acton Vale and Ste. Hyacinthe, respectively) are more environmentally stressed areas based on the freqeuency of chironomid deformities. The occurrence of higher frequencies of deformities below the urban centres (stations 9 and 12) lends credence to the use of deformities in the assessment of environment quality. However, the finding of deformed larvae in areas impacted upon by industrial effluents, municipal effluents, and agricultural (pesticides) inputs as well as in control areas points to the lack of specificity in the response of deformities to environmental stressors. Also, with the low frequency of deformities found at most stations (i.e., <3%), in the present study, it is difficult to either rate areas according to environmental stress or to clearly differentiate between stressed areas and more natural clean areas.

At low frequencies of deformities, the use of deformities to assess water quality is probably not economically practical with respect to other bioassay methods, i.e., the cost and time involved in sampling, sorting, mounting, identifying, and scoring specimens for deformities would place costs at approximately $600 per station based on 200 specimens per station and $25/hour for labour for a <u>trained</u> biologist. However, in such cases the use of deformities in combination with invertebrate community structure may be a more meaningful and economically practical (it costs little to score for deformities once all the other work of mounting and identifying the species is done) means of assessing environmental quality. That is, changes at the organism level, as indicated by deformities, might be more useful than changes at the community level for purposes of environmental monitoring because individual responses occur before community responses and, therefore, may provide an early warning of contaminant stress (Petersen and Petersen, 1983).

Although deformities were not induced in larvae exposed to sediment from station 15 in the laboratory, this approach could be successfully employed to assess the teratogenetic capabilities of suspect sediments. Furthermore, the production of an F_1 generation by mating males, exposed as larvae to suspect sediments with control (non-exposed) females, may be a viable test for genotoxicity.

Further research is required to determine the suitability of chironomid deformities as a bioassay tool for assessing environmental stress. It is recommended that the following areas of research be addressed:

- the specificity of chironomid mouthpart deformities to a variety of toxicants and the dose related response;
- assessment of the extent of chironomid deformities in (known) environmentally stressed areas contaminated by known pollutants as well as in natural "clean" areas;
- the use of chironomid deformities as a laboratory bioassay to test for genotoxicity; and
- the economic viability of a chironomid deformity bioassay in comparison with other bioassays.

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FIGURES

Figure 1. Locations of 1987 sampling sites on the Yamaska River drainage basin.

Figure 2. Representative deformities observed in chironomids collected from the Yamaska River, Quebec. (a-c) Chironomus sp., (a) normal, (b) gap and (c) extra median tooth or lobe; (d-e) Thienemannemyia, (d) normal, (e) extra teeth and (f) asymmetrical teeth; (g-h) Cricotopus trifascia, (g) normal, and (h) extra teeth; and (i) Polypedilum (Pentapedilum) with deformed mandible and antenna (arrows).

Figure 3. Typical deformities observed in median tooth of Chironomus Tentans culture specimens. (a) normal, (b) forked, (c) indented, (d) bi-indented, and (e) tri-indented.

Figure 4. Hypostomal teeth of Chironomus Tentans exhibiting various degrees of wear following exposure to different substrates. (a-c) are normal, (a) sharply pointed exhibiting no wear, (b) slightly worn, (c) moderately worn, and (d) and (e) worn.

Figure 5. Effect of substrate on percentage of Chironomus Tentans in various size classes.

Table 1. Description of Study Sites in the Yamaska River Watershed.

Station	Description
1	Noire River (45°26'N, 72°22.3'W) upstream of Valcourt and about 1.7 km northeast of Lawrenceville. This site is influenced by agriculture and is moderately clean (Environment Quebec 1985 report on the Yamaska River, cited by Metcalfe, 1987a). Here, the river is about 9 m wide and shallow, e.g., approximately 50 cm deep in June and approximately 5 to 20 cm deep on the July sampling occasion. The substrate consisted of sand, gravel and cobble with a few large stones, and was largely covered with *Cladophora*.
5	Yamaska River about 3 km downstream of Granby at Highway 139 (45°21.8'N, 72°46.7'W). Here, the river is influenced by sewage effluent and effluent from light industry and textile and carpet mills in Granby. Here, the river is about 9 m wide and is fast-flowing. In June, the water was 46 to 60 cm deep and turbid, whereas in July depths were from 28 to 38 cm. The substrate was primarily hard-packed cobble and stones. In July, macrophytes (primarily *Potamogeton* and *Cladophora*) covered most of the substrate and a sewage odour was evident. In June, the benthic fauna was dominated by tubificids, with hydropsychid caddisflies, isopods, leeches and *Gammarus* present. In July, tubificids were noticeably less abundant, whereas blackfly larvae dominated the fauna. A colony of swallows, which nested under the bridge, took advantage of the high benthic production that occurred as a result of organic enrichment.
8	Noire River about 5 km downstream of Valcourt (45°22.5'N, 72°30.7'W). Here, the river is influenced by sewage effluent and effluent from light industry. At the site, the river is about 6 m wide, and on the July sampling occasion was about 8 cm deep. The substrate consisted primarily of cobble and bedrock. A treed buffer strip bordered both sides of the stream.
9	Le Renne River about 1.5 km downstream of Acton Vale (45°39'N, 72°35.8'W). This site is influenced by textile mill effluent and sewage effluent. Here, the river is fairly wide (ca. 10 m) and shallow (3 to 30 cm deep). The substrate consisted primarily of bedrock which was covered with algae. Silt-sand deposits were located in areas of slower flow and these areas harboured high numbers of chironomids.
12	Yamaska River downstream of Ste. Hyacinthe (45°38.3'N, 72°56.3'W). Here, the river receives effluents from textile mills and a sewage treatment plant. At the sample site, the river was about 60 m wide and in most places was only 15 to 20 cm deep. Flows were moderate at about 30 to 40 cm/s. The substrate was primarily cobble, with larger rocks. Silt was deposited in slow-flowing areas and intermittent patches of macrophytes were present. A treed buffer strip was located along both banks of the river.

Table 1. (con't)

Station	Description
15	Yamaska River at Val Shefford (45°24.8'N, 72°38.6'W) is about 7 km upstream of Granby, and is downstream of Waterloo, which has significant metal pollution from electroplating operations. Site 15 is located about 13 km downstream of Site 16, and a reservoir is located between the two sites. Silt deposits were found in backwater areas, whereas bedrock, stones, cobble and gravel formed the substrate in the main channel. Chironomids were collected from sediment deposits in a pool area, whereas hydropsychid larvae were collected from a riffle area. The river is about 6 m wide here, and samples were collected from depths of 30 cm to waist deep. A sewage odour was evident in the July sampling occasion.
16	Yamaska River at Warden on Highway 241 (45°22.8'N, 72°30.3'W) is about 3 km downstream of Waterloo and receives metal pollution from electroplating operations. The substrate here was primarily cobble and bedrock, and the river is bordered by a treed buffer strip. The river was about 6 m wide and was about 1 m deep on the June sampling occasion. On this sampling occasion mainly leeches were collected, although a few tubificids, blackflies and hydropsychid larvae were also found. In July, hydropsychid larvae were abundant in areas where the water flowed over bedrock (about 2 to 15 cm deep).
30	a la Barbue upstream from its confluence with the Yamaska River (45°29.51'N, 72°37.21'W). This site is primarily influenced by agricultural inputs with corn fields on both sides of the river. A narrow treed buffer strip is found between the corn fields and the river. Here, the river is about 5 m wide and shallow, generally less than 30 cm deep with a pool about 1.5 m deep in July. The substrate was primarily sand, gravel and cobble overlain by finer silt deposits. The fauna here was primarily chironomids.
31	Ruisseau Runnets close to its confluence with the Noire River (45°35.3'N, 72°38.5'W) and near Roxton Falls. This site is influenced by agriculture. Although a riparian buffer strip about 3 to 5 m wide was left along both sides of the river, cattle were able to graze to the water's edge in places. Three barns were located near the sample site; one about 9 m from the river, another about 20 m and the third about 30 m from the water. The land is flat here so runoff may not be a problem. At this site, the river is about 8 m wide and in July was shallow (ca. 15 to 60 cm) with a substratum primarily of bedrock and large boulders.
32	On the St. Nazaire River near its confluence with the Yamaska River (45°41.1'N, 72°39'W), about 200 m downstream of the concession road leading to Saint Theodore-d'Acton. The St. Nazaire River is impacted upon primarily by agriculture. Although old pasture surrounded the study site, corn fields were nearby. Samples were collected from a riffle area and the downstream end of a large pool. Chironomids were collected mainly from areas of silt deposition below the pool.

Table 1. (con't)

Station	Description
33	Chibouet River at Saint-Hughes (45°47.6'N, 72°49.7'W) near its confluence with the Yamaska River. In July, the river was about 6 m wide and up to about 1.5 m deep. Samples were collected in water from 46 to 107 cm deep. The substrate here was mainly clay. Flow was slow and a dirty brown scum covered much of the water surface, and several dead minnows were observed. A grass-shrub buffer strip bordered the river.
35	In the headwater of the Ruisseau Runnets (45°29.9'N, 72°37.4'W) near Roxton-Sud. This station served as a control site. The stream was 3 to 5 m wide here with a depth generally in the range of 5 to 20 cm. A few small pools of about 50 cm in depth were also present. The substrate was primarily bedrock with many boulders and some cobble. Little siltation or algal growth was evident. Shiner, dace and chub minnows were abundant in the pools, whereas hydropsychid caddisflies were numerous in faster flowing water.

Table 2. Number of Chironomids and Deformities in Samples Collected from the Yamaska River in June 1987.

	Station 1	5	15	16	30	31	32
Tanypodinae							
Ablabesmyia (s.s.) *mallochi*	4						
Natarsia						1	
Procladius							
Conchapelopia/Thienemannimyia	30(2)*	12	6				
Chironominae							
Tanytarsini							
Microspectra	1						
Paratanytarsus							
Rheotanytarsus			2				
Tanytarsus	1						
Chironomini							
Axarus nr. *taenionotus*	1						1
Chironomus (s.s.)	2	8	11(3)		2		
Cryptochironomus	1						
Dicrotendipes neomodestus							
Glyptotendipes (Phytotendipes)			2				1
Harnischia curtilamellata							
Microtendipes	5		(1)			1	1
Phaenospectra	1		2				1
Paratendipes	2						
Polypedilum (s.s.) *convictum*		2(1)	5	1	1		
Polypedilum (Pentapedilum)			1				4
Demicryptochironomus	1						
Stictochironomus		6			75(2)	1	77(1)
Diamesinae						22	
Pagastia			1				
Orthocladiinae							
Brillia	1						
Cricotopus (s.s.) *bicinctus*	17	11				3	2
Cricotopus (s.s.) *nr. triannulatus*	7						
Cricotopus (s.s.) *trifascia*	128(4)						
Cricotopus (s.s.) *cf. vterriensis*	1						
Parametriocneums	1						
Psectrocladius (s.s.) *limbatellus*							
Tvetinia bavarica gr.	1					4	
Tvetinia discoloripes gr.	32(1)	15	2	1		9	
TOTAL NUMBER	245(7)	58(1)	38(4)	2	78(2)	45	87(1)
FREQUENCY OF DEFORMITIES	2.86%	1.72%	10.53%	0%	2.56%	0%	0.33%

*( ) value in brackets represents the number of deformed larvae

Table 3. Number of Chironomids and Deformities in Samples Collected from the Yamaska River in July 1987.

	Station												
	1	8	5	15	15 Aug*	16	12	9	30	31	32	33	35
Tanypodinae													
Ablabesmyia (s.s.) *mallochi*												6	
Natarsia												3(1)	
Procladius	10				1	1						8	18
Conchapelopia/Thienemannimyia	8	28	59(2)*	5(1)		49(2)	24	3		35	3	8	
Chironominae													
Tanytarsini													
Microspectra													
Paratanytarsus		2		1					6				
Rheotanytarsus	5			69	5	1	1		2	1	2(1)		25
Tanytarsus				5	7				59	4			14
Chironomini													
Axarus nr. *taenionotus*							1					129(1)	
Chironomus (s.s.)	2	2	4	12			10(2)	172(9)	154(4)			7	1
Cryptochironomus	2	4	1	3	(1)	1	3			7		9	10
Dicrotendipes neomodestus												2	1
Glyptotendipes (*Phytotendipes*)					9		30	5(1?)				4	
Harnischia curtilamellata	1								1			2	
Microtendipes				2	(1)		1	(1)		28(1)	3	14(2)	2
Phaenospectra	3		4	9	3		5		6(1)			9	26
Paratendipes												1	
Polypedilum (s.s.) *convictum*	21	18	98(2)	11	5	23	100(7)	29	1	37	3	3	7
Polypedilum (*Pentapedilum*)			15	1		4							59
Demicryptochironomus													
Stictochironomus		1						2	12(1)	8	207(4)	2	12
sp.?	2	3	1										
Diamesinae													
Pagastia	2		3	1		54				1			
Orthocladiinae													
Brillia													
Cricotopus (s.s.) *bicinctus*	17	3	8	5		2		6(1)					11
Cricotopus (s.s.) nr. *triannulatus*	13	1	4	1				6		3	2		
Cricotopus (s.s.) *trifascia*				3				2			1		1
Cricotopus (s.s.) cf. *vterriensis*								7					
Parametriocneums													
Psectrocladius (s.s.) *limbatellus*													
Tvetinia bavarica gr.				1		2		3					4
Tvetinia discoloripes gr.	1	3	36	81(1)		11	106(6)			2			26
TOTAL NUMBER	87	83	233(4)	210(2)	32(2)	148(2)	287(15)	236(12)	241(6)	126(1)	222(12)	307(4)	217
FREQUENCY OF DEFORMITIES	0%	0%	1.72%	0.95%	6.25%	1.35%	5.23%	5.08%	2.49%	0.79%	4.95%	1.30%	0%

*( ) value in brackets represents the number of deformed larvae; an additional sample was collected at station 15 in August 1987

Table 4. Number of Net Spinning Caddisfly Larvae, Hydropsychidae, Examined for Deformed Gill Filaments from Various Stations in the Yamaska River

	Station															
	June						July									
	1	5	15	16	31	32	1	5	8	12	15	16	30	31	32	35
Hydropsyche bifida	4	9	15	2	1	1	9	42	2	33	40	60	18	14	72	27
H. betteni	1	74	53		5		3	43	20	3	126	254	33	13	3	98
H. recurvata	3				2		3		3	79	20			14		3
H. slossonae	1		1		6						5	22	1	1		
H. sp.1		1	1		1		30	1	124	9	3	30	16			17
H. sp.2			5						9	1	83		17	1	8	81
H. sp.3			1						1		6		7			7
H. sp.4													4			1
H. sp.5													5			
Damaged			1		2								2		1	2
Cheumatopsyche spp.	3	1			5		2	89	18	19	3	65	4	45	20	9
TOTAL NUMBER	12	85	8	2	23	1	47	135	179	245	286	431	101	88	104	245

Note: Taxonomic evaluation followed Ross (1957) for convenience; a detailed evaluation would have followed Schuster and Etnier, 1978, but was not performed since deformities were not found.

Table 5. Effect of Station 15 Sediment and Control Substrate on Incidence of Deformities and Wear of Teeth per 50 Chironomus Tentans Larvae.

Hypostomal Teeth	Sediment Station 15	Shredded Paper	Clay/Silt	Sandbox Sand	Canary Sand
Total No. of Deformities	8.33 ± 2.48^{a}	$19.67 \pm 2.27^{a,b}$	26.00 ± 2.55^{b}	$16.00 \pm 3.94^{a,b}$	$20.33 \pm 6.34^{a,b}$
Forked Deformities	1.33 ± 0.82^{a}	4.00 ± 0.00^{a}	4.00 ± 0.71^{a}	1.33 ± 0.41^{a}	4.33 ± 1.78^{a}
Single Indentation	1.33 ± 0.82^{a}	$4.67 \pm 2.27^{a,b}$	10.00 ± 5.34^{c}	$8.00 \pm 3.24^{a,b,c}$	$11.33 \pm 4.02^{a,b,c}$
Bi- or Tri-indentation	2.67 ± 0.41^{a}	3.67 ± 0.41^{a}	3.33 ± 1.08^{a}	3.33 ± 1.78^{a}	5.00 ± 3.08^{a}
No. with Broken Teeth	0.00 ± 0.00^{a}	0.67 ± 0.82^{a}	2.67 ± 1.47^{b}	1.33 ± 0.41^{a}	1.33 ± 0.41^{a}
No. with Worn Teeth	12.14 ± 8.34^{a}	0.00 ± 0.00^{b}	2.67 ± 1.47^{b}	1.67 ± 0.41^{b}	0.00 ± 0.00^{b}
Sharply Pointed - No Wear	$1.33 \pm 0.94^{a,b}$	$32.00 \pm 3.94^{a,b}$	$18.00 \pm 7.38^{a,b}$	8.33 ± 3.19^{b}	45.00 ± 1.22^{c}

Mean ± SEM

In rows, values with different superscript letters are significatly different at $p < 0.05$

Table 6. Percent Survival and Biomass of Chironomus Tentans Larvae Reared in Yamaska River Sediment from Station 15 and in Control Substrates

	Sediment Station 15	Shredded Paper Towelling	Clay/Silt	Sandbox Sand	Canary Sand
Biomass (mg/individual)	1.656 ± 0.566^{a}	1.417 ± 0.321^{a}	1.516 ± 0.136^{a}	1.562 ± 0.300^{a}	1.142 ± 0.227^{a}
Survival	98.93 ± 0.86^{a}	$90.40 \pm 6.30^{a,b}$	$89.23 \pm 6.67^{a,b}$	$74.13 \pm 7.64^{a,b}$	71.47 ± 11.79^{b}

$\bar{x} \pm SEM$

Within rows, values with different superscript letters are significantly different at $p<0.05$.

APPENDIX

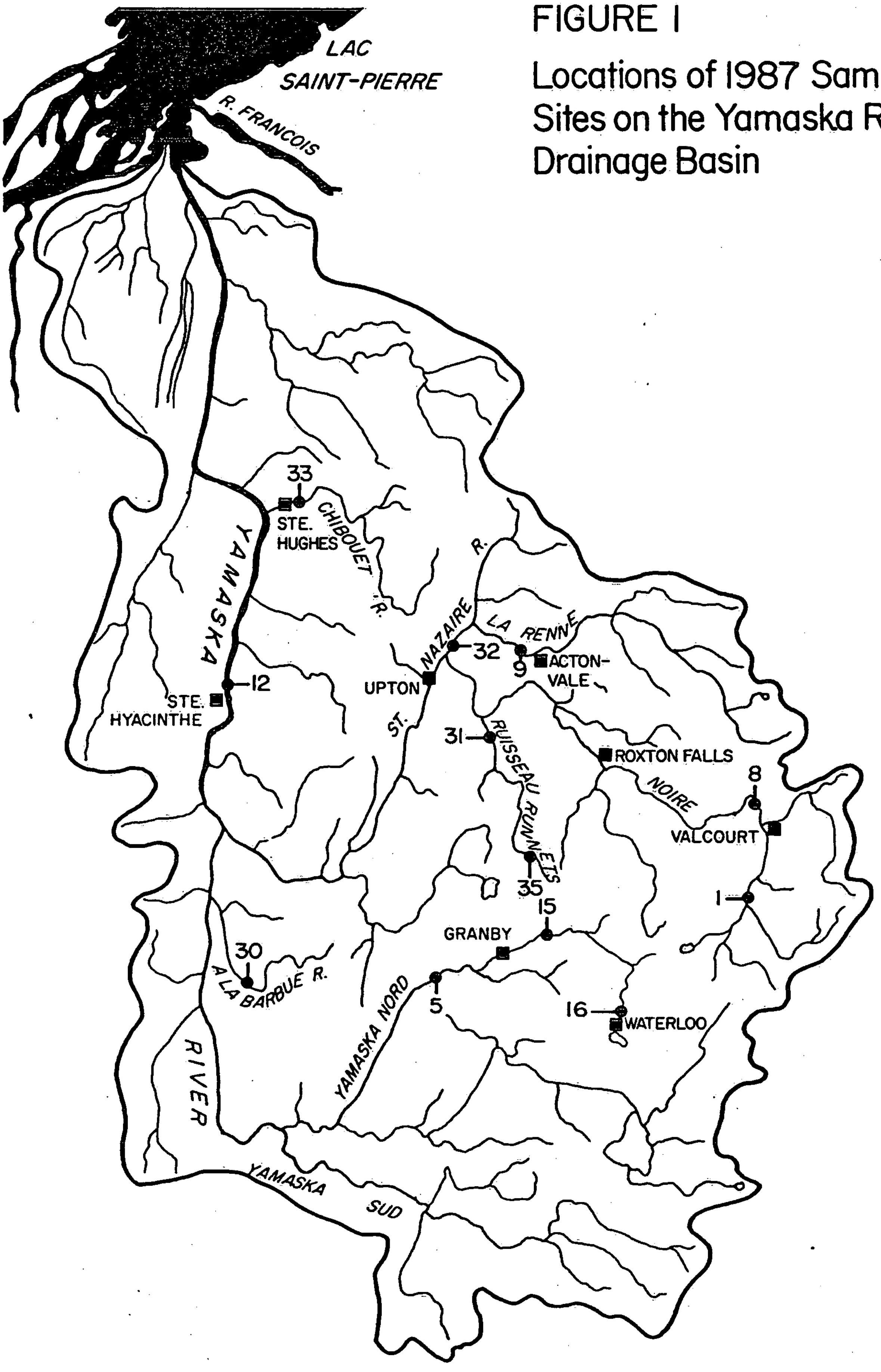
Appendix
Table 1. Trace Metal Concentration (mg/L) in Unfiltered Water Samples Collected from the Yamaska River in July 1987 (Metcalfe, 1987b).

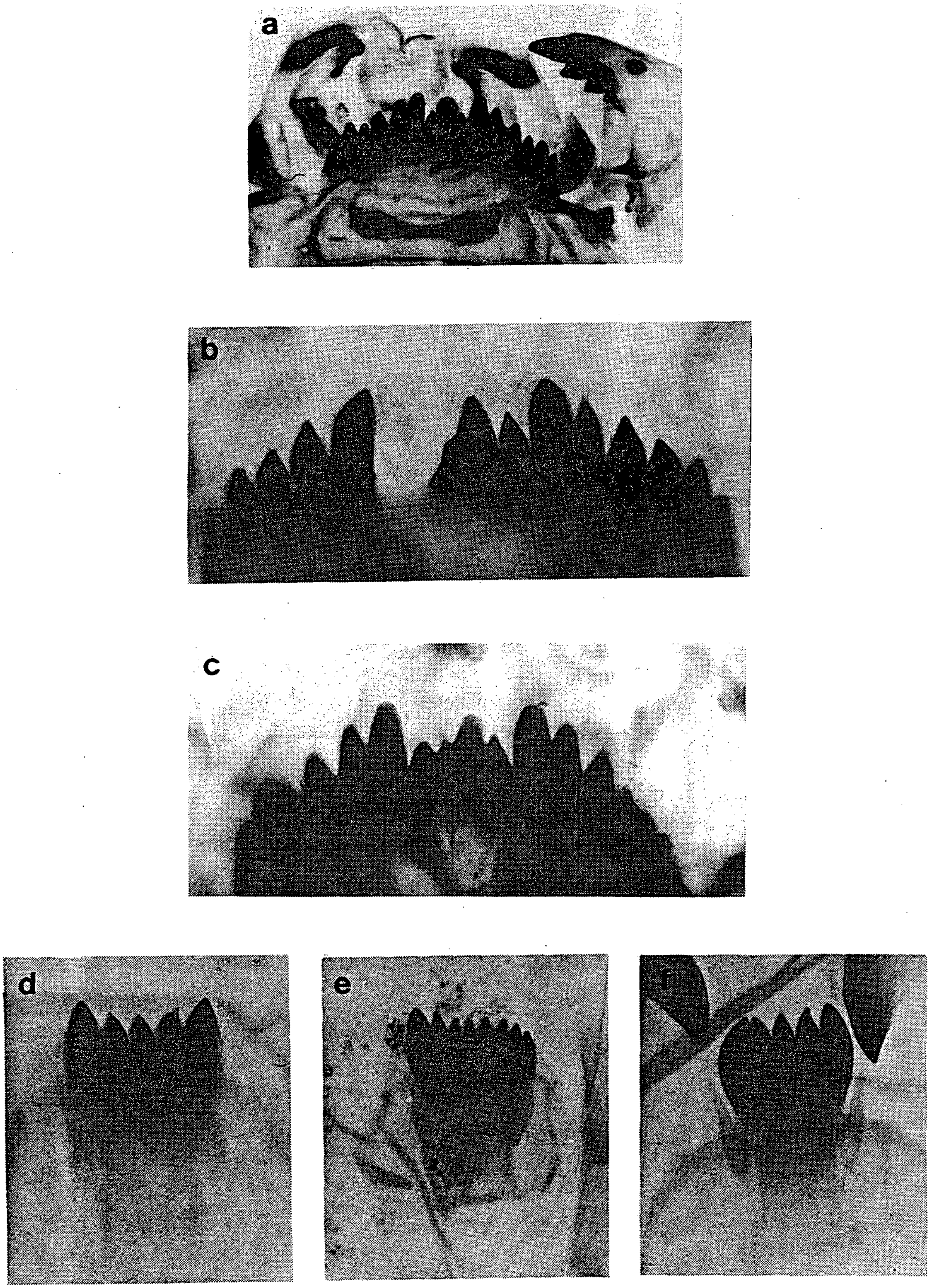
Site	Al	Cd	Co	Cu	Fe	Mn	Ni	Pb	Zn
5	0.2981	0.0002	0.0006	0.0125	0.439	0.096	0.0034	0.0029	0.531
8	0.0673	0.0001	0.0005	0.0048	0.775	0.116	0.0020	0.0007	0.508
9	0.1235	0.0001	0.0008	0.0046	0.421	0.038	0.0016	0.0011	0.313
12	0.1678	0.0001	0.0005	0.0071	0.334	0.039	0.0016	0.0010	0.510
30	1.5	0.0001	0.0016	0.0077	2.35	0.202	0.0051	0.0007	0.536
31	0.1251	0.0001	0.0005	0.0069	0.651	0.033	0.0017	0.0007	0.48
32	0.4465	0.0003	0.0007	0.0086	1.37	0.088	0.0040	0.0014	0.52
33	0.3429	0.0001	0.0006	0.0074	0.739	0.051	0.0026	0.0007	0.49
35	0.1269	0.0001	0.0005	0.0055	0.284	0.025	0.0008	0.0007	0.48
36	0.0886	0.0002	0.0005	0.0055	0.304	0.035	0.0010	0.0007	0.48

Values are for total metal, with the exception of aluminum which is extractable metal.

FIGURE 1

Locations of 1987 Sampling Sites on the Yamaska River Drainage Basin

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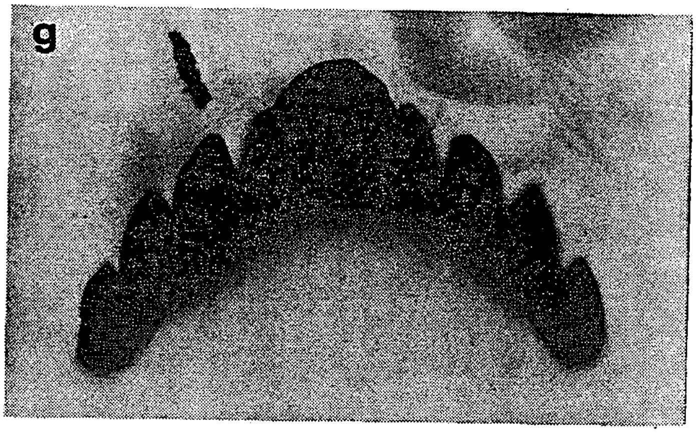
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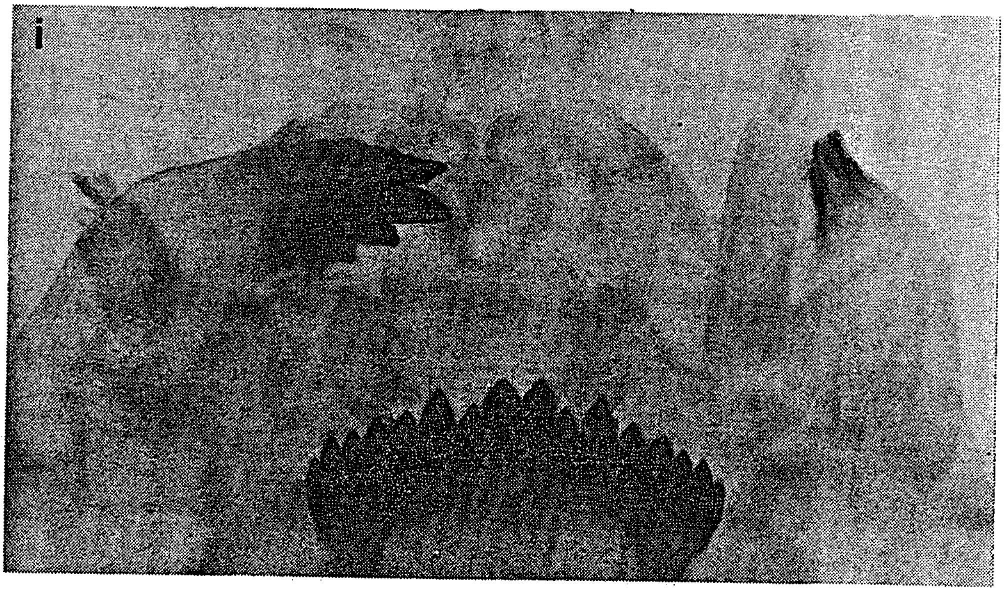
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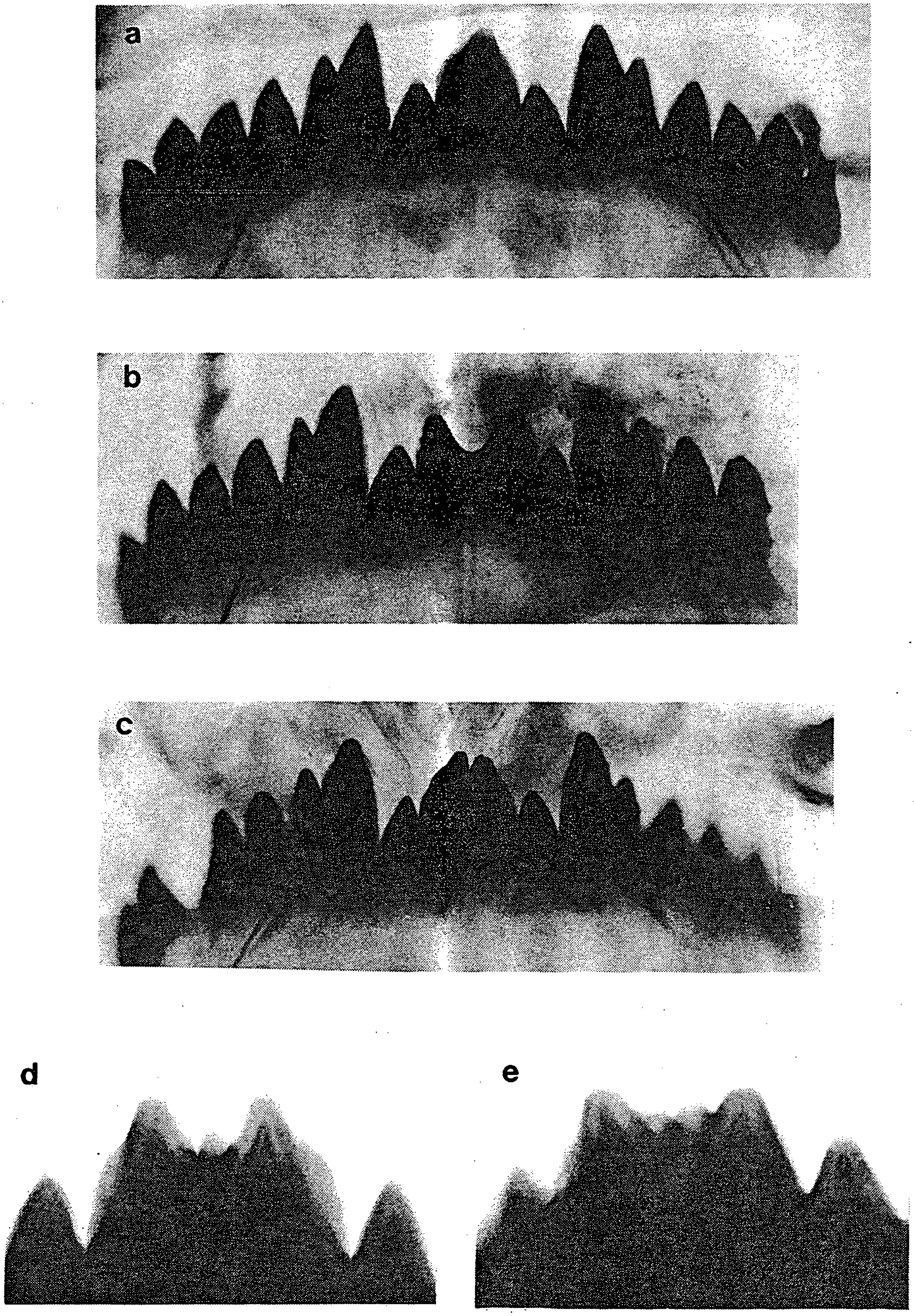
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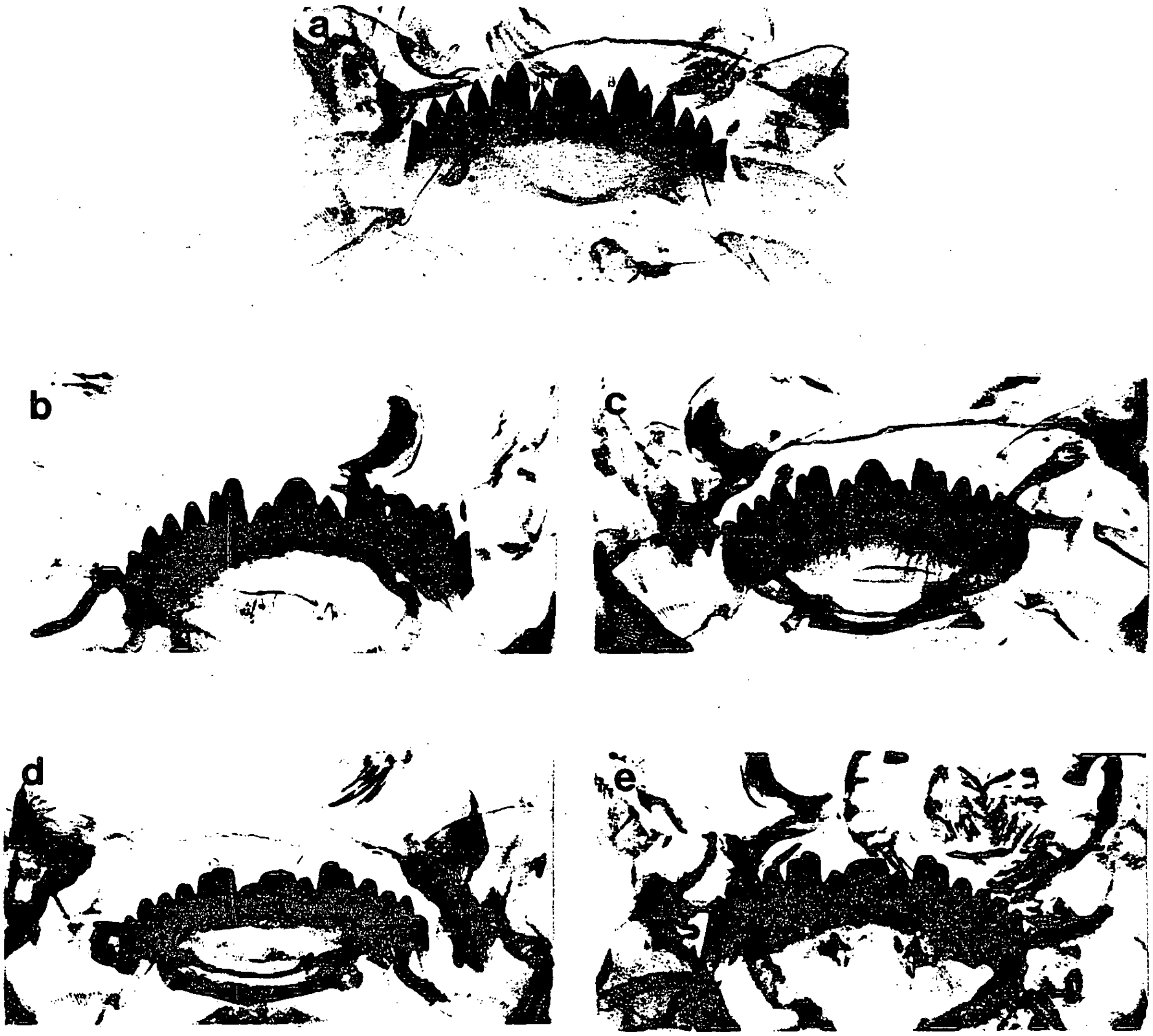
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FIGURE 5 EFFECT OF SUBSTRATE ON PERCENTAGE OF CHIRONOMUS TENTANS IN VARIOUS SIZE CLASSES