USE OF CHIRONOMID DEFORMITIES TO ASSESS ENVIRONMENTAL DEGRADATION IN THE YAMASKA RIVER, QUEBEC

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Abstract. The frequency of morphological deformities in chironomid larvae was used to assess environmental degradation at 12 sites in the Yamaska River, Quebec, that were known to be either impacted by agriculture or urban centres, or were relatively clean and used as reference sites. A total of 2273 chironomid larvae were examined for deformities. The overall frequency of deformities at polluted sites was 2.7%, whereas no deformities were observed at the reference sites. The highest incidence of deformities was found downstream of two urban centres, site 9 at Acton Vale (5.1% deformed) and site 12 at Ste Hyacinthe (5.3% deformed). The frequency of deformities at the agricultural sites ranged from 0.8 to 2.5% and was comparable to sites receiving municipal sewage effluent. The occurrence of higher frequencies of deformities downstream of urban centres indicates that the frequency of deformities increases with environmental degradation.

1. Introduction

In freshwater ecosystems, chironomids are considered to be an ideal bioassay organism since they spend most of their larval stages in surface sediments where they are exposed to toxicants in both water and sediments, their relatively sedentary forging behaviour ensures that their home range is restricted to localized areas, and they have an ubiquitous distribution. The chironomid community structure is used as an index of environmental quality (Saether, 1979).

Examination of chironomid larvae from polluted areas has revealed the presence of specimens with mouthpart deformities (primarily deformities of the hypostoma or mentum, a broad, sclerotized tooth structure). In extremely polluted environments, the frequency of chironomid mouthpart deformities may be as high as about 80% (Hare and Carter, 1976; Warwick *et al.*, 1987). However, the frequency of deformities in polluted areas generally ranges from 1% to about 30% (Dickman *et al.*, 1992; Warwick and Tisdale, 1988; Warwick, 1989, 1990; Wiederholm, 1984) and is often <5% (Cushman, 1984; Tennessen and Gottfried, 1983; Warwick, 1980a,b).

In most studies, it has not been possible to designate specific causative agents with certainty, but agricultural and industrial pollutants have been implicated. More information is required on the incidence of chironomid mouthpart deformities in relationship to various types of pollution. In this study a number of sites, known to be contaminated by a variety of pollutants, were investigated for the presence of deformed chironomid larvae, and environmental degradation at the various sites was ranked on the basis of the incidence of deformed specimens.

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The objective of the present study was to investigate the use of chironomid mouthpart deformities as a practical means of assessing environmental degradation in tributaries of the Yamaska River watershed, Quebec, Canada. The Yamaska River system is known to be polluted by a variety of contaminants including metals (Tessier *et al.*, 1980; Croteau *et al.*, 1984), nutrients from municipal sewage and agricultural runoff (Campbell *et al.*, 1976), pesticides (Muir *et al.*, 1978), dyes (Maguire and Tkacz, 1991) and various other industrial pollutants (Auger *et al.*, 1979). Information on metals, PCBs and pesticides in the Yamaska River is reviewed by Maguire (1991).

2. Methods and Materials

2.1. Study site

The Yamaska River is located in southeastern Quebec between 45°15′ and 46°05′ latitude and 72°12′ and 73°07′ longitude, about 70 km east of Montreal. It is a tributary to Lake St Pierre, which is an enlargement of the St Lawrence River, and has a drainage area of 4843 km^2. The Yamaska River watershed and its water quality have been described elsewhere (Desmeules and Gelinas, 1977; Tessier *et al.*, 1980; Quebec Ministry of the Environment, 1985; Day *et al.*, 1990; Barton and Metcalfe-Smith, 1992; Dutka *et al.*, 1991).

The source of the Yamaska River is in the Appalachian Mountains, whereas the lower reaches flow through the St Lawrence lowlands. The average slope of the Yamaska River is 1.13 km^{-1} (Desmeules and Gelinas, 1977). The basin has a boreal forest climate with mean monthly temperatures ranging from 21 °C in July to –11 °C in January. Mean annual precipitation is 994 mm, and a mean annual flow of 125 m^3 s^{-1} was reported at the confluence with the St Lawrence River (Tessier *et al.*, 1980).

Agriculture is the primary land use in the Yamaska River basin, with about 53% of the land area in agriculture, 42% in forest, 2% in urban area and 3% in other uses (Desmeules and Gelinas, 1977). Corn and hay/alfalfa are the principal crops grown in the basin. The location of our sampling sites is presented in Figure 1. The numbering of the sites is that used by Environment Canada in several other independent studies (Day *et al.*, 1990; Dutka *et al.*, 1991; Barton and Metcalfe-Smith, 1992) to assess the water quality of the Yamaska River. A description of the sites can also be found in these studies (ibid.).

2.2. Methods

Sites investigated in the present study consisted of reference sites (sites 1 and 35), which had no obvious source of pollution, urban sites (sites 5, 8, 9, 12, 15 and 16), which are affected by sewage and light industrial effluents, and agricultural sites (sites 30, 31, 32 and 33), which are affected by pesticides and nutrient runoff (Figure 1). Samples were collected between 7 and 9 July, 1987 during a period of low flow.

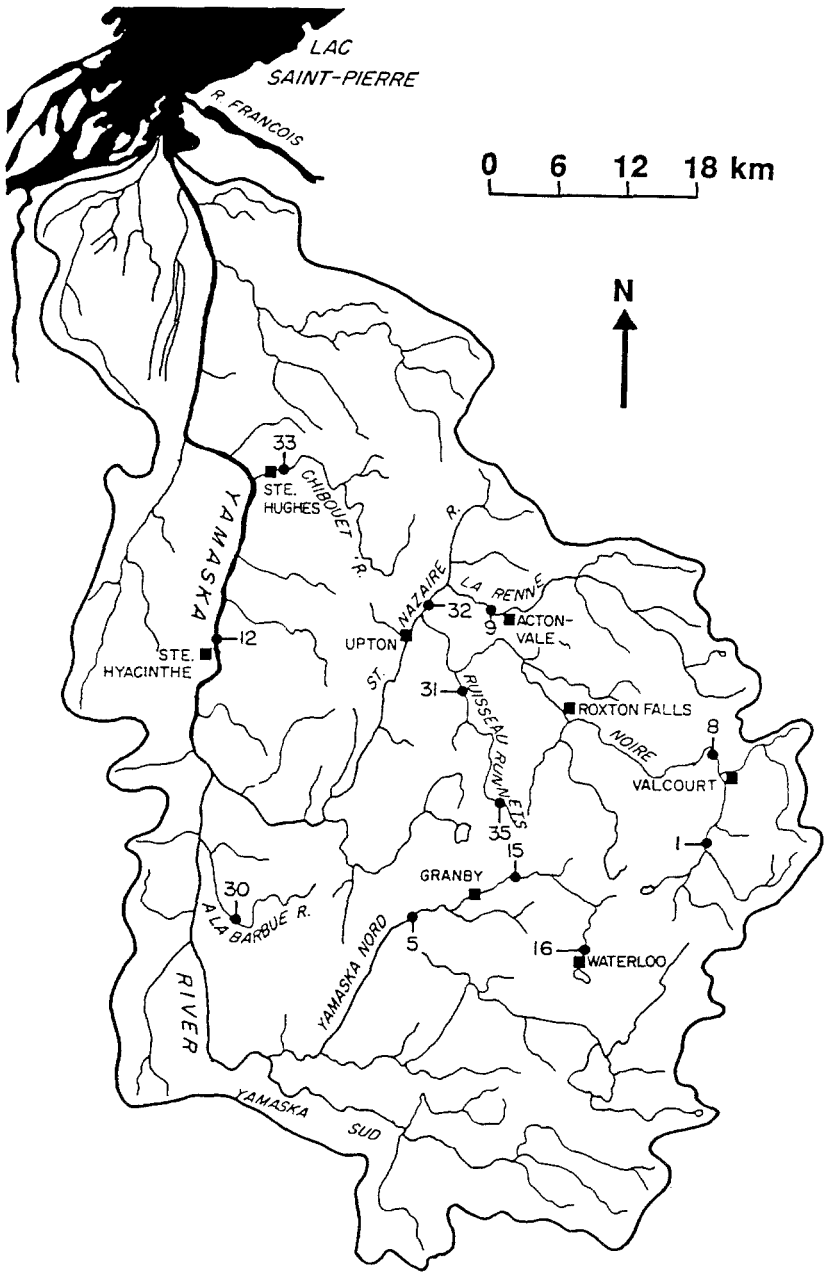

Fig. 1. Location of sampling sites in the Yamaska river drainage basin, Quebec.

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. Samples were sorted for chironomids in the field with the goal of collecting at least 200 larvae at each site. When this was not possible additional samples were collected, preserved in formalin, and sorted later in the laboratory. A sample size of 200 larvae was considered to be adequate based on the published literature (Dermott, 1991; Dickman *et al.*, 1992; Warwick *et al.*, 1987).

Chironomids were cleared in 8% KOH, then placed in glacial acetic acid for 15 min to neutralize the KOH, followed by two 15-min rinses in 70% ethanol. Head capsules were mounted ventral side up in Hoyers media under a dissecting microscope using gentle pressure on the glass cover slip to extrude the mandibles and to provide a clear view of the hypostomal teeth (although this often resulted in mandibles obscuring the view of antennae). Identification of chironomids was to genera using the taxonomic key of Oliver and Roussel (1983).

Chironomid head capsules from third and fourth instar larvae were examined for deformities under a compound microscope, generally at 400× magnification. Emphasis was placed on examining the hypostomal teeth for deformities, although the mandibles, premandibles and antennae (when clearly visible) were also examined, as was the ligula of Tanypodinae. Examination of antennae was carried out only for gross deformities, such as missing or extra segments, or major differences in size of segments between both antennae, as opposed to the more detailed examination described by Warwick (1985). Hypostoma and mandibles were classified as deformed if they exhibited extra teeth, missing teeth including gaps, or were very 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 1000× magnification for evidence of breakage. In addition, a blind test was employed on a subsample of the slides (165 specimens) to determine consistency of scoring between the author and a chironomid taxonomist (Mr. Allan Burt, Beak Consultants Limited, Brampton, Ontario). Agreement between the two scores was 96%.

Statistical analysis of the data was by the G test for goodness of fit with one degree of freedom at $p = 0.05$ level of significance (Sokal and Rohlf, 1981). To allow comparison of the frequency of deformities between sites where no deformities were observed at one of the sites, it was assumed that either one specimen or 1% of the specimens, whichever was less, was deformed.

3. Results and Discussion

A total of 2273 chironomids were collected from the 12 sites and 69% of these were Chironominae (Table I). Representative deformities observed in chironomid larvae

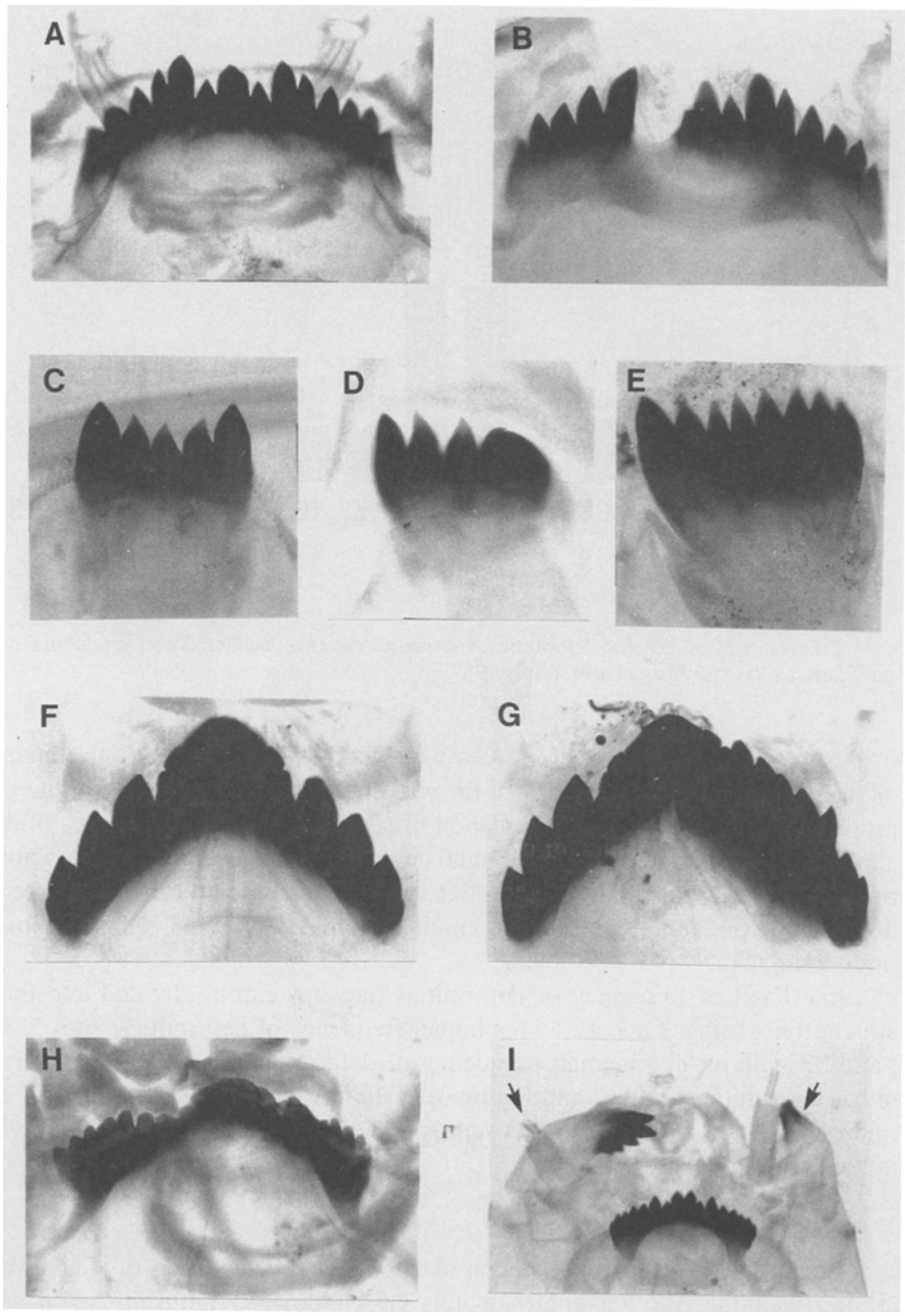

Fig. 2. Representative deformities observed in chironomids collected from the Yamaska River, Quebec: (A,B) *Chironomus* spp., (A) normal and (B) gap; (C–F) *Conchapelopia/Thienemannimyia*, (C) normal, (D) asymmetrical (fused) teeth and (E) extra teeth; (F,G) *Cricotopus trifascia*, (F) normal and (G) extra teeth; (H) grossly deformed specimen and (I) *Polypedilum (Petapedilum)* with deformed mandible and antenna (arrows).

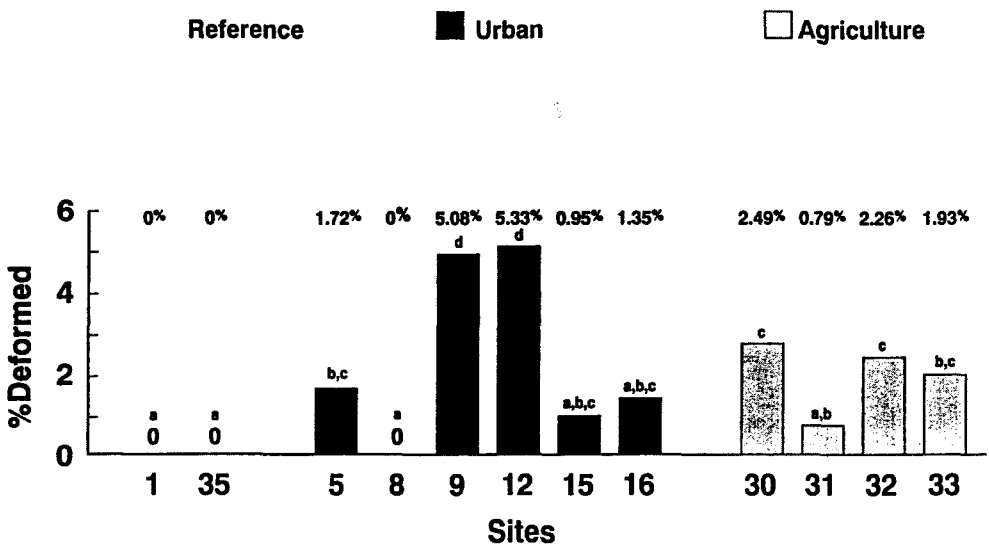

Fig. 3. Frequency of chironomid deformities observed at reference, municipal and agricultural sites in the Yamaska River drainage basin in July 1987.

are illustrated in Figure 2. Deformities were found in 13 of the 26 chironomid genera collected. This is indicative of the widespread occurrence of deformities in chironomid taxa. The poor representation of specific taxa among the sites (Table I) limited the evaluation of environmental degradation by specific taxa. Therefore, environmental degradation at the various sites was assessed by the incidence of deformities in the entire chironomid population collected at each site as opposed to individual taxa.

On the basis of frequency of deformities the sites can be divided into three distinct groups: sites 9 and 12 with a higher frequency of deformities; sites 5, 30, 32 and 33 with an intermediate frequency of deformities; and sites 1, 8 and 35 with no deformities (Table I and Figure 3). A fourth group consisting of sites 15, 16 and 31 formed a continuum between sites with no deformities and site with intermediate frequencies.

3.1. URBAN SITES

Sites 9 and 12 are located downstream of the larger urban centres of Acton Vale and Ste Hyacinthe. Site 9 is approximately 1.5 km downstream from Acton Vale and is affected by textile mill and sewage effluents. Site 12 is downstream of Ste Hyacinthe, which contributes sewage effluent as well as effluents from textile mills and light industry to the river, but is upstream of the sewage treatment plant outlet. At site 9, 5.1% of the larvae were deformed (Table I). Most of the deformities were

TABLE I

Number of chironomids and deformities in samples collected from the Yamaska River in July 1987.

	Station											
	1R*	5U	8U	9U	12U	15U	16U	30A	31A	32A	33A	35R
Tanypodinae												
Ablabesmyia (s.s.) *mallochi*											6	
Natarsia											3(1)	
Procladius	10						1				8	18
Conchapelopia/Thienemannimyia	8	59(2)*	28	3	24	5(1)	49(2)		35	3	8	
Chironominae												
Tanytarsini												
Micropsectra												
Paratanytarsus			2			1		6				
Rheotanytarsus	5				1	69	1	2	1	2(1)		25
Tanytarsus						5		59	4			14
Chironomini												
Axarus nr. *taenionotus*	1				1						129(1)	
Chironomus (s.s.)	2	4	2	172(9)	10(2)	12		154(4)			7	1
Cryptochironomus	2	1	4		3	3	1		7		9	10
Dicrotendipes neomodestus											2	
Glyptotendipe (Phytotendipe)				5(1?)	30						4	
Harnischia curtilamellata	1							1			2	
Microtendipes				(1)	1	2			28(1)	3	14(2)	2
Phaenopsectra	3	4			5	9		6(1)			9	26
Polypedilum (s.s.) *convictum*	21	98(2)	18	29	100(7)	11	23	1	37	3	3	7
Polypedilum (Pentapedilum)		15				1	4					59
Demicryptochironomus												
Stictochironomus			1	2				12(1)	8	207(4)	2	12
sp.?	2	1	3									

TABLE I (continued)

	Station											
	1R*	5U	8U	9U	12U	15U	16U	30A	31A	32A	33A	35R
Diamesinae												
Pagastia	2	3				1	54		1			
Orthocladiinae												
Brillia												
Cricotopus (s.s.) *bicinctus*	17	8	3	6(1)		5	2					11
Cricotopus (s.s.) *triannulatus*	13	4	1	6		1				3	2	
Cricotopus (s.s.) *trifascia*				2		3				1		1
Cricotopus (s.s.) *vterriensis*				7								
Parametriocnemus												
Psectrocladius (s.s.) *limbatellus*							2					
Tvetinia bavarica gr.				3		1	11					4
Tvetinia discoloripes gr.	1	36	3		106(6)	81(1)						26
Total number	88	233(4)	65	236(12)	281(15)	210(2)	148(2)	241(6)	126(1)	221(5)	207(4)	217
Frequency of deformities	0%[a]	1.72%[bc]	0%[a]	5.08%[d]	5.33%[d]	0.95%[abc]	1.35%[abc]	2.49%[c]	0.79%[ab]	2.26%[c]	1.93%[bc]	0%[a]

* Values in parentheses represents the number of deformed larvae; R = reference site; U = urban site; and A = agricultural site.

[a,b,c,d] Values in the row for frequency of deformities with different letters are significantly different at $p < 0.05$. Statistical analysis was by one-way goodness of fit using the G test with one degree of freedom (Sokal and Rohlf, 1981).

seen in *Chironomus* larvae (5.2% were deformed). Single deformed specimens were also found in *Glyptotendipes* and *Microtendipes* (Table I). At site 12, 5.3% of the larvae were deformed. Here, 20% of the *Chironomus*, 7% of the *Polypedilum* and 5.7% of the *Tvetinia discoloripes* gr. were deformed (Table I).

The frequency of deformities at site 5, downstream from Granby, was no different from that at agricultural sites 30, 32 and 33. At site 5, 1.7% of the chironomid larvae were deformed. Here deformities were found in *Polypedilum* and *Conchapelopia/Thienemannimyia* (Table I). Site 5 receives municipal sewage treatment plant effluents and heavy metals from several metal processing plants in Granby. For example, 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 farther downstream, to inputs from Granby.

A low frequency of deformities was observed at urban sites 15 and 16. At site 15, the Yamaska River at Val Shefford, which is located approximately 16 km downstream from Waterloo, 1.0% of the larvae were deformed, although 20% of the *Conchapelopia/Thienemannimyia* larvae were deformed (Figure 2f). At site 16 located about 3 km downstream from Waterloo, which receives metal pollution from electroplating operations, 1.4% of the larvae were deformed. No deformities were observed at site 8 downstream from Valcourt, which receives effluents from light industry.

3.2. AGRICULTURAL SITES

The presence of deformities at the agricultural sites decreased in the order site 30 > site 32 > site 33 > site 31. The occurrence of deformities at these agricultural sites (Tables I, Figure 3) suggests that pesticides may be having an impact on the benthic fauna. In a study of atrazine in these watersheds, Muir *et al.* (1978) found that concentrations were highest in June and July during the herbicide spray season and when heavy rainfall was frequent, whereas 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, 16.3 and 10.1% of the cultivated area of the St Nazaire (site 32), a la Barbue (site 30), Chibouet (site 33) and Runnets Rivers (site 31) respectively were planted in corn (Muir *et al.*, 1978). Thus, the consistently higher incidence of deformities at site 30 is associated with a greater portion of cultivated land in corn (25.1%), and the lowest frequency at site 31 is associated with the smallest portion (10.1%) of cultivated land in corn.

3.3. RANKING OF SITES

In the present study, the frequency of deformities ranged from 0 to 5.3% and at most sites was <2% (Table I). No deformities were observed at sites 1, 8 and 35 and a low number (<1%) of deformities was observed at sites 15 and 31 (Table I), so these sites can be considered to be nonpolluted. Assuming a background level of deformities of <1% (Table I; Warwick, 1980b; Wiederholm, 1984), sites with a frequency of deformities of >1% can be considered to be polluted. This includes sites 5, 9, 12,

16, 30, 32 and 33. The overall frequency of deformities at these sites was 3.19%. The low frequency of deformities observed in our study of generally between 1% and 5% for urban areas is comparable to the findings of others (Warwick, 1980a,b; Weiderholm, 1984; Cushman, 1984; Pettigrove, 1989; Tennessen and Gottfried, 1983). In the Yamaska River, frequencies of deformities of 1% to about 3% probably indicate areas that are slightly to moderately polluted, whereas the 5% levels below urban centres probably indicates moderately to severely polluted conditions.

Based on the frequency of deformities, environmental degradation at the urban sites is ranked in the following order of decreasing severity: site 12 > site 9 > site 5 > site 16 > site 15 > site 8. This order corroborates the findings of Barton and Metcalfe-Smith (1992), who assessed environmental degradation in the Yamaska River using benthic macroinvertebrate bioindices based on samples collected at the same time as those in the present study. They found that sites 9 and 5 were their most environmentally degraded sites using the total biotic index, whereas site 8 was their least impacted urban site (Figure 6A of Barton and Metcalfe-Smith, 1992). In their study, site 12 was intermediate between sites 9 and 5 and site 8. Our study site 12 was upstream from theirs, and was possibly more affected by Ste Hyacinthe. Barton and Metcalfe-Smith (1992) did not sample sites 15 or 16, nor did they sample our reference sites so a comparison is not possible. Based on the frequency of chironomid deformities, environmental degradation at the agricultural sites is in the following order of decreasing severity: site 30 > site 33 > site 32 > site 31 > site 35. This is also in general agreement with the findings of Barton and Metcalfe-Smith (1992), who found that degradation at the agricultural sites was in the order site 30 > site 33 > site 31 > site 32. Thus, our two independent studies using different approaches are in general agreement in the order of ranking of environmental degradation at both the urban and agricultural sites.

3.4. Deformity as a bioassay

Monitoring environmental stress at the organism level may be more useful than at the community level because individual responses occur before community responses and, therefore, may provide an early warning (Petersen and Petersen, 1983). In this respect, the frequency of chironomid deformities may be an ideal bioassay tool in certain situations. This is supported by the recent finding of higher body burdens of contaminants in deformed larvae than normal larvae (Dickman *et al.*, 1992; Janssens de Bisthoven, 1992). However, more information is required on the occurrence of deformities in response to pollutants before deformity can become a useful biotic index. What pollutants cause deformities? Is there a concentration dependent response in frequency and/or severity of deformities? Is deformity a sensitive bioassay?

It has been suggested that different types of chironomid deformities may indicate specific types of environmental pollution and that the frequency of deformities increases with the severity of pollution (Warwick, 1980a). In our study, deformities

found in chironomids collected at agricultural sites versus urban sites were similar in appearance and do not support the hypothesis that deformities are specific to particular environmental pollutants. However, the presence of higher frequencies of deformities downstream of larger urban centres supports the suggestion that the frequency of deformities increases with severity of environmental degradation. The widespread occurrence of deformities in the Yamaska River system suggests that this is a very degraded ecosystem.

In the present study, samples were collected at seven sites in June during a high discharge event and at site 15 in August. Because of the low number of chironomids collected on these occasions the data were not presented. However, the data are presented elsewhere (Bird, 1989). It was apparent from the findings of this study that more information is required on the background levels of deformity. In June, deformed specimens were found at reference site 1, which raises the question: were these deformities representative of background levels in the Yamaska River, or were the deformed larvae washed downstream from a more impacted area? Interpretation of the findings was also confounded by the fact that sample size and taxonomic assemblages vary among the sites (Table I), and all taxa are not susceptible to the same level and type of contaminant exposure. Furthermore, temporal variation also occurred in the incidence of deformities (Bird, 1989), although it is worth noting that these difficulties are encountered in other bioassays, e.g. in standard laboratory bioassays (*Daphnia magna* and Microtox toxicity tests) on the Yamaska River sediment samples (Dutka *et al.*, 1991), in invertebrate community structure (Barton and Metcalfe-Smith, 1992), and in the SOS test performed on sediment samples from the Welland River, Ontario (Lan *et al.*, 1991).

On the basis of frequency of deformities to assess environmental degradation, the 12 sites were divided into four groups consisting of two more degraded urban sites, four moderately degraded urban and agricultural sites, three relatively clean sites, and three sites that spanned the range between clean and moderately degraded sites. Agriculture appears to have as severe an impact on the river as municipal and industrial effluents.

In conclusion, higher frequencies of deformities were associated with the level of pollution at the sites and the ranking of the sites was in general agreement with the total biotic index of environmental degradation (Barton and Metcalfe-Smith, 1992). However, further information is required on the concentration-related response (frequency and severity) of deformities to pollutants and the background level of deformities in natural areas. The use of chironomid deformities as a laboratory bioassay also requires assessment.

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