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Baseline Incidence of Mouthpart Deformities in Chironomidae (Diptera) From The Laurentian Great Lakes, Canada

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ABSTRACT. *Larval chironomid mouthpart deformities are used as indicators of anthropogenic stress. However, there are limited data on the incidence of naturally occurring deformities. Chironomid larvae were collected from 252 reference sites throughout the Great Lakes by Environment Canada from 1991 through 1993. Overall incidence of mentum deformities was 2.27% for* Procladius *(SE = 0.46, n = 1055), 2.15% for* Chironomus *(SE = 0.51, n = 839), 1.27% for* Heterotrissocladius *(SE = 0.57, n = 393), 1.38% for* Tanytarsus *(SE = 0.61, n = 363), and 3.25% for* Polypedilum *(SE = 1.07, n = 277). The most common deformity was one missing tooth in the mentum. Deformity frequency was highest in Northern Channel and Georgian Bay of Lake Huron. However, incidences were homogeneous among regions (G-test, p > 0.05). In examining contaminated conditions, a result greater than one 95% confidence interval above these reference deformity frequencies should be considered significantly elevated from baseline levels.*

INDEX WORDS: *Chironomidae, mouthpart, deformity.*

INTRODUCTION

Many taxa of larval chironomids develop in intimate contact with sediments, and are therefore exposed to substrate-bound contaminants (Heinis *et al.* 1990). Numerous studies have indicated a strong association between chironomid deformities and contamination (Dickman *et al.* 1992; Diggins and Stewart 1993; Hudson and Ciborowski 1996a,b; Janssens de Bisthoven *et al.* 1995; Lenat 1993; Martinez *et al.* 2001, 2002; Meregalli *et al.* 2002; Warwick 1985, 1988, 1996; Wise *et al.* 2001). Morphological deformities of the chironomid mentum and mandibles are the main biomarkers of degraded environmental conditions, though various other mouthparts, the antennae, and pigmentation have been used as well (Hamilton and Saether 1971; Hare and Carter 1976; Warwick 1988, 1990b; Janssens de Bisthoven *et al.* 1995). However, interpretation of deformity data requires results be compared with those from suitable reference sites.

Although few, if any, aquatic environments completely lack contaminants, reference areas are defined on the basis of the physical, chemical, and biological characteristics of the areas which represent the least degraded constituency available. Because there is insufficient knowledge of the natural levels of chironomid mouthpart deformities (Janssens de Bisthoven *et al.* 1995), due to small sample sizes and varying definitions of deformities, baseline levels have been reported from 0.0% (Hamilton and Saether 1971, Bird 1994) to > 40% (Warwick 1990b, Janssens de Bisthoven *et al.* 1992). Research is needed to determine true variability in background levels of deformities.

The purpose of this study was to document the baseline levels of morphological deformities in chironomids collected from a broad geographic region and from a large number of locations characterizing

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FIG. 1. Map showing reference sites throughout the Great Lakes.

reference conditions. Natural incidence of mouthpart deformities, among individual genera and structures, and spatial variation among reference sites were examined. Because deformities are assumed to be associated with anthropogenic stress, and not natural stresses, it is expected that there will be no systematic spatial variation in the incidence of deformities among reference sites. As well, the observed incidence of deformity is expected to be low (< 5%), based on the incidence previously reported in uncontaminated areas (Burt and Ciborowski 1999).

METHODS

Larvae were collected, prepared, and identified by Environment Canada as part of a larger study on benthic community composition in the Great Lakes basin (Reynoldson *et al.* 1995, Reynoldson and Day 1998, Reynoldson *et al*. 2000). Samples were taken from 1991 to 1993 at 252 nearshore reference sites withing 21 independent ecodistricts throughout the Great Lakes, which were judged to be representative of unpolluted conditions (Fig. 1). Reference sites were distinguished on the basis of 43 geographic, sediment and limnological variables (Table 1). Reference sites were located at least 5 km from known point-source discharges as described in the Ontario Intake and Outfall Atlas (Ontario Ministry of the Environment 1990) and within 2 km of shore (Reynoldson *et al.* 2000). Sites were at a depth of less than 50 m and known or suspected to have fine grained sediments (Bailey *et al.* 1995). Detailed site-specific information on sediment and water quality parameters is available from the Environment Canada electronic data archive (T.B Reynoldson, Environment Canada, pers. comm.).

Organisms were collected by inserting five 10-cm-long Plexiglas tubes (i.d. 5.5 cm) into the sediment collected with a box corer. Contents of each sample were sieved through a 250 µm mesh sieve and the materials retained were preserved in 10% formalin before being transferred to 70% ethanol. A low power stereo microscope was used to sort samples. Chironomids were subsequently slide mounted using polyvinyl lactophenol mounting medium and

TABLE 1. Environmental variables used to distinguish Great Lakes reference sites (adapted from Reynoldson* et al. *2000).

Geographic Variables	Limnological Variables	Sediment Variables
Latitude	Water depth	Particle size (% gravel, sand, silt, clay,
Longitude	Dissolved oxygen	mean size, 75th and 25th percentiles)
Lake basin	pH	Major elements (oxides of Si, Ti, Al,
Ecodistrict	Temperature	Fe, Mn, Mg, Ca, Na, K, P)
Date	Alkalinity	Nutrients (TP, TN, loss on ignition,
	Total phosphorus	TOC)
	Kjeldahl nitrogen	Metals (Totals for V, Cr, Co, Ni, Cu,
	Nitrate-nitrite nitrogen	Zn, As, Cd, Pb) \

identified to genus. These organisms were then examined for morphological deformities. Deformities were recorded at 40X through 1,000X with a Kyowa Medilux-12 compound microscope.

In order to account for differences in sensitivity among genera (Warwick 1988, Martinez *et al.* 2002), baseline levels of deformities were tabulated in five separate genera: *Chironomus, Procladius, Tanytarsus, Polypedilum,* and *Heterotrissocladius*. The baseline level of deformities for each of these five genera was tabulated to provide data on individuals from across a range of feeding habits (Saether 1979, Martinez *et al.* 2002). Representative genera were selected for study based on abundance data compiled by Environment Canada (T.B. Reynoldson, pers. comm.). A total of 2,927 chironomid larvae was examined for deformities. The most abundant genus was *Procladius,* with 1,055 individuals, followed by *Chironomus* with 839, *Heterotrissocladius* with 393, *Tanytarsus* with 363, and *Polypedilum* with 277 individuals. These five genera were collected from 211 of the 252 sampling sites. Individuals that were poorly mounted or damaged were excluded from the study.

Deformities in the mentum or ligula and mandibles were recorded, as these are among the most common structures to have deformities (Vermeulen 1995). The mentum, present in *Chironomus*, *Polypedilum*, *Tanytarsus,* and *Heterotrissocladius*, is a broad, sclerotized, toothed structure used in collecting, scraping, and shredding food (Armitage *et al.* 1995). The *Procladius* ligula is a five-toothed, heavily sclerotized organ found at the anteromedial margin of the head capsule (Oliver and Roussel 1983). The basic structure of the mandibles is the same for all genera, with a row of teeth falling along the inner margin. Each of the paired mandibles was examined for deformities in each individual. Only those individuals that displayed a mentum or mandible with missing or extra teeth were defined as deformed (Dickman *et al.* 1992, Hudson and Ciborowski 1996a). Examples of a deformed *Chironomus* mentum and mandible are illustrated in Figure 2. This conservative definition avoids the subjectivity inherent in some other classification schemes (Warwick and Tisdale 1988, Lenat 1993).

Incidence of deformity was expressed as the proportion ± 1 standard error (SE) of deformed larvae at each site. Standard error was determined from the binomial theorem as $\sqrt{(pq/n)}$, where p is the proportion deformed, q is 1-p, and n is the sample size. Heterogeneity of the frequency of deformities among sites in five geographic areas was examined using the G-statistic goodness-of-fit test (Sokal and Rohlf 1981), and Yates' correction applied to those groups with $n < 5$ (Zar 1996).

The incidence of deformity for each genus was examined separately for each Great Lake, which provided sufficient sample sizes for analysis of spatial variation.

RESULTS

Deformed menta and mandibles were observed in all five genera examined. The overall incidence of deformities throughout the Great Lakes is summarized in Table 2.

Mentum Deformities

All genera exhibited relatively low levels of mentum deformities (Table 2). The most common deformity for *Chironomus*, *Polypedilum*, *Tanytarsus*, and *Heterotrissocladius* was the absence of a lateral tooth of the mentum. In *Procladius,* the most common aberration was the absence of the median tooth in the ligula. Incidence of deformities across the Great Lakes is recorded in Table 3. The frequency

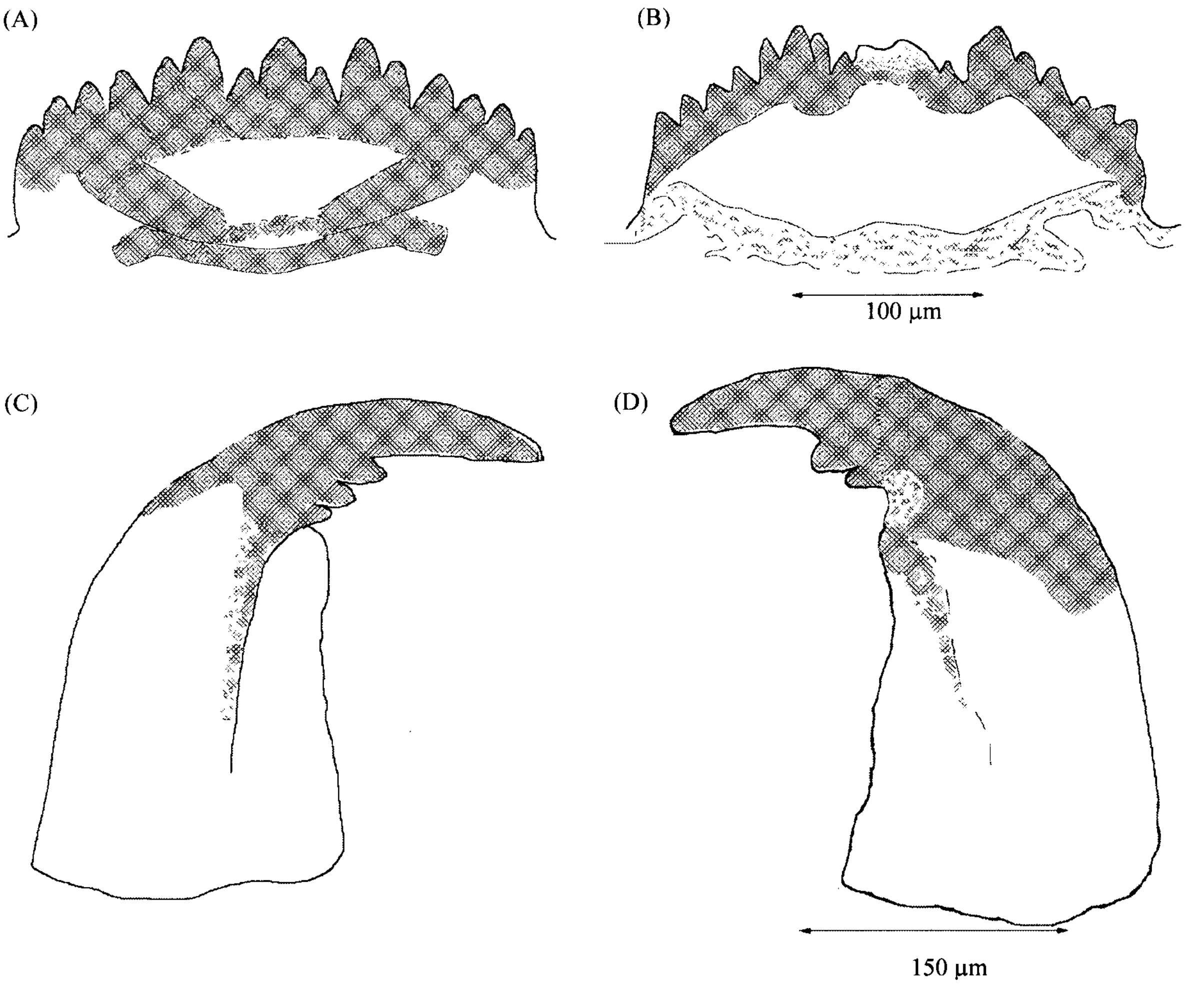

FIG. 2. Ventral view of* Chironomus *mouthparts. (A) Normal mentum. (B) Deformed mentum with an extra lateral tooth. The median tooth is not considered deformed by our conservative definition; See text. (C) Normal left mandible. (D) Deformed right mandible with missing tooth. (Figs. A and C adapted from Oliver and Roussel 1983.)

of mentum deformities was higher in the Northern Channel and Georgian Bay than elsewhere, but the difference was not significant ($p > 0.05$). All genera displayed homogeneity in mentum deformities among regions (*Chironomus:* G = 7.89, df = 4, $p > 0.05$; *Procladius:* G = 5.03, df = 3, $p > 0.05$; *Polypedilum:* G = 3.51, df = 2, $p > 0.05$; *Tanytarsus:* G = 1.98, df = 2, $p > 0.05$; *Heterotrissocladius:* G = 2.51, df = 2, $p > 0.05$).

Mandible Deformities

The frequency of deformity in the mandibles was lower than that observed in the mentum (Table 2). Similar to the mentum, the most common mandibular deformity involved the loss of a tooth. No significant among-region variation (Table 4) was observed in any of the genera examined (*Chironomus*: G = 2.54, p > 0.05; *Procladius*: G = 1.27, p > 0.05; *Polypedilum*: G = 0.00, p > 0.05; *Tanytarsus*: G = 0.76, p > 0.05; *Heterotrissocladius*: G = 0.05, p > 0.05).

DISCUSSION

While most mentum deformities in the present reference study occurred within the lateral teeth, at contaminated sites deformities are most commonly reported in the median teeth (Cushman 1984, Wiederholm 1984, Dermott 1991, Janssens de Bisthoven *et al.* 1992, Bird 1994, Bird *et al.* 1995). Medial Köhn gaps are a particularly common defor-

TABLE 2. Incidence of deformities (%±SE) in the mentum and mandibles of five genera collected from Great Lakes reference sites.

Genus	Deformed Mentum (%±SE)	Upper 95% Confidence Limit	Deformed Mandibles (%±SE)	Upper 95% Confidence Limit	*n*
Chironomus	2.15 ± 0.51	3.15%	1.55 ± 0.43	2.39%	839
Procladius	2.27 ± 0.46	3.17%	1.04 ± 0.31	1.65%	1,055
Polypedilum	3.25 ± 1.07	5.35%	0.72 ± 0.51	1.72%	277
Tanytarsus	1.38 ± 0.61	2.58%	1.65 ± 0.67	2.96%	363
Heterotrissocladius	1.27 ± 0.57	2.39%	0.51 ± 0.36	1.22%	393

mity associated with contamination (van Urk *et al.* 1992, Hudson and Ciborowski 1996a). This suggests that deformities observed in the lateral teeth of the mentum may be due primarily to natural developmental variation, while median tooth deformities and Köhn gaps are more likely the result of contaminants. Though a study by Janssens de Bisthoven *et al.* (1995) did not reveal any pollutant-specific deformity responses, that study did not discriminate between different deformity types or areas of the mentum. Further research is neccessary in this area to elucidate relationships.

There are few reports of elevated levels of mandibular deformities in the literature, perhaps because extreme stress may be necessary for induction (Warwick 1988). The most common deformity in the mandibles of larvae collected from the Great Lakes reference sites was a missing tooth. In examining *Procladius* larvae from the St. Clair River, Dermott (1991) observed that there were few deformities of the mandible aside from a wrinkled outer margin. All other studies utilizing deformities of the mandible have not described the specific aberrations (Hamilton and Saether 1971; Hare and Carter 1976; Wiederholm 1984; Warwick 1990a, b; Janssens de Bisthoven *et al.* 1992, 1995; Bird 1994; Bird *et al.* 1995; Jeyasingham and Ling 1997; Pardalis 1997).

Spatial Variation in Mouthpart Deformities

Though the reference sites represent a diverse array of environmental and geological conditions,

TABLE 3. Incidence of mentum deformities (%±SE) among Great Lakes water bodies for each of the genera examined. Numbers in parenthesis represent sample size.

Lake	*Chironomus*	*Procladius*	*Polypedilum*	*Tanytarsus*	*Heterotrissocladius*
Superior	0.0 (1)	0.0 (2)	N.A. (0)	0.0 (13)	0.0 (124)
North Channel	0.81 ± 0.80 (124)	5.75 ± 2.49 (87)	2.84 ± 1.40 (141)	1.09 ± 1.08 (92)	5.88 ± 4.03 (34)
Georgian Bay	5.33 ± 2.60 (75)	1.98 ± 0.59 (555)	3.00 ± 1.70 (100)	1.48 ± 1.04 (135)	2.17 ± 1.52 (92)
Lake Huron	0.0 (1)	N.A. (0)	13.33 ± 8.78 (15)	0.0 (53)	0.0 (34)
Lake Michigan	4.76 ± 2.73 (84)	0.0 (16)	0.0 (2)	0.0 (30)	0.96 ± 0.96 (104)
Lake St. Clair	N.A. (0)	0.0 (1)	0.0 (1)	0.0 (1)	N.A. (0)
Lake Erie	2.06 ± 0.73 (389)	1.55 ± 0.77 (258)	0.0 (13)	50.0 ± 35.36 (2)	N.A. (0)
Lake Ontario	0.61 ± 0.6 (165)	3.17 ± 1.56 (126)	0.0 (5)	2.70 ± 2.66 (37)	0.0 (5)

TABLE 4. Incidence of mandible deformities (%±SE) among Great Lakes water bodies for each of the genera examined. Numbers in parenthesis represent sample size.

Lake	*Chironomus*	*Procladius*	*Polypedilum*	*Tanytarsus*	*Heterotrissocladius*
Superior	0.0 (1)	0.0 (2)	N.A. (0)	0.0 (13)	0.81 ± 0.81 (124)
North Channel	1.61 ± 1.13 (124)	2.30 ± 1.60 (87)	1.42 ± 1.00 (141)	1.09 ± 1.08 (92)	0.0 (34)
Georgian Bay	1.33 ± 1.32 (75)	1.08 ± 0.44 (555)	0.0 (100)	1.48 ± 1.04 (135)	1.08 ± 1.08 (92)
Lake Huron	0.0 (1)	0.0 (10)	0.0 (15)	1.89 ± 1.87 (53)	0.0 (34)
Lake Michigan	3.57 ± 2.02 (84)	0.0 (16)	0.0 (2)	3.33 ± 3.28 (30)	0.0 (104)
Lake St. Clair	N.A. (0)	0.0 (1)	0.0 (1)	0.0 (1)	N.A. (0)
Lake Erie	1.03 ± 0.51 (389)	0.78 ± 0.55 (258)	0.0 (13)	0.0 (2)	N.A. (0)
Lake Ontario	1.82 ± 1.04 (165)	0.79 ± 0.79 (126)	0.0 (5)	2.70 ± 2.66 (37)	0.0 (5)

these differences have no significant impact on the incidence of deformities. This suggests that elevated chironomid mouthpart deformities when observed are due to causes other than to variation in natural environmental characteristics.

Incidence of Baseline Deformities

Chironomid mouthpart deformities have become increasingly used as an indicator of environmental stress. However, the incidence of deformities observed in contaminated sites must be significantly higher than observed in reference sites if one is to conclude that the contamination is having a negative impact. A meta-analysis by Burt and Ciborowski (1999) described the results of 28 reports utilizing deformities in chironomids as an indicator of contamination. Of these, 12 studies did not tabulate the incidence of deformities in any reference area, and all but four failed to find a significant increase in the magnitude of deformities in the contaminated sites. This was attributed mainly to the use of small sample sizes, which led to large standard errors in the estimate of incidence of deformity. Nevertheless, a meta-analysis revealed a very strong association between contaminants and deformities (Burt and Ciborowski 1999).

Previous authors have suggested that an incidence of deformity of 3% is the typical background level in the lower Great Lakes (Dermott 1991, Hudson and Ciborowski 1996a). This study has shown the overall mean reference deformity frequencies to be less than 3.25%, though specific water bodies (North Channel) have incidences above 5%. Because the incidence of deformity is represented by a proportion, the strength of a result is based upon sample size. Hudson and Ciborowski (1996a) recommended that for detection of a doubling of their estimated background levels to be judged statistically significant at $p < 0.05$, a minimum sample size of 125 individuals be used.

Using the incidence of deformity observed at reference sites in the Great Lakes, the binomial theorem was used to back-calculate the minimum sample sizes needed for each genus if the observed incidence of deformity was 5% and the results were statistically significant ($p = 0.05$). An incidence of 5% mentum deformities in any sample size of 92 *Procladius*, 86 *Chironomus*, 48 *Heterotrissocladius*, 52 *Tanytarsus*, or 998 *Polypedilum* would indicate a statistically significant increase over the baseline incidence. Based on these results, the recommendation of Hudson and Ciborowski (1996a) would be more than sufficient for a 5% incidence to be significant in all genera, with the exception of *Polypedilum*. The incidence of baseline mentum deformity is similar for each of *Procladius, Chironomus*, *Heterotrissocladius*, and *Tanytarsus*. If individual numbers are low in reference sites, it is possible to pool results into a composite for these genera to create a larger sample size and strengthen statistical comparison. It is not recommended that results be pooled for contaminated sites, however, as generic differences in susceptibility to deformity have been observed with increased contamination (Burt 1998, Warwick 1990a).

The incidence of mentum deformity in all genera pooled from the Great Lakes reference sites is 2.1 ± 0.2% (n = 2,927). This is elevated from results observed in streams, rivers, and wetlands where the baseline incidence is generally less than 1% (0.79 ± 0.22% (n = 1619): Swansburg *et al*. 2002; 0.0% (n = 305): Bird 1994; and 0.6 ± 0.42 (n = 335): Whelly 2000). As a result, fewer organisms would be required to assess deformities in the Great Lakes than in other environments.

The baseline deformity frequency recorded in this study is spatially homogeneous, and overall sample sizes exceed most reported previously, providing a much smaller standard error. One may now determine the statistical significance of elevated mentum deformity evaluations in any Great Lakes site by comparing the incidence of deformities in a study site, with the baseline incidence reported herein. This may be done by using either a goodness-of-fit test or the normal approximation for comparing two proportions. A conservative approach consists of calculating the lower 95% confidence limit of the incidence of mentum deformities at a study site. If it is higher than the upper 95% confidence limit calculated from the reference data in this study, 2.73% for *Procladius*, 2.65% for *Chironomus*, 1.84% for *Heterotrissocladius*, 1.98% for *Tanytarsus*, and 4.31% for *Polypedilum*, then one may conclude that there is a significant elevation in the incidence of mentum deformities ($p < 0.05$).

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