Using *Chironomus* (Chironomidae: Diptera) mentum deformities in environmental assessment.

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RESUMO: Uso de deformidade em mento de larvas de *Chironomus* (Chironomidae: Diptera) em diagnóstico ambiental. A análise de incidência de deformidades em mento de *Chironomus* foi empregada com a finalidade de se verificar a ocorrência de efeito crônico sobre a biota devido a presença de contaminantes nos sedimentos e discutir sua aplicação em programas de monitoramento. Amostras de sedimento foram obtidas na margem deposicional do rio Tietê, município de Itú, no inverno/97 e no verão/98, para a realização de análises químicas, ecotoxicológica e biológica, tendo sido consideradas deformidades: falta de dente, dente extra e "gap". A comunidade bentônica apresentou densidades altas de Tubificidae e *Chironomus* e o resultado do teste de toxicidade foi sempre positivo. Quimicamente, apenas o Zn ocorreu com teor preocupante, no inverno. A freqüência de deformidades encontrada foi de 3,7% no inverno e 8,2% no verão, em ambos os casos superior àquela esperada em um ambiente livre de contaminantes. O resultado do teste de toxicidade indicou a presença de contaminantes biodisponíveis, que devem estar provocando efeito agudo sobre populações bentônicas mais sensíveis, enquanto que a incidência de deformidade evidenciou um efeito crônico, potencialmente deletério, para as populações mais resistentes de *Chironomus*. A avaliação da incidência de deformidade em mento de *Chironomus* mostrou-se adequada como medida *in situ* de efeitos crônicos de contaminantes e seu uso em programas de monitoramento aplica-se a bacias em recuperação ou em início de degradação.

Palavras-chave: deformidade, toxicidade, bentos, monitoramento, diagnóstico ambiental.

ABSTRACT: Using *Chironomus* (Chironomidae: Diptera) mentum deformities in environmental assessment. The deformity incidence in *Chironomus* mentum was investigated to evaluate chronic effects in the biota and its usefulness in monitoring programs. Winter/97 and summer/98 samples were taken at the Tietê river depositional bank for chemical, ecotoxicological and biological analysis. It was considered as deformity: gap, excess and loss of teeth. The benthic community showed high densities of Tubificidae and *Chironomus* and the toxicity test results always exhibited acute effect. Zinc, during the winter, was the only chemical that exceeded the severe effect threshold. The deformity incidence in *Chironomus* mentum was 3.7% during the winter/dry and 8.2% during the summer/wet, surpassing the number for clean sites. Ecotoxicological results showed the presence of contaminants, which promoted acute effect on sensible populations while the deformity incidence showed a chronic and potentially deleterious effect on tolerant *Chironomus* population. The measure of *Chironomus* mentum abnormalities was considered useful to assess *in situ* chronic effects and it must be applied in monitoring network at basins subjected to restoration programs or newly received industrial and agricultural activities.

Key-words: deformity, toxicity, benthos, monitoring, environmental assessment.

Introduction

Although Brinkhurst and co-workers (1968 *apud* Warwick *et al.*, 1987 and Janssens de Bisthoven *et al.*, 1998) had already reported the occurrence of deformed larvae in their study concerning the benthos of St. Lawrence Lake, the first paper to deal with these anomalies in *Chironomus* populations was that of Hamilton & Saether (1971 *apud* Warwick *et al.*, 1987; Lenat, 1993). But just in the end of 1980's a great number of papers about larvae deformities was published. In North America, most papers are centralized at the Great Lakes and focused on spatial scale, while in Europe these studies have been developed in small lakes, canals and rivers, focusing on temporal scales (Warwick *et al.*, 1987; Janssens de Bisthoven *et al.*, 1998). In Brazil, researches in chironomids larvae deformities have just begun.

Most of chironomid deformity studies relate the frequency and place (mainly mentum and antenna) of these morphological anomalies with *in situ* chronic effects promoted by inorganic (metals) and organic (PCBs, PAHs and pesticides) contaminants (Cushman, 1984; Diggins & Stewart, 1993; Hudson & Ciborowski, 1996a). The deformity frequency could make inclusively evident the presence of other unobserved effects rendering nonviable population (Cushman, 1984). Historical assessments concerning to the degradation of some places can also be performed using morphological deformity studies (Warwick *et al.*, 1987).

Once high deformity frequency occurs exclusively due to the presence of contaminants, this measure, together with the community structure metrics could distinguish the biological response to eutrophication, domestic waste, thermal effluent and habitat physical degradation which do not cause those anomalies (Diggins & Stewart, 1993; Lenat, 1993; Janssens de Bisthoven *et al.*, 1998).

Although the deformity development mechanisms could theoretically be associated to mutations, interference with transcription and translation, disruption of cell division and metabolic disturbance (Bird *et al.*, 1995; Janssens de Bisthoven *et al.*, 1998), it is becoming clear that at least asymmetric anomalies have somatic or phenotypic origin (Cushman, 1984; Dickman *et al.*, 1992; Bird *et al.*, 1995). So, the deformity does not have hereditary characteristic and its frequency makes evident the presence of teratogenic agents. On the other hand, symmetric deformities, such as median tooth cleft, can be associated to mutations due to, for example, inbreeding of laboratory chironomid cultures (Bird *et al.*, 1995).

Chironomus genus is particularly interesting for deformity studies because it inhabits all kinds of aquatic habitats, has broad distribution, can tolerate poor environmental conditions (like low oxygen concentrations) than other genera and at the same time it is sensitive to morphological deformities (Warwick, 1990; Hudson & Ciborowski, 1996a). This sensitivity is associated with the *Chironomus* alimentary habit, since it ingests sediments, exposes itself to contaminants tied to fine organic (detritus) and inorganic (clay) particles. In fact, every time other genera were tested together with *Chironomus*, the last always presented higher deformity frequencies (Warwick, 1990; Hudson & Ciborowski, 1996b).

This work aims to verify the sensitivity of the *Chironomus* mentum deformity frequency as a measure of *in situ* chronic effect, and to discuss its use in biomonitoring networks.

Materials and Methods

Samplings were done twice a year (winter/dry-97 and summer/rainy-98 seasons) at a depositional riverbank of the Tiete River, at Itu district. At each period, three replicates for chemical, ecotoxicological and biological analyses were taken using a modified Petersen grab (600 cm²).

Chemical analyses determined Cd, Pb, Cu, Cr, Hg, Ni, Zn, PCBs, DDT, DDE, HCB and TDE concentrations. Metals were analyzed with a flame atomic absorption spectrophotometer after extraction in aqueous medium, as described for solid waste analysis by USEPA (1986). Samples were dried at 105°C, pulverized and passed through an 80-mesh sieve. Metal extraction was performed from a digested 0,5g sub-sample of the sieved sample.

Regarding organochlorine and PCBs, the sample was dried and extracted with a mixture of hexane: acetone (1:1 v/v). The extract was purified in an alumina and silica gel column. The final extract was analyzed by gas chromatography using electron capture detector. This procedure was based on the 8080 EPA method for organochlorine and PCB pesticides in solid wastes (USEPA, 1986). The PCB quantification was made by congeners, according do DIN (1991).

The acute toxicity test with the amphipod *Hyalella meinerti* was runned as an indicator of toxic chemicals presence in the sediments. This test followed ASTM method (1988). Young individuals were exposed to the sediment sample during 10 days in static system. After this period the death rate was assessed in relation to a reference condition (a free contaminant environment).

Zoobenthic community was studied as ecological quality indicator of the habitat. Samples were preserved *in situ* with 4% neutral formalin. At laboratory samples were washed throughout a 0.5 mm mesh sieve and the retained material was maintained at 70° GL ethanol and stained with Bengal Rose. The coarse vegetal particles were previously took off by naked eye selection and the remainder passed through a stereo microscope. For the determination of the Oligochaeta-Tubificidae densities, sub-samples were done and only the individuals retained in a quarter of Petri dish were counted. The organisms were identified to the least possible taxonomic level. The *Chironomus* head capsules were mounted in semi-permanent slides using CMC-9 medium (Master's Company, Inc.). 646 larvae of the dry season and 474 larvae of the wet season were analyzed, surpassing the minimum number of 125 larvae proposed by Hudson & Ciborowski (1996b) to demonstrate that a 100% increase in deformity frequency is statistically significant. We considered as morphological deformities: gap, missing and additional teeth (Fig. 1). Anyway, the results were compared using the non-parametric Mann-Whitney's "U" test (Siegel, 1975).

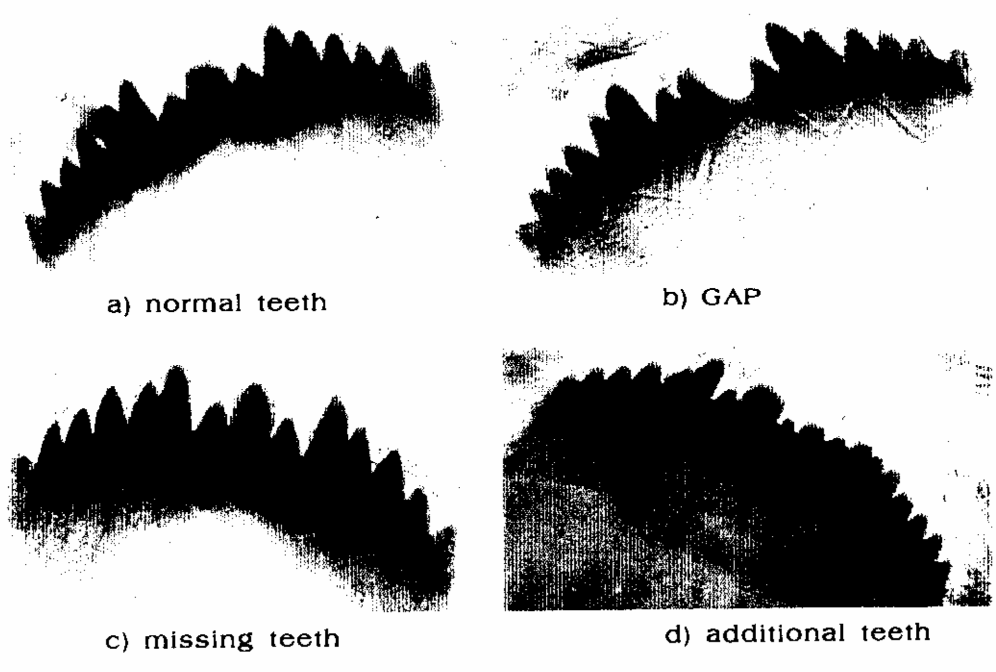

Figure 1: Normal (a) and deformed (b, c e d) mentum.

Results and Discussion

The benthic community (Tab. I) (CETESB, 1998) showed high densities of Oligochaeta-Tubificidae and *Chironomus.* This structure is an indicative of a non-equilibrium state although it could be impossible to define what kind of stress could be promoting the community richness impoverishment and the high dominance of a tolerant population. In general, communities with these features reflect environments receiving high loads of domestic waste, but they are being associated with industrially polluted environments too (Hudson & Ciborowski, 1996a; Diggins & Stewart, 1993).

The tolerance of Oligochaeta-Tubificidae and *Chironomus* populations to habitat alterations promoted by domestic waste, like oxygen depletion and silting increase is well known (Hart & Fuller, 1974), but more information about the capacity of some species (e.g., *Limnodrilus hoffmeisteri* and *Chironomus tentans*) to generate metal resistance are being reported (Klerks & Weis, 1987).

Table I: Zoobenthic densities (ind.m^{-2}) (mean ± standard deviation) at the dry and wet seasons (CETESB,1998).

	DRY	WET
Turbellaria	6 ± 10	0
Gastropoda	1,211 ± 571	39 ± 39
Tubificidae	332,995 ± 249,273	100,949 ± 27,200
Hirudinea	1,195 ± 913	464 ± 336
Chironomini	11,143 ± 4,799	27,363 ± 14,168
Orthocladiinae	11 ± 19	0

The toxic contaminant presence was made evident by the positive toxicity test results (Tab. II) (CETESB, 1998) which are reflecting an acute effect on more sensitive populations of the benthic community.

Table II: Sediment chemical and ecotoxicological results during the dry and wet seasons (CETESB, 1998).

	DRY	WET
Cadmium (µg.g^{-1})	1.13*	0.99*
Lead (µg.g^{-1})	33.4*	25.0
Copper (µg.g^{-1})	56.7*	11.3
Chromium (µg.g^{-1})	56.2*	30.3*
Mercury (µg.g^{-1})	0.04	0.05
Nickel (µg.g^{-1})	26.5*	17.6*
Zinc (µg.g^{-1})	274**	78.3
PCBs (µg.Kg^{-1})	6.873	1.123
DDT (µg.Kg^{-1})	3.567*	N.D.
DDE (µg.Kg^{-1})	2.023*	0.607
HCB (µgKg^{-1})	1.387	0.150
TDE (µg.Kg^{-1})	0	3.310
H. meinerti toxicity (% mort.)	67.5	40.0

N.D. = not detected

* threshold effect (Smith *et al.*, 1996)

** severe effect (Smith *et al.*, 1996)

In the absence of a proper Brazilian criteria establishing contaminant concentrations in sediment compatible with aquatic life, we have used the more restrict set of North American values, defined by many authors, and compiled by

Smith *et al.* (1996). Using the more restrict North American standards it was intended to be more conservative. However it would be preferable to apply limit concentrations in accordance to our systems, since factors linked to biodisponibility of contaminants (such as pH, alkalinity, redox potential, organic matter and acid volatile sulfides) could be distinct.

Results were discussed taking into account the threshold effect level, defined by the concentration below which adverse effects to benthic aquatic organisms rarely occur, and severe effect level, the concentration above which adverse effects to benthic aquatic organisms frequently occur (Smith *et al.*, 1996).

In this context, just Zn (Tab. II) (CETESB, 1998), in the dry season, occurred above the severe effect limit, while Cd, Pb, Cu, Cr, Ni, DDT and DDE occurred above the threshold effect limit, in the dry season, and Cd, Cr and Ni in the wet season.

The deformity frequency in *Chironomus* mentum (Tab. III) was 3.7% in the dry season and 8.2% in the wet season. Published data have shown deformity frequency below 3% in pristine environment (Hudson & Ciborowski, 1996a). In the Piracicaba river, Callisto *et al.* (in press) associated a 5-10% frequency range to mining and pulp industry effluents. The present also showed that industry and/or agricultural contaminants would be causing deleterious effect, although in a chronic level, in *Chironomus* populations. According to Hudson & Ciborowski (1996b) criteria a negative effect over benthic population was actually observed at wet season, when the deformity frequency surpassed more than twice a natural frequency. In the other hand the deformity incidence in the dry season could be due to natural causes.

Table III: Deformity incidence (%) in *Chironomus* larvae mentum during dry and wet seasons.

	DRY (n = 646)	WET (n = 474)
GAP	0.5	1.7
MISSING	1.8	5.3
ADDITIONAL	1.4	1.3
TOTAL	3.7	8.3

According to "U" test the deformity frequency in the dry season was statistically lower than that observed in the wet season (U = 0; r = 0.05). Few works have discussed seasonal variation in the deformity frequency. Janssens de Bisthoven *et al.* (1998) found higher rate of deformed *Chironomus* larvae in the winter than in the summer and he associated this result to population dynamics. Although our results have been contradictory in relation to the work above mentioned, differences in the population dynamics could explain it too, but specific studies concerning the seasonal influence on the life history of the studied *Chironomus* population would be necessary. Since one could expect that the increased discharge in the rainy season dilute contaminants concentrations derived from continuous point sources , attenuating their effects over aquatic biota, another assumption to explain the present results would be the influence of a diffuse contaminant input that could happen in the beginning of the wet season but again specific studies need to be done.

The most frequently seen deformity was missing teeth (Tab. III), like Hudson & Ciborowski (1996b) have found and although we did not consider median cleft and broken teeth as deformities, they were common. In both cases, frequencies were higher in the wet season, agreeing with the final deformity percentage result. It is possible that those anomalies could be related to the presence of contaminants, but as they can have mechanical origin, they would not be suitable as indicator of teratogenic pollutants presence.

Deformity incidence can be an useful tool to biomonitoring, because it can be employed not only in the detection of contaminant concentrations causing deleterious effects on the biota, but also as monitor of recovery processes (Warwick, 1990). In monitoring programs, this measure could increase the value of community structure indices and would indicate the sites where more intensive ecotoxicological investigations must be performed (Diggins & Stewart, 1993). In a monitoring network, deformity frequency might be used where the pollutant effect is not acute and, obviously, just in sites where inhabitant *Chironomus* populations achieve enough high density so that the capture of 125 specimens do not increase sampling cost (Hudson & Ciborowski, 1996b).

The deformity incidence assessment using *Chironomus* mentum was an interesting measure of *in situ* sub-lethal effects on benthic populations, enriching the community structure and ecotoxicological information, as observed by many authors. The use of deformity frequency in monitoring programs could be useful in basins where recovering processes or initial degradation by agricultural and industrial activities are occurring.

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References

ASTM. 1988. Proposed standard guide for conducting solid-phase sediment toxicity tests with freshwater invertebrates. American Society for Testing and Materials, Philadelphia. 51p.

Bird, G.A., Schwartz, W.J. & Joseph, D.L. 1995. The effect of ^{210}Pb and stable lead on the induction of menta deformities in *Chironomus tentans* larvae and on their growth and survival. Environ. Toxicol. Chem., 14:2125-2130.

Callisto, M., Marques, M.M. & Barbosa, F.A.R. (in press). Deformities in larval *Chironomus* from the Piracicaba River, Southeast Brazil. Verh. Internat. Verein. Limnol.

CETESB. 1998. Monitoramento integrado – bacias do alto e médio Tietê: avaliação da qualidade – água, sedimento e peixes. Relatório final. Out/98. Contrato de prestação de serviços 020/97 – SABESP. CETESB, São Paulo. 312p.

Cushman, R.M. 1984. Chironomid deformities as indicators of pollution from a synthetic coal-derived oil. Freshwater Biol., 14:179-182.

Dickman, M., Brindle, I. & Benson, M. 1992. Evidence of teratogens in sediments of the Niagara river watershed as reflected by chironomid (Diptera: Chironomidae) deformities. J. G. Lakes Res., 18:467-480.

Diggins, T.P. & Stewart, K.M. 1993. Deformities of aquatic larval midges (Chironomidae: Diptera) in the sediments of the Buffalo River, New York. J. G. Lakes Res., 19:648-659.

DIN. 1991. German standard methods for examination of water, wastewater and sludge. (DIN 38414 Part 20).

Hart Jr, C.W. & Fuller, S.L.H. (eds) 1974. Pollution ecology of freshwater invertebrates. Academic Press, New York. 389p.

Hudson, L.A. & Ciborowski, J.J.H. 1996a. Teratogenic and genotoxic responses of larval *Chironomus salinarus* group (Diptera: Chironomidae) to contaminated sediment. Environ. Toxicol. Chem., 15:1375-1381.

Hudson, L.A. & Ciborowski, J.J.H. 1996b. Spatial and taxonomic variation in incidence of mouthpart deformities in midge larvae (Diptera: Chironomidae: Chironomini). Can. J. Fish. Aquat. Sci., 53:297-304.

Janssens de Bisthoven, L. Nuyts, P., Goddeeris, B. & Ollevier, F. 1998. Sublethal parameters in morphologically deformed *Chironomus* larvae: clues to understanding their bioindicator value. Freshwater Biol., 39:179-191.

Klerks, P.L. & Weis, J.S. 1987. Genetic adaptation to heavy metals in aquatic organisms: a review. Environ. Pollut., 45:173-205.

.enat, D.R. 1993. Using mentum deformities of *Chironomus* larvae to evaluate the effects of toxicity and organic loading streams. J. North Am. Benthol. Soc., 12:265-269.

Siegel, S. 1975. Estatística não-paramétrica. McGraw-Hill, São Paulo. 350p.

Smith, S.L., MacDonald, D.D., Keenleyside, K.A., Ingersoll, C.G. & Field, L.J. 1996. A preliminary evaluation of sediment quality assessment values for freshwater ecosystems. J. G. Lakes Res., 22:624-638.

USEPA. 1986. Test methods for evaluating solid waste: laboratory manual physical/ chemical methods. Washigton, Environmental Protection Agency. 1986.

Warwick, W.F. 1990. The use of morphological deformities in chironomid larvae for biological effects monitoring. Environment Canada. 34p. (NHRI Paper, 43; Scientific Series, 163).

Warwick, W.F., Fitchko, J., McKee, P.M., Hart, D.R & Burt, A.J. 1987. The incidence of deformities in Chironomus spp from Port Hope Harbour, Lake Ontario. J. G. Lakes Res., 13:88-92.