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Taxonomic and nontaxonomic responses to ecological changes in an urban lowland stream through the use of Chironomidae (Diptera) larvae

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ABSTRACT

Biotic descriptors — both taxonomic (diversity indices, species richness, and indicator species) and nontaxonomic (biomass, oxygen consumption/production, and anatomical deformities) — are useful tools for measuring a stream's ecological condition. Nontaxonomic parameters detect critical effects not reflected taxonomically. We analyzed changes in Chironomidae populations as taxonomic parameters and mentum deformities as a nontaxonomic parameter for evaluating a South-American-plains stream (Argentina). We performed samplings seasonally (March, June, September, and December; 2005) and physical and chemical measurements at three sampling sites of the stream (DC1 at river source, through DC3 downstream). The specimens collected in sediment and vegetation were analyzed to investigate mouth deformities in Chironomidae larvae. We identified a total of 9 taxa from Chironomidae and Orthocladiinae subfamilies. Shannon's diversity index for Chironomidae decreased from 1.6 bits ind^{-1} (DC1) to 0.3 bits ind^{-1} (DC3). The total density of the Chironomidae exhibited a great increase in abundance at site DC3, especially that of *Chironomus calligraphus.* Chironomidae taxonomic composition also changed among the three sites despite their spatial proximity: *C. calligraphus*, *Goeldichironomus holoprasinus*, *Parachironomus longistilus*, and *Polypedilum* were present at all three; *Corynoneura* and *Paratanytarsu* at DC1 only; *Cricotopus* at DC1 and DC3; *Apedilum elachistus* notably at DC2 and DC3; and *Parametriocnemus* only at DC2. *C. calligraphus* individuals from DC1 showed no mentum deformities; only 2 from DC2 exhibited mouth-structure alterations; while specimens from DC3 presented the most abnormalities, especially during autumn and late winter. Type-II deformities (supernumerary teeth and gaps) were the most common. Anatomical deformities are sublethal effects representing an early alert to chemically caused environmental degradation. Mentum deformities in benthic-Chironomidae larvae constitute an effective biological-surveillance tool for detecting adverse conditions in sediments and evaluating sediment-quality-criteria compliance. Taxonomic (community composition) and nontaxonomic (condition of larval mouth parts) descriptors, used together, can indicate a stream's ecological state.

1. Introduction

A combination of biotic descriptors can serve as a useful tool for measuring the ecological condition of streams. Hill et al. (2001) recognized two kinds of parameters for the biological evaluation of waters: taxonomic (diversity indices, species richness, and indicator species) and nontaxonomic (biomass, oxygen consumption and production, and anatomical deformities). Both parameters can provide information on the ecological status of a given stream site under study. The usefulness of nontaxonomic parameters resides in their ability to detect effects that are not reflected in the taxonomic analysis. For instance, some toxic pollutants cause sublethal effects that are not immediately detected by the taxonomic descriptors, but become evidenced by changes in the oxygen production, growth and reproduction rate, biomass, behavioral modifications, and anatomical deformities.

Recent studies based on nontaxonomic parameters have proven their usefulness in evaluating the environmental quality of aquatic systems (Pascoe et al., 2000; Bartsch et al., 2000; Fellows et al., 2003; De Lange et al., 2004). Responses to the effects of sediment contamination have been studied in macroinvertebrate communities as well as in individual species both *in situ* (Pinel-Alloul et al., 1996) and at the laboratory (Sibley et al., 1999).

The nontaxonomic responses of organisms within the Pampean plains have been investigated only a little, with the information being limited to functional responsive studies in the laboratory on either an individual or community level (Rodrigues Capítulo, 1984a,b; de la Torre et al., 1999, 2000, 2002; Olguín et al., 2000, 2004; Demichelis et al., 2001) and with few surveys being conducted in the field (Tangorra et al., 1998; Graça et al., 2002; Sierra and Gómez, 2007; Gómez et al., 2008).

Certain members of the Chironomidae family are well known as indicators of ecological conditions (Paggi, 1999, 2003), with some

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larvae being especially useful for detecting polluted environments through cephalic-capsule structural alterations (Wiederholm, 1984; Warwick, 1985). *Chironomus* deformities, in particular, have a great potential for reflecting the sublethal effects of polluted sediments, in addition to indicating the pollution-sequence history (Janssens de Bisthoven et al., 1992, 1998; Lindegaard, 1995; Diggins and Stewart, 1998; Reynolds and Ferrington, 2001) as well as the presence of complex mixtures of contaminating substances that may cause synergistic or opposite effects (Vermeulen, 1995).

Chironomus calligraphus Goeldi 1905, is the most commonly found species in the neotropics (Spies et al., 2002; Nazarova et al., 2004), it having been recorded mainly at the limnotopes of the Pampean plains in Argentina (Paggi, 1979). This species is widely distributed because of its ability to colonize and develop under a range of ecological circumstances and its relatively broad tolerance to adverse environmental conditions (Spies et al., 2002). Thus, *C. calligraphus* can maintain sizeable populations in sites with strong anthropic pressure and thereby constitute a nuisance to humans (Spies, 2000).

The objectives of the present study were to analyze changes in Chironomidae populations and to utilize mentum deformities in their larvae as taxonomic and nontaxonomic parameters, respectively, for evaluating the ecological well-being of an urban lowland stream within the Argentinian Pampean plains that is subjected to differing degrees of pollution.

2. Materials and methods

The Don Carlos Stream (34°55′–34°50′S, 58°00′–58°03′W) is located in the northeast of the Buenos Aires Province, Argentina. Don Carlos is a small stream, of length 9 km, which flows through the Pampean plain and into the Río de la Plata estuary (Fig. 1). The composition of the riverbed is mainly clay, silt, and sand, with a lesser proportion of gravel (Cortelezzi, 2010). The presence of organic matter varies depending both on the natural contributions from nearby pastures and on the surrounding anthropogenic activity. The scant slope in the rivers of this basin, however, together with an irregular discharge of their water owing to the uneven seasonal rainfall, causes a standstill in the water flow during the summer dry season. This stagnation of water results in an increased transparency and enhanced light penetration that favor the development of many submerged and floating macrophytes within the pooled areas (Rodrigues Capítulo et al., 2003).

For the present study 3 sampling sites were selected: site 1 (DC1) near the source of the river and exposed to agricultural activity, site 2 (DC2) 800 m downstream just beyond the input of a major textile-industry discharge within an urban stretch; and site 3 (DC3) 1100 m farther downstream from DC2. This last site is exposed to sewage effluents and to outflows from textile and metallurgical factories, it has been canalized and its bed and lateral banks cemented.

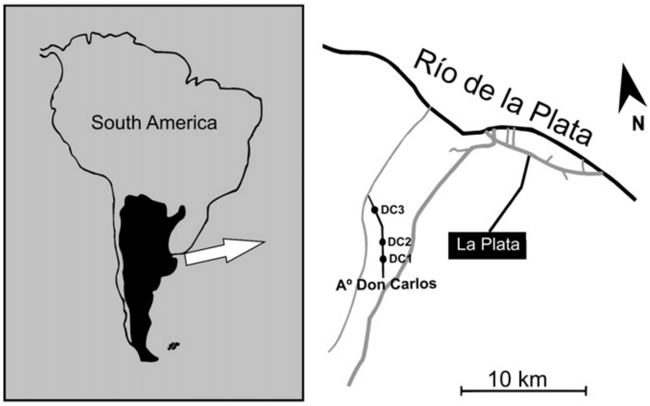

Fig. 1. Map of the study area showing the Don Carlos Stream and sampling locations.

We performed samplings seasonally (March, June, September, and December; 2005) and collected sediment and vegetation samples in duplicate at the three sampling sites of the stream (DC1, DC2, and DC3). We collected the sediment with an Ekmann dredge (100 cm^2), removed vegetation within the area subsumed by a 1300-cm^2 plexiglas square, and trapped the phytophilous individuals on 250-μm-mesh sieves. The following physical and chemical measurements were made: the stream speed (Cole-Parmer CZ-32922-10 Flow meter), width, and depth; temperature and pH (Hanna HI 8633), conductivity (Lutron CD-4303), turbidity (Turbidity meter 800-ESD), and dissolved oxygen (Ysi 52 dissolved oxygen meter). Water samples for the analysis of dissolved inorganic nutrients were filtered immediately through glass fibre filters (Whatman G/FC) and, together with the samples for BOD_5 and COD, these were stored at 4 °C until arrival at the laboratory. Soluble reactive phosphorus ($P–PO_4^{-3}$), ammonium ($N–NH_4^+$), nitrate ($N–NO_3^-$), nitrite ($N–NO_2^-$), biological oxygen demand (BOD_5) and chemical oxygen demand (COD) were determined according to Mackereth et al. (1978) and American Public Health Association (APHA) (1998). Organic matter (OM) percentage in sediments was calculated by weight loss after ignition at 500 °C during 4 h from a subsample (20 g fresh weight).

Triplicate samples of water and sediment of each sample site were collected for determination of Cd, Zn, Cu, Pb, Ni, and Cr and analyzed by atomic absorption spectrophotometry following acid digestion of samples (VARIAN SpectrAA 55 Atomic Absorption Spectrometer, Environmental Protection Agency, EPA, 1986). The water hardness was determined according to American Public Health Association (APHA) (1998); (M 2340 C — EDTA Titrimetric Method). The methods used for the analysis of each heavy metal can be seen in Table 1.

At the laboratory, the Chironomidae larvae collected were cleared with 10% (w/v) KOH, dehydrated stepwise with 80%, 96%, and 100% (v/v) aqueous ethanol, and finally mounted on a slide in Canadian balsam for their observation under the light microscope. The chironomids were expressed in number of individuals m^{-2} for each sampling site using the average of the five replicates. Chironomidae diversity was estimated using the Shannon Weiner Index (H′) (Shannon and Wiener, 1949).

The Chironomidae genera were identified by means of the keys cited in Wiederholm (1983); Paggi (2001), and Epler (2001). The incidence and degree of deformity were quantified under a binocular

Table 1
Methodology for the determination of heavy metals in water and sediment in the Don Carlos stream.

Water	Unit	Detection limit	Method
Pb	μg I^{-1}	0.002	EPA SW 846 M 3OIOAM 7420 EAA
Zn	μg I^{-1}	0.007	EPA SW 846 M 3010 AM 7950 EAA
Cd	μg I^{-1}	0.0006	EPASW846M7I3OEAA
Cu	μg I^{1}	0.005	EPASW 846 M 3OIOAM 7210 EAA
Cr	μg I^{-1}	0.005	EPA SW 846 M 7196 A UV spectrophotometry
Ni	μg I^{-1}	0.006	EPA SW 846 M 3010 AM 7520 EAA
Hardness	CaCQ3 mg l^{-1}	1	Standard Method M 2340 C — EDTATitrimetric
Sediment	Unit	Detection limit	Method
Pb	mg kg^{1}	0.5	EPASW846M742OEAA
Zn	mg kg^{-1}	0.75	EPA SW 846 M 7950 EAA
Cd	mg kg^{-1}	0.125	EPA SW 846 M 3050 A M 7130 FAA
Cu	mg kg^{-1}	1	EPASW846M72IOEAA
Cr	mg kg^{-1}	0.5	EPA SW 846 M 3050 A M 7130 EAA
Ni	mg kg^{-1}	1.125	EPA SW 846 M 7520 FAA

Olympus BH-2 microscope at 400×. In spite of the careful analysis to determine the Chironomidae taxa and their deformities, *C. calligraphus* was the only species that was present in large numbers at the sampling sites. Therefore, individuals of this species at the fourth instar were selected for analysis and quantification of deformities. In order to evaluate *C. calligraphus* mentum abnormalities, only those characteristics different from the normal structure were considered: those alterations resulting strictly from mechanical wear though use were excluded.

Mouth deformities were investigated in each specimen and the type of deformity established according to the classification by Lenat (1993) and Reynolds and Ferrington (2001):

Type I mild deformity, but distinguishable from normal tooth wear.
Type II conspicuous deformity — supernumeraries present or teeth missing.
Type III extreme deformity — combination of type-II deformities.

To evaluate if the differences in density of species between the sampling sites were significant, the ANOVA on Ranks and the Tukey Test were employed because the data were not normally distributed and/or had unequal variances.

3. Results

3.1. Analysis of physicochemical parameters

The main physical and chemical characteristics of the sampling stations are shown in Table 2. Phosphate and nitrate increased upstream with agricultural activity, while ammonium, COD and BOD_5 increased downstream with urban and industrial activities (textile and metallurgical factories). The organic matter had the highest values in DC3, thus reflecting the impoverishment of water quality downstream.

Upon analysis of the heavy metals in the water, only Cu and Zn were detected at the sampling sites exceeding the limits specified as the maximum permissible established by the Argentine Dangerous Wastes Law, N° 24051 (1993) for protection of freshwater life. Moreover, the Pb concentration was much higher than the above mentioned limits in DC3 (Table 3).

All the metals analyzed, except for Cd, were detected in the sediment — *i. e.*, Cr, Cu, Zn, Pb, and Ni. The Zn was higher upstream while Cu and Cr were elevated downstream. Especially notable were the high concentrations of Pb and Ni at DC3: there, those two elements widely exceeded the mean values for the lithosphere according Frink (1996) (Table 4).

Table 2
Physicochemical variables, average and standard deviations (SD), measured at the three sampling stations in the Don Carlos Stream (n = 4).

	DCI	DC2	DC3
T° (°C)	15.0 (4.7)	20.4 (2.8)	18.8 (3.7)
Conductivity (μS cm^{-1})	685 (269)	955 (184)	991 (164)
pH	7.8 (0.3)	7.8 (0.2)	7.8 (0.4)
OD (mg r^{-1})	4.0 (1.3)	2.4 (1.1)	3.8 (1.3)
$N0_2^-$ (mg l^{-1})	0.1 (0.1)	0.2 (0.3)	0.2 (0.3)
$N0_3^-$ (mg l^{-1})	1.5 (1.5)	1.3 (1.1)	0.6 (0.7)
NH_4^+ (mg l^{-1})	0.1 (0.1)	0.6 (0.3)	0.8 (0.7)
$P0_4^{-3}$ (mg l^{-1})	1.0 (0.2)	0.2 (0.2)	0.3 (0.1)
BOD_5 (mgO_2 l^{-1})	8.2 (3.1)	21.7 (16.3)	19.0 (15.8)
COD (mgO_2 l^{-1})	18.2 (6.8)	29.7 (22.0)	28.7 (23.7)
Turbidity (NTU)	22.4 (6.1)	23.6 (34.0)	5.1 (2.3)
Organic matter (%)	5.4 (1.5)	4.9 (4.3)	12.3 (2.5)

Table 3
Concentrations of heavy metals and hardness (mean ± SD) in the water of the Don Carlos Stream at the three sampling stations and maximum permissible according to Argentine Dangerous Wastes Law, N° 24051 (1993) for protection of freshwater life.

	Water			Maximum permissible amount for protection of freshwater life	
	DC1	DC2	DC3		
Pb (μg l^{-1})	8 (10)	<2	60 (50)	2	4
Zn (μg l^{-1})	110 (130)	20 (10)	40 (10)	30	30
Cd(μg l^{-1})	<0.6	<0.6	<0.6	2	3
Cu (μg l^{-1})	31 (4)	3 (6)	6 (1)	0.8	1.3
Cr(μg l^{-1})	<5	<5	<5	2	2
Ni (μg l^{-1})	6 (5)	<6	50 (20)	65	110
Hardness ($CaCO_3$ mg l^{-1})	97.0 (1.1)	120.7 (1.9)	132.7 (5.3)	60–120	120–180

3.2. Chironomidae throughout a polluted gradient

In the present study the following 9 taxa, representing both the Chironominae and the Orthocladiinae subfamilies, were recorded:

Chironominae: *C. calligraphus* Goeldi, 1905, *Goeldichironomus holoprasinus* (Goeldi) Fittkau, 1965, *Parachironomus longistilus* Paggi,1977, *Polypedilum* Kieffer, 1912, *Apedilum elachistus* Townes, 1945, and *Paratanytarsus* Thienemann and Bause, 1913.

Orthocladiinae: *Corynoneura* Winnertz, 1846, *Cricotopus* van der Wulp, 1874, and *Parametriocnemus* Goetghebuer, 1932.

The total density of the Chironomidae family exhibited a great increase in abundance downstream reaching mean values of 46,550 ind/m^2 at site DC3. Shannon's diversity index (1963) when applied to the Chironomidae varied inversely with the density of the taxa, from 1.6 bits ind^1 at DC1 to 0.3 bits ind^1 at DC3 (Fig. 2).

The relative density of the Chironomidae also changed particularly at sites DC2 and DC3 when compared to site DC1; but the most notable difference was the pronounced increase in the number and proportion of *C. calligraphus*: While this species accounted for only 6% of the total individuals at site DC1 (8 ind/m^2), *C. calligraphus* represented an average of 91% at DC2 (471 ind/m^2) and 94% at DC3 (8786 ind/m^2; Fig. 3).

The taxonomic composition of the Chironomidae furthermore varied among the three sites despite their close physical proximity: *C. calligraphus*, *G. holoprasinus*, *P. longistilus*, and *Polypedilum* were present at all three sites; while *Corynoneura* and *Paratanytarsus* were recorded at only DC1, *Cricotopus* at both DC1 and DC3, *A. elachistus* notably at DC2 as well as DC3, and *Parametriocnemus* at site DC2 only.

Table 4
Amounts of heavy metals in streambeds sediments (mean ± SD) of the Don Carlos Stream at the three sampling stations and average natural amount by sedimentary rocks and soils according to Frink (1996).

	Sediment			Average natural amount by sedimentary rocks and soils
	DCI	DC2	DC3	
Pb (mg kg^{-1})	5.2 (2.4)	29.6 (48.5)	679.5 (629.6)	19.6
Zn(mg kg^{-1})	80.3 (40)	36.3 (13.6)	4.5 (3.6)	40
Cd (mg kg^{-1})	<0.125	<0.125	<0.125	0.34
Cu (mg kg^{-1})	25 (7.8)	56 (72.8)	50 (21.2)	29.2
Cr(mg kg^{-1})	5.9 (3.5)	10.2 (11.6)	7.9 (6.4)	129
Ni (mg kg^{-1})	13.0 (1.3)	68.2 (112.4)	330.7 (260.9)	11

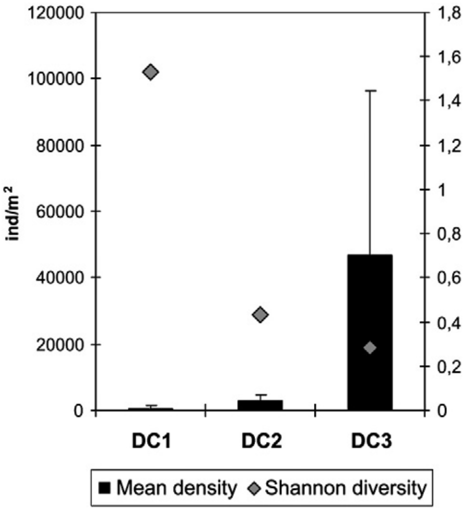

Fig. 2. Density of Chironomidae and the Shannon diversity values at the 3 sampling sites of the Don Carlos Stream.

3.3. *C. calligraphus* mentum deformities

More than 2000 *C. calligraphus* larvae from the Don Carlos Stream were counted and analyzed. As to mentum deformities in this species, individuals from DC1 (n = 12) had no alterations in their mouth structures. At site DC2, of the 51 individuals collected, only 2 exhibited mentum deformities. Site DC3, however, harbored the greatest number of *C. calligraphus* mentum abnormalities, especially during the autumn (n = 1584) and late winter (n = 223) samplings. The abundance of Chironomidae was very low during summer (n = 55) and no deformities were found during that season (Table 5).

The most common deformities registered were of type II (supernumerary teeth and dental gaps, Fig. 4). Mild asymmetries were, however, well represented (Table 6).

Table 5
Summary of the number of larvae sampled and the number and percentage of mentum deformities registered at the DC3 sampling site on the Don Carlos Stream.

DC3	N° larvae	N° deformities	% Deformities
Autumn	1584	42	2.65
Winter	223	4	1.79
Spring	192	18	9.38
Summer	55	0	0.00
Deformities average in DC3			3.13%
Total N° larvae			2054

4. Discussion

4.1. Taxonomic responses

Over 90% of the individuals collected in this study belonged to the Chironomini tribe of the Chironominae subfamily, and as such represented a typical fauna pattern in the lowland areas of South America (Fittkau, 1971, 1986; Fittkau and Reiss, 1979). The Chironomini tribe occupies a large habitat range and can be found in both oligotrophic and eutrophic environments — in rivers and streams of different sizes and with different levels of pollution (Martinez et al., 2002). The Orthocladiinae were generally scarce mainly because of their ecological requirements for rivers and streams with high slopes, high levels of oxygen, and the predominance of a hard substrate (Pinder, 1986). Nevertheless, the detection of *Parametriocnemus* in most of the affected sites in this study was quite unusual since some species of this genus have been reported to be sensitive to organic contamination (Epler, 2001).

Despite the predominance of Chironomidae is common in many freshwater systems (Klein and Trivinho-Strixino, 2005; Bass, 1986; Cohen, 1986), the high density of larvae may indicate environmental disturbances (Coimbra et al., 1996; Marques et al., 1999). Especially, Chironomus larvae have been observed in great amount in eutrophic environments (Frank, 1963; Learner and Edwards, 1966; Dévai, 1988; Tate and Heiny, 1995; Botts, 1997; Janssens de Bisthoven and Gerhardt, 2002). These studies agree with our investigation, the increase in total density of Chironomidae at the sites with the highest level of contamination (highest values of conductivity, COD and BOD_5) and impacted physical habitat (dredging, canalized) was a result of the significant rise in *C. calligraphus*. While in the least stressed site (DC1) this species represented less than 10% of the individuals, at the two sites with a much higher contamination (DC2 and DC3), this species exceeded 90% of total Chironomidae. This finding indicates the necessity of avoiding generalizations in defining the entire Chironomidae family as an indicator of polluted environments: indeed, the data here would argue that only the *Chironomus* genus constitutes a reliable indicator (Marques et al., 1999).

In relation to richness, within Argentina, Marchese and Paggi (2004) reported the presence of 20 Chironominae genera and 7 Orthocladiinae genera in the catchment area of the Río Paraná and Río de la Plata rivers. The low density of those taxa registered in our study (6 Chironominae and 3 Orthocladiinae) is coincident with the values cited by Marques et al. (1999), who recorded only 5 taxa in the most polluted sites in southeast Brazil. The low richness registered in this study would stem from the strong anthropic impact on the plains urban-stream systems in this area.

The paucity of ecological studies on the relationship between the distribution of Chironomidae and the gradient of environmental

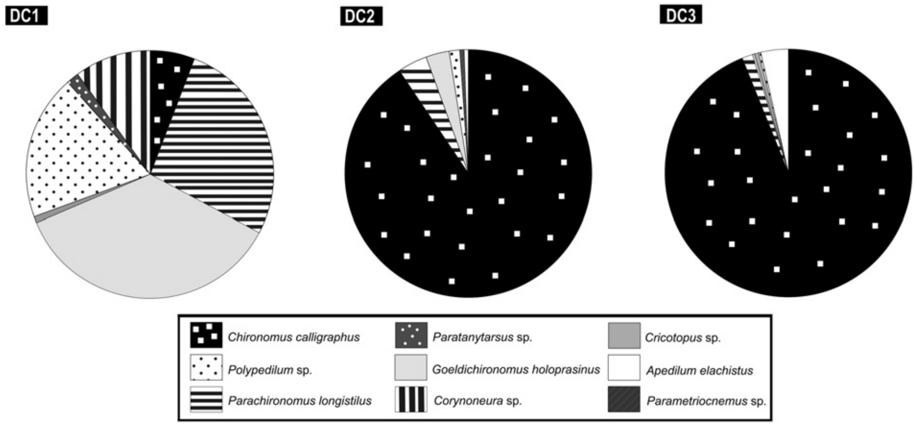

Fig. 3. Relative abundance of Chironomidae collected at each site on the Don Carlos Stream.

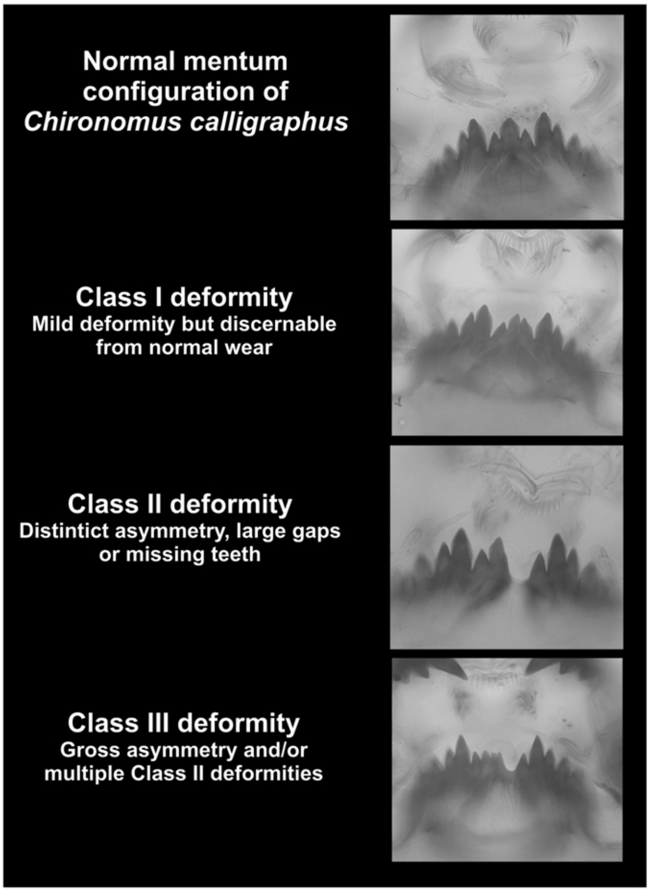

Fig. 4. Types of deformity registered in *C. calligraphus* larvae collected in the Don Carlos Stream (Buenos Aires, Argentina).

pollution for this region makes a comparison of these results with those from other studies impossible. We can, however, cite the presence of *Corynoneura* and *Paratanytarsus* at the least impacted site and of *A. elachistus* at the sites of greater organic and industrial contamination.

In order to make a correct assessment of the environmental status of lowland lotic systems based on the taxonomic responses of Chironomidae, we need further bionomic studies on species within this region in order to generate a larger amount of relevant empirical data.

4.2. Nontaxonomic responses

Several studies have reported the presence of deformities in *Chironomus* larvae at sites with a strong anthropic impact (Warwick and Tisdale, 1988; van Urk et al., 1992; Callisto et al., 2000; Heylen and De Pauw, 2002; Nazarova et al., 2004). According to Kuhlmann et al. (2000), *Chironomus* is particularly useful for the study of deformities because this genus can be found in any type of aquatic system, is thus widely distributed, and can furthermore tolerate adverse environmental conditions (such as low oxygen concentrations). Moreover, *Chironomus* is prone to morphological deformities. This proclivity is associated with the type of food that the genus consumes — *e. g.*, the sediment, whose ingest exposes the specimens to organic contaminants in the form of fine particles (detritus) as well as to inorganic particles (clay). By contrast, populations of other genera that not exhibit great deformities because exposure to metal contaminants would likely be lethal for a large number of individuals, thus diminishing the surviving population down to only the strongest members (Martinez et al., 2002).

Unfortunately, only a few studies have been found in the literature in relation to the heavy metal distribution in water and sediment in streams from the Pampean plain of Argentina. Previous research was focused on the analysis of heavy metals in the bottom river sediment (Manassero et al., 1998; Ronco et al., 2001; Camilión et al., 2003; Ronco et al., 2008). Most of the rivers and streams in urban areas of Buenos Aires Province contain at present a high load of urban and industrial wastes (Herkovits et al., 1996; Castañé et al., 1998). The impact produced by the human activities on these watercourses results in the alteration of the natural balance of the systems (Rendina et al., 2001). In coincidence with our study, Camilión et al. (2003) registered an increased Zn and Cu concentration at the sites where intensive agricultural practices have been performed. Moreover, in sites DC2 y DC3, Pb and Ni exceeded the average natural amount by sedimentary rocks and soils, and Pb surpassed widely the maximum permissible amount for protection of freshwater life. Especially in DC3, the Pb exceeded 34 times the values of sedimentary rocks and soils, and in the water, the Pb exceeded 15 times the permissible values for aquatic life protection. According to Krantzberg and Stokes (1989) the chironomid populations may have the ability to regulate the uptake of heavy metals. These authors, in laboratory experiments, have suggested the chironomids may regulate Cu, Ni, Mn and Zn, but not Pb and Cd. This would explain the great number of deformities registered in site DC3 where the values of Pb are very high. Studies on rivers and lakes in the Northern hemisphere have estimated that a frequency of deformities above 8% of the total number of larvae can indicate unfavorable environmental conditions (Warwick, 1988); this percentage would furthermore be the threshold indicating excessive concentrations of contaminants (Janssens de Bisthoven et al., 1992; Warwick, 1990, 1991, 1992; van Urk et al., 1992). Studies along these lines carried out in South America have shown that the percentages of deformities in response to environmental adversity are lower than those registered at other latitudes. In this study, the percentage of deformities coincides with the data of Callisto et al. (2000) from southeast Brazil (3%). In both researches, heavy-metal values were similar to or higher than the maximum concentrations considered to be safe by international environmental legislation.

The frequency of *Chironomus* mentum deformities in the Tieté river, São Paulo, Brazil (Kuhlmann et al., 2000) showed similar values to those registered in our study in both their dry (3.7%) and wet (8.3%) seasons. During the summer, the number of *C. calligraphus* larvae recorded here diminished, and no deformities were recorded; which finding would be directly related to the temperature-dependent life cycle of this species, with continuously overlapping generations of short life cycles occurring in the spring and summer followed by one or two generations of longer life cycles during the winter (Zilli et al., 2008). Studies performed by Nazarova et al. (2004) in Colombia reported higher values for *Chironomus* deformities (12%) than those registered in this study. According to this author, the low heavy-metal values registered would point to the existence of other influences acting synergistically to increase the percentage of deformities. However, we consider that a number of unquantified stressors could have resulted in such toxicological responses.

Table 6
Percentage of each type of mentum deformity registered at sampling site DC3 of the Don Carlos Stream.

DC3	Types of deformities		
	Class I	Class II	Class III
Autumn	14	26	2
Winter	0	4	0
Spring	5	12	1
Summer	0	0	0
	29%	66%	5%

With respect to the nature of the deformities found, Hamilton and Saether (1971) suggested that the various types of abnormalities could be related to different kinds of contaminants — *e. g.*, gaps would be induced by heavy metals (Köhn and Frank, 1980; Janssens de Bisthoven et al., 1995). This type of deformity was, in fact, the most abundant in our study, in accordance with the high concentrations of Pb and Ni registered. Nevertheless, a wide variety of chemical products act at the same time on the biota of polluted ecosystems (Statham and Lech, 1975; Hellawell, 1986; De March, 1988; Calamari and Vighi, 1992; Kraak, 1992). We assume that the exposure of Chironomidae larvae to a combination of contaminants has resulted in a unique situation causing especially high levels of deformities in this stream.

Deformities are sublethal effects and may, in general, be considered an early alert to the environmental degradation caused by chemical contaminants (Warwick, 1990). Mentum deformities in benthic Chironomidae larvae, in particular, appear to be an effective biological surveillance tool for the detection of adverse conditions in sediments as well as for the evaluation of the degree of compliance with the criteria for acceptable sediment quality.

We conclude that a combination of descriptors – both taxonomic (community composition) and nontaxonomic (the condition of larval mouth parts) – are useful indicators for characterizing the ecological state of a given study area.

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