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AQUATIC TOXICOLOGY

# Mentum Deformities of Chironomid Larvae as an Indicator of Environmental Stress in Büyük Menderes River, Turkey[1]

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**Abstract**– River basins are important for both industrial and agricultural activities. Pollution of air, water and soil is increasing owing to an insufficient number of treatment facilities; thus, most industrial and domestic wastewater either is directly discharged into water or is improperly treated. Here *Chironomus* spp. mentum deformities were used to determine environmental stress sources. A total of 4701 chironomid larvae were collected from 31 stations located in the Büyük Menderes River Basin. The mean mentum deformity incidence was 2.82%, and the frequency of deformities varied from 0 to 14.7%, with the highest frequencies calculated for the Dokuzsele (14.7%) and Banaz (9%) streams. The feature common among both stations is that they receive wastewater from textile, tannery and agricultural facilities. Our results show that mentum deformities are at least five times more pronounced at the most highly polluted sampling stations and indicate that mentum deformities of chironomid larvae are strongly related to ammonium-N and Cl is positively associated with agricultural and household wastewater.

*Keywords:* non-biting midges, waste water, head capsule, malformation

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## INTRODUCTION

Chironomids (Chironomidae, Diptera) have long larval stages in water and sediments, making them ideal biological indicators. Furthermore, their suitability in toxic monitoring, such as in identifying the effects of pollutants in water ecosystems [39, 49], has been reported by many authors [2, 3, 12, 22, 29, 41, 57, 61, 64].

The deformation of chironomid mouthparts has been reported by many countries: in Canada – [38, 62], in USA – [36, 37], in Denmark – [56] and [6], in Finland – [27], in Belgium – [8, 40, 57, 59], in Spain – [50], in Brazil – [46], in Malaysia – [3], in Argentina – [13], in France – [4], in Germany – [23], in Italy – [17, 19, 20] and in Ethiopia – [7].

Many studies have reported the possible associations between mentum deformities (MDs) of chironomids and aquatic pollution [3, 7, 17–20, 36, 37, 46, 50, 57, 63]. These deformities differ according to the density and type of pollution. As a result, the number of studies that have used MDs to detect toxic stress has increased in recent years [3, 7, 17–20, 36, 37, 46, 50, 57, 63]. Previously [36] developed an index called the Toxic Score (TS), which considers only *Chironomus* MDs. However, very few studies have reported correlations between pollutant stress and structural deformities in chironomid larvae [4, 24, 33, 53, 54]. This study comprehensively investigated larval chironomid MDs in Turkey. To the best of our knowledge, only one study has previously reported that MDs occurred in 3–8% of *Chironomus* spp. sampled from the Ulubat Lake in 2010 [5]. Therefore, we attempted to determine the significance of *Chironomus* spp. as indicators of environmental stress. The specific objective of the study was to monitor MDs in *Chironomus* spp. larvae collected from the Büyük Menderes River Basin and deduce a probable connection between pollution and MDs.

## MATERIALS AND METHODS

### *Study Area and Sampling Sites*

The Büyük Menderes River, with headwaters near Dinar, has access to the Aegean Sea through the Büyük Menderes Graben Basin located in southwest Turkey. Furthermore, this river is 584 km long, and the total area of its basin is 25000 km$^2$. Its flow ranges from 16 to 225 m$^3$/s, and its water temperature

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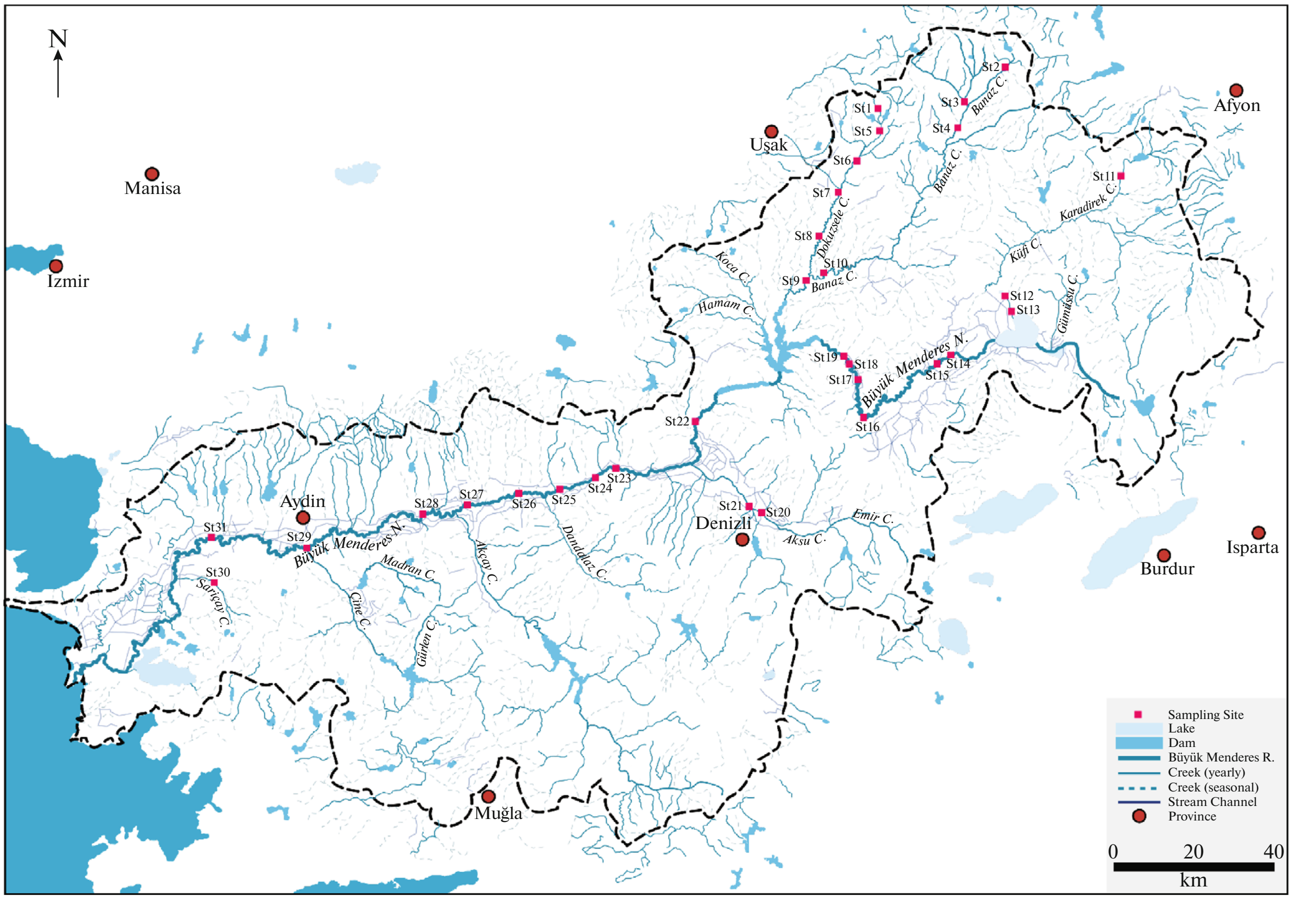


**Fig. 1.** Map of the Büyük Menderes River Basin and sampling sites.

remained 17.4–25.9°C during the study period depending on the season and altitude. The sampling stations were selected based on differences in their typology and habitat conditions. The first station was 0.1 km below 1039 ms to 0 ms, 31 stations were identified (Fig. 1). The river receives discharge of agricultural wastewater, urban sewage, heated water and heavy industrial effluent (textile dye, marble and tannery).

### *Field Methods and Laboratory Analyses*

Samples were collected from a river section of approximately 100 m in length at each location using a D-frame dip net from January to December 2012. Nine types of microhabitats were specified at the stations (silt, fine sand, cobbles, gravel, fine gravel, algae with cobbles, macrophytes, wood and leaf litter). Equal effort (~3 min) was spent in each habitat, and samples were preserved in 70% ethanol. Chironomid larvae were picked from the samples and placed in 10% KOH overnight to make the soft tissues transparent. The head capsules were rinsed with distilled water, dehydrated, mounted on permanent slides in Euparal. Specimens were identified using a binocular microscope (Olympus CX31 with 100×, 200×, 400× and 1000x. Permanent slides were deposited in the collection of the Department of Hydrobiology (University of Pamukkale, Turkey). Studies published by [10, 31, 34, 35, 65, 66] were used to identify larvae. Ecological preferences and species characteristics of chironomid species were quoted from [43–45, 55]. MDs classified into three groups and used them in TS, wherein more severe deformities are assigned a higher weightage by [36]. Class I includes slight deformities, Class II includes moderate deformities such as extra teeth, missing teeth, large cavities and asymmetry and Class III includes strong deformation with at least two Class II characters. TS was calculated as follows:

$$TS = (N_I + 2N_{II} + 3N_{III})/N_T,$$

where $N_I$, $N_{II}$ and $N_{III}$ are the number of larvae showing Class I, Class II and Class III deformities, respectively, and $N_T$ is the total number of larvae.

### *Physicochemical Parameters of Water*

Water temperature, dissolved oxygen (DO) level, electrical conductivity, total dissolved solid (TDS) content, salinity, oxidation–reduction potential and

**Table 1.** Descriptive statistics of the environmental variables

| | $sO_2$ | $dO_2$ | Temp | pH | ORP | Sal | TDS | Cond | Flow | $NH_4$-N | Cl | $NO_3$-N | $NO_2$-N | Fe | $PO_4$ | Mg | Ca |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Min | 0.40 | 0.04 | 10.80 | 6.97 | –139.60 | 0.00 | 207.00 | 2.44 | 0.02 | –0.34 | –2.00 | 0.00 | 0.00 | 0.00 | 0.01 | –0.19 | 0.07 |
| Max | 153.60 | 8675.00 | 31.40 | 8.90 | –26.80 | 43.00 | 2000.00 | 63 200.00 | 1.20 | 5.68 | 154.30 | 32.40 | 0.25 | 0.92 | 5.54 | 1.73 | 3645.00 |
| Mean | 86.59 | 287.58 | 19.20 | 8.07 | –84.99 | 2.72 | 897.52 | 4282.30 | 0.56 | 0.65 | 25.54 | 1.80 | 0.02 | 0.10 | 1.20 | 0.52 | 119.85 |
| St.Dev. | 40.16 | 1556.64 | 5.26 | 0.41 | 22.25 | 9.27 | 613.83 | 13 759.36 | 0.35 | 1.41 | 36.79 | 5.70 | 0.04 | 0.20 | 1.71 | 0.55 | 654.24 |

pH were measured *in situ* using portable instruments. River water composition, including nitrite, phosphate, ferrous, copper, ammonium, sulphate and potassium levels, was determined in the laboratory using a filter photometer. Results of the physicochemical analysis were also assessed according to the Turkish water quality standards. Heavy metals and chemical oxygen demand (COD) were not measured. However, their results are available for previous years [1, 9, 21, 30, 47].

### *Statistical Analyses*

Multivariate analysis was performed using CANOCO 4.5 [51]. Principle components analysis or redundancy analysis (RDA) (according to CANOCO [52]) was performed using a linear method to establish associations between chironomids and environmental variables. We were more interested in the correlation among the counts of individual MDs and between the species and environmental variables (temperature, DO, TDS, salinity, conductivity, oxidation–reduction potential, pH, heavy metals, ammonium, nitrate and nitrite) rather than the exact positions of the stations in the ordination space.

## RESULTS

The environmental variables varied between the clean and polluted regions of the river basin; this is consistent with a decrease in faunal diversity and an increase in chironomid MD rate in polluted conditions. The descriptive statistics of the environmental variables were given in Table. The mean water temperatures in all river basins ranged from 10.8 to 31.4°C. The water pH ranged from 6.97 to 8.9. TDS varied from 207 to >2000 mg $L^{-1}$. Similarly, DO varied from 0.1 to 13.53 mg $L^{-1}$ at some stations that received wastewater from the textile and tannery industries. Salinity at two stations closer to the sea ranged from 0.0 to 43 g/kg. Total organic phosphate content of the water varied from 0.1 to 5.54 mg $L^{-1}$. Ammonium-N content of the water ranged from 0.01 to 5.68 mg $L^{-1}$.

Nitrate-N ranged from 0 to 3.2 mg $L^{-1}$. Nitrite-N varied from 0 to 0.25 mg $L^{-1}$. COD ranged from 0 to 534 mg $L^{-1}$ [47, 1]. Chlorine ranged from 0 to 154.3 mg $L^{-1}$. Mn content in sediment ranged from 380 to 520 ppm. Pb content in sediment varied from 105 to 140 ppm. Cd ranged from 0.53 to 5.78 μg $L^{-1}$. Fe content in sediment ranged from 21 to 25 ppm [1, 9, 21, 30, 47].

A total of 4701 chironomid larvae were collected from 31 stations. The specimens belonged to total 55 taxa and five subfamilies: Subfamily Chironomidae: 24 taxa, Subfamily Diamesinae: 1 taxon, Subfamily Orthocladiinae: 23 taxa, Subfamily Prodiamesinae: 1 taxon and Subfamily Tanypodinae: 6 taxa. The mean incidence of MDs was 2.82%, and the rate of MDs was significantly different among the stations (Kruskal-Wallis, $p < 0.01$). The frequency of MDs varied from 0 to 14.7%, with the highest frequencies calculated at stations St 8 (14.7%; Dokuzsele stream) and St4 (9%; Banaz stream). Both stations were contaminated by textile, slaughterhouse and agricultural wastewater. The type of MDs (Class I, II and III) was not significantly different among the stations (Kruskal-Wallis, $p > 0.05$). In addition, the number of mentum teeth was not significantly different with respect to the types of deformation, such as köhn gap, abrasion, light broken, vigorous broken, fused and extra or missing teeth ($p > 0.05$) (Fig. 2). The RDA ordination biplot confirmed the relationship between the environmental variables and MD rate (Fig. 3). In the RDA model, the first two axes explained 61.5%, and the total eigenvalue was 67.2% of the variance. Ammonium-N and Cl were positively correlated with MD on the first axis. Temperature, DO and salinity were positively correlated on the second axis. However, DO was negatively correlated with temperature, Cl, salinity, ammonium-N and MD. *Chironomus riparius* (Meigen) and *Cricotopus bicinctus* (Meigen) related to the mentum deformation showed a strong positive correlation with species 1, Cl, MD and St 8 stations compared to other *Chironomus* species.

## DISCUSSION

The Büyük Menderes River Basin, one of Turkey's 25 river basins, encompasses an area of 25000 $km^2$. Furthermore, more than 2.5 million people live here, making it the seventh most crowded basin in Turkey. Almost half (45%) of the river basin territory is engaged in agricultural production, accounting for 31% of the chestnut production, ~65% of the fig production and 20% of the olive production in 2010. In addition, cotton, corn, wheat, barley and citrus fruits (oranges and mandarins) are other products are grown in the basin. The main industrial structures in the river basin are leather, textile and food (fig and olive oil pro-

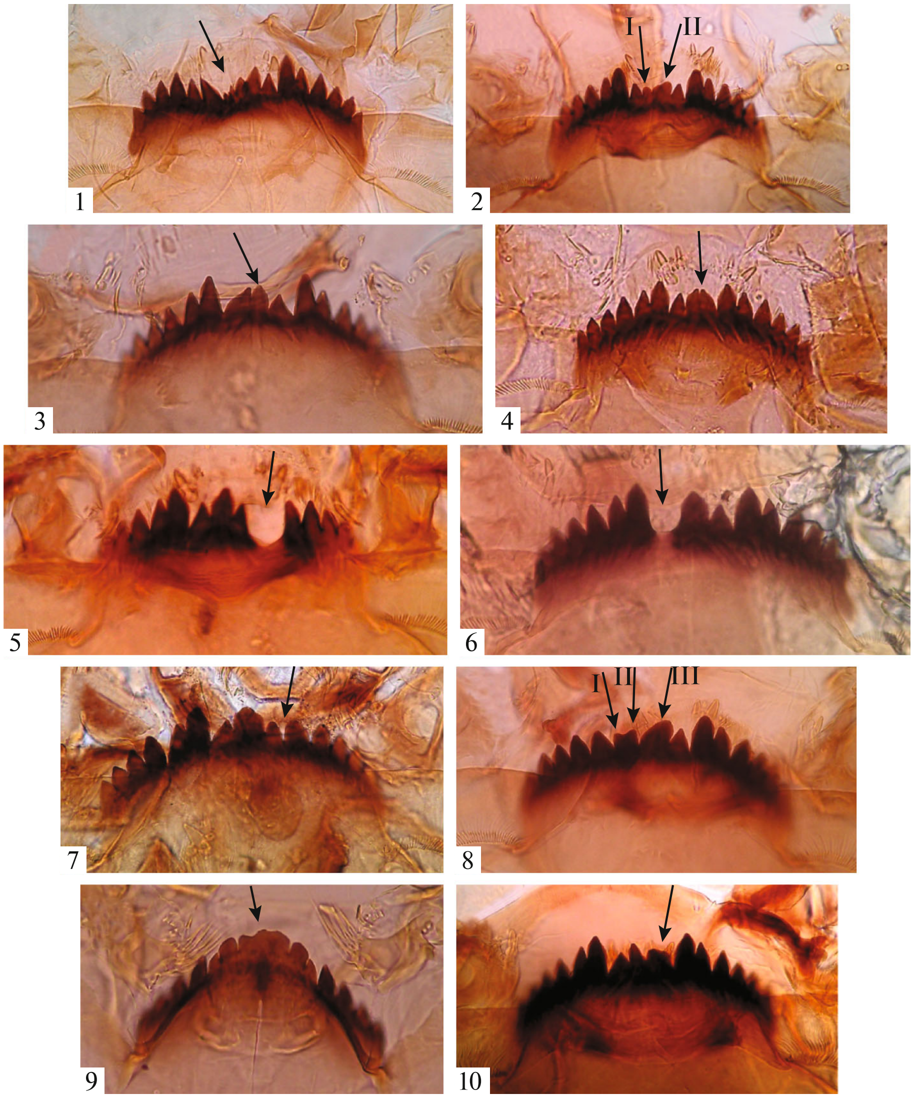


**Fig. 2.** Some form of mentum deformities detected in *Chironomus* spp. in the present study: 1 – extra teeth, 2 – I: extra teeth, II: missed shape, 3, 4 – fused teeth, 5, 6 – Köhn gap, 7 – missing tooth, 8 – I: extra tooth, II: fused tooth, III: missed shape, 9 – poor fracutred tooth, 10 – missed shape. All photos were taken with 40x magnification.

duction) sectors. According to 2010 data, 120000 people work in factories, stratified as approximately 50% in the textile and 10% in the food industry. The leather industry in the basin provides 40% of leather production with 3% of the workers [15]. All these activities have led to air, soil and water pollution as a result of rapid industrialisation and increased agricultural activities in the basin during the last 30 years. The industries and municipalities do not have a sufficient number of treatment plants, and some discharge their toxic effluents directly into the Çürüksu and Dokuzsele streams, which comprise some of the most highly polluted waters in the basin. There are five mixed industrial zones in the basin; that is, areas where there are businesses having different characteristics. However, the biological purification treatment is limited, and the treated water generally does not meet the desired water quality standards. In addition, some factories operating outside of this region directly discharge untreated wastewater.

Chironomidae species live in close association with sediments and are very susceptible to deadly pollutants in these sediments [7, 15] reported that very little is known about how individual larvae react to a toxic environment, It may be useful to identify the biological effects of toxic substances on the organisms in the water. Some chemicals [63] or different chemical blends [42] (quoted from [3]) may cause various deformities. Many researchers have studied chironomid larvae as biomarkers to define polluted freshwater [49] and detect changes in mouth piece deformities [39, 63] and salivary gland chromosomes [41, 42]. The study [62] suggested through a "quantitative dose

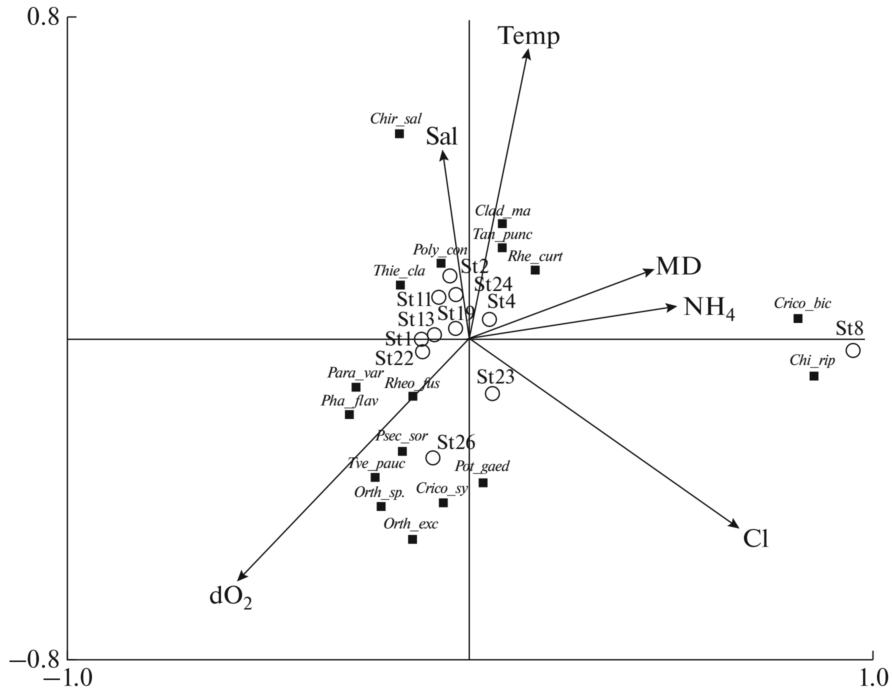


**Fig. 3.** The RDA graph shows the relationship between environmental parameters, Chironomids and mentum deformity (MD) rate; St, station; Sal, salinity, Temp, temperature; $dO_2$, dissolved oxygen; Cl, chlorine; *Chi_rip*, *Chironomus riparius*; *Chir_sal*, *Chironomus salnarius*; *Clad_ma*, *Cladotanytarsus mancus*; *Crico_bic*, *Cricotopus bicinctus*; *Crico_sy*, *Cricotopus syltvestris*; *Orh_sp*, *Orthocladius* sp.; *Orth_exc*, *Orthocladius excavatus*; *Para_var*, *Parachironomus varus*; *Pha_flav*, *Phaenopsectra flavipes*; *Poly_con*, *Polypedilum convictum*; *Pot_gaed*, *Potthastia gaedii*; *Psec_sor*, *Psectrocladius sordidellus*; *Rheo_fus*, *Rheocricotopus fuscipes*; *Rhe_curt*, *Rheotaytarsus curtistylus*; *Tan_punc*, *Tanypus punctipennis*; *Thie_cla*, *Thienemanniella clavicornis*; *Tve_pauc*, *Tvetenia paucunca*.

response" model that pollutants affect the distinct head capsule construction of Chironomids and that dissimilar structures are affected at different doses (i.e. from antennal deformities at low concentrations to anomalies in more sclerotized parts at higher concentrations). Some researchers have only investigated MDs in biological monitoring studies [50]. Previously [40] reported a significant linear regression between TS and the frequency of MDs ($p = 0.012$), and the observed total variance was 83%. Several studies have reported correlations between the pollutant level and frequency of MDs [5, 25, 32, 58]. Previously [58] stated that TS may be adequate to evaluate MDs in biological monitoring studies. The incidence of MDs reported in various studies is 60% [58], 56% [32], 42% [40] and 10.3% [28]. Some authors have reported direct relationships between heavy metal levels and deformities in mouthparts of various *Chironomus* spp. [4, 14, 16–18, 48, 54]. In addition, some authors [24, 27, 36, 50, 53, 57] determined that mouthpart deformities are strongly related to toxic wastes, including combinations of heavy metals and organic pollution. These results are consistent with our results that the Büyük Menderes River Basin produces very high levels of industrial, agricultural and household wastewater, which is often discharged untreated into rivers. High amounts of boron, cadmium, chromium, lead, zinc and iron have been detected in the water, sediments and soil of the Büyük Menderes River Basin [21, 26, 30, 47]. In addition, many years of monitoring have found high levels of COD and ammonium nitrogen and low levels of DO. Our results clearly indicate that polluted sites had a four or five times higher frequency of MDs related to water quality than non-polluted sites. MDs were strongly related to ammonium-N and Cl, which are strongly associated with agricultural and household wastewater. DO was negatively correlated with temperature, Cl, salinity, ammonium-N and MDs, suggesting that non-polluted streams had fewer MDs. MDs were also strongly associated with *Chironomus riparius* and *Cricotopus bicinctus* at the station U8. *Chironomus riparius* often inhabits organically polluted running water [14, 43]. Similar results were obtained in this study because *C. riparius* was detected at stations with high concentrations of organic pollutants and very few macroinvertebrates were recorded at stations contaminated with industrial and organic wastewater and possibly heavy metals. A strong statistical association was observed between organic pollution and MDs of *Chironomus* spp. at the station St 8. Larval *Cricotopus* spp. is eurythermic and particularly associated with eutrophic conditions in moving or standing water. These species prefer to live on macrophytes [10]. When the relationship between the physicochemical properties of the station where the *C. bicinctus* species were detected and the mentum deformation is evaluated that we estimate that mechanical deformation due to feeding may lead to MDs rather than toxic contamination.

## CONCLUSION

This study shows that the frequencies of *Chironomus* MDs might be useful in detecting pollution conditions in rivers. In addition, these frequencies are a potential tool to assess pollution in aquatic ecosystems receiving anthropogenic, agricultural and industrial discharge. Future studies should investigate the damage done to Chironomid larvae by toxic substances in the environment, and laboratory experiments should be conducted to directly assess the effects of various toxins on larvae.

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## COMPLIANCE WITH ETHICAL STANDARDS

*Conflict of interests*. The authors declare that they have no conflict of interest.

*Statement on the welfare of animals*. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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