

# Mouthpart deformities in Chironomidae (Diptera) as bioindicators of heavy metals pollution in Shiroro Lake, Niger State, Nigeria



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## ABSTRACT

In this study, mouthpart deformities in Chironomid larvae (Diptera) were investigated in relation to sediment contamination in the Shiroro Lake in Nigeria. Metals and chironomids were sampled monthly at three stations (A–C) between August 2013 and January 2014. Across the stations, zinc ranged (3.9–75 mg/g), manganese (1.29–1.65 mg/g), lead (0.00–0.10 mg/g), iron (101–168 mg/g) and copper (0.13–0.17 mg/g). The metal ions did not differ significantly ( $P > 0.05$ ) between the sampling stations. However, zinc and iron ions were significantly different between the sampling seasons ( $P < 0.05$ ). Thirteen chironomid species were recorded, with *Chironomus* sp., *Polypedilum* sp. and *Ablabesmyia* sp. dominating the assemblage structure. Mouthpart deformities were significantly higher at Station A compared with Station C, and seasonally significantly higher during dry season compared with wet season. Elevated incidences of deformity were recorded in *Chironomus* spp larvae as compared to other genera therefore for further studies in this region assessments should be based solely on *Chironomus* species and ignoring the rest. Strategies need to be developed to reduce the contaminations and the biological effects.

## 1. Introduction

The growing human activities on the catchment of the Shiroro Lake are threatening the functionality of the lake's ecosystem (Kolo and Oladimeji, 2004). In particular, the expanding industrial activities on the catchment have increased the likelihood of entry of toxic metals into the lake, with potential to cause ecological and human health impairments (Mugidde, 1993; Scheren et al., 2000). Bioaccumulation, bioconcentration and biomagnification can amplify the negative effects of metals contamination on ecosystem function and structure. Therefore, appropriate tools for monitoring the health of aquatic ecosystems are needed to inform decision-making.

In Nigeria, similar to the majority of African countries, aquatic ecosystem health monitoring relies chiefly on the measurements of physico-chemical variables alone. Physico-chemical analyses can indicate the quantities of materials in the environment at specific points and time, but they cannot be used to evaluate effects, either acute or chronic, of contaminants on aquatic organisms. Furthermore, physico-chemical methods are often restricted by limits of detection and yet, exposure to even low concentrations of metals may elicit biological effects such as deformities in individual organisms (Ochieng et al.,

2008; Arimoro, 2011; Arimoro et al., 2015).

The sustainable management of aquatic ecosystem health requires biologically-based methods and approaches that are sensitive to chronic effects of pollutants. The frequencies and patterns of morphological deformities in chironomid larvae have been used to indicate the effects of pollutants such as low concentrations of metal ions (Nazarova et al., 2004). Although chironomid deformities have been used to evaluate environmental quality in Europe (Planello et al., 2015), North America (Martinez et al., 2002) and elsewhere in Africa e.g. South Africa and Uganda (Ochieng et al., 2008; Odume et al., 2012); its application in Nigeria has not been fully explored. Given the growing human population and associated activities on the catchment of the Shiroro Lake which may lead to influx of toxic metals into the lake, coupled with the absence of appropriate biomonitoring tools applied for regular environmental monitoring programmes in Nigeria, this study aimed at creating a baseline for using mouthpart deformities in chironomid larvae for evaluating contaminant effect in the Shiroro Lake. This is particularly important because deformities have been used internationally as a means of evaluating in-stream toxic effects occasioned by pollutants including metals (Martinez et al., 2002; Odume et al., 2012).

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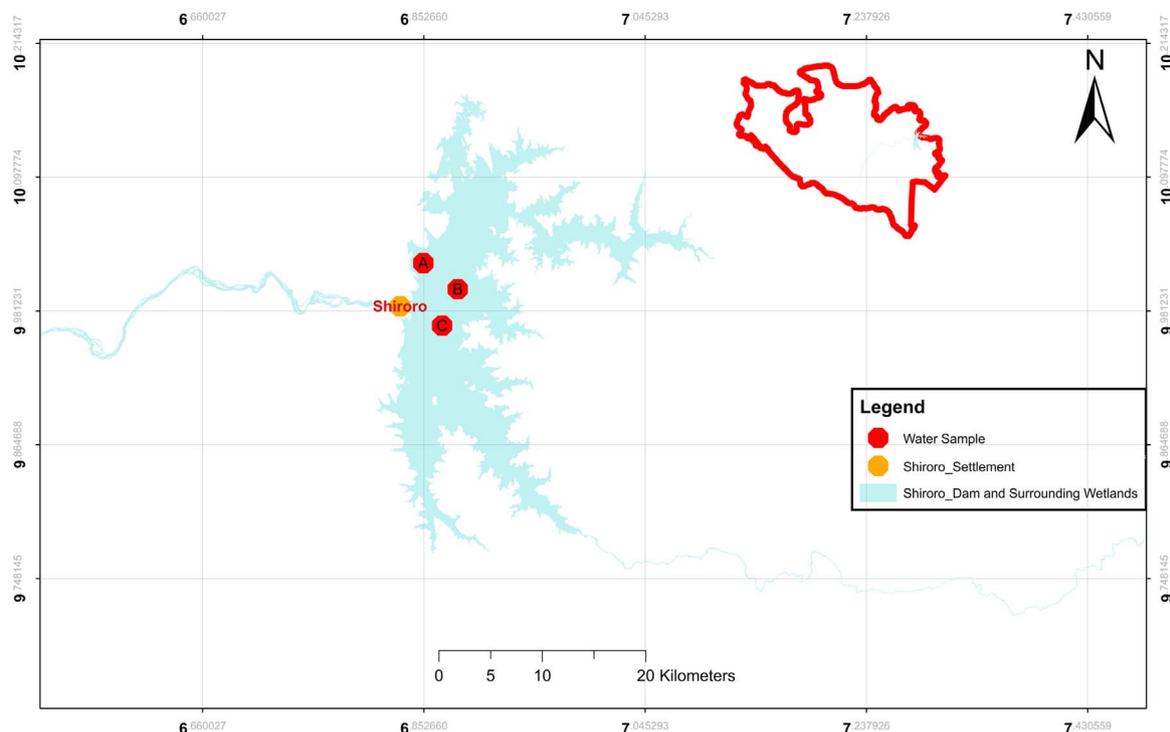


Fig. 1. Map of Shiroro Lake showing the location of the sampling stations: Stations A–C and the positions of Nigeria within Africa and that of the lake within Nigeria.

## 2. Materials and methods

The Shiroro Lake is located on latitude 9° 59'7" North and Longitude 6° 54'58" East. Of the 15 tributaries on the Shiroro watershed, the major ones are Rivers Dinya, Sarkin Pawa, Guni, Erina, and Munyi. The majority of the tributaries flow southwards but a few of them flow from north to west, and south to east. The Shiroro Lake is of the rock-fill type and stands 115 m high above the original riverbed elevation. The climate is hot and tropical, and rainfall usually exceeds evapo-transpiration. The maximum annual rainfall is usually about 195 mm. The mean maximum and minimum temperatures are about 38 °C and 26 °C, respectively. Subsistence fishing activities are a daily affair in the lake. Organic pollution comes mostly from activities of market women and nomadic herdsman and metal contaminations come mainly from the surrounding industries, including battery, fertilizers, pesticides and boat engines. Three sampling stations (A–C) were selected within the lake for the collection of chironomid larvae and physico-chemical analysis (Fig. 1). The stations were selected on the basis of the intensity of human activities (visual observations) likely to impact water quality, and based on this criteria, more human activities were taking place at Station A, followed by Station B and then Station C. Although none of these stations could meet the requirements of a reference station, Station C was selected as the less-impacted of the three stations.

Chironomid larvae were collected monthly between August 2013 and January 2014 (months August–October, wet season; November–January, dry season) using a D-frame net with mesh size 110 µm. Nine sub-samples were collected per station per sampling occasion. Collected larvae were preserved in 70% ethanol, transported to the laboratory for sorting, species identification and screening for deformities. Larvae were mounted according to Odume and Muller (2011) and identified according to the keys described by Wiederhilm (1983), Cranston (1996) and Harrison (2003). Deformities in the mouthparts were screened under an Olympus compound microscope at magnifications of either X10 or X40. Photos were taken using the software ANALYSIS® FIVE soft imaging systems. Only specimens showing clear deformity types including missing teeth, extra teeth, fused teeth and

asymmetry were considered deformed (Odume et al., 2012).

Sediments samples per station per sampling event were analysed for zinc, manganese, lead, copper, and iron according to APHA (1992) and Kruis (2005). Sediments were collected using the Ekman sampler, six (6) total for each site with three (3) at each site in each season. To avoid cross-contamination between the stations, the sampler was thoroughly washed with deionised water before it was used for another station. Briefly, the collected sediments were dried to a constant weight in an oven at 65 °C, and digested using strong hydrochloric acid, perchloric acid and nitric acid. The absorbance of the specific metals in a clear supernatant from digested materials was measured using an atomic absorption spectrophotometer (ALPHA Model). The concentrations of the specific metals in sediments were calculated using regression equations derived from standard solutions. All chemical reagents were of analytical grade, sourced from BDH Chemicals, Nigeria.

A two-way ANOVA and the Tukey's post-hoc test were used to ascertain spatio-temporal significant differences ( $P < 0.05$ ) in the concentrations of the metal ions. Incidences of mouthpart deformities calculated separately for each chironomid genus were expressed as the percent deformed individuals compared with the total number of larvae observed for deformities for the genus. Overall percent incidences of deformities was calculated as the percent number of larvae deformed compared with the total number of larvae screened for per station and season. One-way ANOVA followed by Bonferroni post-hoc test was used to ascertain spatio-temporal differences in the incidences of deformities between the stations, and the seasons. Deformities were arc-sine transformed prior to ANOVA analysis. Statistical analyses were performed using Statistica version 12.0.

## 3. Results and discussion

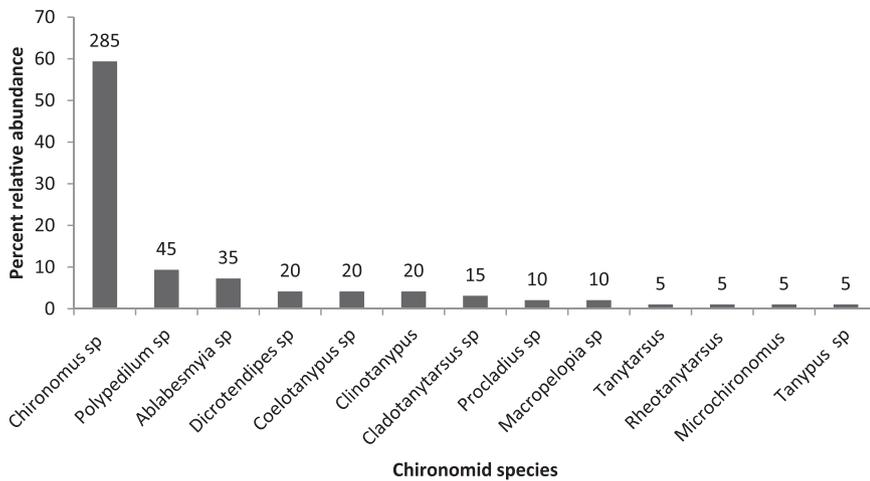
The concentrations of zinc and iron were significantly different between the sampling months, but no significant difference was observed between the stations in terms of the analysed metals (Table 1).

Trace metals are essential for the optimal functioning of organisms, but elevated concentrations in both sediment and water could be detrimental to biota (Golovanova, 2008) and some metals could be very

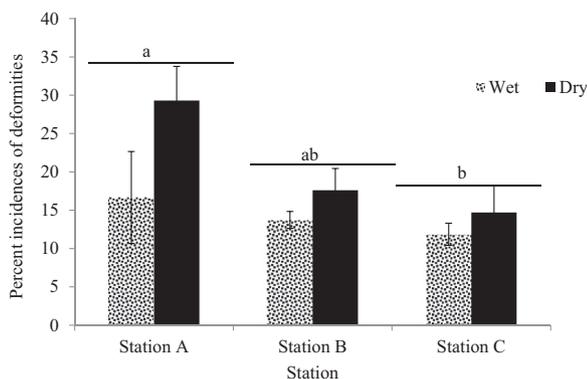
**Table 1**  
Mean and standard deviation (SD) (mean ± SD), ranges (in parenthesis) of metal concentrations in the sediments of the Shiroro Lake (August 2013–January 2014).

| Metal (mg/g) | Stations                    |                             |                              | Two-way ANOVA |         |         |          |
|--------------|-----------------------------|-----------------------------|------------------------------|---------------|---------|---------|----------|
|              | A                           | B                           | C                            | F value       |         | P value |          |
|              |                             |                             |                              | Seasons       | Station | Seasons | Stations |
| Zinc         | 27.87 ± 27.19<br>(4.1–75)   | 21.13 ± 18.35<br>(3.9–44)   | 23.20 ± 18.24<br>(4.1–45.50) | 16.48         | 2.97    | < 0.05  | 0.1      |
| Manganese    | 1.43 ± 0.09<br>(1.29–1.52)  | 1.44 ± 0.12<br>(1.3–1.60)   | 1.44 ± 0.15<br>(1.33–1.65)   | 1.46          | 0.03    | 0.28    | 0.97     |
| Lead         | 0.18 ± 0.41<br>(0–1)        | 0.033 ± 0.026<br>(0–0.05)   | 0.08 ± 0.03<br>(0.05–0.1)    | 0.81          | 0.54    | 0.57    | 0.6      |
| Iron         | 124.83 ± 30.99<br>(101–168) | 123.67 ± 30.56<br>(101–165) | 127.5 ± 30.72<br>(104–168)   | 434.61        | 3.56    | < 0.05  | 0.07     |
| Copper       | 0.16 ± 0.01<br>(0.15–0.17)  | 0.15 ± 0.01<br>(0.14–0.16)  | 0.15 ± 0.14<br>(0.13–0.17)   | 0.87          | 2.42    | 0.54    | 0.14     |
| Cadmium      | BDL                         | BDL                         | BDL                          | BDL           | BDL     | BDL     | BDL      |
| Nickel       | 0.11 ± 0.11<br>(0.09–0.12)  | 0.12 ± 0.12<br>(0.10–0.13)  | 0.12 ± 0.14<br>(0.11–0.13)   | 0.56          | 1.32    | 0.37    | 0.16     |
| Chromium     | BDL                         | BDL                         | BDL                          | BDL           | BDL     | BDL     | BDL      |

Note: BDL-below detection limit.



**Fig. 2.** Percent relative abundance of chironomid genera collected in the Shiroro Lake during the study period (August 2013–January 2014) from the three stations combined. The value on top of each bar is the absolute number (abundance) of each genera collected from the three stations combined.



**Fig. 3.** Spatio-temporal variations in the percent incidences of deformities between the sampling stations and seasons in the Shiroro Lake during the study period (August 2013–January 2014). Different small alphabet letters over the bars indicate stations that were statistically significantly different ( $P < 0.05$ ), while bars having an alphabet letter in common indicate stations that were not statistically different ( $P > 0.05$ ). The sampling seasons i.e. dry and wet seasons were statistically significantly different ( $P < 0.05$ ).

toxic. For instance, copper and zinc ions are only toxic at relatively high concentrations, whereas cadmium and mercury are toxic to many biota even at a very low concentration (Bashkin, 2002; Imam et al., 2013). The concentrations of metals such as zinc, copper and lead could be deemed elevated when compared to the threshold effect concentrations

**Table 2**  
Percent incidence of deformities in the mouthpart of chironomid genera in Shiroro Lake observed during the study period (August 2013–January 2014) across the three stations combined.

| Species                    | % deformed |
|----------------------------|------------|
| <i>Tanytarsus</i> sp.      | 0.0        |
| <i>Rheotanytarsus</i> sp.  | 0.0        |
| <i>Tanyptus</i> sp.        | 0.0        |
| <i>Microchironomus</i> sp. | 0.0        |
| <i>Procladius</i> sp.      | 10.0       |
| <i>Macropelopia</i> sp.    | 10.0       |
| <i>Cladotanytarsus</i> sp. | 13.3       |
| <i>Dicotendipes</i> sp.    | 15.0       |
| <i>Coelotanypus</i> sp.    | 15.0       |
| <i>Clinotanytus</i> sp.    | 15.0       |
| <i>Ablabesmyia</i> sp.     | 20.0       |
| <i>Polypetillum</i> sp.    | 22.2       |
| <i>Chironomus</i> sp.      | 59.3       |

for freshwater ecosystems determined by the Ohio Environmental Protection Agency (2010) for copper (31.6 mg/kg), lead (35.8 mg/kg), and zinc (121 mg/kg). For example, copper is roughly five times the USEPA standard at all sites, lead exceeds the standard greatly (6x) only at Site A, but zinc exceeds the standard by two orders of magnitude at all sites.

Similarly, compared with sediment study undertaken in the nearby

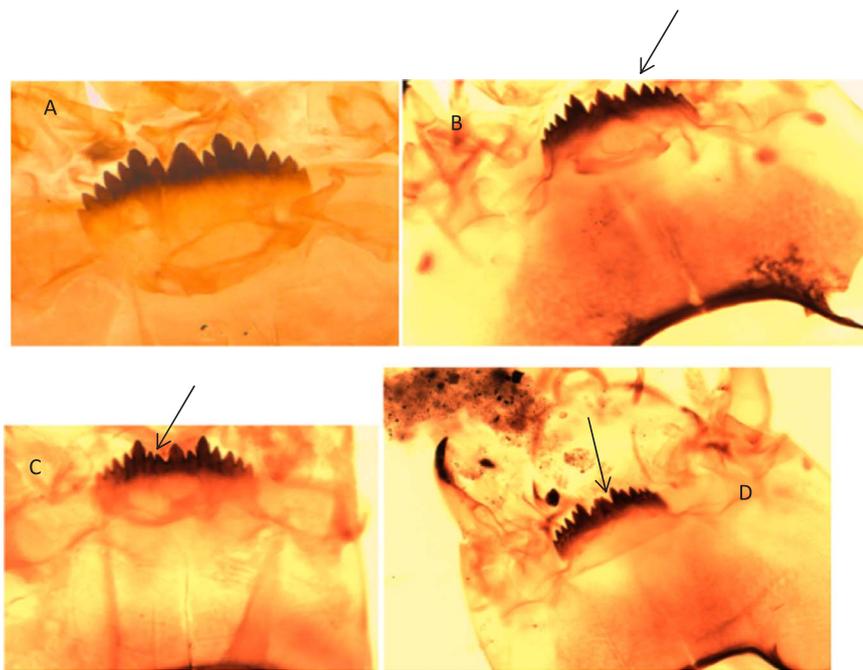


Fig. 4. Illustrations of normal *Chironomus* sp. mentum (A), and a mentum with missing tooth (B), extra tooth (C) and fused teeth (D). The arrow B indicates a position where the second right lateral tooth is missing, that in C indicates an extra left median tooth, and that in D indicates fusion of the three median teeth.

Kaduna River (Omozokpia et al., 2015), the concentrations of metals recorded in the present study could be considered elevated as they were higher than those recorded in the Kaduna River. For instance, Omozokpia et al. (2015) reported a mean of 116.83 mg/Kg for manganese, 21.33 mg/kg for Zinc, 2.00 mg/kg for copper in upper Kaduna River. The mean of Iron in the present study is rather high (12,483 mg/kg) and similar to the findings of those obtained from sediments collected in Panama (17,788.12 mg/kg) by Greaney (2005). The relative high values of heavy metals obtained in this study could be that run-off from industrial activities on the catchment, including the oils discharge from boats, pesticides from farmlands and fertiliser processing factories were contributing to the observed high values of metals in the lake. The combined run-off from these catchment activities together with domestic effluent discharges flowing into the lake are therefore sources of concern. In a similar work by Arimoro et al. (2015), it was reported that more chironomid larvae (*Chironomus* spp.) were deformed in the mentum compared with other structures. The increased deformity incidences corresponded with increased concentrations of Ni, Pb, Cu, Fe, Cr and Zn.

Thirteen genera were collected from the Shiroro Lake (Fig. 2). *Chironomus* sp., *Polypedilum* sp. and *Ablabesmyia* sp. dominated the species assemblage structure during the study. *Chironomus* sp. accounted for over 50% of the relative abundance of the genera. When divided among three stations though, only *Chironomus* (and total chironomids) produce enough animals to calculate meaningful deformity rates. The overall percent incidences of deformities were highest at Station A compared with Stations B and C. ANOVA indicated that the overall incidences of deformities at Station A were significantly higher than those at Station C (Fig. 3). No statistical significant differences were observed between Station A and B, and between Stations B and C. The incidences of deformities were significantly higher during the dry season compared with the rainy season ( $P < 0.05$ ). The significant differences indicated by the deformities between the stations suggested that the three stations were not equally impacted although this difference was not indicated by the metal analysis. The results therefore support the argument for complementing physico-chemical analysis with a biological response analysis since in the present study, deformities picked up differences that would have been missed when relied on metal results alone. Furthermore, none of the three stations could be regarded as a reference station given the range of the surrounding

impacts as well as the percent incidences of deformities greater than 8% which is usually taken as threshold of in-stream contamination (Nazarova et al., 2004).

Mouthpart deformities were found mostly in *Chironomus* sp. (59.3%), *Polypedilum* sp. (22.2%), *Ablabesmyia* sp. (20.0%), *Dicrotendipes* sp. (20.0%) and *Coelotanypus* sp. (20.0%) (Table 2). No incidence of deformities were observed in *Tanytus* sp. *Tanytarsus* sp. and *Rheotanytarsus* sp. Overall, only two species i.e. *Chironomus* sp. and *Polypedilum* sp. showed incidences of deformities greater than 8% in relative percentage. The chironomid assemblage in the Shiroro Lake showed a variety of deformity types. These include missing teeth, fused teeth, extra tooth and lateral gaps. The commonest types of deformities observed include missing teeth, extra teeth and fused teeth (Fig. 4).

The elevated incidences of deformities (i.e. > 8%) recorded at the three stations can be taken as an indication of in-stream toxic stress in the Shiroro Lake. Several laboratory experimental works have demonstrated the potential of metals such as Pb, Cu, Zn, Mn, and Cd as causative agents of deformities in chironomids (Janssens de bisthoven et al., 1998; Martinez et al., 2003). In the present study, the relatively elevated concentrations of the analysed metals were likely to be at least, partly responsible for the induction of deformities observed in the mentum of the chironomids.

Species that were more deformed live in close association with the soft bottom sediment, which could expose them to sediment-bound metals and other toxicants, thus making them more vulnerable to effects of sediment associated toxins than species that are less associated with sediments. These species (such as *Chironomus* spp. and *Polypedilum* sp.) have been found to be more deformed in other studies (Odume et al., 2012).

Given the observed deformities in the larvae of chironomid appropriate strategies need to be developed urgently with regard to the management of in-flow of run-offs from the surrounding catchment activities. The observation of deformities in the larvae of chironomids suggests a biotic assemblage that has been chronically impaired. Furthermore, the observed elevated incidences of deformities is a source of concern because chironomids perform useful ecological function in the aquatic ecosystems, including nutrient cycling, organic matter processing and serving as an important link between primary producers and secondary consumers. The metal concentrations observed also suggested that the surrounding catchments activities need

to be monitored closely with a view to regulating them to minimise waste-inflow into the surrounding rivers and lakes.

From the observations in this study, mouthpart deformities are recommended to be used routinely in biological assessment especially in tropical water bodies polluted with heavy metals. Furthermore, this study has singled out *Chironomus* a relatively large and distinctive genus as been the most sensitive to sediment quality. Therefore, we recommend that for further studies in this region, assessments should be based solely on *Chironomus* species and ignoring the rest. Similarly, it is clearly more effective to sample in the dry season than the wet season as this is the period with very high incidence of mouth deformity.

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