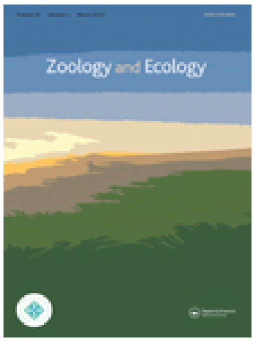

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Environmental drivers of head capsule deformities in *Chironomus* spp. (Diptera: Chironomidae) in a stream in north central Nigeria

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In most developing countries, freshwater pollution is threatening the sustainability and functionality of vital ecosystems. The lack of standardised tools for monitoring river health is worsening the situation in Nigeria. In this study, deformities were screened in *Chironomus* spp. as an indication of non-lethal effects of aquatic pollution in the Oinyi River close to which a cement factory was situated. *Chironomus* larvae collected at three sampling stations were screened for head capsule deformities. Physicochemical variables and selected heavy metal concentrations were measured at each station. Station 1 (upstream of the cement factory) served as a control site, while Station 2 (immediately downstream of the cement factory) was impacted by cement effluent. Station 3 (3 km downstream of the cement factory) was selected as a system recovery site. Elevated incidences of deformity were recorded at Station 2 (43.17%) compared with Stations 1 (10.83%) and 3 (24.33%). More larvae were deformed in the mentum compared with other structures. The increased deformity incidences at Station 2 corresponded with increased concentrations of Ni, Pb, Cu, Fe, Cr and Zn and increased electrical conductivity, suggesting that cement effluents may have contributed to the observed deformities. The screening of deformities provided insights into the cement effluent effect, and the method could be developed further for river health monitoring in Nigeria.

Gėlavandenių telkinių tarša daugelyje besivystančių šalių kelia grėsmę svarbiausių ekosistemų tvarumui ir funkcionavimui. Upių būklės tyrimams skirtų standartizuotų metodų trūkumas Nigerijoje prisideda prie situacijos blogėjimo. Mes tyrėme dėl vandens taršos poveikio atsiradusias *Chironomus* genties uodų rūšių organų deformacijas. Tyrimai atlikti Oinyi upėje, prie kurios pastatytas cemento fabrikas. Trijose upės vietose (stotyse) buvo surinkti *Chironomus* lervų mėginiai ir ištirtos jų galvos kapsulės deformacijos. Kiekvienoje stotyje išmatuoti vandens fiziniai ir cheminiai parametrai bei kai kurių sunkiųjų metalų koncentracijos. Kontrolinė stotis buvo aukščiau cemento fabriko, antroji – vietoje, kur patekdavo cemento fabriko nutekamieji vandenys, trečioji – už 3 km žemyn upe nuo cemento fabriko – pasirinkta tikrinant ekosistemos atsistatymą. Antrojoje stotyje užfiksuotas padidėjęs deformacijų skaičius (43,17%) lyginant su pirmąja (10,83%) ir trečiąja (24,33%) stotimis. Daugiausia rasta lervų smakro (*mentum*) deformacijų. Padidėjęs deformacijų skaičius antroje stotyje susijęs su padidėjusiomis Ni, Pb, Cu, Fe, Cr ir Zn koncentracijomis bei padidėjusiu elektros laidumu. Deformacijų atsiradimui galėjo turėti įtakos cemento fabriko nutekamieji vandenys. Tyrime naudotas metodas gali būti naudojamas upių būklės monitoringui Nigerijoje.

Keywords: cement industry; chironomid; deformities; heavy metals; industrial pollution; water quality

Introduction

Deteriorating surface water quality is threatening the sustainability and functionality of aquatic ecosystems (Chapin et al. 2005). The threat to freshwater ecosystems is exacerbated in Sub-Saharan Africa by the lack of appropriate biomonitoring tools sufficiently sensitive to enable the detection of sub-lethal effects of aquatic pollution (Odume 2014). Biomonitoring of aquatic pollution in most Sub-Sahara African streams relies mostly on changes in the assemblage structure of resident biota (Arimoro and Ikomi 2008; Arimoro 2009; Odume and Muller 2011). A biomonitoring tool based on changes in assemblage structure, focusing mainly on presence–absence and relative abundance–diversity as measures of assessing environmental quality would not enable the detection of sub-lethal effects on resident biota because such changes manifest at the population and community levels (Dickens and Graham 2002). Morphological deformities in chironomid species are an in-stream sub-lethal organismal response to deteriorating water and sediment quality. Deformities are thus indicators of early warning signals of aquatic pollution (de Bisthoven and Gerhard 2003; Ochieng, Steveninck, and Wanda 2008; Arimoro 2011; Di Veroli, Selvaggi, and Goretti 2012; Odume et al. 2012; Planello et al., forthcoming).

Freshwater chironomids are ideal bioindicators because they are among the most diverse, species-rich

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group of aquatic macroinvertebrates with an extraordinary ecological range (Ferrington 2008; Wright and Burgin 2009; Cortelezzi et al. 2011; Luoto 2011). Because chironomid larvae live in close association with river sediments, larval morphological deformities have been used as indicators of sub-lethal effects of exposure to both sediment and water contaminants (Ochieng, Steveninck, and Wanda 2008; Odume et al. 2012). Thus, screening deformities in chironomids is a rapid, easy and cost-effective tool for monitoring aquatic ecosystem health. Observed incidences of deformities greater than 8% have been considered elevated above the natural reference level and are usually taken as an indication of environmental stress (Nazarova et al. 2004; Ochieng, Steveninck, and Wanda 2008). Although the thickening of the body wall in chironomids exposed to chemical contaminants was the earliest report of deformities (Hamilton and Saether 1971), morphological structures on the head capsule, particularly those of the mouthparts and antennae, have imparted the greatest amount of information on deformities. For this reason, mouthparts and antennae have been screened for deformities in this study.

The Oinyi River, where the present study was undertaken, was impacted by cement effluent from a cement manufacturing factory. Cement effluent is a complex mixture of chemicals including potassium and sodium hydroxide, chlorides, sulphates and calcium carbonate. Therefore, cement effluent could impact important water quality variables including temperature, electrical conductivity, suspended solids, chemical oxygen demand (COD) and water colour as well as heavy metal concentrations (Arimoro 2009). Potential effects of the cement effluent on the biological assemblage of the Oinyi River have not been studied previously. The present study is aimed at examining the head capsules of *Chironomus* spp. for deformities as in-stream sub-lethal responses to water and sediment stress in the studied river system.

Materials and methods

Description of the study area and sampling sites

The Oinyi River located in Kogi State, Nigeria, originates in the Ayedebunu River and flows through the rocky plains and tropical rainforest of Canaja before opening into the confluence of the Niger and Benue Rivers in Lokoja and Ajankolo (Figure 1). The river catchment is situated between latitude 7.92 (7°55′0N) and longitude 6.43 (6°25′60E) and is about 177 km southwest (223°) of Abuja, the Nigerian Federal Capital Territory (FCT). A mini dam, the Obajana dam, was built on the river, and a cement factory, which discharges effluent into the river, was located close to the dam.

The river catchment is characterised by tropical climate with two distinct seasons – wet (May–October) and dry (November–April). The mean annual relative humidity is 81%, and the mean annual temperature is about 29 °C. Seasonal flooding usually occurs during the wet season, particularly between the months of June and July.

The study was undertaken bimonthly between October 2010 and September 2011 at three selected sampling stations along the river length. Station 1 was upstream of the cement effluent discharge point and the in-stream microhabitat was characterised by sandy and rocky substrates. The riparian vegetation at the station was composed mainly of grass, herbs and shrubs. Since there are no long-term historical water quality data on the river, the reference site was carefully selected to provide baseline information upon which the effects of the cement effluent could be ascertained. In Nigeria, there is no formal river health water-quality monitoring programme, and thus, no available national data on historical water-quality trends in the Oinyi River. However, although Station 1 was situated upstream of the cement effluent discharge point, domestic activities, including laundry and bathing which were observed at the site, could influence the water quality.

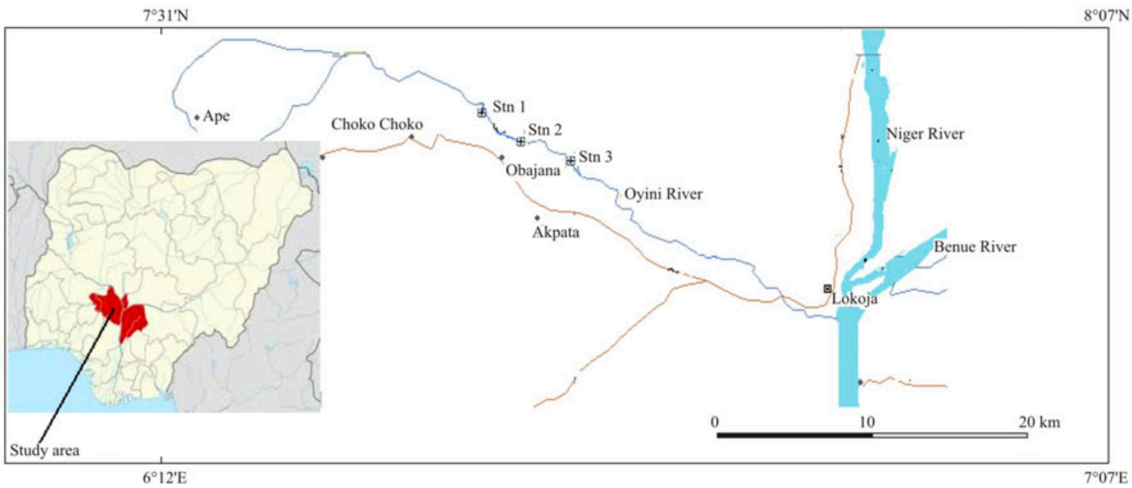

Figure 1. Map of the Oinyi River showing the study stations. Inset: Location of Kogi State in Nigeria.

Station 2 was immediately downstream of the cement effluent discharge point and was selected so that the effects of the effluent on both the biotic and physico-chemical quality of the river can be monitored. In addition, other human activities at this station include fishing, laundry and bathing. Sands, rocks and macrophytes were the main biophysical habitats at the station. Grass, herbs and shrubs dominated the riparian vegetation.

Station 3 was about 3 km further downstream of the cement effluent discharge point and was selected so that the potential system recovery can be monitored. Fine sediment and aquatic vegetation were the main biophysical sampling habitats at the station. Trees and shrubs characterised the riparian zone.

Physicochemical variables

At each sampling station, temperature, pH, electrical conductivity (EC) and total dissolved solids (TDS) were measured using the Hanna HI 991300/1 multi-probe metre. Dissolved oxygen (DO) and turbidity were measured using the YSI 55 dissolved oxygen metre and portable turbidity metre HI 93102, respectively. In the laboratory, water samples collected from each station per sampling event were analysed spectrophotometrically according to APHA (1992) for nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), ammonia-nitrogen (NH_3-N) and orthophosphate-phosphorus (PO_4-P). Five-day biochemical oxygen demand (BOD_5) and COD were also determined according to APHA (1992).

Heavy metals analysis

One gram of the bottom sediment from each station was digested with a mixture of hydrofluoric, nitric, perchloric and sulphuric ($HF–HNO_3–HClO_4–H_2SO_4$) acids. The clear digest was diluted to 50 ml with distilled water and then analysed for cadmium, chromium, copper, lead, iron, manganese, nickel and zinc, using the air-acetylene flame atomic absorption spectrophotometre (AA) (Perkin-Elmer A3100). The atomic absorption spectrophotometer was fitted with D2 background correction devices.

Habitat quality

The qualitative habitat evaluation index (QHEI) is a physical habitat index designed to provide a subjective quantitative evaluation of important lotic macrohabitats (Rankin 2006). The six metrics that constitute the index, substrate, in-stream cover, channel morphology, riparian zone, pool quality and riffle quality, and gradient, are assessed, scored and summed to give a maximum score of 100 (Rankin 2006). At each sampling station, each metric was scored separately and the individual scores summed to provide the total QHEI per station per sampling occasion. At each sampling station, a reach of 25 m was selected and habitat characterisation undertaken, including the description of stream hydraulics, aquatic plants, width, depth, flow and substrate type. Depth was measured in the sampling reaches using a calibrated stick. Mid-channel flow velocity was measured on three occasions by timing a float as it moved over a distance of 10 m and then averaged (Gordon, Mc Mahon, and Finlayson 1994). Substratum composition in each 25 m sampling reach was estimated visually as a percent of silt, loam, clay and sand (Ward 1992).

Chironomid sampling and data analysis

Chironomid larvae were collected by sweeping a hand-held net with a mesh size of 250 μm through different substrates including sediments, stones and vegetation within an approximately 25 m^2 wade-able portion of each sampling station. Larvae were preserved in 70% ethanol and transported to the laboratory for sorting, mounting, identification, and deformity screening. The larvae of *Chironomus* spp. were mounted in Euparal and identified based on the keys described by Harrison (2003). Larval mounting was so undertaken that the head capsule is ventral side up. During mounting, the head capsule was gently pressed to achieve maximum visibility of the antennae, mentum, mandibles, premandibles and pecten epipharygis (hereafter referred to as pecten). Morphological deformities were screened under a compound microscope. Depending on the size of the head capsule and the structure being screen, a magnification of either ×20 or ×100 was used in accordance with Jeyasingham and Ling (2000), de Bisthoven and Gerhard (2003), and Odume et al. (2012).

During each sampling event, the head capsules of 100 randomly selected individuals from each of the three sampling stations were screened for deformities. Broken or ambiguous abnormalities were considered normal. Abnormalities considered to be deformities in the mouthparts included missing teeth, Köhn gap, extra teeth, fused teeth and asymmetry. In the antenna, abnormalities in the lauterborn organ, ring organ, style and blade were considered deformities. Deformities were not classified based on apparent severity in this study because such classification system has been criticised by Hämäläinen (1999) who argued that it was redundant with the percent incidences of deformities. The incidence of deformities per structure was calculated as a percentage of deformed larvae to the total number of larvae screened.

Significant differences in physicochemical variables, heavy metals and incidences of deformities between the three sampling stations were ascertained using one-way ANOVA undertaken in SPSS 15 for windows. When ANOVA indicate significant differences between the stations, the Tukey's (HSD) *post hoc* test was used to indicate stations that differed. Prior to ANOVA, percent incidences of deformities were arcsine transformed.

Results and discussion

The means and standard deviations of the measured physicochemical variables at each of the sampling stations are shown in Table 1. The physicochemical results revealed that the drivers of deteriorating water quality in the Oinyi River were mainly related to non-organic stressors. This was evident in the elevated concentrations of EC, TDS and turbidity at Station 2 compared with Stations 1 and 3. The elevated concentrations of EC, TDS and turbidity observed at Station 2 can be attributed to effluent discharges from the cement factory emptying into the river at Station 2. Increased levels in EC and TDS could be detrimental to biota because of their role in osmotic balance, and if elevated beyond acceptable limits, they could affect internal fluid regulation (Dallas and Day 2004). Similarly, the increased turbidity at Station 3 could be detrimental to biota relying on external gills for respiration because of increased risk of gill clogging (Bilotta and Brazier 2008). The concentrations of DO and BOD_5, which generally provide indication of organic pollution, revealed that organic pollution was unlikely the main driver of differences in water quality between the sampling stations. For example, DO was generally high at all the stations and was not significantly different between the sampling stations (Table 1). Although based on the concentrations of BOD_5 and nutrients, ANOVA indicated statistically significant differences between the sampling stations; these variables were generally low and are therefore, unlikely to cause significant changes (e.g. eutrophication) in the river ecosystem. Generally, based on the analysed physicochemical variables, there seemed to be a system recovery at Station 3, further downstream of the cement effluent discharge point. Improvement in the values of variables, such as EC, turbidity, TDS, DO and colour, indicated system recovery at Station 3 (Table 1).

Apart from Mn, the concentrations of the heavy metals including Ni, Zn, Cu, Pb and Fe were generally higher in the sediments of Station 2 compared with Stations 1 and 3 (Table 2). However, the highest

Table 1. Environmental factors measured at the sampling stations in the Oinyi River, showing habitat quality and physicochemical variables during the study period (October 2010–September 2011).

Habitat characteristics and physicochemical variables	Station 1	Station 2	Station 3
Riparian vegetation	Native	Native	Native
Land use impact	Agriculture	Agriculture and cement factory	Forestry/agriculture
Substrate type	Silt/sand	Silt/sand	Sand/clay
Canopy cover (%)	60	60	70
Qualitative habitat evaluation index (QHEI)	80	54	67
pH	7.075 ± 0.0126^{a}	7.23 ± 0.0095^{a}	6.96 ± 0.037^{a}
	(7.04–7.12)	(7.2–7.26)	(6.8–7.02)
Electrical conductivity (µS/cm) *	111.6 ± 0.46^{a}	210.65 ± 0.41^{b}	106.95 ± 0.13^{a}
	(110–113.1)	(209–211.7)	(106.6–107.5)
Colour (Pt.Co)*	2.47 ± 0.147^{a}	5.51 ± 0.13^{b}	3.63 ± 0.20^{c}
	(2–2.89)	(5–5.83)	(3–4.1)
Turbidity (NTU)*	15.38 ± 0.13^{a}	22.44 ± 0.11^{b}	14.44 ± 0.16^{a}
	(15–15.76)	(22–22.7)	(14–14.86)
TDS (mg/l)*	(45–48)	(51–54)	(48.9–51.6)
	56.42 ± 0.21^{a}	107.57 ± 0.29^{b}	53.67 ± 0.32^{a}
DO (mg/l)	(55.8–57)	(107–108.8)	(52.7–54.6)
	6.63 ± 0.09^{a}	6.11 ± 0.02^{a}	6.39 ± 0.05^{a}
BOD_5 (mg/l)	(6.4–7.01)	(6.02–6.17)	(6.3–6.6)
	2.80 ± 0.03^{a}	4.70 ± 0.03^{b}	2.14 ± 0.03^{a}
COD (mg/l)*	(2.7–2.89)	(3.6–5.78)	(2.05–2.23)
	24.18 ± 0.18^{a}	14.37 ± 0.21^{b}	13.01 ± 0.07^{a}
NH_4–N (mg/l)*	(23.7–24.87)	(13.6–15.02)	(12.8–13.2)
	0.014 ± 0.002^{a}	ND	0.008 ± 0.0026^{b}
NO_2–N (mg/l)*	(0.01–0.02)	0.05 ± 0.011^{b}	(0–0.015)
	0.013 ± 0.002^{a}		0.013 ± 0.0015^{a}
NO_3–N (mg/l)*	(0.01–0.02)	(0.02–0.09)	(0.01–0.02)
	0.03 ± 0.005^{a}	0.06 ± 0.007^{b}	0.045 ± 0.006 *ab*
PO_4–P (mg/l)*	(0.01–0.04)	(0.04–0.09)	(0.03–0.06)
	0.64 ± 0.025^{a}	0.24 ± 0.02^{b}	1.67 ± 0.14^{c}
Temperature (°C)	(0.57–0.73)	(0.19–0.3)	(1.32–2.05)
	25.92 ± 0.36^{a}	25.74 ± 0.28^{a}	25.05 ± 0.31^{a}
Flow velocity (m/s)*	(24.8–27)	(25–26.6)	(24–26)
	0.24 ± 0.04^{a}	0.11 ± 0.03^{b}	0.19 ± 0.06^{c}
	(0.24–0.35)	(0.08–0.16)	(0.14–0.29)

Note: Physicochemical variable values are mean ± SD, range in parenthesis.
*Significant differences indicated by ANOVA. Different superscript letters in a row show significant differences ($p < 0.05$) indicated by Tukey honest significant difference tests.

Table 2. Concentrations (mean ± standard deviation) of analysed heavy metals in the Oinyi River during the study period (October 2010–September 2011).

	Station 1	Station 2	Station 3
Fe (mg/kg)*	204.77 ± 1.50[b] (200–208.6)	93.11 ± 0.21[a] (92.4–93.8)	214.17 ± 0.35[b] (213–215.4)
Mn (mg/kg)*	51.37 ± 0.19[a] (50.8–52.02)	115.27 ± 0.16[b] (114.8–115.8)	42.71 ± 0.22[a] (42.01–43.2)
Zn (mg/kg)*	1.443 ± 0.015[a] (1.4–1.49)	6.16 ± 0.03[b] (6.07–6.26)	1.36 ± 0.02[a] (1.3–1.42)
Cu (mg/kg)	1.71 ± 0.01 (1.67–1.74)	3.34 ± 0.014 (3.3–3.39)	1.84 ± 0.02 (1.8–1.89)
Cr (mg/kg)	0.31 ± 0.01 (0.25–0.35)	0.37 ± 0.02 (0.3–0.45)	0.36 ± 0.01 (0.32–0.4)
Cd (mg/kg)	0.155 ± 0.02 (0.1–0.2)	0.19 ± 0.01 (0.15–0.24)	0.17 ± 0.02 (0.12–0.23)
Pb (mg/kg)	0.38 ± 0.02 (0.3–0.44)	0.5 ± 0.02 (0.4–0.55)	0.33 ± 0.01 (0.29–0.38)
Ni (mg/kg)	4.74 ± 0.03 (4.65–4.8)	5.6 ± 0.13 (5.2–6)	3.85 ± 0.08 (3.5–4.03)

Note: Metal concentrations are mean ± SD, range in parenthesis.
*Significant differences indicated by ANOVA. Different superscript letters in a row show significant differences ($p < 0.05$) indicated by Tukey honest significant difference tests.

concentration of Mn was recorded at the downstream station (i.e. Station 3). The relatively high levels of heavy metals at Station 2 compared with Stations 1 and 3 were indicative of the effluent altering the sediment and water chemistry of the studied river system. Alteration of water and sediment chemistry could have detrimental effects on benthic organisms, including *Chironomus* spp.

Chironomus spp. live in soft-bottom sediments where they feed on detritus and attached algae. Thus, their modes of life predispose them to effects of sediment-bound heavy metals (Armitage, Cranston, and Pinder 1995). Of the different causative agents of deformities in chironomids, heavy metals, including Zn, Cu, Mn, Pb, Ni, As and Cd, have been extensively implicated as the main causes of observed deformities both in the field and in laboratory experiments (de Bisthoven, Vermeulen, and Ollevier 1998; de Bisthoven and Gerhard 2003; Martinez et al. 2004; Nazarova et al. 2004; Ochieng, Steveninck, and Wanda 2008). Consequently, in the present study, a high incidence of deformities (43.17%) recorded at Station 2 compared with the other two stations (<25%) (Figure 2), could be attributed to the effects of the relatively elevated concentrations of heavy metals at Station 2.

Of the structures screened for deformities in this study, more larvae were deformed in the antennae, mentum and pecten than in the mandibles and premandibles (Figure 3). Examples of deformities observed in the mentum are shown in Figure 4. The antenna is a highly sensitive organ prone to easily develop morphological deformities when exposed to even low concentrations of pollutants (Warwick 1985, 1988; Bhattacharya et al. 2005). The mentum and pecten are less sclerotised compared with the mandibles and premandibles. The mentum has been reported to be more susceptible to deformity-inducing agents than the heavily sclerotised structures such as mandibles and premandibles (Odume 2011). This probably explains why more larvae were deformed in the mentum than in the mandibles and premandibles. The incidences of a single larva having multiple deformed structures were highest at Station 2.

Furthermore, the observed deformities represent sub-lethal effects of pollutants resulting mostly from the cement effluent. Although the organic matter content seemed to favour chironomid abundance, the chemical components seemed to induce elevated incidences of deformities in the head capsule of *Chironomus* spp. larvae examined in this study. The observed deformities, apart from being sub-lethal effects, could be considered important endpoints of ecological significance with potential effects on acquiring and processing resources because most of the deformities were associated with

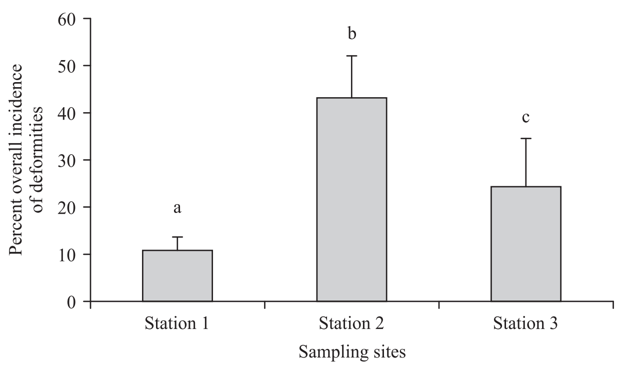

Figure 2. Percent overall incidence of deformities in *Chironomus* spp. collected at three sampling station in the Oinyi River during the study period (October 2010–September 2011). $N = 600$ specimens per station. ANOVA indicated significant differences between the stations as shown by different letters above each bar ($F = 19.1$, $p < 0.001$).

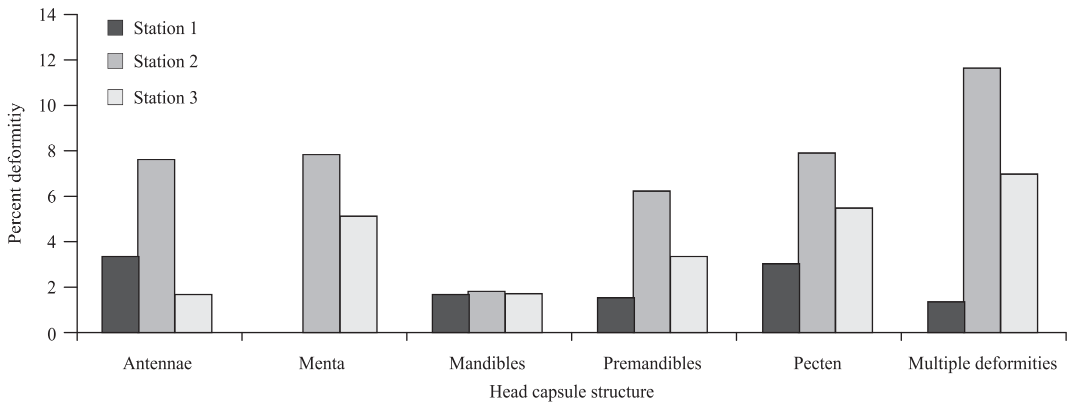

Figure 3. Percent incidence of deformities in the head capsule structures of *Chironomus* spp. collected at three sampling stations in the Oinyi River during the study period (October 2010–September 2011).

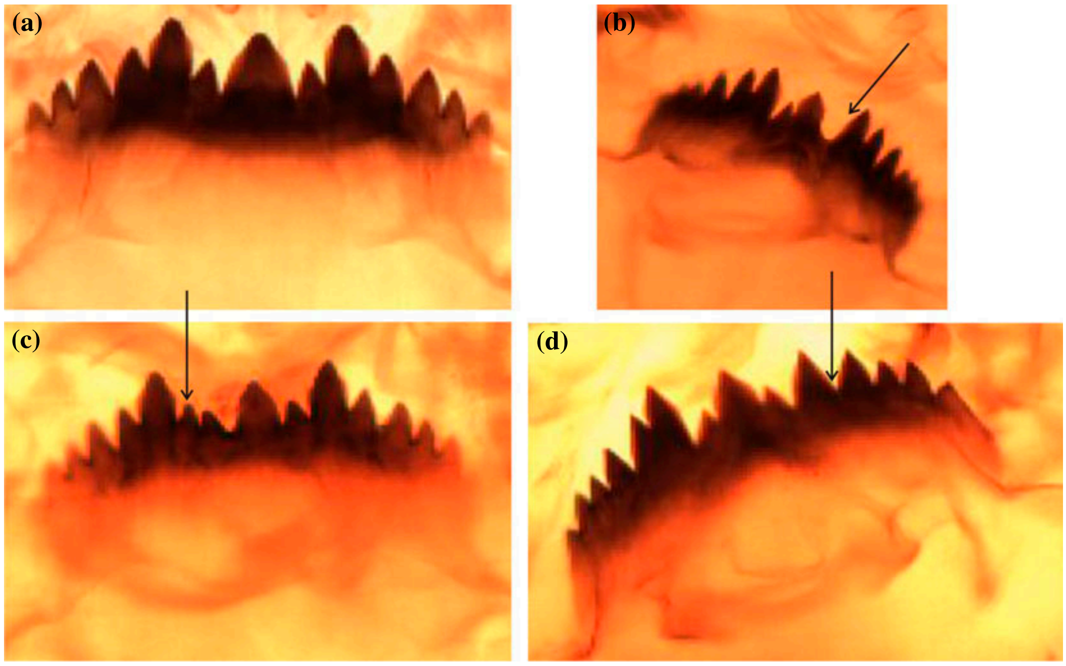

Figure 4. *Chironomus* mentum: (a) normal, (b) mentum with a missing lateral median tooth, (c) mentum with multiple deformities, a gap and extra tooth and (d) missing second lateral tooth.

mouthparts, which are usually employed in feeding. The mentum, mandibles and premandibles are used in combination with other structures for resource processing such as leaf fragmentation (Armitage, Cranston, and Pinder 1995). Deformities in these structures may therefore impact on these important ecological functions performed by *Chironomus* spp.

In conclusion, *Chironomus* spp. are widely distributed and abundant in tropical rivers and streams in Sub-Saharan Africa. Screening morphological deformities in this genus could serve as an easy and cost-effective means of monitoring the health of aquatic ecosystems, particularly in Nigeria where there are no well-developed and standardised tools for monitoring river health. It is important to note that correlation is not a causal relationship, and laboratory cause–effect experiment would be needed to confirm the observed field-based result in this study. Nevertheless, the screening of deformities offers insight into biotic effects of the cement effluent, signifying the need to mitigate the effluent effects through the deployment of appropriate technologies to improve the effluent quality before it reaches the receiving river system.

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