Incidence of Larvae Deformities in *Chironomus* Species (Diptera: Chironomidae) as Bio-Indicators of Water Quality in Lake Victoria Basin, Kenya

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Abstract

Lake Victoria is the second largest freshwater body in the world and one of the richest ecosystems in terms of biodiversity but is facing a major environmental concern originating from pollution, degradation and overfishing. This study sought to determine the incidence of larvae deformities in Chironomus species (Diptera: Chironomidae) as bio-indicators of water quality in Lake Victoria Basin, Kenya. Chironomids were sampled upstream and downstream of Rivers Nzoia and Mbogo for tests in the laboratory. They were exposed to dilutions of kraft paper-mill and sugar cane factory effluents for 21 days under controlled conditions. Results for the deformity effluent toxicity tests were presented as means (±Standard Error of Mean, SEM). Data for the tests expressed as the proportion deformity was first transformed by the Probit analysis to check for normality before analysis. Two way ANOVA for the deformity tests was run with the station and concentration as the main factors with the station x concentration interaction effect and where there was no interaction then 1-way ANOVA was run with the main individual factors only (i.e. effluent, station and concentration). Post hoc Tukey test was then performed to identify means of concentrations for deformities of the Chironomus midges differing from one another. All pair wise multiple comparisons of stations and effluent concentrations was done to determine whether there existed any effect. One-Way ANOVA was also, used to determine significant difference between the control and the effluent concentrations. All analysis was done in Sigma Plot and the significant differences were inferred at $\alpha=0.05$ level. There were significant effects ($p<0.05$) between effluents on abnormality of Chironomus species. The study concluded that Chironomus species are sensitive to sugar cane processing and paper mill effluents, hence can be used as test organism in monitoring the health of riverine ecosystems in LVB for management purposes.

Key Words: Bioindicators, *Chironomus* species, effluents, Sensitivity and ecotoxicology

INTRODUCTION

The ecological integrity of riverine ecosystems is affected by interrelated biotic and abiotic factors. Further, these factors can be broken down into classes of chemical and physical water quality variables, flow regime, habitat structure, biotic interactions and energy sources

(Karr and Chu, 2000). The compromised integrity of these systems has a direct impact on the resident biota. Therefore, making critical observations of such aquatic fauna and flora may give a glimpse of the state of environment of that ecosystem (Mafalda *et al*., 2007).

In this regard, macroinvertebrates of the species Chironomus have been identified as the most suitable candidates for the assessment and monitoring of riverine systems. Several reasons have been adduced for their robustness in biomonitoring (Myslinski and Ginsburg, 1977; Lynch *et al*., 1988; Hare and Campbell, 1992; Nyakeya *et al*., 2009; Masese *et al*., 2013; Nyakeya *et al*., 2017):

i. They are ubiquitous – are readily found in diverse groups in many of the freshwater types;
ii. Are sedentary hence represent local conditions;
iii. They are benthic thus in close association with sediments;
iv. They bioaccumulate pollutants and at the same time tolerate low to moderate toxicant concentrations;
v. Pollutant concentrations in chironomids are closely related to those in their environment;
vi. Are long-lived thus easily integrate with contaminants over a reasonable period of time hence can be used for monitoring programmes of the environment;
vii. Since they are the immature stages of the life-cycle, body concentrations are not affected by reproductive cycles or sexual differences;
viii. They are at the lower trophic level, making them vital agents of toxicants entry into the food chains.

Pollutants getting into aquatic systems either from point or non-point sources get suspended in the water or may be trapped in the sediments via absorption, precipitation and by ion exchange processes (Liu, 2005). Chironomus midges in the sediments while ingesting their food they are able to metabolize the different contaminants (Harkey *et al*., 1994), causing them abnormalities in their body parts. This abnormality is referred to as deformity - deviation from normal configuration (Warwick, 1985 a,b). Such abnormalities in different parts of the body could represent sub lethal effects and can be considered as early warning signs of pollution by pollutants (Warwick, 1980).

The frequency of deformity in chironomids could inform researchers and managers of aquatic resources about the extent of pollution (Hamilton & Saether, 1971; Warwick, 1985a,b) that could be classified as either low, minimum or extreme depending on the body appendages affected (i.e. Alessandra Di Veroliar, 2008). However, while carrying out studies that involve chironomid midges sampled in the wild they may show some signs of deformities due to natural condition. In such a scenario, caution must prevail when making a professional judgment in relation to environmental stress. It is suggested that in such an occurrence deformity incidences should never exceed 8% (Warwick, 1980).

Biomonitoring of the environment by aquatic organisms has received wider appreciation by scientists. Many of the studies could be traced to way back in the 1970s (Hamilton and Saether, 1971) and since then there has been an appreciating number of scientists working in this field (e.g. Karr *et al*., 1986; Ochieng *et al*., 2008; Raburu *et al*., 2010; Odume *et al*., 2012).). There is, however, still a limited amount of information about their use in freshwater monitoring. The aim this study, therefore, was to determine the incidence of

larvae deformities in *Chironomus* species (Diptera: Chironomidae) as bio-indicators of water quality in Lake Victoria Basin, Kenya.

MATERIALS AND METHODS

Study Area

Chironomid midges for laboratory tests were sampled on two locations in the Rivers Nzoia and Mbogo, a tributary of R. Nyando within the LVB, i.e. polluted site (C for River Nzoia and D for River Mbogo) and pristine site (R for River Nzoia and S for River Mbogo). Station R of R. Nzoia had a canopy cover, murky water and a sandy substratum while, station C received paper kraft effluents from Webuye Factory. The water was too turbid, no riparian vegetation and the substratum was muddy. The point source of pollution for R. Nzoia is Webuye Paper mill which, whereas R. Mbogo receives effluent from the Chemelil Sugar factory. The reference sites were located upstream in the forest from the two factories while the impacted stations were just downstream from the point of the factories' discharge.

Field Sampling

Fourth instar larvae were used in the effluent deformity test. This is because they are easy to identify. Sampling took place in sediment banks, stones and wood debris that created a suitable habitat for the midges. The sediment was then sieved by nets of mesh size of 400 µm and larvae of the genus *Chironomus* were collected and placed in clear plastic vials. They were then taken to the laboratory for acclimatization in a controlled climate room for 24 hours before they were exposed to the effluent dilutions. Industrial effluents for the toxicity tests were sampled from Webuye Paper mill Factory and Chemelil Sugar Factory treatment ponds/lagoons.

Collection of Effluents and Preparation

Liquid effluent samples from the factories were sampled at a point between the final treatment and the discharge outfall. Samples were then transported to the laboratory and either used immediately or kept at 0-4^{o}C until used to inhibit microbial degradation, chemical transformations, and loss of highly volatile toxic substances. However, samples once collected were used within the 36-h period.

Laboratory acclimatization of the test organisms

Midges were acclimatized based on validated protocols (Fonseca and Rocha, 2004; Santos *et al*., 2007) i.e. tray length (45 cm x 6 cm x 35 cm) with a mesh cage on the top with a control, sand layer at the bottom (1 cm, sieved through 0.5 mm mesh size and sterilized at 550ºC for 2 h), and 4 litres of dechlorinated water (USEPA, 1999). Dechlorinated water was also used for toxicity testing of the *Chironomus* species.

Bioassays/Bio-assessment

Five industrial effluent sample concentrations with control for each test replicated four was used for the test of the *Chironomus* midges (USEPA, 2002). Ten live (midges) test organisms were exposed to each industrial effluent sample concentration (i.e. six concentrations x ten midges per concentration for each test). Serial dilutions of the effluent for each test was done with a factor of 0.5 i.e. 100%, 50%, 25%, 12.5% and lastly 6.25% (USEPA, 2002). Ten midges in all the experiments were added to 0.5 l beakers, containing the appropriate serial effluent dilutions, 1.0 ml of food suspension. A temperature of 25^{o}C and a 12:12, light: dark regime was maintained. The experiment ran for 21 days (Fonseca

and Rocha, 2004). Consequently, additional feeding of suspended chick mash was added to provide an extra source of food. This was done three times a week.

Identification of the Chironomids

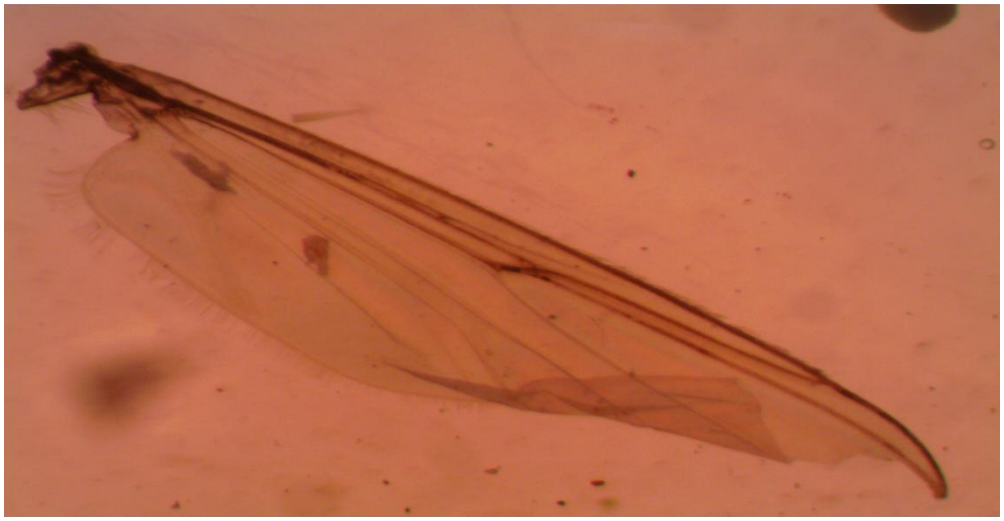
The Chironomid midges were identified to the lowest level possible using identification keys (Epler, 1995). Larval specimens sampled from the four stations of the two rivers and stored in 70% ethanol were observed under a dissecting microscope in order to ascertain the physical features before the head capsule was removed for further investigations (Plate 1).

X5 magnification

Plate 1: Fourth instar Chironomid larvae used in this study

The head capsule of each individual was removed while observed under a dissecting microscope (GallenKamp) using a needle and a sharp thin metal plate. The head capsule was then immersed in a 10% potassium hydroxide (KOH) for 24 hours in order to remove the soft obscuring tissues. It was then removed from the 10% KOH and mounted on the slide using Euparal mountant with the ventral side facing upward. The mentum teeth were examined and counted as well as the antennae segments. This was done in order to assist in ascertaining the species of the midges.

The wings of the reared Chironomid adults were also removed while observed under the dissecting microscope and mounted on the slides using Euparal mountant and observed under the compound microscope (Plate 2) with different ranges of magnification (i.e. x5, x10 and x20) in order to determine the species of the midges under study.



X40 magnification

Plate 2: A wing of an adult *Chironomus* species

Deformity

The deformity test to *Chironomus* species midges (Def50) i.e. the actual toxic concentration in the paper and pulp mill and sugarcane effluents that was observed was done for a period of twenty one days. Chironomus midges were placed in 150 ml dechlorinated water containing 100%, 50%, 25%, 12.5% and lastly 6.25% paper and pulp mill and sugarcane effluent, with four replicates per concentration, and 10 larvae per replicate. The experiment was inspected regularly to remove any dead larvae in the concentrations. At the end of the experiment period, the larvae were taken and stored in 70% ethanol to check for any deformity in each concentration.

In order to observe for mentum and the mandible deformity, the larval specimens preserved in 70% ethanol were used. The head capsule of each individual specimen was carefully removed while observed under a dissecting microscope (GallenKamp) at a magnification of x5 and x10 using a needle and a sharp thin metal plate. The clean head capsule was then put in a 10% potassium hydroxide (KOH) for 6-24 hours in order to remove the soft obscuring tissues. After 6-24 hour period the head capsule was removed cautiously and mounted on the slide using Euparal mountant with the ventral side facing upward. In order to expose the mouth parts properly, the head capsule was depressed gently.

The slides were then observed for deformities under the Binocular Microscope (Motic BA200) at a magnification of x40 depending on time availability. For scrutiny and verification of the specimens for mouth part and mandible deformities, the specimens were photographed using a digital camera and analysis for deformities done in the Toup-View (Version 3.7) programme.

Deformities were then classified using a protocol that assigned each specimen to a specific morphological class (modified from Janssens De Bisthoven *et al*., 1998; Alessandra Di Veroliar, 2008):

I. Deformity Class 1, Def. CL 1 – specimens without any morphological deformity an indication of unimpacted site.

II. Deformity Class 2, Def. CL 2 – specimens with weak deformity: one additional or missing tooth, one or two round teeth, weak asymmetry, one bifid tooth, two joined teeth, hence low level of pollution.

III. Deformity Class 3, Def. CL 3 – specimens with strong deformity): Kohn gap, very round teeth, two or more additional or missing teeth, no developed teeth, three or more joined teeth. This indicates medium level of pollution.

IV. Deformity Class 4, Def. CL 4 – specimens with very strong deformity): missing antennae and the mandibles. This is an indication of high level of pollution.

V. Tot Def - This is the total sum of the other three classes namely Def. Cl4, Def. Cl3 and Def. Cl2).This class therefore gave the general outlook of deformities as they occurred in the midges sampled from the four stations within the two rivers and exposed to two forms of effluents namely paper and pulp and sugar cane.

This is because different morphological structures in the *Chironomus* species respond differently to levels of pollution such that the pectin, mentum and the mandibles can be signs of low, medium and high levels of pollution respectively (Janssens De Bisthoven et al., 1998; Alessandra Di Veroliar, 2008).

Lastly to estimate the number of the deformed chironomids regardless of the class in both the paper pulp mill and sugarcane effluents, the proportion of the deformed individuals or deformity incidence was applied as indicated below:

$$DI = d/n$$

Where d = the number of deformed larvae, and

n = the number of larvae examined.

Data analysis

Results for the deformity effluent toxicity tests were presented as means (±Standard Error of Mean, SEM). Data for the tests expressed as the proportion deformity was first transformed by the Probit analysis to check for normality before analysis.

Two way ANOVA for the deformity tests of the *Chironomus* species midges was run with the station and concentration as the main factors with the station x concentration interaction effect and where there was no interaction then 1-way ANOVA was run with the main individual factors only (i.e. effluent, station and concentration). *Post hoc* Tukey test was then performed to identify means of concentrations for deformities of the Chironomus midges differing from one another. All pair wise multiple comparisons of stations and effluent concentrations was done to determine whether there existed any effect.

One-Way ANOVA was also, used to determine significant difference between the control and the effluent concentrations. All analysis was done in Sigma Plot and the significant differences were inferred at α=0.05 level.

RESULTS

***Chironomus* species identified in the study**

Four species were identified and classified in this study. They included *Chironomus decorus, Chironomus riparius, Chironomus stigmaterus* and *Goeldichironomus c.f. natans*.

Deformity of *Chironomus* species

Under the deformity class Def. Cl4 station C of R. Nzoia recorded the least number of midges that were deformed (1±1%) while station D of R. Mbogo had the highest cases of

deformities under this category (7±2%). Stations S and R registered 6%±2 and 3%±2 respectively (Figure 1).

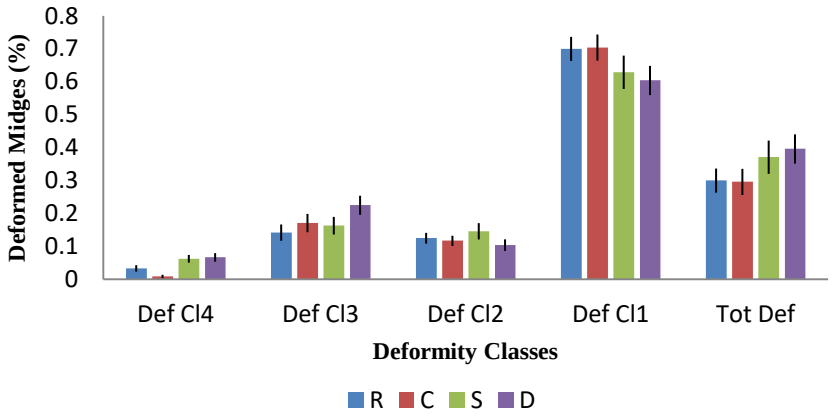


Figure 1: Mean (±SEM) deformity classes of *Chironomus* species for the four stations from rivers Mbogo and Nzoia during the study period.

There was an increase in the mean percentage in deformities in all the stations under the deformity class Def. Cl3 as compared to Def. Cl4. Station D of R. Mbogo had the highest instances of deformities (i.e. 23% ±5) followed by station C (17% ±5) of R. Nzoia, then S of R. Mbogo (16% ±5) and lastly R of R. Nzoia which recorded 14% ±4 deformities. Station S of R. Mbogo registered the highest percentage (15% ±5) of midge deformities in the deformity class Def. Cl2 followed closely by station R with a percentage value of 13% ±4, then C (12% ±4) both of R. Nzoia and lastly station D that recorded 10% ±3.

Of significance in this study also, was the deformity class Def. Cl1 and total Def. Total deformity indicated the impacted midges whereas Def. Cl1 showed the midges that were not affected by the two effluents. Station D of R. Mbogo recorded the highest number of total deformities (40% ±4) compared to stations R and C of R. Nzoia which registered 30% ±6 and 30% ±7 respectively (see Plate 4 for abnormal mentum and Plate 3 for deformed mandible and antennae). On the other hand, station S of R. Mbogo was second with 37% ±7 of total deformed midges under study. Going by the above results on total deformity, the reverse is true with regard to Def. Cl1 hence stations R and C of R. Nzoia had the highest cases (70% ±7 each) of midges that were not affected by the paper and pulp effluents followed by station S and D of R. Mbogo with 63% ±7 and 60% ±4 respectively (See Plate 5 for normal mentum, mandible and antennae).

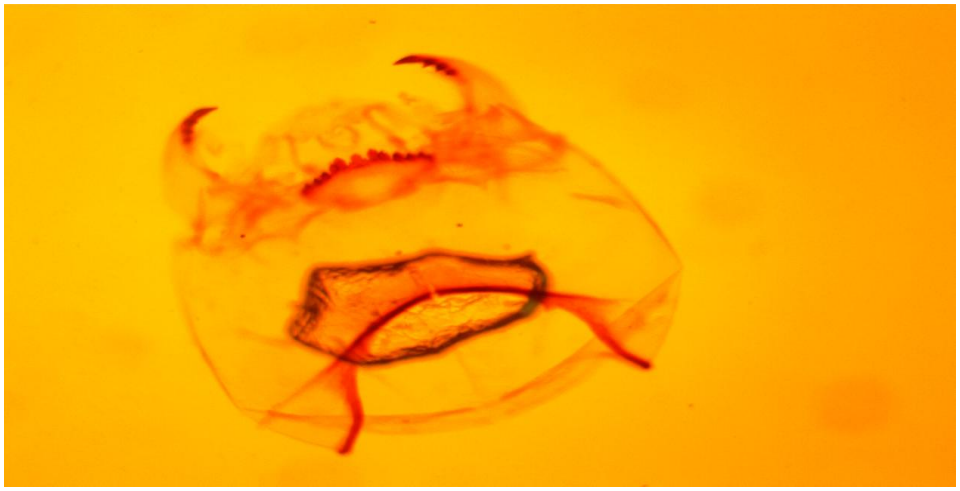


Plate 3: Normal mentum with a deformed mandible and antennae

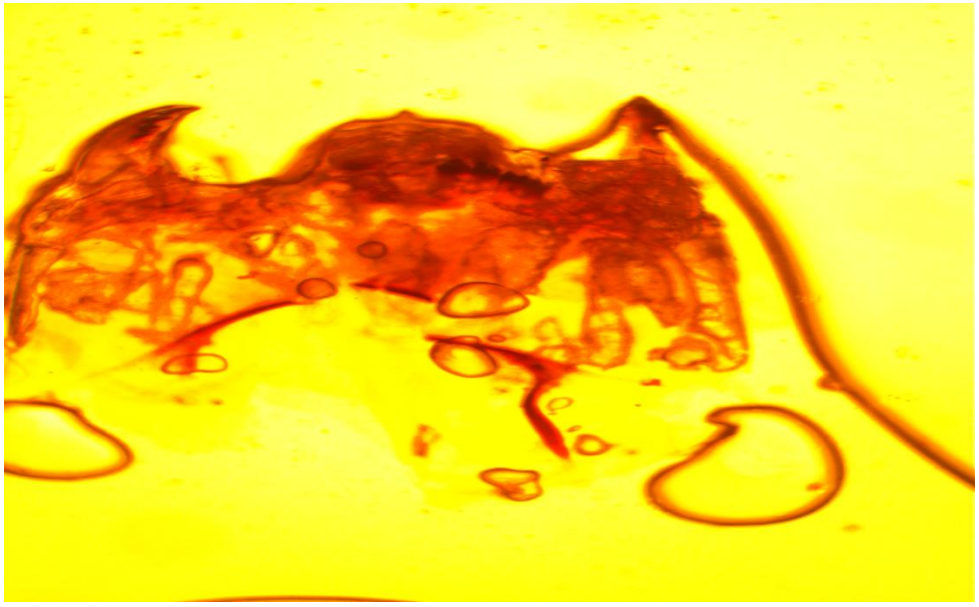


Plate 4: Abnormal mentum (Kohn gap i.e. middle teeth absent) Mg x40

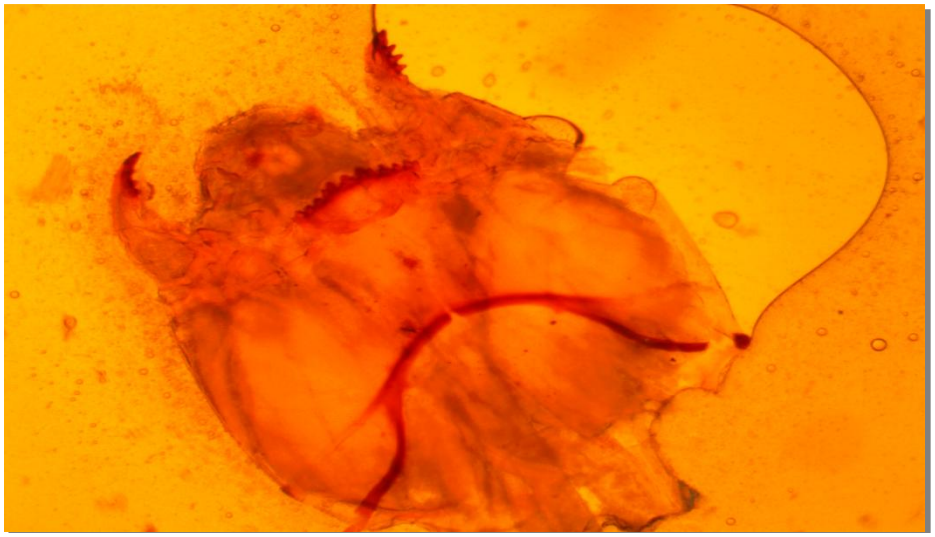


Plate 5: Normal mentum, mandible and antennae, Magnification x40

Results on the Two-Way ANOVA for the Def. Cl4 showed a significant difference in the means among the stations (F=7.254, p=<0.001) and the concentration (F=3.896, p=0.004) as compared to station x concentration which had no effect. There was no significant difference found in the means among the station x concentration of Def. Cl3, but there was a relationship among the stations (F=3.964, p=0.011) and the concentrations (F=25.985, p=0.004). However, under Def. Cl2, only the concentration displayed a relationship (F=13.825, p=0.001) but there were no interactions in the means among stations and the station x concentration combined (Table 1).

There was an interaction in the means among the station (F=5.467, p=0.002) and the concentration (F=56.854, p=0.001) under total deformity class but there was no interaction recorded in the station x concentration combined. Similar results were observed for Def. Cl1 as those in total deformity (Table 1).

Table 1: Summary of Two-Way ANOVA for the deformity classes Def. Cl4, Def. Cl3, Def. Cl2, Def. Cl1 and total Deformity (*=p<0.05).

Source of Variation	DF	SS	MS	F	P
Def. Cl 4					
Station	3	0.0536	0.0179	7.254	<0.001*
Concentration	5	0.048	0.0096	3.896	0.004*
Station x Concentration	15	0.0157	0.00105	0.425	0.967
Residual	72	0.178	0.00247		
Total	95	0.295	0.0031		
Def. Cl 3					
Station	3	0.0908	0.0303	3.964	0.011*
Concentration	5	0.993	0.199	25.985	<0.001*
Station x Concentration	15	0.0867	0.00578	0.756	0.72
Residual	72	0.55	0.00764		
Total	95	1.72	0.0181		
Def. Cl 2					
Station	3	0.0221	0.00736	1.493	0.224
Concentration	5	0.341	0.0682	13.825	<0.001*
Station x Concentration	15	0.0917	0.00611	1.239	0.264
Residual	72	0.355	0.00493		
Total	95	0.81	0.00852		
Def. Cl 1					
Station	3	0.183	0.0609	5.467	0.002*
Concentration	5	3.168	0.634	56.854	<0.001*
Station x Concentration	15	0.138	0.00919	0.824	0.648
Residual	72	0.803	0.0111		
Total	95	4.292	0.0452		
Total Deformity					
Station	3	0.183	0.0609	5.467	0.002*
Concentration	5	3.168	0.634	56.854	<0.001*
Station x Concentration	15	0.138	0.00919	0.824	0.648
Residual	72	0.802	0.0111		
Total	95	4.292	0.0452		

*Denotes significant difference

Deformity incidence of the *Chironomus* species sampled from the two rivers increased with an increase in effluent concentration in both effluents. However, comparing the two effluents deformity incidence in sugar cane effluent remained at 2.5% in both the control

and the 6.25% concentration before increasing steadily in the subsequent concentrations (Figure 2).

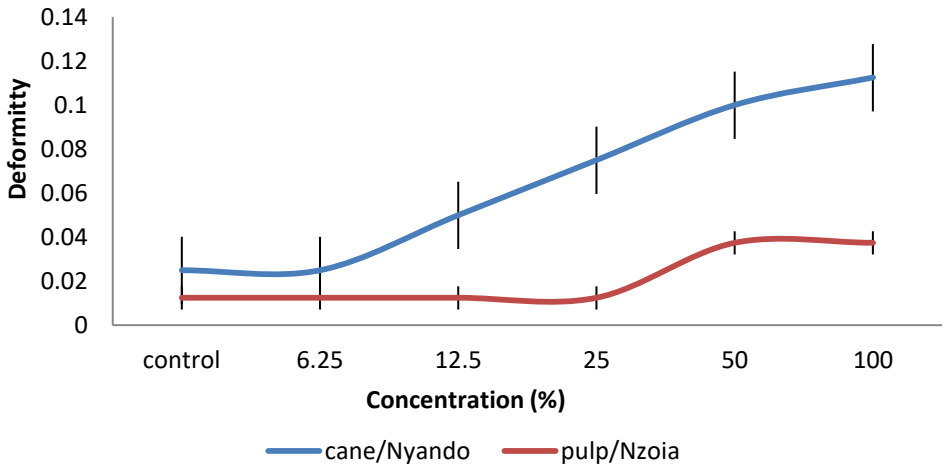


Figure 2: Mean (±SEM) Deformity incidences of *Chironomus* species under different dilutions of sugar cane effluents and paper and pulp effluent during the study period

The highest level of deformity under sugar cane effluent was recorded in 100% concentration with a deformity incidence of 11.25% followed by 10.0%, 7.5%, 5.0% for concentrations 50%, 25%, and 12.5% respectively (Fig. 2). under the paper and pulp effluent the control, 6.25%, 12.5% and 25% concentrations recorded 1.25% deformity incidence each before increasing to 3.75% for both 50% and 100% concentrations. In general midges exposed to paper and pulp effluent concentrations had low levels of deformity incidence as compared to those exposed to sugar cane effluents.

Relationship among the concentrations for deformity of the exposed *Chironomus* species

Post hoc Tukey test (Table 2) for deformity showed a significant difference (although weak) between the Chironomid midges in the control and all other effluent concentrations (F=121.4, p=0.006).

Table 2 *Post hoc* Tukey test among means of different concentrations for deformity (p<0.05)

Concentration (%)	Deformity
0	0.019±0.02a
6.25	0.019±0.04a
12.5	0.031±0.02ab
25	0.04±0.06ab
50	0.07±0.02ab
100	0.075±0.08b

A,b,c,d,e shows an effect

DISCUSSION

Under normal circumstances, Chironomus midges sampled from their wild habitat may display some form of abrasions on their mentum or mandibles due to the nature of feeding especially on the sediment substrates. However, it is argued that such abrasions should not exceed 8% (Alessandra Di Veroliar, 2008). Therefore, if taken to the laboratory for experimental purposes where they are exposed to certain levels of pollutants and they display deformity incidence exceeding the stated level (i.e. of 8%) then chances are that they have been impacted by anthropogenic activities.

In this study, total deformity i.e. the total sum of all other deformity classes (Def. Cl4-Def. Cl2) except Def. Cl1 which was the midges that did not show any form of deformity, revealed that the chironomid midges sampled from the four stations of the two rivers were affected by the two effluents because their level of morphological deformity incidences exceeded 8% (figure 1). The two stations of R. Mbogo had the highest deformity incidence compared to stations R and C of R. Nzoia and there was significant difference between stations and total deformity giving a clear indication of different levels of pollutants being exhibited in the effluents hence explaining the fact that effluents can cause adverse effects on aquatic organisms. The results of this study are, therefore, in agreement with Diggings and Stewart (1993) who reported elevated frequencies of abnormalities among several species of Chironomidae including Proclaims, Polypedilum, Cryptochironomus, and Dicroendipes under the influence of industrial effluents.

Of interest, however, was the same level (i.e. 30% each) of total deformity incidence that was witnessed in midges sampled from both the two stations of R. Nzoia and exposed to paper and pulp effluent. It was anticipated that midges sampled upstream could have had higher incidence of deformity compared to those taken downstream because of the nature of their habitat. Or alternatively chironomids sampled downstream where the river received effluents would have had higher incidence of deformity given that their habitat was already contaminated. This was not to be and according to Jeyasingham and Ling (1997) increased exposure to contaminants does not always result in a higher incidence of deformity. In fact a combination of toxicants may bring about both synergistic and antagonistic effects on an organism and in this case midges sampled downstream may have experienced antagonistic effects. Warwick (1985a) also, reported that both the incidence of deformities and the severity of response in *Chironomus* species were inversely related to exposure to the insecticide DDE.

However, it has been argued that chironomids from polluted sites are usually characterized by greater deformity incidence (be either fluctuating asymmetry index values or general mouth part abnormalities) than those from unpolluted sites, not only for metals or organic pollution but also for complex mixtures of various types of pollution (Vermeulen, 1995; Janssens de Bisthoven, 1995; Groenendijk, 1998)

Def. Cl4 was was highest in C*hironomus* species sampled from station D (7±2%) of R. Mbogo while the least was recorded at station C (1±1%) of R. Nzoia. This would be explained by the presence of contaminants of severe adverse effects in the sugar cane effluents unlike paper and pulp effluents. The sugarcane effluents are channeled in a long narrow trench along the sugar cane plantation to the receiving R. Mbogo and chances are that toxicants from other sources such as insecticide sprayed on the growing of sugar cane may find their way into the effluent trench. Therefore, such pollutants as organochlorine

insecticide Endosulfan are a major toxicant to many aquatic organisms including benthic macoinvertebrates such as chironomids (Dallas and Day, 2004). The high level of pollution displayed by the midges sampled from R. Mbogo could also be explained by the synergistic effects of the pollutants. Of interest, however, is the fact that at the time of this study, Webuye Paper Mill factory was not in operation justifying its low pollution level as compared to Chemelil Factory that was operational.

CONCLUSIONS

From the results of this study, deformity incidence in *Chironomus* species was similar among individuals sampled in R. Nzoia but it increased downstream in R. Mbogo. On the other hand, deformity in *Chironomus* species from R. Nzoia was not affected by increase in the effluent concentration. It however, increased from a concentration of 25% and then improved off at 50%. There was a significant difference observed between the deformity of *Chironomus* species exposed to the two effluents hence the null hypothesis was rejected.

RECOMMENDATIONS

Based on the findings of the study, it is recommended that chemical analysis of sugar cane and paper pulp effluents be studied in order to identify the responsible toxicants for the adverse effects in terms of deformities observed on the *Chironomus* species.

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