

A Study of the Morphological Structure of Chironomidae (Diptera) in a Tropical Urban Polluted Water System

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ABSTRACT: In this study, the Olezoa stream in Yaounde was assessed to determine the morphological structure of Chironomid genera that are present in the aquatic ecosystem. The biological analysis revealed a low diversity of larva and pupal exuviae. The tribe of Chironomini revealed 03 genera which are: *Chironomus*, *Goeldichironomus* and *Nilothauma*. The chironomidae developmental stages exhibit a variety of adaptations in polluted areas which could be: morphological, physiological and behavioral all of them occurring simultaneously within each developmental form and the different genera. Gelatinous egg masses which were found floating on the surface of water or attached to the aquatic vegetation of Obili Lake have an elongated- ribbon like shape. Each mass possesses an average of 50 ± 7 egg rows arranged linearly; with each egg row containing an average of 16.8 ± 1 eggs. Each egg has an oval shape with a characteristic brown colour. It measures between 4.32-4.96mm in length with an average length of 4.73 ± 0.25 mm in the samples assessed.

Keywords: Chironomid, Olezoa Stream, Larva Instars, Pupal Exuviae

I. INTRODUCTION

Dipterans also known as true flies are the most abundant insect group that are found in an aquatic milieu and also the most diversified aquatic macroinvertebrates collected in many fresh water habitats. Dipterans commonly known as true flies are holometabolous insects which are undergoing complete metamorphoses with four distinct developmental forms which are: egg, larva, pupa and adult (Pinder, 1995). They can be predators, parasite or filterers, with the parasitic dipterans causing and transmitting certain diseases to their host such as humans in the course of a blood meal (Bouchard, 2009). Dipterans are the most diversified and abundant macro- invertebrates collected in many fresh water habitats ranging from marshes, pools, ponds, lakes, streams and rivers with either a slow or fast moving water current in an aquatic milieu. They are highly represented by their larvae and pupae whose sizes vary from small to large (Williams & Feltmate, 1992). Egg-laying behavior is diverse in the aquatic members of this holometabolous order. Some lay eggs just below the surface of plants or on mineral substrates; others deposit eggs in gelatinous masses below, or above the water surface on

emergent objects (Williams & Feltmate, 1992). Their larvae and pupae can be found excavating or digging mud, colonizing rocks or attached to aquatic vegetation and substrates. Their adults are mostly terrestrial and are characterized by highly specialized mouth pads for sucking fluids which can either be nectar or blood, a pair of anterior wings, while the posterior wings are reduced to pegs or halteres (Elouard, 1980).

These organisms are identified and classified based on the morphology of their larvae. These larvae are maggot like or worm like, and their body lengths varies from 2-60mm long as described by Bouchard (2009). Some possess an obvious head capsule which is either reduced or obscured. Their larvae generally lack segmented legs and wing pads on the thorax. Their common diagnostic character includes the number and location of prolegs, the shape of the terminal processes and the head condition (e.g., well defined and reduced head capsule). Based on these descriptive characteristics by (Williams & Feltmate, 1992) we will distinguish two groups or sub orders of Diptera which are; the nematocera, having a well distinguished or differentiated head capsule. For

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instance we have: Culicidae (mosquito), Chironomida (non-biting midges) among others and Brachycera with a reduced or retracted/withdrawn head capsule into the thorax. Some examples are: the Muscidae (house fly), Dolichopodidae (long-legged flies) among others.

Chironomids like other Dipterans are holometabolous. They are the most abundant aquatic dipterans inhabiting all types of fresh water. They spend most of their lives in the larval stage (Wiederholm, 1983 and 1986). They are commonly known as non-biting midges whose wide distribution in combination with the widely varying species – specific ranges of tolerance make them a highly valuable tool for surveying and monitoring polluted habitats (Armitage *et al.*, 1995)

Their life cycle comprises of four distinct stages, whereby, the immature stages of most of the species develop in an aquatic habitat or ecosystem. Their adults lay eggs on water surfaces in the form of gelatinous egg masses that slowly sink when dropped onto the surface of water bodies with their gelatinous bodies taking up water. Egg masses can be found attached or fixed to objects at the time of ovi-position (Langton, 1995). Thienemann (1954) stated that, the time taken for each egg to develop till the point of hatching lies between 2.5–6 days, this is strongly influenced by temperature. These eggs hatch into a miniature adult known as the first larva instars, which undergoes three successive moults to give the fourth larva instars. Most morphological and taxonomical observations are made on the final instars, with most of the structures appearing to be present in the earlier instars (Olafsson, 1992). These fourth larva instars, attaches themselves with a silken secretion to the surrounding substrate and pupation occurs giving rise to the pupa. Once the developing adult is mature, the pupa frees itself from the silken chamber and swims to the surface of the water where the adult can emerge from the pupa skin or exuviae (Armitage *et al.*, 1995). This exuviae floats on water surface and tends to accumulate in areas rocks, riparian

vegetations or fallen trees downstream that are in contact with the water surface.

I. MATERIAL AND METHODS

Sampling period and Site description

This research work was realised over a period of seven months beginning from the month of November 2013 to May 2014 with biostatistical assessment in 2016. It all started with the prospection of the site during the month of November which permitted for the selection of the different sampling points based on a number of factors. A total of three points were chosen along the stream denoted as upstream (Ol₁), lake (Ol₂) and downstream (Ol₃) as seen on figure 1 below. From December 2013 to May 2014 the sampling proper for both the physicochemical and biological parameters were carried out once each month. The sampling point, upstream (Ol₁) has as geographical coordinate's latitude 3°51'33" N and longitude 11°30'8" E with an altitude of about 747m, situated about 500m after the source precisely below the school of post and telecommunications. The station is characterized by the presence of farmlands, homes, vegetations of which we can cite *Panicum*, *Pennisetum purpureum* (commonly known as elephant grass), *Sida rhombifolia*, *Echinochloa pyramidalis*. Water crosses latrines, poultry farms, piggery and garbage. The second point is the Lake (Ol₂) situated some 1km from the source with geographical coordinates 3°51'23" N and 11°29'43" E and has an altitude of about 731m. This lake measures about 60m large, 100m long and about 1.5m deep, it is used for fish culture such as, *Oreochromis*, *Hemichromis*, *Heterotis* and *Clarias* around the lake is a huge garbage pit, farmlands, homes and a pisciculture centre. This lake suffers a great deal of eutrophication. Downstream (Ol₃) has an altitude of about 720m, with geographical coordinates of 3°51'52" N and 11°29'45" E. The station is situated about some 3km from the source below the lake around the bridge at ENSPT. We observe the presence of suck-away pipes from latrines that empty themselves directly into the stream, and scattered distribution patterns of houses and hostels.

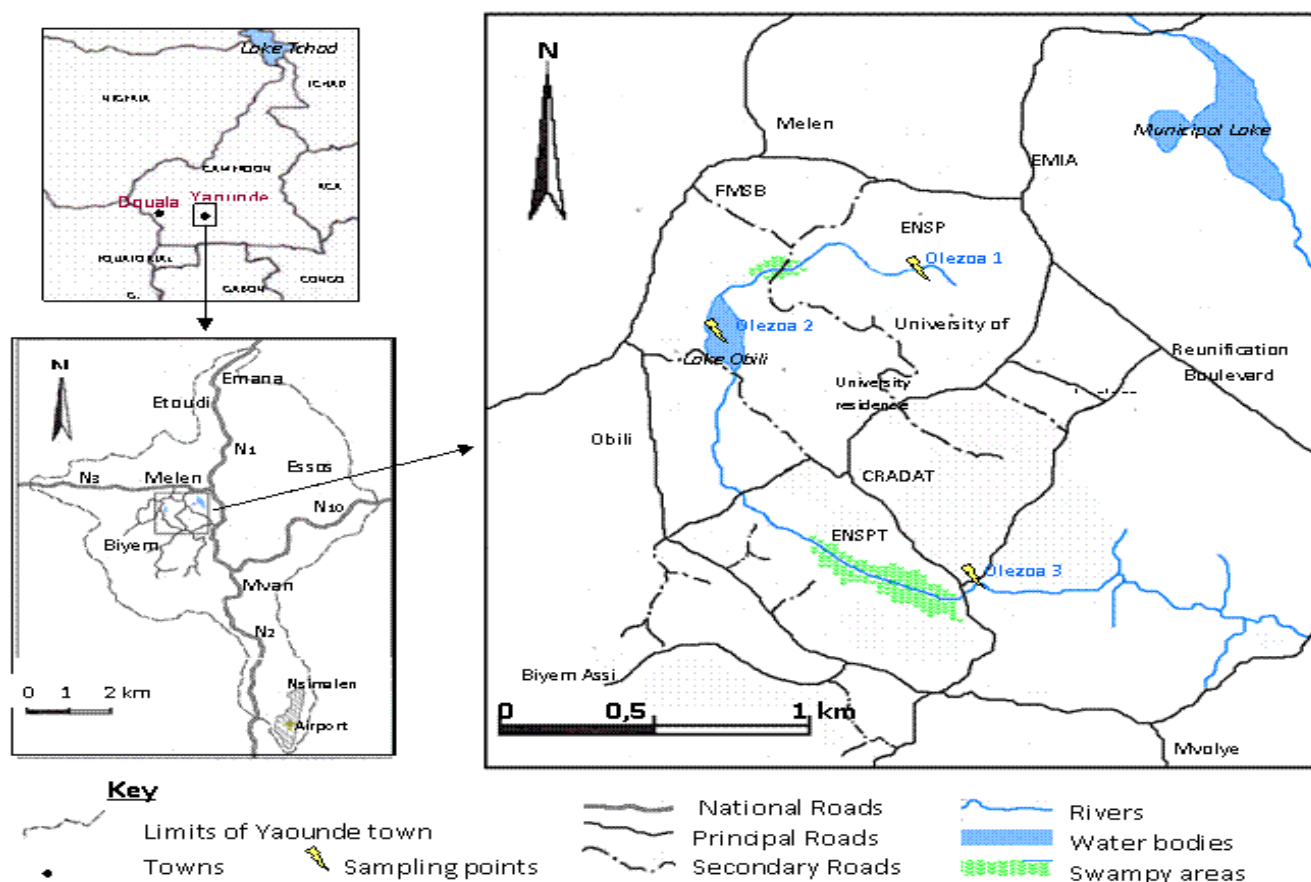


Figure 1: Map of Yaounde showing the different sampling points (Source: Plan guide of Yaounde, INC, 2014).

Measuring of physico-chemical parameters: this was achieved following the recommendation of APHA, 1998; Rodie *et al.* (2009). Hence, on the field, parameters like temperature ($^{\circ}\text{C}$), pH (CU), electrical conductivity ($\mu\text{S}/\text{cm}$), turbidity (FTU) where measured using appropriate instruments. Oxygen was fixed with a Winkler reagent. Back in the laboratory, other parameters such as colour (Pt.Co), alkalinity (mg/L), oxydability (mg/L), nitrate (mg/L), orthophosphates (mg/L), oxygen (mg/L), Carbondioxide (mg/L) where measured.

II.1 Collection of larvae and pupalexuvia

On the field with the help of a hand net with very fine mesh about $300\mu\text{m}$ the aquatic vegetation of the stream was swept across upstream and downstream. At lake, the hand net was submerged until it touched the benthic zone, a mixture of the specimens and mud was collected. This was latter washed using a $250\mu\text{m}$ mesh sieve. The specimens were then picked out and conserved in 100ml polythene bottle containing 10% formalin before their transportation to the laboratory (Soumiet *al.*, 2012). In the laboratory the specimens brought from the field were washed in a $250\mu\text{m}$ mesh sieve with tap water and conserved in 70 % alcohol. The measurement of larvae instars

and pupalexuvia was based on their lengths from their anterior to their posterior extremes (Olafsson, 1992).

III: RESULTS AND DISCUSSION

III.1. Spatio-temporal evaluation of physicochemical parameters:

Table 1 below shows the mean, minimum and maximum values for physico-chemical parameters during the study period. We notice that, water temperature of the stream varies between 20.5°C and 26.8°C . The highest temperature value of 26.8°C was recorded at Ol_1 in the month of May, while the lowest value of 20.5°C was recorded at Ol_2 still in the month of May. Turbidity values fluctuate between 119 and 461 FTU. The lowest value of 119 FTU was recorded at Ol_1 and Ol_2 in the month of February while the highest value of 461 FTU was recorded at Ol_3 in the month of January. The values of suspended solids ranges from 107 to 825 mg/L with the lowest value being recorded at Ol_2 in the month of March while the highest value of 825 mg/L was recorded at Ol_3 in the month of January. Values of colour vary between 266 and 2800 Pt.Co. The lowest value of 266 Pt.Co was recorded at Ol_3 in the month of December while the highest of 2800 Pt.Co was recorded at Ol_2 in the month of May. pH varied

slightly across the three sampling points ranging between 6.40 to 7.52 CU. The minimum value of 6.40 CU was recorded at Ol₁ in the month of March while a maximum value of 7.52 CU was recorded at Ol₃ in the month of May. Also, TDS values fluctuate between 108 and 266 mg/L. The least value was recorded at Ol₂ in the month of April and May, while the highest value was recorded at Ol₁ in the month of April. Dissolved oxygen values ranges between 0.6 and 6.3 mg/L with the lowest value of 0.6 mg/L recorded at Ol₃ in the month of February while the highest value of 6.3 mg/L was recorded still in the month of February at Ol₁. On the other hand, values of dissolved carbondioxide fluctuate between 0 and 35.2 mg/L. The lowest value was recorded at Ol₁ in the month of March while, the highest value of 35.2 was also recorded at Ol₁ in the month of April. The values of electrical conductivity fluctuate between

194 and 505 μ S/cm. The least value was recorded at Ol₃ in the month of January, while the highest value 505 μ S/cm was recorded at Ol₁ in the month of February. The values of alkalinity ranges between 48 and 196 mg/L. the minimum and maximum values were recorded at Ol₂ in the month of March and May respectively. Nitrates and orthophosphates ranges from 0.2 to 10.9 mg/L and 0.03 to 2.67 mg/L respectively. Their minimum values (0.2 mg/L of nitrates and 0.03 mg/L of orthophosphates) were both recorded at Ol₂ in the month of March while their maximum values (10.9 mg/L of nitrates and 2.67 mg/L of orthophosphates) were also recorded at Ol₃ in the month of February. Oxydability values range between 0.2 to 15.6 mg/L with a minimum value of 0.2 mg/L recorded at Ol₂ while it maximum value of 15.6 mg/L was recorded at Ol₃ in the month of March.

Table 1: mean, minimum and maximum values for physicochemical parameters during the study period.

Parameters	Minimum	Maximum	Mean	\pm Std. Deviation
Temperature ($^{\circ}$ C)	20.5	26.8	24.811	1.5412
Turbidity (FTU)	119	461	214.17	109.261
Suspendedsolid (mg/L)	107	825	247.39	172.397
TDS (mg/L)	108	266	169.89	51.430
Colour (pt.Co)	266	2800	917.17	643.507
pH (CU)	6.40	7.13	6.8083	0.24030
Electricalconductivity (μ S/cm)	194	505	312.76	95.447
Dissolvedoxygen (mg/L)	0.6	6.3	1.783	1.3192
Dissolvedcarbondioxid (mg/L)	0.00	35.20	9.5689	9.43704
Alkalinity (mg/L)	48	196	97.11	43.366
Phosphate (mg/L)	0.03	2.67	0.8353	0.83071
Nitrate (mg/L)	0.2	10.9	2.875	3.8882
Oxidability (mg/L)	0.20	15.60	8.9556	4.81129

III.2. morphological structure of the egg mass

Gelatinous egg masses (figure 2A) which were found floating on the surface of water or attached to the aquatic vegetation of the second station (lake) have an elongated- ribbon like shape. Each mass possesses an average of 50 ± 7 egg rows arranged linearly, with each egg row containing an average of 16.8 ± 1 eggs. Each egg has an oval or ovoid shape with a characteristic brown colour (figure 2, B₁ and B₂). It

measures between 4.32-4.96mm in length with an average length of 4.73 ± 0.25 mm. Mature eggs possesses two distinct regions; an exterior transparent region enclosed in a dark or intense pigmented region known as the chorion which gives rise to the larva. Each egg hatches in to an L₁ larva called larvulae (figure 2, C) which remains inside the gelatinous egg mass until their disintegration.

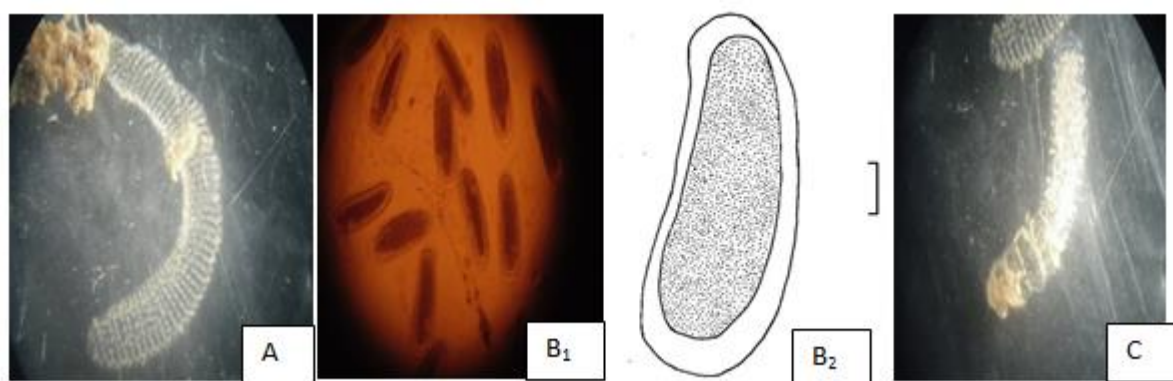


Figure 2: Morphology of: A = Egg mass, B1 and B 2 = Egg structure and C = Larvulae.

III.3. Development of the chironomid larva instars

Generally, the development of the larva comprises four embryonic forms which are known as instars. Morphologically Larva instars are similar in structure figure 3 A, but differ by their body length. The variation in body length of each larva instars per month and per sampling station is summarized on table 2. The morphological structure of the larva led to the identification of the chironomid up to the sub-family level. All larva forms have a characteristic deep red or brick red colour due to the presence of haemoglobin called erythrocrucorin (figure 3 B). Meanwhile, newly hatched first instars larvae are

transparent with a dark internal tube running from their thorax to their abdomen. Each larva is divided in to three main segments: head, thorax and abdomen, with each segment bearing a pair of taxonomical characteristics. The head bears a pair of non – retractile antennae, eyespots, mandibles and labial plates which can be used to separate individuals into the various sub-families (figure 3 C). Their thorax is sub – divided into three segments which are pro, meso and meta thoraxes which bears the anterior prolegs. Their abdomen counts 08 segments which end with an anal lobe that bears a pair of posterior prolegs, anal tubules and pre anal papillae.

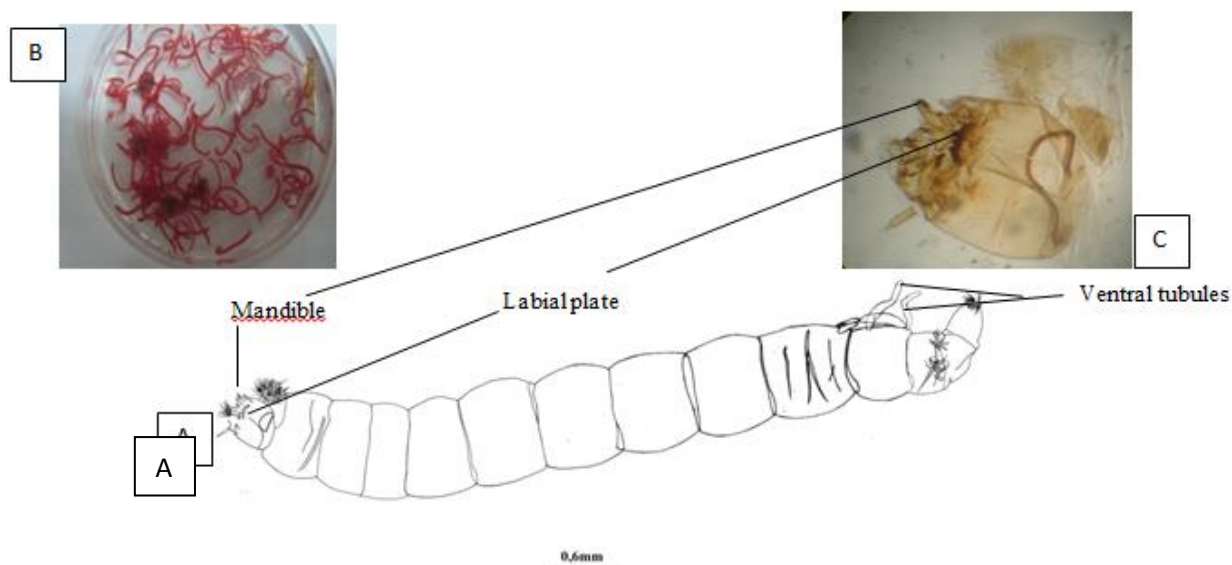


Figure 3: Larva morphology; A = individual larvae showing their characteristic red colour, B = larva structure, C = larva head showing their mandibles.

Table 2, Minimum, maximum and average of length variation of developmental stages.

	Number	L1			L2			L3			L4			Exuviae		
		min	Max	average	min	max	aver	min	max	average	min	max	average	min	max	average
Upstream	Dec	2.27	5.92	4.45±0.1	3.68	7.68	5.04±0.12	7.04	10.4	8.25±0.11	8.96	13.2	10.21±0.13	5.52	7.84	6.76±0.07
	Jan				4	6.24	4.99±0.18	5.28	8.8	7.21±0.13	7.36	11.52	9.22±0.13	4.8	7.2	6.13±0.08
	Feb				6.08	10.56	8.25±0.13	6.08	10.6	8.25±0.13	7.84	13.12	10.08±0.15	5.6	9.44	6.74±0.1
	Mar	2.24	3.03	2.64±0.4	3.52	7.2	5.40±0.09	5.54	8.8	6.84±0.12	8.84	12.48	10.62±0.16	5.63	11.52	8.64±0.15
	Apr	1.44	1.76	1.60±0.16	3.04	6.08	4.80±0.24	5.6	9.28	7.9±0.12	8.16	11.84	10.25±0.13	5.6	8.48	7.17±0.1
	May	2.72	2.88	2.80±0.8	4	6.24	5.23±0.35	5.92	8.8	7.30±0.15	8.32	12.32	9.93±0.17	6.4	8.32	7.59±0.06
Lake	Dec										9.12	14.08	11.16±1.11	4.96	10.4	7.54±0.24
	Jan				7.68	8.48	6.2±0.24	9.44	9.74	9.6±0.16	10.4	11.36	11.01±0.16	5.44	9.12	6.82±0.13
	Feb				4.96	6.08	5.55±0.16	7.04	9.44	8±0.31	8.8	14.56	11.77±0.67	5.44	9.76	7.67±0.16
	Mar				3.68	7.36	5.18±0.2	6.24	11.36	8.74±0.18	9.28	10.84	13.74±1.93	5.44	9.76	7.28±0.14
	Apr				4.64	6.56	5.72±0.24	6.4	8.32	7.28±0.16	7.04	13.6	9.04±0.3	5.28	9.28	6.69±0.16
	May	2.08	3.2	2.77±0.35	4	6.24	3.8±0.14	5.12	7.2	6.11±0.24	8.16	12.8	9.89±0.41	4.96	8	6.13±0.14
Downstream	Dec							8	9.76	8.85±0.28	8.8	11.2	9.6±0.35	5.44	7.92	6.77±0.26
	Jan				6.08	6.4	6.24±0.16	6.08	8.96	8.13±0.23	7.52	11.52	10.06±0.25	6.56	8.32	7.44±0.88
	Feb				4.64	6.88	5.12±0.25	5.6	7.68	6.92±0.27	5.92	8.48	7.34±0.32	6.72	8.8	7.89±0.28
	Mar				5.44	5.28	5.36±0.08	5.6	7.84	6.77±0.33	8.16	14.88	11.26±0.55		11.68	
	Apr															
	May							4.64	7.68	6.33±0.15	7.04	11.68	9.84±0.14	6.72		7.81±0.16

III.4. morphological description of the chironomid Pupal Exuviae

Pupalexuviae were collected on the water surface; their body is differentiated into three main segments: head, thorax and abdomen of which the thoracic region is most often fused with the head region to form the cephalothorax.

Morphological description of chironomid genera identified.

a) Genus: Chironomus

Their head region bears cephalic tubercles with a bulbous base and a tubular apex. The abdomen counts 09 tergites with the last modified into an anal

lobe and filaments used for swimming. Tergite I is bare, while II-VIII bears shagreen with either an anterior, posterior, median or lateral patches. Tergite II possesses median shagreen with a continuous hook row (1/2). Tergites III-V possess median shagreens with a slightly darker posterior, conjunctives III/IV and IV/V with short slender setae. Tergites VI bear a heavier anterior and posterior patch of shagreen meanwhile; Tergites VII and VIII bear light anterior and lateral patches of shagreens respectively. The base of their bulbous cephalic tubercles has a tubular apex. Their anal spurs are long dark, counting around 45 anal lobe filaments (figure 4).

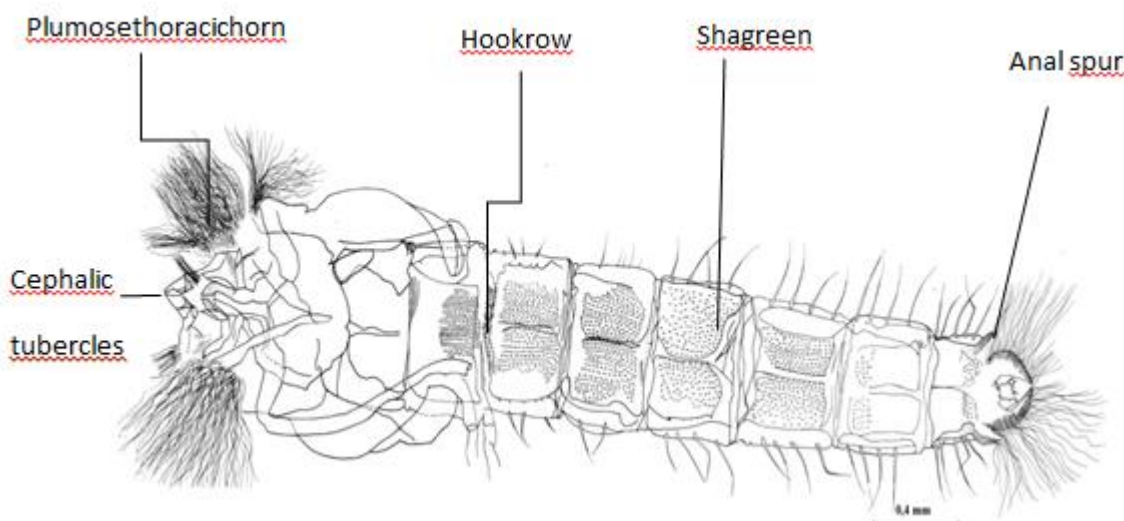


Figure 4: Morphological structure of Chironomus

b) Genus:Goeldichironomus

The head region bears cephalic tubercles which are small and pointed with frontal setae. Their thorax bears basal rings which are medially constricted. The upper portion of the thorax is granulose with 04 dorso-central setae which are spaced out between one another. The abdomen counts 09 tergites with the last being modified into an anal lobe. The first tergite is bare and the remaining 07 possess shagreen which

can be anterior, posterior or medial. Tergite II bears shagreen with continuous hook-row ($\frac{3}{4}$ widths). Tergites V-VII bears 4 slender L setae, while Tergite VIII bear 5 slender L- setae that occupy varying positions around the anal spur, with a gap between the first two and last three setae and a dark brown globular three point anal claw with a complete single row of anal fringe (figure 5).

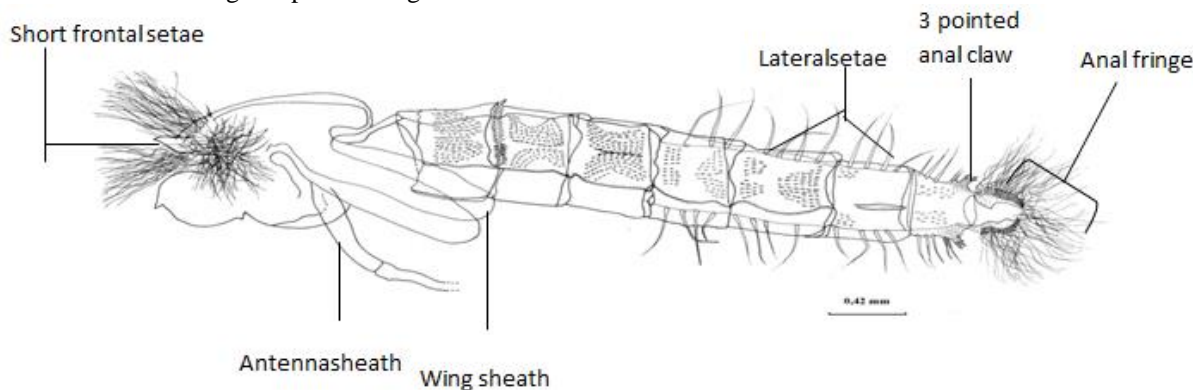


Figure 5: Morphology of Goeldichironomus

c) Genus:Nilothauma

The head region does not bear a cephalic tubercle but frontal setae are present. Their thorax is granulose with a median suture and a distinct banded antennal sheath. Their abdomen count 9 tergites with the first being bare: Tergites II-VIII possess shagreen which may be anterior, posterior or median. Tergite II bear continuous hook row occupying $\frac{1}{2}$ widths. Tergites

II-V with continuous dark shagreen while Tergites VI and VII possess each an hourglass shaped shagreen, segment V-VIII bears 4 slender L- setae. Tergite VIII has median circular shagreen with its posterior area having a characteristic dark brown pigmentation with a 5- pointed dark anal claw. Anal lobe possesses a complete anal fringe with about 32 taeniae as seen on figure 6.

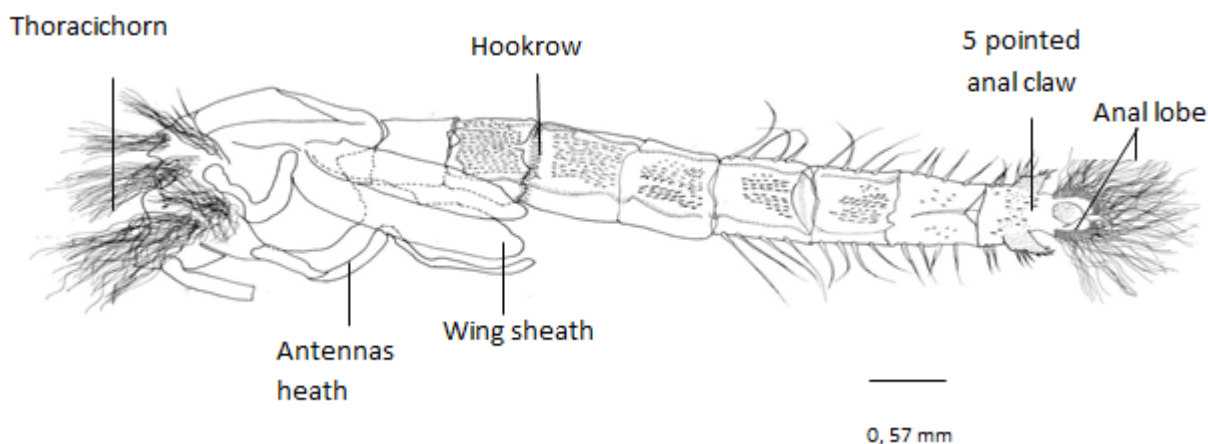


Figure 6: Morphology of Nilothauma

DISCUSSION

III.2.1. Physicochemical characteristics of Olezoa stream

The study show that, temperature varies slightly across the different sampling points along the stream

with mean temperature of $24.81 \pm 0.41^\circ\text{C}$. The temperature value of the stream is influenced by the tropical environmental temperature (Dajoz, 2006),

The mean pH value of 6.81 ± 0.1 CU was influenced by dissolve carbon dioxide (9.57 ± 3.84 mg/L) coming from the dissolution of atmospheric carbon dioxide gas and biological activities taking place in the stream which both favors the conversion of carbonates to bicarbonate ions that acidifies the milieu.

The relatively high values of electrical conductivity (313.72 ± 15.72 μ S/cm) and TDS (169.89 ± 84 mg/L) as seen in figure 7 and 8, attests for the high rate of mineralization of the stream. The test of Kruskal-Wallis and that of Mann-Whitney shows that, there is a significant difference in TDS across the three stations. This enriches the milieu with free ions that originates from household wastes and effluents coming from the population living along the banks of the stream. The addition of CaCO_3 (917.17 ± 218.29 mg/L) at the level of the lake influences the turbidity and the colour of the stream. As stipulated by Rodier (1996), the high conductivity values could be due to a high concentration in ionic and nutrient substances coursed by fast mineralization rate (Ajeegah *et al.*, 2013). Conductivity values between $200\text{--}333$ μ S/cm and $333\text{--}666$ μ S/cm indicates an average rate and a fast rate of mineralization respectively (Rodier *et al.*, 2009). The test of Kruskal-Wallis and that of Mann-Whitney shows that, there is a significant difference in conductivity O_1 and O_2 . The value of suspended solids (247.39 ± 56.13 mg/L) was relatively high along the stream due to the presence of house hold wastes that is emptied directly into the stream by the population. This value recorded is higher than that recorded by Ngoko in 2011, which was about 30.64mg/L. The average values of Nitrates (2.88 ± 2.06 mg/L) and orthophosphates (0.84 ± 0.34 mg/L) recorded confirms the high degree of mineralization of all organic wastes, fertilizers, animal wastes as well as domestic wastes deposited in the stream. It can also be due to the complete oxidation of Nitrogen, Ammonium and phosphorus compounds into Nitrates and phosphates respectively. The mean value of oxydability (8.96 ± 1.60 mg/L) is higher than the normal value of 1mg/L stipulated by Rodier (2009) for surface water. As a result, the Olezoa stream is very rich in degradable organic matter that is coming from household wastes and effluents from the latrines of the population living nearby the stream.

The physico-chemical analysis of the water quality in the stream shows that the water is lowly oxygenated, highly mineralized, slightly acidic and rich in organic matter. Hence, the stream is highly polluted and not good for human consumption without treatment. This is according to the findings of Ajeegah *et al.* (2013).

III.2.2. Biological characteristics

The morphological analysis present a low biodiversity of the chironomidae genera and developmental forms as presented in figures 3-6. The tribe of Chironomini identified revealed 03genera which are: Chironomus, Goeldichironomus and the Nilothauma genera. With a general structure as described by (Langton, 1991).

The biodynamic of their different developmental stages attest for the monthly effects on their development and also the conditions which govern within each station, favouring the installation of each developmental form as presented in figures 3-6. Due to the fact that the chironomidae developmental stages exhibit a variety of adaptations in polluted areas which could be: morphological, physiological and behavioral, occurring simultaneously within each species (Arslan *et al.*, 2010). Physiological adaptations such as: the presence of haemoglobin (erythrocrurin) enables them to exist even in complete absence of dissolve oxygen gas for a period of 30 to 120 days (Arslan *et al.*, 2010), their ability to combine the mechanisms of dormancy and anaerobic metabolism during extreme anoxic conditions has greatly favoured their development in a polluted milieu such as the urban stream (Brodersen *et al.*, 2004). Morphologically, their pupae possess highly specialized respiratory organs or thoracic horns with an apical respiratory surface or plastron plate which is in direct contact with the aerial environment at the water surface which favours respiration. Their posterior segment is modified to an anal lobe which ends with fringes that facilitates undulatory movements, propelling respiratory currents through the tube to ensure an effective respiratory movement (Langton, 1995).

CONCLUSION

We noted the presence of well adapted and most resistant chironomid genera such as chironomus, Goeldichironomus and Nilothauma. These identified genera are indicatives of certain environmental pollution in the stream studied which could serve as an empirical tool for their assessment. Their distribution in the stream was influence by some physico-chemical parameters. Chironomid mouth part deformities serve as a useful tool for assessing aquatic pollution. With goeldichironomus been the most abundant and most resistance. Hence, we suggest that a multimetric index of biotic integrity focused on the chironomidae Family might be useful to evaluate the integrity of streams. Future studies can be made on the reproduction and post – embryonic development of chironomidaespecies on a small scale in the laboratory in order to obtain information on their life cycle and duration of each

developmental form. Carry out studies to precise on the bioecology, systematics and morphodynamics of chironomids in tropical aquatic ecosystem and determine the trophic status of chironomids in tropical aquatic ecosystems

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