Study of seasonal distribution of chironominae (Chironomidae: Diptera) in Al- Battar river north Al Kut city / Iraq.

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التوزيع الموسمي لأنواع البرغش غير الواخز لعويلة Chironominae (Diptera: Chironomidae) في نهرالبتار شمال مدينة الكوت

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المستخلص

درس تاثير العوامل الفيزيائية والكيميائية على وفرة وتنوع عويلة البرغش غير الواخز Chironominae في نهر البتار شمال مدينة الكوت ولمدة ثمانية أشهر للفترة من ايلول 2012 الى نيسان 2013 وتم تشخيص ثمانية انواع وهي *Chironomus plumosus* الذي كان اكثر الانواع وفرة وبنسبة 42.5% ويليه *Chironomus sp* 17.8% , *Einfeldia sp* 13.6% , *Chironomus riparius* 10.3%, *Chironomus staegeri* 10.3% , *Lauterborniella sp.* 2.6% , *Parachironomus sp* 1.4% , *P.hirtalatus* 1.1%.

اظهرت نتائج التحليل الاحصائي PCAوجود ارتباطات بين انواع البرغش غير الواخز والعوامل البيئية ,فالأنواع *C. plumosus, C.staegeri, C.riparius and Einfeldia sp.* ارتبطت ارتباطا ايجابيا معنويا عند مستوى معنوية (p<0.01 , 0.05) مع عمق الماء وتركيز NH4-N ومعنويا سلبيا مع العكورة اظهرت هذه الانواع ارتباطا"سلبيا" غير معنويا"مع درجة الحرارة وتركيز NO3-N. ان النوع *Chironomus plumosus*(اكثر الانواع وفرة)ارتبط ارتباطا"ايجابيا"غيرمعنويا"مع الملوحة ,الاملاح الذائبة الكلية ,التوصيلية الكهربائية و الاس الهيدروجيني وارتباطا" سلبيا" غير معنويا" مع الاوكسجين المذاب .

النوع *Parachironomus sp* اظهر ارتباطا" ايجابيا" معنويا"مع الملوحة ,الاملاح الذائبة الكلية, التوصيلية الكهربائية و الاس الهيدروجيني وقد سجل هذا النوع خلال شهر ايلول فقط. النوع *P.hirtalatus* اظهر ارتباطا" سلبيا" معنويا"مع عمق الماء وتركيزNO3-N.

النوع *Lauterborniella sp* ارتبط ايجابيا معنويا مع الاوكسجين المذاب وارتبط هذا النوع ارتباطا" ايجابيا" غير معنويا"مع الحرارة,الملوحة,الاملاح الذائبة الكلية ,التوصيلية الكهربائية وNH4-N,NO3-N وعلاقة سلبية غير معنوية مع الاس الهيدروجيني pH ,عمق الماء والعكورة .النوع *Chironomus sp* اظهر علاقات ايجابية وسلبية لكن غير معنوية مع العوامل البيئية فارتبط ايجابيا مع الحرارة ,عمق الماء ,الملوحة,الاملاح الذائبة الكلية ,التوصيلية الكهربائية وارتبط سلبيا مع العكورة , الاوكسجين المذاب , NH4-N, NO3-N و الاس الهيدروجيني .

Abstract

The influence of physical and chemical parameters on the abundance and diversity of subfamily Chironominae(Chironomidae: Diptera) in Al-Battar river north Al-kut city was studied over a period of eight months(Septemper 2012- April 2013) . Eight species of Chironominae were identified *Chironomus plumosus* (Linnaeus) was the most dominant made up 42.5% ,*Chironomus sp.* 17.8% ,*Einfeldia sp*. 13.6%, *Chironomus riparius* Meigen 10.3%,*Chironomus staegeri* Lundbeck 10.3%, *Lauterborniella sp*. 2.6% ,*Parachironomus sp.* 1.4% and *Parachironomus hirtalatus*(Beck & Beck) 1.1%.

The results of this study showed that were significant correlations between the species and several environmental parameters.*C. plumosus, C.staegeri, C.riparius and Einfeldia sp*. this group showed significantly positive correlation ($p<0.01$ and 0.05) with water depth and NH4-N and showed significantly negative correlation($p<0.01$ and 0.05) with water turbidity. Also this group showed negative correlation but not significant with water temperature and NO3-N. *C.plumosus* showed positive correlation but non significantly with (pH, EC,TDS and NaCL) and showed no significant negative correlation with DO. One species *Parachironomus* sp. showed significant positive correlation with pH, EC, TDS and NaCL and this species recorded only in September.

Parachironomus hirtalatus showed significant negative correlation with water depth and NO3-N. *Lauterborniella sp.* showed significant positive correlation with dissolved oxygen. Also showed positive correlation but non significantly with temperature, NO3-N, NH4-N, EC, TDS and NaCL and negative correlation but non significantly with pH, turbidity and water depth .*Chironomus sp.* showed positive and negative correlation but non significantly with ecological factors, so showed positive correlation with EC, TDS, NaCL, water depth and temperature. This species showed negative correlation with pH, DO, turbidity, NO3-N and NH4-N.

Introduction

Chironomidae are a relatively primitive (phylogenetically speaking) group of flies (Diptera) in the suborder Nematocera. Commonly called non biting midges, or “blind mosquitoes”, as adults and “bloodworms” as larvae, Chironomids are closely related to mosquitoes (Culicidae) and biting midges (Ceratopogonidae). Unlike their nasty relatives, female Chironomids do not bite (1).The common name of non-biting midges derives from the weak development of the mandibles in the adult, in contrast to their close relatives, the biting midges or sand flies (Ceratopogonidae) in which the female mouthparts are often designed for taking ablood meal (2). Chironomidae constitute the most diverse group of aquatic insects: their larvae are aquatic, but the adults terrestrial (some species have terrestrial larvae) (3). The Chironomidae are usually the most abundant macroinvertebrate group, in numbers of species and individuals, encountered in the majority of freshwater aquatic habitats. In addition, Chironomids have invaded the sea, being found along coastlines worldwide and occurring at least 30 m down in the ocean, and the land (1).The variability of environmental conditions under which Chironomids are found is more extensive than for any other group of aquatic insects (4). Because of their diversity and their specific sensitivity to environmental changes, Chironomids are widely used in ecological investigations (5), therefore, they are also used in monitoring water quality of lakes and rivers (6). The classification of lakes as oligotrophy, mesotrophyand eutrophy can be performed on the basis of Chironomid communities (8).

At present Chironomid larvae are used in environmental tests owing to their wide range of sensitivity to different environmental factors such as temperature, pH, depth, food, freezing, salinity, oxygen, pollutants and etc. The external morphology of the larvae (mandibles, antenna, submentum, and epipharynx) is very sensitive to these parameters (9). When Chironomids continuously exposed to stress or pollution, late instars of some Chironomid larvae frequently develop deformities in the mouthparts, especially the mentum (10).Morphological abnormalities in the heads capsule of Chironomid larvae collected in polluted environments suggest a relationship between these deformities and pollution (11). The deformities are reported to be more frequent in more-polluted aquatic ecosystems, and some studies have used their frequency as an indicator of severe pollution (11; 12).

Chironomids are one of the most widely distributed insect groups in the world. Insects in the family Chironomidae are holometabolous, passing through four distinct stages are egg, larval, pupa, and adult stage (13). Their larvae stage is the most critical, the most responsive to environmental stress, and the most metabolically active stage of their life (8). Chironomid larvae are directly exposed to pollutants, and their tissues can accumulate various pollutants, which later are transferred to fish and ducks and then to humans (8).Chironomid adults are considered nuisances when large emergences occur in close proximity to human habitations (1).

The aims of this study

1- Identification of Chironominae species in Al-Battar river north Al-Kut City/Iraq.

2- Correlation of some ecological factors with abundance of Chironominae species.

3 - Effects of ammonium-nitrogen (NH_4-N) and nitrate-nitrogen (NO_3-N) on the abundance of Chironominae species.

2. Materials and Methods

2.1. Study area Al-Battar River

The river is located north of Al-Kut City, near Al-Thahab Al-Abiadh Village, originates from Tigris River, the river has (10)km length, maximum 2.5 m depth and it has 10-15m width. The river is divided into two branches Al-Battar-right and Al-Battar-left and subdivided into several outlets for irrigating of many agricultural areas (figure 1).In this study, Al-Battar river is divided into three permanent stations, the first station at the beginning of the river ,the second station at the right branch and the third station at the left branch. This river characterized by high amount of plants and algae.

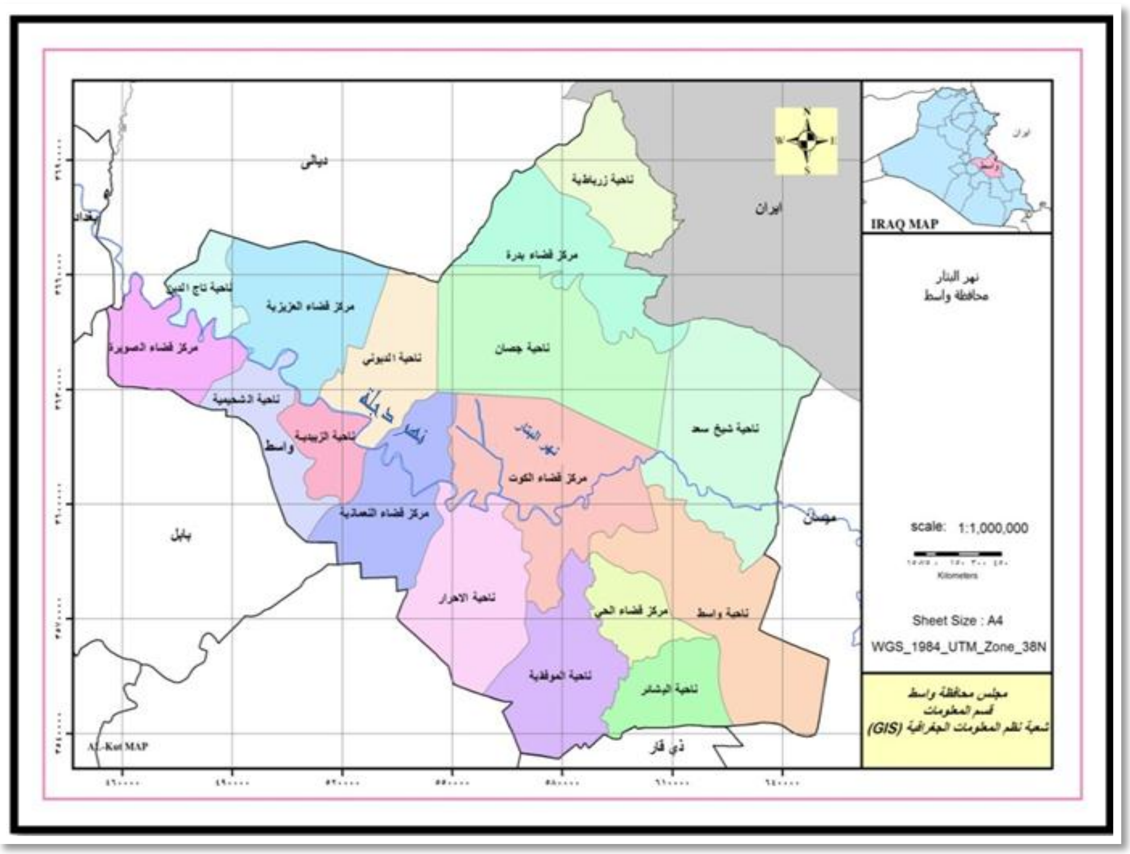


Figure (1): Location of Al-Battar river north Al-Kut City /Iraq

2.2. Chironomid larval sampling

Quantitative samples of Chironomid larvae were randomly collected every month from three stations in Al-Battar river. A Standard Metallic Dipper D shaped (15 cm in width and 3 cm in height) equipped with a 1.2m long handle was used for collecting the larval samples (14).Ten samples were collected in each station (total 30 samples). All benthic materials collected during each sampling were transferred to labeled plastic bags (13×18cm) and transported to the laboratory on ice. In the laboratory, each sample was washed with tap water through a sieve (300μm pore).The residue on the sieve was transferred into a white plastic pan and sufficient tap water was added to the pan to examine midge larvae. The larvae were collected with a dropper and transferred to small vials. These larvae were preserved in 80% ethanol for subsequent taxonomic identification and counting.

2.3. Ecological parameters of water

During sampling, measurements of physico-chemical parameters were made in situ at three randomly selected locations at each sampling site, such as water depth was measured by using standard metallic ruler, water temperature was measured with simple mercury thermometer, dissolved oxygen (DO) was measured with dissolved oxygen meter (Martini instruments) model MI 605,electrical conductivity (EC)**,** water pH, salinity (NaCL) and total dissolved solids (TDS) were measure by using multi parameter (Martini instrument) model Mi 180. To analyze selected chemical parameters, three separate samples from each location were randomly collected in labeled 500ml plastic bottles.All water samples were transported to the laboratory in an ice chest and kept at 4°C until analyzed for water turbidity, whereas water turbidity was measured with turbidity meter model (Hach 2100). All turbidity measurements were accomplished in Wasit Ecology Office.

2.4. Sediment samples

At each station , three sediment samples were randomly collected by using standard metallic dipper and these samples dried by using nylon(25-30°C) in the laboratory and then applied to sieving out with 2mm. Ammonium –N and Nitrate-N were estimated, based on Kjeldahl method (Extraction and Steam distillation) (15).

2.5. Identification of Chironominae larvae

Permanent slide mounts of Chironomid larvae were prepared for the purpose of identification of Chironomid larvae (3-4th instar). The preserved larvae were transferred to a petri dish containing 10% KOH solution and left in the solution for 24-48 h to digest the larval muscle.Thereafter, the permanent slide mounts of the larvae were prepared following the method of (1). The slide mounted larvae were identified to genus or species using appropriate taxonomic keys (1; 2).The identification of larvae were confirmed in the Collage of Science, Basrah University/Iraq.

2.6. Estimation of Ammonium–N and Nitrate-N

2.6.1. Extraction

Soil samples (40) g was mixed with 200 ml of 2M KCl in a conical flask (250 ml) and shake for 1h in shake electric device spins at 200-300 cycle / minute. The suspension was filtered by using filter papers No.42 (15).

2.6.2. Steam distillation

Ammonium –N:- 50 ml of extract was transferred into distillation flask. Ten ml of fresh boric acid solution was placed in the receiving flask and insert it under the condenser. 0.5 g of MgO was added to the extract. Pass steam and collect about 50 ml of distillate. Remove the receiving flask and retain for titration. Disconnect the steam supply. Place another receiving flask under the condenser for the analysis of nitrate –N (15).

Nitrate-N:- 0.5 g of Devarda' s alloy were added to the extract in the distillation flask. Immediately reconnect the steam line and distil a further 50 ml of distillate (15).

Calculation

The content of nitrogen, (NH4 –N %) was calculated using the formula:-

$$NH4\text{-} N (\%) = \frac{(V1 - V0) \times N \times 14}{Wt}$$

Where:
N = Normality of HCl (0.01 M)
V1 = Volume of HCl acid used in the titration of the sample (ml).
V0 = Volume of HCl acid used in the titration of the blank (ml).
Wt = sample weight (g), Atomic weight of Nitrogen =14

Concentration of NO3-N is calculated in the same manner but must enter a value of Devarda' s Alloy in the equation.

2.7. Statistical analysis

The one-way ANOVA ($p<0.05$) was used to test the differences in means of Chironomid larval abundances among various sampling occasions. The nonparametric Kendall's tau-b correlation was used to assess the influence of physicochemical variables on abundance of Chironomidae by using the SPSS (Statistical Package for Social Science), Version 17.0. Principal Component Analysis (PCA) program investigate the distribution of Chironominae species.

3. Results

3.1. Chironomid taxa abundance and distribution

Eight species belonging to the subfamily Chironominae were collected at all sampling occasions .Among the tribe Chironomini, *Chironomus plumosus* was the most dominant made up 42.5% ,*Chironomus sp* 17.8% , *Einfeldia sp* 13.6%, *Chironomus riparius* 10.3%, *Chironomus staegeri* 10.3%, *Lauterborniella sp*. 2.6% ,*Parachironomus sp.* 1.4% and *P.hirtalatus* 1.1% were identified in Al-Battar river. Figure (2) showed the abundance of *C.plumosus* was obvious. It was found in the samples of all months expected in October, the highest density was reported in January (7.3 larvae/dip) and the low density was reported in April (0.33 larvae/dip).*Chironomus sp.*was found in the samples of the months (Octoper, January, February and April),the high density was reported in January (1.96 larvae/dip) and the low density (0.53 larvae/dip) in April. *Einfeldia sp* was found in January and February with (2.7 and 1.7 larvae/dip) respectively. *C.riparius* was recorded in December, January and February with (0.5, 1.4 and 1.43 larvae/dip) respectively.

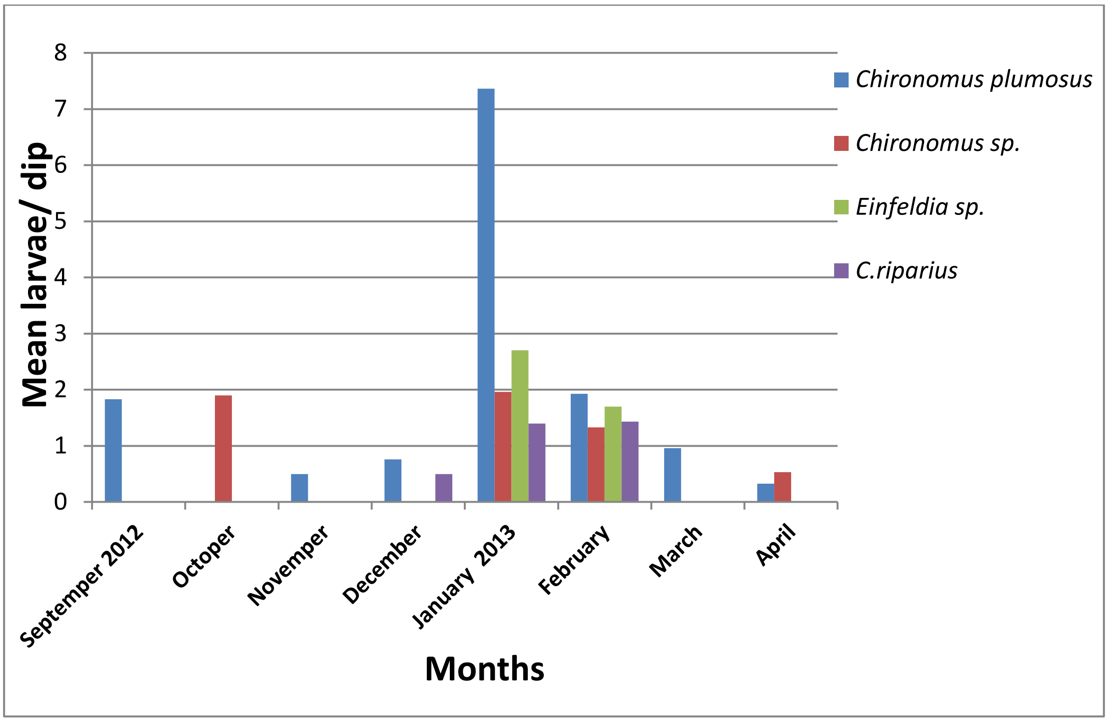


Figure (2): Larval density of Chironomid species sampled monthly (September 2012 April2013) in Al-Battar river.

Fig.(3) showed that *C.staegri* was recorded in December, January and February with (0.4, 1.96 and 0.93 larvae/dip) respectively. *Lauterborniella sp*. was recorded in November with (0.86 larvae/dip). *Parachironomus sp*. appears in September with (0.46 larvae/dip). *P.hirtalatus* was recorded in October with (0.36 larvae/dip).

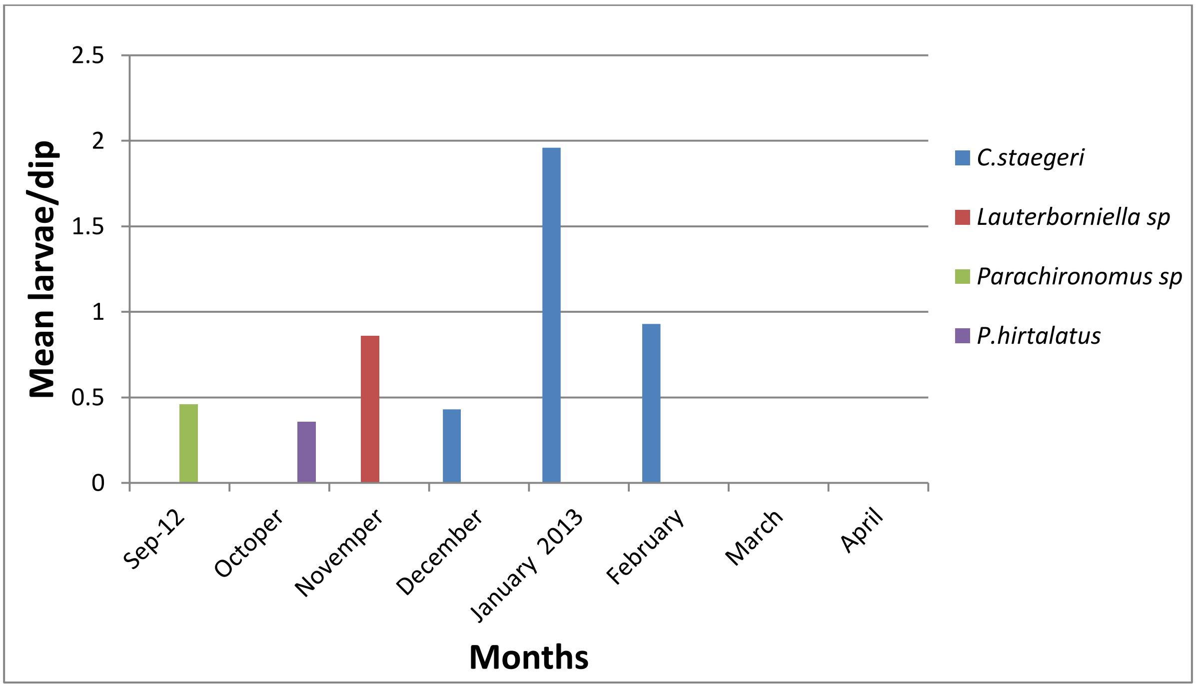


Figure (3): Larval density of Chironomid species sampled monthly (September 2012- April 2013) in Al- Battar river.

3.2. Physical and chemical parameters of water and sediment

Table (1) showed the correlation coefficients (nonparametricKendall's tau-b correlation test at ($P < 0.05$) between the physicochemical parameters and Chironomid taxa abundance. *Chironomus plumosus* ($P<0.01$), *C.staegeri*, *C.riparius* and *Einfeldia sp.* ($P<0.05$) showed statistically significant positive correlation with water depth. *Parachironomus hirtalatus* ($P < 0.01$) showed statistically significant negative correlation with water depth. *C.plumosus* ,*C.staegeri* and *C.riparius* ($P < 0.05$) showed significant positive correlation with NH4-Nconcentration in sediment, while *P.hirtalatus* had correlation with NO3-N in sediment. *Lauterborniella sp.* showed statistically significant positive correlation with dissolved oxygen concentration in water. *C.staegeri* ($P < 0.01$) and *C.riparius* ($P < 0.05$) showed statistically significant negative correlation with turbidity. *Parachironomus sp*. had significant positive correlation with pH, electrical conductivity, total dissolved solids and salinity (NaCl) respectively. *Chironomus sp*. showed statistically correlated with environmental parameters but not significantly values.

Table (1): The effect s of water and sediment parameters on Chironomid species abundance during the study period (September 2012 to April 2013) in Al-Battar river. **P*<0.05 ; ***P*<0.01

Parameter	*C. plumosus*	*Parachironomus sp.*	*Chironomus sp.*	*Lauterborniella sp.*	*C.staegeri*	*C.riparius*	*P.hirtalatus*	Einfeldia sp.
PH	0.015	0.352*	-0.176	-0.022	0.102	0.042	0.037	-0.125
Electrical Conductivity µs/cm	0.115	0.428*	0.231	0.015	0.009	-0.005	-0.045	0.256
Total dissolved solids (ppm)	0.100	0.443*	0.181	0.089	-0.009	-0.060	-0.060	0.235
Salinity (NaCl) %	0.086	0.441*	0.136	0.158	-0.056	-0.108	-0.045	0.171
Water depth (cm)	0.397**	-0.271	0.038	-0.082	0.335*	0.348*	-0.485**	0.394*
Dissolved oxygen (ppm)	-0.015	-0.022	-0.075	0.386*	0.014	0.000	0.171	-0.087
Turbidity (NTU)	-0.239	0.157	-0.113	-0.133	-0.415**	-0.373*	0.237	-0.320
Temperature ºC	-0.171	-0.209	0.095	0.168	-0.249	-0.273	0.080	-0.064
NO3-N	-0.023	0.207	-0.090	0.190	-0.109	-0.066	-0.350*	-0.017
NH4-N	0.363*	-0.102	-0.088	0.062	0.378*	0.391*	-0.210	0.273

4. Discussion

Many studies have demonstrated that the physical and chemical parameters of the water influence Chironomid composition and abundance (16). Chironomid larvae comprise a prominent part of the benthic macrofauna because of their high species richness and adaptability to different environmental conditions (7). Distributions of midge taxa have been related to salinity (17), in this study. *Parachironomus sp* showed positive correlation with salinity, as showed in figure (4).

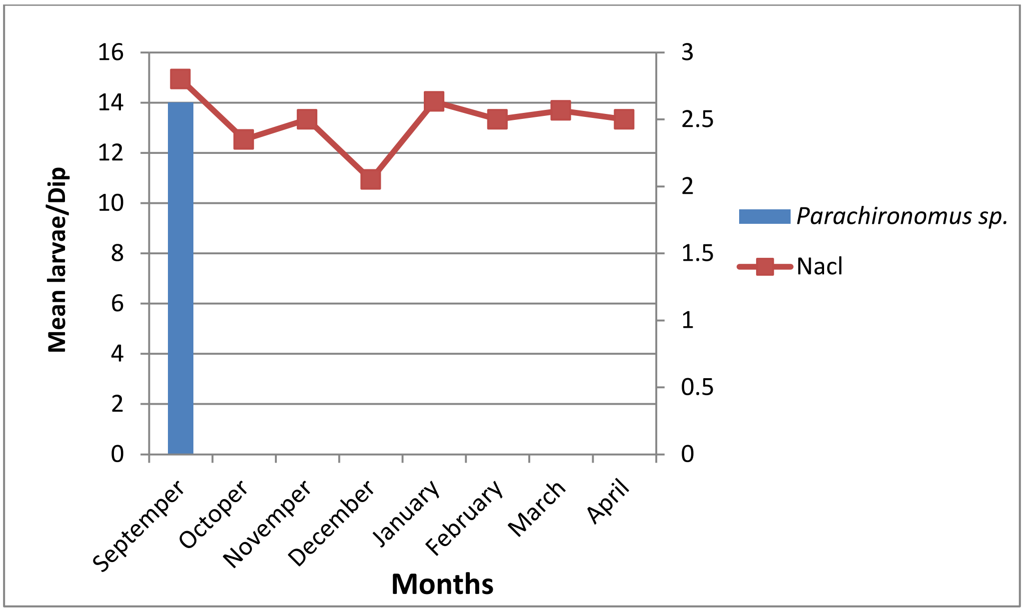


Fig (4): The effect of Salinity (NaCL) on *Pararchironomus sp.*

A survey in the Yukon Territory similar to the one conducted here determined that midge distributions were also correlated with pH (18), *Parachironomus sp* showed positive correlation with pH, as showed in figure (5). pH directly affects the physiology of aquatic organisms by influencing ionic balance and enzyme function (19).In this study . *Chironomus plumosus, C.staegeri, C.riparius and P.hirtalatus* showed positive correlation but not significantly with pH while *Lauterborniella sp, Chironomus sp* and *Einfeldia sp.* showed negative correlation but not significantly with pH because Chironomid taxa that are tolerant of low pH level stend to be large-bodied, able to maintain internal pH-balance(20) and possess hemoglobin which provides greater buffering capacity (21).

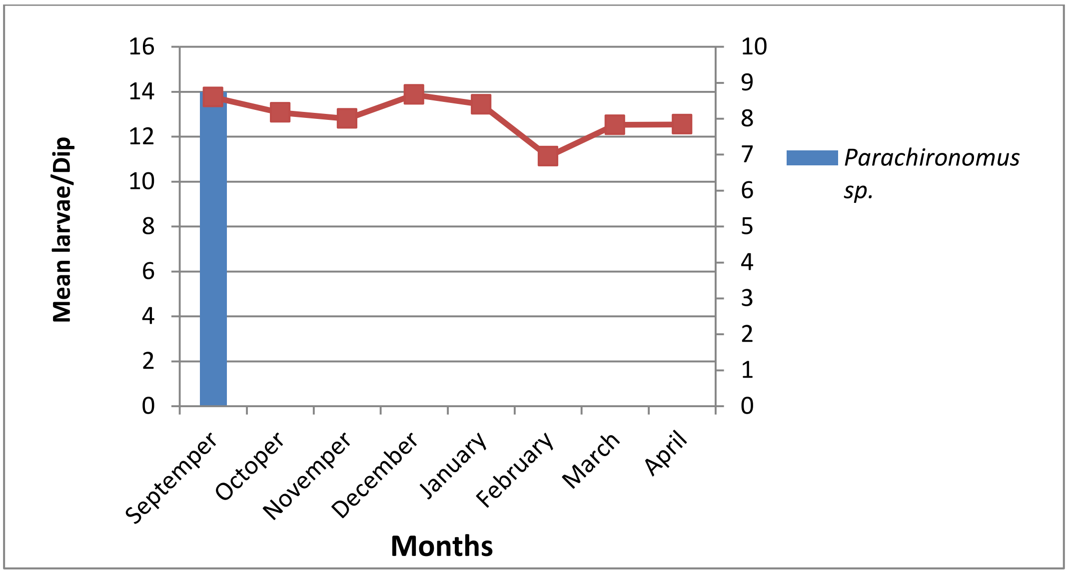


Fig (5): The effect of pH on *Parachironomus sp.*

Addition of fertilizers to aquatic ecosystems increase nitrogen and phosphorous levels that can affect Chironomid community composition (22).The positive correlation of *Chironomu splumosus*, *C.staegeri* and *C.riparius* figure (6) with NH4-N agree with (18) found that total Kjeldahl nitrogen was a significant factor in midge distributions in the Yukon Territory and agree with (10) found that species of Chironominae such as *Chironomus kiiensis*, *C.javanus* and *P. trigonus*, showed a positive relationship with most of the organic pollutants such as TOM,ammonium-N and nitrate-N.

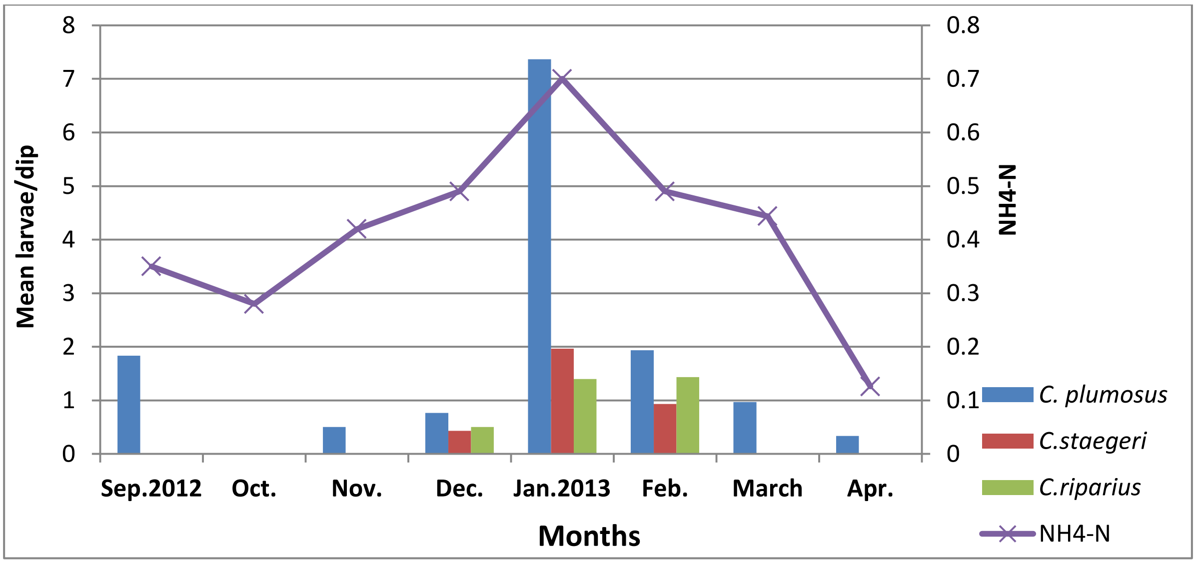


Fig (6): The effect of NH4-N on *Chironomus plumosus*, *Chironomus riparius* and *Chironomus staegeri*.

While *Parachironomu shirtalatus* showed significant negative correlation with NO3-N as showed in figure (7).

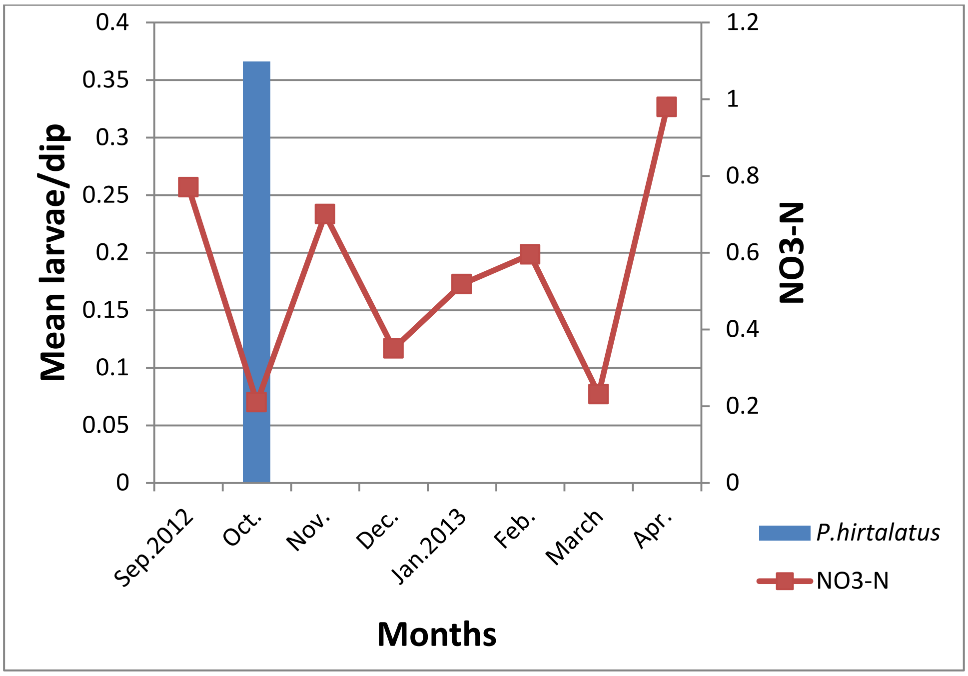


Fig (7): The effect of NO3-N on *Parachironomus hirtalatus*.

The positive correlation of *Chironomusbplumosus*, *C.staegeri*, *C.riparius* and *Einfeldia sp.* figures (8,9,10) with water depth agree with (10) found that *Chironomus* species preferred deeper and wider rivers as their abundances correlated positivelywith river depth ($P < 0.01$).

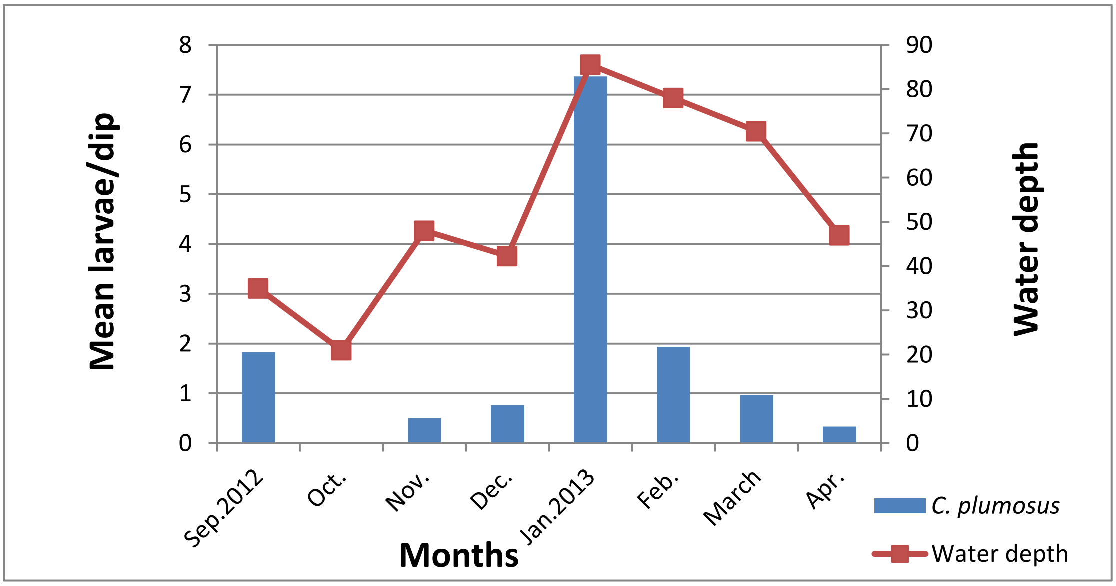


Fig (8): The effect of water depth on *Chironomus plumosus*.

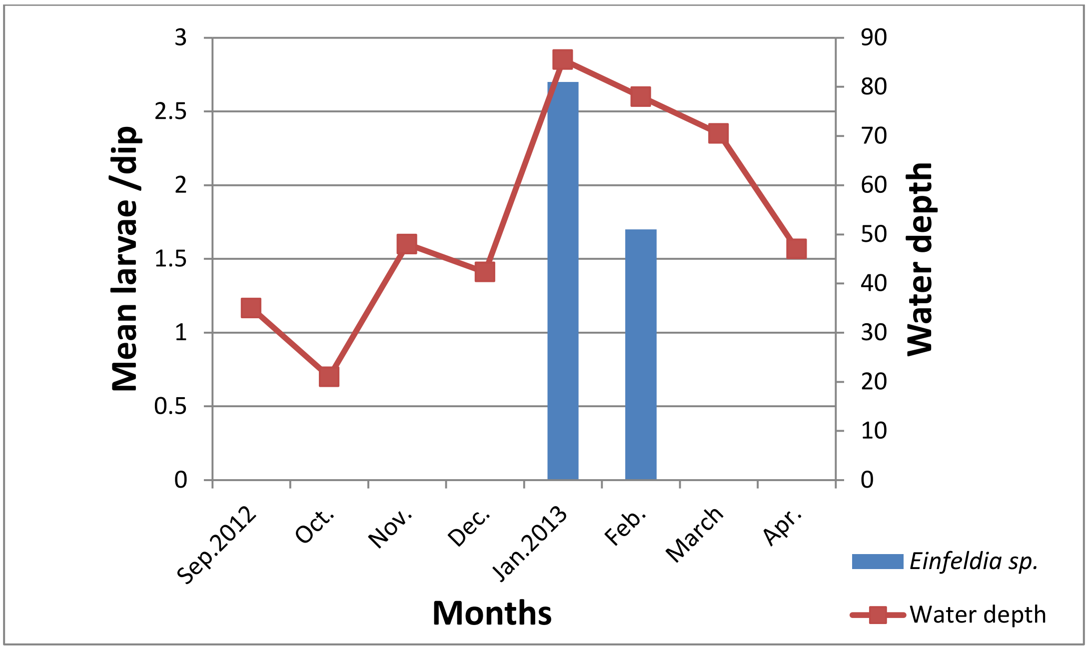


Fig (9): The effect of water depth on *Einfeldia sp*.

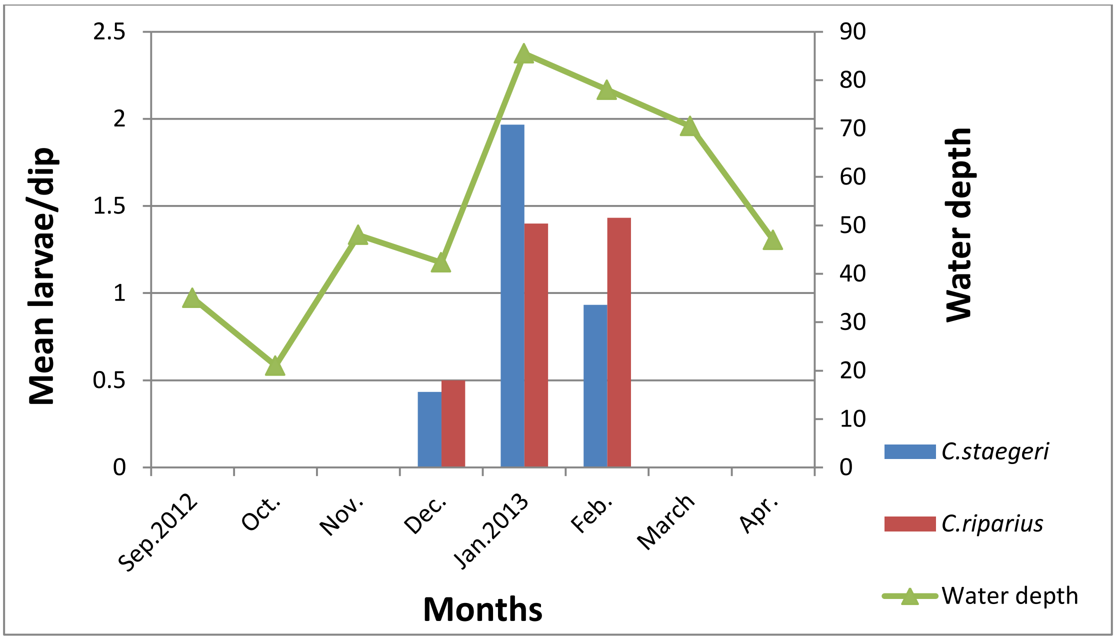


Fig (10): The effect of water depth on *C.staegeri* and *C.riparius*

(10) reported that Chironomids species are considered as tolerant species because they have the ability to survive in extreme environmental conditions with low amounts of dissolved oxygen and high concentrationsof pollutants, in this study *Chironomus plumosus*, *Parachironomus sp. Chironomus sp.* and *Einfeldia sp.* had negative correlation with D.O concentration while *Lauterborniella sp* had significant positive correlation with D.O. as showed in figure (11), DO concentrations is probably that level of decomposer activity versus photosynthetic activity was also acontributing factor in the seasonal changes observed in DO concentrations.

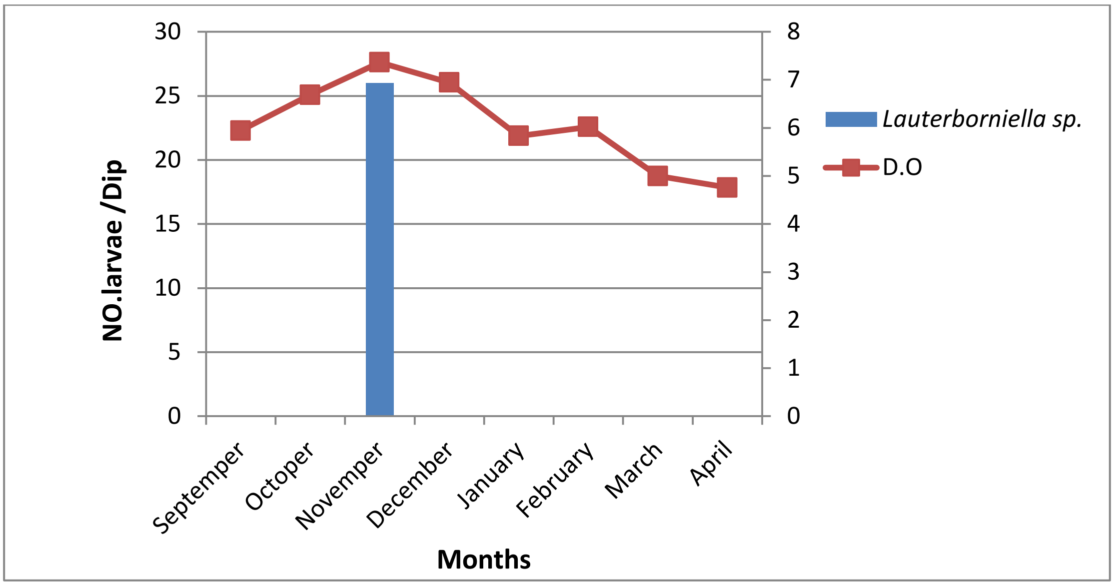


Fig (11): The effect of D.O on *Lauterborniella sp.*

Parachironomus sp. had significant positive correlation with electrical conductivity and total dissolved solids as showing in figure (12,13).High electrical conductivity levels indicating high concentration of dissolved solids ,suggest the presence of significant phytoplankton biomass at the sediment (23).

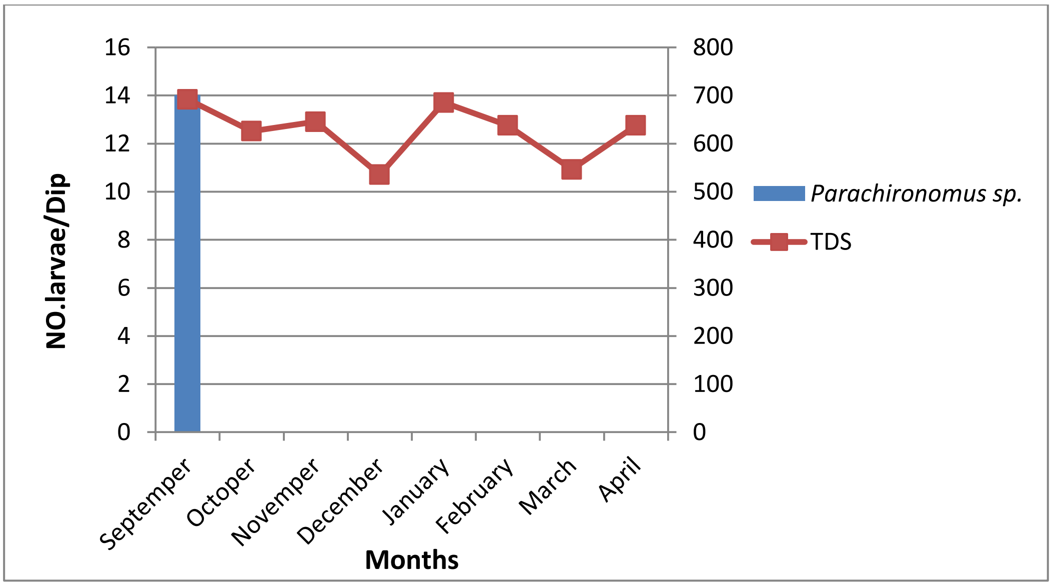


Fig (12): The effect of TDS on *Parachironomus sp.*

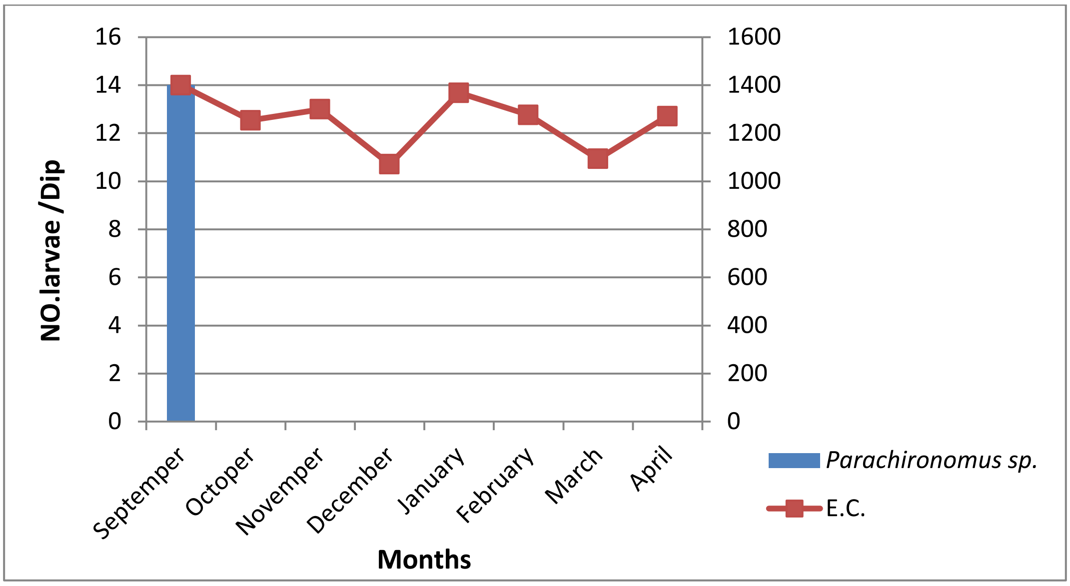


Fig (13): The effect of EC on *Parachironomussp*

Turbidity showed significant negative correlation with two species *C.staegeri* (P< 0.01) and *C.riparius* (P< 0.05) as showed in fig. (14). similarly with (24) turbidity in rivers is caused by erosion of materials from the contributing watershed. It may be created from a wide variety of eroded materials, including clay, silt, or mineral particles from soils, or from natural organic matter created by the decay of vegetation.

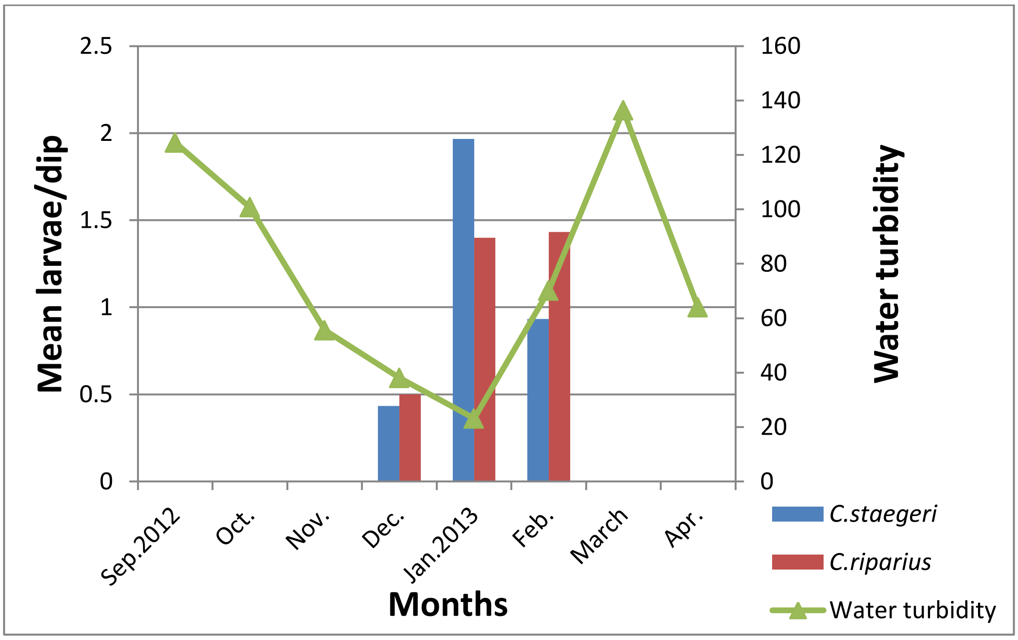


Fig (14): The effect of turbidity on *C.staegeri* and *C.riparius*.

In this study, temperature did not show any significant correlation with abundance of Chironomid larvae, and *Chironomus sp*. did not show any significant correlation positive or negative with any parameters during the period of this study.

5. Response of individual species

The results of the statistical analysis (Principal Component Analysis PCA) showed that the highest density of a species occur at optimum values of the environmental factors. The eigen value being an estimate of the optimal values. The species were ordinated in a similar way with increasing value for accumulated factors in the component with some variation in species order.

In this study found nine parameters such as (pH, DO, EC, TDS, salinity (NaCL) , water depth, Turbidity, NO3-N and NH4-N) had significantly positive and negative correlation. Also showed the relationship between species in this study. As showed from ordination diagrams Fig.(15), Chironomid species in Al- Battar river were divided in to five groups:

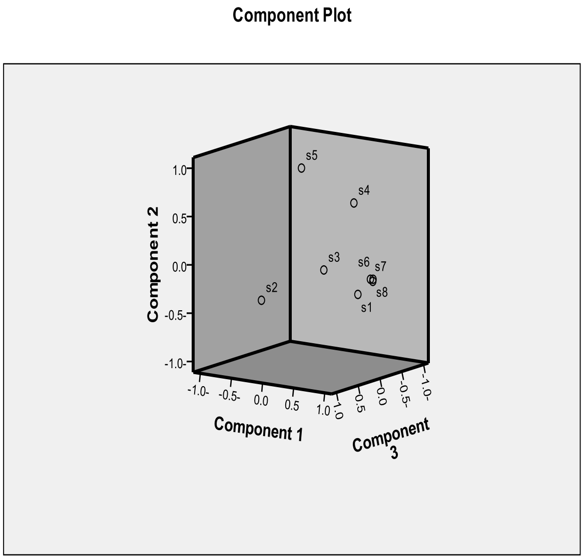
1-**First group**: included four species (*C. plumosus, C.staegeri, C.riparius and Einfeldia sp*) this group showed significantly positive correlation ($p<0.01$ and 0.05) with water depth and NH4-N and showed significantly negative correlation ($p<0.01$ and 0.05) with turbidity. Also this group showed negative correlation but not significant with temperature and NO3-N.

2-Second group: included one species (*Lauterborniella sp.*) this group showed significant positive correlation with dissolved oxygen. Also showed positive correlation but non significantly with temperature, NO3-N, NH4-N, EC, TDS and NaCL and negative correlation but non significantly with pH, Turbidity and water depth.

3-Third group: included (*Chironomus sp.*) this group showed positive and negative correlation but non significantly with any ecological factors, so showed positive correlation with EC, TDS, NaCl, water depth and temperature and showed negative correlation with pH, DO, Turbidity, NO3-N and NH4-N.

4-Fourth group: included one species (*Parachironomus hirtalatus*) showed significant negative correlation with water depth and NO3-N.

5-Fifth group*:* included one species *Parachironomus* sp. showed significant positive correlation with pH, EC, TDS and NaCL.



Component Matrix[a,b]

	Component		
	1	2	3
s1	.834	-.253-	.112
s2	-.261-	-.331-	.762
s3	-.307-	-.238-	-.736-
s4	.708	.667	.024
s5	-.098-	.955	.077
s6	.968	-.127-	-.040-
s7	.913	-.114-	-.063-
s8	.975	-.104-	-.035-

Extraction Method: Principal Component Analysis.

Fig (15): Principal Component Analysis (PCA) in dependent of distribution to eight species of Chironominae .

S1=*Chironomus plumosus* ,S2=*Parachironomus sp*, S3=*Lauterborniella sp.*, S4=*Chironomus sp.* S5=*Parachironomus hirtalatus* S6=*Chironomus staegeri*, S7=*Chironomus riparius*, S8=*Einfeldia sp.*

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