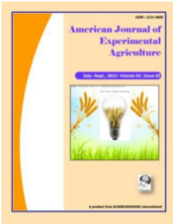

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# Diversity and Community Structure of Benthic Insects in Fish Farm Ponds in Southern Côte d'Ivoire, West Africa

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***Authors' contributions***

*This work was carried out in collaboration with all authors. Author MLY designed the study, wrote the protocol and the first draft of the manuscript. Author BCA managed the analyses of the study. Author PK managed the literature researches. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** The aims of this study were to determine diversity and community structure of benthic insects in fish farm ponds.
**Study Design:** Monthly samplings have been conducted from December 2007 to November 2008 in five fish farms (Layo, Banco, Azaguié, Anyama I and Anyama II) in southern Côte d'Ivoire. In each farm, sediments were collected in three ponds using a van Veen grab. In each pond, environmental variables such as transparency, temperature, pH, dissolved oxygen and conductivity were measured *in situ*.
**Methodology:** Samples were taken in six replicates which were pooled to constitute a sample for each pond.

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**Results:** A total of 31 taxa belonging to 18 families and 7 orders were recorded. Benthic Insects fauna is clearly dominated by Chironomid Diptera. *Nilodorum fractilobus*, *Chironomus imicola*, S*tictochironomus* sp., *Tanypus fuscus* and *Ceratopogon* sp. were the dominant taxa. Anyama II station recorded the maximum values of Shannon-Wiener diversity index and evenness. Insect community structure was visualized using Canonical Correspondence Analysis to show the affinities of each species for selected environmental parameters.
**Conclusion:** The species reported for the first time contributed to the list of insects' species from Ivorian aquatic ecosystems.

*Keywords: Fish farm ponds; benthic insects; diversity; community structure.*

## 1. INTRODUCTION

Insects play an important role in aquatic ecosystems functioning [1]. They are an important component of invertebrate assemblages in aquatic ecosystem. With their abundance and diversity, insects dominate fresh water ecosystem [2]. Many studies on aquatic insects in Côte d'Ivoire were undertaken in running water and were also carried out in the northern part of the country. Indeed, in the southern part, there is very sparse literature on aquatic insects [3-9]. Few studies have been carried out on the ecological and systematic aspects of aquatic entomofauna. Aquatic invertebrates are extremely diverse and important to fishpond ecosystem as a major food resource for many predatory organisms [10]. In fact, fishpond ecosystems are important hotspots for macrofauna biodiversity [11-14]. However, very little attention has been given to natural or artificial ponds ecosystems in Côte d'Ivoire, while there is lack of studies on diversity and community structure of aquatic insects in artificial ponds. The aim of the present study is to determine the benthic insect abundance, diversity, evenness, and degree of similarity and their relationship to environmental variables in fishponds of southern Côte d'Ivoire.

## 2. MATERIALS AND METHODS

### 2.1 Study Site

This study was undertaken in five fish farms in the southern region of Côte d'Ivoire characterized by two seasons (dry and rainy seasons). The dry season extends from December to March and from August to September while the rainy season extends from April to July and from October to November. Sampling sites were assigned to habitat types according to environmental and ecological features: Aquaculture Testing Station of Layo (05º19'N; 04º18'W), fish farms of Banco (05º23'N; 04º03'W), Azaguié (05º39'N; 04º05'W), Anyama I (05º33'N; 04º03'W) and Anyama II (05º34'N; 04º02'W) (Fig. 1). They were assigned to habitat types according to environmental and ecological features. Banco site is located in the National Reserve of Banco which is mainly constituted of primary forests. In Azaguié, Anyama I and Anyama II, ecosystems are constituted by agricultural landscape, while at Layo site, immediate environment is characterized by habitations. The main water supplies were different in the sites: ponds in Anyama I and Azaguié were fed respectively by a man-made lake and a stream, ponds in Banco by Banco River, ponds in Anyama II by groundwater and ponds in Aquaculture Testing Station of Layo by coastal aquifer. Ponds located in this last site were fed by brackish water (salinity ranging from 0 to 10mg.$L^{-1}$ [15] while in the four others sites, ponds were supplied with fresh water. The ponds in the five fish farms were permanent and were shallow (depth<1 m). In each fish farm, three ponds which area varied from 280$m^2$ to 500$m^2$ were randomly selected. Bottom sediment were mostly composed by sand in Layo and Azaguié stations, by silt in Banco, sand and clay in Anyama I while in Anyama II station, silt and sand were the dominant substrates. All ponds contained tilapia *Oreochromis niloticus* Linnaeus, 1758 except Banco where ponds were abandoned. Fish were reared at a density of 3-5 fish/$m^2$ (Layo), 6 fish/$m^2$ (Azaguié and Anyama II) and 7 fish/$m^2$ (Anyama I) and fed on rice bran.

### 2.2 Sampling Procedures

In each pond, monthly samples were collected in six replicates using a van Veen grab of 0.09 $m^2$ internal area, from December 2007 to November 2008. The six samples were pooled, sieved through 1 mm aperture size sieve and the remaining materials were preserved in plastic bottles containing 10% formalin. The total area sampled per station is 1.62 $m^2$ (3 ponds x 6 replicates x 0.09 $m^2$). In the laboratory, specimens were sorted and identified under a stereo binocular microscope to the lowest possible taxonomic level (species, genus, family), by use of systematic and classification keys [3,16,17,18]. Insects were counted and the number of each species was expressed as organisms.$m^{-2}$. Biomass (dry weight; mg.$m^{-2}$) was estimated after desiccation to constant weight for 24 h at 60 °C according to [19]. Mean densities (individuals.$m^{-2}$) and biomass were calculated for each season and for overall total study period.

### 2.3 Measurement of Environmental Variables

On each sampling date, environmental variables such as transparency, temperature, pH, dissolved oxygen and conductivity were measured *in situ* between 08.00 and 10.00 a.m. Water temperature, pH and electric conductivity were measured using a multiparameter digital meter (WTW pH/Cond 340i). Dissolved oxygen concentration was measured with a WTW Oxi 92 oxygen meter and water transparency was measured using a 20-cm-diameter Secchi disk. Water samples were collected on every sampling day, filtered through GF/C Whatman® filters, frozen upon arrival at laboratory. Analyses of dissolved inorganic nutrients (ammonium, $NH_4^+$; nitrite, $NO_2^-$ and soluble reactive phosphorus, $PO_4^{3-}$) from filtered samples were carried out according to [20]. The substrate rate was visually estimated in percentage of sand, clay and silt according to [21].

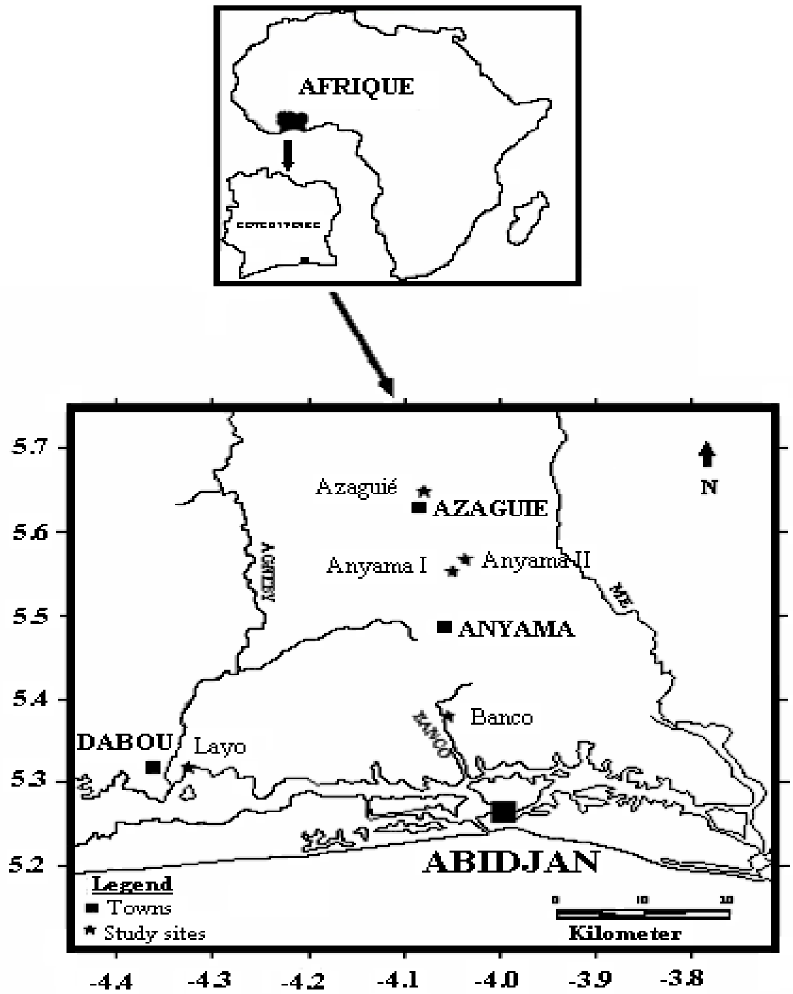


**Fig. 1. Location of the study area showing the different sampling stations**

## 2.4 Statistical Analysis

In each sampling station, abundance, density, Shannon-Wiener diversity index (bits) [22], and Pielou Evenness [23] were calculated. Shannon-Wiener diversity index was used to quantify taxonomic richness and distribution of taxa in the communities. Evenness was used to determine aquatic insect distribution, regardless of species richness. Coefficient of similarity among stations was estimated following [24]. Sorensen index was used to assess the similarity of insect communities between different stations. Analysis of variance (ANOVA) was used to determine effects of stations and seasons on environmental variables, Shannon-Wiener diversity, evenness, density and biomass. Before performing the comparison test, the normality of data was checked by Kolmogorov-Smirnov test. Data were log10 (X+1) transformed prior to analysis.

Comparison of data collected at different stations was made using one-way ANOVA and Tukey's *post hoc* test. Dominance (Do) was described on the basis of relative abundance. Relationships between the distribution of aquatic insects and environmental variables in all sampling stations were determined by Canonical Correspondence Analysis (CCA) using CANOCO 4.5 software. The importance of CCA was tested by the Monte Carlo test at *P-value*=0.024 (F- ratio=2.93) for 499 permutations. At least, 0.2% obtained Taxa of the total abundance in benthic environment were included in the analysis. These taxa were considered as principal taxa (16 taxa). Eight environmental parameters were returned for the analysis.

# 3. RESULTS AND DISCUSSION

## 3.1 Physical and Chemical Variables

The variations of environmental parameters are given in Fig. 2. Temperature oscillated between 27.2ºC (Banco) and 28.9ºC (Azaguié). The highest values of temperature were recorded during the dry season in March at the stations of Layo, Banco, Azaguié and Anyama I and in August at station of Anyama II. The highest values of dissolved oxygen were recorded in March at the stations of Layo, Banco, and Anyama I, in July at Azaguié station, in September and October (Anyama II). The mean values of pH fluctuated between 6.75±0.19 (Banco) and 7.08±0.12 (Anyama I). The highest values of pH were recorded during the dry season in September at stations of Layo, Banco and Anyama II and during the rainy season in October (Azaguié), and in November and in July at station of Anyama I. The highest value of electrical conductivity (3037.83±2980.25 µS/cm) was recorded at the station of Layo due to its brackish water source. In the others stations, conductivity varied from 35.85±2.88µS/cm (Banco) to 71.22±14.10µS/cm (Anyama I). Water transparency fluctuated between 21.65±6.84cm (Layo) and 30.14±4.25cm (Banco). The highest values of nitrite were recorded during the dry season in March at stations of Layo, Banco, Azaguié and Anyama II, and during the rainy season in November at the station of Anyama I. The average values of ammonium oscillated between 0.12±0.16mg/l (Anyama II) and (0.31±0.36mg/l (Banco). The highest values of water ammonium were recorded during the dry season in January at the station of Banco and in September at station of Layo then during the rainy season in November at Azaguié, Anyama I and Anyama II stations. Phosphorus content varied from 3.16±7.94 mg/l (Anyama I) to 5.56±8.35mg/l (Layo). The highest values were obtained in November (Layo), October (Banco), June (Azaguié) and December (Anyama I). In ponds, nutrients are released by mineralization of rice bran. The highest values of nutrients registered in Layo station were due to the high and regular distribution of fish feed, in contrast to the other stations where fish received less feed. Indeed, feed Inputs were often expensive. This fact caused breaks in the distribution of the fish feed which was not given in optimal amount. The stations of Layo and Banco had old-age ponds that accumulated sediments. Cleaning of these ponds was no longer made. So this accumulation of sediments could increase the nutrient content in these ponds, particularly for Banco station.

## 3.2 Taxonomic Richness

The list and dominance of the benthic insect species are given in Table 1. A total of 31 taxa belonging to 18 families and 7 orders were recorded. It's one of the first report on aquatic insects in fishponds in Côte d'Ivoire. Three taxa (*Macroplea* sp., *Valleriola* sp. and Corydalidae) were reported for the first time in the Ivorian aquatic ecosystems and contribute to the list of insect' species of the Côte d'Ivoire aquatic ecosystems. In all stations, benthic insect fauna was dominated by Diptera which is typical of many freshwater systems [25].

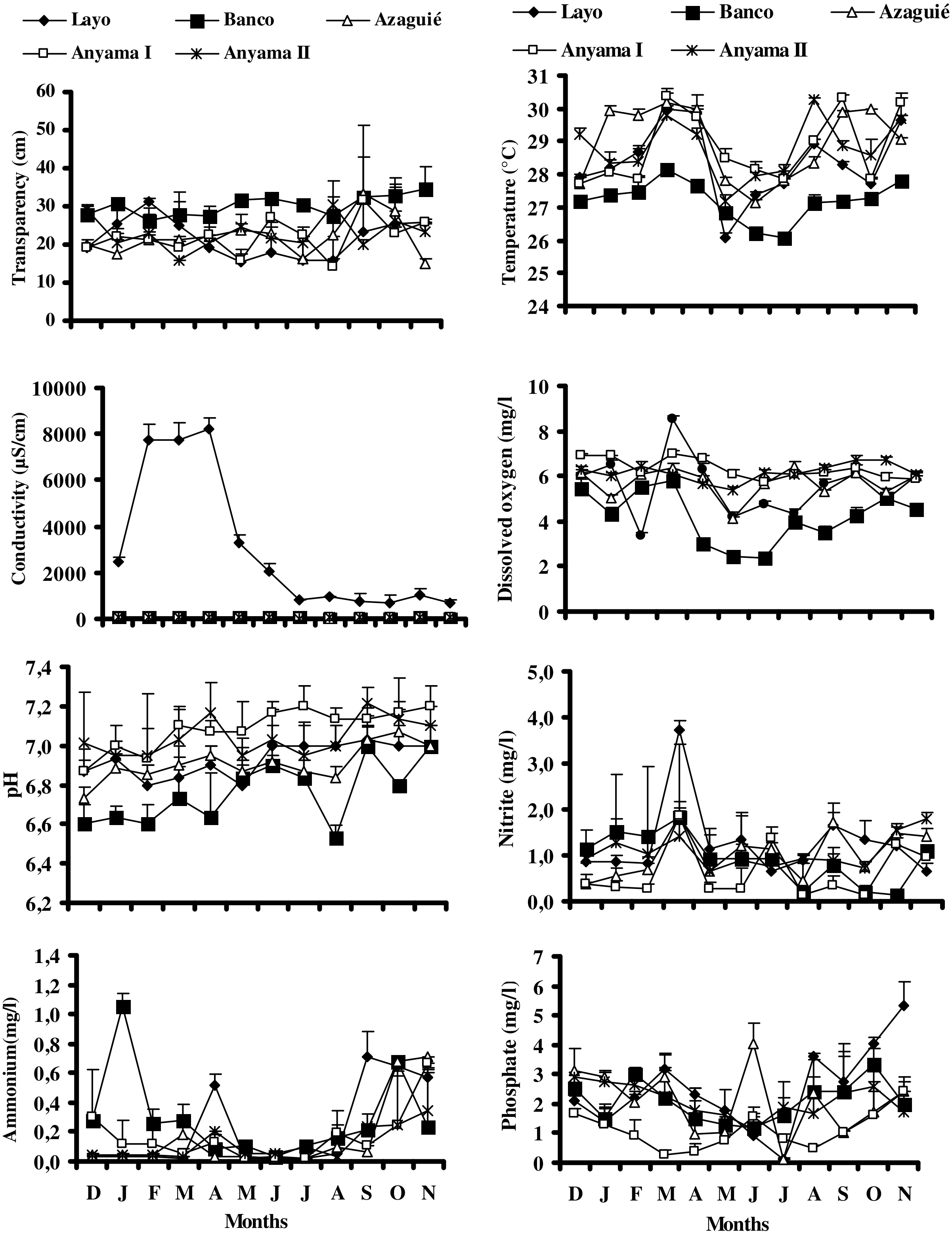


**Fig. 2. Temporal variation of environmental variables in the different stations**

**Table 1. List of benthic insects and dominance in percentage in the different stations during december 2007 to november 2008**

| Taxa | Stations | | | | |
|---|---|---|---|---|---|
| | **Layo** | **Banco** | **Azaguié** | **Anyama I** | **Anyama II** |
| **Ephemeroptera** | | | | | |
| Caenidae | | | | | |
| *Caenis* sp. | | | 0.15 | 2.33 | 0.47 |
| Polymitarcyidae | | | | | |
| *Povilla adusta* | | 0.59 | 0.04 | 0.07 | 0.21 |
| **Odonata** | | | | | |
| Gomphidae | | | | | |
| *Ictinogomphus* sp. | | | | 0.55 | |
| Libellulidae | | | | | |
| *Orthetrum* sp. | | | 0.04 | 0.34 | |
| *Libellula* sp. | | 0.21 | 0.07 | 0.21 | 0.37 |
| *Pantala flavescens* | | | 0.04 | | |
| *Brachythemis* sp. | 10.56 | 0.70 | 1.15 | 1.44 | 0.79 |
| **Hemiptera** | | | | | |
| Hydrometridae | | | | | |
| *Hydrometra* sp. | | | | 0.07 | |
| Leptopodidae | | | | | |
| *Valleriola* sp. | | | | 0.07 | |
| **Megaloptera** | | | | | |
| Corydalidae | | 0.05 | | | |
| Hydroptilidae | | | | | |
| *Hydroptila* sp. | | 0.05 | | | |
| **Diptera** | | | | | |
| Chironomidae | | | | | |
| *Ablabesmyia dusoleili* | | 0.48 | | | 0.90 |
| *Chironomus imicola* | 46.13 | 4.83 | 7.28 | 8.44 | 15.87 |
| *Clinotanypus claripennis* | | 3.92 | 1.55 | 2.54 | 2.32 |
| *Cryptochironomus* sp. | | 0.27 | 2.48 | | |
| *Nilodorum fractilobus* | 40.85 | 4.83 | 25.38 | 1.65 | 7.06 |
| *Nilodorum brevipalpis* | | 2.20 | 1.18 | 0.75 | 1.05 |
| *Polypedilum* sp. | | 4.67 | 0.96 | 0.82 | 0.32 |
| *Stenochironomus* sp. | | 0.16 | | 0.07 | |
| *Stictochironomus* sp. | | 51.93 | 10.27 | 38.78 | 16.50 |
| *Tanypus fuscus* | 1.41 | 12.19 | 40.08 | 30.75 | 30.15 |
| *Cricotopus kisantuensis* | | | | | 0.42 |
| Ceratopogonidae | | | | | |
| *Ceratopogon* sp. | 1.06 | | 1.92 | 3.16 | 17.24 |
| Chaoboridae | | | | | |
| *Chaoborus anomalus* | | 7.63 | 7.31 | 7.62 | 5.96 |
| Tabanidae | | | | | |
| *Tabanus* sp. | | 0.48 | | | 0.11 |

*La= Layo; Ba=Banco; Az= Azaguié; An I= Anyama I; An II= Anyama II Number in bold: the dominant taxa per station*

Chironomidae was the family who had the highest number of taxa (11 taxa). In addition, Chironomidae, with 87.29% of Dipterans in terms of abundance dominated quantitatively the Diptera taxa. This situation was reported by [26]. Similar result was observed by [27] in a temporary pond in southern Nigeria, by [4] in Agnéby river (Côte d'Ivoire) and by [28] in Biyémé river (Cameroon). [29] reported that the Chironomidae populations are numerically predominant, both in lotic and lentic environments, due to their tolerance of extreme conditions. Moreover, [30] reported that the life environmental conditions of Chironomids are more extensive than those of any other group of aquatic insects. Their wide ecological amplitude is related to their very extensive array of morphological, physiological and behavioural adaptations [29]. Our results showed that some genera of Diptera such as *Tanypus, Nilodorum* and *Chironomus* were recorded in different stations. According to [25], genera *Tanypus* and *Chironomus* have worldwide distributions and occur in freshwater systems. In addition, *Tanypus fuscus* is reported as ecologically tolerant [31] and has an extensive geographical range [32]. By contrast, especially *Valleriola* sp., *Hydrometra* sp. (Hemiptera), *Hydroptila* sp. (Trichoptera) and Corydalidae (Megaloptera) are represented by only one specimen. These species are not particularly abundant in this study. Pond habitats may be marginal for these species [33].

### 3.3 Abundance, Density and Biomass

The highest and the lowest insect abundance were recorded respectively at the stations of Azaguié and Layo (Table 2). The spatial distribution patterns of benthic insect's density ($F_{4, 180}$= 16.40; *P*=.00) and biomass ($F_{4, 180}$= 6.65; *P*=.00) showed significant difference among stations. Benthic insect density and biomass (Table 3) were significantly higher in the dry season compared to the rainy season at the stations of Anyama I ($F_{1,180}$ = 6.47; *P*=.01) and Anyama II ($F_{1,180}$ = 6.43; *P*=.01). Insect density varied spatially and temporary in response to environmental variables such as conductivity at the station of Layo. The seasonal variation showed that the highest density of benthic insects was recorded in the dry season at the stations of Anyama I and Anyama II, due to presence of *Chironomus imicola, Clinotanypus claripenis, Stictochironomus* sp. and *Tanypus fuscus* in these stations. In contrast, densities of *Chironomus imicola* and *Nilodorum fractilobus* (Layo), *Polypedilum* sp., *Stictochironomus* sp. and *Chaoborus anomalus* (Banco) and *Nilodorum fractilobus* (Azaguié) were higher during the rainy season. During the dry season, biomass was significantly higher at all stations except Azaguié. This situation is due to the high biomass of *Macroplea* sp., *Brachythemis* sp., *Clinotanypus claripennis*, *Tabanus* sp (Banco), *Brachythemis* sp. and *Nilodorum fractilobus* (Layo), *Ictinogomphus* sp., *Chironomus imicola*, *Nilodorum fractilobus* (Anyama I), *Chironomus imicola* and *Tanypus fuscus* (Anyama II) in dry season.

### 3.4 Diversity and Similarity Indices

Shannon-Wiener diversity ($F_{4,180}$ = 29.80; *P*=.00) and Evenness ($F_{4, 180}$ =2.42; *P*= .04) indexes showed significant difference among the stations (Table 2). The highest values of Shannon-Wiener diversity and Evenness indexes were recorded at Anyama II station. According to [34], the station of Anyama II had diverse taxa with balanced abundances. This is the consequence of its ecological heterogeneity and stability. Our results are similar to those obtained by [27] regarding the spatial variation of Shannon-Wiener diversity index and Evenness. Seasonal variation showed significant difference in Shannon-Wiener index in Azaguié station (Table 3) indicating that ponds ecosystems presented fewer disturbances during the dry season in this station. The Sorensen similarity index showed that Azaguié and Anyama II were strongly similar (QS=77.77) (Table 4). This index revealed that there was a minimum similarity between Layo and the others stations. This observation might be due to the low specie richness harvested in this station. In the other hand, the insect population identified in the others stations had high similarity.

**Table 2. Spatial variation of number of taxa, abundance, density, biomass, Shannon-Wiener diversity index (bits), and evenness among stations (mean ± (SD))**

| Parameters | Stations | | | | |
|---|---|---|---|---|---|
| | Layo | Banco | AzAzaguié | Anyama I | Anyama II |
| Number Of taxa | 5 | 19 | 18 | 19 | 18 |
| Abundance | 284 | 1863 | 2707 | 1456 | 1897 |
| Density ($ind.m^{-2}$) | 87.65$^{a}$ (46.89) | 575.00$^{b}$ (438.22) | 835.49$^{c}$ (598.03) | 449.38$^{b}$ (397,66) | 585.49$^{bc}$ (327.27) |
| Biomass ($mg.m^{-2}$) | 0.89$^{a}$ (0.75) | 1.97$^{b}$ (1.51) | 3.38$^{c}$ (3.33) | 2.32$^{b}$ (2.47) | 2.14$^{b}$ (1.16) |
| Shannon- Wiener Index | 0.86$^{a}$ (0.52) | 1.88$^{bc}$ (0.57) | 1.89$^{bc}$ (0.49) | 1.65$^{b}$ (0.59) | 2.13$^{c}$ (0.49) |
| Evenness | 0.68$^{a}$ (0.36) | 0.76$^{ab}$ (0.17) | 0.74$^{ab}$ (0.14) | 0.75$^{ab}$ (0.13) | 0.83$^{b}$ (0.11) |

*$^{a, b, c, d}$ letters in the same row show differences among stations (P=.05)*

### 3.5 Relationships between Environmental Variables and Benthic Insect Communities

Most of the data variability was explained by the two first axes of Canonical Correspondence Analysis (Fig. 3) (57.4 % axis I and 28.4 % axis II). The first axis separates stations and species from more salty and sandy environments (Layo farm), in the right side of thaxis, from freshwater stations with sediments composed essentially of silt and clay, in the left side of this axis. No clear separation is noticed between dry and rainy seasons. The differences among farms are larger than the differences among seasons since the farms stations are positioned near each other, independently of season. High values of pH, temperature and dissolved oxygen were observed in Anyama II and Azaguié stations, whereas transparency and silt are quite important in Banco and Anyama I stations. In Layo station, high conductivity values were registered and the substrate is mostly composed by sand. Brachythemis sp. and Chironomus imicol, projected near the right side of axis I, reveal some preference by brackish and sandy environments, while Ceratopogon sp and Tanypus fuscus select environments with higher temperature and pH values and higher dissolved oxygen concentrations. All other taxa, such as Stictochironomus sp, Clinotanypus claripennis and Polypedilum sp. apparently preferred lower values of the previously mentioned variables, but transparent waters and sediments with higher silt content. Chaoborus anomalus preferred sediments with higher clay content.

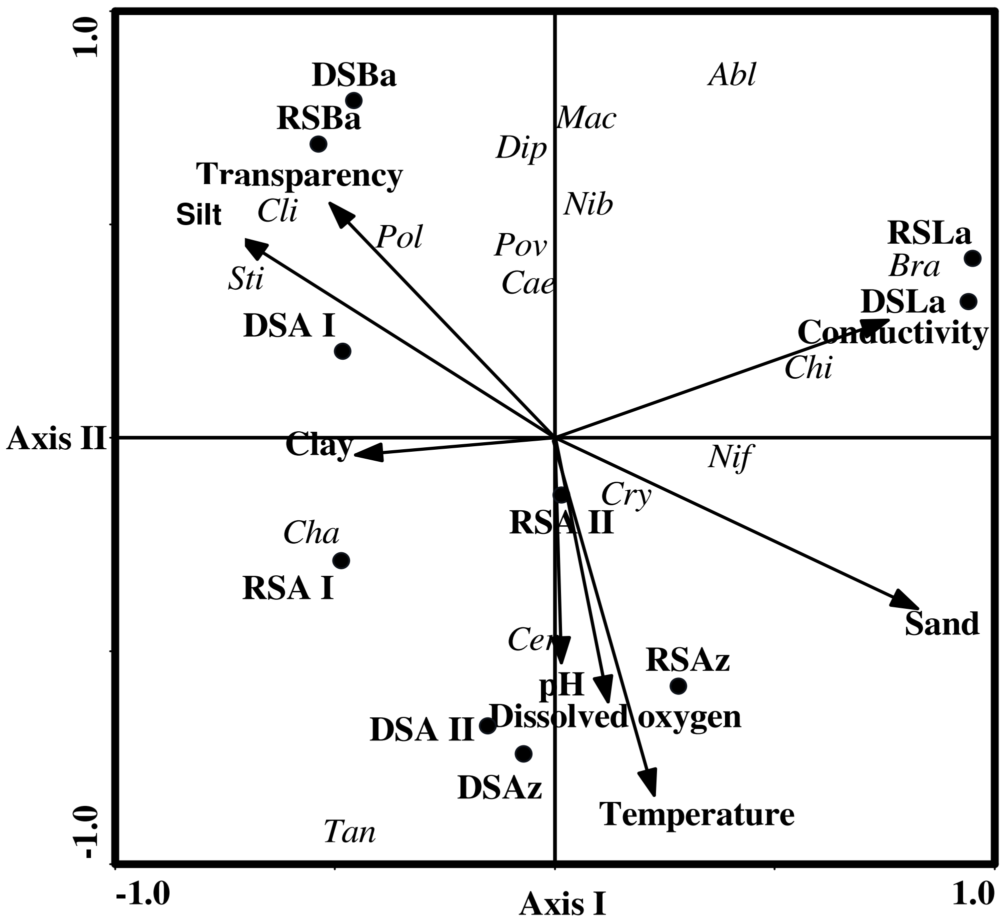


**Fig. 3. Canonical correspondence analysis carried out with selected environmental variables and with the dominant insect taxa. Taxa codes**

*Abl = Ablabesmyia dusoleili, Bra= Brachythemis sp., Cae= Caenis sp., Cer= Ceratopogon sp., Cha= Chaoborus anomalus, Chi= Chironomus imicola, Cli= Clinotanypus claripennis, Cry= Cryptochironomus sp., Dip= Dipseudopsis capensis, Mac= Macroplea sp., Nif= Nilodorum fractilobus, Nil= Nilodorum brevipalpis. Pol= Polypedilum sp, Pov= Povilla adusta, Sti= Stictochironomus sp.,Tan= Tanypus fuscus, Stations codes: DS= dry season; RS= rainy season; La= Layo; Ba= Banco; Az= Azaguié; AI= Anyama I; AII= Anyama II*

**Table 3. Seasonal variation of abundance, density, biomass, Shannon-Wiener diversity index and evenness of aquatic insects among stations (mean ± (SD)). DS= dry season; RS= rainy season**

| Parameters seasons | Stations | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Layo | | Banco | | Azaguié | | Anyama I | | Anyama II | |
| | DS | RS | DS | RS | DS | RS | DS | RS | DS | RS |
| Density (ind.m$^{-2}$) | 83.33[a] (45.93) | 91.97[a] (48.77) | 570.98[a] (352.48) | 579.01[a] (520.66) | 824.07[a] (351.20) | 846.91[a] (782.76) | 606.17[b] (487.47) | 292.59[a] (189.44) | 714.19[b] 402.44) | 456.79[a] (153.24) |
| Biomass (mg.m$^{-2}$) | 0.91[a] (0.84) | 0.87[a] (0.67) | 2.17[a] (1.21) | 1.77[a] (1.76) | 3.20[a] (1.56) | 3.56[a] (4.50) | 3.42[b] (2.94) | 1.22[a] (1.15) | 2.61[b] (1.34) | 1.67[a] (0.73) |
| Shannon- Wiener index | 0.95[a] (0.54) | 0.77[a] (0.50) | 2.03[a] (0.60) | 1.74[a] (0.53) | 2.09[b] (0.47) | 1.69[a] (0.44) | 1.78[a] (0.57) | 1.52[a] (0.60) | 2.08[a] (0.42) | 2.17[a] (0.56) |
| Evenness | 0.71[a] (0.35) | 0.65[a] (0.37) | 0.77[a] (0.16) | 0.74[a] (0.18) | 0.77[a] (0.11) | 0.72[a] (0.17) | 0.74[a] (0.13) | 0.76[a] (0.14) | 0.82[a] (0.09) | 0.84[a] (0.13) |

[a, b,] *letters in the same row show differences among seasons within station (P=.05)*

**Table 4. Sorensen similarity index of benthic insect's communities recorded in the different station**

| | Layo | Banco | Azaguié | Anyama I | Anyama II |
|---|---|---|---|---|---|
| Layo | | 33.33 | 43.47 | 41.66 | 43.47 |
| Banco | | | 64.86 | 63.15 | 75.67 |
| Azaguié | | | | 75.67 | 77.77 |
| Anyama I | | | | | 70.27 |
| Anyama II | | | | | |

In this study, the pattern distribution according to environmental variables indicates that Ceratopogon sp. and Tanypus fuscus were associated to high value of pH, temperature, and dissolved oxygen. [8] reported significant relationships between species composition and pH, temperature, and dissolved oxygen in the Soumié, Ehania, Tanoé and Eholié rivers in southern Côte d'Ivoire. Similar result was observed by [4] in Bia River in southern Côte d'Ivoire. [27] Observed a significant positive correlation between density and water temperature in temporary pond in Okomu Forest Reserve, in southern Nigeria. According to [35], temperature is one of the most important environmental factors controlling aquatic insect density. In addition, high water temperatures might accelerate the feeding activity of benthic predators Brachythemis sp., Nilodorum fractilobus and Chironomus imicola.

## 4. CONCLUSION

The present study has reported 31 benthic insect taxa in the different stations. Three of these taxa (*Macroplea* sp., *Valleriola* sp. and Corydalidae) were recorded for the first time in the Ivoirian aquatic ecosystem. Diptera is the most diversified and numerically abundant group. Indeed, Chironomidae is quantitatively the most important family among Diptera. The insect communities are quite diverse and stable among stations except Layo. Consequently, these communities are similar among the stations except Layo. In addition, community parameters are correlated to environmental variables such as water temperature and chemistry, and to pond substrate.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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