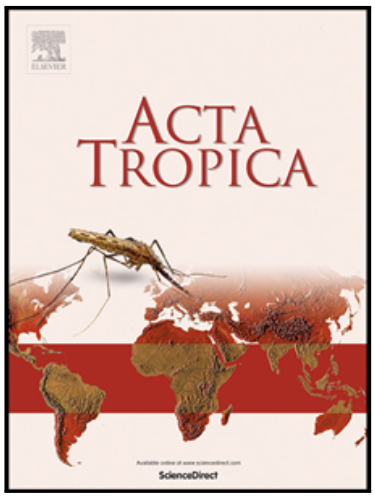

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Physicochemical factors affecting the diversity and abundance of Afrotropical *Culicoides* species in larval habitats in Senegal

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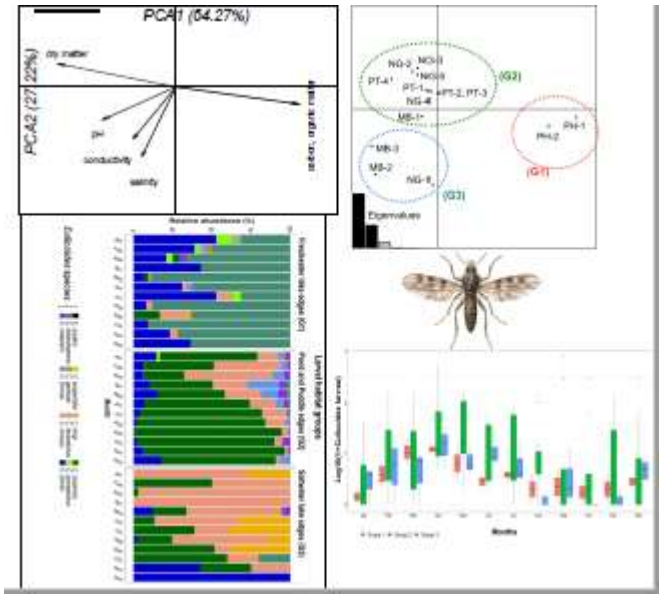
Graphical abstract



Abstract

Biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae) are the biological vectors of arboviruses of global importance in animal health. We characterized the physicochemical parameters that determine the density and composition of the main *Culicoides* species of veterinary interest in larval habitats of the Niayes region of Senegal. For this purpose, we combined larval and substrate sampling in the field in different habitat types with adult emergence and physicochemical analyses in the laboratory. Three major habitat types were identified, conditioning the predominant species of *Culicoides* and pH and the amount of organic matter were positively correlated with the abundance of larvae and emerging *Culicoides*, as opposed to salinity. The diversity of emerging *Culicoides* was positively correlated with pH while it was negatively correlated with salinity. *Culicoides distinctipennis* was the predominant species in the larval habitat group of freshwater lake edges. In the larval habitat group of pond and puddle edges, *C. oxystoma* and *C. nivosus* were predominant; both species were again most abundant in the larval habitat group of saltwater lake edges. These variabilities in physicochemical parameters support the distribution of different *Culicoides* species in different habitat groups. These results make it possible to implement effective, selective and environmental-friendly control measures but also to improve current models for estimating the abundance of adult vector populations at a local scale.

Keywords: *Culicoides*, African horse sickness, larval habitat, physicochemical parameters, Niayes area, Senegal

Introduction

Improving the efficiency of animal production is essential for achieving sustainable agricultural development and food security. Livestock play a direct role in food security in developing countries and is an important source of income. Animal traction using equidae (horses, mules, and donkeys) is still widely used in the Afrotropical region and there is increased interest in horseback riding for recreation. With the increased trade and environmental changes, this region is now facing health risks related to the emergence/re-emergence of *Culicoides*-borne diseases that impact livestock production or equine breeding. Indeed, certain species of *Culicoides* (Diptera: Ceratopogonidae) are involved in the transmission of pathogens of veterinary importance worldwide (Mellor et al., 2000; Mullen, 2009; Simonsen et al., 2011). These biting midges are de facto biological vectors of African horse sickness virus (AHSV) affecting equidae as well as bluetongue virus (BTV), epizootic hemorrhagic disease virus (EHDV) and Schmallenberg virus (SBV) affecting wild and domestic ruminants (Purse et al., 2015). In the Afrotropical region, AHSV is endemic and mortality rates frequently exceed 90% in naïve horses (Carpenter et al., 2017; Mellor and Hamblin, 2004). With similar symptoms to AHS, the equine encephalosis (EE) caused by the equine encephalosis virus (EEV) is endemic in sub-Saharan Africa where virus circulation has been shown in The Gambia very close to Senegal (Oura et al., 2012). AHSV was first described in South Africa (Meiswinkel et al., 2004), where it is transmitted by *Culicoides imicola* Kieffer and *C. bolitinos* Meiswinkel (Du Toit, 1944; Paweska et al., 2003; Venter et al., 2000). Outbreaks of African horse sickness (AHS) occurred between 2006 and 2008 in Namibia (Scacchia et al., 2009) and in 2007 in Senegal (Diouf et al., 2013) leading to 1,169 dead horses and considerable economic losses in the latter country, estimated at 1.4 million euros (Akakpo et al., 2011). In Senegal, 53 species of *Culicoides* have been recorded including two proven vectors of AHSV: *C. imicola* and *C. bolitinos* (Diarra et al., 2014; Fall et al., 2015a), plus other species suspected as potential vectors due to their abundance and attractiveness to horses such as *C. oxystoma* Kieffer (Bakhoum et al., 2016b; Diarra et al., 2015; Fall et al., 2015c), but to date, their formal implication as a biological vector has not been confirmed.

To protect horses from the risk of transmission of AHSV, several prevention and control methods could be used to decrease *Culicoides* populations or at least reduce host-vector contact; for a review of *Culicoides* control technics (Carpenter et al., 2008; Cilek and Hallmon 2005; Cilek et al., 2003; Clements and Rogers 1968; Doherty et al., 2004; Kline

and Roberts 1981; Venail et al., 2015; Venail et al., 2011). So far, control methods have mainly focused on adult *Culicoides* while methods against larvae are not developed/evaluated due in particular to the lack of knowledge on the larval habitats of *Culicoides* species of veterinary interest (Carpenter et al., 2008). Indeed, the success of the control of immature stages of *Culicoides* depends primarily on the identification of their larval habitats, their spatial distribution, their seasonal dynamics and the ecological factors affecting the choice of these larval habitats. Although the larval ecology of some *Culicoides* species of veterinary interest seems well known in the Palearctic region (Foxi and Delrio, 2010; Harrup et al., 2013; Uslu and Dik, 2007; Zimmer et al., 2008; Zimmer et al., 2014), this is not the case for species from the Afrotropical region where studies on the biology and ecology of the main vectors remain rare and are mainly carried out in South Africa (Nevill, 1967). In particular, the development cycle of the immature stages of tropical *Culicoides* is poorly known, as well as the preferences of the main species in terms of larval habitat, the ecological factors involved in these choices or the dynamics of emergence. In general, females lay eggs in a semi-aquatic or moist substrate rich in organic matter and larval development is preferably in the upper layer of these habitats (Uslu and Dik, 2006). Larvae feed on organic matter and microorganisms such as algae, bacteria, and protozoa or small invertebrates such as nematodes or insect larvae (Blanton and Wirth, 1979; Mullen and Hribar, 1988). Depending on the species, the habitats of the immature stages of *Culicoides* can be very different, such as freshwater marshes and swamps, shallow margins of ponds, streams, and rivers, peat bogs, beaches, around irrigation systems and leaking water troughs, tree holes and other natural cavities in decaying wood, waterlogged pastures, rotting fallen fruits, highly alkaline or saline inland water collections, and animal dung (Bakhoum et al., 2016a; Blackwell et al., 1994; Braverman et al., 1974; Dipeolu and Ogunrinade, 1976; Foxi and Delrio, 2010; Gonzalez et al., 2013; Harrup et al., 2013; Jenkins and Young, 2010; Labuschagne, 2016; Lubega and Khamala, 1976; Nevill, 1967; Poddar et al., 1992; Ray and Choudhury, 1988). However, many factors remain unknown regarding the ecology of the immature stages. Some *Culicoides* species are highly adaptable and can be found in a wide range of habitats, while others are poorly adaptable and require specific requirements such as coprophilous or fruit-feeding species (Nevill, 1967; Zimmer et al., 2014). Although poorly documented, some physicochemical parameters are important for larval development, such as pH (Blackwell et al., 1999; Blackwell et al., 1994; Harrup et al., 2013; Uslu and Dik, 2010), organic matter content and mineral composition (Schmidtmann et al., 2000; Uslu and Dik, 2010).

The knowledge of substrates adapted to the larval development of *Culicoides* is important, particularly for the main species of veterinary interest. This is an essential prerequisite for the implementation of vector control methods targeting the larval habitats of *Culicoides*. In Senegal, *Culicoides* larval habitats belonging to 10 *Culicoides* species have recently been described. *Culicoides* larvae and emerging adults have been found at the edges of ponds, puddles and lakes (Bakhoum et al., 2016a). This is the first time that *Culicoides* larval habitats have been identified in this country severely affected by AHS, but several factors remain unknown, such as the chemical parameters favourable to larval development of the *Culicoides* species of interest, as well as the diversity of larval habitats, their spatial distribution and seasonal dynamics.

Here we conducted a physicochemical soil analysis of fourteen larval habitats in Senegal to define the physicochemical factors that affect the abundance and diversity of *Culicoides* in larval habitats. The spatial and temporal dynamics of emerging *Culicoides* are also described. Besides, the importance of larval habitat type and physicochemical conditions in determining the occurrence of *Culicoides*, particularly species of veterinary interest, is discussed.

Materials and Methods

Description of the study area. This study was conducted in the southern part of the Niayes area. The Niayes area in Senegal is a 25- to 30-km wide coastal band that extends 180 km from Dakar to the southern tip of the Senegal River Delta. The climate is oceanic, with relatively constant humid winds and low thermal amplitudes (Sagna, 2000). There are two main seasons in this area: the rainy season (July to October) and the dry season, sub-divided into the cold dry season (November to February) and the hot dry season (March to June); the vegetation is diversified and mainly composed of steppes and shrub savanna. Rainfall in the Niayes rarely exceeds 350 mm/year (Faye et al., 1995). *Culicoides* sampling was carried out at four sites in the southern part of the Niayes area: Parc de Hann, Mbao, Niague and Pout. A total of fourteen larval habitats distributed over these four sites were monitored twice monthly for one year from January to December 2015 (Figure 1).

Larval habitats sampling. Larval habitats were sampled using an 8 cm diameter sampling cylinder driven into the ground to a depth of 6 cm. For each larval habitat, four samples were collected. The first was used to study the presence of midge larvae in the lab using a direct

flotation technique in a saturated sugar solution (850 g/litre) (Bakhoum et al., 2016a). *Culicoides* larvae were then collected, counted and preserved in 70° ethanol. The remaining three substrate samples were used for laboratory emergence followed by a collection of adults. The soil samples were individually placed in 200 ml plastic pots covered with a net before being transported to the laboratory (insectarium) to monitor adult emergence. The emergence pots were maintained for 35 days at a temperature of 25 ± 1°C, relative humidity of 80± 10% and a light-dark photoperiod of 12:12h, conditions favourable for *Culicoides* emergence. The surface of the substrate was sprayed every two days with demineralized water to prevent desiccation. Each day, the emerging adults were collected using a mouth aspirator and then stored in 70° ethanol. Adult *Culicoides* were identified using a stereomicroscope (10-40x) and reference identification keys (Boorman, 1989; Boorman and Dipeolu, 1979; Cornet and Brunhes, 1994; Glick, 1990; Labuschagne, 2016). Larvae resulting from the direct flotation technique were identified by their DNA barcode sequences (Bakhoum et al., 2018).

Physicochemical measurements. During each larval sampling session, approximately 20 mg of additional ground sample was collected in each larval habitat, placed in two plastic pots with lids and transported to the laboratory. In the laboratory, six physicochemical parameters were analysed, such as carbon content (%), conductivity (ms/cm), dry matter content (%), organic matter content (%), pH and salinity (%). The analysis of these parameters was carried out by the food analysis service of the Chemistry Department of the National Livestock and Veterinary Research Laboratory of the Senegalese Institute for Agricultural Research (LNERV-ISRA, BP 2057, Dakar, Senegal).

Data analysis. All statistical analyzes were performed using R software (R Development Core Team 2008, URL http://www.R-project.org/).

Partial triadic analysis (PTA) were performed using the *ade4* package (Dray and Dufour, 2007) and adegraphics package (Siberchicot et al., 2017) to compare the variation in the assemblage of physicochemical parameters between various larval habitats and to determine the temporal stability of this structure. Based on the logic of Principal Component Analysis (PCA), PTA is a multivariate method that analyzes matrices in three-dimensional data tables (Thioulouse and Chessel, 1987; Thioulouse et al., 2004). In our study, a matrix (larval habitat $_x$ values of physicochemical parameters) with a third dimension representing time (months). The PTA is designed to study simultaneously several sub-matrices of quantitative data and to detect within the structure any pattern common to these different sub-matrices to extract a multivariate structure that is expressed through the different times

(months). The analysis consists of three successive steps called interstructure, trade-offs and trajectories. The interstructure provides the individual contribution of the matrices of physicochemical parameters of each month, to the common ecological structure over time and gives a weight to each matrix according to their importance. The compromise calculates an average matrix (compromise table); with a weighted mean of the different matrices of physicochemical parameters. The trade-off table provides a structure that is then analyzed by the PCA to reveal the common structure between observations for an inferential approach to validate the trade-off (Lazraq et al., 2008).

The effectiveness of temporal sampling in larval habitats was determined by plotting species accumulation curves according to a randomization procedure (1000 permutation) using the *vegan* package (Oksanen et al., 2013). A species accumulation curve derives as a plot of a cumulative number of species found as a function of sampling effort (Ugland et al., 2003). Each species is considered independently of its abundance or rarity. The number of non-sampled species was extrapolated by estimating different indices of richness.

For each larval habitat group, species richness (S) was determined as the number of insect species collected and the diversity of communities was assessed using the Shannon index (H) calculated with the "*diversity*" command of the *vegan* package. Furthermore, the number of non-sampled species (NS) was extrapolated using the "*estimateR*" command of the *vegan* package. The apparent density (*AD*) was estimated for emerging *Culicoides* as the number of specimens (sp) collected per larval habitat (h) and per day (d). The frequency of the emerging *Culicoides* species distribution in larval habitats was estimated using the *iNEXT* package (Hsieh et al., 2013). The dominant species index (*d*) was estimated using the Berger-Parker equation: $d = Ni/N$, where *Ni* is the number of individuals of the i^{th} species and *N* is the total number of individuals sampled (all species). This index ranges from 0 to 1, and values close to 1 indicate a strong dominance of certain species.

Generalized Linear Mixed-Effects Models (GLMM) (McCullagh and Nelder, 1989) were used to assess the relationships between physicochemical parameters and the abundance of *Culicoides* larvae and emerging *Culicoides* for each group of larval habitats. The models were fitted by maximum likelihood (Laplace Approximation). We used Poisson regression mixed-effect models with random effects on the larval habitat groups (G1, G2 and G3) and level of sampling months. Two-thirds of the sample was used for the training dataset and the remaining 1/3 of the sample for the test dataset. The full model contained the untransformed continuous physio-chemical parameters: carbon content, conductivity, dry matter content, organic matter content, pH and salinity; and the different larval habitat groups. The final

models were obtained using a backwards-stepwise selection-based procedure, such that variables that did not contribute significantly to explaining the variation in *Culicoides* larvae or emerging *Culicoides* were successively eliminated based on the Akaike Information Criterion (AIC) (Akaike, 1973). The root means square error (RMSE) was used for validation.

Results

Classification of larval habitats

A partial triadic analysis (PTA) was carried out to study the longitudinal variations of the physicochemical parameters found in the larval habitats monitored during the study and to identify clusters of similar larval habitats (Figure 2). As shown by the interstructure, which represents the structure of the physicochemical parameters during the study period, the measured parameters (values of carbon content (%), conductivity (ms/cm), dry matter content (%), organic matter content (%), pH and salinity (%)) were globally stable throughout the year (Figure 2A). The first two axes of the Principal Component Analysis of the compromise table (Figures 2B, 2C) showed a clear separation between three groups of larval habitats and explained 91% of the total inertia. The first group of larval habitats (G1) corresponded to two sampling sites on the edge of freshwater lakes in the Parc de Hann. This group of larval habitats was characterized by the highest level of organic matter (mean = 8.70%) and carbon (mean = 5.04%); and an acidic pH (mean = 6.05) (Table 1). The second group of larval habitats (G2) consisted of nine larval habitats distributed between two localities (Pout and Niague) and all corresponded to semi-permanent pond and puddle edges. This group was characterized by the highest value of dry matter content (mean = 71.17%), neutral pH (mean = 7.25) and the lowest salinity (0.21%) (Table 1).

Finally, the third group corresponded to three larval habitats of saltwater lake edges distributed between two localities (Mbao and Niague) with the highest values of conductivity (mean = 15.07 ms/cm) and salinity (mean = 1.52%), but also a basic pH (mean = 8.14) (Table 1). The cos2 values revealed that the compromise table was representative of the individual matrices, ranging from 0.77 to 0.90. Similarly, the table weights were similar, ranging from 0.26 to 0.31 (Figure 2D; Table S1). These values, therefore, indicated that the assemblages of physicochemical parameters varied moderately from month to month.

Abundance of larvae and emerging Culicoides.

During the one-year sampling period (twice a month from January to December 2015), a total of 4,716 *Culicoides* larvae were collected by direct flotation carried out on substrate samples from fourteen larval habitats (Table 1). Bakhoum et al. (2018) identified 906 cox1 sequences of *Culicoides* larvae corresponding to eight *Culicoides* species: *C. distinctipennis* Austen (n = 32), *C. enderleini* Cornet and Brunhes (n = 26), *C. imicola* (n = 1), *C. kingi* Austen (n = 17), *C. nivosus* de Meillon (n = 195), *C. oxystoma* (n = 605), *C. pycnostictus* Ingram and Macfie (n = 1), and *C. similis* Carter, Ingram and Macfie (n = 29).

Furthermore, 11,268 *Culicoides* specimens (5,200 ♂ / 6,068 ♀) belonging to 12 species emerged in the laboratory from the substrate samples collected from these larval habitats and preserved for identification (Table 1).

Inventory and diversity of emerging Culicoides from larval habitats.

The 11,268 emerging *Culicoides* from larval habitats were distributed into 12 species as follows: *C. oxystoma* (n = 7,346), *C. nivosus* (n = 2,134), *C. similis* (n = 706), *C. imicola* (n = 419), C. *distinctipennis* (n = 294), *C. enderleini* (n = 210), *C. kingi* (n = 102), *C. pycnostictus* (n = 24), *C. leucostictus* Kieffer (n = 21), *C. gambiae* Clastrier and Wirth (n = 7), *C. exspectator* Clastrier (n = 3) and *C. austeni* Carter, Ingram and Macfie (n = 2). Species accumulation curves (as an estimate of species richness) plotted for 14 samplings indicated that the sampling was representative of different larval habitats (Figure S1). *Culicoides oxystoma* and *C. imicola* were observed in all larval habitat groups, whereas *C. austeni* was observed in only one larval habitat group (Table 2). The highest apparent density (*AD*) of *Culicoides* was observed in the larval habitat group G2 with a value of 48.49 specimens/ larval habitat/day (Table 1), which underlines that pond edges and puddles are the most productive habitats. In addition, *Culicoides* diversity was highest in this group with a specific richness (S) value of 12 and a Shannon index (H) of 1.01, followed by larval habitat group G1 (AD=6.92, S= 11, and H= 1.04) and larval habitat group G3 (AD=6.42, S= 7, and H= 1.19) (Table 1). Species composition and dominance index (*d*) revealed that in the larval habitat group of freshwater lake edges (G1), *C. distinctipennis* was the predominant species (*d*= 0.651) followed by *C. similis* (*d*= 0.244) (Table 2). In the larval habitat group of pond and puddle edges (G2), *C. oxystoma* and *C. nivosus* were predominant with dominance index values of 0.692 and 0.177 respectively (Table 2). Both species were again most abundant in the larval habitat group of saltwater lake edges (G3), but with the highest index for *C. nivosus* (*d*= 0.574) followed by *C. oxystoma* (*d*= 0.186) and *C. kingi* (*d*= 0.154) (Table 2). Seven of

the 12 species were found in all habitat groups (*C. distinctipennis*, *C. enderleini*, *C. imicola*, *C. kingi*, *C. nivosus*, *C. oxystoma* and *C. similis*), only four species were found in G1 and G2 (*C. exspectator*, *C. gambiae*, *C. leucosticus* and *C. pycnostictus*) and *C. austeni* was only found in G2 (Table 2).

Spatio-temporal dynamics of larvae and emerging Culicoides.

The spatio-temporal variations of *Culicoides* larval density were strong (p-value<0.001) (Figure S2). Regarding the emergence of the adults over the one year, a significant pick was observed in September, corresponding to the rainfall mid-season (Figure 3). Analysis of the spatio-temporal dynamics of each emerging *Culicoides* species in the three larval habitat groups revealed significant variations of their density and species composition assemblages over time (Figure 4). Changes in *Culicoides* species dominance were observed within and between larval habitat groups. In larval habitat group G1, *C. distinctipennis* was observed throughout the year, as well as *C. oxystoma* and *C. nivosus* in larval habitat group G2 (Figure 4). Although these species were observed in these two larval habitat groups throughout the study, the highest densities were observed between March and May. The density of *C. distinctipennis* peaked between August and December. For *C. oxystoma*, the highest densities were observed between July and December, in contrast to *C. nivosus* whose highest densities were observed in February and March (Figure 4). In larval habitat group G3, *C. nivosus* was the most represented species throughout the year, except in December when only *C. oxystoma* was present. The highest densities were observed in January and between March and May (Figure 4).

Physicochemical parameters related to the abundance of larvae and emerging Culicoides

The abundance of *Culicoides* larvae was positively correlated with pH (z value= 23.09, p-value< 2e-16) and organic matter (z-value= 21.13, p-value< 2e-16). However, it was negatively correlated with salinity (z-value=-4.92, p-value=8.55e-07) (Table 3). As for the abundance of emerging *Culicoides*, it was positively correlated with pH (z-value= 33.21, p-value< 2e-16) and organic matter (z-value= 37.09, p-value< 2e-16). Furthermore, it was negatively correlated with salinity (z-value=-27.17, p-value< 2e-16) and dry matter (z-value=-15.37, p-value< 2e-16). With regard to the diversity of emerging *Culicoides*, pH was positively correlated with specific richness (z-value= 2.71, p-value=0.007) while salinity was negatively correlated with specific richness (z-value=-5.40, p-value=6.65e-08) (Table 3).

Discussion

The larval ecology of *Culicoides* species remains one of the most neglected areas of study in their life cycle. One reason may be that data are almost impossible to generate by standardised methods and on a quantitative basis since most species of biting midges cannot be bred in the laboratory. Although numerous field studies describing the larval habitats of *Culicoides* species of interest have been performed in Europe, India and Africa (Bakhoum et al., 2016a; Blackwell et al., 1994; Braverman et al., 1974; Dipeolu and Ogunrinade, 1976; Foxi and Delrio, 2010; Gonzalez et al., 2013; Harrup et al., 2013; Jenkins and Young, 2010; Labuschagne, 2016; Lubega and Khamala, 1976; Nevill, 1967; Poddar et al., 1992; Ray and Choudhury, 1988), important factors such as the influence of physicochemical parameters on the abundance and diversity of *Culicoides* species at the larval sites have been received little attention. In general, however, it has been reported that larval development of *Culicoides* is optimal in a nutrient-rich habitat (Blanton and Wirth, 1979; Mullen and Hribar, 1988; Zimmer et al., 2014). In an earlier study of larval habitats in the Niayes area in Senegal, it was found that some Afrotropical *Culicoides* species developed in a range of wet substrates such as the edges of ponds, puddles, freshwater lakes and saltwater lakes (Bakhoum et al., 2016a). In the present study, longitudinal entomological monitoring of *Culicoides* larval habitats was performed and the physicochemical characteristics correlated with the dynamics and diversity of emerging *Culicoides* species were described. Our results showed that pH and organic matter content were positively correlated with the abundance of larvae and emerging *Culicoides*. In contrast, salinity was negatively correlated with the development of larvae and emerging *Culicoides*. According to their physicochemical characteristics, the partial triadic analysis clustered the larval habitats observed in the study area into three groups (G1, G2, and G3) with varying abundance and diversity of *Culicoides*. The first larval habitat group (G1) with the highest rates of organic matter and carbon and an acidic pH was located in the Parc de Hann. This locality contains a freshwater lake surrounded by a dense forest that produces a significant amount of organic matter through the decomposition of leaf and wood. This decomposition of organic vegetative materials probably leads to the acidic pH that characterizes this habitat, which can select the species that can develop in such conditions. This habitat presents a low apparent density of *Culicoides* but a high species richness. The dominant species found in this habitat are *Culicoides distinctipennis* and *C. similis*. These two species are probably specific to this forest area in association with a high organic matter

content. *Culicoides distinctipennis* occur throughout Senegal and are abundant in the forest area in the forest area of Bandia south-east of Dakar, Saboya in the Sine Saloum area, Balingore and Dioulacolon in the south of Senegal, Niokolo-Koba Park in eastern of Senegal, Madina-Ndiatebe and Ndioum-Kalo in the Senegal River valley (Cornet, 1969). It is also a widespread species in tropical Africa (Cornet, 1969; Glick, 1990; Labuschagne, 2016) and its ecological preferences for larval habitats appear to be related to the amount of organic matter, as has been demonstrated in the Harare region (formerly Salisbury) in Zimbabwe, where *C. distinctipennis* was dominant along drainage canals in a muddy intermediate organic matter environment (Braverman, 1978).

The second group of larval habitats (G2) consisted of the edges of ponds and puddles and contained the greatest species richness. This group was characterized with the highest dry matter content, neutral pH and the lowest salinity. The dominant species in this group were *C. oxystoma* and *C. nivosus*, observed throughout the year from January to December 2015, with peaks between July and December for *C. oxystoma* and in February and March for *C. nivosus*. *Culicoides oxystoma* is described as a species associated with aquatic and semi-aquatic habitats, such as paddy fields, riverbanks, pond edges or estuaries (Poddar et al., 1992; Ray and Choudhury, 1988; Yanase et al., 2013). Previous studies conducted at Mbao, Niague and Pout (localities where the larval habitats of the G2 group were located) showed an abundance of *C. oxystoma* in *Culicoides* adult collections (Diarra et al., 2014; Diarra et al., 2015; Fall et al., 2015a). Braverman (1978) showed in Zimbabwe that the main breeding sites of *C. nivosus* were around puddles rich in organic matter where it was associated with other species such as *C. pycnostictus*, *C. distinctipennis*, *C. tropicalis* Kieffer, *C. shultzei* group, *C. zuluensis* de Meillon and *C. neavei* Austen. In our study for larval habitat group G2, *C. oxystoma* and *C. nivosus* were associated with 12 species including *C. distinctipennis*, *C. enderleini*, *C. imicola*, *C. kingi* and *C. similis*.

Finally, the third group (G3) with the highest values of conductivity and salinity, and a basic pH had the lowest species richness. This group included the larval habitats of saltwater lake edges distributed between Mbao and Niague. In Mbao, these were two lagoons that were connected to the sea and subject to the influence of the tides. In Niague, the larval habitat was also connected to the sea. In this group, three dominant species were found: *C. kingi*, *C. nivosus* and *C. oxystoma*. *Culicoides oxystoma* has been described in previous studies as a species that breeds preferentially in salt mud in India (Poddar et al., 1992; Ray and Choudhury, 1988) but it does not appear to be exclusive and could be also found in freshwater habitats such as paddy fields, stream bans, pond edges (Yanase et al., 2013) as already

mentioned in Japan. Indeed, in our study, *C. oxystoma* specimens emerged from all larval habitats of group G2 (pond and puddles edges) and represented 69.2% of the *Culicoides* specimens emerged from this group. With regard to *C. kingi*, previous studies have shown that this species prefers very salty mud in Africa (Cornet, 1969; Lubega and Khamala, 1976). However, this species can also be present in low salinity mud. Thus, in our study, 29% of *C. kingi* specimens emerged from the larval habitat group G2 and 1% from the larval habitat group G1. To our knowledge, this study showed for the first time that *C. nivosus* is associated with larval habitats such as the shores of saltwater lake.

The relative abundance of *C. oxystoma* and *C. imicola* was higher in larval habitat group G2 than in larval habitat groups G1 and G3. *Culicoides imicola* larvae have previously been reported to develop favourably in moist (but not waterlogged) nutrient-rich soils (Foxi and Delrio, 2010; Nevill, 1967). Accordingly, Nevill (1967) postulated that pupae of the subgenus Avaritia drown when submerged and thus these species are restricted to moist, but not waterlogged, substrates. This seems to be confirmed by the fact that almost all *C. imicola* specimens emerged from larval habitat group G2 with the highest value of dry matter content, neutral pH and the lowest salinity. For *C. distinctipennis*, densities were relatively high in group G1 and almost zero in the other groups. As for *C. nivosus* and *C. kingi*, their relative abundance was highest in larval habitat group G3. *Culicoides similis* was not specific to any larval habitat type and this species, even if found at low densities, could develop in a wide range of larval habitats and physicochemical conditions from acid to basic pH and from fresh to saltwater. Our results suggest a high capacity of this species to develop in different types of habitats, which is probably a consequence of its ecological flexibility. *Culicoides similis* can therefore be qualified as "generalist" species and present a high phenotypic plasticity as compared to *C. austeni* which seems to be a more "specialist" species. The ability to colonize or not fresh and/or saltwater habitats reveal important information about their physiology. Indeed, to survive in saline conditions, insect's larvae must regulate their water balance (*i.e.* osmoregulation) and remain confined in water with an osmotic concentration higher or equal than that of the larval haemolymph (Bradley, 1987). The diversity of emerging *Culicoides* was positively correlated with pH while it was negatively correlated with salinity. This supports the distribution of the different *Culicoide*s species emerged from the different habitat groups with different physicochemical parameters. Studies performed in the UK showed that *C. impunctatus* was strongly correlated with substrate moisture levels and peatlands at acidic pH (5.0–6.5) (Blackwell et al., 1999; Blackwell et al., 1994). This species known as the Scottish midge proliferates in nutrient-poor peat bog areas of Scotland and northern England

and is a major nuisance in the summer and a hindrance to outdoor activities and leisure industries (Hendry and Godwin 1988). In the UK, Harrup et al. (2013) found that the presence of emerging *Culicoides* and more particularly *C. obsoletus* in larval habitats was strongly correlated with substrate moisture level and neutral or alkaline pH. By analysing species-specific habitat preferences and the relationships between the abundance of *Culicoides* in larval habitats and their physicochemical characteristics, dungheaps appeared as the most important substrate for the development of *Culicoides obsoletus* on cattle farms in Germany. The study showed that abundance of *C. obsoletus* in dungheaps was further related to increasing substrate moisture (Steinke et al., 2016).

In our study, species such as *C. austeni*, *C. exspectator* and *C. gambiae*, which were found in small numbers in larval habitat group G2, are not necessarily rare as adults in the same study sites (Fall et al., 2015a). Thus, it is possible for these species that only their marginal larval habitats have been sampled and that their main breeding site has yet to be discovered. Although some species such as *C. kingi*, *C. nivosus*, and *C. oxystoma* were present in low densities in larval habitat group G1, they were, however, abundant in larval habitat groups G2 and G3 where their ecological conditions appear to be better. In Senegal, out of 53 recorded *Culicoides* species, 41 were found in the same Niayes area during a longitudinal study from July 2011 to October 2012 using OVI light suction traps and horse-baited traps (Fall et al., 2015a). In this study, 12 of these 41 *Culicoides* species were found in *Culicoides* specimens emerging from larval habitats. This may indicate that the unrecorded species use larval habitats other than the edges of ponds, puddles, freshwater lakes and saltwater lakes. Furthermore, the conditions of field collection but also the transport and holding at the insectarium of substrate samples from larval habitats to monitor the emergence of adult *Culicoides* could influence the selection of the species found.

Interestingly, three main species of veterinary interest, namely, *C. oxystoma*, *C. imicola*, and *C. kingi* were found together in all larval habitat groups. *Culicoides imicola* is considered to be the major vector species for orbiviruses of animal health importance, such as BTV affecting ruminants (Venter et al., 2006, 1998) and AHSV affecting equidae (Paweska et al., 2003; Venter et al., 2000, 2009; Venter and Paweska, 2007). In the case of *C. kingi*, it is involved in the transmission of *Onchocerca gutturosa*, a widespread parasite of cattle in Sudan (Sinnary and Hussein, 1980). *Culicoides oxystoma* is a well-known vector of bovine arboviruses such as Akabane virus in Asia (Oem et al., 2013; Yanase et al., 2005) and is suspected to be the vector of Epizootic Haemorragic Disease Virus (EHDV) in Israel (Morag et al., 2012). These vectors could play an important role in the transmission of AHSV in the

Niayes area of Senegal. Previous studies in this area have shown that *C. imicola* and *C. oxystoma* are strongly attracted to horses (Bakhoum et al., 2016b; Fall et al., 2015b; Fall et al., 2015c) in contrast to *C. kingi* which appeared to favour cattle but can also feed on horses in the absence of cattle (Bakhoum et al., 2016b). Consequently, the ability of these 3 species of veterinary interest to develop in a wide range of larval habitat could be one of the main constraints to the implementation of vector control methods targeting larval habitats in order to prevent the animal arboviroses they transmit.

In the present study, analysis of the spatio-temporal dynamics of emerging *Culicoides* species revealed significant variations in density over time within groups of larval habitat. Our results highlight that the ubiquity of certain *Culicoides* species such as *C. imicola*, *C. kingi* and *C. oxystoma* contributes to increasing the risk of transmission of pathogens of veterinary interest, namely AHSV in the Niayes area of Senegal. In the Niayes area, the larval habitats of these species are the puddle and pond edges. Such habitats are dispersed, extensive, natural (hosting important non-target fauna) and for some, temporary with strong fluctuations depending on rainfall or human activities through agricultural practices. These characteristics are a challenge for larval control, unlike other *Culicoides* that only develop in very specific and delimited larval sites such as dung or manure species. Further studies are needed to clarify the spatial and temporal productivity of these habitats. The majority of ubiquitous *Culicoides* species that can be controlled at the larval stage live in microhabitats that have been accurately defined by long-term studies such as *C. sonorensis* in North America (Schmidtmann et al., 2000). Recent field studies conducted in Italy against *C. imicola* have shown the potential of biorational larvicides (diflubenzuron and *Bacillus thuringiensis* var. israelensis (H-14)) with low environmental impact as a control tool that can be part of an integrated strategy including other innovative methods directed against adults or to reduce host-vector contact (Meloni et al., 2018).

Author contributions

M.T.B., G.G. and A.G.F. designed the study. M.T.B. analysis the data. A.G.F, M.T.S., M.F., M.C., C.G. contributed to the manuscript written by M.T.B., G.G., J.B. and T.B. All authors read and commented the final manuscript version.

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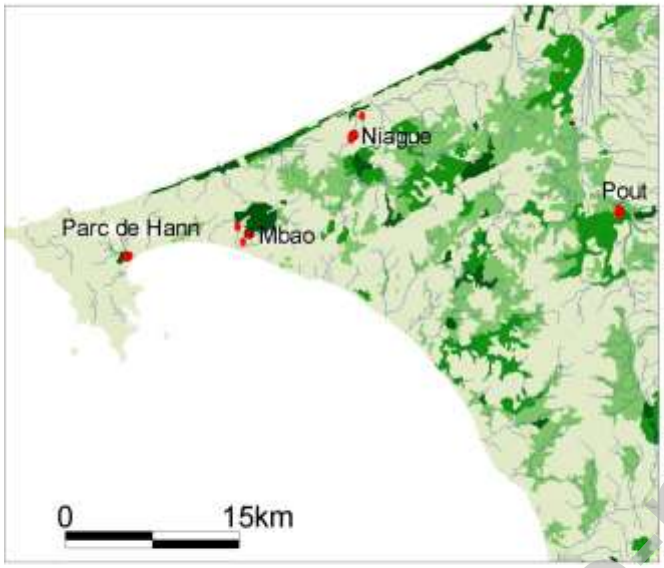

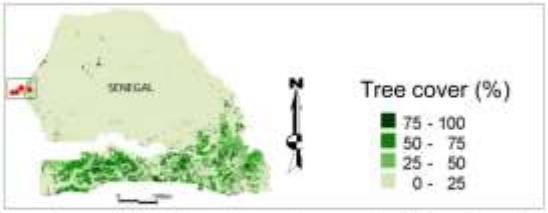

Locality	Larval habitat	Latitude	Longitude	Type of Larval habitat
Mbao	MB-1	14.74511	-17.33286	Pond edge
	MB-2	14.73427	-17.32978	Saltwater lake edge
	MB-3	14.73431	-17.326633	Saltwater lake edge
Niague	NG-1	14.833854	-17.247484	Saltwater lake edge
	NG-2	14.82199	-17.25184	Pond edge
	NG-3	14.8223	-17.25139	Pond edge
	NG-4	14.82394	-17.25113	Pond edge
	NG-5	14.82383	-17.251178	Pond edge
Parc de Hann	PH-1	14.72946	-17.43185	Freshwater lake edge
	PH-2	14.72878	-17.43101	Freshwater lake edge
Pout	PT-1	14.766433	-17.035385	Puddle edge
	PT-2	14.76633	-17.036605	Puddle edge
	PT-3	14.766037	-17.035527	Puddle edge
	PT-4	14.766309	-17.034959	Puddle edge

Figure 1. Geographic location of the four localities in the south of the Niayes area in Senegal (Parc de Hann, Mbao, Niague, and Pout) where larval habitats have been studied

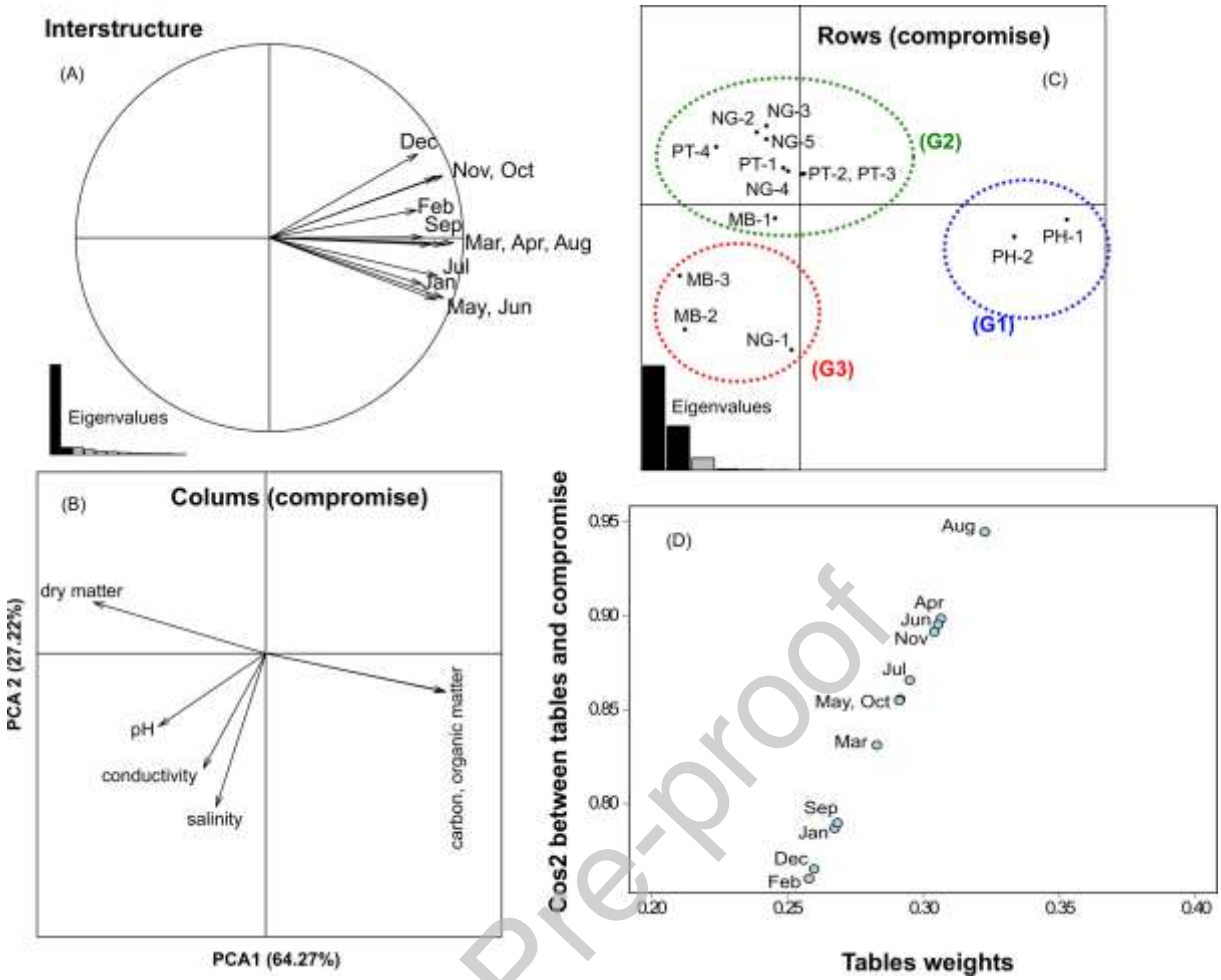

Figure 2. Interstructure month ordination from vector correlation coefficients (A), ordination plots of the first two axes (PCA1 = 64.27% and PCA2 = 27.22%) of the Principal Component Analysis of the compromise table (B and C), and table weights and cos2 values for each month represented in a scatter plot (D)

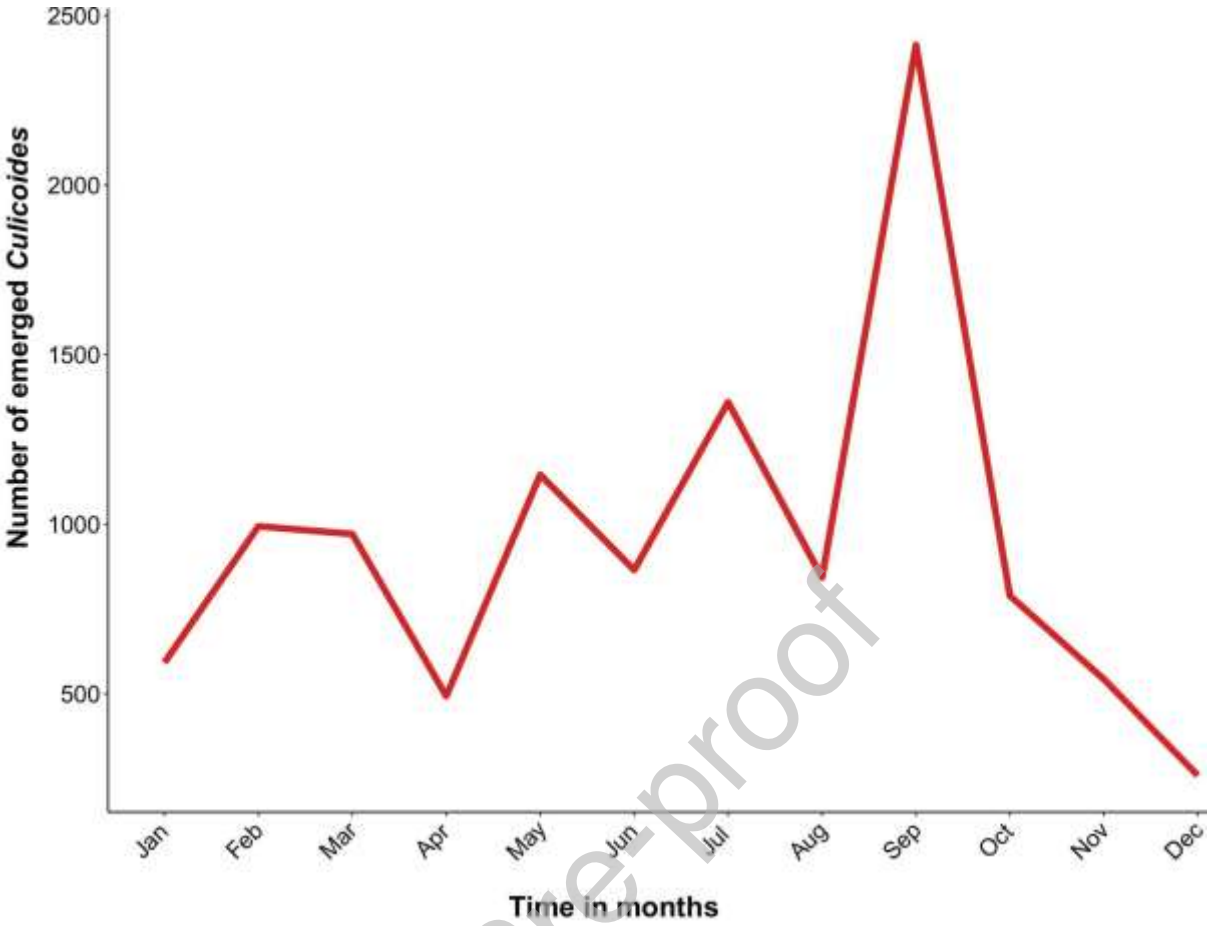

Figure 3. Variation of emergence of the adults over the one year period

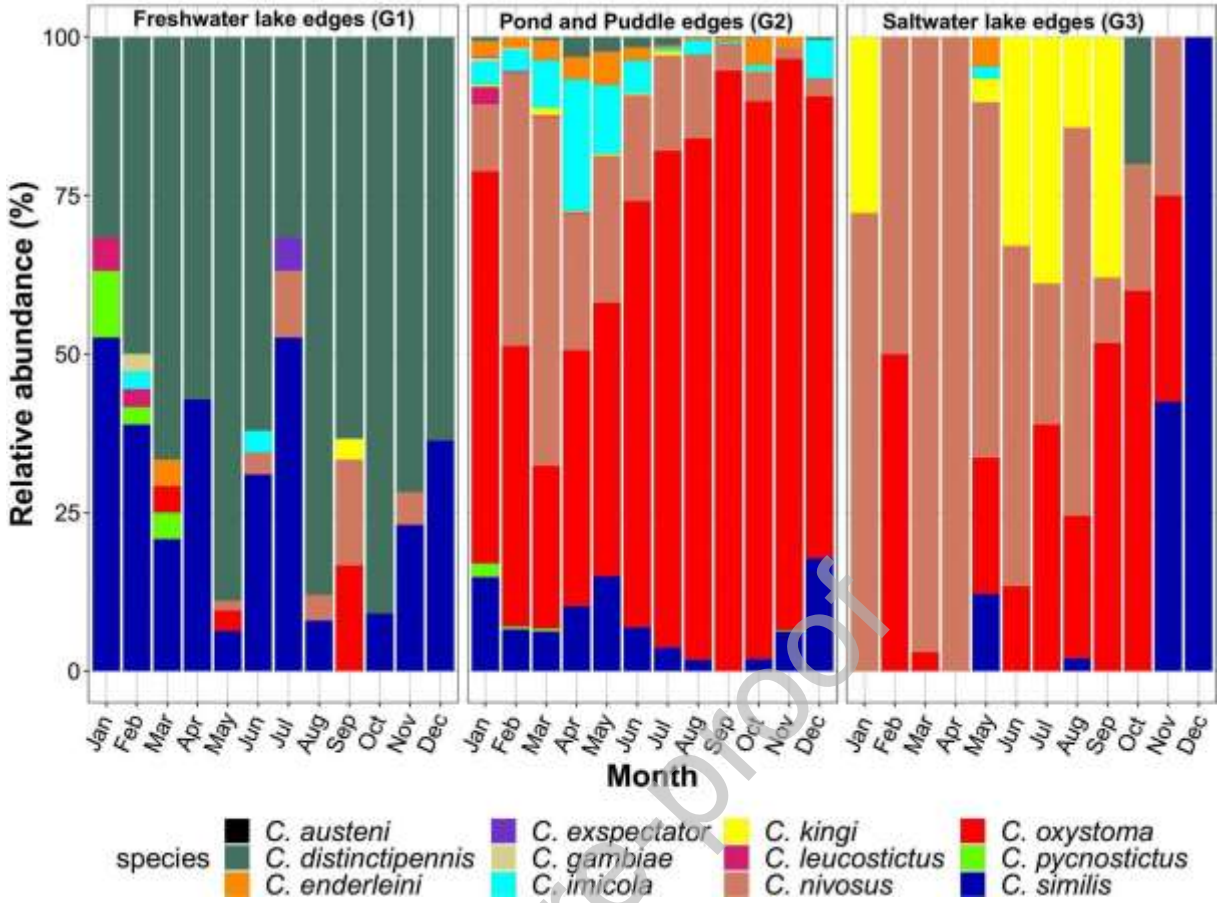

Figure 4. Temporal variations in emerging *Culicoides* species dominance in the three larval habitat groups sampled from January to December 2015

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Group of larval habitats

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Table 1. Characteristics of the three groups of larval habitats and diversity of emerging *Culicoides* in the different groups

		Group 1 (PH-1 and PH-2)	**Group 2** (MB-1, NG-2, NG-3, NG-4, NG-5, PT-1, PT-2, PT-3, and PT-4)
Characteristics	Type of larval habitat	Freshwater lake edge	Pond and Puddle edges
	Dray matter	24.06 ± 2.21	71.17 ± 2.74
	pH	6.05 ± 0.67	7.25 ± 0.2
	Conductivity	1.58 ± 0.29	3.18 ± 2.25
	Salinity	0.29 ± 0.09	0.21 ± 0.09
	Carbon	5.04 ± 0.65	1.10 ± 0.21
	Organic matter	8.70 ± 1.13	1.92 ± 0.38
	Number of emerged *Culicoides*	332	10474
	Culicoides larvae	96	4469
Diversity	AD (sp/h/d)	6.92	48.49
	Specific richness observed (S)	11	12
	Shannon index (H)	1.04	1.01
	Specific richness estimated (NS)	11.75 ± 1.41	12 ± 0.23

Table 2. Composition and density of emerging *Culicoides* species in each larval habitat group. The index d value of the dominant species is marked in bold

	Number of specimen				**Dominance index d**		
	G1	**G2**	**G3**	**Sex ratio**	**G1**	**G2**	**G3**
C. austeni	0	2	0	2♀	0.000	0.000	0.000
C. distinctipennis	216	77	1	130♂ / 164♀	**0.651**	0.007	0.002
C. enderleini	1	204	5	27♂ / 183♀	0.003	0.019	0.011
C. exspectator	2	1	0	1♂ / 2♀	0.006	0.000	0.000
C. gambiae	1	6	0	1♂ / 6♀	0.003	0.001	0.000
C. imicola	2	415	2	197♂ / 222♀	0.006	0.039	0.004
C. kingi	1	30	71	29♂ / 73♀	0.003	0.003	**0.154**
C. leucostictus	2	19	0	14♂ / 7♀	0.006	0.002	0.000
C. nivosus	14	1,855	265	931♂ / 1,203♀	0.042	**0.177**	**0.574**
C. oxystoma	8	7,252	86	3,547♂ / 3,799♀	0.024	**0.692**	**0.186**
C. pycnostictus	4	20	0	11♂ / 13♀	0.012	0.002	0.000
C. similis	81	593	32	312♂ / 394♀	**0.244**	0.057	0.069

Group 1 (G1) = freshwater lake edges; Group 2 (G2) = pond and puddle edges; and Group 3 (G3) = saltwater lake edges

Table 3. Relationships between physicochemical parameters and the abundance of emerging *Culicoides* species based on Generalized Linear Mixed-Effects Models using a Poisson regression mixed-effect model with random effects at the larval habitat groups (G1, G2 and G3) and level of sampling months (2/3 of the sample was used for the training dataset and the remaining 1/3 of the sample for the test dataset). (*p-value <0.05; **p-value<0.01***p-value< 0.001)

	Fixed effects				
		Estimate	Std. Error	z value	Pr(>|z|)
***Culicoides* larvae**	(Intercept)	-8.23	1.05	-7.84	4.46e-15 ***
	pH	1.14	0.05	23.09	< 2e-16 ***
	Organic matter	0.40	0.02	21.13	< 2e-16 ***
	Salinity	-0.28	0.06	-4.92	8.55e-07 ***
	Dry matter	0.00	0.00	0.24	0.808
Abundance of emerged *Culicoides*	(Intercept)	-3.55	1.25	-2.83	0.005 **
	pH	0.92	0.03	33.21	< 2e-16 ***
	Organic matter	0.40	0.01	37.09	< 2e-16 ***
	Salinity	-1.93	0.07	-27.17	< 2e-16 ***
	Dry matter	-0.02	0.00	-15.37	< 2e-16 ***
***Culicoides* diversity**	(Intercept)	0.14	0.69	0.20	0.84326
	pH	0.19	0.07	2.71	0.007 **
	Organic matter	-0.01	0.04	-0.13	0.89653
	Salinity	-0.65	0.12	-5.40	6.65e-08 ***
	Dry matter	0.00	0.01	-0.66	0.51077

Author Statement

M. T. Bakhoum, G. Gimonneau and A. G. Fall designed the study. M. T. Bakhoum analysis the data. A. G. Fall, M. T. Seck, M. Fall, M. Ciss, C. Garros contributed to the manuscript written by M. T. Bakhoum, G. Gimonneau, J. Bouyer and T. Baldet All authors read and commented the final manuscript version.

Declaration of interests

☑ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: