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Distribution and larval habitat characterization of *Anopheles moucheti*, *Anopheles nili*, and other malaria vectors in river networks of southern Cameroon

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ABSTRACT

Despite their importance as malaria vectors, little is known of the bionomic of *Anopheles nili* and *Anopheles moucheti*. Larval collections from 24 sites situated along the dense hydrographic network of south Cameroon were examined to assess key ecological factors associated with these mosquitoes distribution in river networks. Morphological identification of the III and IV instar larvae by the use of microscopy revealed that 47.6% of the larvae belong to *An. nili* and 22.6% to *An. moucheti*. Five variables were significantly involved with species distribution, the pace of flow of the river (lotic, or lentic), the light exposure (sunny or shady), vegetation (presence or absence of vegetation) the temperature and the presence or absence of debris. Using canonical correspondence analysis, it appeared that lotic rivers, exposed to light, with vegetation or debris were the best predictors of *An. nili* larval abundance. Whereas, *An. moucheti* and *An. ovengensis* were highly associated with lentic rivers, low temperature, having Pistia. *An. nili* and *An. moucheti* distribution along river systems across south Cameroon was highly correlated with environmental variables. The distribution of *An. nili* conforms to that of a generalist species which is adapted to exploiting a variety of environmental conditions, Whereas, *An. moucheti*, *Anopheles ovengensis* and *Anopheles carnevalei* appeared as specialist forest mosquitoes.

1. Introduction

The spatial and temporal distribution of Anopheline mosquitoes is dependent on the availability of suitable aquatic habitats where the immature stages can develop. Rivers constitute an important larval habitat for several species of Anophelines of medical importance. Two of the most important vectors of human malaria in the equatorial forest domain of central Africa, *Anopheles nili* and *Anopheles moucheti*, occur in sympatry along river networks where their immature stages are usually found at the edge of large rivers or islands within. These mosquitoes are found in forest villages and have a broad distribution from Nigeria to Uganda, with *An. nili* extending its range in the savannas of Western and Eastern Africa (Gillies and De Meillon, 1968). *An. moucheti* depends strongly on human blood and tends to bite and rest indoors, whereas *An. nili* is largely exophilic and feeds on a variety of vertebrates, yet with a high proportion of blood meals taken on humans. Despite the public health importance of both *An. nili* and *An. moucheti* (Fontenille and Simard, 2004; Awono-Ambene et al., 2004; Antonio-Nkondjio et al., 2002, 2006), there is still little knowledge of their larval biology. A good understanding of larval habitat selection can provide relevant information about areas that are at higher risk of malaria transmission. Such knowledge could improve control strategies targeting these vectors. It is generally recognized that *An. nili* larvae are usually found in the vegetation or dense shade along the edges of fast running streams, whereas *An. moucheti* larvae are mainly found associated to floating vegetation of slow-moving streams or rivers (Gillies and De Meillon, 1968). A quantitative framework formalizing such traditional observations, however, is still lacking. In addition to *An. nili* and *An. moucheti*, several other anophelines co-occur in the same aquatic habitats, and some of these species have been found to contribute significantly to malaria transmission (Antonio-Nkondjio et al., 2006). Given the current trend of expansion of human settlements and deforestation, it is possible that an increasing number of people will be at risk of malaria due to transmission by these vectors. Moreover, human-induced large-scale environmental changes such as the construction of hydroelectric dams, several of which are planned to be built in south Cameroon during the next decade, could contribute to increase the epidemiological role of these mosquitoes by extending the availability of suitable lar-

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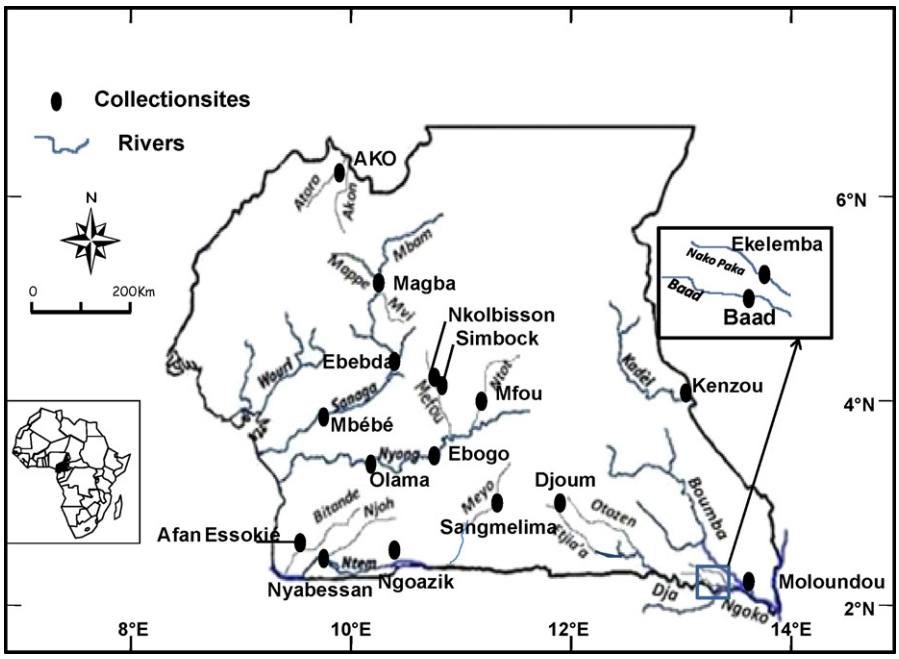

Fig. 1. Distribution of collection sites along river networks across south Cameroon.

val habitats and by allowing the expansion of their distribution range.

Although *An. nili* and *An. moucheti* are still susceptible to most of the insecticides used for bed-net impregnation (Etang et al., unpublished data), this advocated control tactics might prove less efficient due to the high exophagy and partial endophily of these forest vectors, inasmuch the indoor-spraying campaigns with DDT and dieldrine of the 1950s in southern Cameroon (Livadas et al., 1958). Because of the menace of insecticide resistance and the heterogeneity of the vectorial system across Africa, an optimized integrated approach using an array of tools including indoor residual spraying or larviciding to supplement the use of insecticide-treated nets (ITNs) is envisaged for sustainable vector control (Fillinger et al., 2008; N'Guessan et al., 2007). Whatever the control strategy of choice, a good understanding of the influence of environmental factors on vector bionomics, and their spatial and temporal distribution and dynamics is important for the implementation of efficacious and sustainable vector control. The main objective of this study was to determine the spatial distribution of larval habitats and associated environmental parameters that influence the occurrence and abundance of anopheline mosquitoes of medical importance along river networks in the forest of southern Cameroon.

2. Materials and methods

2.1. Study sites

Anopheline larvae were sampled in 24 sites situated along the dense hydrographic network of south Cameroon: Simbock (3°49′N, 11°28′E) and Nkolbisson (3°52′N, 11°27′E) along river Mefou; Ebebda (4°21′N, 11°16′E) and Mbebe (4°10′N, 11°04′E) along river Sanaga; Ebogo (3°25′N, 11°25′E) and Olama (3°26′N, 11°17′E) along river Nyong; Magba (5°57′N, 11°13′E) along rivers Mappe, Mvi and Mbam; Ako (6°49′N, 10°43′E) along rivers Atoro and Akon; Nyabessan (2°24′N, 10°23′E) along rivers Ntem and Ndjoh; Ngoazik (2°18′N, 11°18′E) along river Ntem; Moloundou (2°02′N, 15°12′E) along rivers Boumba and Ngoko; Djoum (2°40′N, 12°40′E) along rivers Etjia'a and Otozen; Kentzou (4°09′N, 15°02′E) along river Kadei; Baad (1°59′N, 15°43′E) along river Baad; Afan-Essokié (2°22′N, 9°59′E) along river Bitande; Ekelemba (1°49′N, 15°54′E) along river Nako-Paka; Sangmelima (2°56′N, 11°58′E) along river Meyo; and Mfou (3°58′N, 11°56′E) along river Ntot (Fig. 1). Magba and Ako are situated in a highland area, whereas all other study sites are located within the Congo-Guinean phytogeographic zone characterized by a typical equatorial climate with two rainy seasons extending from March to June and from September to November. Mean annual rainfall is >1500 mm in all sites. Collections were conducted from November 2006 to March 2007, a period encompassing the long dry season (50–100 mm rainfall per month) during which rivers are on the decay, providing optimal breeding opportunities for members of the *An. nili* and *An. moucheti* groups (Antonio-Nkondjio, 2003).

2.2. Habitat characterization

Larval habitats were characterized either visually or by the use of hand-held field equipment (HI98204, Hanna Instruments). Nominal environmental variables used to characterize each river network were: light exposure (sunny or shady), river flow (lotic or lentic), turbidity (turbid or clear), substrate (muddy, sandy or gravel), debris (present or absent), and vegetation (presence of *Pistia* sp., *Paspalum* sp. or no vegetation). Continuous variables included temperature, pH, conductivity and redox potential measured at the time of larval sampling between 10 and 15 cm depth.

2.3. Larval collections and mosquitoes identification

Larva collections were made following WHO malaria entomology field sampling protocols (WHO, 1975), sampling in each site for 1–2 h. For each sample, we took 80–100 dips along the edges of the river using a standard 350 ml dipper. Larvae were stored in bottles containing water from the habitat, and were then brought back to the laboratory for species identification. Third and fourth instar larvae were identified using morphological identification keys (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987). Sibling species

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Table 1
Characteristics of river networks in south Cameroon.

Rivers	Physical properties		Water properties							Vegetation/debris
	River size	Light exposure	Lentic/Lotic	Clear/turbid	Substrate	pH ±0.2	*T*, °C ±1	Conductivity ±0.02	Redox ±2	
Sanaga	Big	Sunny	Lotic	Turbid (reddish)	Muddy	7.2	24.9	80	187	*Pistia stratiotes*/floating debris
Mefou	Small	Sunny	Lotic	Turbid (dark)	Muddy	6.6	24.4	77	135	*Paspallum* sp.
Nyong	Big	Sunny	Lentic	Turbid (dark)	Muddy	5.9	25.3	22	202	*Pistia stratiotes*
Ntot	Small	Shady	Lotic	Clear/slightly turbid	Sandy	4.6	21.2	14	310	*Paspalum*
Etjia a	Small	Shady	Lotic	Clear/slightly turbid	Sandy	6.6	20.6	25	112	*Paspalum* sp.
Otozen	Small	Shady	Lotic	Clear/slightly turbid	Sandy	6.5	24.1	19	162	*Paspalum* sp.
Meyo	Small	Shady	Lotic	Clear/slightly turbid	Muddy	5.5	21.5	18	208	*Paspalum* sp.
Ntem	Big	Sunny	Lentic/slightly lotic	Turbid (dark)	Muddy	5.4	23.3	14	207	*Pistia stratiotes*/*Paspalum* sp.
Njoh	Medium	Shady	Lentic	Turbid (dark)	Muddy	5.5	23.8	22	199	*Pistia stratiotes*/*Paspalum* sp.
Bitande	Small	Shady	Lotic	Clear	Sandy	4.5	25.6	18	216	*Paspallum* sp.
Kadei	Big	Sunny	Lotic	Slightly turbid	Muddy	8	26.6	20	112	Floating organic debris
Nako-Paka	Small	Shady	Lotic	Clear	Gravel	7.7	25.2	156	127	Dead leaves
Baad	Small	Shady	Lotic	Clear	Gravel	7.6	24.5	364	131	Dead leaves
Ngoko	Big	Sunny	Lotic	Turbid (dark)	Sandy/Muddy	7.1	24.9	119	100	Jacinth/floating organic debris
Boumba	Big	Sunny	Lentic	Turbid (dark)	Sandy	7.6	26.3	127	181	Floating organic debris
Atoro	Medium	Sunny	Lotic	Clear	Sandy	7.5	30.2	79	149	*Paspalum*
Akon	Small	Sunny	Lotic	Clear	Sandy	7.4	27.7	75	153	*Paspalum*
Mappe	Big	Sunny	Lotic	Turbid (reddish)	Muddy	6.3	24.7	43	164	*Paspalum* floating debris
Mbam	Big	Sunny	Lotic	Turbid (reddish)	Muddy	7	26.7	78	182	*Paspalum*
Mvi	Medium	Sunny	Lotic	Clear/slightly turbid	Sandy	7.5	30.6	77	148	Floating organic debris

Big: >40 m, medium: between 10 and 40 m, small: <10 m. Redox: oxydo redox potential.

of the *An. nili* and *An. moucheti* groups were further identified by molecular analysis (Kengne et al., 2003, 2007).

2.4. Statistical analysis

Statistical analyses were done using the software CANOCO version 4.5 (Ter Braak and Smilaurer, 2002). Nominal variables were defined by series of indicators or dummy variables, one dummy variable for each category. A dummy variable takes the value of one when the sample belongs to a given corresponding category and the value zero otherwise. Prior to our analysis, species data were squareroot transformed to prevent a few abundance values from unduly influencing the analysis. Detrended correspondence analysis (DCA) was used to determine the correlation between species distribution or species composition in collection sites and environmental variables or covariates. Canonical correspondence analysis (CCA) was then used to extract environmental variables that could explain mosquito distribution in river networks via an ordination diagram. This multivariate method is able to detect unimodal relationships between several species and external variables. It is particularly suited for a forward selection of environmental variables in order to determine which variables best explained the species data (Ter Braak, 1986). Statistical validity of resulting environmental axes of the model and of the selected environmental variables explaining the variation of mosquito species abundance were evaluated by means of unrestricted Monte Carlo permutation tests ($n = 499$).

3. Results

A total of 20 rivers systems were sampled in 24 sites in the south Cameroon forest region. The characteristics of each river network sampled are shown in Table 1. Although most of the river surface was generally exposed to sunlight, the edge was often in shade because of surrounding trees. Water turbidity rose from very turbid in big rivers to clear in small streams. Larvae were abundant in river sections close to villages or human habitations and abundance decreased sharply when moving away from habitations. In all of these river networks larvae were sampled near the edges.

A total of 2269 anopheline mosquito larvae at the late instars were collected. Up to nine species were identified: *An. moucheti*, *An. nili* ss, *Anopheles ovengensis*, *Anopheles carnevalei*, *Anopheles somalicus*, *Anopheles funestus*, *Anopheles paludis*, *Anopheles marshallii* and *Anopheles cinctus*. The *An. paludis* specimens may also refer to *Anopheles ziemanni* and *Anopheles coustani* since their larvae are morphologically indistinguishable (Gillies and Coetzee, 1987). Among the most abundant species, were *An. nili* representing 47.6% of the identified and *An. moucheti* accounting for 22.6% (Table 2).

Ordination by Detrended Correspondence Analysis (DCA) was used to analyze the distribution and mutual relationships between species and samples. The graphic representation showed some degree of clustering between species: at one extreme are species that are characteristic of the deep evergreen rainforest, such as *An. moucheti*, *An. ovengensis*, and *An. cinctus*, whereas at the other extreme are species that can also be found at the forest/savanna transition and northern up in the more arid savannas, such as *An. funestus*, *An. nili*, and *An. somalicus*. *An. paludis* and *An. marshallii* lie between these clusters (Fig. 2).

The ordination diagram performed under the DCA accounted for 50.4% of the total variance. But, by applying the CCA species-environment correlations increased considerably with the first two axes explaining over 86% of the total variance. The first axis defined a gradient with fast-flowing, clear, more acid and cooler water having sandy substrates and shady sites with no debris and emergent *Paspalum* vegetation at one extreme (right end of the axis), and at

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Table 2
Anopheles mosquitoes larvae species composition in river networks across south Cameroon based on morphological identification of late instar larvae.

Vegetation	Rivers	Collection sites	Anopheline species								
			An. moucheti	*An. nili*	*An. ovengensis*	*An. carnevalei*	*An. somalicus*	*An. funestus*	*An. paludis*	*An. cinctus*	*An. marshallii*
Deep forest	Nyong	Olama	108	0	0	0	0	0	8	0	0
Deep forest	Nyong	Ebogo	100	0	0	0	0	0	18	0	7
Deep forest	Etjia'a	Djoum	0	0	0	0	0	0	2	30	0
Deep forest	Otozen	Djoum	7	0	0	0	0	0	0	15	0
Deep forest	Meyo	Sangmelima	5	0	0	0	0	0	0	92	0
Deep forest	Ntem	Ngoazik	93	0	11	0	0	0	10	0	0
Deep forest	Ntem	Nyabessan	39	0	53	0	0	0	16	0	0
Deep forest	Njoh	Nyabessan	88	0	3	0	0	0	4	1	0
Deep forest	Bitande	Afan-Essokié	0	6	0	28	0	0	0	51	0
Deep forest	Nako-Paka	Ekelemba	0	6	0	40	0	0	10	1	0
Deep forest	Baad	Baad	0	0	0	3	0	0	9	0	0
Deep forest	Ngoko	Moloundou	19	5	0	0	0	0	0	0	0
Deep forest	Boumba	Moloundou	0	12	0	50	0	0	1	0	0
Degraded forest	Sanaga	Mbébé	0	90	0	0	1	0	8	3	28
Degraded forest	Sanaga	Ebebda	0	100	0	0	0	0	9	0	0
Degraded forest	Mefou	Nkolbisson	0	500	0	0	0	0	9	0	0
Degraded forest	Mefou	Simbock	50	119	0	0	0	0	0	0	0
Degraded forest	Ntot	Mfou	0	0	0	0	0	0	2	52	0
Degraded forest	Kadei	Kenzou	4	38	0	0	0	0	37	0	0
Savanna	Atoro	Ako	0	130	0	0	0	11	0	0	0
Savanna	Akon	Ako	0	35	0	0	0	35	11	0	0
Savanna	Mappe	Magba	0	27	0	0	1	2	1	0	0
Savanna	Mbam	Magba	0	0	0	0	0	0	0	0	0
Savanna	Mvi	Magba	0	13	0	0	0	0	2	0	0
		Total	513	1081	67	121	2	48	157	245	35
		%	22.6%	47.6%	2.9%	5.3%	0.09%	2.1%	6.9%	10.8%	1.5%

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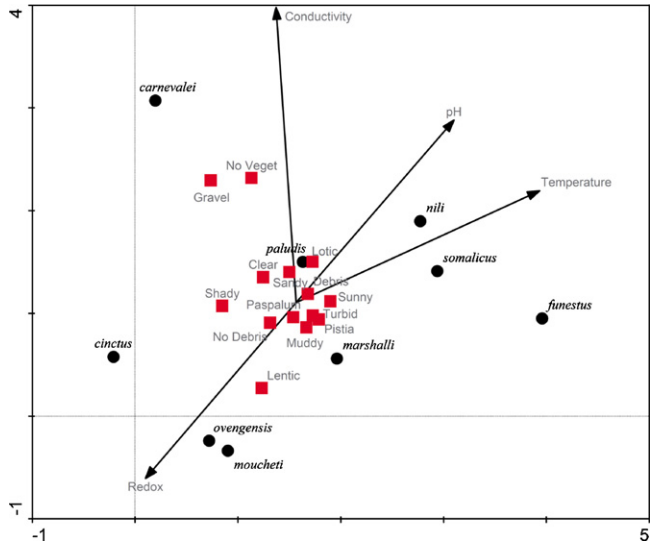

Fig. 2. Detrended correspondence analysis (DCA) diagram showing the distribution of species in relation with environmental variables. The first axis is horizontal, second vertical. Qualitative predictor variables (■) mesurable variables (arrows).

the opposite end sites characterized by slow-moving, more basic and warmer water, with muddy substrates exposed to the sun and floating *Pistia* vegetation (left end of the axis). The second environmental gradient was essentially associated to absence of vegetation in the water, and higher values of conductivity or lower values of redox potential (Fig. 3). Five variables were significantly associated with species distribution ($P = 0.002$): pace of flow, light exposure, vegetation (present/absent), debris (present/absent) and temperature. Using CCA it appeared that lotic rivers exposed to sunlight, with vegetation or debris were the best predictors of *An. nili* larval abundance. Whereas, *An. moucheti* and *An. ovengensis* were highly associated with lentic rivers, low temperature, and the presence of *Pistia* sp. A strong differentiation pattern between the larval biology of the four members of the *An. nili* group was detected. *An. nili* and *An. somalicus* were associated with warmer and lotic rivers situated in degraded forested areas, *An. carnevalei* was found in shady and lotic rivers with low temperature.

Unimodal response curves (Fig. 4) for the probability of occurrence along measurable variables fitted by correspondence analysis permitted to detect *An. nili* and *An. moucheti* optima for temperature, pH, Redox potential and conductivity. For these analysis, the software proceeds by assuming environmental measurable data

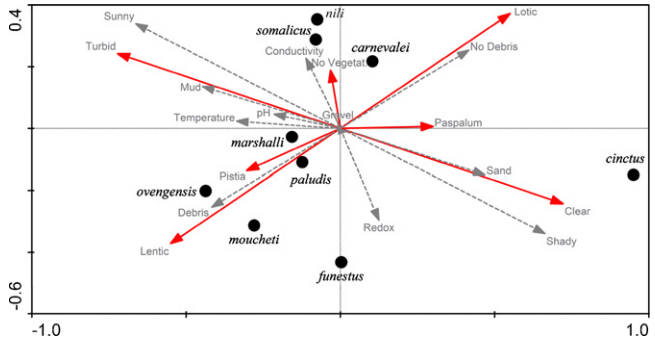

Fig. 3. Canonical correspondence analysis (CCA) diagram showing the ordination of anopheline species along the first two axes and their correlation with environmental variables. The first axis is horizontal, second vertical. Direction and length of arrows shows the degree of correlation between mosquito larvae and the variables (example: *An. cinctus* is positively correlated with shady waters having no debris and clear and negatively correlated by the exposition to sun and temperature).

to be independent Poisson variables with expected x values and applies a loglinear model known precisely as the Gaussian response curves that gives a species responses (tolerance) along a gradient (Gauch, 1982). These species presented large differences in their tolerance to these factors. No optimum was detected for *An. nili* for temperature meaning that the sampled interval was not amply contained in the interval of the optima.

4. Discussion

An. nili and *An. moucheti* distribution along river networks across south Cameroon was highly correlated with environmental variables. *An. moucheti* was the only member of the group identified in all study sites. Within the *An. nili* group, all four members of the group were collected: *An. nili*, *An. carnevalei*, *An. somalicus* and *An. ovengensis*. *An. nili* larvae were collected in sympatry with *An. somalicus* and *An. carnevalei*. These data were in full agreement with the distribution of adult anopheline mosquitoes in south Cameroon (Hervy et al., 1998; Antonio-Nkondjio et al., 2006). Of the nine species sampled during this study, no *Anopheles gambiae* was collected although adult *An. gambiae* specimens have been recorded from the area. These findings are in agreement with the known biology of the species which does not use river systems as breeding sites. However it could exploit residual marshes created in the river bed during the dry season for it immature stages development (Edillo et al., 2006).

The use of both CCA and DCA permitted to approximate the maximum likehood solution of explicit unimodal response models in one latent variable using logistic linear for presence/absence data and loglinear for poison counts. The method proceeds by using a two-step approach termed as indirect gradient analysis: extract from the species data the dominant pattern of variation in community composition and attempt to relate this pattern to the environmental variables (Gauch, 1982). The use of DCA permitted to remove nonlinear dependencies between axes such that species show a unimodal response curves or surfaces with respect to these axes (Ter Braak, 1986). Although approximation is best when the following conditions are respected: the maxima and tolerances of the response curves are equal, the species optima and the sample values of latent variables are equally spaced or if optima are uniformly distributed. Some violations to these assumptions were recorded with *An. nili* and *An. moucheti* failing to give a shaped response curve for temperature. As Hill and Gauch (1980) stated, the first two conditions (C1 and C2) are not likely to hold in most natural communities but the usefulness of correspondence analysis in practice relies on its robustness against violations to these conditions. Differences in species distribution were strongly associated with the characteristics of each river system. However as was reported in other studies (Simard et al., 2009), local descriptors variables related to human activity were found to modify species distribution along the same river system. For instance although *An. nili* and *An. moucheti* could overlap in areas colonized by one another they were never collected in both high proportions in the same site except in few cases such as Simbock where deviation of the Mefou river network for the creation of fishing lakes contributed in increasing significantly the abundance of *An. moucheti* while upstream (Nkolbisson) *An. nili* was the predominant species. This observation was consistent with the fact that although both species do not share similar habitat requirements, they could take advantage of anthropogenic changes to expand. Five parameters were described as key discriminate factors by CCA: pace of flow, light exposure, vegetation (present/absent), debris (present/absent) and temperature. *An. nili* and *An. funestus* appeared to be positively correlated with water temperature which reflected greater exposure to the sun of rivers flowing through

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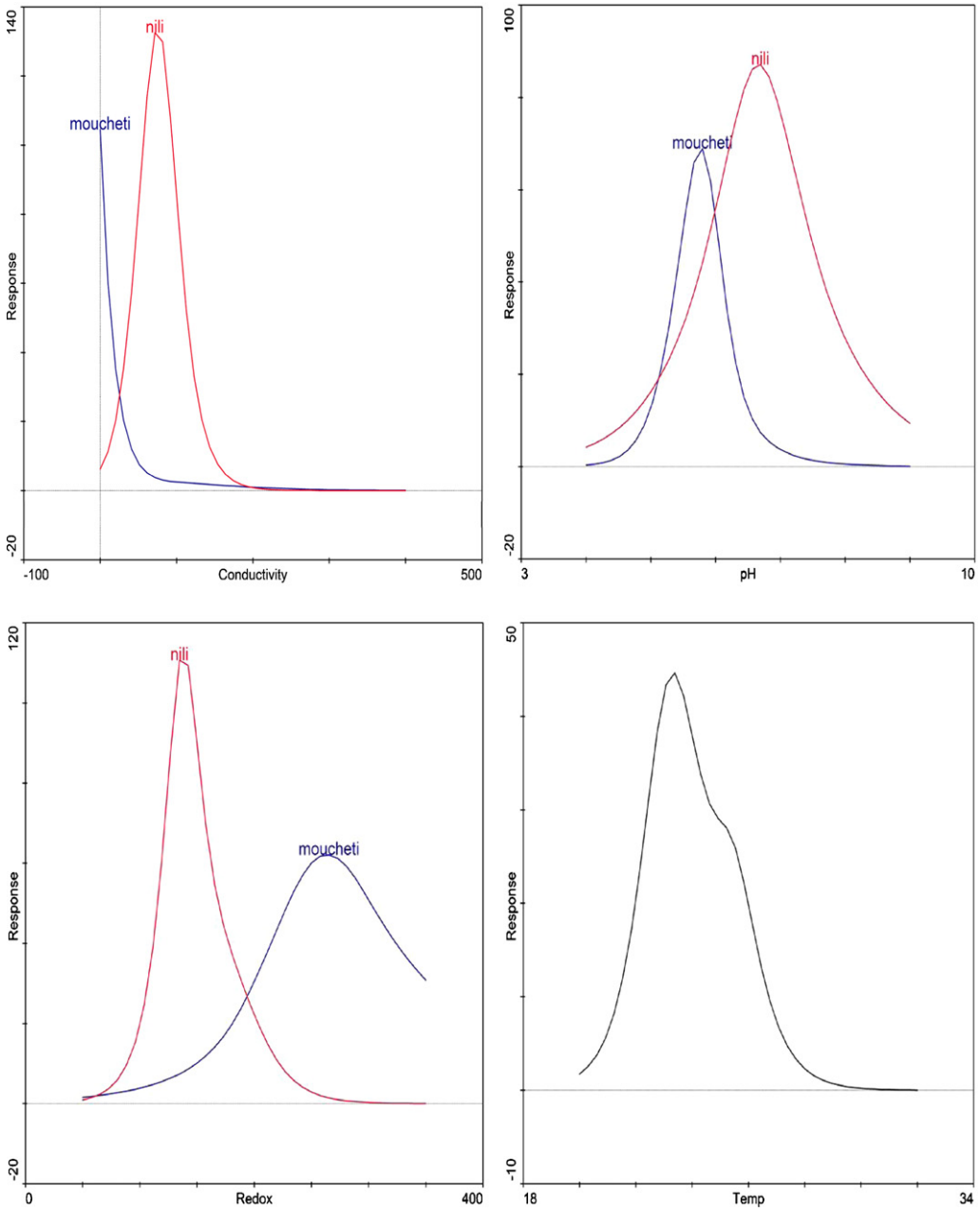

Fig. 4. Unimodal response curves for the probability of occurrence of *An. moucheti* and *An. nili* in relation with: conductivity, pH, oxydo redox potential and temperature (Redox = oxydo redox potential, Temp = temperature).

periforested or savanna areas. Whereas *An. moucheti*, *An. ovengensis*, *An. carnevalei* and *An. cinctus* showed a negative correlation with water temperature which is consistent with vegetation covering river systems in forested areas. The characteristic of the river running flow (lotic/lentic) was a key discriminating factor for the segregation of *An. moucheti* and *An. nili*. Within *An. nili* group, the characteristic of the river flow lentic/lotic further improve separation of larval preference of *An. ovengensis* and *An. carnevalei* all found in deep forested areas. Anopheline larvae were abundant in areas with an emergent vegetation or floating debris. The possible explanation to this may be determined by the oviposition behavior of gravid females. *An. gambiae* females preferentially select small open habitat for oviposition probably because larval predation is less prevalent in temporary habitats than it is in large and permanent habitats (Bentley and Day, 1989; Service, 1977). However, even though the presence of an emergent vegetation could lower predator risk and reduce the risk of been driven by rivers running flow, this area also concentrate a number of other invertebrate species which could be important as predators or competitors for anopheline larvae (Carlson et al., 2004). Although significant associations were reported between some mosquitoes larva and the presence of some aquatic plants (Gillies and De Meillon, 1968), it is common to find high density of larva in crypts or rocks at edges of rivers without any vegetation supporting further investigations into the relationship between mosquitoes larva and their close environment.

Although we used over 10 variables for our analysis only five were found to be significantly associated with species distribution. The remaining variables were not quantified due to the fact that they were not related strongly to the principal axes and thus cannot account for main part of the variation in species composition and may not be easy to detect in an indirect gradient analysis. These

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limitations can only be overcome by methods of direct gradient analysis in which species occurrences are directly related to each environmental variable (Gauch, 1982). However, when the number of species is large, separate regression analyses for each species may be impractical. Moreover, separate analyses cannot be combined easily to get an overview of how species composition varies with multiple environmental variables and a multivariate method based on a common response model is required.

This study allowed us to more finely separate types of habitats showing interdependency between environmental variables and the distribution of *An. nili* and *An. moucheti* in the south Cameroon forest region. Thus the distribution of *An. nili* conforms to that of a generalist species which is adapted to exploiting a variety of environmental conditions. However, *An. nili* was abundant in the humid savanna area and highly degraded forest area and scarce through the deep forest zone. In this area, it was replaced by other members of its group namely *An. carnevalei* and *An. ovengensis*. Its scarcity through this area may be likely due to the fact that this species does not adapt to deep forest areas and was consistent with recent microsatellites analysis showing a low genetic diversity of it populations in forested areas compared to those collected in highly degraded forest or savanna areas (Ndo et al., unpublished data); Whereas, *An. moucheti*, *An. ovengensis* and *An. carnevalei* appeared as specialist forest mosquitoes.

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