PRIMARY RESEARCH PAPER

Environmental drivers for *Coquillettidia* mosquito habitat selection: a method to highlight key field factors

Julien Sérandour · John Willison ·
Wilfried Thuiller · Patrick Ravanel ·
Guy Lempérière · Muriel Raveton

Abstract A crucial component for developing insect management strategies is the understanding of the ecological parameters involved in habitat selection by proliferating species. The key ecological drivers underlying habitat selection in the mosquito *Coquillettidia* sp. have been investigated *in natura*. Vegetation analysis suggested that the most suitable habitats were ponds with a high vegetation cover maintaining a high degree of humidity in the air. The optimal biotope for *Coquillettidia* was associated with the presence of larval host plants such as *Typha* sp., *Phragmites* sp., and *Juncus* sp. Water quality was also found to be a key factor in larval habitat distribution. The presence of larvae was significantly correlated with physico-chemical factors and the optimal water characteristics were neutral pH, low salt concentration, and a relatively low level of suspended particulate matter. A significant correlation was observed between chemical cues and the *Coquillettidia* distribution pattern. For instance, 2,6-di-*tert*-butyl-*p*-cresol was positively correlated to larval habitat, whereas high lauric acid and heptadecanoic acid concentrations may be limiting factors. This study underlines the fact that mosquito habitat selection is driven by a complex process based on discriminating levels of several ecological factors. Multivariate analysis helps understand such processes, which is this case will assist in managing expanding populations of a species that threatens human health.

Handling editor: S. A. Halse

J. Sérandour · W. Thuiller · P. Ravanel ·
M. Raveton (✉)
Laboratoire d'Ecologie Alpine, UMR CNRS 5553,
Université Joseph Fourier, BP 53,
38041 Grenoble Cedex 09, France
e-mail: muriel.raveton@ujf-grenoble.fr

J. Willison
Laboratoire de Chimie et Biologie des Métaux, UMR
5249 UJF/CEA/CNRS, Institut de Recherches et
Technologies en Sciences du Vivant, CEA-Grenoble,
17 rue des Martyrs, 38054 Grenoble Cedex 9, France

G. Lempérière
Laboratoire GEOPHEN, UMR CNRS 6554, Université de
Caen-Basse-Normandie, BP 8156, 14032 Caen Cedex,
France

Introduction

In organisms with no parental care, like insects, the development of pre-imaginal stages depends strongly on the suitability of the habitats in which they have been deposited. Adults would be expected to choose the most favorable habitats, this being a critical factor both for the survival of the progeny and in population dynamics (Clements, 1999; Reiskind & Wilson, 2004).

A number of studies have investigated behavioral selection of habitat by female mosquitoes with a view to facilitating mosquito control. Field studies have demonstrated that mosquitoes have the ability to discriminate between suitable habitats for egg deposition, thus determining the distribution of larval populations (Bentley & Day, 1989). This breeding site selection is the result of the recognition of physical and chemical key factors. Physical factors (optical density, temperature, reflectance) have long been recognized as critical for the choice of breeding site (Clements, 1999). Moreover, for many mosquito species, this site selection is influenced by the presence of chemical substances of a wide range of origins (Bentley & Day, 1989). Chemical signals have been widely investigated in laboratory studies, resulting in a long list of molecules acting either as attractants and stimulants, such as terpenes (Davis & Bowen, 1994), fatty acid esters (Ganesan et al., 2006) and pheromones (Prasad & Anbarasan, 2007) or as deterrents and repellents, for example, salts (Navarro et al., 2003), carboxylic acids (Hwang et al., 1982),

and phytochemicals (Sukumar et al., 1991). Studies of the criteria involved in mosquito habitat selection have been carried out mainly on species of public health concern, such as *Culex* sp., *Toxorhynchites* sp., *Anopheles* sp., *Aedes* sp., and *Wyeomyia* sp. (Clements, 1999) and show that site selection is strongly species dependent.

The proliferating mosquito genus, *Coquillettidia* (Diptera: Culicidae) (Brothers, 2005; LaPointe, 2007) has been linked to a more recent public health issue. This genus is a potential vector of West Nile virus (Mandalakas et al., 2005; Cupp et al., 2007), which is a re-emergent disease in Europe (Romania, Czech Republic, Italy) (Hubálek & Halouzka, 1999; Murgue et al., 2001). Since 1999, new equine and human cases have been detected in Southern of France (Fig. 1a). The spatial distribution of *Coquillettidia* sp. is in part correlated with areas experiencing West Nile fever (Fig. 1a). An efficient control of these mosquito populations appears essential. However, control proves extremely difficult because the occurrence of larval habitat is difficult to determine with larvae

Fig. 1 Location of The Bourget Lake in France (**a**) and the 20 wetlands where *Coquillettidia* sp. larvae were collected between 2003–2007 (**b**). Superscript a: West Nile fever is a reemerging disease in South of France according to Hubálek & Halouzka (1999) and Murgue et al. (2001). Superscript b: Results obtained from sociological study of mosquito nuisances. The *Coquillettidia* sp. adult numbers (average/year) were measured in 24 h trapping periods (Claeys-Mekdade & Sérandour, 2009)
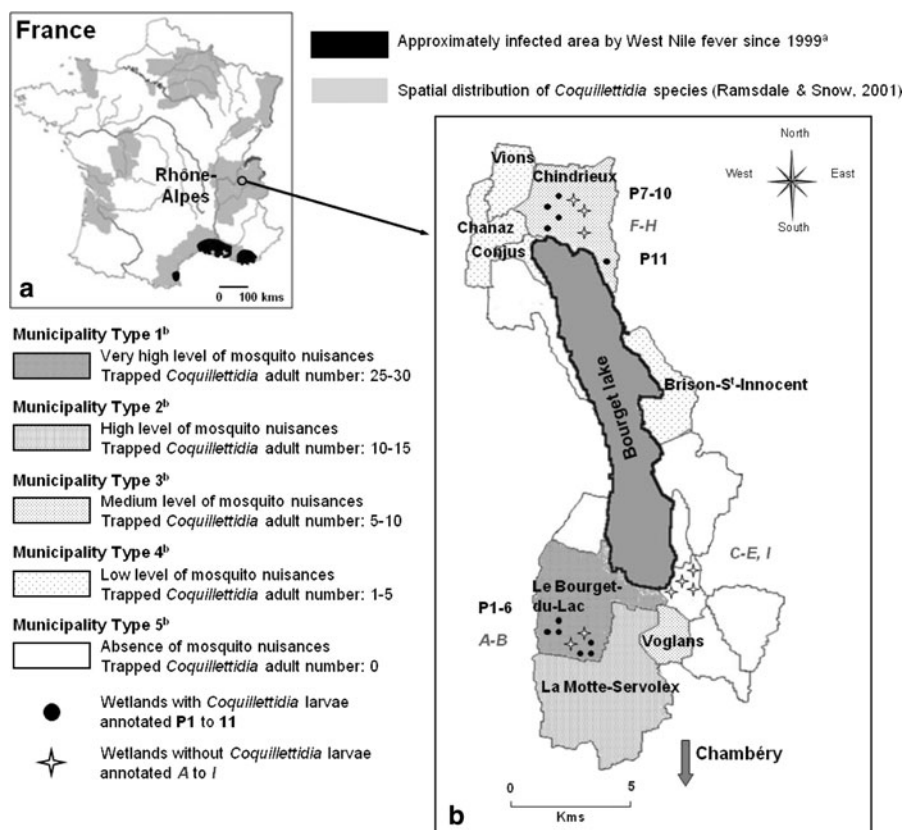
undetectable from the water surface (Brothers, 2005; LaPointe, 2007). The larval and pupae stages are localized at the bottom of densely overgrown aquatic habitats where they remain attached to root macrophytes in deep nutrient-rich and hypoxic sediments (Sérandour et al., 2006, 2008). This insect–plant interaction appears to be regulated by the need of the continuously submerged larvae to find oxygen in the aerenchymal channels of roots (Laurence, 1960). *Coquillettidia* habitats have been very little studied compared to those of other mosquito genera. Early studies focussed on the nature and distribution of host plants (Batzer & Sjogren, 1986; Callahan & Morris, 1987) and the influence of abiotic conditions, such as water chemistry and environmental factors has only been addressed recently with respect to *Coquillettidia* habitat description (Callahan & Morris, 1987; Bosak et al., 2001; Medlock et al., 2005).

Determination of the key environmental factors involved in the colonisation of aquatic habitats by *Coquillettidia* is important in evaluating how the interaction of abiotic and biotic parameters influences habitat selection and organism distribution. Multiple environmental factors may influence habitat selection and cross-interactions between these factors may modulate this selection. Based on a field survey of adult and larval *Coquillettidia* distribution across a broad region (Rhône-Alpes, France; Fig. 1a, b), 20 wetland plots were selected to test the importance of ecological, physical, and chemical factors in habitat selection by *Coquillettidia* mosquitoes (Fig. 1b). In this study, multivariate analysis was used to investigate the environmental correlates of site selection by *Coquillettidia*, in order to determine which environmental variables are the most suitable for predicting larval establishment. In particular, the following two questions were addressed: (i) what is the importance of vegetation cover and abundance to site selection by mosquitoes? (ii) what are the most critical factors explaining habitat suitability for larvae?

Materials and methods

Study areas

The occurrence of *Coquillettidia* larval populations was recorded in all the permanent wetlands in the vicinity of The Bourget lake (Savoie, France) between 2003 and 2007 (Fig. 1b). This study provided a map of the mosquito distribution and allowed us to select 20 natural plots in a homogeneous climatic and geomorphologic area (210 km^2, average altitude: 230 m, geographical localization: 45°N–5°E). The selection of plots was based on the presence/absence of *Coquillettidia* larvae (plots noted P1–P11 and *A–I*, respectively). *Coquillettidia* larvae were sampled following the Morozov method where specimens were collected by pulling up host plants and manually sorting the larvae from the collected aqueous sediment (Service, 1993). The geographical locations of the wetlands are given in Fig. 1 and their descriptions in Table 1. Previous sociological studies in this area revealed the existence of nuisances linked to mosquito biological cycle (Claeys-Mekdade & Sérandour, 2009). *Coquillettidia* adults were captured (light trap

Table 1 Site descriptions (Bourget lake, Savoie, France) and *Coquillettidia* distribution

Plot	Insolation	*Coquillettidia* larvae	Bank vegetation (CI)		Trench plants (CI)
			Trees	Herbaceous plants	
P1	Sunny	Yes	3	2	2
P2	Shady	Yes	3	1	2
P3	Shady	Yes	3	1	5
P4	Shady	Yes	3	2	2
P5	Sunny	Yes	3	2	3
P6	Sunny	Yes	4	1	3
P7	Shady	Yes	4	1	2
P8	Shady	Yes	4	1	1
P9	Sunny	Yes	3	4	2
P10	Sunny	Yes	2	3	3
P11	Sunny	Yes	2	3	1
A	Shady	No	2	1	2
B	Shady	No	4	2	2
C	Shady	No	3	1	1
D	Shady	No	3	1	1
E	Sunny	No	+	2	2
F	Shady	No	2	1	2
G	Sunny	No	2	2	2
H	Sunny	No	+	2	2
I	Sunny	No	+	1	3

CI cover index was expressed in function of plant cover percentage, (+) = 0–1%, (1) = 1–5%, (2) = 5–25%, (3) = 25–50%, (4) = 50–75%, (5) = 75–100%

Fig. 2 Pond characteristics and parameters measured *in natura*
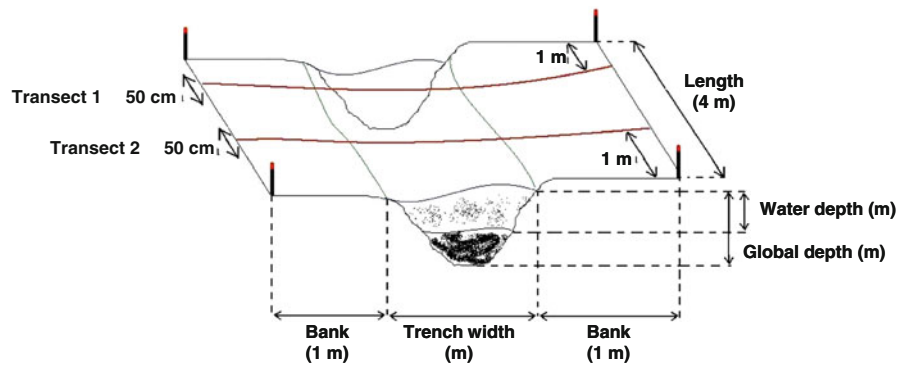
experiments) in the districts with a very high to low mosquito nuisance, but were absent in other districts (Fig. 1b).

Each plot was delimited with a length range of 4 m in which plant species distribution and determination were noted along two transects (50 cm width) (Fig. 2). Plant abundance was expressed in terms of percentage coverage (Cover Index, CI) using a standardized protocol (Braun-Blanquet scores; Bell et al., 2008) and divided into six categories with the following CI: (+) = 0–1%, (1) = 1–5%, (2) = 5–25%, (3) = 25–50%, (4) = 50–75%, (5) = 75–100%. Abiotic parameters were measured and water samples were collected in the centre of the station delimiting by the two transects.

Sampling methods

Climatic factors (temperature, humidity) were measured at three different points in each plot using a thermohygrometer (Carl-Roth) placed at 10 cm above the water surface and at 50 cm above the bank soil. Physical characteristics of each site (trench depth, water flow) were also measured in triplicate.

Water parameters i.e. temperature (°C), dissolved O_2 concentration (mg l^{-1}), conductivity (μS cm^{-1}) and pH, were measured in triplicate at 5 cm below the surface using a multi-parameter probe designed for field use (SenTix® 41-3; Multi 350i; WTW). The relationship between atmospheric temperature and water temperature was regularly assessed in order to normalize temperature measurements made at different times of day.

Water samples (15 ml, triplicates) were collected 5 cm below the water surface to measure the levels of Suspended Particulate Matters (SPM), nitrates and

nitrites concentrations. Samples were filtered with 0.5 μm cellulose filter papers (Millipore). SPM retained on the filter were desiccated (80°C, 24 h) and weighed. The SPM were expressed in mg l^{-1}. Nitrate (Spectroquant 1.14776.0001 kit, Merck) and nitrite (Spectroquant 1.14773.0001 kit, Merck) concentrations were determined in the filtered water samples.

Chemical analyses

Water samples of 100 ml were collected from each plot at 5 cm below the water surface, then filtered (0.5 μm, cellulose filter paper, Millipore) and freeze-dried in the laboratory. The powder obtained after lyophilization was resuspended in 50 μl acetonitrile and 100 μl BSTFA-TCMS (99:1) reagent (Supelco). The derivatization (silylation) reaction was carried out as described previously (Sérandour et al., 2008).

GC–MS analyses were carried out on a HP6840/HP5973 apparatus (Agilent Technologies, Les Ulis, France) equipped with an MDN-12 fused silica capillary column (30 m, 0.25 mm internal diameter, 0.25 μm film; Supelco). The oven temperature was held at 70°C for 4.5 min, then increased to 240°C at a rate of 50°C min^{-1} and held for a further 20 min (Injector temperature: 250°C; Detector temperature: 280°C). Mass spectral analyses were carried out using the NIST/EPA/NIH Mass Spectral Library, Version 2.0d, 2005.

Statistical procedures

A statistical screening procedure was used to test plant species abundance and coverage in selected stretches of water. The abundance index for each

plant species was compiled in a contingency table in order to remove minor plant species from the data set. The contribution of major group (tree and herbaceous) cover was assessed using a non-Gaussian test (Mann–Whitney, Statview 4.57.0.0). Correspondence Analysis (CA, ter Braak, 1985) ordinations were performed on the species association data characterizing *Coquillettidia* ponds (XLSTAT 2007.5).

The correlation between the presence/absence of *Coquillettidia* larvae and the physico-chemical parameters of the ponds was evaluated. The parameters were first classified into two groups, corresponding to ponds with or without *Coquillettidia*. The Gaussian distribution of the data was analyzed using a Shapiro–Wilk test (XLSTAT 2007.5). Data with a Gaussian distribution were tested using the Student t test (Statview 4.57.0.0) and non-Gaussian data were tested using the Mann–Whitney test (Statview 4.57.0.0). These tests identified environmental factors that might be involved in the distribution of *Coquillettidia* larvae in the selected plots.

A Canonical Correspondence Analysis (CCA, ter Braak, 1986) was performed on the plant species associations and environmental factor variables (XLSTAT 2007.5) which have previously been found to be correlated with the presence of *Coquillettidia* larvae, in order to obtain an overall description of *Coquillettidia* ponds.

Results

Testing for plants factors

The taxa present both in the bank and trench vegetation of each plot were identified. The composition of the bank vegetation in each plot was directly influenced by the plant community of the surrounding countryside (Table 1). Plots P1, P2, P3, P4, P5 were wet meadows with a strong percentage cover of Graminaceae (*Glyceria fluitans*, *Dactyle* sp., *Poa trivialis*). Plots P7, P8, P9, P10, A, B had a high level of tree cover (*Populus* sp). Plots G, H were characterized by the presence of species from recently formed wetlands (*Rumex* sp., *Iris* sp., *Hippuris* sp.). Plots C, D, E, F, I, P11 were dominated by species typical of *Phragmites* wetlands (*Phragmites* sp., *Carex* sp.).

Bank tree cover seemed to be a parameter describing *Coquillettidia* habitats, since a strong tree cover

index (CI: 3–4) was determined for sites containing larvae (Mann–Whitney, $U = 22.5$, $P = 0.031$). On the other hand, the CI of bank herbaceous plants did not seem to affect the selection of habitat (Mann–Whitney, $U = 38.5$, $P = 0.36$). On the banks, 18 dominant plant taxa were determined, which corresponded to species commonly found in aquatic ecosystems. The CA with bank plant species did not allow us to identify a plant species group characteristic of plots colonised by *Coquillettidia* (Fig. 3a).

Trench plants corresponded to 11 taxa with a variable degree of cover (Table 1). As a consequence, the aquatic plant CI did not seem to be a key factor of habitat selection (Mann–Whitney: $U = 37.5$, $P = 0.31$). The trench plant species CA revealed that some specific taxa, such as *Sparganium erectum* and *Carex* sp., were present only in plots without *Coquillettidia* (Fig. 3b). Some plant species were present in both plot types: *Juncus effusus*, *Juncus inflexus* and *Phragmites australis*. The plots with *Coquillettidia* were characterized by species such as *Juncus effusus* (10 out of 11 occurences), *Typha latifolia* (8/11), and *Phragmites australis* (7/11). At least one of these three species was found in sites with *Coquillettidia* larvae. The CA allowed the identification of plant assemblages typically found in *Coquillettidia* habitats: *J. effusus–T. latifolia* (8 occurrences on 11), *J. effusus–P. australis* (6/11), *J. effusus–T. latifolia–P. australis* (5/11).

Testing for pond environmental factors

Environmental parameters statistically selected as potential key factors were as follows: water depth and flow, temperature (index: air temperature/water temperature), relative humidity above bank and trench. Significant correlation between environmental factors and the presence/absence of larvae revealed that some of these parameters might be characteristic of *Coquillettidia* habitats (Table 2).

The water depth in the 20 selected ponds varied from 5.7 to 40 cm. However, water depth did not seem to play a role in the *Coquillettidia* larval distribution (Table 2). Water movement ($6–10$ m min^{-1}) seemed to be a limiting factor for *Coquillettidia* habitat, as no larvae were found in plots with moving water (Table 2).

Air humidity was also measured above the bank and trench (10 cm above the water surface). Bank

Fig. 3 Factorial Correspondence Analysis ordination diagram of the sites studied (*Coquillettidia* plots: *filled circle* P1–P11; non-*Coquillettidia* plots: *filled square* A–I) and vegetation taxa (*open triangle*) constituted of bank (**a**) and trench (**b**) ponds

Author's personal copy
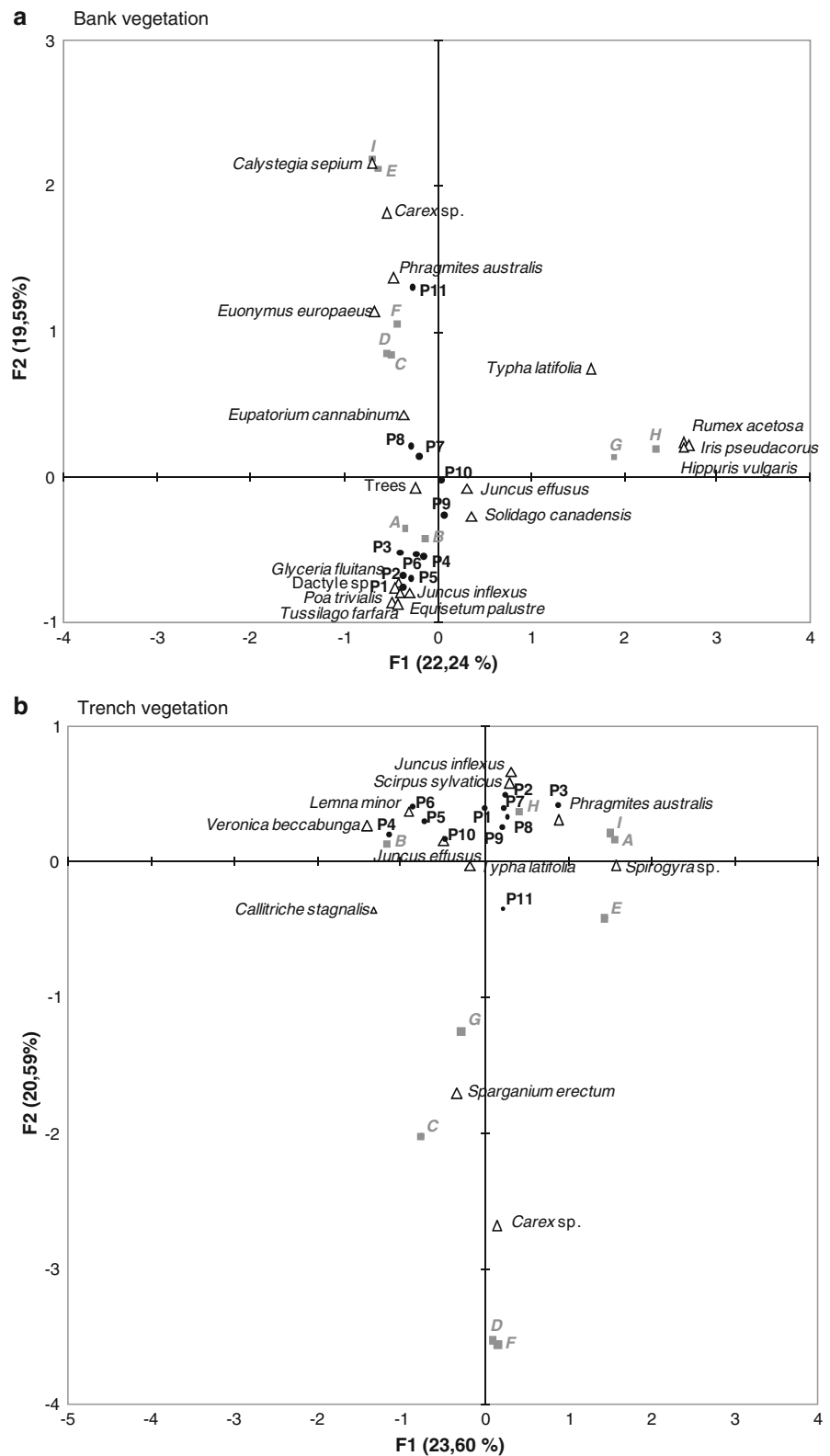
humidity varied from 21 to 66%, showing an average of $50 \pm 11.8\%$ in ponds with *Coquillettidia* and $33 \pm 10.3\%$ in ponds without *Coquillettidia*. This factor seemed to be correlated with the presence or absence of *Coquillettidia* larvae ($t = 3.324$, 17 ddl, $P = 0.004$). Moreover, the relative humidity above the trench was also a significant factor (Table 2). Overall, air humidity (bank and trench) was higher in ponds colonized by *Coquillettidia* larvae.

Testing for water physico-chemical factors

The mean oxygen concentrations measured below the water surface (5 cm) were low, ranging from 0–7.8 mg l^{-1} (Table 2). In ponds with *Coquillettidia*, oxygen water concentrations were slightly lower (1.59 ± 2.2 mg l^{-1}) than in ponds without *Coquillettidia* (2.61 ± 2.9 mg l^{-1}) but this difference was not significant. SPM levels were measured in water with an average of 124 ± 27.1 mg l^{-1} in ponds with *Coquillettidia* and 260 ± 206.2 mg l^{-1} in ponds without *Coquillettidia*. In some plots without *Coquillettidia*, some extreme concentrations were observed; with higher concentrations reaching 491 ± 123 and 711 ± 339 mg l^{-1}. A high concentration of SPM seemed to be a significant limiting factor for the presence of larvae ($t = -2.174$, 18 ddl, $P = 0.043$).

Conductivity (linked to water salinity) was another factor of water quality determinant for the presence of larvae. Indeed, conductivity measurements differed greatly between plots, giving conductivity values of 515 ± 66.5 µS cm^{-1} and 947 ± 656 µS cm^{-1} in plots with and without *Coquillettidia*, respectively (Table 2). Salinity/conductivity significantly influenced the presence of larvae in ponds (Mann–Whitney, $U = 21.5$, $P = 0.033$).

GC-MS analyses of water samples from the 20 selected ponds enabled us to establish a list of the major compounds that could be detected (Table 3). These molecules were tested (Mann–Whitney) in order to establish if their presence/absence or concentration in water samples were correlated to *Coquillettidia* larvae presence/absence. Most of the compounds did not seem to be correlated to the *Coquillettidia* larvae habitat ($P > 0.05$; Table 3).

Compounds from the fatty acid group were detected in all ponds and mostly comprised both medium (C_5–C_9) and long (C_{10}–C_{18}) chain molecules. The fatty acid group as a whole did not influence the presence/absence of *Coquillettidia* larvae (Mann–Whitney, $U = 40$, $P = 0.47$). However, testing each fatty acid compound separately showed that low concentrations of lauric acid (Mann–Whitney, $U = 21$, $P = 0.043$) and heptadecanoic acid (Mann–Whitney, $U = 17$, $P = 0.040$) might be characteristic of ponds with *Coquillettidia*. Finally, the presence of a third compound, 2,6-di-*tert*-butyl-*p*-cresol, seemed to be correlated with the *Coquillettidia* habitat: (Mann–Whitney, $U = 18$, $P = 0.017$).

Table 2 Significance of environmental factors with respect to *Coquillettidia* distribution

Parameter	Plots P1–P11			Plots A–I			*Coquillettidia* presence/absence × parameter
	Mean	Minimum	Maximum	Mean	Minimum	Maximum	
Conductivity (µS cm^{-1})	514.9	383	606	946.7	483	2143	[a]$U = 20$, $P = 0.025$
Trench humidity (%)	49.07	26	69	33.76	20	51	[a]$U = 20$, $P = 0.041$
SPM (mg ml^{-1})	124	76	153	260	100	711	[b]$t = -2.174$, 18 ddl, $P = 0.043$
Water flow (cm s^{-1})	0	0	0	4.28	0	10	[a]$U = 33$, $P = 0.044$
Nitrates (mg l^{-1})	2.39	0	6.86	5.18	0	10.7	[b]$t = -2.059$, 18 ddl, $P = 0.054$
Nitrites (µg l^{-1})	1.1	0	7.8	14.6	0	99	[b]$t = -1.404$, 18 ddl, $P = 0.178$
Water depth (cm)	19.81	5.3	40	12.19	2.7	39	[b]$t = 1.507$, 18 ddl, $P = 0.149$
Oxygen (mg l^{-1})	1.59	0	7	2.61	0	7.8	[a]$U = 42.5$, $P = 0.595$
Temperature atmosphere/water	1.76	1.4	2.1	1.82	1.3	2.2	[b]$t = -0.430$, 17 ddl, $P = 0.672$
pH	7.39	7.1	7.6	7.38	6.9	7.9	[b]$t = 0.034$, 18 ddl, $P = 0.973$

[a] Non-Gaussian distribution of values (Shapiro–Wilk); *P*-value determined using Mann–Whitney test

[b] Gaussian distribution of values (Shapiro–Wilk); *P*-value determined using Student's *t* test

Table 3 Analysis of aquatic semiochemicals implicated in *Coquillettidia* habitat selection

Molecules	*Coquillettidia* larvae presence/absence × molecule	
	U	P value
Diethylene glycol	4	0.48
1-Phenyl-1-ethanol	1	1
Glycerol	22.5	0.32
Hexadecan-1-ol	3	0.64
Phenol	0	0.32
2,6-Di-*tert*-butyl-*p*-cresol	**18**	**0.017***
1.4-(1,1,3,3-Tetramethylbutyl)phenol	34	0.23
Oxalic acid	0	0.22
Acetic acid	0	1
Carbodiimide	0	1
n-Butylamine	31	0.92
Urea	0	0.22
2,4,6-Trimethylpyridine	6	0.08
o-Toluic acid	48	0.91
p-Hydroxybenzoic acid	2	1
Phthalates as group	38	0.38
1,2-Diethyl phthalate	42	0.57
1,2-Diisobutyl phthalate	19	0.12
1,2-Didodecyl phthalate	27	0.91
Fatty acids as a group	40	0.47
Methylmalonic acid	8	0.35
Levulinic acid	1	0.65
Lactic acid	21	0.42
Caproic acid	4	0.83
Nonanoic acid	35	0.27
Capric acid	43	0.62
Laurie acid	**21**	**0.043***
Myristic acid	49	0.97
Pentadecanoic acid	42	0.57
Palmitic acid	48	0.91
Heptadecanoic acid	**17**	**0.04***
Stearic acid	36.5	0.32
Unsaturated fatty acids as a group	47	0.84
Palmitelaidic acid	58	0.55
Linoleic acid	30	0.56
Oleic acid	36	0.51
Fatty acid esters as a group	33	0.21
1-Glyceryl laurate	45	0.73
1-Glyceryl myristate	27	0.09
2-Monopalmitine	48.5	0.94

Table 3 continued

Molecules	*Coquillettidia* larvae presence/absence × molecule	
	U	P value
1-Monopalmitine	42.5	0.59
2-Monostearine	46	0.79
2,3-Bis monostearine	40	0.47

Data were analysed using the Mann–Whitney test

Significance levels: * $P < 0.05$

Discussion

The potential risk of *Coquillettidia* population expansion highlights the need to characterize their larval habitat for effective control (Brothers, 2005; LaPointe, 2007). Larvae and pupae of this genus are relatively immobile and do not obtain air at the water surface, as do other genera, but rather they obtain it from the underwater roots of aquatic plants to which they are able to adhere (Laurence, 1960). Therefore, their detection in natural wetlands is extremely difficult.

One of the main goals of this study was to determine the role of vegetation cover (notably identify key plant species) in habitat selection by *Coquillettidia* mosquitoes. For this purpose, the distribution of trees, helophytes and hydrophytes was determined in the natural sites studied. A key factor appears to be a high Cover Index (3–4) of the canopy sheltering the habitat from wind, and variations in temperature and sunlight. Hydrophytes (genera: *Lemna*, *Veronica*, *Alisma*, *Glyceria*) did not seem to be a key factor in characterizing *Coquillettidia* habitats. A high coverage of the water surface by *Lemna minor* might be a parameter supporting the female choice for oviposition. This plant species is not a suitable support for larvae because the roots are too thin and are located in the upper water layer (Sérandour et al., 2006). However, the abundance of floating vegetation cover might be a relevant factor by modifying the spectral reflectance of the water and thus affecting the optical response of gravid females, as has been shown with *Anopheles arabiensis* (Jacob et al., 2007), *Aedes aegypti* (Clements, 1999), and *Wyeomyia* sp. (Frank, 1986). Significant negative *Coquillettidia*–vegetation associations were also measured with helophytes such as

Sparganium erectum and *Carex* sp. which have been identified as potential host plants by Callahan & Morris (1987). Habitats of *Coquillettidia* larvae seem to be characterized by at least three major genera of helophytes (*Typha*, *Juncus*, *Phragmites*) in the studied area. All these species are known to be host plants for the larval stage and have been identified in *Coquillettidia* ponds in Denmark (Wesenberg-Lund, 1920), in Great Britain (Marshall, 1938) and in the French Mediterranean district. In the study area, the association of *T. latifolia–J. effusus–P. australis* was the main vegetation parameter associated with higher numbers of *Coquillettidia* larvae. This plant association and its high coverage were a significant indicator of the *Coquillettidia* habitat permitting to aquatic stages a reliable fixation and oxygen source for their survival.

Plant associations and cover level were essential parameters in the initial selection of plots for study in terms of the biological status and maturity of marshes. This simple analysis is not precise enough to clearly identify *Coquillettidia* habitats. Therefore, CCA was performed using data on the presence/ absence of *Coquillettidia* larvae together with a selection of physico-chemical parameters (Fig. 4). The CCA revealed that a high level of atmospheric

humidity, most likely maintained by the vegetation cover on the bank, was a factor supporting *Coquillettidia* female choice. This factor is not specific to *Coquillettidia* species, and it is an ecological parameter involved in the selection of oviposition site for several mosquito species such as *Aedes* (Madeira et al., 2002) and *Toxorhynchites* (Jordan, 1992).

The water flow (mean 6–10 m min^{-1}) was a significant negative factor for the presence of larvae, although Batzer & Sjogren (1986) succeeded in collecting some *Cq. perturbans* larvae from areas of water with a similar flow. An explanation for the absence of larvae in flowing water flows may be that eggs and larvae are carried away before they are able to reach and attach to the deeper root layer. On the other hand, stagnant and eutrophic waters characterized by high SPM concentrations seemed unsuitable for the establishment of *Coquillettidia* larvae. Finally, the salinity/conductivity of the marsh water was also involved in the colonisation by mosquitoes since a high level of salinity was a negative factor.

Under laboratory conditions, water salinity did not have a negative impact on the oviposition behavior of *Cq. richiardii* gravid females (EID Montpellier-France, personal communication). Nevertheless, ecological studies demonstrated that *Coquillettidia* sp.

Fig. 4 Ordination biplot of Canonical Correspondence Analysis (CCA) with sites studied (*Coquillettidia* plots: *filled circle* P1–P11; non-*Coquillettidia* plots: *filled square* A–I) and environmental factors. Length of the vectors indicates the strength of the correlation between categories of factors and sites studied. Axis 1 allowed us to determine environmental factors characterizing non-*Coquillettidia* habitat
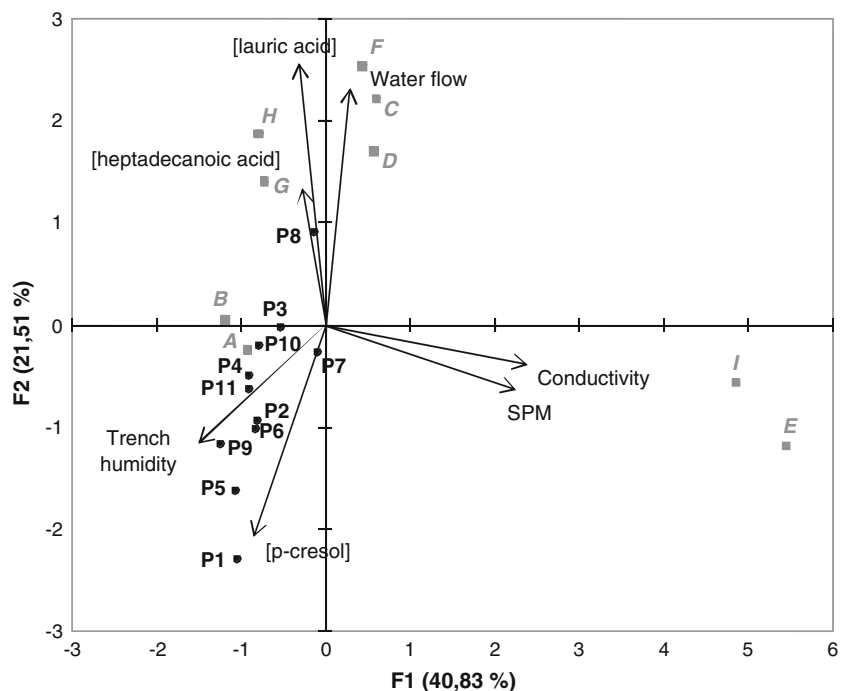
were strongly associated with vegetated freshwater pools (Medlock et al., 2005). Several studies have demonstrated that ovipositing mosquitoes from different genera (*Culiseta*, *Aedes*, *Anopheles*) could tolerate a variety of salt concentrations but the larvae failed to develop correctly in salt water (Bentley & Day, 1989; Carver et al., 2009). In the case of *Coquillettidia*, survival of the larvae might be affected by salt water, as suggested by laboratory experiments which have shown that egg hatching and development of larvae were perturbed by salt water (EID Montpellier-France, personal communication).

The CCA showed that high concentrations of lauric acid and heptadecanoic acid in water marshes were related to the absence of larvae. Fatty acids and ester compounds are known to be involved in the ovipositional responses of mosquito females (Ganesan et al., 2006). Some studies demonstrated that lauric acid showed a significant positive response at different concentrations on *A. aegypti* females whereas the ester form had a deterrent/repellent effect (Ganesan et al., 2006; Sharma et al., 2008). On the other hand, fatty acids may have larvicidal effects, as demonstrated for *Culex quinquefasciatus* (Kannathasan et al., 2008). Therefore, high levels of lauric acid might have similar effects on *Coquillettidia*, acting as an ovipositing attractant but also having an impact on larval development. Previous studies concerning attraction for the host-seeking with *A. aegypti* females demonstrated that long-chain fatty acids could increase the attraction by synergistically acting with other fatty acids (Bosch et al., 2000). Therefore, we would suggest that heptadecanoic acid might have an impact on the oviposition behavior of *Coquillettidia* female even if not all the fatty acids detected seemed to have an attractant or repellent effect. This is the first time that the observed absence of larvae in water has been correlated with high levels of heptadecanoic acid.

In the literature, a group of middle-range volatiles of plant origin (*p*-cresol, phenols) are known to be ovipositional stimulants for various mosquito genera: *Culex*, *Aedes*, *Anopheles*, and *Toxorhynchites* (Bentley & Day, 1989; Allan & Kline, 1995; Collins & Blackwell, 2002; Geetha et al., 2003). 2,6-di-*tert*-butyl-*p*-cresol, a molecule that is chemically similar to p-cresol, was found to be always present in *Coquillettidia* habitats. This molecule might act as ovipositional attractant/stimulant.

Conclusion

The approach used in this study is a descriptive one designed to highlight key ecological factors. In the Rhône-Alpes region, *Coquillettidia* habitats are mainly permanent ponds and drains. These habitats are characterized by: a poorly diversified vegetation cover (*Typha* sp., *Phragmites* sp., *Juncus* sp.); a bank canopy with high cover maintaining a high air humidity; neutral, oligo-haline water with low levels of SPM; and the presence of plant-generated semiochemical cues (e.g. 2,6-di-*tert*-butyl-*p*-cresol). Additional laboratory studies are required to test if correlations between larval presence/absence and water chemical factors (semiochemicals, SPM, salinity) found in this field study are in fact mechanisms that ovipositing mosquitoes use to select larval habitats.

The screening procedure described in this report has allowed us to identify the key parameters characterizing *Coquillettida* larval habitats. This method should be a useful tool for characterizing *Coquillettidia* habitats at any geographical site. At present, we are in the process of launching a more extensive field campaign in which the key environmental factors will be measured and correlated with the presence/absence of *Coquillettidia*. A more predictive approach will then be used (Guisan & Thuiller, 2005) to map the potentially suitable habitats for *Coquillettidia* sp. onto a continuous grid system to be used for eradication and management programs.

References

Allan, S. A. & D. L. Kline, 1995. Evaluation of organic infusions and synthetic compounds mediating oviposition in *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). Journal of Chemical Ecology 21: 1847–1860.

Batzer, D. P. & R. D. Sjogren, 1986. Larval habitat characteristics of *Coquillettidia perturbans* (Diptera: Cilicidae). Canadian Entomologist 118(11): 1193–1198.

Bell, S. S., A. Tewfik, M. O. Hall & M. S. Fonseca, 2008. Evaluation of seagrass planting and monitoring techniques: implications for assessing restoration success and habitat equivalency. Restoration Ecology 16: 407–416.

Bentley, M. D. & J. F. Day, 1989. Chemical ecology and behavioural aspects of mosquito oviposition. Annual Reviews Entomology 34: 401–421.

Bosak, P. J., L. M. Reed & W. J. Crans, 2001. Habitat preference of host-seeking *Coquillettidia perturbans* (Walker) in relation to birds and eastern equine encephalomyelitis virus in New Jersey. Journal of Vector Ecology 26: 103–109.

Bosch, O. J., M. Geier & J. Boeckh, 2000. Contribution of fatty acids to olfactory host finding of female *Aedes aegypti*. Chemical Senses 25: 323–330.

Brothers, D. R., 2005. New distributional records for *Coquillettidia perturbans* (Walker) (Diptera, Culicidae) in Idaho. Journal of Vector Ecology 30: 163–164.

Callahan, J. L. & C. D. Morris, 1987. Habitat characteristics of *Coquillettidia perturbans* in central Florida. Journal of the American Mosquito Control Association 3: 176–180.

Claeys-Mekdade, C. & J. Sérandour, 2009. Ce que le moustique nous apprend sur le dualisme anthropocentrisme/biocentrisme: perspective interdisciplinaire sociologie/biologie. Natures Sciences Sociétés 17: 136–144.

Carver, S., A. Storey, H. Spafford, J. Lynas, L. Chandler & P. Weinstein, 2009. Salinity as a driver of aquatic invertebrate colonisation behaviour and distribution in the wheatbelt of Western Australia. Hydrobiologia 617: 75–90.

Clements, A. N., 1999. The Biology of Mosquitoes, Vol. 2: Sensory Reception and Behaviour. CABI Publishing, Wallingford, UK.

Collins, L. E. & A. Blackwell, 2002. Olfactory cues for oviposition behaviour in *Toxorhynchites moctezuma* and *Toxorhynchites amboinensis* (Diptera: Culicidae). Journal of Medical Entomology 39: 121–126.

Cupp, E. W., H. K. Hassan, X. Yue, W. K. Oldland, B. M. Lilley & T. R. Unnasch, 2007. West Nile virus: infection in mosquitoes in the Mid-South USA, 2002–2005. Journal of Medical Entomology 44: 117–125.

Davis, E. E. & M. F. Bowen, 1994. Sensory physiological-basis for attraction in mosquitoes. Journal of the American Mosquito Control Association 10: 316–325.

Frank, J. H., 1986. Bromeliads as oviposional sites for Wyeomyia mosquitoes-form and color influence behavior. Florida Entomologist 69: 728–742.

Ganesan, K., M. Mendki, M. V. S. Suryanarayana, S. Prakash & R. C. Malhotra, 2006. Studies of *Aedes aegypti* (Diptera: Culicidae) ovipositional responses to newly identified semiochemicals from conspecific eggs. Australian Journal of Entomology 45: 75–80.

Geetha, I., K. P. Paily, V. Padmanaban & K. Balaraman, 2003. Oviposition response of the mosquito, *Culex quinquefasciatus* to the secondary metabolite(s) of the fungus, *Trichoderma viride*. Memorias do Instituto Oswaldo Cruz 98: 223–226.

Guisan, A. & W. Thuiller, 2005. Predicting species distribution: offering more than simple habitat models. Ecology Letters 8: 993–1009.

Hubálek, Z. & J. Halouzka, 1999. West Nile fever-a reemerging mosquito-borne viral disease in Europe. Emerging Infectious Diseases 5(5): 643–650.

Hwang, Y. S., G. W. Schultz, H. Axelrod, W. L. Kramer & M. S. Mulla, 1982. Ovipositional repellency of fatty acids and their derivatives against *Culex* and *Aedes* mosquitoes. Environmental Entomology 11: 223–226.

Jacob, B. G., E. J. Muturi, J. M. Mwangangi, J. Funes, E. X. Caamano, S. Muriu, J. Shililu, J. Githure & R. J. Novak, 2007. Remote and field level quantification of vegetation covariates for malaria mapping in three rice agro-village complexes in Central Kenya. International Journal of Health Geographics 6: 21–32.

Jordan, S., 1992. Cues for oviposition site selection by *Toxorhynchites amboinensis* (Diptera: Culicidae). Journal of Medical Entomology 29: 37–40.

Kannathasan, K., A. Senthilkumar, V. Venkatesalu & M. Chandrasekaran, 2008. Larvicidal activity of fatty acid methyl esters of *Vitex* species against *Culex quinquefasciatus*. Parasitology Research 103: 999–1001.

LaPointe, D. A., 2007. Current and potential impacts of mosquitoes and the pathogens they vector in the Pacific Region. Proceedings of the Hawaiian Entomological Society 39: 75–81.

Laurence, B. R., 1960. The biology of two species of Mosquito, *Mansonia Africana* (Theobald), and *Mansonia uniformis* (Theobald) belonging to the subgenus Mansonioides (Diptera, Culicidae). Bulletin of Entomological Research 51: 491–517.

Madeira, N. G., C. A. Macharelli & L. R. Carvalho, 2002. Variation of the oviposition preferences of *Aedes aegypti* in function of substratum and humidity. Memorias do Instituto Oswaldo Cruz 97: 415–420.

Mandalakas, A. M., C. Kippes, J. Sedransk, J. R. Kile, A. Garg, et al., 2005. West Nile virus epidemic, Northeast Ohio, 2002. Emerging Infectious Diseases 11(11): 1774–1777.

Marshall, J. F., 1938. The British Mosquitoes. British Museum (Natural History), London.

Medlock, J. M., K. R. Snow & S. Leach, 2005. Potential transmission of West Nile virus in the British Isles: an ecological review of candidate mosquito bridge vectors. Medical and Veterinary Entomology 19: 2–21.

Murgue, B., S. Murri, S. Zientara, B. Durand, J. P. Durand & H. Zeller, 2001. West Nile outbreak in horses in Southern France, 2000: the return after 35 years. Emerging Infectious Diseases 7(4): 692–696.

Navarro, D. M. A. F., P. E. S. de Oliveira, R. P. J. Potting, A. C. Brito, S. J. F. Fital & A. E. G. Sant'Ana, 2003. The potential attractant or repellent effects of different water types on oviposition in *Aedes aegypti* L. (Dipt., Culicidae). Journal of Applied Entomology 127: 46–50.

Prasad, K. R. & P. Anbarasan, 2007. Stereoselective synthesis of (-)-6-acetoxyhexadecanolide: a mosquito oviposition attractant pheromone. Tetrahedron Asymmetry 18: 2479–2483.

Ramsdale, C. D. & K. R. Snow, 2001. Distribution of the genera *Coquillettidia*, *Orthopodomyia* and *Uranotaenia* in Europe. European Mosquito Bulletin 10: 25–29.

Reiskind, M. H. & M. L. Wilson, 2004. *Culex restuans* (Diptera: Culicidae) oviposition behaviour determined by larval habitat quality and quantity in south-eastern Michigan. Journal of Medical Entomology 41: 179–186.

Sérandour, J., D. Rey & M. Raveton, 2006. Behavioural adaptation of *Coquillettidia* (*Coquillettidia*) *richiardii* larvae to underwater life: environmental cues governing plant-insect interaction. Entomologia Experimentalis et Applicata 120: 195–200.

Sérandour, J., S. Reynaud, J. Willison, J. Patouraux, T. Gaude, P. Ravanel, G. Lempérière & M. Raveton, 2008. Ubiquitous water-soluble molecules in aquatic plant exudates

determine specific insect attraction. PloS ONE 3(10): e3350.

Service, M. W., 1993. Mosquito Ecology. Field Sampling Methods, 2nd ed. Elsevier, Chapman and Hall, London.

Sharma, K. R., T. Seenivasagan, A. N. Rao, K. Ganesan, O. P. Agarwal, R. C. Malhotra & S. Prakash, 2008. Oviposition responses of *Aedes aegypti* and *Aedes albopictus* to certain fatty acid esters. Parasitology Research 103: 1065–1073.

Sukumar, K., M. J. Perich & L. R. Boobar, 1991. Botanical derivatives in mosquito-control—a review. Journal of the American Mosquito Control 7: 210–237.

ter Braak, C. J. F., 1985. Correspondence analysis of incidence and abundance data: properties in terms of a unimodal response model. Biometrics 41: 859–873.

ter Braak, C. J. F., 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. Ecology 67: 1167–1179.

Wesenberg-Lund, C., 1920. Contribution to the biology of the Danish Culicidae. In København, A. F. (ed.), Mémoires de l'Académie Royale des Sciences et des Lettres du Danemark, Copenhague, Section des Sciences, 8ème série, VII, No 1. Høst & søn, Denmark: 1–210.