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ORIGINAL PAPER

Monitoring of *Culicoides* at 20 locations in northwest Germany

Ellen Kiel · Gabriele Liebisch · René Focke · Arndt Liebisch

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Abstract Entomological monitoring was carried out from April 2007 to May 2008 at 20 locations in the areas of Lower Saxony, Schleswig-Holstein, Hamburg, and Bremen. A total number of 26 *Culicoides* species were sampled by light traps during the first week of every month. *Culicoides* diversity was highest in summer, achieving more than 20 species and genera per month. Numbers of *Culicoides* were highest in spring and summer with a maximum of 325,000 individuals in May 2008 at a single location. During the winter, the number of individuals decreased considerably, but few individuals of *Culicoides* were present even during the coldest months in January and February with *Culicoides obsoletus* remaining the only species complex. The total number of Ceratopogonidae and the number of individuals from *C. obsoletus* complex and *Culicoides pulicaris* complex were significantly correlated with temperature almost at any date and location.

Introduction

The data we present in this publication originate from 20 out of 28 locations chosen for entomologic monitoring of *Culicoides* in the areas of Lower Saxony, Schleswig-Holstein, Hamburg, and Bremen. The first occurrence of bluetongue disease (BTD) in Germany dates back to 21 October 2006. At that time, the first infections were diagnosed on a farm in the Aachen area. Despite wide-ranging restricted areas established by the authorities soon after the onset of the disease, BTD spread quite rapidly north and especially eastwards.

In spring 2007, an entomological monitoring project was started in 89 geographic areas of Germany in order to gain adequate data on the distribution of *Culicoides* species, of which at least the *Culidoides obsoletus* and *Culicoides pulicaris* complex were considered to be potent BT virus vector species. Additionally, this monitoring program was planned to provide useful information on the biology and vector capacity of *Culicoides* species.

Prior to the start of this monitoring project in March 2007, BTD had already been diagnosed in Lower Saxony. While screening BT virus antibodies during a specific survey, 21 infections were noticed in winter 2007/2008. BTD specialists and veterinary medics thus presume the infection of cows to date back to the previous year (Dr. Schmedt auf der Günne, LAVES, acromatic, November 2008).

Materials and methods

All traps were placed in the immediate vicinity of the predominant residences of cows. Thirteen light traps were placed either adjacent to the barn (0–3 m distance) or in its entrance area (two traps). Another five traps were placed 5 and 19 m away from the barn. These traps were also considered to be close to the cows, as they stood adjacent to the dirt road which was used by the cows at least twice a day (Table 1).

E. Kiel (✉) · R. Focke
Carl von Ossietzky Universität Oldenburg,
Campus Haarentor,
26111 Oldenburg, 0049-441-4719, Germany
e-mail: ellen.kiel@uni-oldenburg.de

R. Focke
e-mail: Rene.focke@uni-oldenburg.de

G. Liebisch (✉) · A. Liebisch
ZeckLab, Labor für klinische Diagnostik und Prüfung,
Up'n Kampe 3,
30938 Burgwedel, Germany
e-mail: liebisch@zecklab.de

Table 1 Location of light traps

Location/ code	Light trap exposure			
	Inside barn	Close to barn (0–3m)	Distance to barn (m)	Position
HB HB			5	Adjacent to barn/ next to dirt road
HH HH		x		Adjacent to barn/next to barn windows
NI AUR		x		Adjacent to barn entrance
NI BRA		x		Barn entrance
NI CE	x			Barn entrance
NI CLP			6	Next to dirt road
NI CUX			5	Next to dirt road
NI DH		x		Adjacent to barn entrance
NI EL		x		Adjacent to barn/ next to dirt road
NI H		x		Wall of barn/next to dirt road
NI HI	x			Barn entrance
NI NOH			19	Next to dirt road
NI OHZ		x		Adjacent to barn/next to barn windows
NI OS		x		Adjacent to barn to entrance
NI SHG		x		Outside barn/wall of barn
NI STD		x		Adjacent to barn (next to barn windows)
NI VEC			12	Next to dirt road
NI WST		x		Adjacent to barn (next to barn windows)
SH IZ		x		Adjacent to barn (next to barn windows)
SH OD		x		Adjacent to barn (next to barn windows)

Light trapping = first to seventh day of every month

All traps were installed at 1.5 m above the ground. In the immediate vicinity of the trap, a photo sensor and a data logger were installed. The photo sensor was adjusted to illuminate the black light lamp at sunset. The data logger recorded air temperature and humidity in 4-h intervals. The traps had to be shielded against rain as effectively as possible. Therefore, they either were fixed to a free-standing wooden construction, which was stuck in the ground next to the barn (Fig. 1, left picture) or were fixed directly to a wall of the barn (Fig. 1, right picture).

In April 2007, light traps were emptied only once at the end of the catch period (first week) at every location. Insects caught until the end of the seventh night were emptied immediately, put into 70% ethanol, and transported to the laboratory. This monthly catch rhythm was maintained for the whole catch period till May 2008 at four locations (NI CE, NI H, NI HI, and NI SHG). At the other locations, from May 2007 to May 2008, insects were emptied and put into 70% ethanol diurnal before sunset just before start of the next catch night (Table 2).

In the laboratory, the material was presorted in order to differentiate Ceratopogonidae from other insects. Individuals of the *C. obsoletus* and *C. pulicaris* species complex were differentiated from other Ceratopogonidae. While males were only counted, females were counted and differentiated according to the criteria: gravidity and no blood ingestion/blood ingestion. Aliquots of the *Culicoides* were sent to Dr. D. Werner (ZALF, Müncheberg) for determination of species level.

Results

From April 2007 to May 2008, a total of 26 *Culicoides* species (det. D. Werner 2007–2008) were collected in our area of investigation (Lower Saxony, Germany). Additionally, we found many undetermined individuals belonging to the genera Atrichopogon and Forcipomyia and huge amounts of other insects (Table 3).

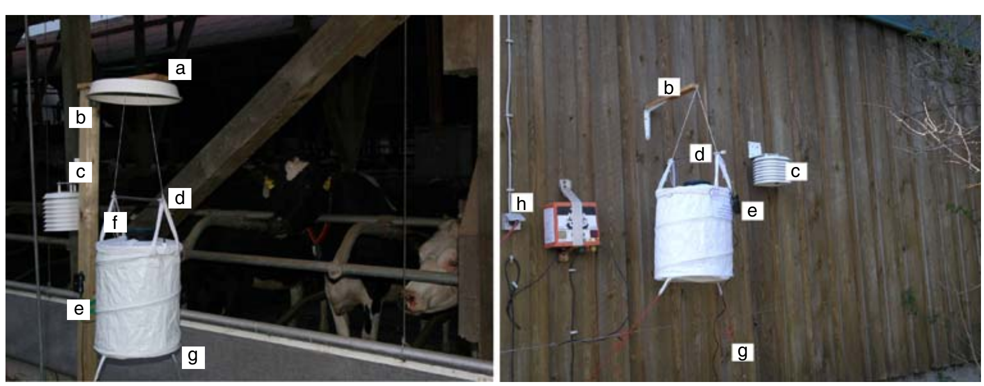


Fig. 1 Configuration of black light traps. *Left* free-standing wooden construction, *right* fixed to the wall of a barn. *a* Rain shield, *b* (wooden) fixture, *c* data logger (air temperature and humidity), height 1.50 m above ground, *d* black light lamp, *e* photo sensor, *f* funnel trap, *g* anchoring chord, *h* power supply (pictures: *left* E. Kiel, *right* G. Liebisch)

Table 2 Catch period and emptying

Location/code	Emptying rhythm during catch period (1week)	
	Once a catch period—end of last night	Diurnal—before sunset
HB HB	April 2007	05/07–05/08
HH HH	April 2007	05/07–05/08
NI AUR	April 2007	05/07–05/08
NI BRA	April 2007	05/07–05/08
NI CE	April 2007–May 2008	
NI CLP	April 2007	05/07–05/08
NI CUX	April 2007	05/07–05/08
NI DH	April 2007	05/07–05/08
NI EL	April 2007	05/07–05/08
NI H	April 2007–May 2008	
NI HI	April 2007–May 2008	
NI NOH	April 2007	05/07–05/08
NI OHZ	April 2007	05/07–05/08
NI OS	April 2007	05/07–05/08
NI SHG	April 2007–May 2008	
NI STD	April 2007	05/07–05/08
NI VEC	April 2007	05/07–05/08
NI WST	April 2007	05/07–05/08
SH IZ	April 2007	05/07–05/08

Culicoides diversity was highest during June and July 2007 (Fig. 2), achieving 21 species and genera per month. Although the number of species and genera subsequently decreased, up to 16 or 17 species and genera per month were caught until October 2007 and again in May 2008. *Culicoides* diversity was lowest in January and February 2007, when *C. obsoletus* remained the only species complex caught in our light traps.

The total amount of individuals caught by light trap investigations at 20 locations in Lower Saxony (Germany) achieved maxima of 140,000 individuals in August 2007 and 325,000 individuals in May 2008. During the winter time, the number of individuals decreased considerably (Fig. 3). However, seven to 256 individuals per month were trapped even in the winter months (December 2007–April 2008). Some Ceratopogonidae were caught even during the coldest months (January and February) at the locations CE, DH, EL H, HB, HH, NI, VEC, and WST.

While the *C. obsoletus* complex dominated in summer, more individuals of the *C. pulicaris* complex were trapped in spring (Fig. 4). However, when interpreting these results, it has to be considered that most of the *C. pulicaris* individuals originated from two locations only: NI AUR and HH HH.

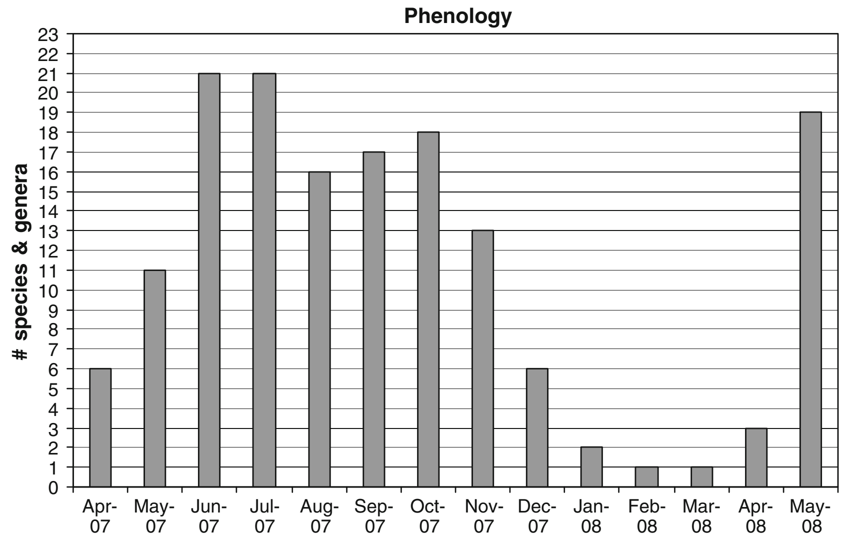
The highest number of *Culicoides* was achieved at the location NI AUR. In May 2008, a total of approximated 237,000 *Culicoides* was caught at this location and 90% of the individuals belong to the *C. pulicaris* complex (Fig. 5). There was no other location and no other sampling date with such high numbers and such a clear dominance of the *C. pulicaris* complex. At the location HH HH, the individuals of the *C. pulicaris* complex achieved about 60% of the fauna, while the number of individuals of the *C. obsoletus* complex was much lower, although they dominated at all other locations. The total numbers of other species of the Ceratopogonidae were also much lower.

However, the sex ratio in the *C. pulicaris* complex was often unbalanced. From August 2007 to November 2007 and in May 2008, only about 40% of the individuals were

Table 3 List of species and genera (naming according to http://www.iah.bbsrc.ac.uk/bluetongue/culicoides/index_species.html, 1 November 2007)

Species and genera		
Genus *Culicoides*		
C. achrayi	*Silvaticulicoides*	(Kettle & Lawson, 1955)
C. albicans	*Oecacta*	(Winnertz, 1852)
C. brunnicans	*Oecacta*	(Edwards, 1939)
C. chiopterus	*Avaritia*	(Meigen, 1830)
C. circumscriptus	*Beltranmyia*	(Kieffer, 1918)
C. clastrieri	*Oecacta*	(Callot, Kremer & Deduit, 1962)
C. dewulfi	*Avaritia*	(Goetghebuer, 1936)
C. fagineus	*Culicoides*	(Edwards, 1936)
C. fascipennis	*Silvaticulicoides*	(Staeger, 1839)
C. festivipennis	*Oecacta*	(Kieffer, 1914)
C. furcillatus	*Oecacta*	(Callot, Kremer & Deduit, 1962)
C. impunctatus	*Culicoides*	(Goetghebuer, 1920)
C. lupicaris	*Culicoides*	(Downes & Kettle, 1952)
C. newsteadi	*Culicoides*	(Austen, 1921)
C. nubeculosus	*Monoculicoides*	(Meigen, 1830)
C. obsoletus	*Avaritia*	(Meigen, 1818)
C. poperinghensis	*Oecacta*	(Goetghebuer, 1953)
C. pulicaris	*Culicoides*	(Linnaeus, 1758)
C. punctatus	*Culicoides*	(Meigen, 1804)
C. puncticollis	*Monoculicoides*	(Becker, 1903)
C .riethi	*Monoculicoides*	(Kieffer, 1914)
C. scoticus	*Avaritia*	(Downes & Kettle, 1952)
C. simulator	*Oecacta*	(Edwards, 1939)
C. subfasciipennis	*Silvaticulicoides*	(Kieffer, 1919)
C. truncorum	*Oecacta*	(Edwards, 1939)
C. vexans	*Oecacta*	(Staeger, 1839)
Genus *Atrichopogon*		
Genus *Forcipomyia*		

Fig. 2 Number of species and genera collected at 20 locations in Lower Saxony (Germany)



females (Fig. 7). While in April and May 2007 and in April 2008, exclusively females without blood ingestion were trapped, the proportion of females trapped with blood ingestion increased during summer. From June to August 2007 and from September 2007 to January 2008, almost 10–30% and 15–50%, respectively, of the females already had ingested blood (Fig. 7).

The sex ratio in the *C. obsoletus* complex was more or less balanced with only minimal surplus of males in some months. During the whole period of investigation, we found females that already had ingested blood with the lowest numbers in spring (≤10% of females in April, May 2007, and March 2008) and about 20–90% of the females in the other months (Fig. 6). The maximum of engorged or partly engorged females (90% of females) could be observed in November 2007. Figures 6 and 7 again highlight that the few individuals caught in winter predominantly belong to

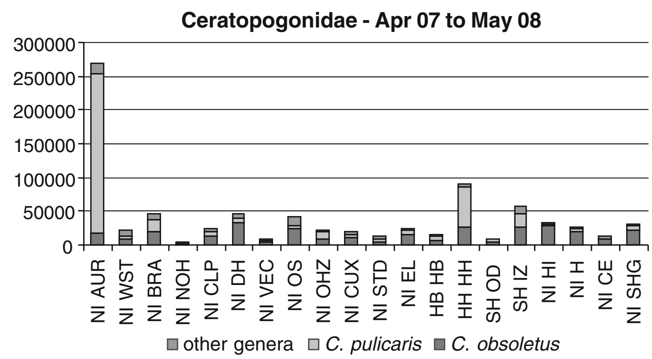


Fig. 4 Total number of Ceratopogonidae at every sampling location in Lower Saxony (Germany). The species complexes *C. obsoletus* and *C. pulicaris* were differentiated. Light trap investigations were carried out during the first seven nights of every month

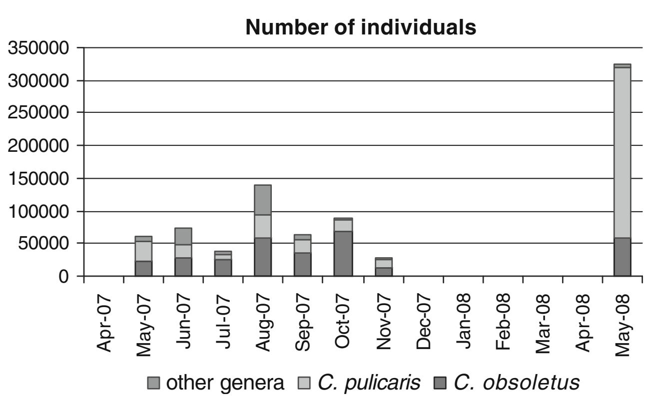


Fig. 3 Total amount of Ceratopogonidae caught by light trap investigations in 2007 and 2008 at 20 locations in Lower Saxony (Germany). The species complexes *C. obsoletus* and *C. pulicaris* were differentiated. Light trap investigations were carried out during the first seven nights of every month

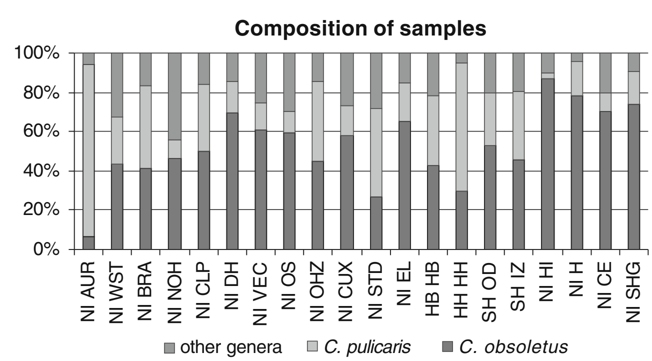


Fig. 5 Faunal composition concerning the species complexes *C. pulicaris*, *C. obsoletus*, and other genera. Percent individuals caught from April 2007 to May 2008 by light trap sampling in Lower Saxony (Germany). Light trap investigations were carried out during the first seven nights of every month

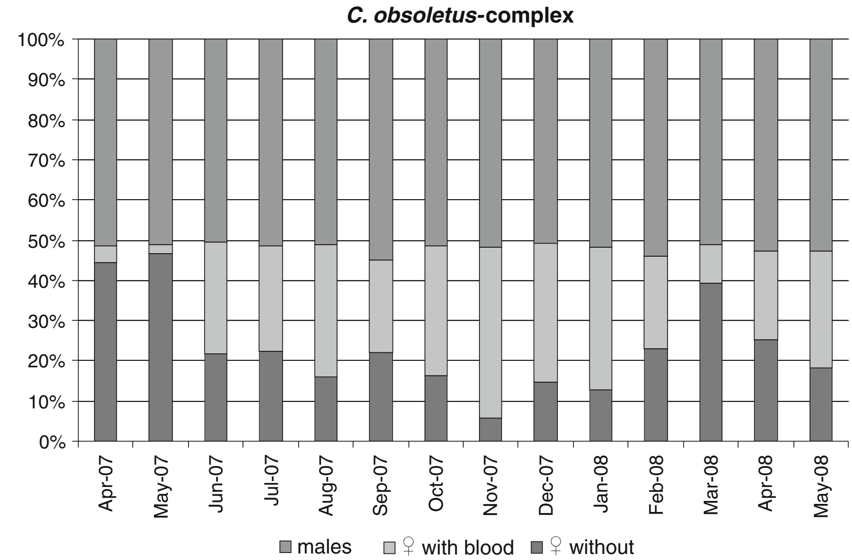


Fig. 6 Sex ratio among individuals of the *C. obsoletus* complex. Females were differentiated according to blood meal ingestion. Data cover the total period of investigation (April 2007 until May 2008) at 20 locations in Lower Saxony (Germany)

the *C. obsoletus* complex. At least in January and February, no individuals of the *C. pulicaris* complex were found.

The total number of Ceratopogonidae and the number of individuals from both species complexes were significantly correlated with temperature at almost any date and location. Significant correlations exist between Ceratopogonid numbers and mean, maximum, and minimum temperature (Table 4).

Discussion

Havelka and Aguilar (1999) named a total of 332 species of Ceratopogonidae as members of the German Ceratopogonid fauna, 57 of which are *Culicoides*. Except for *Culicoides puncticollis* (Becker), all of the 20 species we found are named on this list, i.e., were already known to occur in Germany. According to the Institute for Animal Health (Great Britain) (2008), *C. puncticollis* (Becker) occurs infrequently in Northern Europe but is more common in the Mediterranean, the Middle East, and North Africa.

Although not all our samples could be determined to species level, we estimate that more than 20 species are present in our study area. Currently, it is not clear whether *Culicoides lupicaris* (Downes and Kettle) can be separated from *C. delta* (Edwards). Havelka and Aguilar (1999) mention both of them to occur in Germany. On the other hand, Borkent (2008) treats *C. lupicaris* as a synonym of *C. deltus*. According to the information provided by the Institute for Animal Health (Great Britain) (2008), *C. lupicaris* and *C. delta* may likely be treated as synonyms (http://www.iah.ac.uk/bluetongue/culicoides/deltus.html).

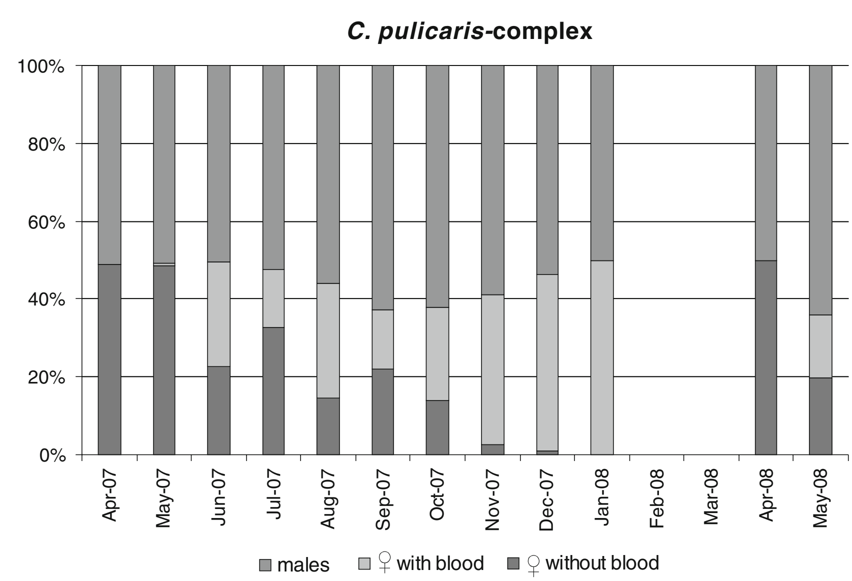


Fig. 7 Sex ratio among individuals of the *C. pulicaris* complex. Females were differentiated according to blood meal ingestion. Data cover the total period of investigation (April 2007 until May 2008) at 20 locations in Lower Saxony (Germany)

Table 4 Temperature dependency of *C. obsoletus* and *C. pulicaris* complexes

		Cerato_total	*C. obsoletus*	*C. pulicaris*
NI AUR	Temp_mean	0.776	0.784	0.727
	Temp_max	0.751	0.762	0.637
	Temp_min	0.599	0.673	0.546
NI WST	Temp_mean	0.695	0.611	0.731
	Temp_max	0.559	*0.488*	0.623
	Temp_min	0.681	0.591	0.687
NI BRA	Temp_mean	0.846	0.814	0.833
	Temp_max	0.872	0.835	0.849
	Temp_min	0.771	0.746	0.771
NI NOH	Temp_mean	0.816	0.762	0.629
	Temp_max	0.749	0.709	0.714
	Temp_min	0.887	0.86	0.632
NI CLP	Temp_mean	0.802	0.798	0.749
	Temp_max	0.857	0.826	0.839
	Temp_min	0.82	0.847	0.784
NI DH	Temp_mean	0.66	0.647	0.614
	Temp_max	0.713	0.686	0.699
	Temp_min	0.66	0.647	0.614
NI VEC	Temp_mean	0.808	0.716	0.689
	Temp_max	0.754	0.709	0.685
	Temp_min	0.623	0.578	0.533
NI OS	Temp_mean	0.869	0.811	0.883
	Temp_max	0.839	0.74	0.821
	Temp_min	0.719	0.635	0.712
NI OHZ	Temp_mean	0.645	0.623	0.636
	Temp_max	0.564	*0.507*	0.551
	Temp_min	*0.497*	*0.519*	*0.506*
NI CUX	Temp_mean	0.778	0.75	0.81
	Temp_max	0.729	0.708	0.786
	Temp_min	0.483	0.476	0.495
NI STD	Temp_mean	0.886	0.88	0.843
	Temp_max	0.896	0.9	0.871
	Temp_min	0.637	0.67	0.633
NI EL	Temp_mean	0.748	0.696	0.738
	Temp_max	0.656	0.548	0.716
	Temp_min	0.678	0.706	0.628
HB HB	Temp_mean	0.738	0.711	0.74
	Temp_max	0.58	0.584	0.592
	Temp_min	0.711	0.72	0.737
HH HH	Temp_mean	0.552	0.585	0.582
	Temp_max	0.582	0.576	0.609
	Temp_min	*0.517*	0.536	0.567
SH OD	Temp_mean	0.858	0.746	0.858
	Temp_max	0.858	0.802	0.83
	Temp_min	0.603	*0.449*	0.631
SH IZ	Temp_mean	0.788	0.693	0.647
	Temp_max	0.727	0.625	0.639
	Temp_min	0.701	0.623	0.576
NI HI	Temp_mean	*0.633*	*0.55*	*0.467*
	Temp_max	0.689	0.61	*0.508*
	Temp_min	0.648	0.564	*0.426*
NI H	Temp_mean	0.835	0.813	0.763
	Temp_max	0.759	0.704	0.68
	Temp_min	0.763	0.741	0.574
NI CE	Temp_mean	0.762	0.762	0.605
	Temp_max	0.654	0.654	*0.459*
	Temp_min	0.664	0.664	0.533
NI SHG	Temp_mean	0.794	0.757	0.756
	Temp_max	0.875	0.856	0.849
	Temp_min	0.741	0.713	0.769

Results of Spearman test for correlations between number of individuals caught with light traps and temperatures during the time of investigation (April 2007–May 2008, days 1 to 7 of every month). Insignificant correlations ($p<0.05$) are italicized

Starting this project, we expected a correlation between air temperature and population development of Ceratopogonidae. It was, therefore, not surprising to find peak numbers of *Culicoides* in August and a significant correlation of air temperature with the number of *Culicoides*.

However, we did not anticipate so many species to be still present in midwinter, when we caught a total of 13 Ceratopogonid species in November 2007 and six Ceratopogonid species even in December 2007. We also did not expect active males and females even in the coldest months of our investigation period, i.e., January and February 2008. Males and females of *C. obsoletus* were trapped during these rainy and stormy months. We conclude that *C. obsoletus* is the most robust species, which might be a year-round active vector of the bluetongue virus, or at least one of them. Further investigation is necessary to determine if *C. obsoletus* is reproducing even in winter or if the adults hibernate at adequate sites.

Further studies will also have to reveal possible effects of trap location on the amount and composition of *Culicoides* fauna caught in our traps. If temperature dependency of *Culicoides* development and activity is actually as strong as our correlations suggest, the weekly sampling we carried out may cause overestimation or underestimation. Consequently, seasonal, annual, and regional variations should be taken into account. The annual deviations are most pronounced if the May samples are compared: The number of individuals in May 2008 was more than five times the height of the May samples of the previous year.

Concerning the temperature dependency, we also have to be aware of regional differences as well as effects of light trap position, possibly causing differences in numbers and composition of our light trap samples. But currently, too little is known on the biology of most of the species we found in our light traps.

Regardless of the species-specific differences in habitat preferences and the regional differences in habitat conditions, we were surprised to notice the dominance of the *C. obsoletus* species complex. At more than half of our study locations and for most of the year, the *C. obsoletus* complex dominated by number. On the other hand, we also wonder what might have caused the huge number of *C. pulicaris* at a few of the other locations.

Referring to literature (Kampen and Kiel 2006; Olbrich 1987) several habitat types should have been suitable breeding places, but niche confinement and niche selection of most species is not known in detail. However, even though the ecological parameters controlling the habitat selection and the population development of certain *Culicoides* species are not known in detail, it has to be assumed that aspects of landscape ecology as well as human impacts could have important effects on the distribution and population development. However, we are currently not in a position to relate *Culicoides* faunal difference to ecological aspects and differentiate natural ecological parameters from the effects of farm management, e.g., manure management.

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