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The phenology and population dynamics of *Culicoides* spp. in different ecosystems in The Netherlands

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Abstract

The Netherlands has enjoyed a relatively free state of vector-borne diseases of economic importance for more than one century. Emerging infectious diseases may change this situation, threatening the health of humans, domestic livestock and wildlife. In order to be prepared for the potential outbreak of vector-borne diseases, a study was undertaken to investigate the distribution and seasonal dynamics of candidate vectors of infectious diseases with emphasis on bluetongue vectors (*Culicoides* spp.). The study focused primarily on the relationship between characteristic ecosystems suitable for bluetongue vectors and climate, as well as on the phenology and population dynamics of these vectors.

Twelve locations were selected, distributed over four distinct habitats: a wetland area, three riverine systems, four peat land areas and four livestock farms. *Culicoides* populations were sampled continuously using CO_2-baited counterflow traps from July 2005 until August 2006, with an interruption from November 2005 to March 2006. All vectors were identified to species level. Meteorological and environmental data were collected at each location.

Culicoides species were found in all four different habitat types studied. Wetland areas and peat bogs were rich in *Culicoides* spp. The taxonomic groups *Culicoides obsoletus* (Meigen) and *Culicoides pulicaris* (Linnaeus) were strongly associated with farms. Eighty-eight percent of all *Culicoides* consisted of the taxon *C. obsoletus*/*Culicoides scoticus*. On the livestock farms, 3% of *Culicoides* existed of the alleged bluetongue vector *Culicoides dewulfi* Goetghebuer. *Culicoides impunctatus* Goetghebuer was strongly associated with wetland and peat bog. Many *Culicoides* species were found until late in the phenological season and their activity was strongly associated with climate throughout the year. High annual variations in

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population dynamics were observed within the same study areas, which were probably caused by annual variations in environmental conditions.

The study demonstrates that candidate vectors of bluetongue virus are present in natural and livestock-farm habitats in the Netherlands, distributed widely across the country. Under favourable climatic conditions, following virus introduction, bluetongue can spread among livestock (cattle, sheep and goats), depending on the nature of the viral serotype. The question now arises whether the virus can survive the winter conditions in north-western Europe and whether measures can be taken that effectively halt further spread of the disease.

Keywords: *Culicoides*; Biting midge; Population dynamics; Surveillance; Bluetongue; Habitat; The Netherlands

1. Introduction

In recent years, the livestock industry in Europe has suffered from a number of outbreaks of infectious diseases that were thought to have been eradicated or, at least, kept well under control. Bovine spongine encephalitis (BSE), swine fever, foot and mouth disease and chicken flu have caused havoc among the livestock industry and huge losses to the European Community. These diseases have also affected the human society, in several cases causing the death of veterinary staff and disrupting traffic and trade by stringent control of transport into and out of disease-affected areas. Many farmers were forced to have their livestock slaughtered, and as a result suffered economic losses and traumas. In contrast to these contagious diseases, vector-borne infectious diseases of livestock have hitherto been restricted to southern Europe. These diseases are often associated with diseases that are common in the (sub)tropics and accidentally introduced into Europe by migration of vectors or transport of infected livestock.

Among the vector-borne diseases of veterinary importance, bluetongue virus (BTV) has received most attention because of its impact on livestock in southern Europe and its ability to spread rapidly by their insect vectors: biting midges belonging to the genus *Culicoides* (Tabachnik, 2004). Although many *Culicoides* species are indigenous to Europe, historic infrequent incursions of BTV in Spain and Portugal were associated with immigration of the African BTV vector *Culicoides imicola* Kieffer (Ortega et al., 1998), often carried by wind from Africa (De Liberato et al., 2003; Sellers, 1980). Only after the introduction of this vector species in Italy did the disease become firmly established in southern Europe (Baylis et al., 2001; Savini et al., 2003). In Italy, the disease has caused serious damage to the sheep industry, requiring stringent control methods. Since then, BTV has spread into Mediterranean islands such as Corsica, Sicily and Sardinia. Recent BTV outbreaks in the Balkans are thought to have arrived from Asian Turkey and may be vectored by local vector species (Purse et al., 2006). Model predictions suggest a widespread distribution of BTV across the Mediterranean basin and into central Europe as far as southern Germany and Austria (Tatem et al., 2003; Wittmann et al., 2001). Climate was considered to be the main limiting factor for spread of the disease into Northern Europe.

Following the widespread reports of BTV in southern Europe in recent years, the Dutch government commissioned a study to investigate the presence and distribution of *Culicoides* spp. in the Netherlands with emphasis on likely areas where such vectors would be readily found and where potential outbreaks of BTV might be expected. The study was conducted in 2005 and 2006. Vector populations of different habitat types have been compared and associated with environmental variables such as climate and vegetation. The data were used to develop a BTV risk model (De Koeijer and Elbers, 2007).

2. Materials and methods

2.1. Area descriptions

As many species of *Culicoides*, the only known arthropod genus associated with BTV transmission, in North-West Europe are closely associated with humid or wet areas (Service, 1971), these areas were considered most at risk for bluetongue virus transmission, especially when large ruminants are present. Selected areas include especially fresh water-associated semi natural areas: wetlands, peat bogs and moors and floodplains, with free-ranging large domestic animals such as horses, sheep and cattle. The presence of wild ungulates is also considered favourable for *Culicoides* as these provide a natural food source for these insects. These different areas are regarded as 'natural' *Culicoides* habitats. A fourth, less natural, habitat is a livestock farm with domestic animals. This habitat was included because of the existing day-to-day interactions between blood feeding arthropods, domestic animals, and people.

Twelve study sites were selected in the Netherlands: one wetland area, four peat areas, three floodplain areas, and four livestock farms (Fig. 1), which are described in detail below. Of these study sites geographical and ecological characters were identified. The ecological characterization of the sites included description of the type of vegetation at various layers (trees, shrubs and herbs), determining the most abundant plant species present, and estimate the proportion of area covered by these species. Additionally, the landscape at each site was described. These data,

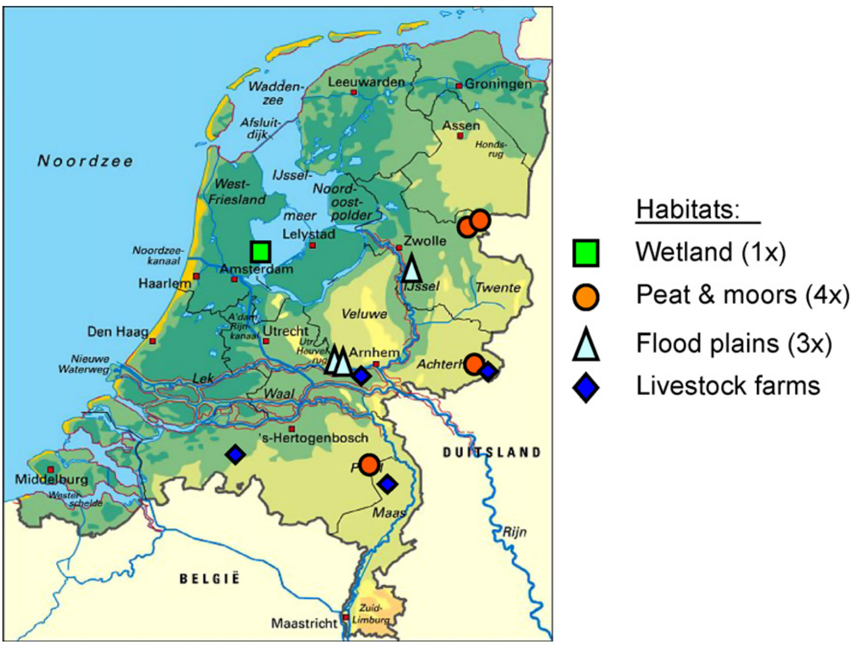

Fig. 1. Geographic distribution of the study areas in The Netherlands.

combined with the entomological data of *Culicoides* spp. collected at these habitats, provide the basis for a risk assessment for transmission of BTV in the Netherlands.

2.2. Wetland (Oostvaardersplassen)

The Oostvaardersplassen is a nature reserve and is an important area for (migratory) birds. Also, there are large herds of Heck cattle, Konik horses and red deer. Migratory birds could potentially bring diseases from southern Europe and Africa to the Netherlands. Many blood-feeding insects are opportunistic feeders (feeding on birds and mammals) and can therefore transmit diseases from migratory birds to the cattle, horses and deer. The area consists of large wet areas with predominantly reed (*Phragmitus australis*) alternated with small, drier areas with willows (*Salix alba*) and nettles (*Urtica dioica*).

2.3. Peat bogs and moors

Like the wetland area, the peat bogs were also very wet and large numbers of mosquitoes (Diptera: Culicidae) and biting midges (Diptera: Ceratopogonidae) are present. Three study areas were selected. In the area ‘‘Engbertsdijkse Venen’’ we selected two sites as one side of this area was very wet (in the South West) and the other side much drier (in the North East).

- Engbertsdijkse Venen South West (Overijssel province)—As mosquitoes and biting midges have been of great nuisance in this area, groundwater levels have been modified to reduce the biting insect populations (Verdonschot, 1996). The water level is maintained by dykes. The vegetation mainly consists of peat moss (*Sphagnum*) and in the drier areas of birch (*Betula*) and *Myrica gale*, which is often associated with biting midges (*Culicoides* spp.).
- Engbertsdijkse Venen North East (Overijssel province)—This area is drier than the southern part of the Engbertsdijkse Venen. *M. gale* is not present, there is less *Sphagnum* and more *Betula* trees. Limousin cattle are grazing the area as a means of vegetation management.
- Korenburgerveen (Gelderland province)—The area consists of flooded forest with alder (*Alnus glutunosa*), ash (*Fraxinus excelsior*) and oak (*Quercus robur*). Other vegetation present is common reed (*P. australis*), blackberry (*Rubus fruticosus*), rowan (*Sorbus aucuparia*) and nettles (*U. dioica*). Vegetation management is achieved by grazing of Galloway cattle.
- De Grote Peel (Noord-Brabant/Limburg province)—This is mostly open peat bog with small stands of birch (*Betula*) forest. Most of the ground vegetation consisted of purple moor grass (*Molinia caerulea*). Animals present include roe deer, sheep and Limousin cattle.

2.4. Flood plains

These consist of river flood plains that are flooded several times a year during times of excessive rainfall in Germany and France. These plains contain many shallow water bodies that could act as *Culicoides* breeding habitat.

- De Duursche Waarden (Overijssel province)—Parts of the area are flooded several times a year. Vegetation consists of several willow (*Salix*) species, purple loosestrife (*Lythrum salicaria*) and bentgrass (*Agrostis stolonifera*). The area is grazed by ponies and Highland cattle.

- Afferdense Waarden (Gelderland province)—Vegetation consists of a few *Crataegus* shrubs, bentgrass (*A. stolonifera*) and water mint (*Mentha aquatica*). The area is grazed by dairy cattle.
- De Blauwe Kamer (Utrecht province)—Vegetation consists mostly grassland interspaced by stands of *Crataegus* and nettles (*U. dioica*). The area is grazed by Konik horses and Galloway cattle.

2.5. Ecological livestock farms

As biting midges were expected to thrive on domestic livestock, we included livestock farms in the study that practiced ecological farming methods. On these farms, the use of pesticides, fertilizer and veterinary drugs is minimized so that natural populations of insects can be expected and the population dynamics of insect populations can be studied under natural circumstances. The selected farms were mixed farms, producing cash crops and raising livestock.

- Ecological farm 'De Brömmels' (Gelderland Province)—A small goat farm where, apart from goats, also ponies ($n = 3$), cows ($n = \pm 10$), sheep ($n = 2$) and pigs ($n = 2$) were present. The farm also includes a camping, where many people are present during summer holidays. Swallow nests (migratory birds), a small fish pond and other small water bodies, were present. The surrounding pastures are alternated with small forest patches. Insect traps were positioned in the meadows about 50 m distance from the farm buildings.
- Ecological farm 'De Voortse Hoeve' (Noord-Brabant province). This farm specializes in pork production, and pigs were the only animals present. As the pigs were both indoors and outdoors, we positioned the insect traps outdoors near the entrance to the stable.
- Ecological farm 'De Waog' (Neer, Limburg province)—The farm produced mostly vegetables, but some sheep were also present. At a short distance from the farm dogs, horses, cows and sheep were present.
- Ecological farms 'De Ravenswaard' (Afferden, Gelderland province)—This dairy farm lies one kilometer from the River Waal. Cows walk outside, or are inside an open barn. One horse was present. Swallow nests (migratory birds) were abundant. Traps were placed in the meadows away from the farm buildings.

2.6. Monitoring methods

Carbon-dioxide baited counterflow traps (Liberty Plus®, American Biophysics Co., North Kingstown, RI, USA) were used for sampling of *Culicoides* spp. These traps have been reported as reliable sampling tools for mosquitoes and biting midges in the USA and Scotland (Kline, 2002; Mands et al., 2004). The traps produce a constant flow of carbon dioxide at 4.5%/min, which mimics the breath of mammals. The traps ran continuously and were emptied once a week.

2.7. Diagnostics of insect samples

Weekly collections were killed at −20 °C, and transferred to 70% ethanol. Samples were identified to the nearest taxon using keys kindly provided by Dr. S. Carpenter, Institute of Animal Health, Pirbright, UK and with information provided by Meiswinkel et al. (2004) and Gomulski et al. (2005).

2.8. Recording of meteorological data

Tinytag® meteorological data loggers (Gemini Data Loggers, Chichester, UK) were used to record humidity (min, max, mean) and temperature (min, max, mean) every 10 min. Because of failure of the data loggers in 2005, data were only available for 2006. To be able to compare data of 2005 and 2006, interpolated data from established meteorological field stations were used.

Temperature data were available from 46 weather stations of MeteoConsult (ALWeer database, Alterra, Wageningen University and Research Centre). Because insects were collected once a week, data were converted to average weekly temperatures and maximum and minimum temperatures per week were indicated. The data of the weather stations were interpolated (Spline) to a climate map of the Netherlands for 2005–2006. This map was used to determine the temperature at the 12 monitoring locations.

2.9. Classification of vegetation structures

An index of the vegetation was made according to the method of Braun–Blanquet (adjusted by Maarel). This index was used to classify the vegetation types (Weeda et al., 2005) of the 12 sampling sites (appendix 7).

3. Results

During the study, fifteen species of *Culicoides* were collected. The key species were:

Culicoides impunctatus (Goetghebuer): A very common *Culicoides* species in the Netherlands (this study) and proven to be orally susceptible to bluetongue in a laboratory study (Carpenter et al., 2006).

Subgenus Avaritia: this subgenus comprises the taxa *C. obsoletus/C. scoticus, C. dewulfi* and *C. chiopterus* as well as the African vector *C. imicola* (Gomulski et al., 2005). *C. obsoletus* and *C. scoticus* have been incriminated as bluetongue vectors in Italy (De Liberato et al., 2005). *C. dewulfi* is the only species from which bluetongue virus was identified during the bluetongue outbreak in the Netherlands of 2006[2] although in a *C. obsoletus* pool in Germany viral DNA was also detected.

Subgenus *C. pulicaris*. This is a species complex, including the species *C. pulicaris* and *C. punctatus*: A known bluetongue virus vector (Caracappa et al., 2003), which also occurs in colder areas like the Netherlands.

A complete list of all species collected during this study is presented in Table 1.

3.1. Wetland

Of the 15 *Culicoides* species found during this study, ten species were found in the wetland area. In late July 2005 the abundance of *Culicoides* was high compared to the other habitats, but it

[2] In one pool of *C. dewulfi*, collected by Drs. Meiswinkel and Goffredo in Limburg Province in September 2006, genetic material of BTV serotype 8 was identified. *Source*: Dr. P. van Rijn, Wageningen University and Research Centre, personal communication.

Table 1
Culicoides spp. collected during the longitudinal study of the 12 study sites, 2005–2006

Species
Culicoides impunctatus
Culicoides minutissimus
Culicoides nubeculosis
Culicoides obsoletus
Culicoides scoticus
Culicoides chiopterus
Culicoides dewulfi
Culicoides festivipennis
Culicoides pulicaris
Culicoides punctatus
Culicoides newstaedy
Culicoides fascipennis
Culicoides maritimus
Culicoides achrayii
Culicoides stigma

was much lower in 2006. This difference in *Culicoides* abundance between 2005 and 2006 at this site is due to the very high number of *C. impunctatus* caught in week 29 of 2005 (Fig. 2). By contrast, numbers of *C. impunctatus* were very low in the corresponding week in 2006. These data show that numbers of *C. impunctatus* can vary much within seasons and between years.

As it was assumed that adult *Culicoides* do not survive cold winters, no surveillance was undertaken during the winter months (November 2005 to February 2006). Weekly sampling was resumed on 1 March 2006. The spring of 2006 was relatively cold, with many occasions of night frost. Adult *Culicoides* spp. were not found until week 18 (first week of May), corresponding with a temperature increase (above 10 °C) in week 18.

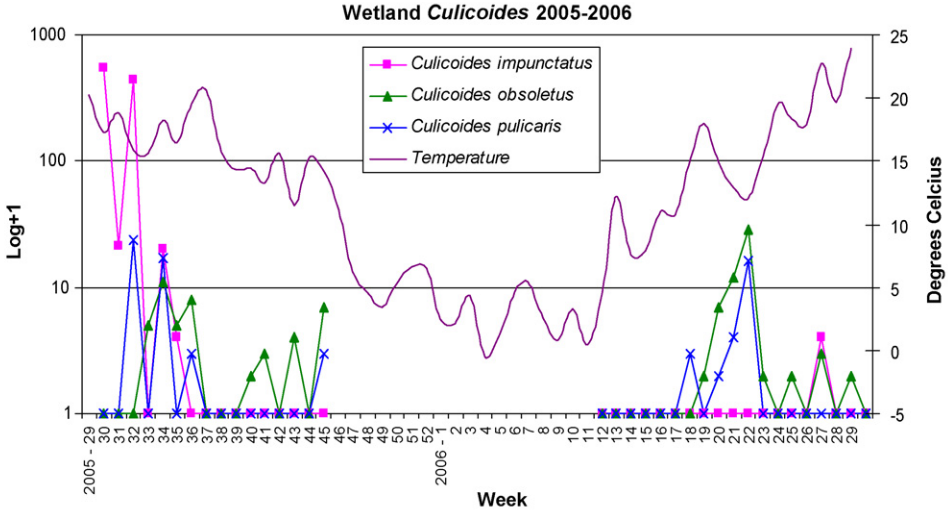

Fig. 2. Wetland: catches of three most abundant *Culicoides* species.

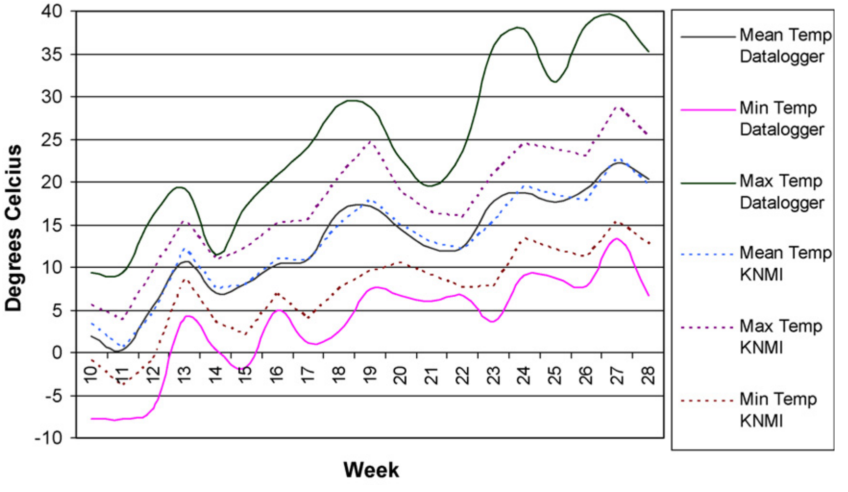

Fig. 3. Wetland (Oostvaardersplassen): mean, maximum and minimum weekly temperatures from data logger compared with meteorological data provided by KNMI during week 10–28 in 2006.

3.2. Meteorological data wetland

As results from the data loggers were not available in 2005, mean temperatures per week presented in Fig. 3 were interpolated from data provided by the national meteorological service (KNMI, de Bilt, The Netherlands). These interpolated data were compared with the data from the data logger collected in 2006. Although the temperatures recorded by KNMI and from our interpolation were very similar, a comparison of maximum and minimum temperatures between the two methods shows a distinctive difference in extreme temperatures (Fig. 3). The mean

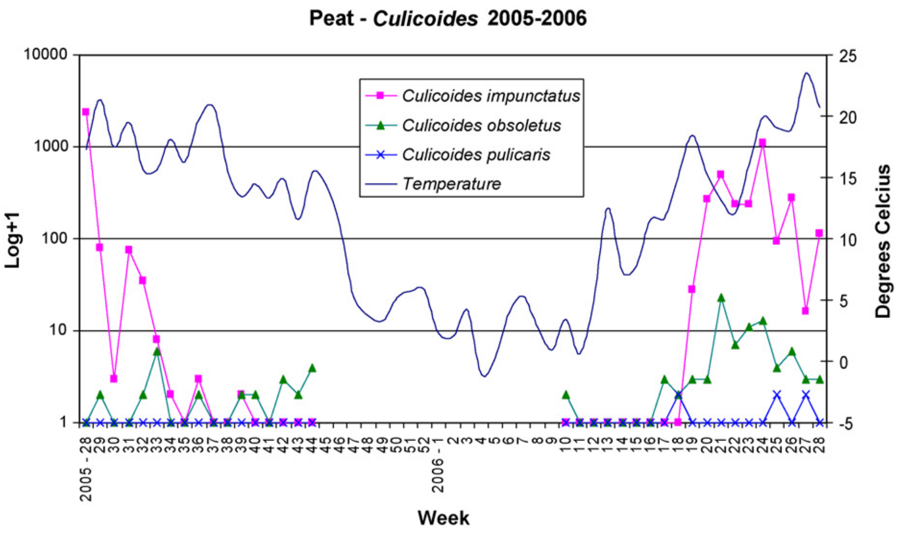

Fig. 4. Peat bogs and moors: catches of three most abundant *Culicoides* species in 2005–2006.

temperatures from the interpolation at microclimate level were similar to those from the meteorological stations but variations in temperature were larger at microclimate level compared to those provided by the national meteorological service. Such variations may be of major importance for insect development and survival.

3.3. Peat bogs and moors

Culicoides species diversity was low compared to other habitats, but densities were higher. *C. impunctatus* was by far the most common species. *C. obsoletus* and *C. pulicaris* were only present in small numbers (Fig. 4). Also between the peat areas the variation in collected numbers of *C. impunctatus* was large. Populations of *C. impunctatus* built op rapidly and decreased again within a few weeks (Week 28–30, 2005) or rose quickly to >100 per trap per week, and stayed high for many weeks. In late July, the abundance of *C. impunctatus* in the Korenburgerveen in 2006 was much higher than in 2005, which further confirms the difficulty of predicting the population dynamics of this species. As in the wetland area, host-seeking midges appeared only at the beginning of May 2006, when the number of *C. impunctatus* rose notably quickly (Fig. 4).

3.4. Floodplains

Culicoides catches were relatively low in the floodplains compared to the other habitats. *C. obsoletus* was the most common species and *C. impunctatus* was found only occasionally (Fig. 5). Numbers were low throughout the second half of 2005. If this is due to flooding of the areas or a seasonal effect is not clear. It is possible that *Culicoides* are nearly absent from the floodplains as larvae would drown during annual flooding. The floodplains are frequently flooded for a period of 7–14 days during high run-off of water from the river Rhine.

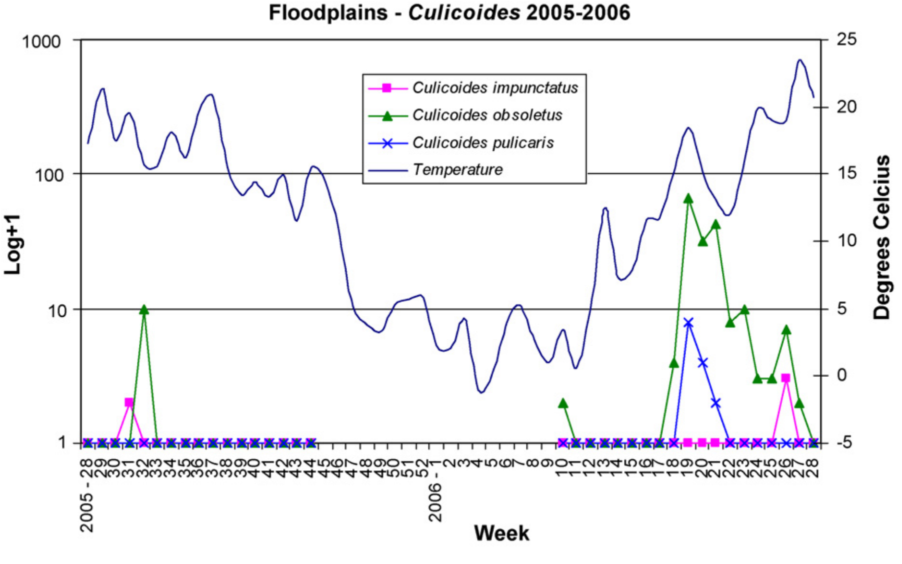

Fig. 5. Floodplains: catches of three most abundant *Culicoides* species in 2005–2006.

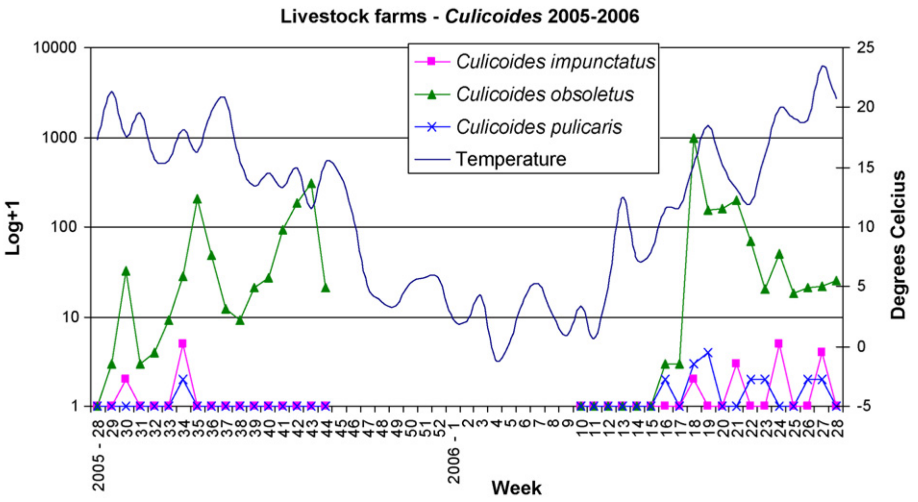

Fig. 6. Livestock farms: catches of three most abundant *Culicoides* species in 2005–2006.

3.5. Livestock farms

The most common *Culicoides* species at the livestock farms were species from the subgenus Avaritia, notably the taxa *C. obsoletus*/*C. scoticus*, *C. dewulfi* and *C. chiopterus*. In contrast with *C. impunctatus*, which was common in other areas but much less abundant on livestock farms throughout the entire sampling period, catches of the said taxa were relatively stable throughout the sampling period and many species were still present in week 44 (1st November) of 2005. In 2006, sampling started in early March. The first *Culicoides* specimens were found in the first week of May when numbers of *C. obsoletus* rose quickly (Fig. 6).

C. obsoletus was found in larger numbers at the livestock farms than at the other habitats and was found more often at the farms with a higher diversity of farm animals (Brömmels and Ravenswaard).

Because of the suspected role of *Culicoides* spp. in bluetongue virus transmission, in particular members of the subgenus Avaritia, biting midges were identified to sibling species

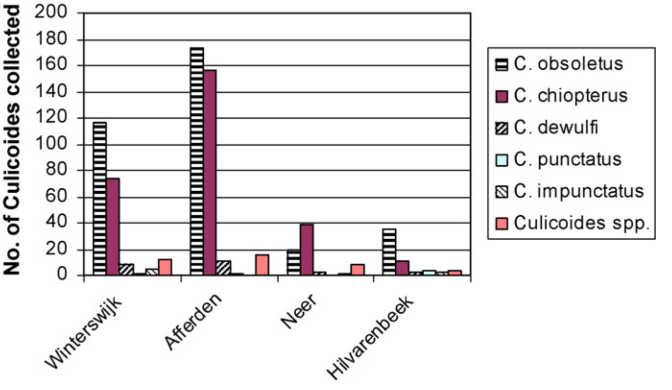

Fig. 7. The species composition of *Culicoides* collected by CO_2-baited traps in livestock farms, May–July 2005.

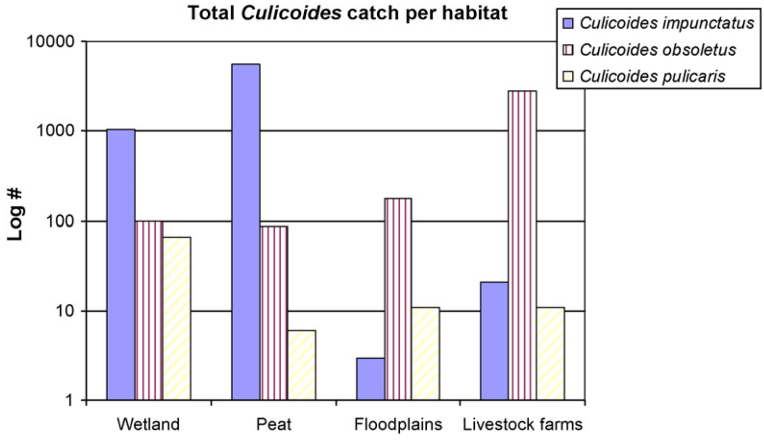

Fig. 8. Total *Culicoides* catch per habitat of the three most common *Culicoides* species.

level as much as possible. Five species were identified (Fig. 7). *C. obsoletus* (which might have included *C. scoticus*) and *C. chiopterus* were the most common species of the *C. obsoletus* taxon, notably in Winterswijk and Afferden. *C. dewulfi*, from which BTV was isolated in September 2006, represented 2.86–3.39% of the catches.

3.6. *Culicoides phenology*

In 2006 in all habitats *Culicoides* spp. became active only by the end of April. The earliest date that we found *Culicoides* in 2006 on the farms was early May. Continuous monitoring since 1 March 2006 revealed only few *Culicoides* spp. up to that time. The majority of species was more abundant between May and 15 June then in the period 15 June to 31 July.

3.7. *Culicoides abundance and habitats*

As mentioned in the paragraphs before, vector abundance and species diversity varied much between the habitats. *Culicoides* species were present in all habitat types, but with different composition of abundance: *C. impunctatus* was found more often in the peat and wetland areas, *C. obsoletus* on the other hand, was found more often on the livestock farms (Fig. 8). As we have shown in the previous paragraphs, *C. obsoletus* on livestock farms consisted of a rich complex of species, dominated by *C. obsoletus* and *C. chiopterus*.

4. Discussion

This study demonstrates that a total of 15 *Culicoides* species were present in the four different habitats across the country, although they were usually collected in relatively low numbers. Because no estimate (by mark-release-recapture studies) of the absolute density of midges was made, we do not know what the real density of biting *Culicoides* was. It became obvious that *C. obsoletus* and *C. pulicaris* were strongly associated with livestock farms. The former species group has been incriminated as a vector of BTV in the 2006 epidemic (Mehlhorn et al., 2007). At least three members of the subgenus Avaritia have been found in the livestock farms: *C. obsoletus* s.s., *C. dewulfi* and *C. chiopterus* (Fig. 6). However, the proportion of *C. dewulfi* in these collections was approximately 3%, and much lower than that of *C. obsoletus*. This suggests that in future the ecology of *C. obsoletus* should be investigated to obtain information on biting behaviour, time of biting and winter phenology. *C. dewulfi* breeding has been associated with animal dung around stables and feeding troughs, but we know little about breeding habitats of other members of the subgenus Avaritia in North Western Europe. Meanwhile, a short but intense surveillance of *Culicoides* species in Limburg in September 2006, using the Onderstepoort light trap (Venter et al., 1996), revealed again that *C. obsoletus* was the dominant species group among all *Culicoides* spp. found (Meiswinkel et al, 2008). More than 88% of all collections consisted of members of this sibling group, including *C. obsoletus* s.s. and *C. dewulfi*. Nine percent consisted of *C. pulicaris*, another species suspected as BTV vector (Carpenter et al., 2006; Purse et al., 2006).

It was interesting that of the four habitat types investigated, the livestock farms displayed the highest proportion of *C. obsoletus* and *C. pulicaris*. Both species are confirmed vectors of BTV in Italy and suspected vectors on the Balkan (De Liberato et al., 2005). Although these species were not expected to be active after the first nights with frost in October, in November 2005 we found *C. obsoletus* still active in one of the farms, suggesting that host seeking was still occurring relatively late in the phenological season.

C. impunctatus was found most abundantly in the Oostvaardersplassen. It was also frequently collected in the peat bogs. This species is highly dominant in Scotland and Ireland, and has been incriminated a potential BTV vector (Carpenter et al., 2006). We have no evidence that it was involved in the BTV outbreak in the Netherlands. It was remarkable that this species was completely absent from Limburg province, approximately 80 km south of National Park de Grote Peel, an area where the species was quite abundant (WT, unpublished data). A possible explanation might be that, since in 2005 the species disappeared from our collections by the end of August (week 37), the collections in Limburg province in 2006 were done too late in the phenological season for this species to be available for trap catches. It is also possible that the marked difference in habitat and soil composition between Limburg and other areas of the Netherlands (Weeda et al., 2005) may have contributed to the absence of *C. impunctatus* in Limburg province.

There was no clear association between temperature and *Culicoides* activity. The spring of 2006 was relatively cold (Fig. 3), and although collections started in early March, the first midges appeared only in the last week of April. In 2005, numbers of all *Culicoides* spp. dropped sharply at the end of August. Although there was a markedly warm period in September 2005, this was not followed by a temporary rise in *Culicoides* numbers (Fig. 2). By the end of May 2006 there were clear peaks of *C. impunctatus* and *C. obsoletus*, which dropped sharply at the beginning of July. We propose that this drop in *Culicoides* catches may have been caused by the exceptionally hot and dry summer in North Western Europe that year; the sharp drop in relative humidity, and simultaneous increase in saturation deficit creating unfavourable conditions for *Culicoides* spp. In particular, *C. obsoletus* was affected by this in all study sites, demonstrating the sensitivity of *Culicoides* population dynamics to environmental factors.

One aspect of *Culicoides* behaviour, with relevance for the 2006 bluetongue epidemic in North Western Europe, is the biting activity of those species that are indigenous to the Netherlands. We have frequently observed high biting intensity, evidenced by intense nuisance experienced by the investigators during daylight hours, particularly on cloudy days. This behaviour is well known from *C. impunctatus*, and we suspect it also to occur in *C. obsoletus* and *C. pulicaris*. Thus, it is possible that biting by *C. obsoletus* s.l. and *C. pulicaris* s.l. is not limited to nocturnal hours at this latitude.

We have used the Liberty Plus carbon dioxide baited trap as our main sampling tool for *Culicoides* and mosquito species. Whereas the sampling efficiency of the Liberty Plus trap for European *Culicoides* species is not known, it has proven a highly effective trap for the sampling of *C. impunctatus* (Mands et al., 2004). A rough comparison of this trap with data from the Onderstepoort light trap (Venter and Meiswinkel, 1994) shows that the latter collects more *Culicoides* specimens than the carbondioxide baited trap (W. Takken, unpublished data). The carbon dioxide baited trap, however, operates 24 h per day, and therefore also catches those midges that are active during daylight hours, unlike the light trap. This might be a considerable advantage when studying the population composition and daily activity of midges, because the indigenous *Culicoides* species in the Netherlands are not restricted to nocturnal activity as we experienced during our studies. Another factor to consider is that we used carbon dioxide as our only volatile bait, whereas a blend of cattle odour might have attracted greater numbers of *Culicoides* compared to carbon dioxide alone. For example, tsetse flies are attracted in far greater numbers to blends of odour simulating cow odour than to single odour components (Vale and Hall, 1985; Willemse and Takken, 1994) and a similar effect may be observed with *Culicoides*, which are often found feeding on cattle.

The study demonstrates that *Culicoides* species are widely present in the Netherlands, and that several suspected BTV vectors appear to be particularly associated with livestock farms. With respect to the 2006 outbreak of bluetongue in the Netherlands, Belgium and Germany, detailed studies on the ecology of indigenous *Culicoides* spp. are needed in order to understand their diurnal rhythm, feeding and oviposition behaviour, as well as how this important group of vectors hibernates in the lowlands.

Conflict of interest statement

None.

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