



WAGENINGEN UNIVERSITY  
LABORATORY OF ENTOMOLOGY

**The overwintering behaviour of adult *Culicoides* species on livestock farms  
in the Netherlands and the effect of indoor insecticidal treatment on  
*Culicoides* species density**

*Thesis submitted in partial fulfilment of the degree Master of Science  
Wageningen University, The Netherlands*

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*“It is not the strongest of the species that survives, nor the most intelligent, but the one most responsive to change”*

**- Charles Darwin -**

## SUMMARY

In the summer of 2006, Bluetongue, a vector-borne viral disease of domestic and wild ruminants appeared in several countries of North-western Europe. Historically, bluetongue virus (BTV) has been absent from Europe, but since 1998 an increasing number of outbreaks have occurred in southern Europe. The origin and route of introduction of the bluetongue disease epidemic in North-western Europe during the summer of 2006 is unknown.

The outbreaks of BTV are caused by virus serotype 8, being an unforeseen arrival of a hitherto not been seen serotype in Europe. The competent vector of BTV is the biting midge *Culicoides* known for its worldwide distribution. The vectors of BTV in North-western Europe are indigenous *Culicoides* species, endemic in areas of bluetongue disease.

It was expected that during the winter of 2006-2007 virus transmission would not take place, as it was assumed that adult *Culicoides* spp. did not overwinter but die when temperature decreases. Ruminant hosts are assumed to be viraemic up to 100 days after infection, which may indicate that a seasonal interruption of vector abundance over 100 days will die out bluetongue virus disease. Nevertheless, the summer of 2007 became as devastating as the summer of 2006, severe outbreaks of bluetongue disease from Belgium, Germany, Luxembourg, France, Denmark, UK and the Netherlands were reported by the end of July.

Overwintering adult *Culicoides* spp., presumably influenced by global warming, may be a possible reservoir of BTV. Several hypotheses are formulated pointing in different directions towards a conceivable way BTV may overwinter. Experts in the Netherlands assume that the overwintering is driven by the presence of low densities of *Culicoides* spp. in winter and high densities of viraemic hosts, acting as reservoirs, at the start of the winter season.

In this study the vector behaviour on livestock farms during winter was studied. Identification of insect collections showed the presence of *Culicoides* species on livestock farms during winter. Both indoor and outdoor activity of midges was studied. During the study, adult midges were found active until the second half of January 2008. This result indicates overwintering of adult *Culicoides* spp. on livestock farms in the Netherlands.

Abundance and activity of *Culicoides* species is strongly influenced by temperature. During the study a correlation of temperature on midge abundance and activity was shown as *Culicoides* spp. were only captured at mean outdoor temperatures above 5°C. If temperature decreases further *Culicoides* species tend to migrate. Low outdoor temperatures, prevailing in winter, seem to force midges indoors as during insect collection days most midges were captured indoors. Relative high indoor temperatures induce survival and activity of midges.

An emergence trap experiment was carried out during the study and showed larval development from composted manure collected indoors. This indicates that certain *Culicoides* species breed indoors and young midges are likely to emerge from indoor breeding areas when temperature increases in spring.

The indoor insecticidal surface treatment carried out by mid February 2008 had no considerably effect on the population densities of midges in livestock barns. The densities before and in time of the treatment were low, more scientific research is needed to study the effect of insecticides on population densities, preferably when *Culicoides* densities are high.

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# 1. INTRODUCTION

## 1.1 History of bluetongue disease in North -western Europe

Never before a devastating outbreak of bluetongue disease has reached this far North in western Europe as it did during the late summer of 2006. Bluetongue, the vector-borne viral disease of domestic and wild ruminants, was first discovered in the southern province of Limburg, the Netherlands on August 14, 2006 (De Koeijer & Elbers, 2007). This was the onset of several bluetongue outbreaks in the Netherlands and the neighbouring countries Belgium, Germany, Luxembourg and France. Historically bluetongue virus has been absent from Europe, but since 1998 an increasing number of outbreaks have occurred in southern Europe (Wilson *et al.*, 2007). Bluetongue is endemic in the tropical regions of southern Africa, the Americas, Australia and southern Asia (Takken, *et al.*, 2007). The competent vector of the bluetongue virus is the biting midge *Culicoides* known for its worldwide distribution. Over 1200 species of *Culicoides* are described. Also in the Netherlands several indigenous species of *Culicoides* can be found.

The origin and route of introduction of the bluetongue disease epidemic during the summer of 2006 is unknown. The captured *Culicoides* spp. during an earlier survey and the serotype of the virus found in North-western Europe excludes several possible routes of introduction. As a result of the mild weather in North-western Europe in 2006, it was assumed that *Culicoides imicola*, abundant throughout Africa and parts of southern Europe, was the abundant competent vector in northern Europe. Nevertheless, the results of the identification of the captured *Culicoides* spp. from the 2006 survey did not come up to these expectations. The biting midge, *Culicoides imicola*, assumed to be responsible for more than 90% of bluetongue virus transmission in southern Europe, was not found at all amongst the collected *Culicoides* spp. in the bluetongue virus infested areas of northern Europe (EFSA, 2007). This result might indicate that the competent vectors of bluetongue virus in North-western Europe are not imported, but indigenous *Culicoides* species, endemic in the areas of bluetongue disease. During the *Culicoides* spp. collection survey in 2006 in the Netherlands 9 potential vector species were found of which *Culicoides obsoletus* and *Culicoides pulicaris* were the most abundant species complexes. Species from the *C. obsoletus* complex were present in 88% of the collections as species from the *C. pulicaris* were present in 9% of the collections (Takken *et al.*, 2007). Both the *C. obsoletus* complex and *C. pulicaris* complex species are indigenous and wide spread throughout the Netherlands and are often found in the surroundings of livestock farms (Takken *et al.*, 2007).

Besides the *Culicoides* species found, the virus serotype found in North-western Europe did also not come up to the expectations. Currently, 24 bluetongue virus serotypes are identified worldwide.

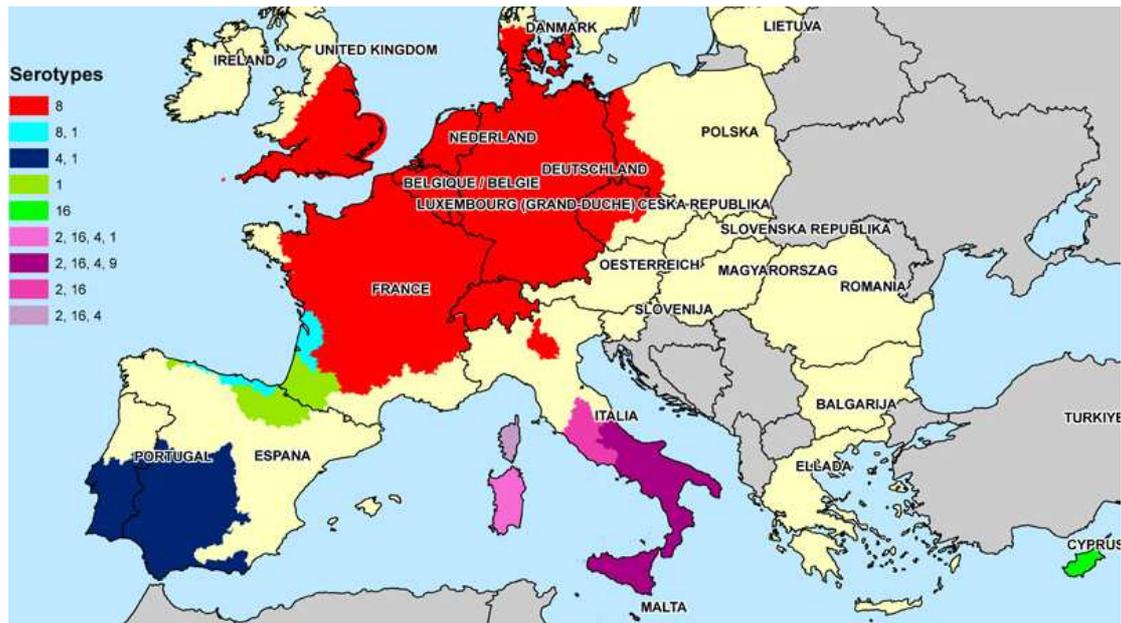


Figure 1. Bluetongue serotype distribution Europe (Defra, 2002)

Before the outbreak in northern Europe, 5 distinct serotypes (1, 2, 4, 9 and 16) of bluetongue virus were identified causing serious disease in ruminants in southern Europe. The North European outbreak of 2006 was caused by virus serotype 8, being an unforeseen arrival of a hitherto not seen serotype in Europe (Meiswinkel *et al.*, 2007). Bluetongue virus serotype 8 has been isolated before in Africa, South-east Asia and the Caribbean, but recent outbreaks of serotype 8 have not been reported since 1980. Thus, to date the source of the bluetongue virus serotype 8 introduction remains unknown (De Koeijer & Elbers, 2007).

It was expected that during the winter of 2006–2007 virus transmission would not take place, as it was assumed that adult *Culicoides* spp. did not overwinter but die when temperature decreases. Ruminant hosts are assumed to be viraemic up to 100 days after infection, which may indicate that a seasonal interruption of vector abundance over 100 days will die out bluetongue virus disease. Nevertheless, the summer of 2007 became as devastating as the summer of 2006, severe outbreaks of bluetongue disease from Belgium, Germany, Luxembourg, France, Denmark, UK and the Netherlands were reported by the end of July (Takken *et al.*, 2007). These developments raise several questions upon bluetongue virus, vectors and environment. It remains unknown where the virus came from. Wilson *et al.* (2007) suggested that the new cases may result from either resumption of transmission, iatrogenic infection, reintroduction of infected adult vector species or viraemic hosts from other enzootic areas or another source.

Indigenous, endemic vectors became competent in transmission of the virus and recent studies of Losson *et al.* (2007) demonstrate overwintering of a small proportion of adult *Culicoides* spp. Overwintering adult midges may be a possible reservoir of bluetongue virus. Contrary to the summer of 2006, the summer of 2007 was relatively cool and wet, which may indicate that high temperatures are not needed for the distribution of bluetongue virus (Takken *et al.*, 2007). Emerging vector-borne diseases such as bluetongue, distributed elsewhere than ever been reported, might be influenced by global warming. The various assumptions made demonstrate the lack of knowledge on bluetongue vector behaviour and virus distribution in northern Europe (Meiswinkel *et al.*, 2007).

## 1.2 Bluetongue disease

The bluetongue virus is a double stranded RNA, non enveloped virus belonging to the genus of the Orbivirus within the family Reoviridae (Purse *et al.*, 2005). Worldwide, 24 serotypes of the bluetongue virus are identified. The virus causes an infectious non-contagious, arthropod-borne disease in ruminants causing morbidity and mortality. Although the virus is able to replicate in all ruminants, severe morbidity and high mortality levels are restricted to certain breeds of sheep and certain species of deer (Purse, *et al.*, 2005). Characteristically, orbiviruses transmitted by *Culicoides* spp. show often a pattern of annual episodes of disease followed by episodes of no disease (Meiswinkel *et al.*, 2007).

The virus transmission cycle starts when an uninfected adult female *Culicoides* midge feeds on a viraemic host. The extrinsic incubation period inside the *Culicoides* virus vector lasts 4 to 20 days depending on temperature. After the incubation period the vector is able to transmit the virus to susceptible ruminant hosts (cattle, sheep goats and deer). The *Culicoides* vector stays infectious throughout her lifespan. It is assumed that transovarial transmission in vectors does not take place. During the latency period infected hosts develop a viraemia which becomes infective to bluetongue vectors after 2 to 4 days (Purse, *et al.*, 2005). The duration of detectable bluetongue viraemia is 50 up to 100 days for sheep and cattle, respectively (Meiswinkel *et al.*, 2007). It is assumed that long term persistence of bluetongue virus in a certain area is only possible if adult vectors are present throughout the year. In this context, vector-free periods longer than the duration of the maximum duration of viraemia in host populations should be able to perish virus transmission as infected hosts have died or recovered before new vectors arrive (Defra, 2002).

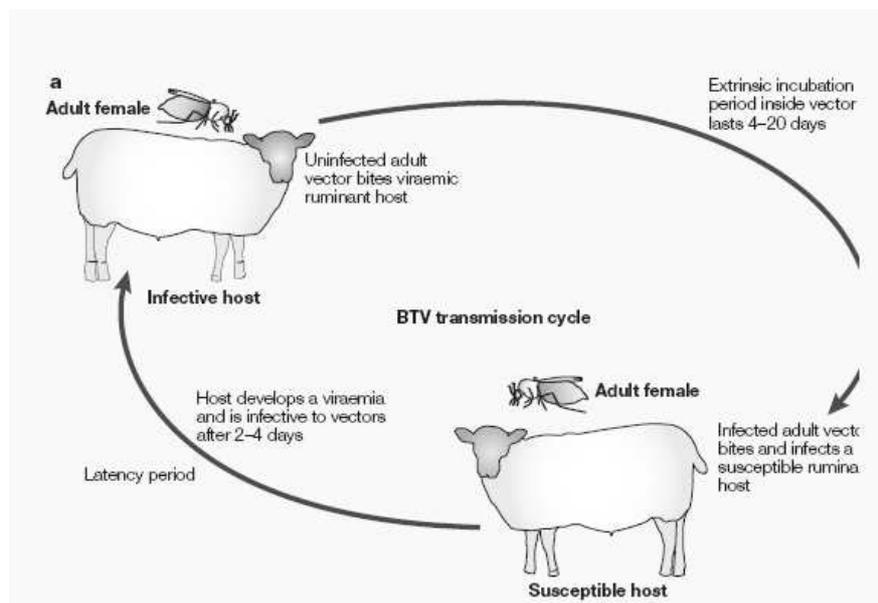


Figure 2. Bluetongue virus transmission cycle (Purse *et al.*, 2005)

Overwintering mechanisms of the virus have been studied recently. Takamatsu *et al.* (2003) studied an overwintering mechanism in the absence of the virus vector. It is shown that certain bluetongue virus serotypes are able to survive 9 up to 12 months in hosts, allowing virus survival throughout vector free periods (Takamatsu *et al.*, 2003). White *et al.* (2005) studied possible overwintering mechanisms of the virus in larval stages of colonized *Culicoides sonorensis*. During the study, bluetongue virus nucleic acid was found, which may indicate the survival of virus in vertically infected immature life stages of some *Culicoides* spp. (White, *et al.*, 2005).

The bluetongue virus serotype 8 epidemic followed her very own pattern. The high numbers of animals and herds that became infected, causing severe morbidity in both sheep and cattle and high mortality in sheep, together with the large area covered by the infection has never been reported before (De koeijer & Elbers, 2007). To date, the immediate causes of this specific virus serotype 8 pattern is unknown.

A wide range of clinical signs of bluetongue disease in infected cattle and sheep can be distinguished. Bluetongue virus serotype 8 associated clinical signs are more prominent in sheep than in cattle (Elbers, *et al.*, 2007). Clinical signs in affected sheep are fever, salivation, erosion of oral cavity, facial oedema, apathy and tiredness, redness of oral mucous membrane and lameness. Cattle show crusts and lesions of nasal mucous membrane, nasal discharge, salivation, fever, apathy and tiredness, purple colouration of teats and lameness. The cyanose colouring of the tongue is often not seen. Besides the clinical signs in cattle, several severe after effects of infection appeared. A large number of cattle showed reproduction disorders such as abortion, still birth and failing conception. The reproduction disorders might indicate vertical transmission of the virus. A recent study indicates vertical transmission of bluetongue virus after experimental infection of in calf ruminants. 10% PCR positive calves were found from a sample of seropositive, PCR negative ruminants (Van Rijn, *personal communication*, 2008). Elbers *et al.* (2007) showed a mean morbidity of 20% in affected sheep flocks and a mean morbidity of 6.8% in affected cattle herds. The mean mortality rate in sheep flocks was 5% compared to a mean mortality of 0.3% in cattle herds. Case fatality ranged between 0 to 100% for both sheep flocks and cattle herds. A case fatality of 50% was shown in 23% of the sheep flocks and in 6% of the cattle herds. Morbidity as well as mortality and case fatality are higher in sheep flocks than cattle herds (Elbers *et al.*, 2007).

### 1.3 Biology and ecology of *Culicoides* species in Europe

The tiny biting midges *Culicoides*, 1 to 3 mm in size, belongs to the family of *Ceratopogonidae* (Diptera: *Ceratopogonidae*). A total of 5500 species, belonging to 78 genera in the family of *Ceratopogonidae*, can be distinguished. Only 4 genera are known to feed on vertebrates, of which, in this context, the genus *Culicoides* is the most important. Species belonging to the genus *Culicoides* are of great veterinary importance, they can be considered as main vectors of animal diseases (Mullen, 2002).

*Culicoides* spp. have a worldwide distribution, occurring in both tropical and temperate regions of the world.

**Table 1. Taxonomy of the genus *Culicoides* indigenous in Europe**

Species complex	<i>Culicoides</i> species	subgenus
Obsoletus	<i>C. obsoletus</i> / <i>C. scoticus</i> / <i>C. dewulfi</i>	Avaritia
Chiopterus	<i>C. chiopterus</i>	Avaritia
Pulicaris	<i>C. pulicaris</i> / <i>C. lupicaris</i> / <i>C. punctatus</i>	Culicoides
Imicola	<i>C. imicola</i>	Avaritia

#### 1.3.1 Life cycle of *Culicoides* spp.

The life cycle of *Culicoides* spp. consists of several stages. The first stage is the egg stage which requires a development period of 2 to 10 days in general. The egg stage is followed by 4 larval instars, a short-lived pupal stage and an adult stage (Borkent, 2005). The total life cycle varies between 2 weeks in favourable tropical environments up to a year in unfavourable arctic environments. The environmental factors temperature, humidity, and

population density are assumed to influence the rate of development of immature *Culicoides* spp. (Borkent, 2005). Oviposition takes place after mating and feeding. Female *Culicoides* spp. lay their eggs in batches in a wide range of moist habitats as the eggs cannot withstand desiccation. The biting midges deposit batches ranging from 30 up to 450 eggs depending on species and size of blood meal.

Four larval stages can be distinguished. The larval development time varies between 2 weeks up to a year depending on species and season. During the winter season many larvae diapause resulting in a larval development time of more than 6 months. During spring or early summer the overwintering larvae pupate. At the end of the short-lived pupal stage the adult *Culicoides* emerges from the pupa. The life span of adult *Culicoides* spp. is assumed to range between 1 to 7 weeks (Borkent, 2005). Most adults survive less than 20 days, occasionally, depending on species, environment and region, adults live up to 90 days (Mellor *et al.*, 2000). In cooler environmental conditions the metabolism of *Culicoides* spp. decreases which in return increases the life span of biting midges.

Adult overwintering has been studied recently. Losson *et al.* (2007) studied the overwintering of *Culicoides* spp. inside a calving unit in Belgium. Results from insect collections indicate either overwintering of a small proportion of adults or new emergence of midges from suitable nearby breeding sites as during the winter of 2006-2007 small numbers of adult *Culicoides* spp. were found.

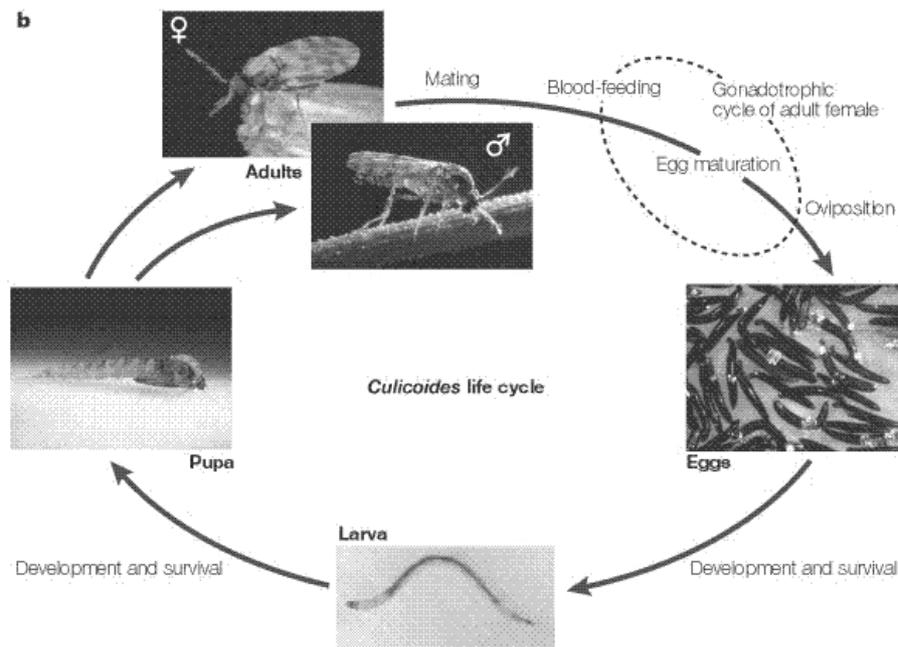


Figure 3. *Culicoides* life cycle (purse *et al.*, 2005)

### 1.3.2 Behavioural patterns

The feeding, resting and oviposition behaviour of the bluetongue vector *C. imicola* bluetongue has been studied as this vector is assumed to be responsible for over 90% of bluetongue virus transmission in several countries. However, the recent outbreak of bluetongue in North-western Europe showed a paucity of information on behavioural patterns of involved *Culicoides* species. Currently it is assumed that *Culicoides* spp. show both nocturnal and diurnal activity. The *Culicoides* spp. found in northern regions of the world show two biting peaks, one after sunrise and one close to sunset. Hours of feeding can

lengthen on days with low light intensities as warm and calm nights extend nightly activities of midges (EFSA, 2007). The biting and blood feeding behaviour of the female *Culicoides* midge is assumed to differ among species, being either exophagic, endophagic or both. In general it is assumed that an adult female midge will feed every 3 to 5 days (EFSA, 2007). Female *Culicoides* bluetongue vectors are zooprophagous, feeding exclusively on animals, preferably on ruminants. Activity of adult females is assumed to be influenced by temperature, light intensity, relative humidity and wind velocity. The resting behaviour is assumed to differ as well, being either endophilic or exophilic or both.

Although activity is assumed to decrease when temperatures go down, it is assumed that overwintering of adult *Culicoides* spp. takes place, possibly in warm livestock barns. It has been demonstrated that small numbers of active adult *Culicoides* spp. can be collected throughout the winter, from November up to March (EFSA, 2007).

Knowledge on feeding and resting behaviour during possible overwintering is still unknown. Members from the *Obsoletus* complex preferably breed in damp soils and composted organic materials such as old manure. Members from the *Pulicaris* complex prefer to breed in wet soils, peat moss and swamps (Defra, 2002). From *C. dewulfi*, member of the *Obsoletus* complex, it is known that it preferably uses animal dung as oviposition site. Other *Culicoides* species found in North Europe are also often associated with livestock and their surroundings (Takken *et al.*, 2007).

Biting midges congregate where there are suitable breeding sites and hosts upon which to feed. Therefore the largest *Culicoides* species populations can be found where ruminant populations are highest (Defra, 2002).

#### **1.4 Epidemiology of bluetongue disease**

To understand the epidemiology and risk of transmission of bluetongue virus in North-Europe, knowledge on the behaviour of vectors throughout the seasons is essential.

Vector borne viral diseases have their own dynamics, different from other viral diseases which are not vector borne. Transmission of vector borne diseases is highly influenced by environmental factors and weather conditions, and more specific, by temperature (De Koeijer & Elbers, 2007). The key parameters biting rate, extrinsic incubation period (EIP) and vector lifespan are all influenced by temperature (Gubbins, *et al.*, 2007). It is generally assumed that bluetongue virus vectors die as soon as temperatures fall below 0°C, but unfortunately there is little knowledge on this. Therefore most parameters used in modelling the transmission of bluetongue virus in northern Europe are estimated due to the paucity of information on both behavioural patterns of vectors and virus development.

The number of *Culicoides* spp. capable of transmitting bluetongue virus depends on vector competence, adult survival rate, biting rate and EIP. To transmit the bluetongue virus a vector must be competent and survive long enough to feed on a host after the completion of the EIP (Defra, 2002). Temperatures play a crucial role in this process as survival, biting rate and EIP are highly temperature dependent. Temperatures between 15°C and 25°C are most favourable. Although the adult survival rate decreases fast at temperatures above 15°C, this is compensated by a decrease in duration of the EIP and an increasing biting rate (Defra, 2002).

Orbitoviruses, such as the bluetongue virus, need a certain temperature for development within the vector to become transmissible to hosts. It is assumed that the virus is unable to develop at temperatures below 10°C. Temperatures between 15°C and 25°C are optimal for bluetongue virus to replicate to transmissible levels (Gubbins *et al.*, 2007). Also a minimum amount of time at these suitable temperatures are needed for completion of the virus development cycle (EIP) within a vector (Defra, 2002). It is assumed that vector EIP ranges between 2 weeks and

4 days at low (<10 °C ) and high temperatures (>25 °C), respectively, while adult vector lifespan is assumed to range between 1 week at high temperatures and several months at low temperatures (De Koeijer, *personal communication*, 2008).

Several hypotheses are formulated pointing in different directions towards a conceivable way BTV may overwinter. Most experts in the Netherlands assume that the overwintering of bluetongue virus is driven by the presence of low densities of *Culicoides* spp. in winter and high densities of vireamic hosts, acting as reservoirs, at the start of the winter season.

The parameters used in modelling are often estimated from earlier studies on different vector spp. than the ones abundant in northern Europe. Besides, extrapolation is often used as studies during winter seasons are scarce. The following parameters for winter conditions are obtained from available literature and personal communication with experts from the Central Veterinary Institute (CVI) and Wageningen University.

**Table 2. Parameters Bluetongue disease for winter conditions**

Parameter	Estimate	Reference
Vector biting rate* (per week winter season)	0.5-1.0	De Koeijer & Elbers
Vector EIP* (weeks)	2.0-4.0	De Koeijer & Elbers
Vector lifespan* (weeks)	1.0-6.0	(2007)
Vector density females	**	Takken (2008)
Livestock infectiousness (weeks)	min.1.5, mean 2.0, max.4.0	De Koeijer (2008)
Livestock EIP (weeks)	0.5	De Koeijer (2008)
Probability of transmission vector to host	0.8-1.0	Gubbins <i>et al.</i> (2007)
Probability of transmission host to vector	0.001-0.15***	Gubbins <i>et al.</i> (2007)

\*(temperature dependent) \*\* (number of female vectors per host per day) \*\*\*(vector competence assumed to range between 0.1 and 15%)

The vector biting rate is estimated to range between 0.5 and 1.0 bites per week during winter based on *Culicoides imicola* data. The biting rate is strongly influenced by temperature as it increases with increasing temperatures (De koeijer & Elbers, 2007).

The extrinsic incubation period (EIP) within the vector depends on temperature as well. The EIP decreases with increasing temperatures and is estimated to range between 2.0 and 4.0 weeks during the winter season.

The expected vector lifespan during winter is estimated to range between 1.0 and 6.0 weeks depending on temperature. Adult vector lifespan increases from 0°C up to 15°C followed by increasing mortality rates when temperature rises (De koeijer & Elbers, 2007). Besides it is assumed that 90% of the vectors die when temperatures fall below 0°C.

Minor changes in survival of a vector can greatly affect the transmission of bluetongue virus as the virus requires an incubation period within the vector before transmission is possible (Gubbins *et al.*, 2007).

Vector densities express a number of female *Culicoides* spp. available per host per day. The trap efficiency is assumed to be 10%, which means that 90% of the present *Culicoides* spp. are not captured during collection.

Host infectiousness is estimated to range between 1.5 and 4.0 weeks during winter, while the host EIP is estimated at 0.5 weeks (De koeijer & Elbers, 2007).

Vector to host transmission ranges between 80% and 100% due to a high concentration of virus in the salivary glands of infectious vectors.

Vector competence or host to vector transmission is estimated to range between 0.1% (estimate for field caught *C.sonorensis*) and 15% (experimental competency estimate of *C.obsoletus*) depending on viral titres in hosts and vector species (Gubbins *et al.*, 2007). Host to vector transmission is therefore less efficient than vector to host transmission. It is assumed

that high abundance and high survival rates of certain species can compensate for low levels of vector competence and therefore still be effective vectors in the field (Defra, 2002).

*C.chiopterus* and *C.dewulfi* are assumed to be responsible for bluetongue virus transmission in the Netherlands. *C. obsoletus* species are not found to be vectors of the virus in the Netherlands so far, as virus isolations are not made from the species captured in the Netherlands. In southern Europe members of the *Obsoletus* complex are vectors of bluetongue virus as virus isolations are made from field-caught species (Defra, 2002). The same species are also competent of transmitting African Horse Sickness Virus (AHSV) as isolations from this virus are made in Spain. Hence, bluetongue virus and AHSV tend to utilize the same *Culicoides* spp. as vectors which can result in emerging vector-borne diseases in other parts of Europe.

## **2. RESEARCH**

### **2.1 Research objective**

The objective of the research is to monitor the behaviour of adult *Culicoides* species during winter in barns on livestock farms in the Netherlands and to assess the impact of insecticidal treatment for *Culicoides* control during the winter.

### **2.2 Research questions**

The overall objective will be reached by answering the following research questions;

- What are the population densities and dynamics of *Culicoides* spp. in livestock barns in winter?
- What is the age structure of *Culicoides* spp. resident in winter?
- Are adult *Culicoides* spp. in livestock barns active during winter?
- What is the feeding and resting behaviour of adult *Culicoides* spp. during winter in the Netherlands?
- What is the correlation between the activity of adult *Culicoides* spp. and environment?
- What is the effect of insecticidal treatment in livestock barns on *Culicoides* spp.?

### 3. MATERIALS AND METHODS

#### 3.1 Description of study sites

The study was conducted on 16 livestock farms in Gelderland and Utrecht (Fig. 4) as in these districts several cases of bluetongue disease were reported in 2006 and 2007 (Annex II).



Figure 4. District participating farms (map-of-netherlands-uk.co)

All selected farms had a dairy cattle branch providing a stable livestock herd during the whole study. Each farm was described in detail according to the exact location, housing system, livestock species kept, breed of livestock, number of livestock kept, farm surrounding and insecticidal usage (Annex I). The information needed for the farm description was obtained using a farmers survey which was held during the collections of insects on the farms.

#### 3.2 Collection of adult *Culicoides* species

During the study adult *Culicoides* spp. were collected using Onderstepoort light traps. The insect collections took place from November 19, 2007 up to April 11, 2008.

The collection took place at all farms included in the study at an interval of 2 weeks per farm. Due to the insecticidal treatment in week 7, close monitoring at an interval of 1 week per farm during week 6, 7 and 8 took place. On each farm 2 traps were operated on 2 consecutive days, being emptied every 24 hours. The traps were randomly assigned to the farms. The Onderstepoort light traps were operated during the whole day to overcome low levels of catches due to the possible diurnal activities of *Culicoides* spp. One trap was placed outdoors and one indoors. The traps were placed at a height of between 1.5 to 2 meters, indoors as close to the livestock as possible and outdoors near to the entrance.

Mobile Onderstepoort black light suction traps were used during the collections of adult *Culicoides* species. Compared to white light, black light is found to be 8 to 10 times more attractive to *Culicoides* spp. (Goffredo & Meiswinkel, 2004). A fine mesh netting surrounding the black light tube excludes larger insects from entering the trap. Below the suction fan a fine meshed gauzed bag is tied up to the frame. The gauzed bag holds the trap, a transparent 200 ml collection beaker filled with 50 ml mixture of water and detergent. A strainer and a separate square of fine gauze separates the day collections from the mixture before put in a storage container. The day collection of insects of each trap is stored in labelled screw lid containers with 70% ethanol. The insect collections did also receive a week number including a or b (e.g. 47a). A and b represent the collection days being either Tuesday and Wednesday or Thursday and Friday of a particular week. A represents the insects collected on Tuesday and Wednesday, b represents the insect collections of Thursday and Friday.

Each collection of insects received an unique code based on farm number, date and trap number. The weekly collections of insects were transferred to Plant Protection Services for identification. The insect collections were analyzed for the presence, absence and abundance of *Culicoides* spp. The age structure of the collected female *Culicoides* spp. was also measured by using the stages nulliparous, parous, freshly blood fed and gravid of the gonothrophic development cycle of the midge as indicators of age.

### 3.3 Environmental parameters

During the collection of *Culicoides* spp. the environmental parameter temperature was measured by meteo data loggers. A total of 16 data loggers were available. Per farm 2 data loggers were operated at a height of 2m. One data logger was placed outdoors near the outdoor light trap, as the other one was placed indoors near the indoor light trap. Every 30 minutes the data loggers measured the temperature. This resulted in a measurement of environmental parameters on 8 farms during the whole study. These farms represent the temperatures of the farms in the nearby districts.

### 3.4 Insecticidal treatment

By mid February 2008 eight from the 16 livestock farms were randomly selected for insecticidal indoor surface treatment using the insecticides permethrin (214g/L) and pyrethrum (23,5g/L). The remaining farms served as a control group treated with water only (Table 3). The insecticide was applied on all surfaces in livestock barns up to 2 m. The presence of livestock during the treatment was permitted. Feed, water and milking equipment was covered over. Before and after the treatment close monitoring of *Culicoides* spp. density took place on all farms to investigate whether there is an effect of the insecticidal treatment on *Culicoides* spp. density in livestock barns.

**Table 3. Insecticidal treatment**

date	farm	barn type	treatment
11-02-08	11	stand	control
11-02-08	5	stand	insecticide
11-02-08	3	stand	insecticide
11-02-08	12	stand	control
11-02-08	2	stand	insecticide
12-02-08	6	cubicle*	insecticide
12-02-08	14	cubicle*	control
12-02-08	9	cubicle*	insecticide

12-02-08	16	cubicle*	control
13-02-08	7	loose**	insecticide
13-02-08	15	cubicle*	control
13-02-08	13	loose**	control
13-02-08	8	cubicle*	control
14-02-08	1	cubicle*	insecticide
14-02-08	10	cubicle*	insecticide
14-02-08	4	cubicle*	control

\*( cubicle on slatted floor with storage for slurry manure) \*\*( loose housing of livestock)

### 3.5 Emergence traps

An emergence trap experiment was set up to study possible larval development and adult emergence from manure. By the end of February 2008 manure was collected at random from 5 out of the 16 participating farms (Table 4). Manure was collected from inside the livestock barns and represented old manure (no fresh manure was used). The manure from each farm was stored in a black bucket (10l) and covered over with a fine white netting in pyramid shape. During the first week the manure samples received no light. After one week a transparent trap bucket was placed on top. From week two onwards the traps received UV light for 24 hours a day. Every 2 days the traps were emptied (Mondays, Wednesdays and Fridays). The emergence traps were stored at an average temperature of 19°C. The collections of insects were transferred to Plant Protection Services for identification.

**Table 4. Sampling schedule emergence traps**

date	sampling farm	activity	cell temperature (°C)
27-02-08	3-6-14	manure collection	
28-02-08	5-16	manure collection	
29-02-08	3-6-14-5-16	UV light on	
03-03-08	3-6-14-5-16	empty out	19
05-03-08	3-6-14-5-16	empty out	19
07-03-08	3-6-14-5-16	empty out	19
10-03-08	3-6-14-5-16	empty out	17
13-03-08	3-6-14-5-16	empty out	18
13-03-08	1-4-9-10-15	manure collection	
21-03-08	1-4-9-10-15	UV light in	
25-03-08	1-4-9-10-15	empty out	15
28-03-08	1-4-9-10-15	empty out	19
31-03-08	1-4-9-10-15	empty out	20
02-04-08	1-4-9-10-15	empty out	19
04-04-08	1-4-9-10-15	empty out	19
01-04-08	2-12	manure collection	
03-04-08	11-7-8	manure collection	
11-04-08	2-12-11-7-8	UV light on	
14-04-08	2-12-11-7-8	empty out	19
16-04-08	2-12-11-7-8	empty out	18
18-04-08	2-12-11-7-8	empty out	20
21-04-08	2-12-11-7-8	empty out	20
23-04-08	2-12-11-7-8	empty out	21
21-04-08	13	manure collection	
29-04-08	13	UV light on	
02-05-08	13	empty out	20
05-05-08	13	empty out	19
07-05-08	13	empty out	20
09-05-08	13	empty out	21
13-05-08	13	empty out	22

## 4. RESULTS

### 4.1 Winter collection of *Culicoides* spp.

The insect collections were analyzed for the presence, absence and abundance of *Culicoides* species. From the 16 participating farms a total number of 101 *Culicoides* spp.(n =101) were captured. The insect collections took place from November 20, 2007 up to April 11, 2008. The total number comprises both the outdoor and the indoor collection of insects.

From the total number of midges collected, 16 *Culicoides* spp. (n =16) were captured in the outdoor traps and 85 (n =85) in the indoor traps.

From the morphological analysis the *Culicoides* spp. collection represented the 4 following species, *C.chiopterus*, *C.obsoletus*, *C.punctatus* and *C.scoticus*.

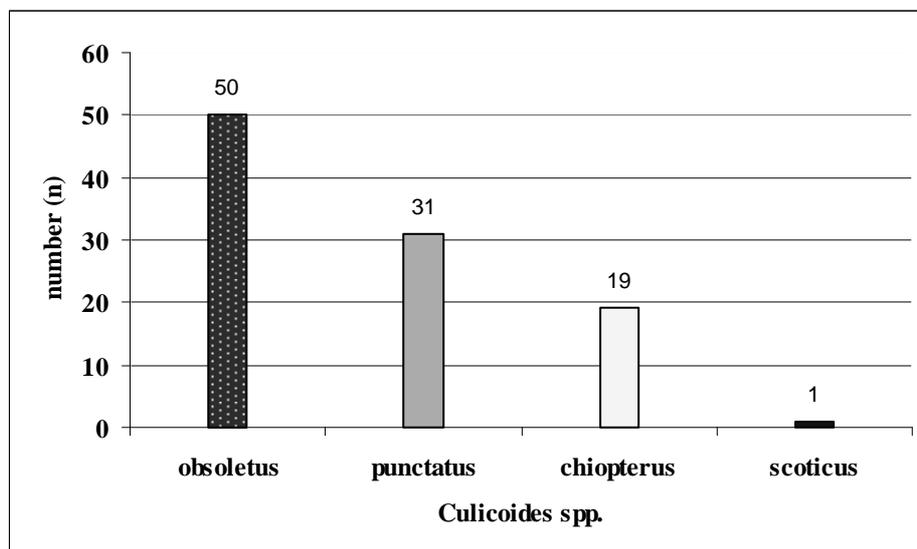


Figure 5. Morphological analysis *Culicoides* spp.

*C.obsoletus* was the most abundant and prevalent species found. From the total 101 *Culicoides* spp. found 50 were *C.obsoletus* (n = 50), 31 *C.punctatus* (n =31), 19 *C.chiopterus* (n =19) and 1 *C.scoticus* (Fig 5).

In total 93 female midges (n = 93) and 8 male midges (n = 8) were captured during the study. The different *Culicoides* species obtained from the insect collections were not evenly distributed over time (Fig. 6).

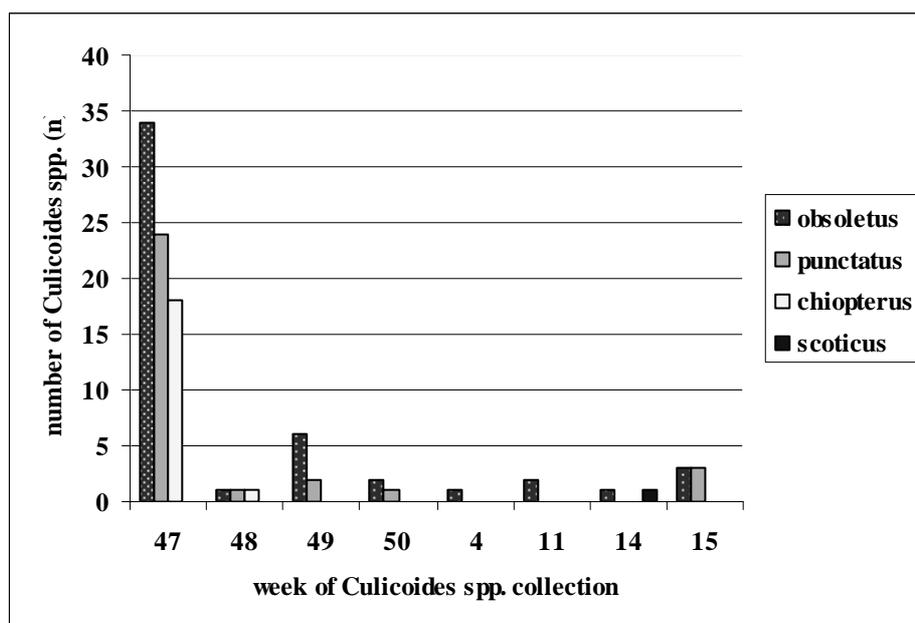


Figure 6. Incidence *Culicoides* spp. per week

*Culicoides obsoletus* species were captured throughout the whole insect collection period (November, 2007 up to April, 2008). *C.punctatus* species were captured during the first 4 weeks of insect collections (week 47, 48 and 49) and after an absence of 17 weeks, in week 15 again. *C.chiopterus* was captured during week 47 and week 48. *C.scoticus* has been absent during the first 20 weeks of insect collection and was captured only once in week 14.

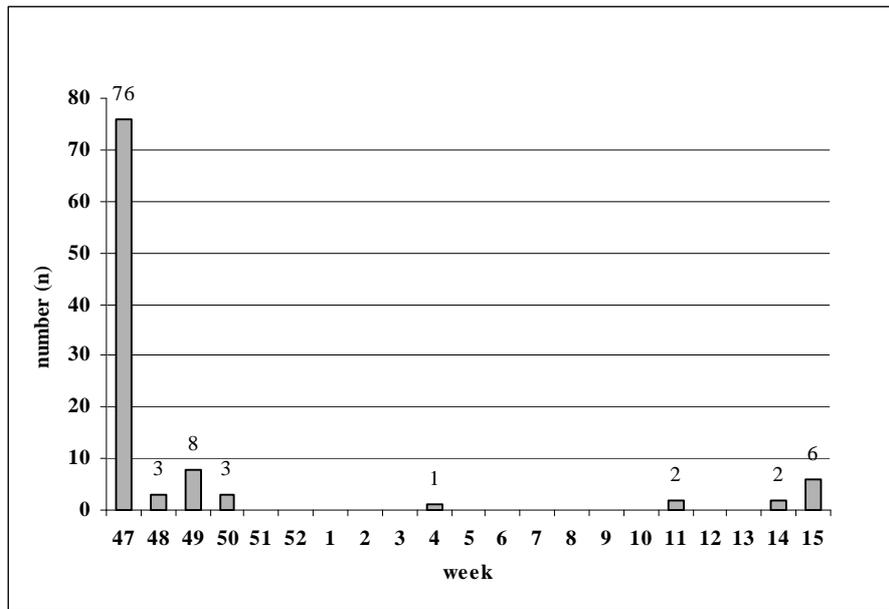
During the winter insect collections *Culicoides* spp. were obtained from 11 out of the 16 farms as on 5 farms no *Culicoides* spp. were captured at all (Table 5).

Table 5. Total number of captured *Culicoides* spp. per farm

farm	district	total nr <i>C. spp.</i>	<i>C.obsoletus</i>	<i>C.punctatus</i>	<i>C.chiopterus</i>	<i>C.scoticus</i>
1	Afferden	5	2	3	0	0
2	De Klomp	0	0	0	0	0
3	Ederveen	4	1	2	1	0
4	Dreumel	5	3	2	0	0
5	Lunteren	12	8	1	3	0
6	Werkhoven	47	20	14	13	0
7	Ooij	0	0	0	0	0
8	Beuningen (GLD)	0	0	0	0	0
9	Pannerden	0	0	0	0	0
10	Puiflijk	1	1	0	0	0
11	Ede	4	2	1	1	0
12	Ederveen	3	2	0	0	1
13	Afferden	1	1	0	0	0
14	Werkhoven	17	8	8	1	0
15	Persingen	0	0	0	0	0
16	Pannerden	2	2	0	0	0

The majority of *Culicoides* spp. found were obtained from 3 farms in 2 districts, namely Werkhoven and Lunteren.

The *Culicoides* collections over time were not evenly distributed (Fig. 7). At the start of the insect collections, week 47, november 20, 2007, a high number of *Culicoides* spp. was captured.



**Figure 7. Weekly total collections of *Culicoides* spp. 2007-2008**

During the first week of trap collections 76 *Culicoides* spp.(n =76) were captured, which is the majority of the total collection. From week 51 up to week 4 no *Culicoides* were captured from the traps. In week 4 one *Culicoides* spp.(n =1) was captured, while in week 11 two *Culicoides* spp. (n= 2) were captured. During the last week of insect collection 6 *Culicoides* spp. were captured.

#### 4.2 Age structure of female *Culicoides* spp.

The age structure of the collected female *Culicoides* spp. was estimated. Three stages of gonothrophic development in females were used as indicators of age. These stages of development include the nulliparous, parous and gravid stage. The freshly blood fed females were count up as well.

**Table 6. Development stage female *Culicoides* spp.**

	nulliparous	parous	gravid	freshly blood fed
Obsoletus	16	8	19	3
Punctatus	7	20	1	0
Chiopterus	0	18	0	1
Scoticus	0	0	0	0
Total	23	46	20	4

Most *Culicoides* spp. found were in the parous stage of gonothrophic development. Almost an even number of nulliparous and gravid females were found. Freshly blood fed females were found 4 times (Table 6).

The development stages of female midges were not evenly distributed over time (Fig. 8) .

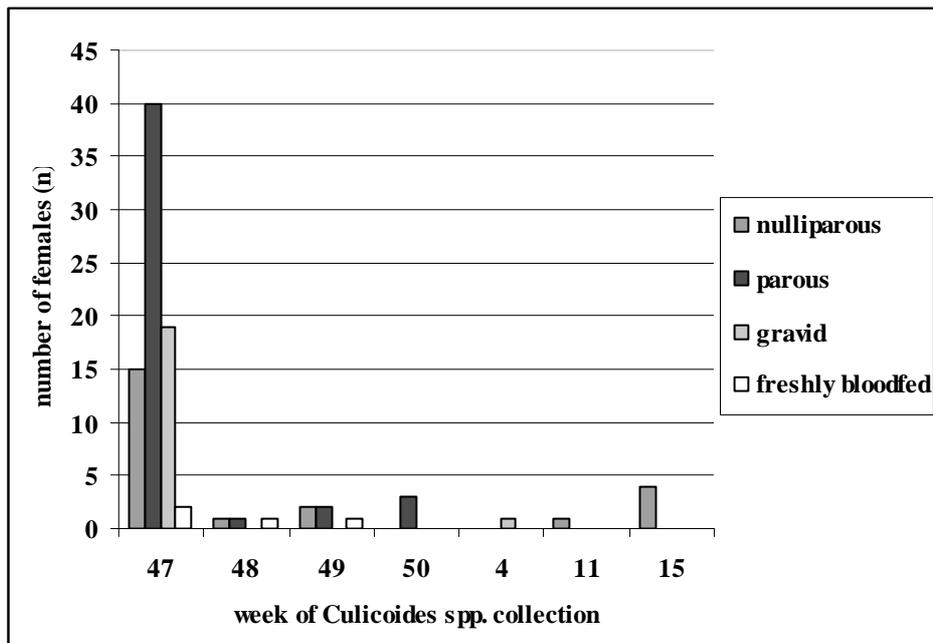


Figure 8. Development stage distribution over time

At the start of the collections in week 47, November 2007, a high parous rate (54%) of female *Culicoides* spp. was found. 20% of the females collected were in the nulliparous stage of development while 26% of the females captured were gravid. Two females captured in week 47 were freshly blood fed. In week 48, November 2007 and 49, December 2007, a parity rate of 50% of parous and nulliparous females was found. In both weeks 1 captured female was freshly blood fed. During week 48 and week 49 no gravid females were captured. In week 50, December 2007, all captured females were found to be in the parous stage of development. In week 4, January 2008, one gravid *Culicoides* spp. was captured. During week 11, March 2008, and week 15, April 2008, all captured females were in the nulliparous stage of development.

#### 4.3 Weekly *Culicoides* spp. collections on farms

At all farms included in the study insect collection took place at an interval of 2 weeks per farm. During week 6, 7 and 8 close monitoring took place at an 1 week interval. On each farm 2 traps were operated on 2 consecutive days, being emptied every 24 hours. This resulted in a total *Culicoides* spp. collection of 101 (n= 101). All biting midges were obtained from 11 farms with the corresponding numbers 1,3,4,5,6,10,11,12,13,14 and 16 (Annex III). The majority of *Culicoides* spp.(n= 47) were obtained from farm 6, followed by farm 14 (n=17), farm 5 (n= 12), farm 4 (n= 5), farm 1 (n= 5), farm 3 (n= 4), farm 11 (n= 4), farm 12 (n=3) and farm 16 (n= 2). From farm 10 and 13 one (n=1) *Culicoides* spp. was obtained.

The majority of *Culicoides* spp. were captured during the weeks 47, 48, 49 and 50 (Annex IV).

A total of 25 *Culicoides* spp.(n=25) were captured during the first 24 hours of insect collection on farm 6 (week 47b). One biting midge was captured in the outdoor trap, the remaining 24 *Culicoides* spp. were captured in the indoor trap. During the following 24 hours (week 47b) another 20 *Culicoides* spp.(n= 20) were captured in the indoor trap. After a 2 week interval 2 *Culicoides* spp. (n= 2) were captured in the outdoor trap on farm 6 (week 50b).

During 2 collection days in week 47b, a total of 16 *Culicoides* spp.(n= 16) were captured on farm 14. During the first 24 hours of insect collection, 13 midges (n= 13) were captured, 8 in the indoor trap and 5 in the outdoor trap. During the following 24 hours of insect collection (week 47b) the remaining 3 midges (n= 3) were captured, 2 were collected in the indoor trap and 1 was collected in the outdoor trap. In week 50b one *Culicoides* spp. (n= 1) was captured in the indoor trap operated on farm 14.

On farm 5 a total of 10 *Culicoides* spp.(n= 10) were captured during the first 2 collection days in week 47a. One midge was captured in the indoor trap during the first 24 hours. During the following 24 hours 5 midges in the indoor trap and 4 midges in the outdoor trap were captured. In week 49b, 2 *Culicoides* spp.(n= 2) were captured on farm 5, one indoors and 1 outdoors.

On farm 4, four midges were captured in week 49b indoors during the first 24 hours of collection while one midge was captured during the second 24 hours of insect collection.

On farm 1 a total of 5 *Culicoides* spp.(n= 5) were captured. One midge was captured in week 49a in the indoor trap during the second 24 hours of insect collection. Another 4 midges were captured indoors in week 15b during the second 24 hours of insect collections on the farm.

On farm 3 a total of 4 *Culicoides* spp.(n= 4) were captured indoors in week 47a. All midges were collected during the second 24 hours of the 2 consecutive days of insect collections on the farm. Also on farm 11 a total of 4 *Culicoides* spp. (n= 4) were captured. In week 48a, 2 midges were captured in the outdoor trap during the first 24 hours of collection. In week 4a and week 11a, 2 midges were captured in the indoor traps. The midge captured in week 4a was obtained during the second 24 hours of collection while the midge captured in week 11a was obtained during the first 24 hours of collection on farm 11.

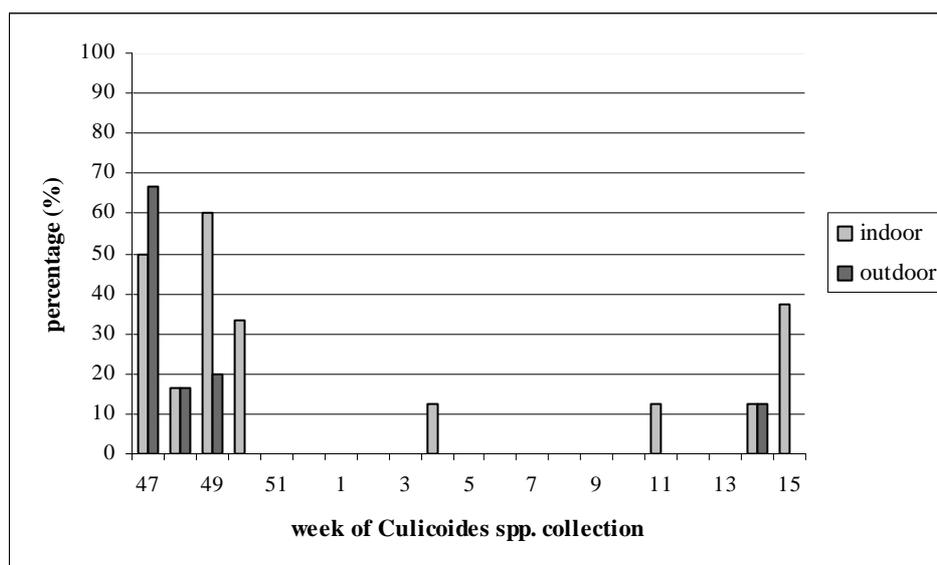
On farm 12 a total of 3 *Culicoides* spp.(n= 3) were captured. One midge was captured in week 47b in the outdoor trap during the second 24 hours of insect collection on the farm. During week 14a 2 midges were captured, one outdoors during the first 24 hours of collection and one indoors during the second 24 hours of insect collection on the farm.

On farm 16 two midges were captured. Both the midges were captured in the indoor traps. The first midge was captured in week 48a during the second 24 hours of insect collection and the second midge was captured in week 11b during the first 24 hours of insect collection on farm 16.

On the remaining 2 farms, 1 midge per farm was captured (n= 1) In week 15b one midge per farm was collected from farm 10 and 13. On both farms the midges were captured in the indoor trap during the first 24 hours of insect collection.

#### **4.3.1 Midge activity on farms**

The percentage of farms with midge activity during a particular week is calculated to compare the weekly collections of *Culicoides* spp. on farms (Fig. 9).



**Figure 9. Weekly percentage of farms with midge activity**

On 50% of the farms included in the insect collections of week 47 midge activity was shown indoors as *Culicoides* spp. were obtained from the indoor traps. In the same week 67% of the farms showed midge activity outdoors. In week 48 midge activity was shown on 17% of the farms indoors and on 17% of the farms outdoors. During week 49, midges from the indoor traps were obtained from 60% of the farms as on 20% of the farms included in the week collections midges were obtained from the outdoor traps. On 33% of farms included in the collections of week 50, midges were obtained from the indoor traps. During week 4 and week 11 midge activity indoors was shown on 13% of the farms included in the collections. In week 15 midge activity indoors was shown on 38% of the farms included in the collections of week 15.

#### **4.4 Environmental parameters**

Midge activity is assumed to be influenced by environmental parameters. Therefore, during the insect collections temperature was measured on 8 participating farms. Both inside and outside, minimum, maximum and mean temperatures were obtained. The data were interpolated to obtain the average values for each region. Besides, average national climate data were obtained from both the Royal Netherlands Meteorological Institute (KNMI) as well as from WUR weather station (met.wau, 2008) (Annex V).

##### **4.4.1 National averages environmental parameters**

The average temperature in the autumn of 2007 (September, October, November) in the Netherlands was 10.3°C which is a common temperature (average 1971-2000) during the autumn season in the Netherlands. In September and October the average temperatures were 13.8°C and 10.1°C respectively, which is slightly below the average temperatures of both months in previous years (1971-2000). November 2007 had an average temperature of 6.9°C. On October 20 the first frost was noted in the Netherlands, in total 7 frost days (minimum temperature < 0.0°C) were noted during the autumn of 2007. The minimum temperature during autumn, -5.5°C, was measured on November 16, 2007. The autumn was relatively dry

with a national precipitation of 181mm. In total 309 sun hours were noted which is average during the autumn (average 1971-2000).

The winter of 2007/2008 (December, January, February) in the Netherlands was relatively mild with a national average temperature of 5.1°C. Historically it has been very mild, as since 1901 only 6 winters were milder than the winter of 2007/2008. The winter of 2006/2007 was the mildest winter ever been recorded with a national average temperature of 6.5°C.

The average temperature in December was 3.8°C which is common for the time of the year, while January 2008 was extremely mild compared to previous years, with an average temperature 6.5°C. Also February was mild with an average temperature of 5.1°C. In total 28 frost days were noted during the winter. January was an extremely wet month with an average precipitation of 85 mm compared to an average of 69 mm in previous years, while February was dry compared to previous years (average 1971-2000). In February only 36 mm precipitation was measured. The sun hours during the winter of 2007/2008 exceeded the average of previous years with a total of 250 hours compared to 175 hours normal (average 1971-2000).

March 2008 had an average temperature of 5.9°C which is common for the time of the year. In total 9 frost days were noted during March which is also the national average of previous years. During March it was extremely wet with an average precipitation of 104 mm compared to 65 mm normal (average 1971-2000). An average of 125 sun hours were noted during March, which is common during early spring (climate data KNMI, 2008).

The average temperature of April 2008 was 8.9°C, which is slightly higher than 8.3°C normal (average 1971-2000). Before April 20, 2008 temperatures did not exceed 15°C. A total of 8 frost days were noted during April which is twice as high as normal. Five consecutive frost days were noted (April 6, 2008 up to April 10, 2008). The average precipitation was 33 mm compared to 44 mm normal and an average of 190 sun hours were noted compared to 162 normal. This makes April 2008 relatively dry and sunny but cold during the first 3 weeks of the month (climate data KNMI, 2008).

Temperatures obtained from the WUR weather station (met.wau, 2008) represent the region of Wageningen (Annex V). Temperatures in the province of Gelderland were slightly higher than the national average temperatures.

#### **4.4.2 Indoor and outdoor temperature distribution on farms**

Indoor and outdoor temperatures were measured on 8 participating farms in the study (Annex VI). The temperature measurements of the 8 farms represent the temperatures of the 8 remaining farms in the study (Table 7). According to district area and barn type the remaining farms were assigned to the temperature measurement farms as corresponding farms.

**Table 7. Temperature measurement**

farm*	barn type	corresponding farm**	mean difference (°C)***
6	cubicle	14	2.3
4	cubicle	1	1.4
11	stand	2-12	6.0
9	cubicle	16	2.2
7	loose	15	2.2
8	cubicle	10	4.0
13	loose	1	1.1
5	stand	3	5.9

\*(farm with data loggers representing corresponding farms) \*\*(corresponding farm according to district area and barn type)\*\*\*(mean difference in temperature indoor versus outdoor)

During the winter of 2007-2008 the mean indoor temperatures were higher than the outdoor temperatures (Annex VI). Depending on barn type, the mean difference between indoor and outdoor temperatures ranged between 1.1°C and 6.0°C (Table 7). The mean difference in indoor temperature compared to outdoor temperature for all farms was 3.1°C. Stand type barns showed the highest mean differences between indoor and outdoor temperatures while loose housing and cubicle type barns showed the lowest mean differences in temperature.

#### 4.4.3 Relation between temperature and activity of *Culicoides* spp. on farms

There is a strong influence of temperature on activity of *Culicoides* species. Midges are assumed active as long as they are captured during the trap collection days. At outdoor temperatures of 5°C and above (Annex VII and Annex III) midges were obtained from the traps. At outdoor temperatures below 5°C there was no activity of *Culicoides* species seen as no midges were captured. Low temperatures slow down the metabolism and thereby the activity of midges, while the survival of an individual midge increases.

It seems that midges in winter are most active in the days of or soon after a temperature peak between 5°C and 10°C (Annex VII and Annex VIII).

If outdoor temperatures are low midges tend to migrate as at decreasing temperatures most *Culicoides* spp. were captured in indoor traps. Temperatures around indoor traps are on average 3.1°C higher compared to outdoor temperatures. The relative high temperatures and the presence of cattle upon which to feed make livestock barns favourable environments to midges to overwinter.

#### 4.5 Insecticidal treatment

To investigate whether there is an effect of the insecticidal treatment on *Culicoides* spp. densities in livestock barns close monitoring of *Culicoides* spp. took place on all farms before and after the insecticidal treatment.

**Table 8. *Culicoides* spp. collection after treatment**

farm	barn type	treatment	date	<i>Culicoides</i> spp.***	date	trap	stage
11	stand	control	11-02-08	1 ( <i>C. obsoletus</i> )	11-03-08	indoor	nulliparous
5	stand	insecticide	11-02-08				
3	stand	insecticide	11-02-08				
12	stand	control	11-02-08	1 ( <i>C. obsoletus</i> ) 1 ( <i>C. scoticus</i> )	01-04-08 02-04-08	outdoor indoor	male male
2	stand	insecticide	11-02-08				
6	cubicle*	insecticide	12-02-08				
14	cubicle*	control	12-02-08				
9	cubicle*	insecticide	12-02-08				
16	cubicle*	control	12-02-08	1 ( <i>C. obsoletus</i> )	13-03-08	indoor	male
7	loose**	insecticide	13-02-08				
15	cubicle*	control	13-02-08				

13	loose**	control	13-02-08	1 ( <i>C. obsoletus</i> )	10-04-08	indoor	nulliparous
8	cubicle*	control	13-02-08				
1	cubicle*	insecticide	14-02-08	1 ( <i>C. obsoletus</i> )	11-04-08	indoor	nulliparous
				1 ( <i>C. punctatus</i> )	11-04-08	indoor	nulliparous
				1 ( <i>C. punctatus</i> )	11-04-08	indoor	male
				1 ( <i>C. punctatus</i> )	11-04-08	indoor	male
10	cubicle*	insecticide	14-02-08	1 ( <i>C. obsoletus</i> )	10-04-08	indoor	nulliparous
4	cubicle*	control	14-02-08				

\*(cubicle on slatted floor with storage for slurry manure) \*\* (loose housing of livestock) \*\*\* (*Culicoides* spp. collected after treatment)

A total of 10 *Culicoides* spp. (n= 10) were collected after the insecticidal treatment of mid February 2008 (Table 8). The first midge captured after treatment was collected on March 11, 2008, 29 days after treatment. This midge was captured from farm 11 which was assigned to the control group during the treatment.

*Culicoides* spp. were captured on 6 out of the 16 farms included in the treatment. Four out of the 6 farms were assigned to the control group and treated with water only. The remaining 2 farms were assigned to the treatment group and treated with insecticides.

Nine *Culicoides* spp. (n= 9) were captured in the indoor traps, 1 *Culicoides* spp. was captured in the outdoor trap.

Only male *Culicoides* spp. and young female *Culicoides* spp. in the nulliparous stage of gonothropic development were collected after the insecticidal treatment.

Due to low *Culicoides* species densities before and in the days of the treatment no effect was shown of the insecticidal treatment on *Culicoides* spp. density in livestock barns.

#### 4.6 Emergence traps

An emergence trap experiment was set up to study possible larval development and adult emergence from manure. It is assumed that certain species of *Culicoides* preferably oviposit in manure of livestock.

A total of 80 manure samples were transferred to Plant Protection Services for insect identification.

**Table 9. Emerged *Culicoides* spp. from emergence trap experiment**

sample	farm	barn type	<i>Culicoides</i> spp.	gender
19	14	cubicle	<i>C. obsoletus</i>	♂
19	14	cubicle	<i>C. obsoletus</i>	♂
19	14	cubicle	<i>C. obsoletus</i>	♂
19	14	cubicle	<i>C. obsoletus</i>	♂
24	14	cubicle	<i>C. obsoletus</i>	♂
29	10	cubicle	<i>C. obsoletus</i>	♂
30	15	cubicle	<i>C. obsoletus</i>	♂
30	15	cubicle	<i>C. obsoletus</i>	♂
35	15	cubicle	<i>C. obsoletus</i>	♂
50	15	cubicle	<i>C. obsoletus</i>	♂

After the experiment 6 manure samples were found positive for the presence of *Culicoides* species (Table 9). In total 10 *Culicoides* spp. (n= 10) emerged from the manure. The emerged

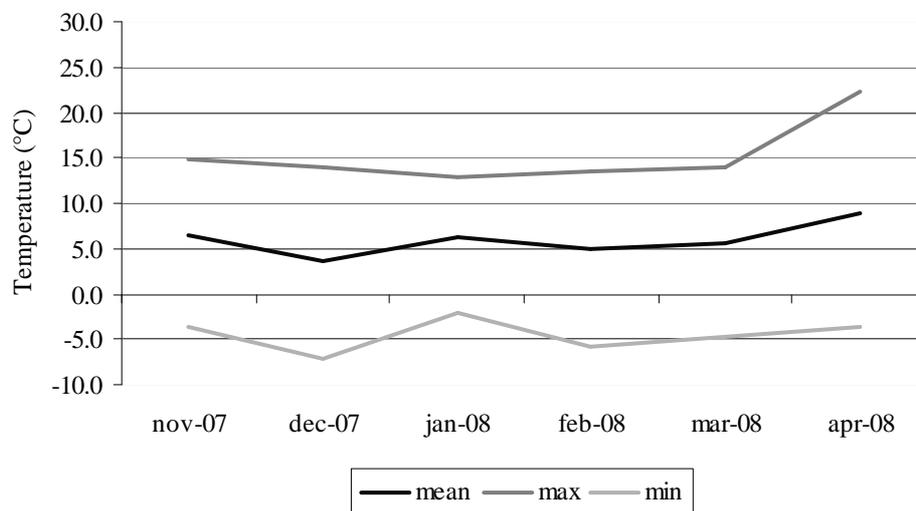
midges originate from manure collected on 3 out of the 16 sampled farms (manure from farm 14, 10 and 15). All farms had cubicle type barns. All *Culicoides* spp. captured were *C.obsoletus* species. From the captured species 8 midges were male and 2 midges were female.

## 5. DISCUSSION

### *Winter collection of Culicoides spp.*

In general midge activity is assumed to decrease when outdoor temperatures fall below 10°C (De Koeijer & Elbers, 2007). Nevertheless, during the study several *Culicoides* spp. were collected at outdoor temperatures below 10°C. In the time of the study midge activity was still observed at outdoor temperatures of 5 °C and above (Annex VII). Besides activity, the expected lifespan of *Culicoides* spp. also depends on temperature. It is assumed that the lowest mortality rates of *Culicoides* spp. can be found at 15°C. Towards 0°C mortality rates are assumed to increase fast while temperatures above 15°C are assumed to cause an increasing mortality of biting midges due to an increasing metabolism (De koeijer & Elbers, 2007).

During the collection of insects in the winter of 2007-2008 the mean outdoor temperature was below 10°C (Fig. 10).



**Figure 10. Outdoor temperature distribution winter 2007 -2008**

In general adult populations of *Culicoides* spp. tend to fall by the end of October. Depending on the prevailing temperature from December up to April, adult midges are either not at all or only in very small numbers found. Due to the low temperatures in winter low density and low activity of *Culicoides* spp. present on farms is expected. Most midges were captured in the traps operated indoors. The average temperatures inside livestock barns are higher than the average temperatures outdoors which may increase survival and activity of present *Culicoides* spp.(Annex VI). The mean difference between indoor and outdoor temperatures ranged between 1.1 °C and 6.0 °C. Barn type seems to influence the mean difference in temperature indoors and outdoors. The temperature measurements during the study showed the highest mean difference between indoor and outdoor temperature on farms with stand type barns (farm 5 and farm 11) (Fig. 34 and Fig. 39). Farms 5 and farm 11 showed a mean difference between indoor and outdoor of 5.9 °C and 6.0 °C, respectively. The temperature measurement on farm 5 showed a minimum indoor temperature of 7.5 °C as on farm 11 the minimum indoor temperature was 7.0 °C. On farm 5 a high number of midges (n = 6) were captured indoors during week 47, November 2007 while on farm 11 one gravid midge was captured indoors in the second half of January 2008. In the context of the correlation between temperature and activity it seems that activity and survival is highly influenced by prevailing

indoor temperatures. This, in return, may result in an increase of overwintering possibilities of adult midges.

The trap efficiency is assumed to be 10 % which implies that only 10% of the *Culicoides* spp. present on farms are captured during the collection days. Although black light traps are found to be more attractive to certain midges than white light traps or CO<sub>2</sub> traps, the trap efficiency of 10% during winter is disputable. Whether this assumption is a reasonable indication of *Culicoides* densities present on farms during winter is unknown due to the paucity of information and scientific research done on the subject of trap efficiency and seasonal *Culicoides* spp. densities.

*C. obsoletus* is the most abundant and prevalent species found during the winter collection of *Culicoides* species on farms, which comes up to the expectation of most abundant species to be found in the surroundings of livestock. Nevertheless, *C. obsoletus* species are assumed not to be responsible for bluetongue virus transmission in the Netherlands as virus isolations to date have not been made from *C. obsoletus* species captured in the Netherlands. Virus isolations from field-caught *Culicoides* spp. in the Netherlands were only made from *C. chiopterus* and *C. dewulfi* (Dijkstra *et al.*, 2008).

The relatively high numbers of *C. obsoletus* species captured might also be due to species dependent attractiveness to the light traps. It is assumed that the level of attractiveness to certain traps is to some extent dependent on midge species. To substantiate the prevailing assumptions further scientific research is needed, in particular on species abundance and density in the field.

The midge collections are not evenly distributed over the farms as from 11 out of the 16 farms *Culicoides* spp. are obtained during the study. The study was conducted on a relative small number of farms and limited area. Nevertheless, *Culicoides* spp. were found on 69% of the farms included in the study. The farms were described in detail according to location, housing system, livestock species kept, breed of livestock, number of livestock kept, farm surrounding and insecticidal usage to see if there is any correlation between midge activity and environment (Annex I). Besides housing system (which is correlated to indoor temperature), no correlation between midge presence, abundance, activity and environment was found during the study as the number of midges captured was too low for any reliable analysis.

Also the collections over time are not evenly distributed as most *Culicoides* spp. were captured in the second half of November 2007, at the start of the collections. Temperatures during these weeks were relatively mild (but below 10 °C) which may have caused the number of *Culicoides* spp. captured (Fig. 11). In November midge populations decline, though, during the study low densities of midges were found throughout November 2007 until mid December 2007. *Culicoides* spp. in North-western Europe are assumed to have 3 generations per year. The first generation of midges starts by the end of April, the second starts in early July, and finally, the last generation starts by the end of August (Takken, *personal communication*, 2008). In this context, the midges captured in November and December are probably remained third generation midges. Shorter and warmer winters will increase the overwintering abilities of adult midges and extend their development seasons, which, in return, will result in more generations of midges per year.

Midge collection became zero at mid December 2007 which may have been caused by the outside temperature decline at this time (Fig. 12).

During the first week of insect collections the percentage of farms with midge activity, from which *Culicoides* spp. were obtained, was high, especially for midges captured in outdoor traps (Fig. 9). This may have been caused by relative high outdoor temperatures during week 47 (Fig. 11).

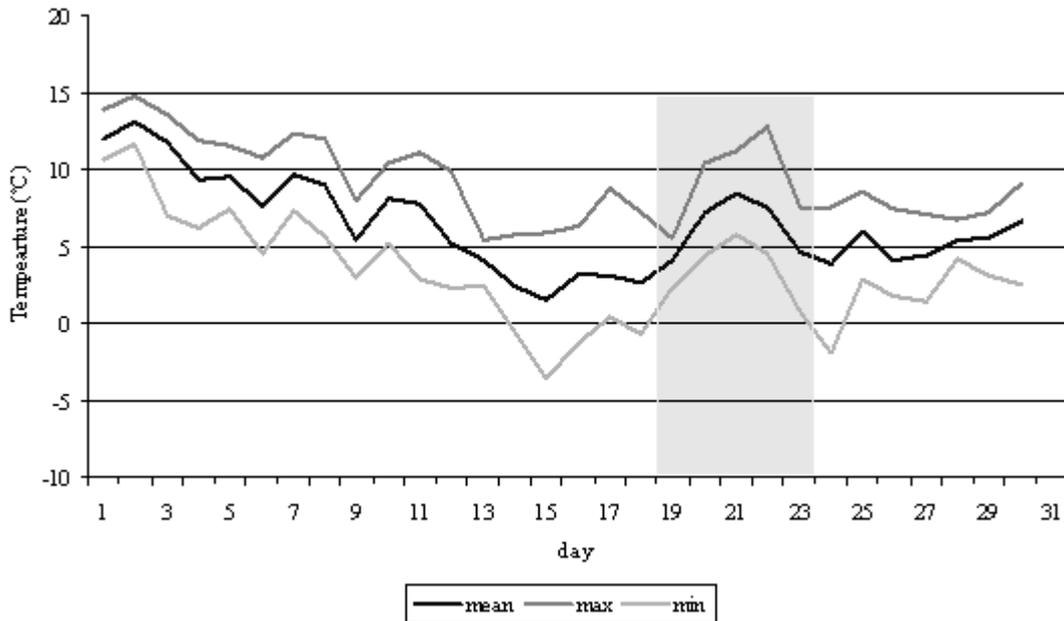


Figure 11. Temperature course week 47 November 2007

In week 48 the percentages of farms with midge activity decreased which may have been caused by the outdoor temperature decrease during week 48. During week 49 the highest percentage of farms with indoor midge activity was reached. Only a small percentage of farms captured midges in the outdoor traps. In week 50 the percentage of farms with midge activity decreased, only *Culicoides* spp. from indoor traps were collected. Week 4, 11 and 14 had the lowest percentage of farms with midge activity. In week 4 and 11 only midges in indoor traps were captured while in week 14 also midges in outdoor traps were captured. In week 15 the percentage of farms with midge activity increased again. During this week only midges in indoor traps were captured.

During the first weeks of collection the percentage of farms with midge activity was high, which may have been caused by relative high outdoor temperatures. As temperature decreases, a decreasing percentage of farms involved in midge collections can be seen (Fig. 9). The percentage of farms with midge activity outdoors decreases as well during winter.

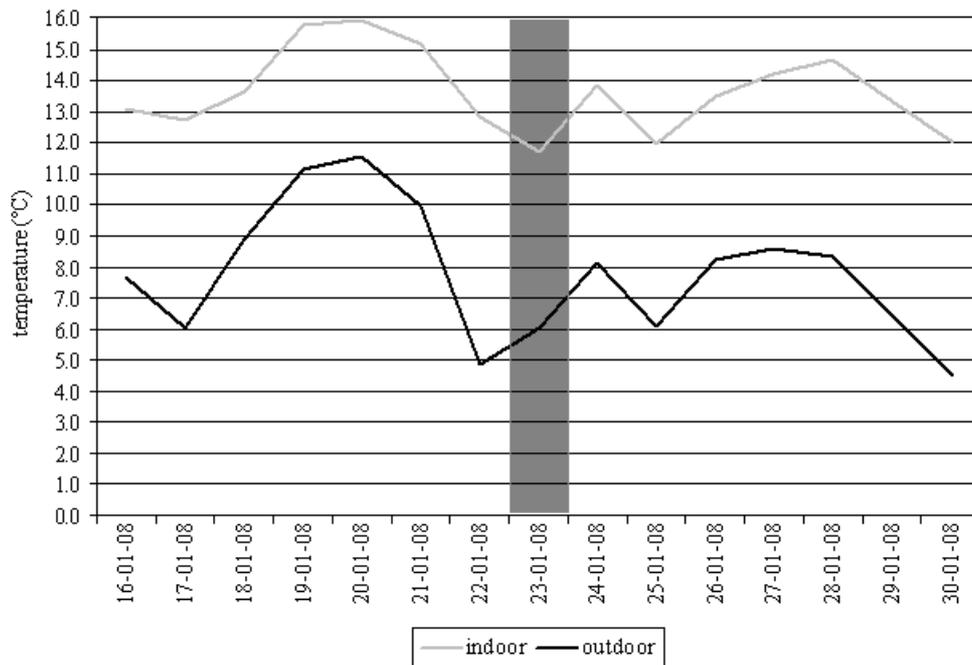
#### *Development stage*

The age structure of the collected females *Culicoides* spp. is estimated as it provides valuable information on the activity of midges throughout the year and thereby the risk of spreading BTV during winter.

Three stages of gonothrophic development in females were used as indicators of age. These stages of development include the nulliparous, parous and gravid stage. Nulliparous females are young, unfed females. Parous and gravid females are older than nulliparous females and are about to, or have laid one or more batches of eggs. As a blood meal is needed for the development of the eggs, the latter two stages have been ingesting blood in previous days and thereby they may have become infected with BTV as well (EFSA, 2007).

At the start of the winter collections a high parous rate was found among the *Culicoides* females, which is indicative for a high survival rate (Fig. 8). A high survival rate of vectors is essential for successful replication of BTV within the vector as well as for the transmission of BTV from vector to host (EFSA, 2007). A high survival rate is assumed to be induced by decreasing temperatures as low temperatures slowdown the metabolism and activity of midges. From November 2007 up to mid December 2007 all development stages of female midges were found as in the second half of January 2008 one gravid female was found

followed by nulliparous females in March and April 2008 (Fig. 8). The gravid female captured in January 2008 indicates the overwintering ability of adult midges. The female was obtained from farm 11 with the stand type housing system known for its high indoor temperatures (Fig.12). The mean temperature difference between indoor and outdoor on farm 11 was 6.0°C, which is the highest mean temperature difference of all farms sampled. Farm 11 had a minimum indoor temperature of 7.0°C during winter, presumably an ideal environment for adult midges to overwinter. Although it is a single catch, this particular female in this particular development stage is one of great importance as they can be the onset of a bluetongue epidemic when overwintering.

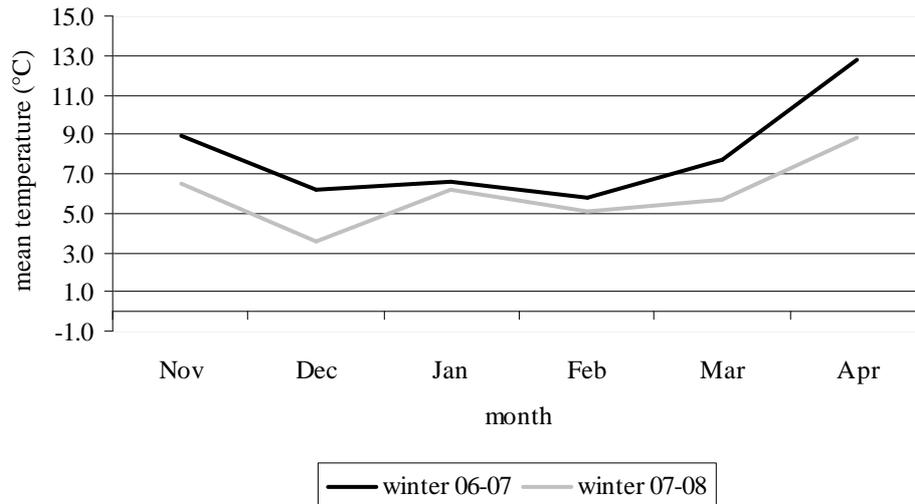


**Figure 12. Temperature distribution farm 11 during *Culicoides* spp. collection January 23 2008**

The midges captured in March 2008 and April 2008 were in the nulliparous stage of gonothrophic development which indicates newly emerged midges instead of adult ones that have overwintered.

*Temperature*

The winter of 2007/2008 was mild with an average outdoor temperature of 5.1°C (December, January and February). The winter of 2006/2007 was even milder with an average temperature of 6.5°C (Fig. 13).

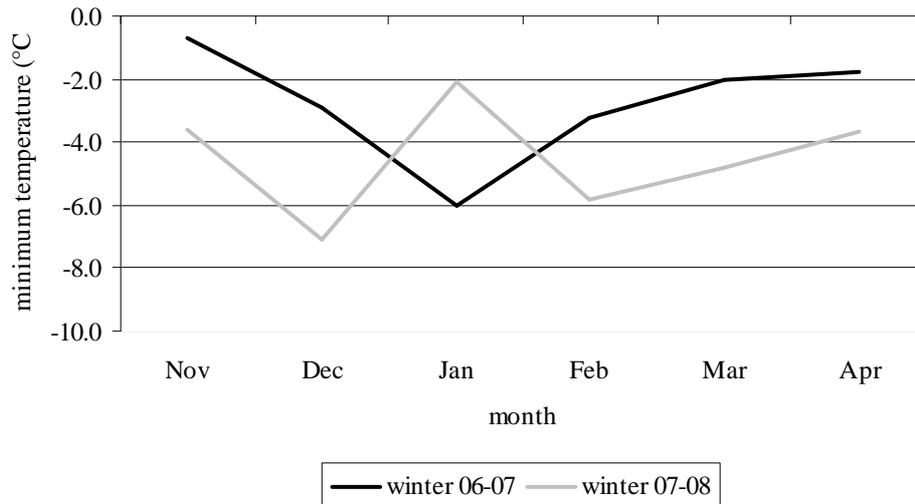


**Figure 13. Mean temperature distribution winter 06-07 and winter 07-08**

The relative high temperatures (but below 10°C) during the previous winter may have increased survival and lifespan of *Culicoides* spp. during winter. Formerly it was assumed that *Culicoides* spp. did not survive the winter season as temperatures were too low, resulting in a midge free season. Former scientific research showed that a small proportion of adult *Culicoides* spp. does survive winter as during earlier surveys midges were found in traps throughout the entire winter season. Also in time of the study (winter 2007-2008) midges were found during supposed midge free periods of the year.

Temperatures inside livestock barns usually do not fall below 0°C, this may induce survival and activity of midges during winter in livestock barns. Indoor and outdoor temperature measurements during winter show a mean difference in indoor temperature compared to outdoor temperature of 3.1°C. The mean difference between indoor and outdoor temperatures ranges between 1.1°C and 6.0 °C. The differences in temperature depends on the barn type. Stand type barns show the highest mean difference between indoor and outdoor temperatures (farm 5 and farm 11). Old fashioned stand type barns are often small in size, isolated and lack proper ventilation. Besides, livestock in stand type barns are leashed, making high indoor livestock densities possible. High densities of livestock in barns increases indoor temperatures, making it a favourable environment for biting midges to overwinter.

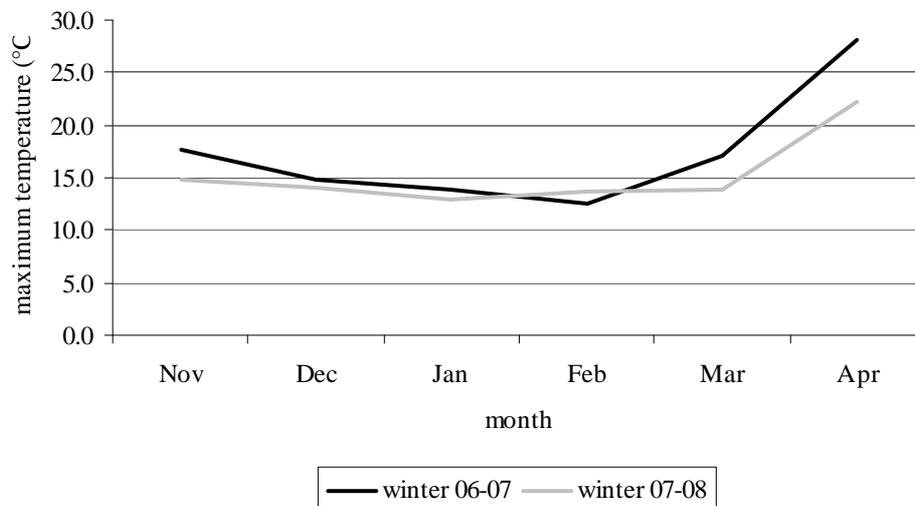
The minimum outdoor temperatures at the start of the winter of 2007-2008 were low compared to the winter of 2006-2007 (Fig. 14). This may have caused high mortality rates among present adult *Culicoides* spp. in early winter resulting in a low species density found throughout the winter.



**Figure 14. Minimum temperature winter 06-07 and winter 07-08**

In November 2007 the minimum outdoor temperature was  $-3.6\text{ }^{\circ}\text{C}$  compared to a minimum of  $-0.7\text{ }^{\circ}\text{C}$  in November 2006. In December 2007 the minimum outdoor temperature reached  $-7.1\text{ }^{\circ}\text{C}$  while in December 2006 the minimum temperature did not exceed  $-2.9\text{ }^{\circ}\text{C}$ .

The low densities of midges found after December 2007 may have been caused by the frost peak in December 2007, supposing that frost causes high mortality rates among *Culicoides* species.



**Figure 15. Maximum temperature winter 06-07 and winter 07-08**

The maximum outdoor temperatures did not exceed  $15\text{ }^{\circ}\text{C}$  during the winter of 2007-2008 (Fig. 15). Even in November 2007 the maximum temperature did not exceed the  $15\text{ }^{\circ}\text{C}$  while the maximum temperatures during November and part of December 2006 did exceed a temperature of  $15\text{ }^{\circ}\text{C}$ . The high maximum temperatures and high minimum temperatures during the winter of 2006-2007 may have caused the higher densities of midges found throughout the winter compared to the winter of 2007-2008.

A temperature effect on *Culicoides* spp. behaviour is undisputable. Nevertheless, closer monitoring of temperatures and behaviour will be needed for more exact cut off values on development and temperature, survival and temperature, feeding and temperature and oviposition and temperature.

### *Temperature and bluetongue disease*

Orbiviruses need a certain temperature for development within the vector to become transmissible to hosts. It is assumed that the virus is unable to develop at temperatures below 10°C. Temperatures between 15°C and 25°C are optimal for bluetongue virus to replicate to transmissible levels (Gubbins *et al.*, 2007). Also a minimum amount of time at suitable temperatures is needed for completion of the virus development cycle within a vector. Supposed global warming will extend the vector season as survival rates increase and vectors stay active for a longer period of time (Defra, 2002). Combined this could result in extended virus development in vectors throughout the year (increased viral transmission season), increased vectorial capacity and a wider geographical area in which bluetongue virus becomes prevalent.

Increasing temperatures resulting in shorter and warmer winters will also improve the overwintering ability of adult *Culicoides* spp. Overwintering adults are likely to increase the spring midge population resulting in even larger populations during summer (Defra, 2002). Overwintering and an extended development season of *Culicoides* species resulting in more generations per year will increase the seasonal occurrence of adult midges and improve the overwintering of bluetongue virus.

### *Insecticidal treatment*

There was no considerable effect of the indoor surface insecticidal treatment on *Culicoides* spp. density inside the livestock barns, presumably due to the low densities of *Culicoides* species before and in the days of the treatment (Annex III). An insecticidal treatment will be an efficient vector control measure when vector densities are high. Nevertheless, more efficient insecticidal treatments such as an ultra low volume (ULV) space spraying are not permitted due to their supposed impact on food safety and health. The effect of insecticidal treatment need to be studied in more detail as it can be part of an integrated control of vector-borne diseases.

### *Emergence trap experiment*

The results of the emergence trap experiment showed larval development at 3 out of the 16 farms sampled (farm 10, 14 and 15) (Table 9). The manure was randomly collected from inside the livestock barns, from areas which were difficult to clean on regular base. This collection method resulted in the use of composted manure in the experiment. Composted manure indoors is presumably an excellent egg development site as it is not cleaned out during winter and besides, oviposition can take place near ruminants to which upon to feed.

The results of the emergence trap experiment show that *Culicoides* species breed, besides outdoors, also indoors. In this context, hygiene on farms plays an important role as larval development does take place on those spots which are difficult to clean out on a regular base. Minimizing indoor breeding sites, by for example, the use of to be determined thorough cleaning methods, needs to be taken into consideration. It becomes even more important as larval development took place from small quantities of composted manure, randomly selected on farms. This may indicate that suitable indoor breeding sites on farms are in abundance resulting in high indoor emergence of *Culicoides* species.

All farms with larval development during the trap experiment had cubicle type barns. As livestock is kept indoors during winter, thorough cleaning of the barn does not take place. Cubicle type barns have slatted floors with storages for slurry manure. Although most manure is cleaned out on a daily base, it is impracticable to avoid manure remaining in cracks and holes of the barns.

Cell temperatures during the experiment did not exceed 22°C. Whether this is a suitable temperature for larval development of indigenous *Culicoides* spp. is unknown.

During the light trap insect collections 1 midge was obtained from farm 10. The midge was captured in April 2008 and had the nulliparous stage of gonothrophic development which may indicate also a newly emerged midge in the field instead of one that had overwintered. On farm 14 a total of 17 midges were captured during the light trap collections. All midges were obtained in November and December 2007, which indicates that no new spring emergence in the field took place until the end of the current study (April 11, 2008). During the entire light trap insect collection period no midges were captured on farm 15. The results of the emergence trap experiment from farm 15 indicate the presence of *Culicoides* species in former times but no activity during the collection days in winter.

Only *C. obsoletus* species emerged from the collected manure which may indicate that these species preferably oviposit in composted manure indoors.

## 6. CONCLUSIONS

Several indigenous (potential) competent bluetongue vectors can be found in the Netherlands. Throughout the summer season *Culicoides* population densities are assumed to be high due to relative high outdoor temperatures. By the end of autumn population densities tend to fall due to decreasing temperatures. Exact numbers on midge densities present throughout the various seasons are unknown. Abundance and activity of *Culicoides* species is strongly influenced by temperature, but an influence of light intensity, relative humidity and wind velocity is assumed as well. During the study a correlation of temperature on midge abundance and activity was shown as *Culicoides* spp. were only captured at mean outdoor temperatures above 5°C. If temperature decreases further *Culicoides* species tend to migrate. Low outdoor temperatures, prevailing in winter, seem to force midges indoors as during insect collection days most midges were captured indoors. Temperatures inside livestock barns are on average 3.1°C higher compared to outdoor temperatures. The difference between indoor and outdoor temperatures ranged between 1.1 °C and 6.0 °C, depending on barn type. Stand type barns had the highest mean difference in temperature, while cubicles had the lowest temperature difference between indoor and outdoor. Relative high indoor temperatures induce survival and activity of midges.

Until mid December 2007 adult *Culicoides* species were found active outdoors as well as indoors. In the second half of January 2008 activity was seen on one of the livestock farms as one female midge was captured indoors after a midge absence of a month. The captured female adult midge was in the gravid stage of gonothropic development which shows adult survival and activity during winter. The presence of parous or gravid females during winter can be the onset of bluetongue disease.

Whether most present *Culicoides* species become inactive or expire during winter is unknown as low temperatures decrease the metabolism and activity of midges. In return, decreasing metabolism and activity could result in an increased survival. It is assumed that adult midges are able to survive over a month in cool environments.

By mid March 2008 activity of adult *Culicoides* species on livestock farms increased again. Activity of midges started indoors as all midges in March were captured indoors. In the first half of April 2008 midge activity was seen both indoors and outdoors. It is assumed that a temperature rise in late spring, from April onwards, will further increase activity and density of *Culicoides* spp., being the onset of the summer populations of *Culicoides* species.

A large proportion of midges captured until mid December were in parous stage of gonothropic development, which indicates a high survival rate. In return, a high survival rate of *Culicoides* species is essential for successful replication of BTV within the vector as well as for the transmission of BTV from vector to host. Though, virus development within the vector does depend on temperature. It is assumed that the virus is unable to develop to transmissible levels at temperatures below 10 °C.

Besides the gravid female midge captured in January 2008, gravid females were only captured during week 47, November 2007.

Freshly blood fed females were only captured during week 47, 48 and 49, November and December 2007. The gravid and freshly blood fed females captured indicate continuing adult survival and activity outdoors as well as indoors. The gravid female captured in January 2008 indicates indoor overwintering of adult midges.

The midges captured in March 2008 and April 2008 were all in the nulliparous stage of development which may indicate newly emerged species instead of overwintered species.

The emergence trap experiment showed larval development from composted manure collected indoors. This indicates that certain *Culicoides* species breed indoors and young midges are likely to emerge from indoor breeding areas when temperature increases in spring.

The indoor insecticidal surface treatment by mid February 2008 had no considerable effect on the population densities of midges. The densities before and in time of the treatment were low, more scientific research is needed to study the effect of insecticides on population densities, preferably when *Culicoides* densities are high.

During the study adult midges were found during supposed midge free periods of the year. This finding might explain the overwintering of bluetongue virus, which is presumably driven by the presence of low densities of *Culicoides* spp. in winter and high densities of viraemic hosts, acting as reservoirs, at the start of the winter season.

The wide range of assumptions made demonstrate the paucity of knowledge on bluetongue vector behaviour and bluetongue virus distribution in northern Europe. Further scientific research will be needed to substantiate the various assumptions in order to control severe future outbreaks of vector-borne diseases.

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# ANNEX

## Annex I

**Table 10. Barn characteristics**

farm	type of housing	size (m <sup>3</sup> )	lockable side	lockable ridge	hygiene
1	cubicle*	2888	yes	no	sufficient
2	stand	1560	closed	no	moderate
3	stand	798	closed	closed	sufficient
4	cubicle*	4813	yes	no	moderate
5	stand	3712	yes	no	poor
6	cubicle*	4557	yes	no	moderate
7	loose housing	1925	yes	no	moderate
8	cubicle*	5850	yes	no	sufficient
9	cubicle*	4900	no	no	moderate
10	cubicle*	11250	yes	no	moderate
11	stand	2340	no	yes	sufficient
12	stand	1300	yes	yes	sufficient
13	loose housing	3820	closed	closed	poor
14	cubicle*	4200	yes	no	moderate
15	cubicle*	4500	yes	no	moderate
16	cubicle*	2080	yes	yes	poor

\*( cubicle on slatted floor with storage for slurried manure)

**Table 11. Livestock characteristics**

farm	herd size cattle	dairy cattle	breed	other livestock spp.	insecticidal use	pour on Butox®	number of treatments during summer of 2007
1	150	80	HF*	horses/sheep	yes	yes	1
2	830	30	MRY**	none	yes	yes	1
3	40	30	HF*	pigs	yes	no	n.a.
4	100	60	HF*	horses	yes	yes	1
5	80	40	HF/MRY	layers	yes	yes	1
6	70	60	HF*	sheep/rodents	yes	yes	1
7	40	n.a.	HF*	sheep	yes	no	n.a.
8	120	100	MRY**	horses	yes	yes	n.a.
9	70	50	HF*	horses	yes	yes	1
10	115	80	MRY**	none	yes	yes	4
11	30	20	HF*	pigs	no	n.a.	n.a.
12	30	25	HF/MRY	pigs	yes	no	n.a.
13			HF*	none	no	n.a.	n.a.
14	120	100	HF*	sheep	no	n.a.	n.a.
15	65	50	HF*	horses	yes	yes	4
16	100	45	HF*	horses	no	n.a.	n.a.

\* (breed Holstein Friesian) \*\*(breed Maas Rijn IJssel)

**Table 12. Farm yard characteristics**

farm	paving	hygiene	open manure heap	trees and shrubs	ditches well maintained	ditches shallow and muddy
1	yes	sufficient	yes	no	yes	yes
2	yes	moderate	yes	yes	yes	no
3	yes	sufficient	yes	yes	yes	no
4	yes	moderate	yes	yes	yes	no
5	yes	moderate	yes	yes	yes	no
6	yes	sufficient	yes	yes	yes	no
7	no	sufficient	yes	yes	yes	no
8	yes	sufficient	no	yes	yes	no
9	yes	sufficient	yes	yes	yes	yes
10	yes	sufficient	yes	yes	yes	no
11	yes	sufficient	no	yes	yes	no
12	yes	sufficient	no	no	yes	yes
13	yes	moderate	yes	no	yes	yes
14	yes	moderate	no	no	yes	no
15	yes	moderate	yes	no	yes	yes
16	no	poor	yes	no	yes	yes

**Table 13. Farm surrounding**

farm	pastureland	arable land	woodland	rivers and small lakes	nature reserve	livestock in area
1	yes	no	no	no	no	sheep
2	yes	yes	yes	no	no	none
3	yes	no	no	no	no	none
4	yes	no	no	yes	yes	sheep
5	yes	yes	yes	no	no	none
6	yes	yes	yes	yes	yes	none
7	yes	yes	no	yes	yes	horses/cattle
8	yes	no	no	no	no	none
9	yes	yes	no	yes	yes	none
10	yes	yes	no	no	no	sheep
11	no	yes	yes	no	no	none
12	no	no	no	no	no	none
13	yes	no	no	no	no	sheep
14	yes	yes	no	no	no	none
15	yes	yes	no	yes	yes	none
16	yes	yes	no	yes	yes	none

## Annex II



Figure 16. Map participating farms (Google Earth 2007)

Table 14. Corresponding farms from figure numbers

Number	Farm	District	Coordinates	Elevation (m)
001	1	Afferden	51°52'48.75"N 5°38'24.51"E	5
002	2	De Klomp	52°02'27.68"N 5°35'27.68"E	7
003	3	Ederveen	52°03'54.06"N 5°35'34.58"E	7
004	4	Dreumel	51°50'44.20"N 5°27'14.88"E	3
005	5	Lunteren	52°07'38.28"N 5°41'06.29"E	13
006	6	Werkhoven	52°01'27.91"N 5°15'27.78"E	2
007	7	Ooij	51°51'05.08"N 5°53'40.84"E	11
008	8	Beuningen	51°50'21.10"N 5°44'45.25"E	5
009	9	Pannerden	51°54'36.41"N 6°01'14.45"E	10
010	10	Puiflijk	51°52'52.33"N 5°34'35.13"E	4
011	11	Ede	52°04'02.01"N 5°37'38.40"E	11
012	12	Ederveen	52°03'52.64"N 5°36'09.07"E	8
013	13	Afferden	51°52'14.14"N 5°39'07.26"E	5
014	14	Werkhoven	52°00'43.49"N 5°15'39.18"E	2
015	15	Persingen	51°50'24.92"N 5°55'04.90"E	8
016	16	Pannerden	51°54'30.59"N 6°06'52.95"E	10

### Annex III

**Table 15. *Culicoides* spp. collection per farm**

Farm	<i>Culicoides</i> spp. (n)	collection date	trap	gender	development stage
1	1	05-12-07	indoor	♀	nulliparous
	4	11-04-08	indoor	♀/♂	nulliparous
3	4	21-11-07	indoor	♀	nulliparous/parous/fed*
4	4	06-012-07	indoor	♀/♂	nulliparous/parous
	1	07-12-07	indoor	♀	parous
5	1	20-11-07	indoor	♀	gravid
	5	21-11-07	indoor	♀	gravid/parous
	4	21-11-07	outdoor	♀	gravid/parous
	1	07-12-07	indoor	♀	blood fed
	1	07-12-07	outdoor	♂	
6	24	22-11-07	indoor	♀	nulliparous/parous/gravid
	1	22-11-07	outdoor	♀	nulliparous
	20	23-11-07	indoor	♀	nulliparous/parous/gravid/fed*
	2	13-12-07	indoor	♀	parous
10	1	10-04-08	indoor	♀	nulliparous
11	2	28-11-07	outdoor	♀	nulliparous/parous
	1	23-01-08	indoor	♀	gravid
	1	11-03-08	indoor	♀	nulliparous
12	1	23-11-07	outdoor	♀	parous
	1	01-04-08	outdoor	♂	
	1	02-04-08	indoor	♂	
13	1	10-04-08	indoor	♀	nulliparous
14	8	22-11-07	indoor	♀	parous/gravid
	5	22-11-07	outdoor	♀	nulliparous/parous
	2	23-11-07	indoor	♀	nulliparous/gravid
	1	23-11-07	outdoor	♀	nulliparous
	1	13-12-07	indoor	♀	parous
16	1	28-11-07	indoor	♀	fed*
	1	13-03-08	indoor	♂	

\*(freshly blood fed females)

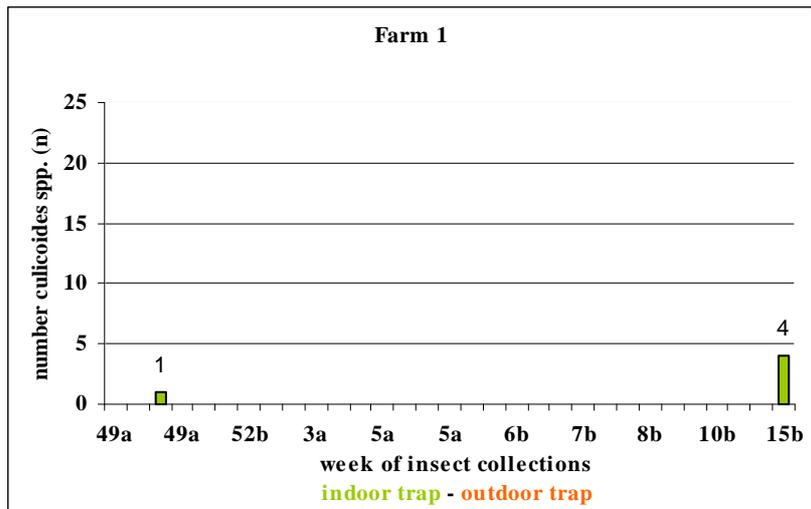


Figure 17. Farm 1 weekly collections of *Culicoides* spp.

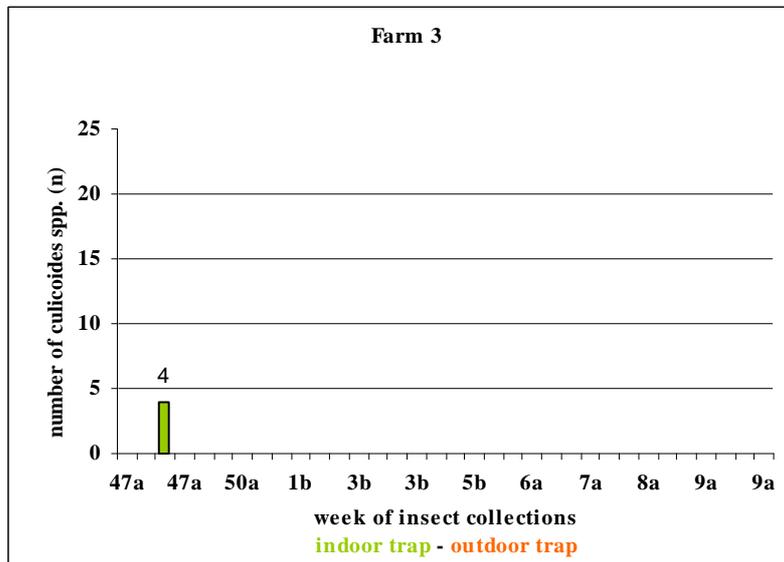


Figure 18. Farm 3 weekly collections of *Culicoides* spp.

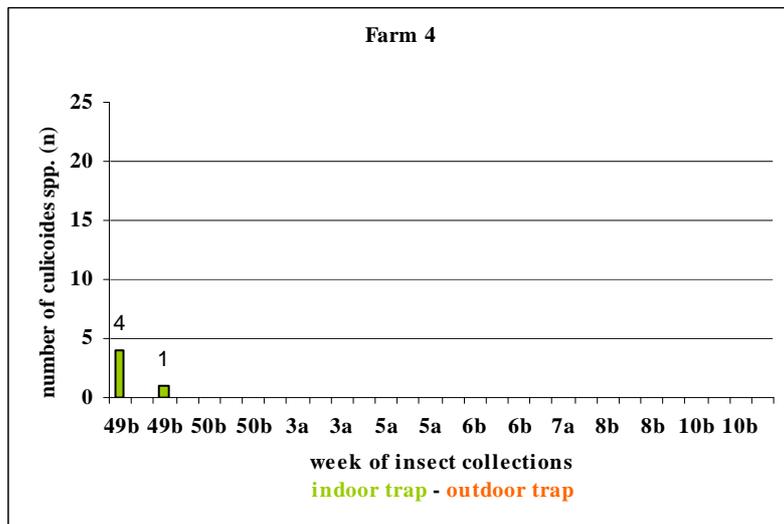


Figure 19. Farm 4 weekly collections of *Culicoides* spp.

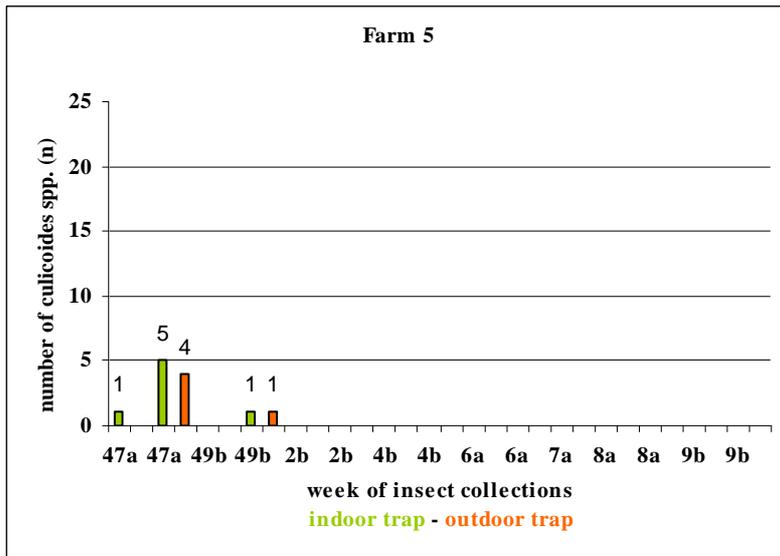


Figure 20. Farm 5 weekly collections of *Culicoides* spp.

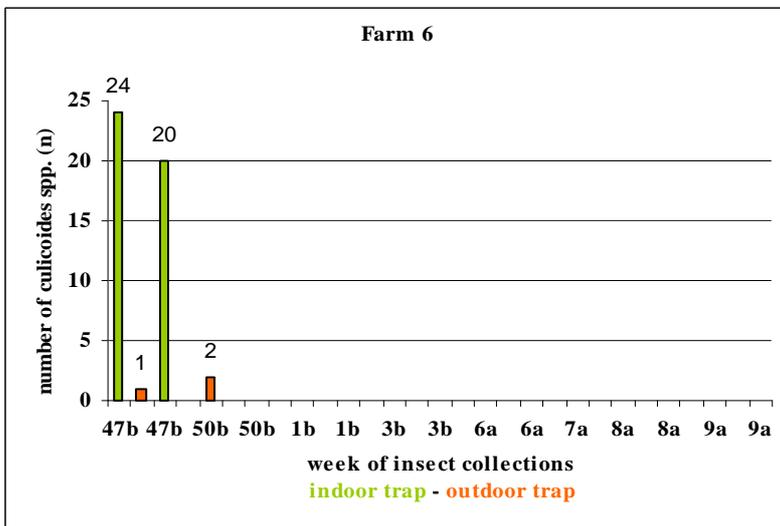


Figure 21. Farm 6 weekly collections of *Culicoides* spp.

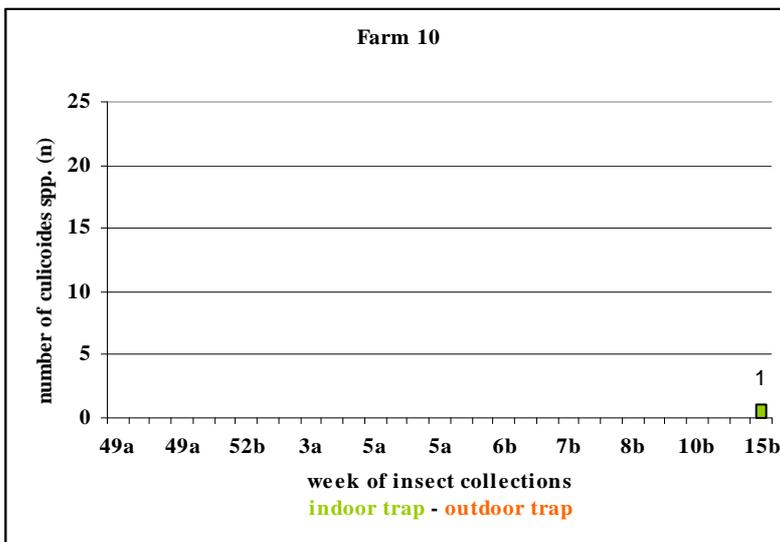


Figure 22. Farm 10 weekly collections of *Culicoides* spp.

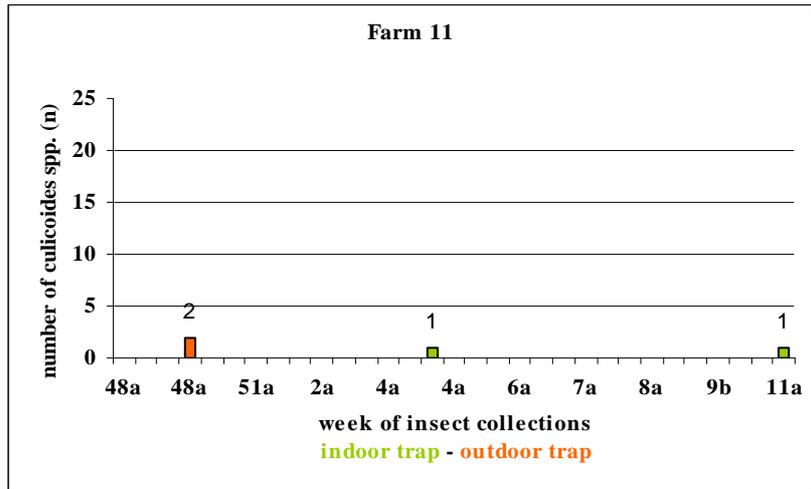


Figure 23. Farm 11 weekly collections of *Culicoides* spp.

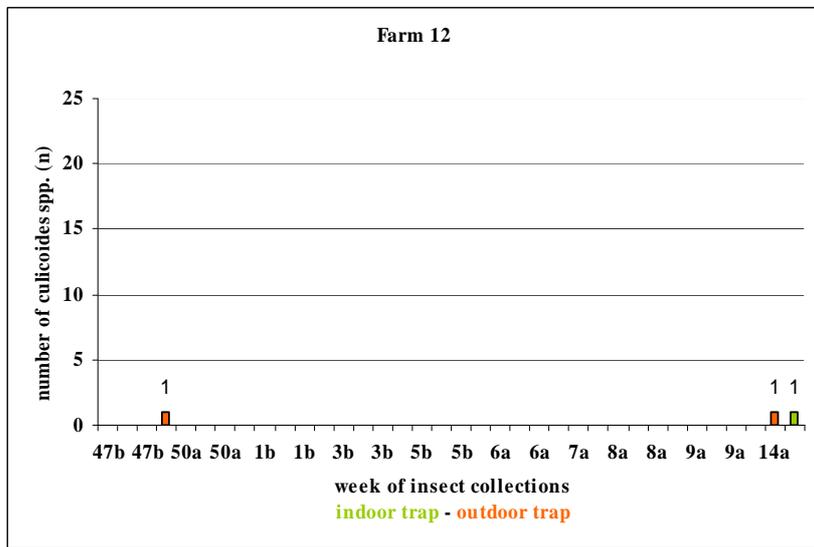


Figure 24. Farm 12 weekly collections of *Culicoides* spp.

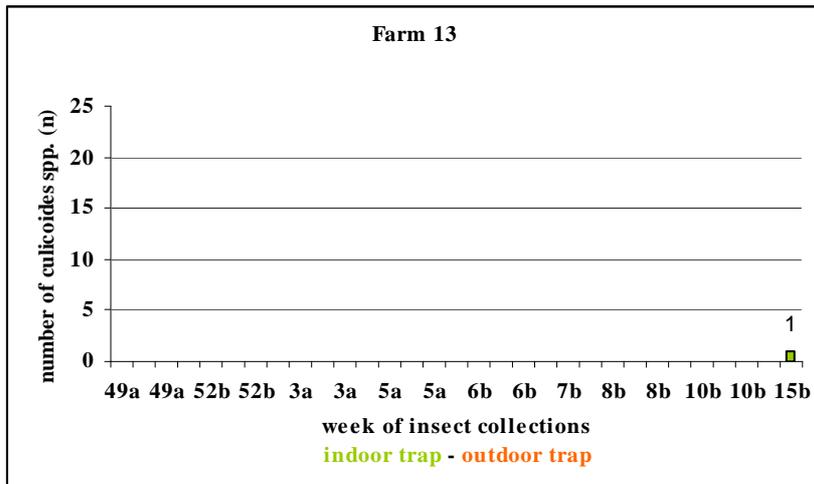


Figure 25. Farm 13 weekly collections of *Culicoides* spp.

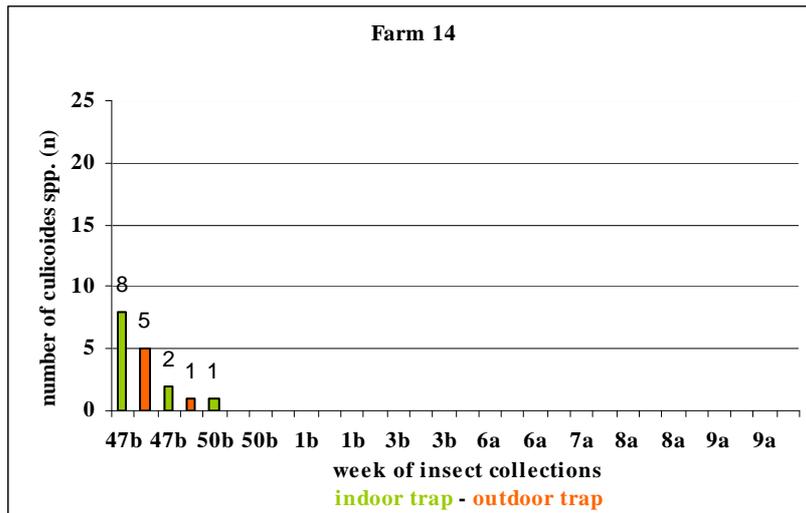


Figure 26. Farm 14 weekly collections of *Culicoides* spp.

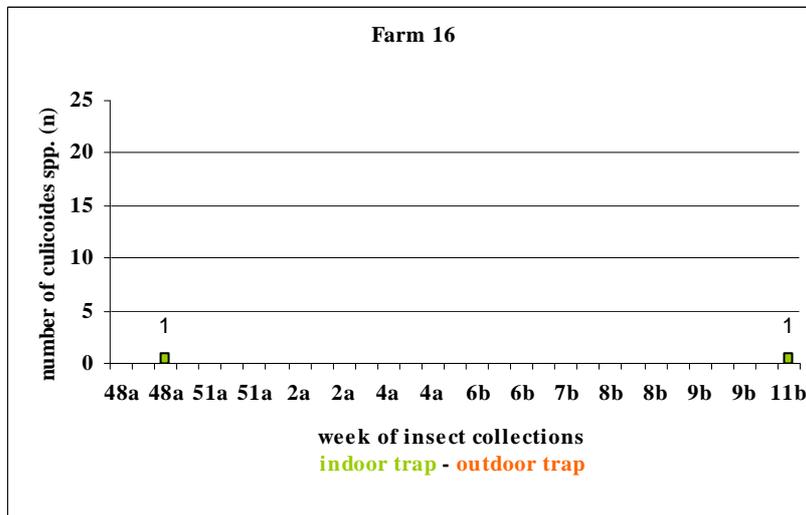


Figure 27. Farm 16 weekly collections of *Culicoides* spp.

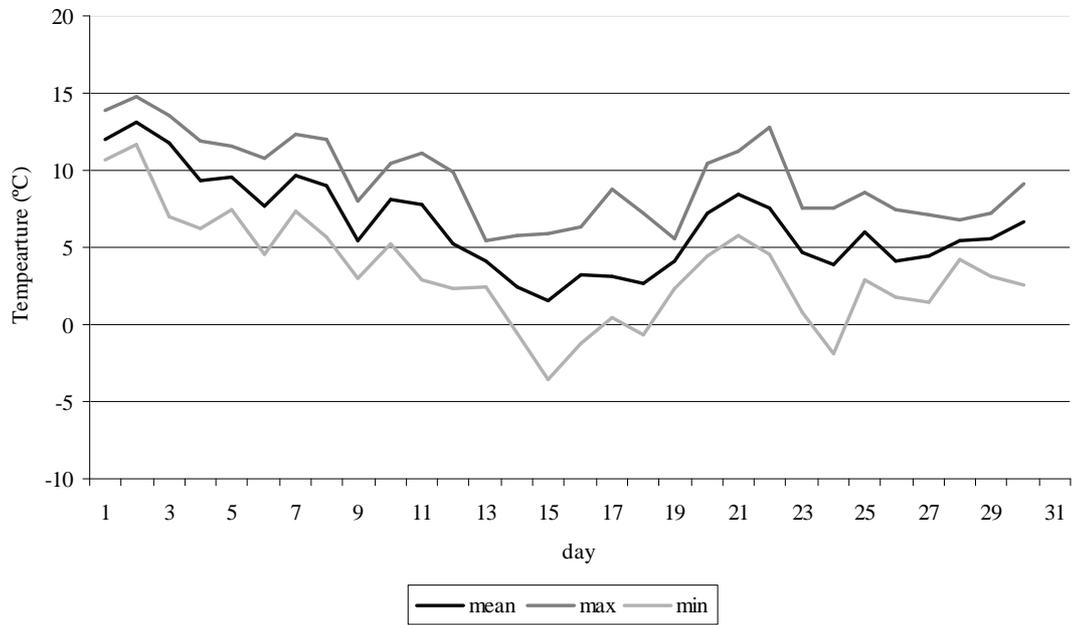


Figure 28. Temperature November 2007 (met.wau)

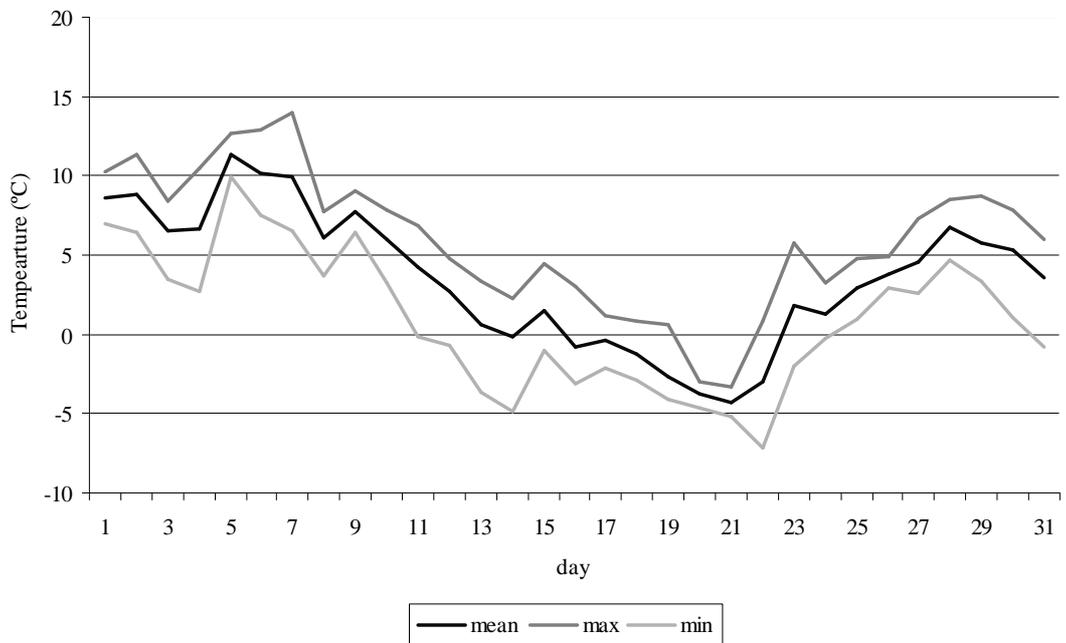
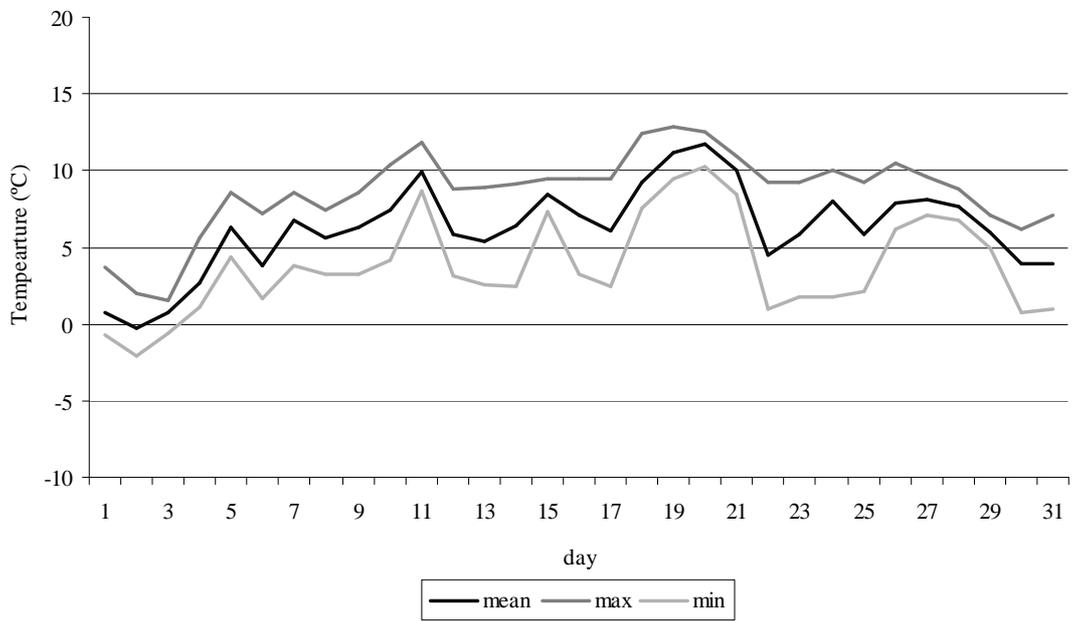
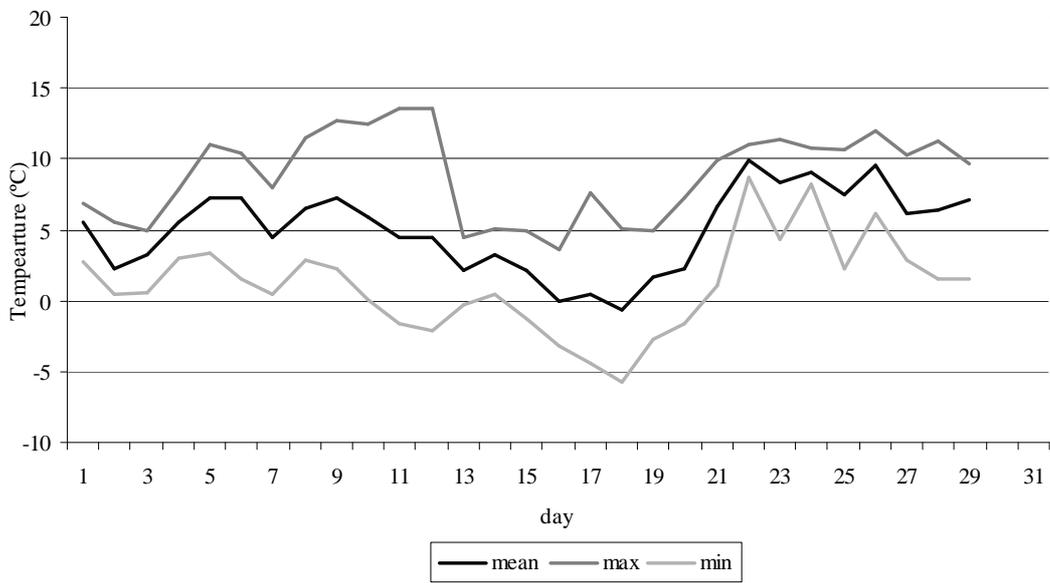


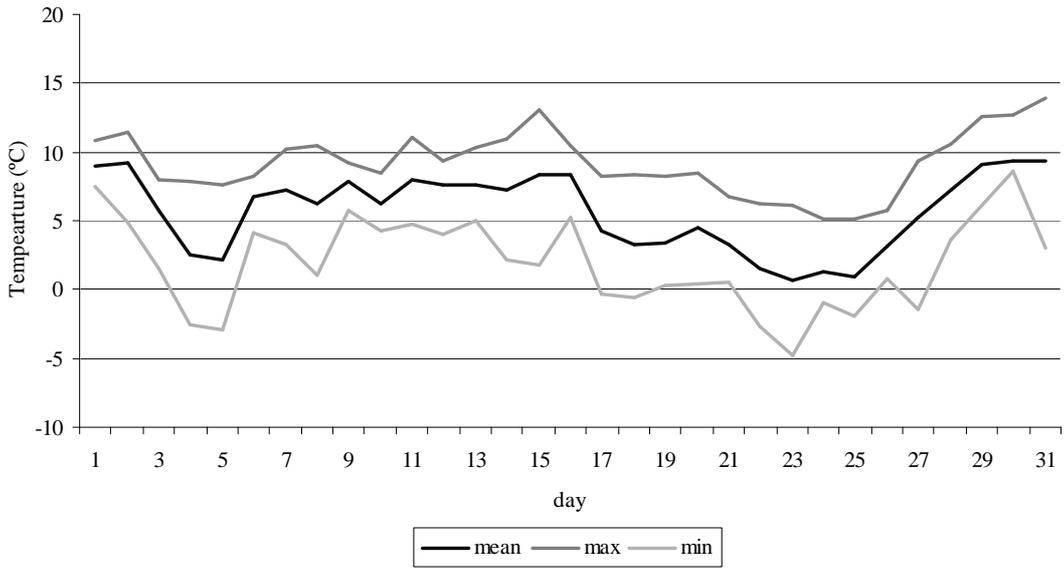
Figure 29. Temperature December 2007 (met.wau)



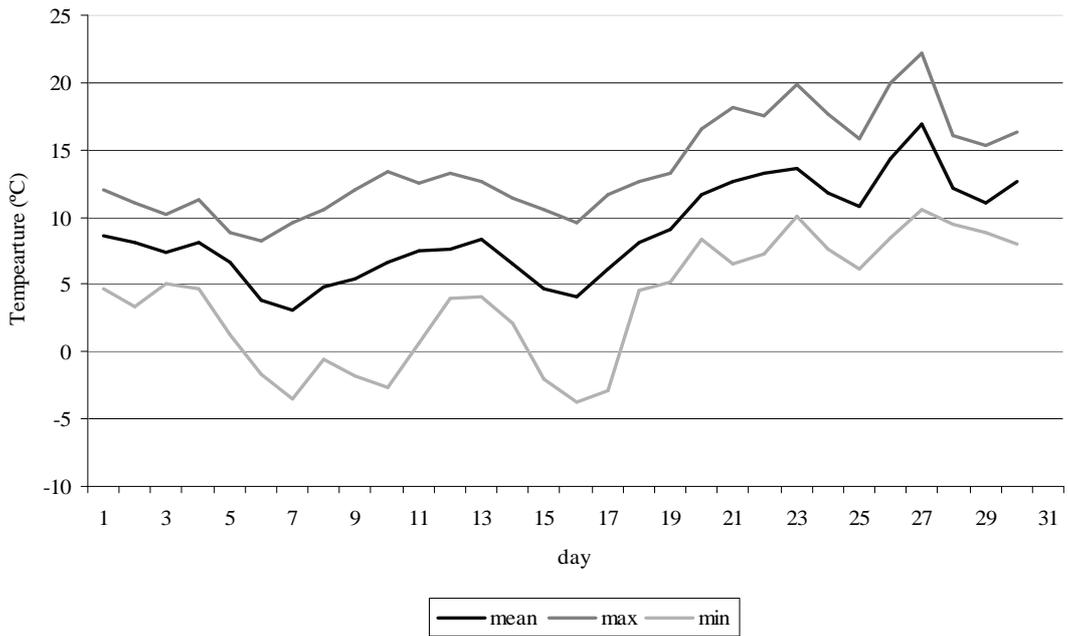
**Figure 30. Temperature January 2008 (met.wau)**



**Figure 31. Temperature February 2008 (met.wau)**



**Figure 32. Temperature March 2008 (met.wau)**



**Figure 33. Temperature April 2008 (met.wau)**

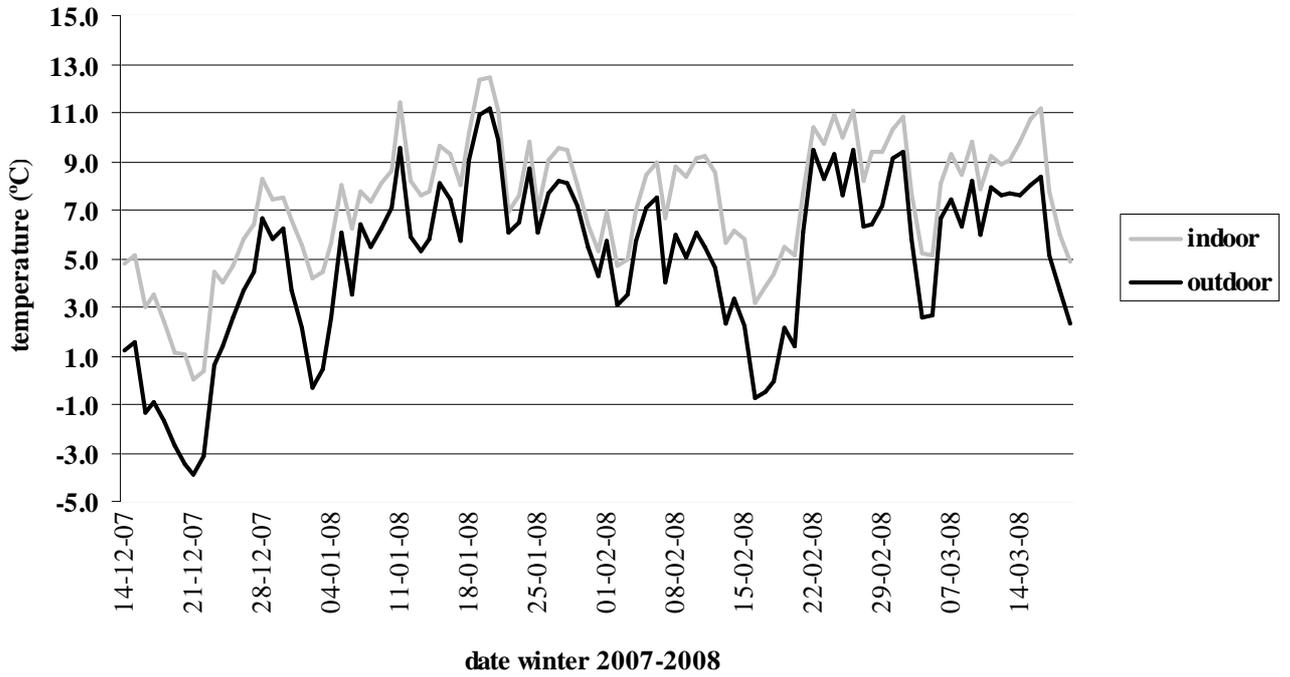


Figure 34. Farm 6 temperature distribution indoor and outdoor

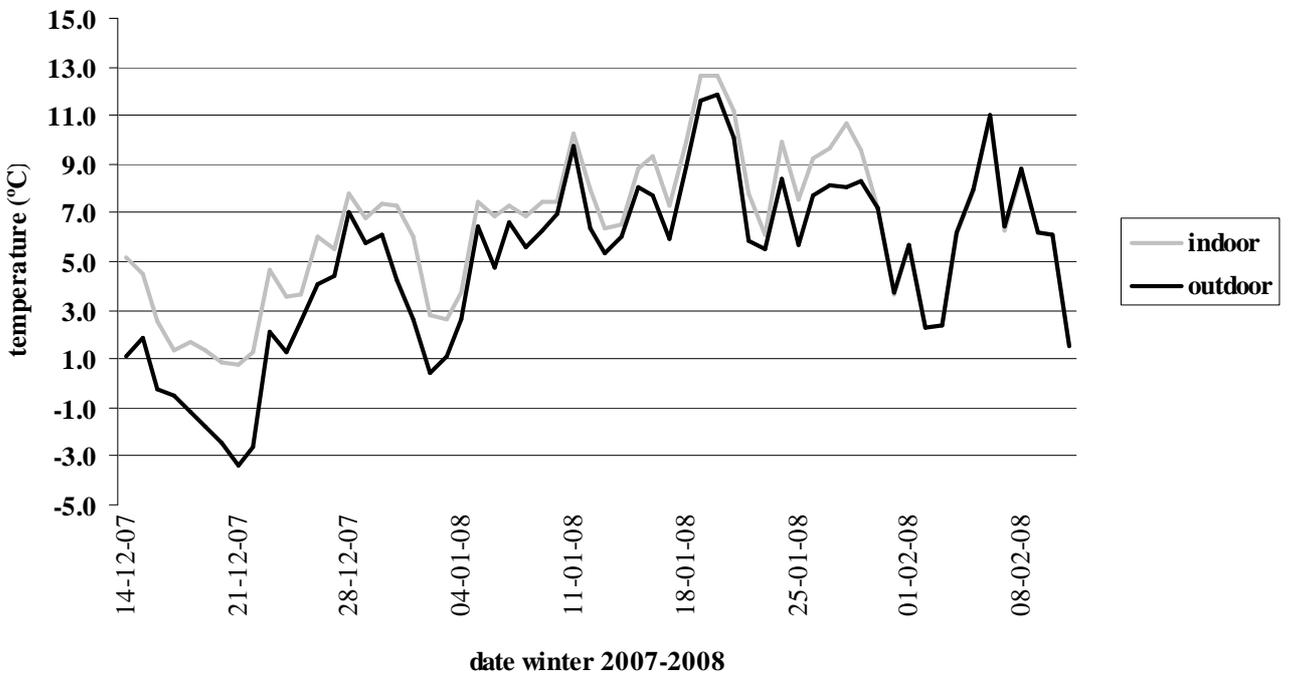
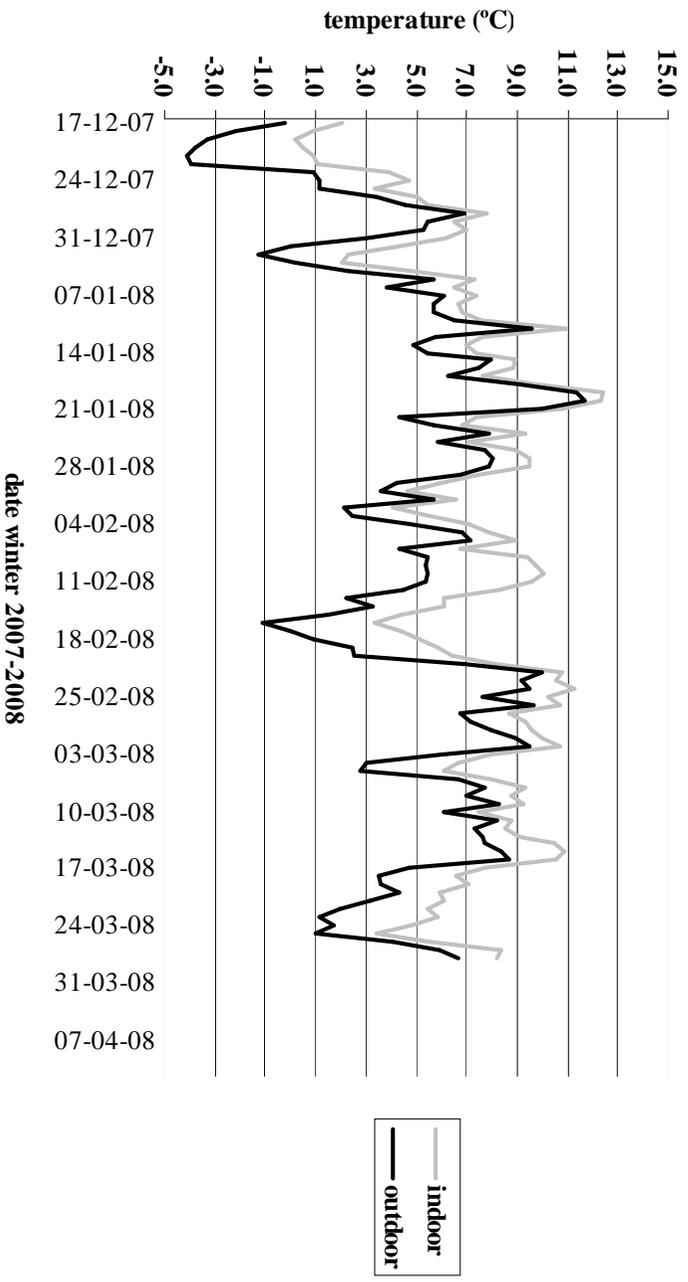
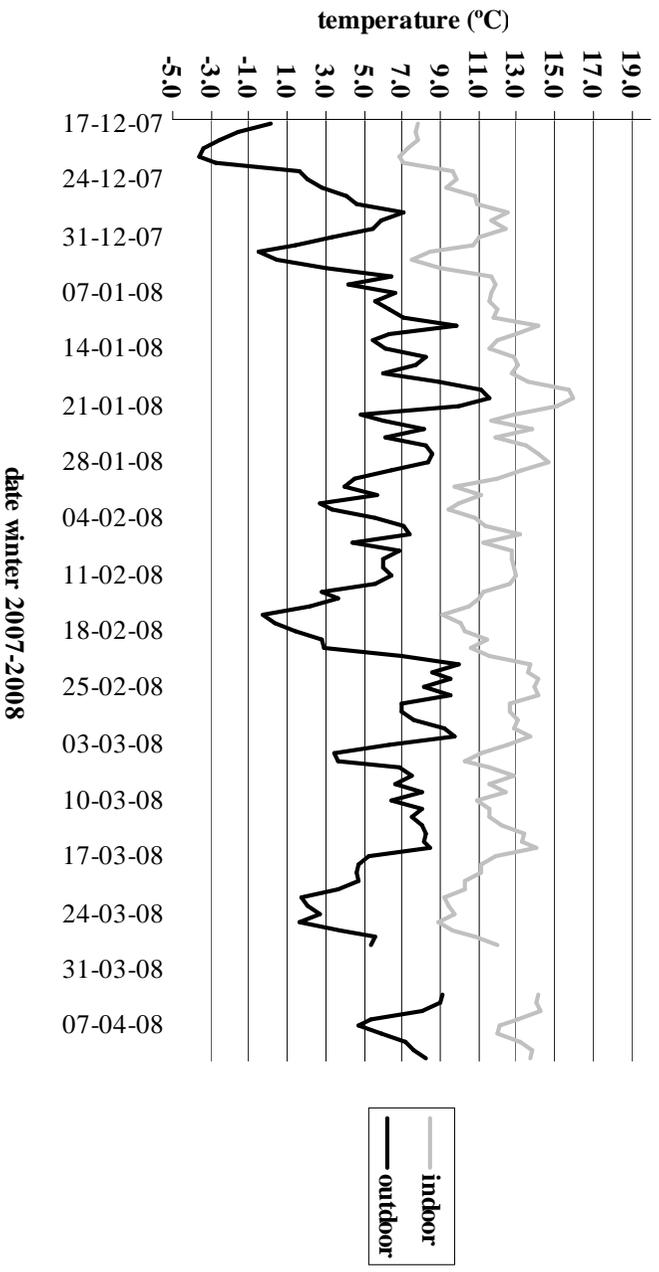
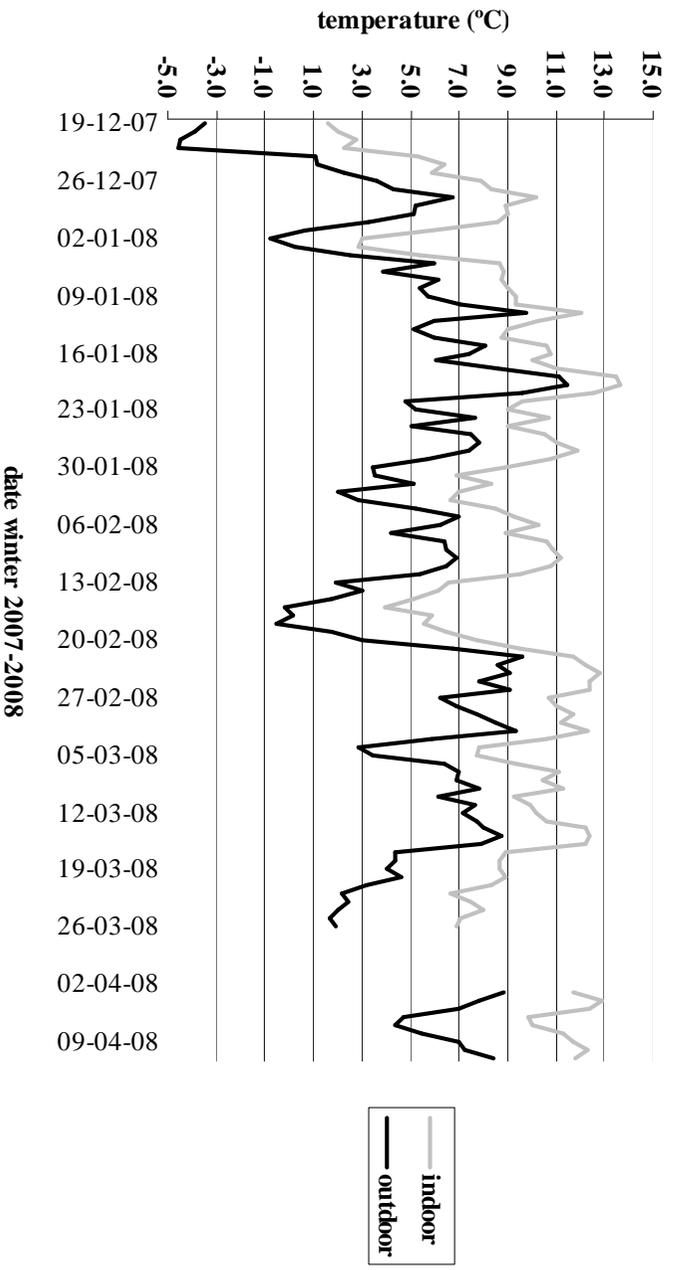
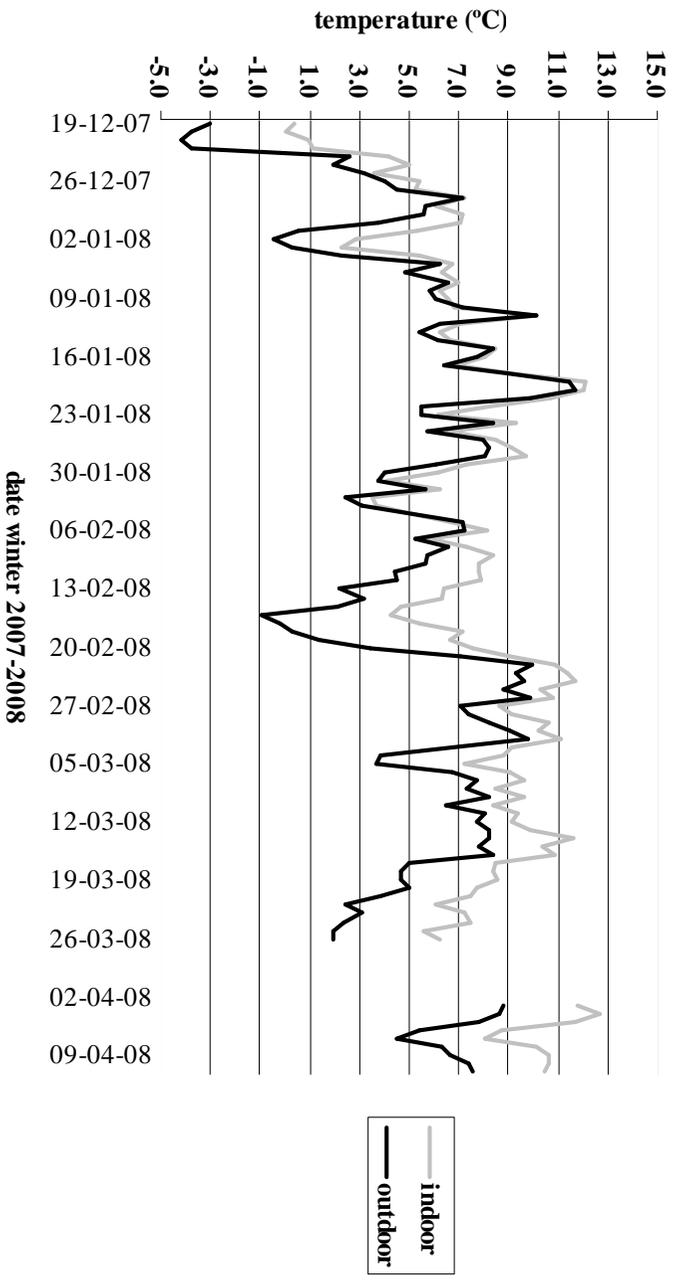


Figure 35. Farm 4 temperature distribution indoor and outdoor





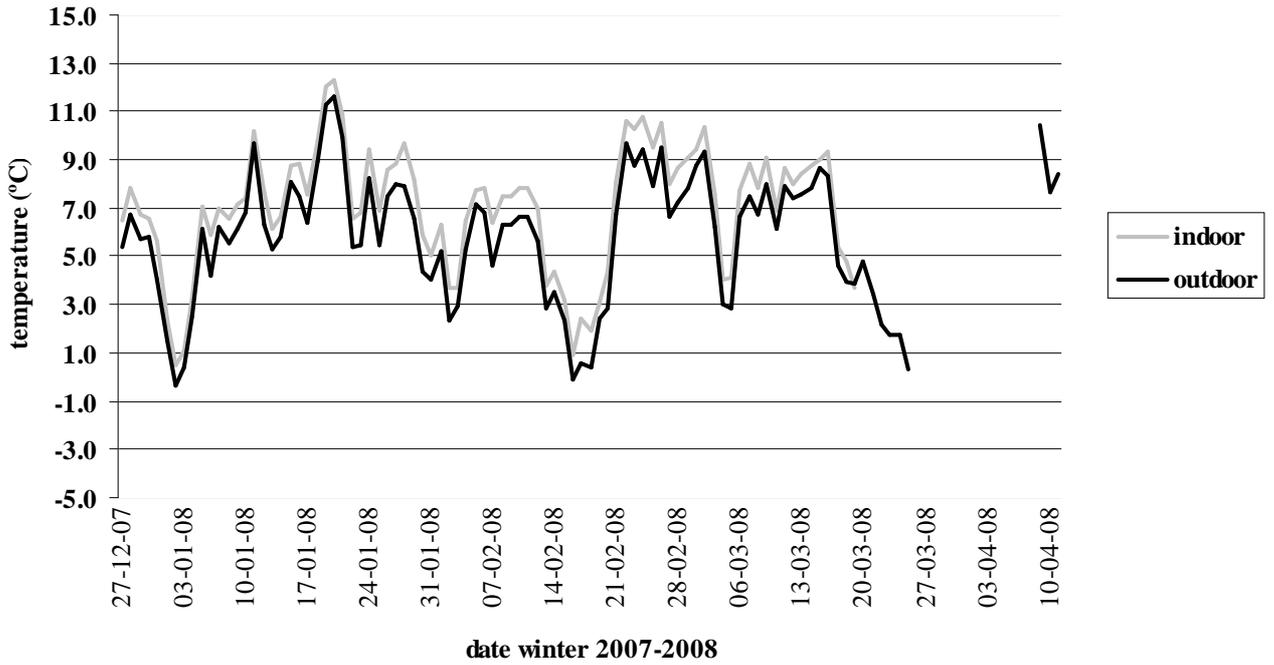


Figure 40. Farm 13 temperature distribution indoor and outdoor

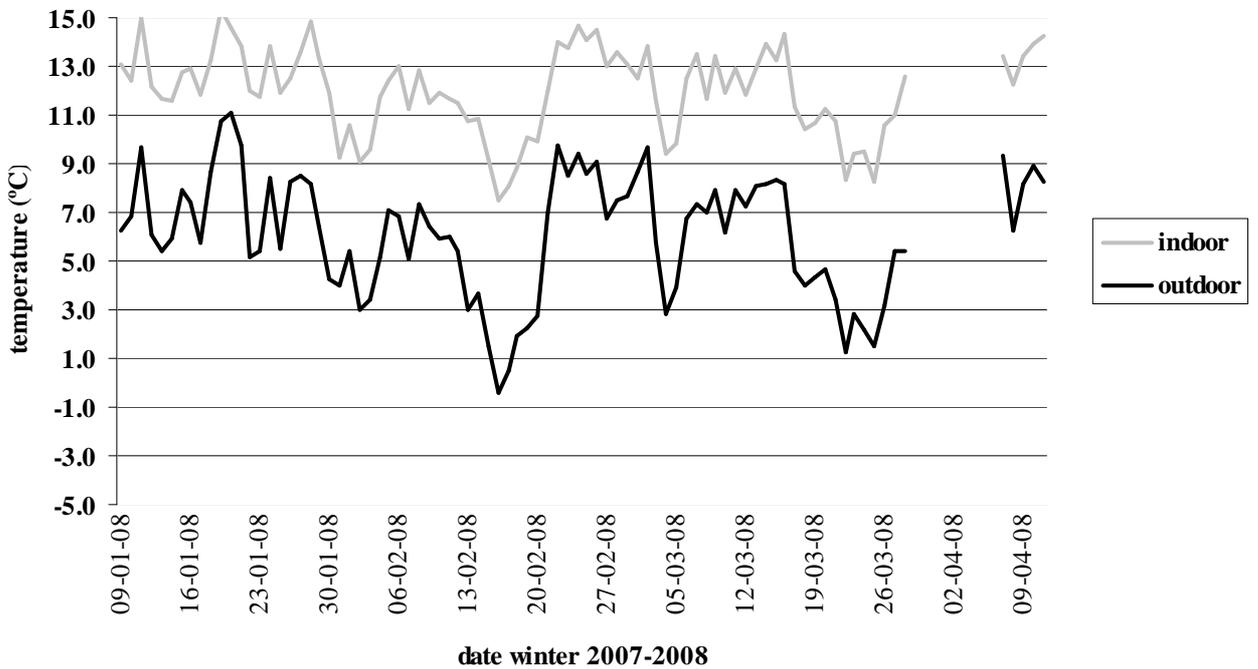


Figure 41. Farm 5 temperature distribution indoor and outdoor

## Annex VII

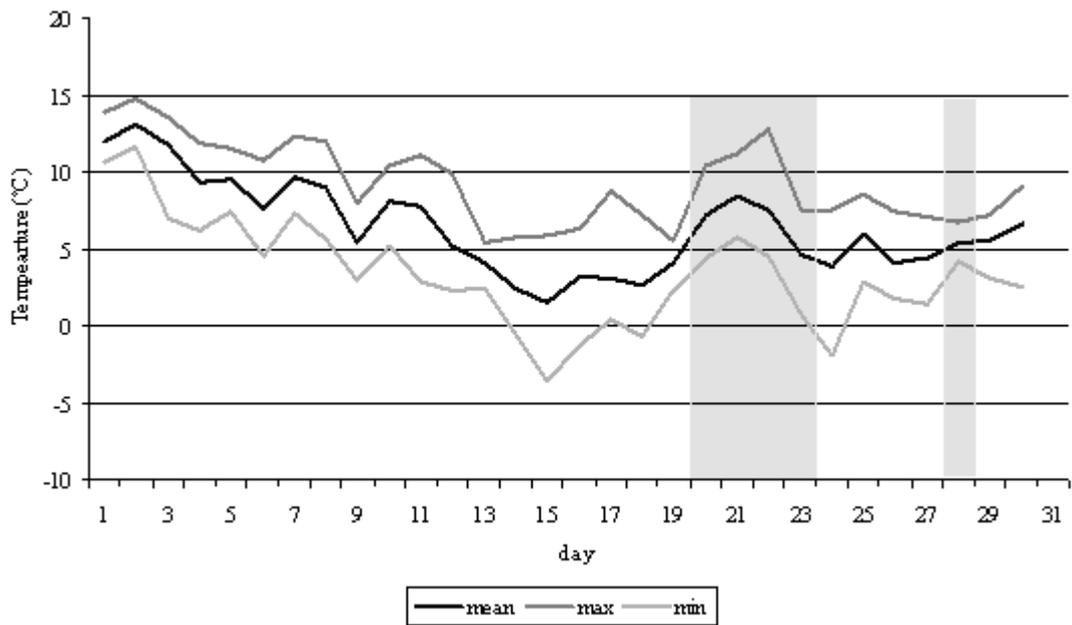


Figure 42. Outdoor temperature course during *Culicoides* spp. collection days week 47- 48 November 2007

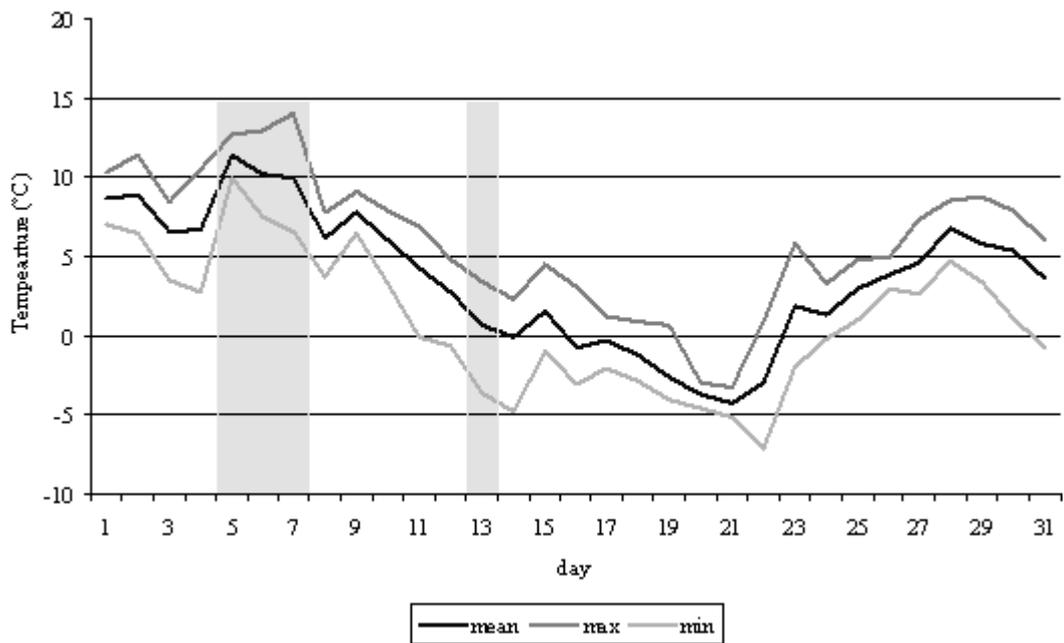


Figure 43. Outdoor temperature course during *Culicoides* spp. collection days week 49- 50 December 2007

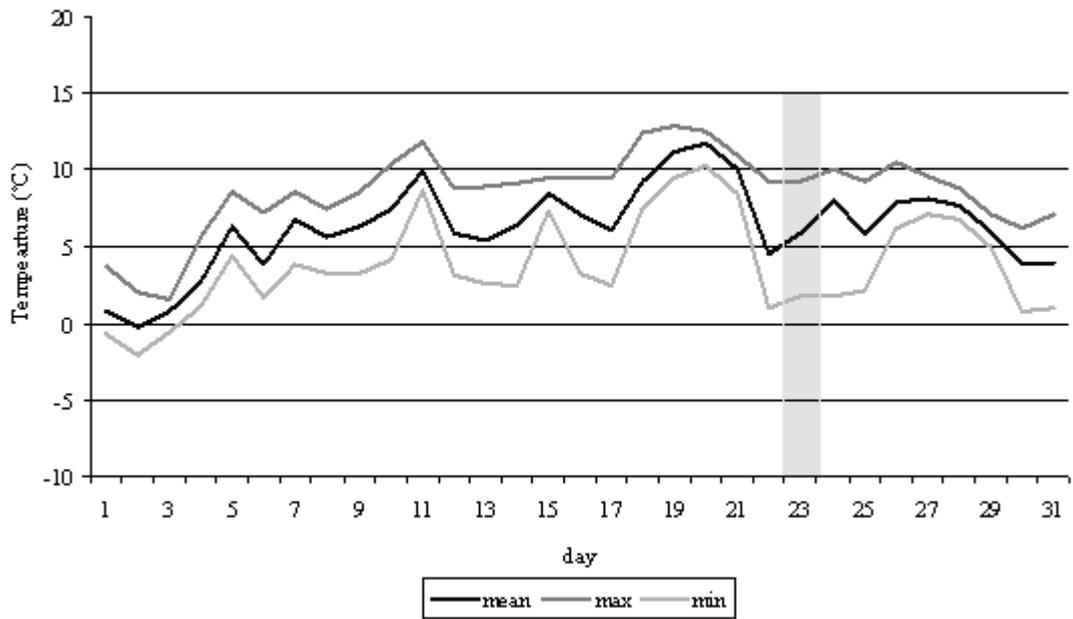


Figure 44. Outdoor temperature course during *Culicoides* spp. collection days week 4 January 2008

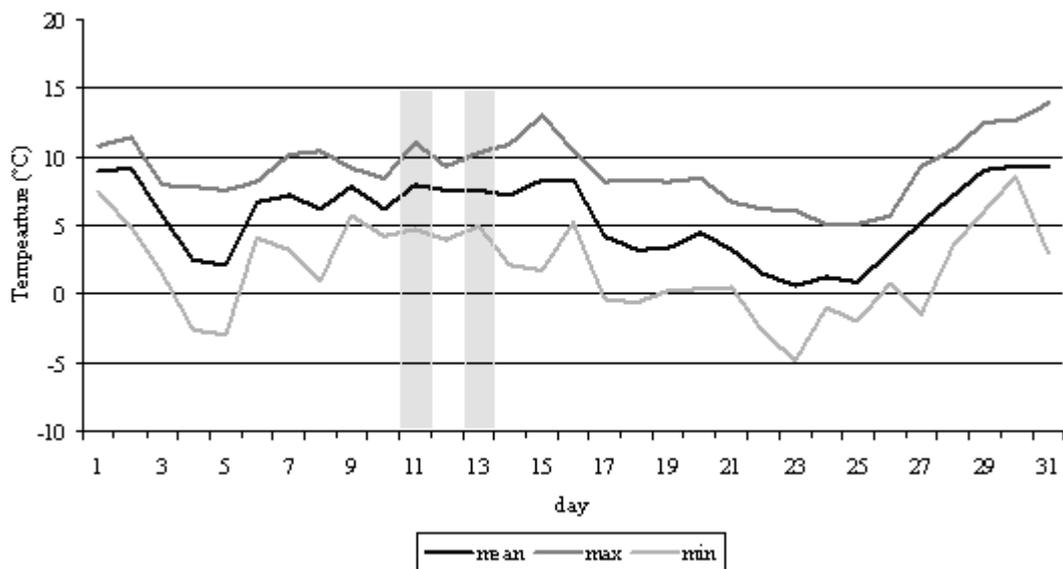


Figure 45. Outdoor temperature course during *Culicoides* spp. collection days week 11 March 2008

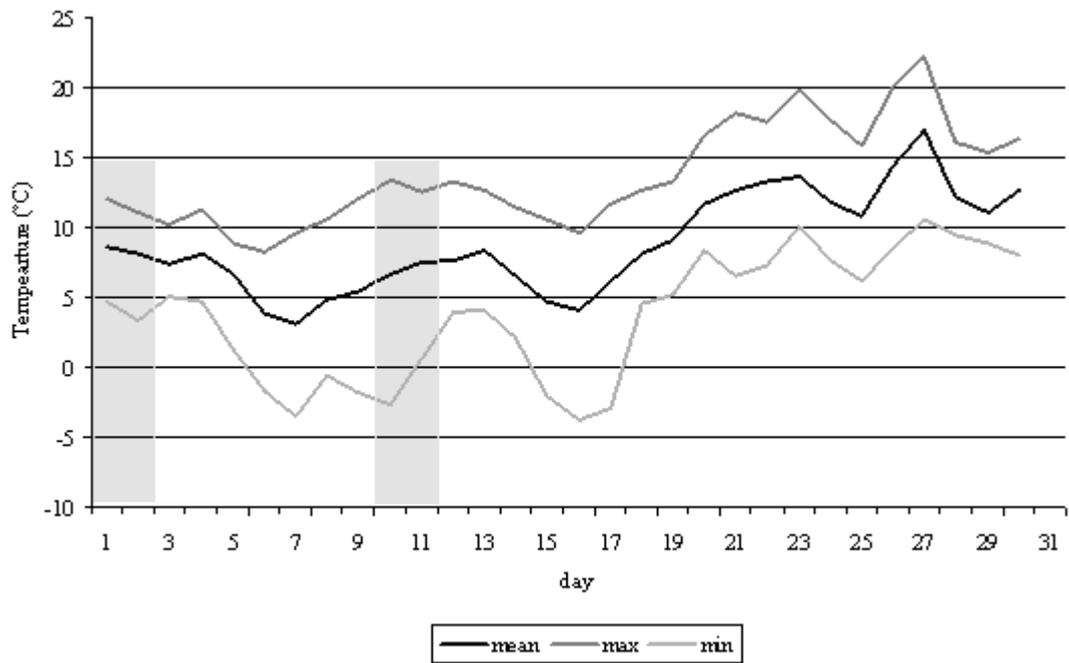
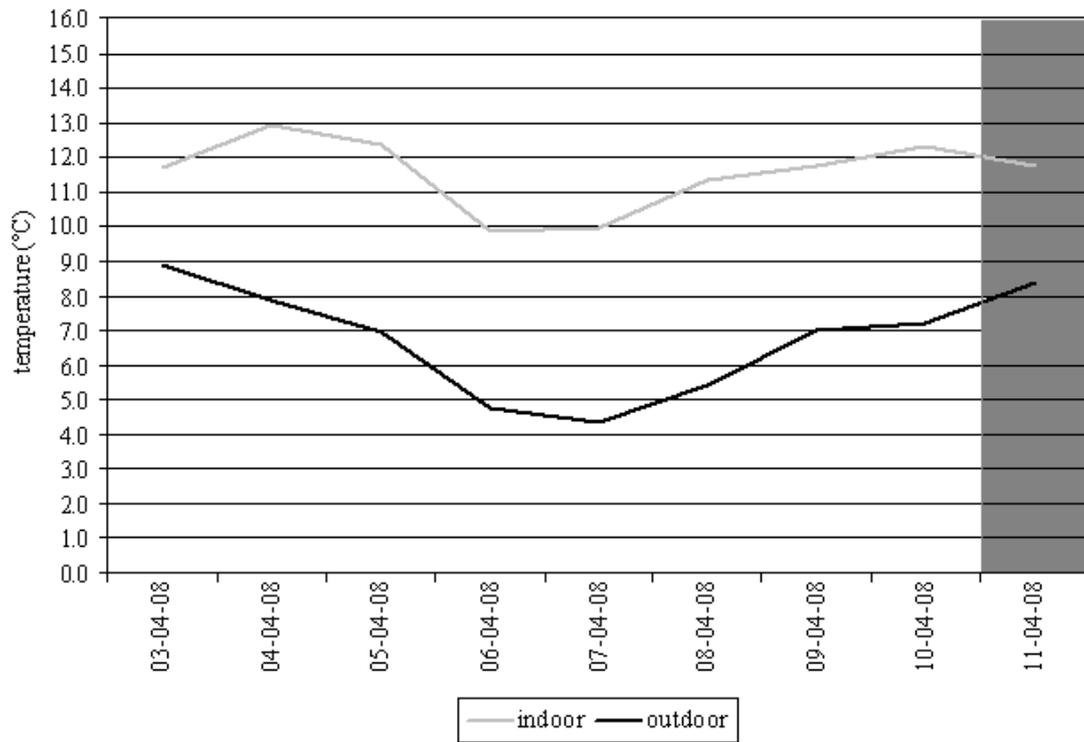
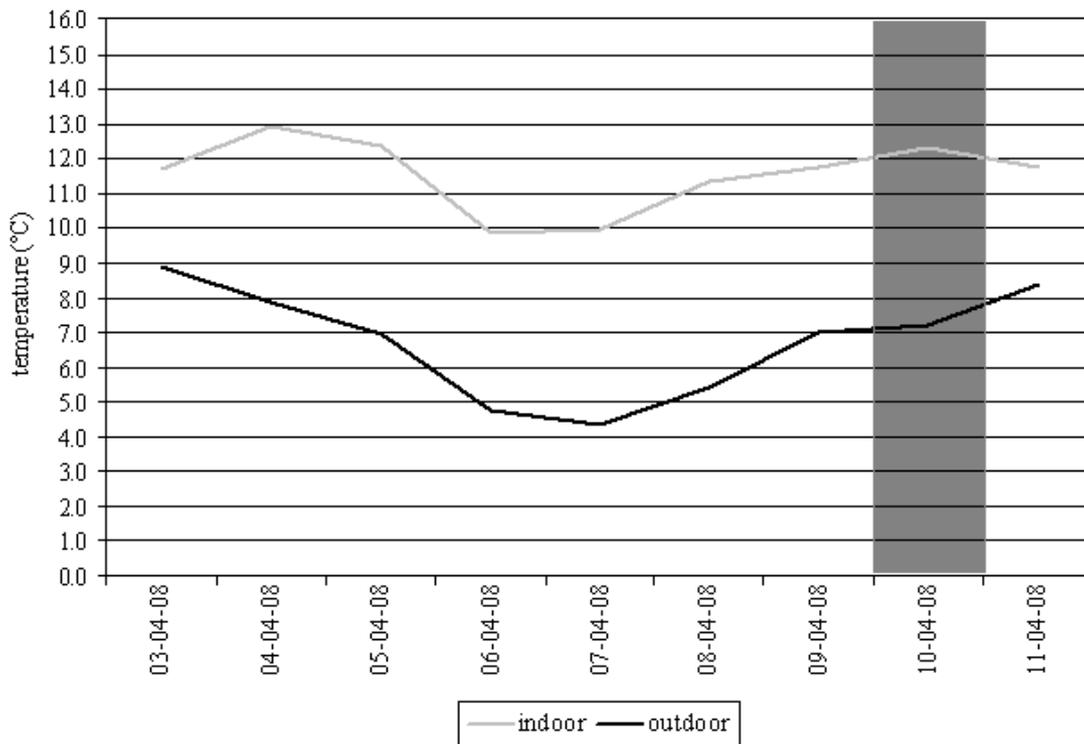


Figure 46. Outdoor temperature course during *Culicoides* spp. collection days week 14-15 April 2008

**Annex VIII**



**Figure 47. Temperature distribution farm 1 during *Culicoides* spp. collection April 11 2008**



**Figure 48. Temperature distribution farm 10 during *Culicoides* spp. collection April 10 2008**

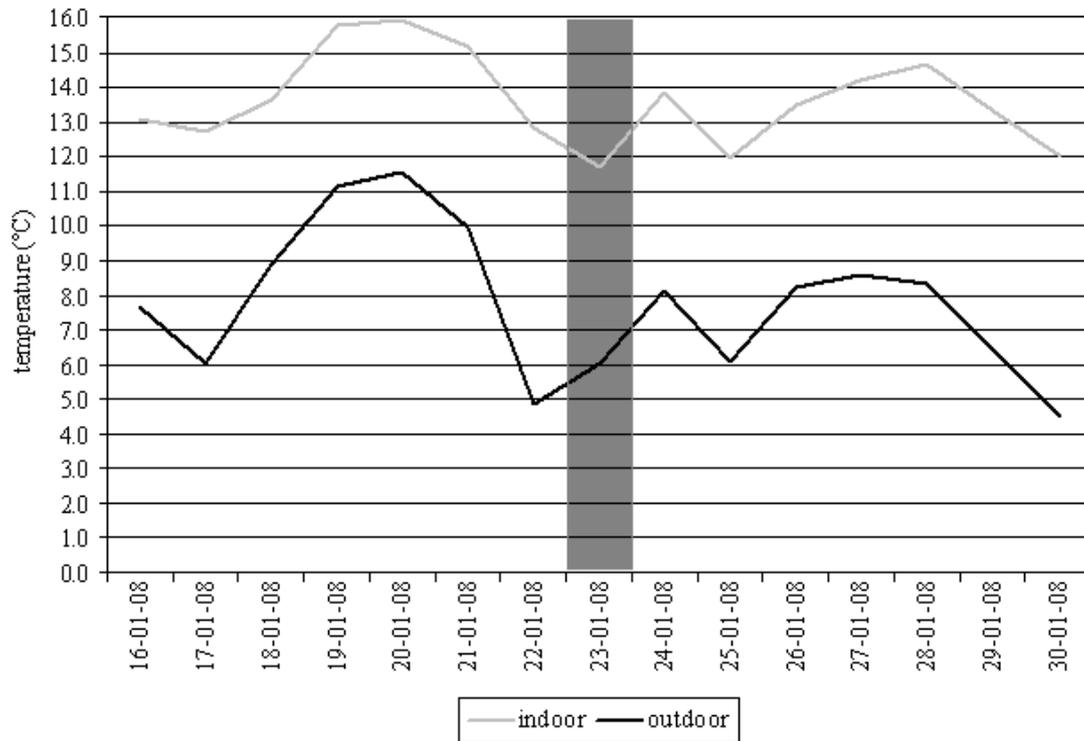


Figure 49. Temperature distribution farm 11 during *Culicoides* spp. collection January 23 2008

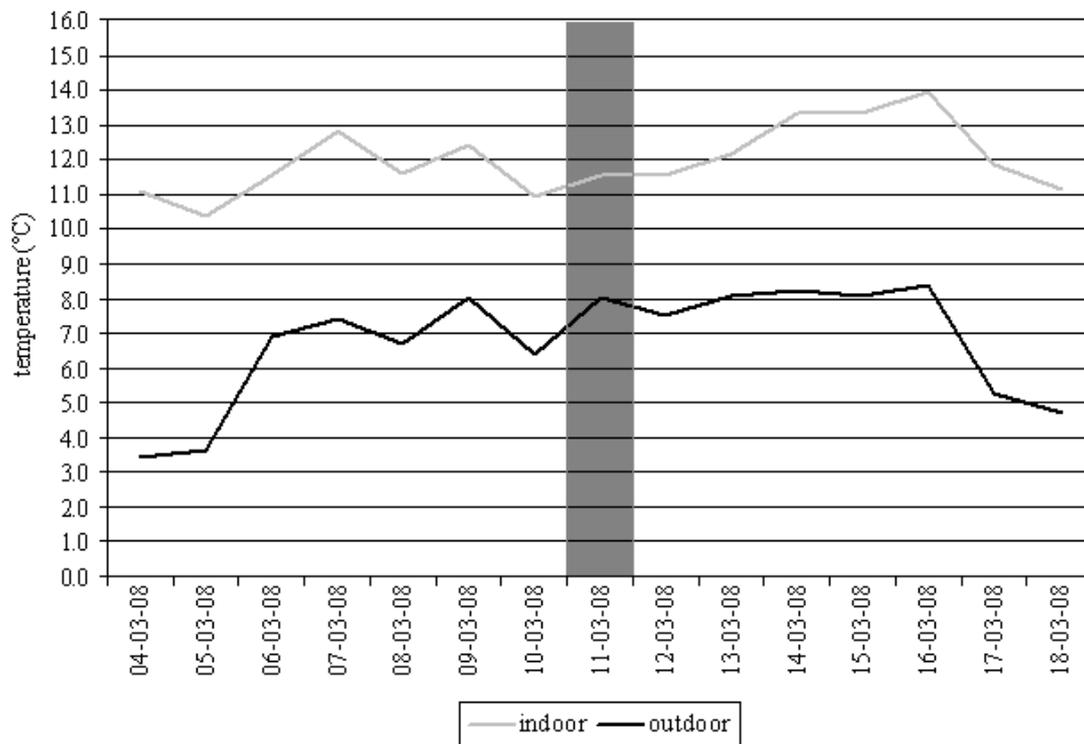


Figure 50. Temperature distribution farm 11 during *Culicoides* spp. collection March 11 2008

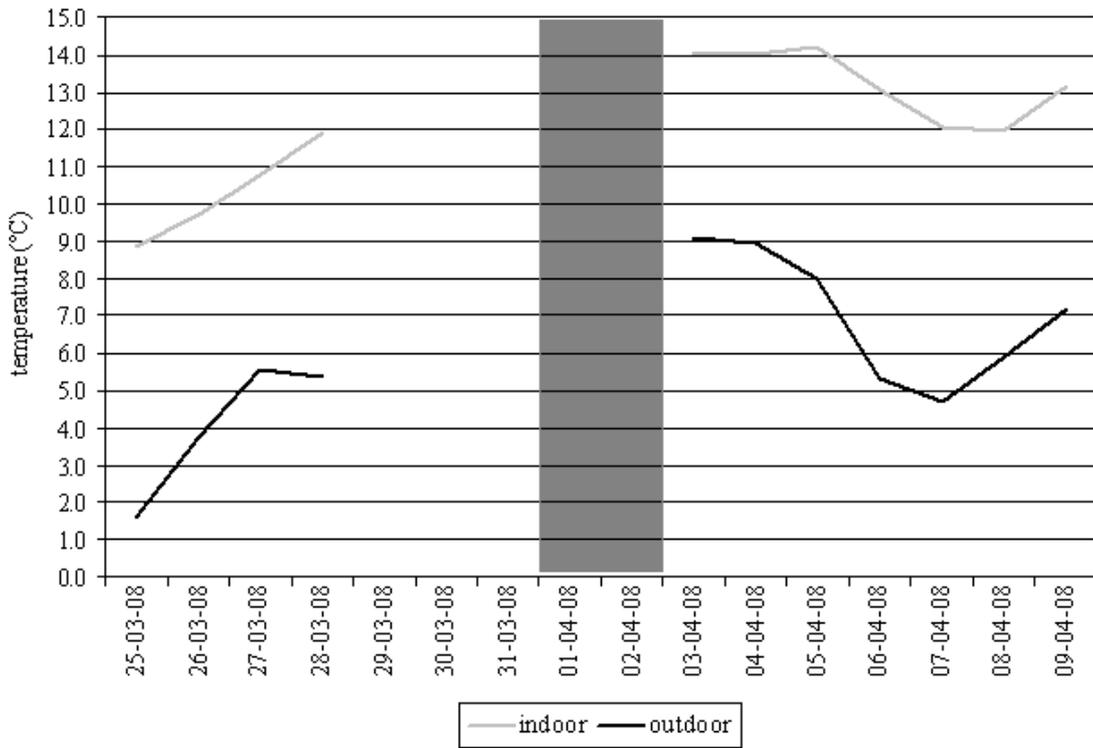


Figure 51. Temperature distribution farm 12 during *Culicoides* spp. collection April 1-2 2008

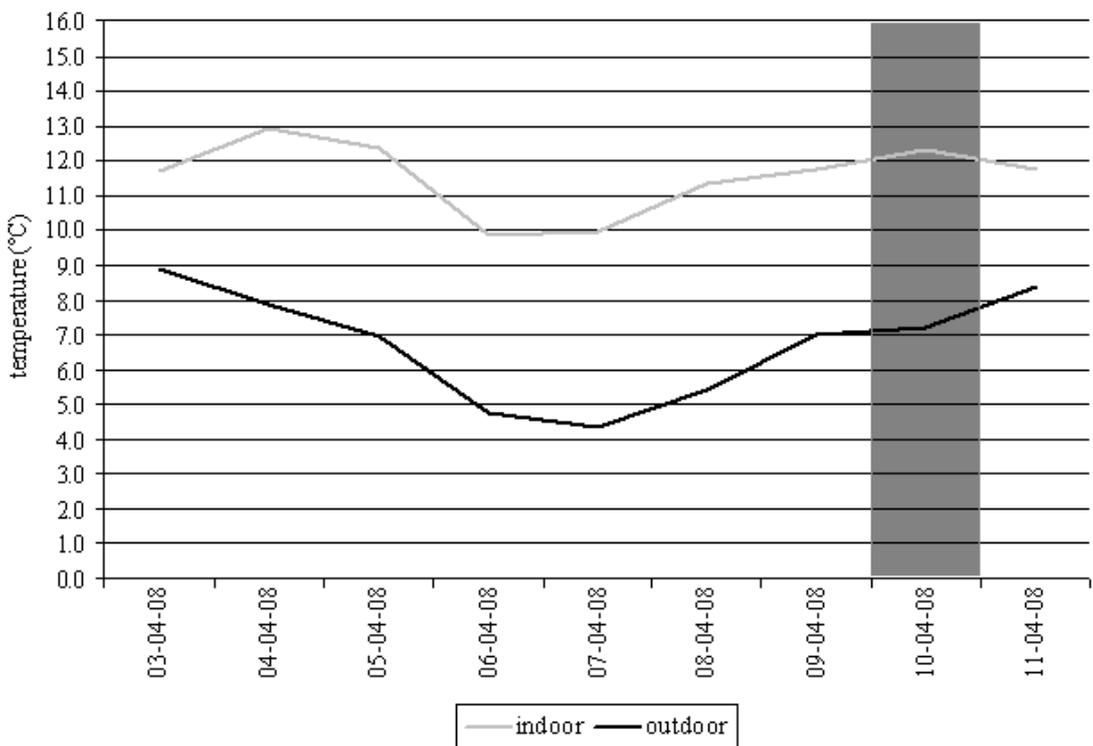
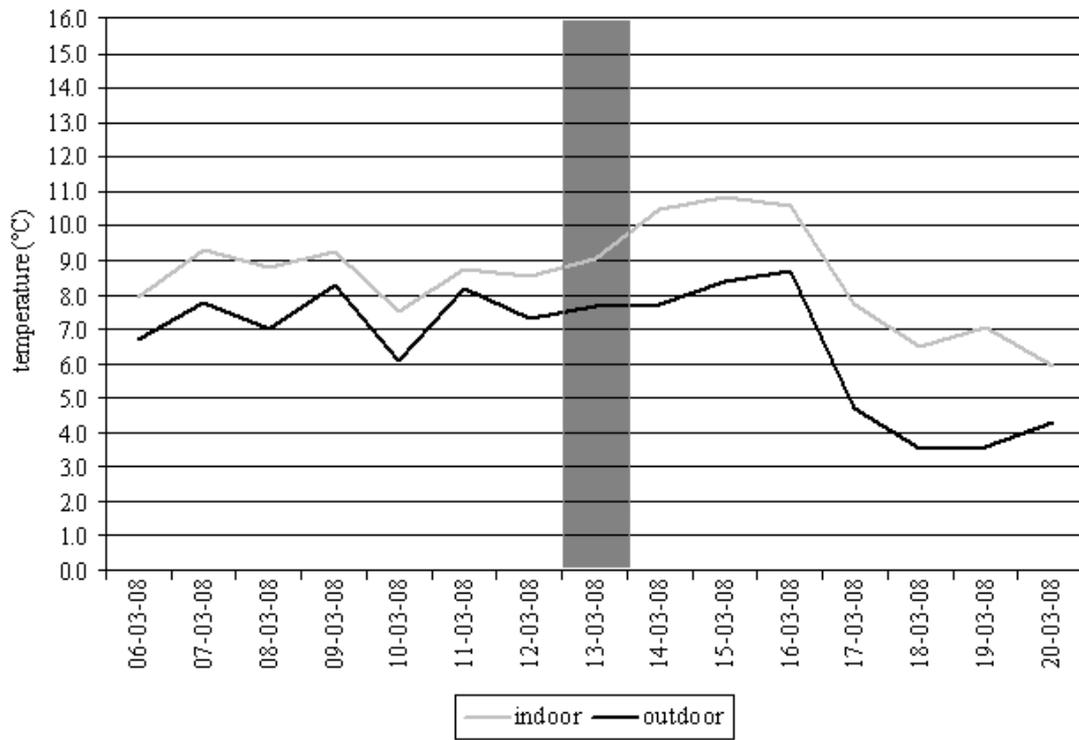


Figure 52. Temperature distribution farm 13 during *Culicoides* spp. collection April 10 2008



**Figure 53. Temperature distribution farm 16 during *Culicoides* spp. collection March 13 2008**