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Department of Agriculture, Fisheries and Food
An Roinn Talmhaíochta, Iascaigh agus Bia

National BTV Vector Surveillance Programme 2007-2009

Annual Report 2008

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BTV
Vector Ecology Unit

Table of Contents

EXECUTIVE SUMMARY

- The 2008 BTV Vector Surveillance Programme involved weekly field sampling over the complete year at the 34 randomly distributed sites previously investigated from April to December 2007.
- Improvements to the efficiency of the field sampling, specimen transport and to laboratory protocols, resulted in collection of environmental data and, subject to seasonal activity patterns of insects, *Culicoides* samples on 94% of the total potential sampling events (1,768).
- It is estimated that 433,357 *Culicoides* were sampled in the 34 sites and that 39,649 of these occurred at 10 index sites for which full taxonomic determinations of the 19 species/species complexes present were made.
- The duration of the *Culicoides* activity season, April (week 17) to December (week 51) was similar in 2008 to that recorded in 2007.
- Differences in the mean monthly *Culicoides* abundances were noted between 2008 and 2007. These were shown to be related to differences in meteorological conditions during the two years analysed. Cold, wet, windy conditions were associated with reduced *Culicoides* catches.
- The majority (95%) of the *Culicoides* specimens sampled in 2008 can be regarded as potential vectors and, compared to 2007, those in the *C. obsoletus* complex were proportionally less abundant in May when compared with the *C. pulicaris* complex. However, in summer/autumn of 2008 *C. obsoletus* complex midges were more abundant than the *C. pulicaris* complex, in contrast to this period in 2007 when similar numbers of both groups were recorded in these months.
- Differences between 2007 and 2008 environmental conditions were reflected in the overall annual population dynamics of *Culicoides*. However, considerable week to week variations in total *Culicoides* counts and activities of particular species/species complexes occurred in 2008 and these were also strongly influenced by changes in weather conditions (wind speed, rainfall, etc.).
- The 8 most abundant species / species complexes recorded in 2008 (*C. dewulfi*, *C. pulicaris*, *C. obsoletus*, *C. achrayl*, *C. impunctatus*, *C. reconditus* and *C. festivipennis*) were also the most abundant in 2007.
- A modified sampling programme, involving 7 former index sites and 3 additional ones, selected to ensure high overall abundance and biodiversity of the *Culicoides* samples, is recommended for 2009. Reduction in the number of sites will allow for use of replicate and novel traps at the 2009 sites, with a view to investigating *Culicoides* habitats and behaviour in greater detail.

INTRODUCTION

Bluetongue is an infectious, non-contagious, arthropod borne disease affecting ruminants. Though the natural host range is strictly limited to ruminants, sero-conversions without disease have been reported in carnivores (Alexander, C. *et al.*, 1994). The causative agent is Bluetongue Virus (BTV) and this is transmitted amongst vertebrate hosts by certain species of *Culicoides* midges (Mellor *et al.*, 2000). The disease has at least 24 known serotypes and different vector species have been implicated in transmission of blue tongue disease, which occurs widely through out the warmer regions of the world. *Culicoides imicola* Kieffer has been generally regarded as being the major BTV vector associated with recurring outbreaks of BTV increasingly recorded in the Mediterranean basin since 1998, though recent studies have implicated other species and species complexes of *Culicoides* (Calvete *et al.*, 2008).

Bluetongue was reported, for the first time, in several Northern European countries in August 2006. Initially observed in the area where Belgium, the Netherlands and Germany share borders, the disease was rapidly and widely disseminated with outbreaks subsequently being reported in France and Luxembourg in 2006 (Méroc *et al.*, 2008). In 2007 and 2008 outbreaks were also reported in the UK. The virus involved was shown to the BTV-serotype 8, a form not previously reported in Europe and which prior to the present epidemic has only occurred in Africa, Central America, Malaysia and India/Pakistan (Méroc *et al.*, 2008) (Fig. 1).

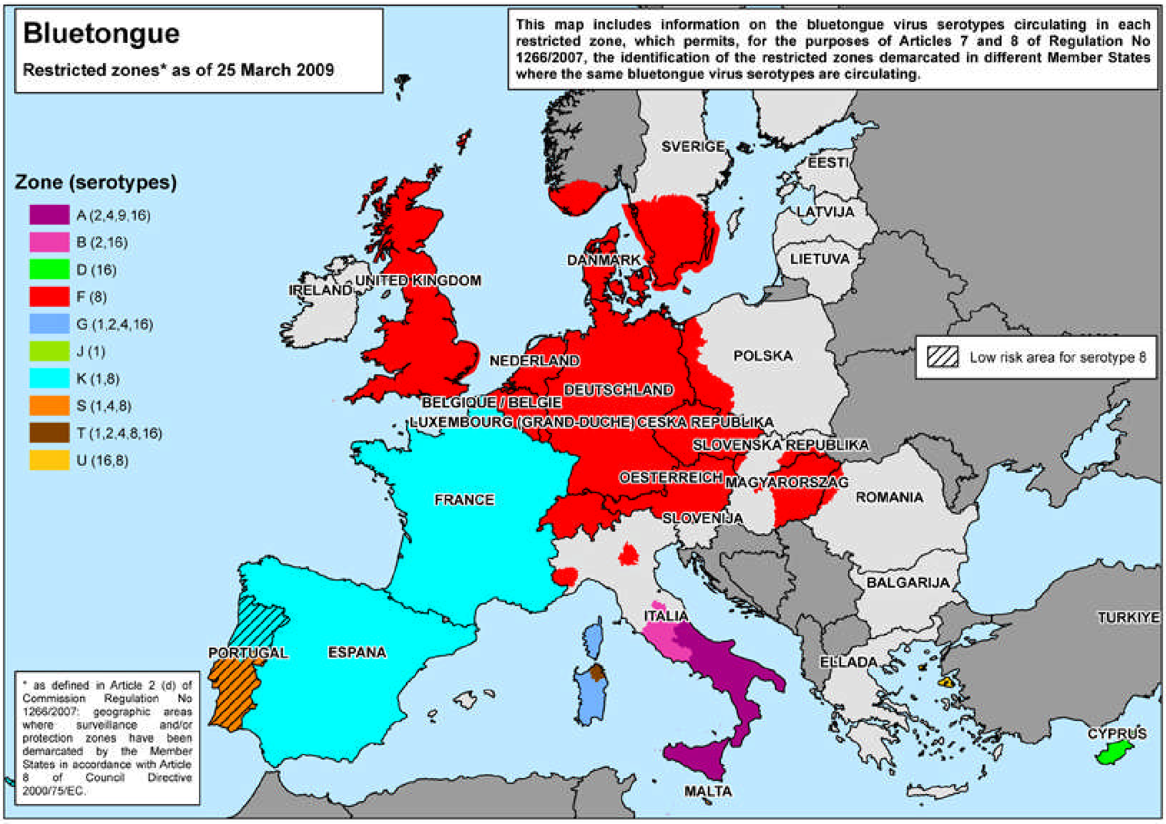


Fig. 1 Map illustrating the current spread of Bluetongue serotypes across Europe (http://ec.europa.eu/food/animal/diseases/controlmeasures/bluetongue_restrictedzones-map.jpg).

The unprecedented outbreak of BTV-serotype 8 disease in North Europe was unexpected, because of previous research on the role of climatic variables and because the midge species (*C. imicola*) previously implicated as a major vector of BTV in southern Europe was not recorded in northern and central European countries. However, because BTV is a serious notifiable agricultural disease which quickly affected livestock such as cattle and sheep, the outbreaks prompted swift action at EU and member state levels. Monitoring and surveillance programmes, as appropriate, were initiated and these involved standard field/laboratory protocols. In Ireland, following meetings in September 2006, a review of existing data on *Culicoides* species known to occur in the country was undertaken and a three year (2007-2009) BTV vector surveillance programme was scheduled to begin in the spring of 2007.

This second annual report describes the results of the BTV vector surveillance work undertaken in 2008. A previous report (McCarthy *et al*. 2008) described the results of the 2007 work and an additional interim reports (2007 and 2008) have given mid-season reviews of the sampling programmes. As in 2007, the 2008 BTV vector surveillance has involved extensive sampling of midges by DAFF staff from District Veterinary Offices throughout the country, with sample processing and analyses being undertaken as the Vector Ecology Unit's laboratory in NUI, Galway.

This report presents results of weekly sampling of *Culicoides* at 34 sites, distributed randomly, around the country, at which total midge abundances were recorded. In addition, a more detailed analysis of the spatio-temperal variations in *Culicoides* species and species complexes is presented for 10 index sites. A comparison is made with the results obtained in 2007 and recommendations of changes in 2009 field surveys are presented. A more detailed statistical analysis of the variations in abundances of particular species/species complexes, and of their relationship to environmental parameters, will be undertaken later in 2009.

RESULTS

Completion of 2007 sample processing

During the 2007 national BTV vector surveillance sampling programme a series of 34 randomly selected sites were sampled weekly by staff from the nearest DVO and samples of insects, including *Culicoides* and various other small insects were dispatched each week to the newly established Vector Ecology Unit at the National University of Ireland, Galway. Over 400,000 *Culicoides* specimens were obtained, as well as very large numbers of other insects, and 91,453 of these were identified in 2007 to species or species complex level. The specimens fully identified represented all the samples from the index sites, as well as samples from all 34 sites for selected weeks. In addition, full *Culicoides* counts were undertaken, without further taxonomic determinations, on samples for 9 non-index sites. The locations of the sampling sites are illustrated in Fig.2. The annual report for the 2007 survey (McCarthy *et al*. 2008) was therefore based on a partial analysis of the potential set of samples available for taxonomic composition / total midge abundance determinations in the laboratory. Thus, in 2008 the residual samples from the 15 remaining sites were examined and total *Culicoides* estimates were obtained. Details of the additional 2007 results were presented in an interim 2008 report and the data will be further analysed later. As recommended in the 2007 annual BTV vector surveillance report, a new counting protocol was adopted during 2008, in which full counts are no longer undertaken for samples with excessively large numbers of *Culicoides*. In the latter cases, a sub-sampling procedure is adopted and this enabled the sample processing rate of the Vector Ecology Unit to be significantly increased. Thus, the NUIG C*ulicoides* data-base now includes all relevant data for 2007 and 2008.

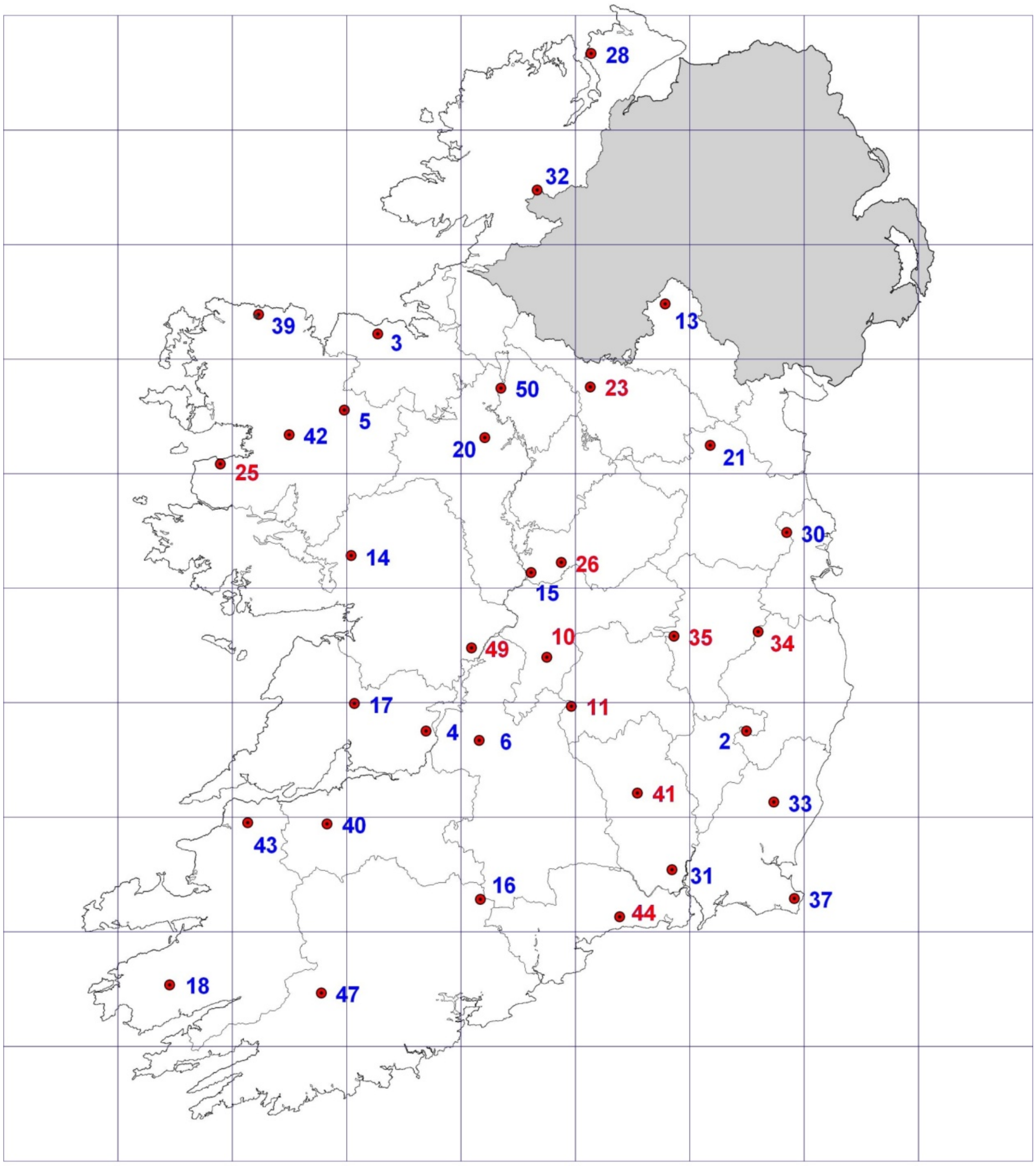


Fig. 2. Location of the 34 sampling sites (with the 10 index sites highlighted in red font at which weekly *Culicoides* collections were made in 2007 and 2008).

The 2008 *Culicoides* survey results, with comparisons to those for 2007

Trapping programme and site selection

During 2008 the *Culicoides* sampling programme has been continued at the same 34 sites that were sampled in 2007 and the same 10 index sites have also been retained. Some initial technical problems occurred at a small number of sites. However, in Table 1 a summary of the trapping activities at each site is presented for the period January to end of December is presented. This documents the extent to which weekly records of trapping conditions and insect samples have been sent to the Vector Ecology Unit in NUI, Galway from the 34 sampling sites. As can be seen (Table 1) the response rate from the DVO trapping staff was initially good for most locations but poor response rates were noted for certain sites (e.g. 11, 30, 47) and these seem to have largely been attributable to unresolved technical problems with traps. However, the situation has been greatly improved following adoption of a new communication protocol in which DVO trapping teams that have not supplied data or samples are individually contacted at the end the relevant sampling week.

Field sampling protocols

The standard UV light traps used in the 2007 survey were deployed at the 34 sampling sites (Fig. 1). Improvements to trap maintenance and spare parts supply lines were implemented in 2008 and this contributed to the overall success of the field sampling programme (Table 1). Data loggers (Tiny Tag) recording both temperature and relative humidity were positioned close to the traps at the 10 index sites. Temperature data loggers (HOBO ware) were similarly positioned at the remaining 24 sites. Improvements were also made to the packaging and transportation of samples to the Vector Ecology Unit laboratory and, in contrast to 2007, this resulted in no loss of samples during 2008. These improvements included new, more durable specimen jars which are placed into heavy duty plastic bags which are then sealed thus protecting the sample and the field record form. The specimen jar and plastic bag are then placed in cardboard boxes (Speci-Box V) which clearly comply with postal regulations for the transportation of specimens. Labels with unique identification numbers for each sampling event were also supplied to be placed on the specimen jar and the field record form in order to avoid any mix-ups along the sampling/transportation/laboratory line.

Samples and environmental data received

A total of 1,661 returns (i.e. insect samples and/or weekly field reports) were received by the Vector Ecology Unit in 2008. This represented 94% of a possible maximum of 1,768 (i.e. 34 x 52 weeks). However, when weeks 1 and 52 which performed particularly badly with regard to returns are excluded the return percentage of 94% increases to 96.6%. The good response rate by field crews in 2008 resulted in receipt of 1,099 insect samples. The 2008 response rate was much better than 2007. In 2007 the survey operated, apart from some limited sampling at a few sites, from mid-April to late December (Weeks 17-51 inclusive). The overall response rate in 2007 (92.1%) was lower than that obtained (98.6%) in the same period in 2008 and for the

overall 2008 survey period. No sites had a full (100%) response rate in 2008, however, if weeks 1 and 52 (Christmas holidays) are excluded 15 sites had a full response rate. The weekly response rate has not fallen below 82% from January to December and by comparison with other comparable surveys this is considered to be exceptionally good trapping crew performance. Further details of the 2008 trapping programme response rate are presented in Table 1.

Environmental conditions were not as favourable for fieldwork in 2008 as in 2007. However, gaps in the data series for some sites were largely accounted for by trap failure, difficulties with battery chargers or other minor technical problems.

Table 1. A record of trapping results and/or samples received by the NUIG laboratory from the 34 trapping sites during 2008 (0=No completed forms or samples received; 1=either or both forms/samples received).

Week No. \ Site No.	2	3	4	5	6	10	11	13	14	15	16	17	18	21	23	25	26	28	30	31	32	33	34	35	37	39	40	41	42	43	44	47	49	50		%
1	0	0	0	0	1	0	0	1	1	0	1	0	1	1	1	0	0	0	0	1	0	1	0	0	1	0	1	1	0	1	0	0	0	0	1	38.24
2	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	2	91.18
3	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	3	88.24
4	1	1	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	4	88.24
5	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	5	91.18
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	6	91.18
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	7	91.18
8	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	8	91.18
9	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	9	91.18
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	10	94.12
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	11	94.12
12	1	1	0	1	1	1	1	1	1	0	1	0	1	1	1	1	0	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	12	82.35
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	13	94.12
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	14	97.06
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	15	97.06
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	16	100.00
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	17	97.06
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18	100.00
19	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	19	97.06
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	20	100.00
21	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	21	97.06
22	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	22	100.00
23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	23	100.00
24	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	24	97.06
25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	25	100.00
26	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	26	97.06
27	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	27	100.00
28	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	28	97.06
29	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	29	97.06
30	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	30	97.06
31	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	31	100.00
32	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	32	100.00
33	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	33	100.00
34	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	34	100.00
35	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	35	100.00
36	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	36	100.00
37	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	37	100.00
38	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	38	97.06
39	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	39	100.00
40	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	40	100.00
41	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	41	97.06
42	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	42	97.06
43	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	43	100.00
44	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	44	100.00
45	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	45	97.06
46	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	46	100.00
47	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	47	100.00
48	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	48	100.00
49	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	49	100.00
50	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	50	91.18
51	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	51	94.12
52	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	52	14.71

Laboratory processing of samples

Insect samples received at the Vector Ecology Unit laboratory are logged electronically on arrival, the contents and field report forms are examined and stored and all records are coded for ease of retrieval and analysis. The initial sample processing involves sorting by hand, using low power binocular microscopes, and removal of all non-*Culicoides* insects trapped. These specimens are retained in a separate archival system for possible identification in future.

All *Culicoides* samples are retained and stored in ethanol in the dark in laboratory cupboards and use of a code system in the electronic database facilitates specimen retrieval. It is estimated that, including samples counted by sub-sampling, the total number of *Culicoides* trapped during 2008 was approximately 433,357. 90 samples have been processed using sub-sampling methods developed by Van Ark & Meiswinkel, (1992). Of these 90 samples, 56 were used for total *Culicoides* count purposes only and the other 34 samples were fully identified. Taxonomic keys provided by Rawlings (1996) and Campbell and Pelham-Clinton (1960) were adapted to local faunal composition and these were used together with a set of digital photo micrographs, for routine lab analysis.

Revised Culicoides counting protocols

The sub-sampling protocol, adopted for completion of the 2007 sample processing and for the full 2008 survey, which is currently in use for the BTV surveillance programme is essentially that proposed by Van Ark and Meiswinkel (1992). This involves sub-division of samples into 8 parts on large Petrie dishes, counting of one of these sub-divisions and then deciding whether to undertake a full count of the entire sample or to extrapolate from the sub-sample and to obtain an estimate of the total numbers of midges present. A similar procedure applies, whether generic *Culicoides* counts or individual species counts, for determination of sample species composition, is being undertaken.

Database management

A dedicated BTV vector database, established in 2007 at N.U.I., Galway, is used to store all available information on Irish *Culicoides*. The software was up-dated in 2008 to Microsoft Access© 2007 in a Windows XP© environment. Every *Culicoides* sampling event record is traceable by means of unique numbers generated using the following: site number, week number and year. Responses (i.e. receipt of samples and / or reports on weekly site trapping) are logged on arrival of the daily post at the laboratory. At this stage internal report forms are assigned to each sample and field reports are used to input environmental variables to the database. All data in the field reports are subjected to scrutiny and where needed observations are standardised before being input into the database. Hard copies of all field reports are retained and filed systematically (chronologically and on a site basis) for future reference.

Sample processing involves generation of total midge counts for all sites / weeks and in the case of 10 index sites (and other full taxonomic analyses) counts for each species / species complex sampled are recorded. In this case the process is tracked by means of the internal reports, on which each laboratory participant identifies by individual codes, their contributions to the data being recorded. Once the sample is fully processed the internal report information is input into the database and hard copies of the internal reports are attached to the relevant field reports for systematic filing in the VEU archive. Errors in reports or differences associated with sub-sampling protocols are signalled by cross-referencing between abundance data-sets and cumulative numbers generated from individual species counts. This and other quality control measures contribute to the overall efficiency of the laboratory sample processing operations.

Electronic data-loggers have been deployed, for temperature at 24 non-index sites and for temperature and relative humidity at index sites, for the 2008 programme. Additional site-specific information concerning environmental conditions will therefore be available for the overall 2008 statistical analyses when all the data-loggers are fully downloaded in 2009.

Species recorded

A checklist of the 29 species of *Culicoides* recorded from Ireland (Ashe *et al.* 1998) is presented in Table 2, in which the 22 species recorded in the 2007and 2008 surveillance programmes are highlighted. An additional species, *Culicoides furcillatus*, was recorded for the first time in 2008. Of the 22 species recorded so far, some species (*C. stigma, C. heliophilus, C. parroti, and C. furcillatus*) have yet to be confirmed. As in 2007 considerable variation in species richness (number of species) present in individual samples was observed in 2008, reflecting both within site and between site variations. The number of species varied from site to site ranging from 10 to 15 (mean 12.5). In this survey, the *Obsoletus* species complex is treated in two senses, i.e. when it is regarded as including the 4 species of the sub-genus *Avaritia* it is referred to as the *C. obsoletus* species complex but when, as is now frequently the case in European studies, it includes only *C. obsoletus*, *C. scoticus* and females of *C. chiopterus* it is referred to as *C. obsoletus*. Occasionally, (subject to taxonomic caveats) the term *C. obsoletus* is used in the strict sense (s.s.) and *C. scoticus* data are treated separately (e.g. Figs. 9, 10 and Appendix II).

Table 2. Checklist of Irish *Culicoides* species with those recorded to date in the BTV Vector Surveillance programme highlighted (*).

SPECIES	
Culicoides (Avaritia)	***chiopterus*** **(MEIGEN)***
	dewulfi **GOETGHEBUER***
	obsoletus **(MEIGEN)***
	scoticus **DOWNES & KETTLE***
Culicoides (Beltranmyia)	***circumscriptus*** **KIEFFER***
Culicoides (Culicoides)	***delta*** **EDWARDS***
	grisescens **EDWARDS***
	impunctatus **GOETGHEBUER***
	newsteadi AUSTEN
	pulicaris **(LINNAEUS)***
	punctatus **(MEIGEN)***
Culicoides (Monoculicoides)	***nubeculosus*** **(MEIGEN)***
	parroti **KIEFFER***
	riethi KIEFFER
	stigma **(MEIGEN)***
Culicoides (Oecacta)	*brunnicans* EDWARDS
	duddingstoni **KETTLE & LAWSON***
	festivipennis **KIEFFER***
	furcillatus **CALLOT, KREMER & PARADIS***
	heliophilus **EDWARDS***
	kibunensis TOKUNAGA
	pictipennis **(STAEGER)***
	poperinghensis GOETGHEBUER
	reconditus **CAMPBELL & PELHAM-CLINTON***
	segnis **CAMPBELL & PELHAM-CLINTON***
	vexans (STAEGER)
Culicoides (Silvaticulicoides)	***achrayi*** **KETTLE & LAWSON***
	fascipennis **(STAEGER)***
	pallidicornis KIEFFER

Comparisons of overall abundances of Culicoides in 2007 and 2008

In Fig. 3 the results for 2007 and 2008 are compared, based on full season data sets. However, because of the late start in the 2007 season the number of zero counts appears to be much lower in case of 2007. In 2007, the sampling programme effectively lasted from mid-April (week 17) to late December (week 51) and during this time weekly counts varied from 0 to 28,592. The mean weekly count ($\bar{x}$±S.E.) per trap was 412.02 ±56.3 (N=977). When comparable data for the same period (weeks 17 to 51 inclusive) in 2008 are combined and weekly counts varies from 0 to 65,536 value ($\bar{x}$ ±S.E.) was 382.46±86.04. Comparison of frequency distributions of weekly counts for 2007 and 2008 showed that they were very highly significantly different (Mann-Whitney U test, U=48; P≤0.001).

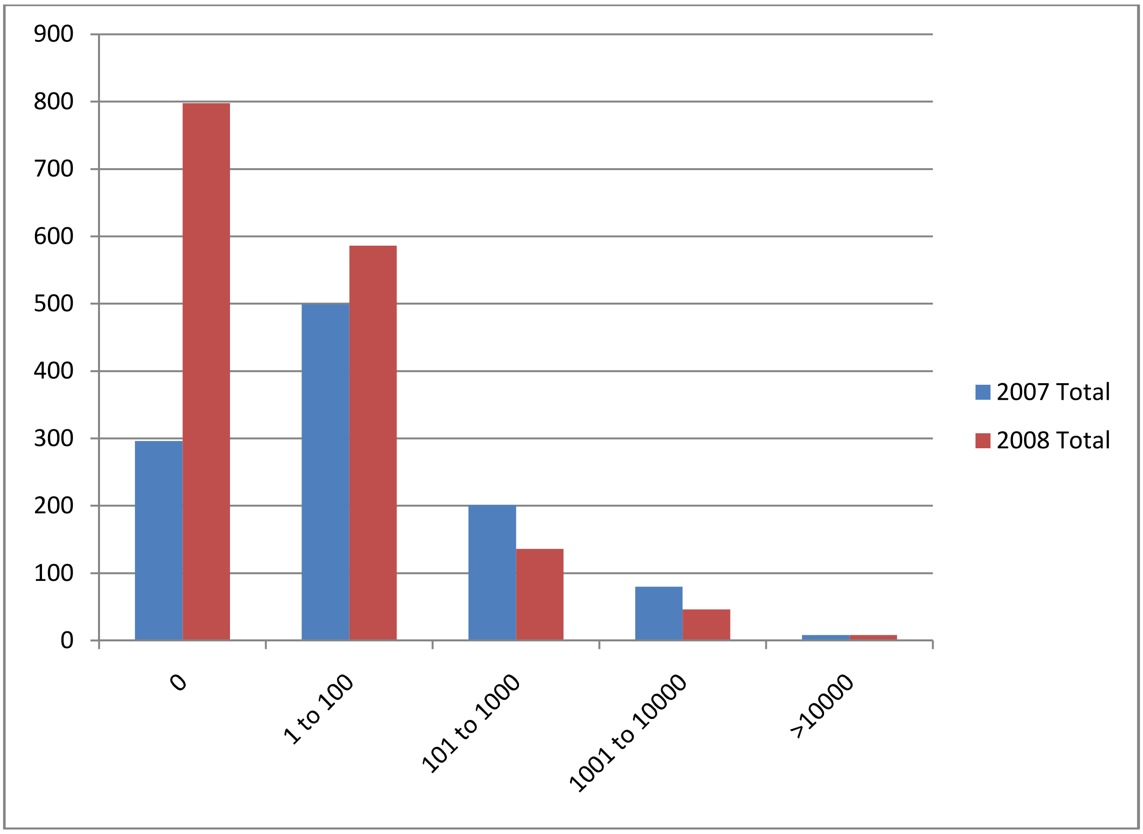


Fig. 3 Comparison of 2007 (April to December) and 2008 (full year) weekly sample sizes from 34 sites.

Comparison of sites 2007 and 2008

Table 3. Total *Culicoides* numbers for the 34 sites sampled in 2008.

Site No.	N
2	534
3	4458
4	9987
5	18068
6	257
10*	111
11*	14162
13	64106
14	5341
15	2592
16	11665
17	7296
18	33691
21	8124
23*	183199
25*	2453
26*	23360
28	491
30	6649
31	790
32	4192
33	196
34*	765
35*	848
37	3499
39	964
40	1679
41*	3919
42	1815
43	2195
44*	6784
47	6270
49*	2416
50	481

*Index Site

In Table 3 the cumulative weekly total counts of 34 sampling sites are presented and as can be seen the values varied from 111 to 183,199 ($\bar{x}$±S.E.= 12745.8±5582). The results can be compared with those obtained in 2007 when site totals varied from 335 to 56,270 ($\bar{x}$±S.E.= 11841.3±2744). The results are also presented, as percentage frequency distributions, in Fig. 4. As can be seen in 2007 the 4 sites (17, 21, 23 and 26) each recorded more than 10% of the cumulative annual total, but none exceeded 15%. In contrast in 2008 only two sites (13 and 23) had catches exceeding 10% of the total but in the case of one of these (23) the catch was 42.27% of the total number of midges recorded. This site (23) had the highest cumulative *Culicoides* abundance values in both years.

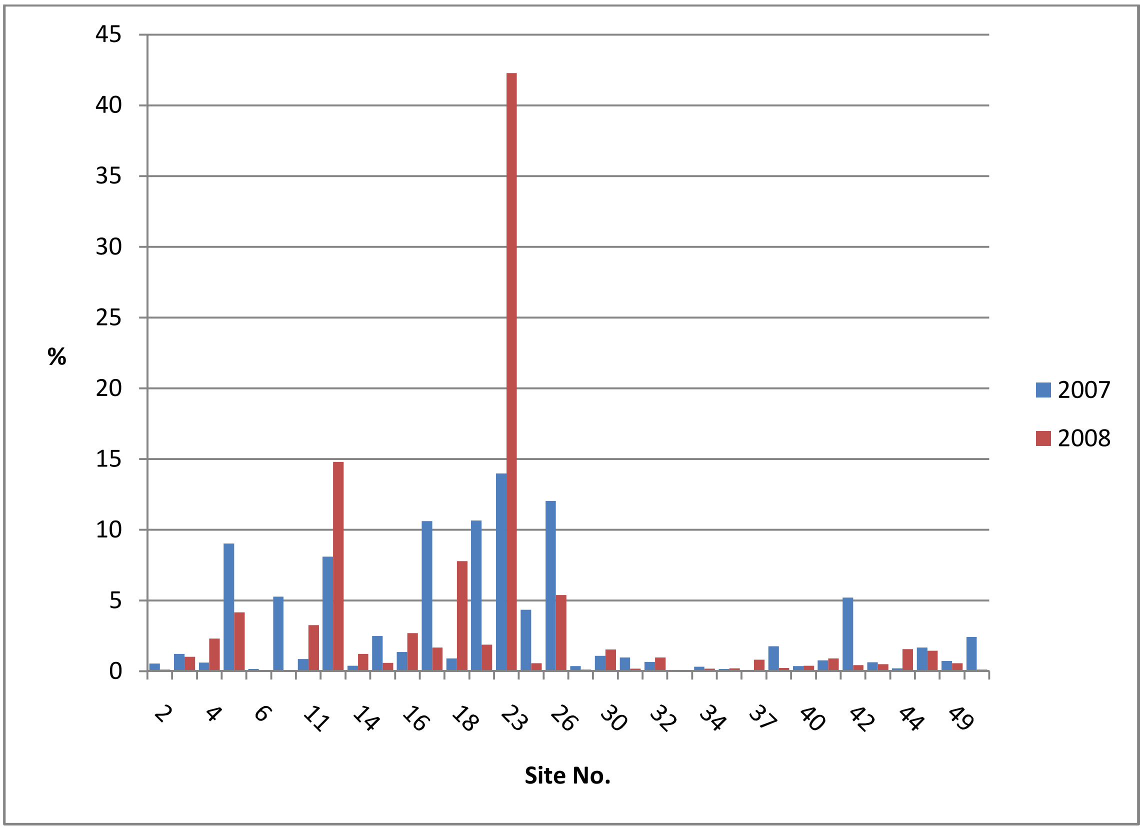


Fig. 4. Percentage frequency histogram comparing 2007 and 2008 *Culicoides* site abundances.

Comparison of the cumulative weekly counts for these sites in respect of the two years (Wilcoxon Test T=252.0, Z=0.44, P≤0.05) showed that the data sets were not significantly different. Likewise, examination of a scatter plot for 2007 and 2008 (Fig. 5) and the results of a non-parametric correlation (Spearman r S=0.4630, P≤0.01) showed that, though considerable between year variation in abundances was observed for many sites, they can be ranked with respect to their midge abundances and that certain sites can therefore be anticipated to provide good catches again in 2009.

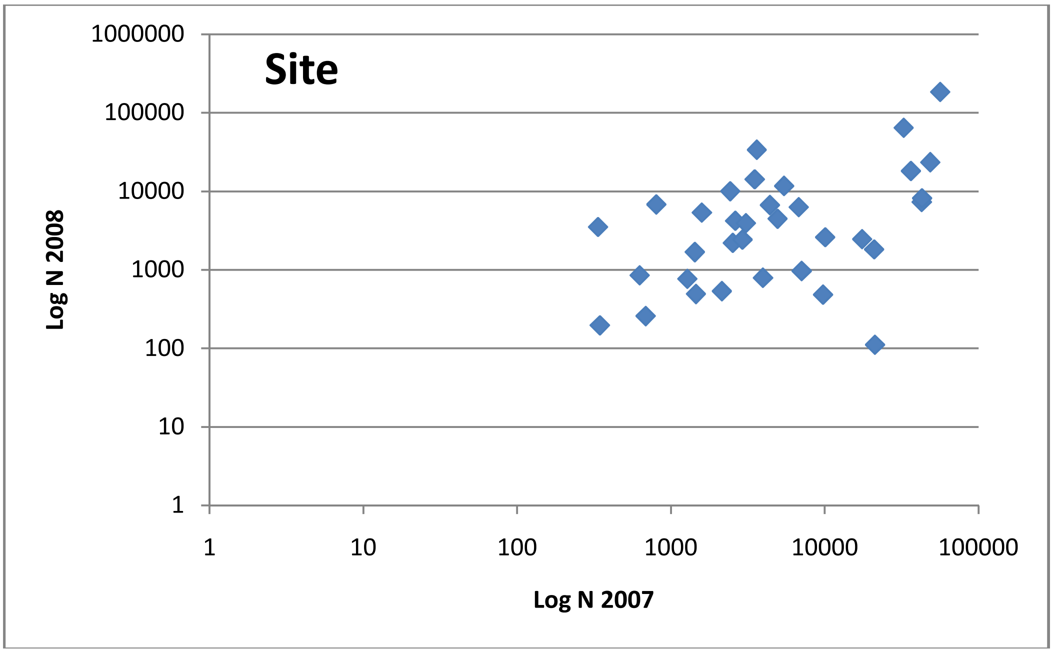


Fig. 5. Comparison of 2007 and 2008 total *Culicoides* abundances at sites (combined site catches for year).

In Fig. 6 the distribution of sites with their different abundance levels (100-1,000, 1,000-10,000 and >10,000) are indicated as a map of Ireland and it can be seen that the sites with the highest midge (>10,000) numbers are well represented in the central axis of the island. However, some western locations (site 5, Mayo and site 18, Kerry) are also included in this abundance category. Sites with intermediate or low in abundance values were widely distributed around the country and no clear pattern can be discerned.

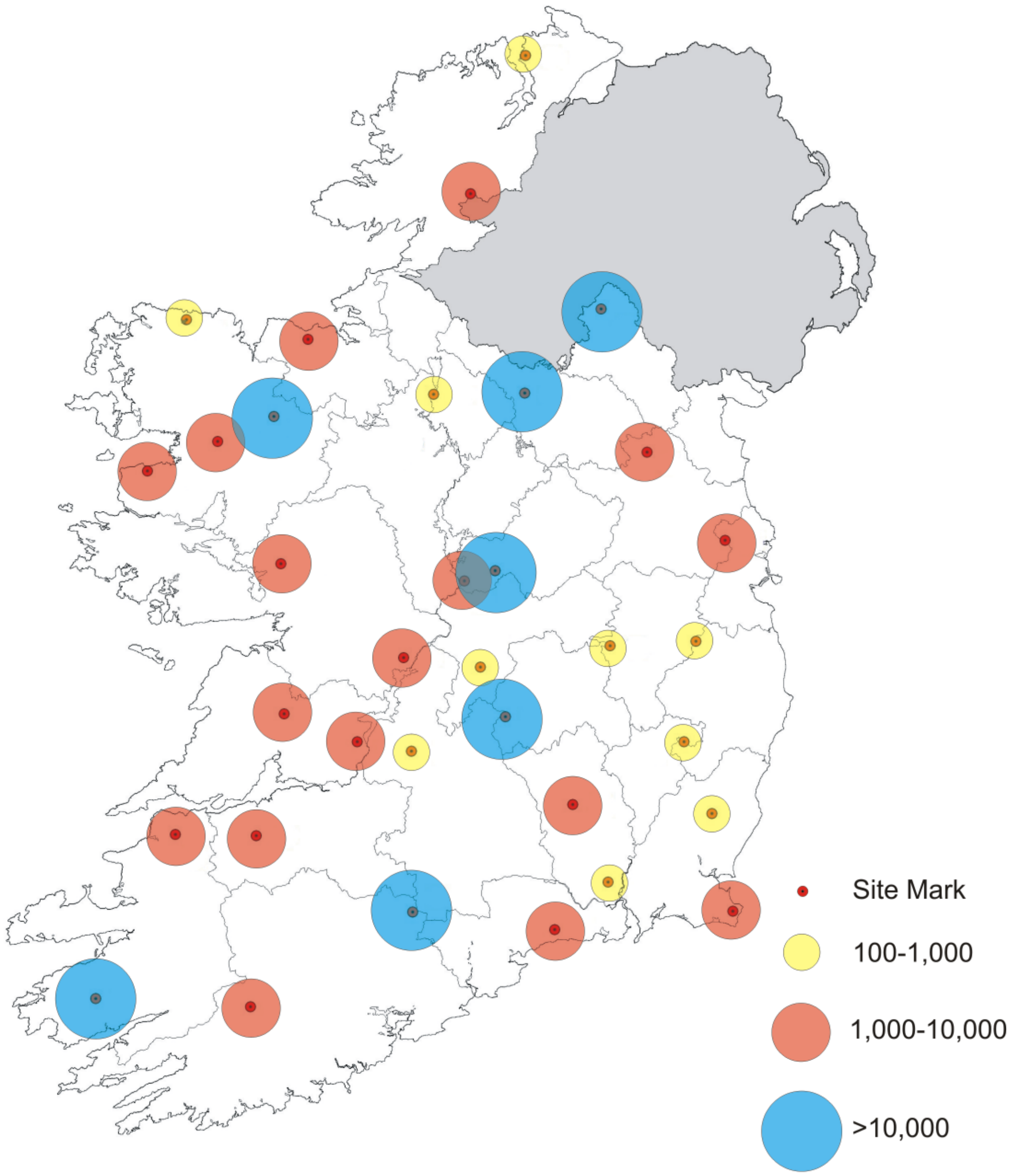


Fig. 6. Map of Ireland showing distribution of *Culicoides* sampling sites with different abundance levels (annual totals).

Seasonal variation in Culicoides abundance, 2007 and 2008

In Fig. 7 the seasonal variation in weekly *Culicoides* counts (all 34 sites) are illustrated for both the 2007 and 2008 surveys. As can be seen the periods of midge activity were broadly similar though surveillance began a little later (mid-April) in 2007, whereas in 2008 a full year sampling programme was possible. *Culicoides* can typically be trapped in the field from April to December inclusive in Ireland, and though higher numbers occur from May to early October the catch statistics (all sites combined) illustrates how variable the patterns are from week to week and between years. Peaks in *Culicoides* abundance that numbered more than 10% of the annual total occurred three times in 2008, whereas this occurred only once in 2007. Similarly in 2008 a single site peak represented more than 20% of the total *Culicoides* sampled that year but no such proportionally great peak was observed in 2007. The within and between year variation in weekly total counts illustrates the need for good time series for analyses of *Culicoides* population dynamics. Weekly variations in total *Culicoides* numbers recorded at each of the 34 sampling sites are illustrated in Appendix I, where the considerable between site variations in 2008 can be clearly observed. Likewise, the weekly total counts for each of the 19 taxa (species or species complexes) recorded in 2008 are similarly illustrated in Appendix II, where the extent of between week variation for species/species complexes and the considerable differences between the seasonal activity patterns for these taxa can also be noted. These results confirm observations made in 2007 concerning between site and between taxa variations in weekly abundances.

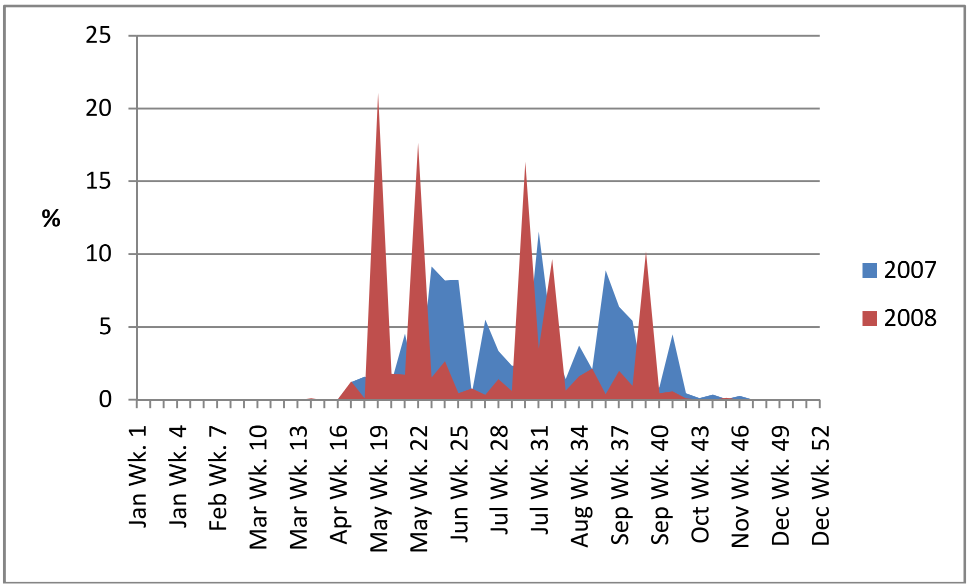


Fig. 7. Seasonal variation in *Culicoides* abundance for all 34 sites in 2007 and 2008.

Differences between sites can be considerable both in terms of numbers for different species/species complexes and overall midge abundance. Major peaks in abundance, such as was noted in site 23 where a total *Culicoides* count (estimated) of 66,536 was recorded can make interpretation of overall seasonal patterns (using combined site data) difficult. Likewise, irregular peak abundances for individual

species/species complexes, as seen in Appendix II make it difficult to interpret variation in overall *Culicoides* dynamics.

A comparison between the results obtained in 2007 and 2008 with respect to the relative abundances of the various taxa was made using log-transformed abundances. The results are illustrated in Fig. 8 in which it can be seen that the rank order of the numerically dominant species in the combined weekly catch at the 10 index sites were broadly similar in both years. However, evidence of between year variations can also be noted. Thus, for example, *C. dewulfi*, *C. pulicaris* and *C. punctatus*, were relatively more abundant in 2008. In contrast, high numbers of other taxa (e.g. *C. achrayi and C. impunctatus*) occurred in 2007.

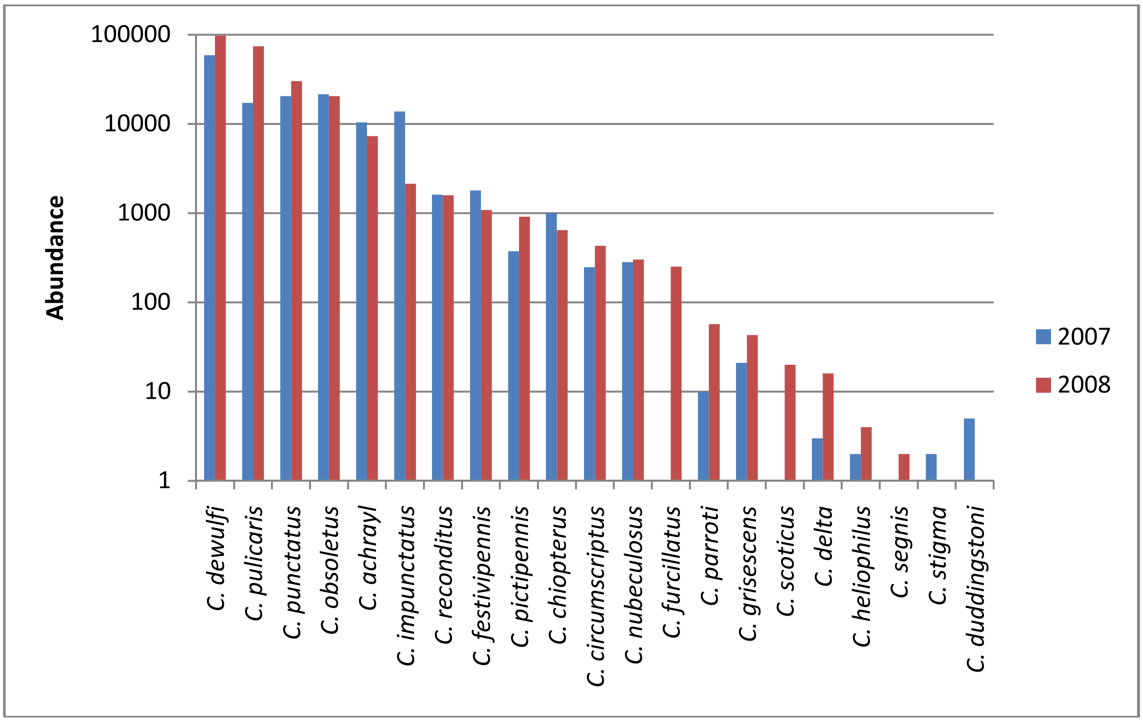


Fig. 8. Log-transformed abundance data for 2007 and 2008 *Culicoides* species.

In Fig. 9 the relationship between overall abundances of the 19 taxa and their estimated geographical range in Ireland (i.e. numbers of index sites at which they occur) is illustrated. As can be seen the more abundant species/species complexes are those that occurred at all or most of the index sites.

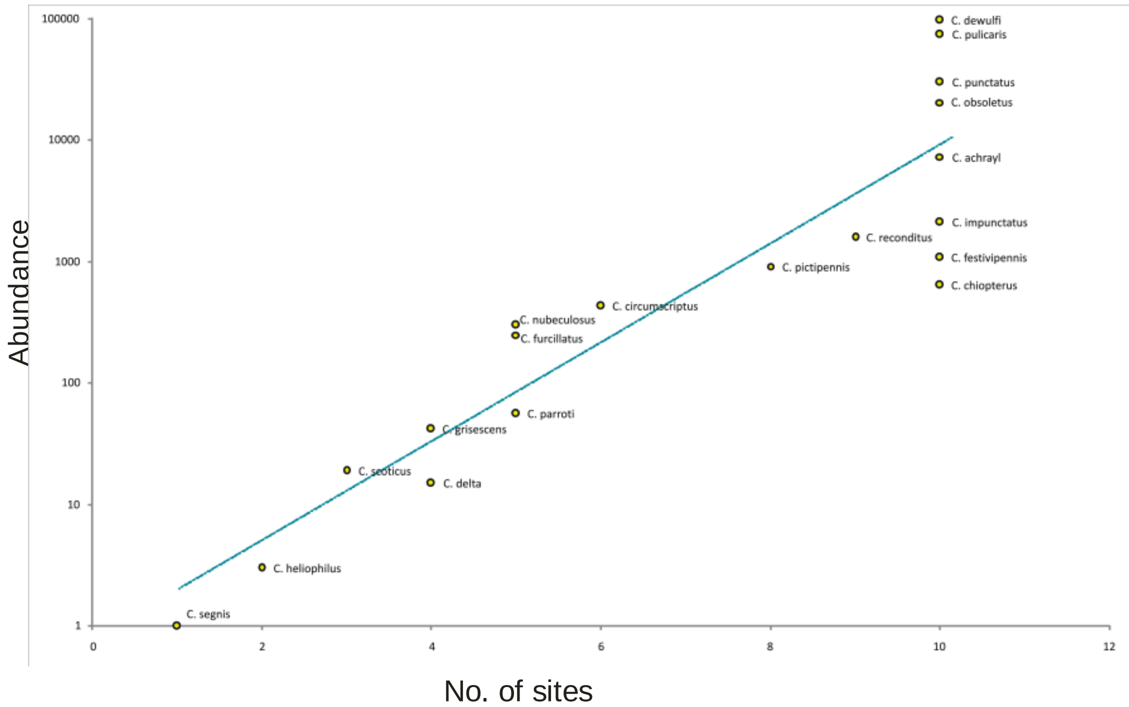


Fig. 9. The relationship between species abundance and geographical distribution of Irish *Culicoides* observed in the 2008 survey.

Species richness

The total number of species/species complexes record in 2008 was 19. This can be compared with the results obtained in 2007, when 21 taxa were involved. The main differences noted involved 3 rare species recorded only in 2007 and one additional rare species encountered in 2008. These differences are not considered to be of any great significance, with respect to the BTV Surveillance Programme objectives. The fact that other species in the Irish check list (N=29) have not been sampled indicates that some species are not amenable to UV light trap sampling. However, rarity, localised distributions and questionable taxonomic identifications of some species in the past are also likely to be factors. The number of species recorded per week at particular sites varied (0 to 15).

Composition of species assemblages

In 2008 full identifications, subject to certain taxonomic constraints detailed in 2007, were made in respect of the 10 index sites sampled. A proportion (18.4%) of the total number of weekly index site samples (N=490) had to be analysed using sub-sampling protocols. However, the total number of actual species identifications was still very high (N=39,649) and the process was very time consuming. The direct counts

and estimated counts for the sub-sampled *Culicoides* samples collectively represented an estimated 238,071 midge specimens.

The overall percentage composition of the sampled *Culicoides* catch of 2008 is illustrated in Fig. 10. The relative abundance of species/species complexes is also similarly illustrated for each of the 10 sites, Appendix III.

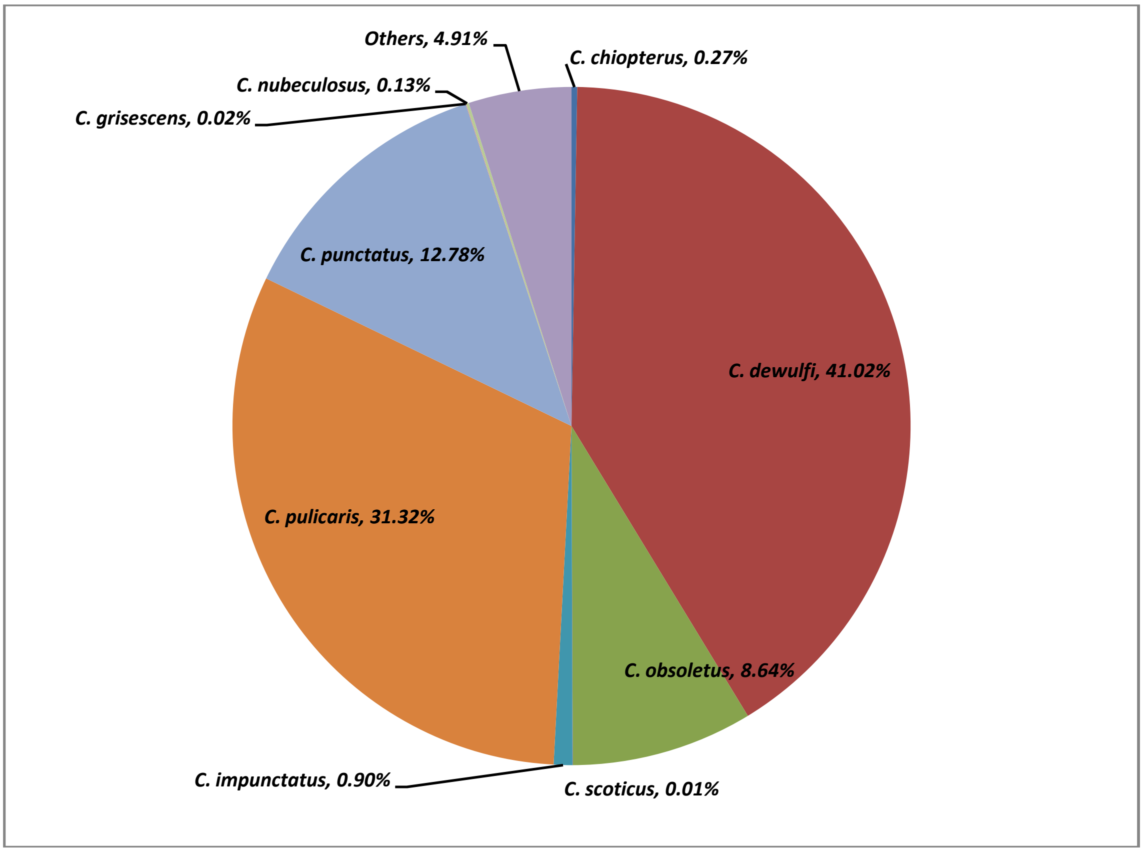


Fig. 10. The percentage composition of the combined catches at the 10 index sites during 2008, with respect to the more abundant species.

Environmental conditions

In 2008 data loggers were deployed at the 34 sampling sites. However, all these have not been fully downloaded pending the end of the vector free period. Analysis of the relevant temperature and relative humidity data from the loggers will be undertaken later. Some technical difficulties have been encountered with apparent failure in some relative humidity loggers, that need to be addressed. However, Met Éireann data has enabled some preliminary analysis of the effects of weather on *Culicoides* activity. Likewise, field records of environmental conditions by trap operators are available for analysis of the effects of environmental variables on weekly/annual catch levels and midge species assemblage composition. A summary of weather records compiled by trap operators in 2008 is shown in Fig. 11. These data indicate that fluctuations between weeks and between trap setting and removal times. The extent of fluctuation in rainfall, between setting and removal of traps was more extreme than was the case for cloud cover and wind intensity.

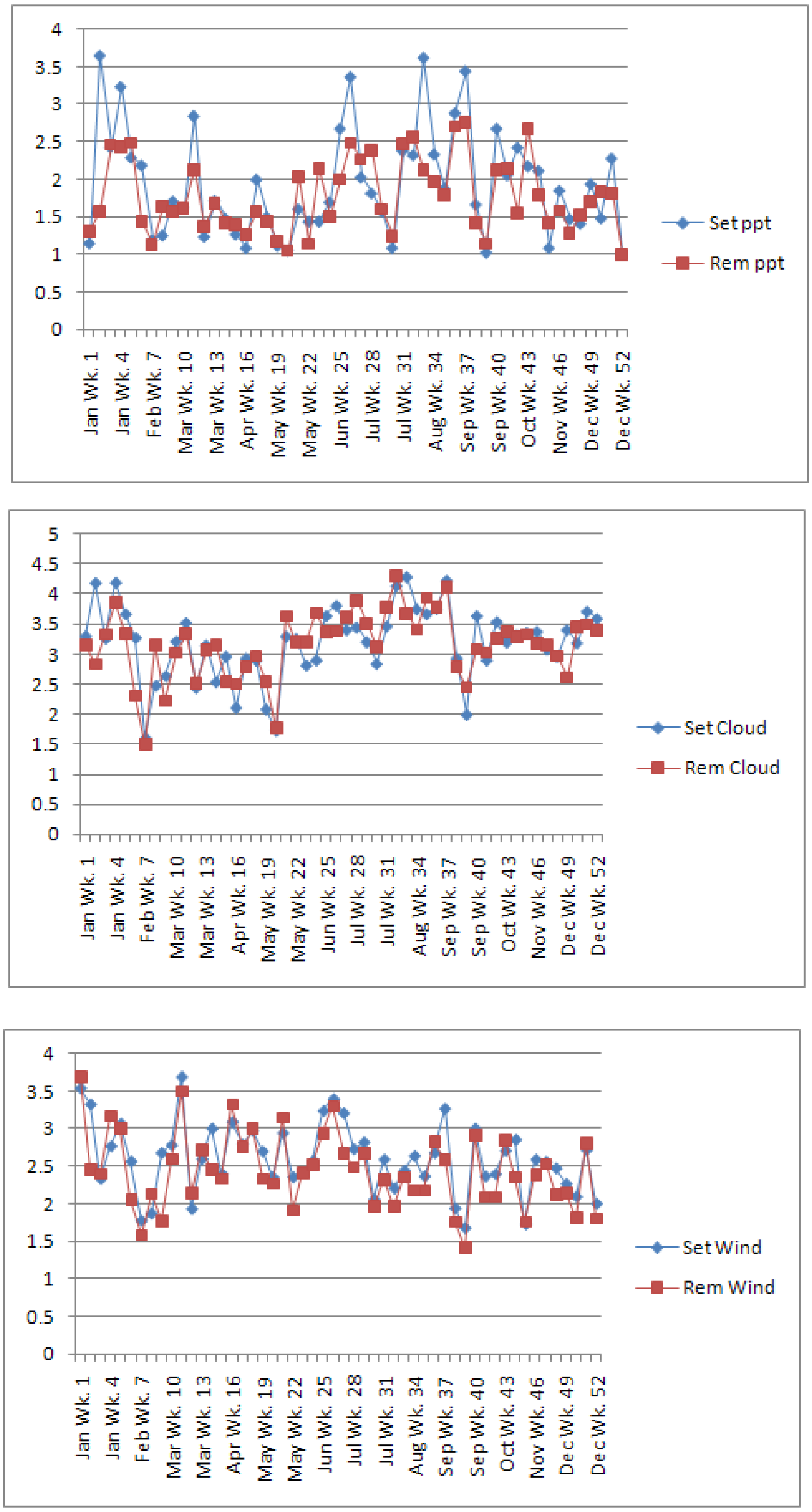
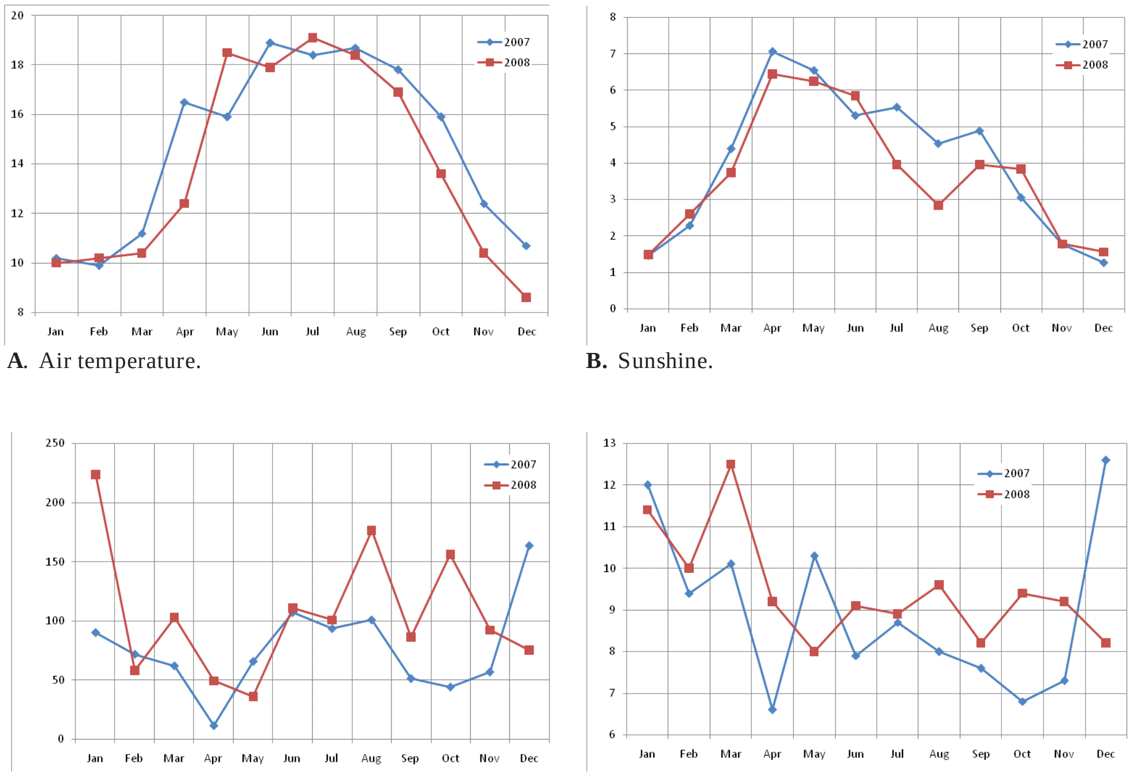


Fig. 11. DVO personnel weather score data for 2008. Data shown are the average scores of all 34 sites for days on which traps were set and removed.

The environmental conditions experienced in 2008 differed in several important respects from those recorded in 2007. Differences between the two years can be seen in Fig. 12 in which Met Éireann monthly averages for air temperature, sunshine, precipitation and wind are summarised. It can be seen, for example, that whereas summer and early autumn temperature conditions were similar in 2007 and 2008, May 2007 was considerably cooler than May 2008. Likewise, the low levels of sunshine recorded in 2008 for July to September are evident. The extremely wet autumn months of 2008 can be contrasted with the much drier conditions in those months of 2007. Similarly in respect of wind speeds, it can be seen that higher levels were noted in March/April of 2008 and from late summer to mid-winter, though in 2007 the December levels were much higher than those for December 2008.



A. Air temperature. **B.** Sunshine.

C. Rainfall. **D.** Wind speed.

Fig. 12. Met Éireann monthly mean data for (A) air temperature, (B) sunshine, (C) rainfall, (D) wind speed for 2007 and 2008.

In Fig. 13 the annual cycles of *Culicoides* abundance (total monthly site values) are illustrated for April to December 2007 and from January to December 2008. The results are presented as log transformed data to limit the variation associated with periodic large fluctuations in numbers and the between week/site variation described previously. As can be seen, the abundance levels are similar during the period July to September in the two years. However, in June 2008 the *Culicoides* abundance was less than in 2007. This can be seen with respect to the meteorological summaries above (Fig. 12) in which it can be noted that this month was warmer, drier and less windy in 2007. Likewise, in October 2008 the mean monthly *Culicoides* abundance was lower than for that month in 2007. The weather conditions in 2008 (Fig. 12) show that this was a colder, windier and wetter month than was October 2007.

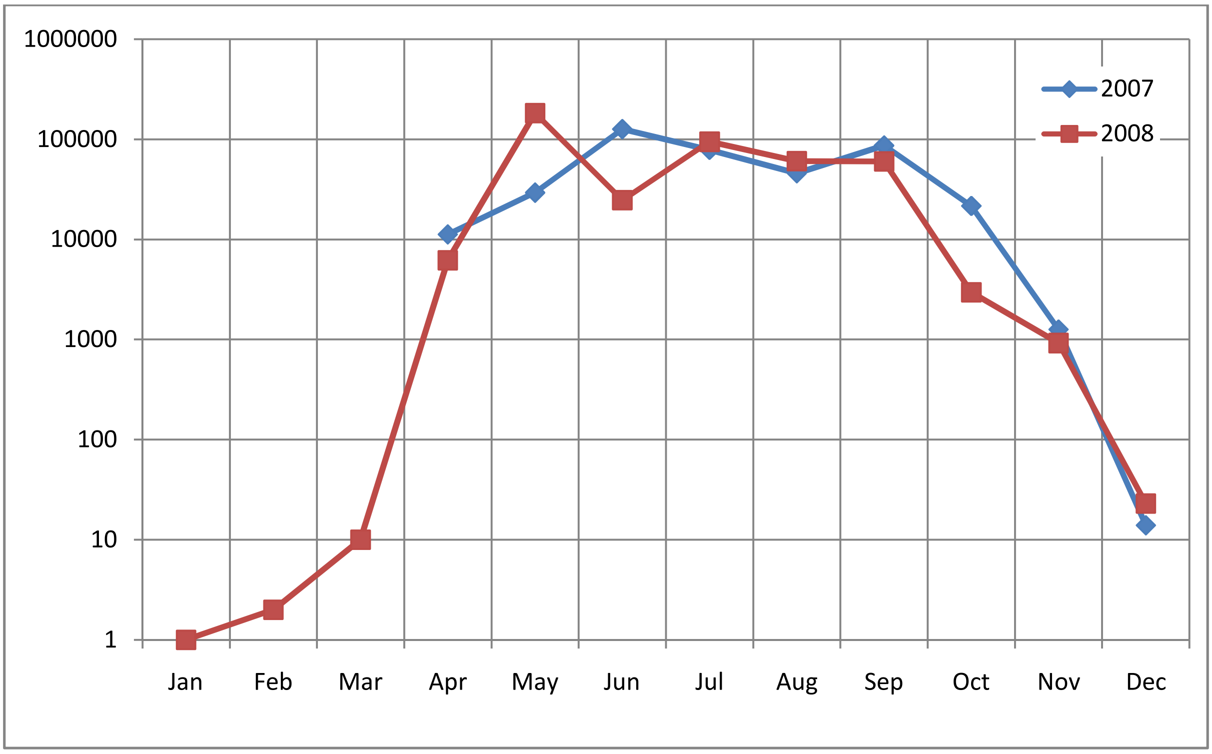


Fig. 13. *Culicoides* log transformed abundance for 2007 and 2008 (2007 survey started in April).

Interpreting weekly variations in *Culicoides* abundance and in species composition of the catches will require analyses of the full data set for the 2007/2009 period. Individual site analyses may eventually be more revealing with respect to short term fluctuations. In Fig. 14 the relative contribution made by a single site (23) to the overall seasonal pattern in 2008 can be observed.

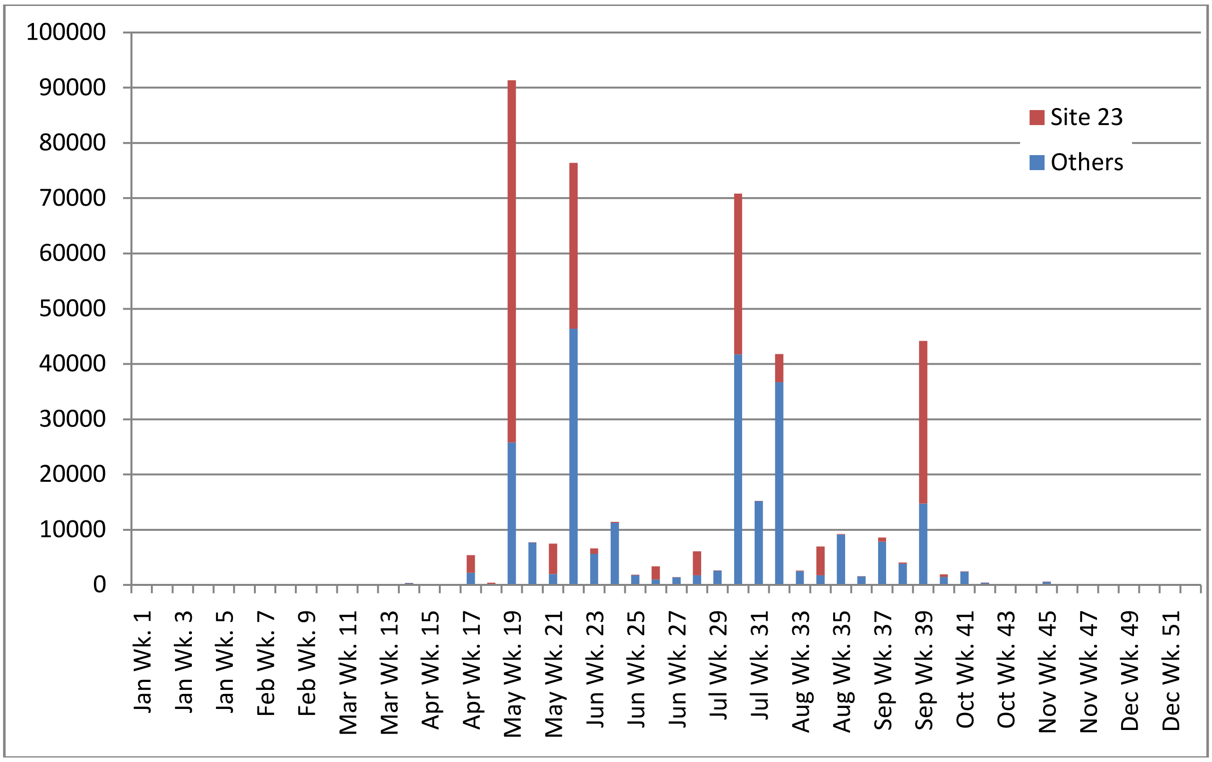


Fig. 14. Weekly variation in *Culicoides* abundance in 2008, with results for site 23 compared with those for the other sites combined.

Potential BTV vectors in Ireland

In this report the term *Obsoletus* complex is used to include all members of the subgenus *Avaritia*, though some taxonomists now exclude *C. dewulfi* from this complex. As can be seen, the *Obsoletus* complex represented by *C. dewulfi*, *C. obsoletus*, *C. scoticus* and *C. chiopterus* comprised more than half of the total catch. *C. dewulfi* was the dominant species in the *Obsoletus* Complex and it comprised 41% of the total index site samples in 2008. In view of the established role that this species and other members of the *Obsoletus* Complex has played in BTV transmission in northern and central Europe the very high numbers (48%) of these potential vector species in 2008 is a matter of concern. Likewise, in the case of the *Pulicaris* Complex, represented by *C. pulicaris*, *C. punctatus*, *C. impunctatus*, *C. grisescens* and *C. nubeculosus*, also known to have vector potential, they represented a very high proportion of the index site samples, 45% of the total *Culicoides* samples for the index sites. The 3 main species, *C. pulicaris*, *C. punctatus* and *C. impunctatus* were all well represented and they may also be of significance in the event of a BTV outbreak in Ireland. When combined, the *Obsoletus* and *Pulicaris* Complexes represented 96% of the total samples. In figure 15 the seasonal variation in abundance of the two major vector complexes (Obsoletus Complex and Pulicaris Complex) are illustrated for both 2007 and 2008. Whereas in 2007 the results for the two complexes are remarkably similar, some differences in relative abundances were observed in 2008 during July to September when the Obsoletus Complex numbers were higher than those of the Pulicaris Complex. It’s not clear whether this reflects differences in environmental conditions during the wetter, windier, weather experienced in 2008 versus 2007.

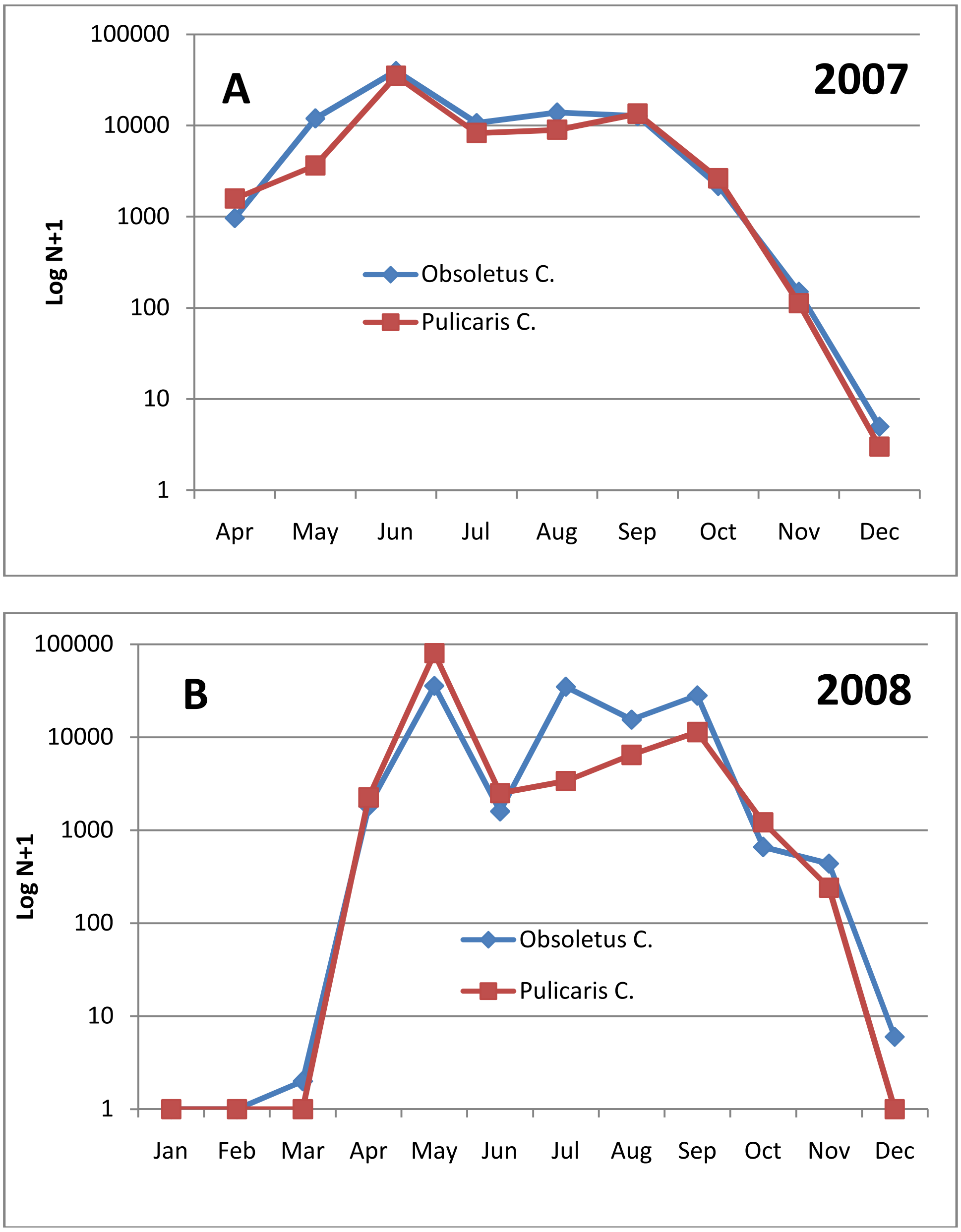


Fig. 15. Seasonal variation in abundance for potential vector complexes for (A) 2007 and (B) 2008.

Vector free period

The vector free period (VFP) is defined in accordance with the criteria outlined in the Official Journal of the European Union (Article 9 (3)) defining the VFP as the total absence of *C. imicola* specimens and less than 5 parous *Culicoides* per trap. *C. imicola* has a more southerly geographical range and has not been detected in Ireland to date and is therefore not a consideration for this programme at the present time. In November and December *Culicoides* abundance decreased dramatically and samples were examined retrospectively for parous females. Once the aforementioned criteria was met, i.e. less than 5 parous females per trap, the VFP was deemed to be in effect. The 2007/2008 VFP (Fig. 16) commenced in the first week of December (week 49) where 4 *Culicoides* were trapped at site 41 (Kilkenny). In the previous week a total of 43 *Culicoides* were trapped however only 2 sites trapped more than 5 *Culicoides*. Sites 5 (Mayo) and 13 (Monaghan) trapped 20 and 8 *Culicoides* respectively and the sample from site 5 contained 10 parous females and site 13 contained 5 parous females. The VFP ended in the first week of April 2008 (week 14) where 9 sites submitted samples with more than 5 parous females. The sample from site 17 (Clare) contained 218 *Culicoides* specimens. The 2008/2009 VFP commenced in mid-December (week 51) where 2 *Culicoides* were trapped at site 16 (Cork). In the previous week, samples from 2 sites containing *Culicoides*, 14 trapped at site 16 (Cork) and 1 at site 43 (Kerry). The sample received from site 16 contained 5 parous females. Defining the VFP by a complete cessation in *Culicoides* activity reduces the VFP in 2007/2008 by 2 weeks (week 51) and by 3 weeks in 2008/2009 (week 1, 2009). Likewise, by using this criteria, the VFP ends earlier in 2008/2009 by 8 weeks (week 5) where 1 *Culicoides* was trapped at site 43 (Kerry).

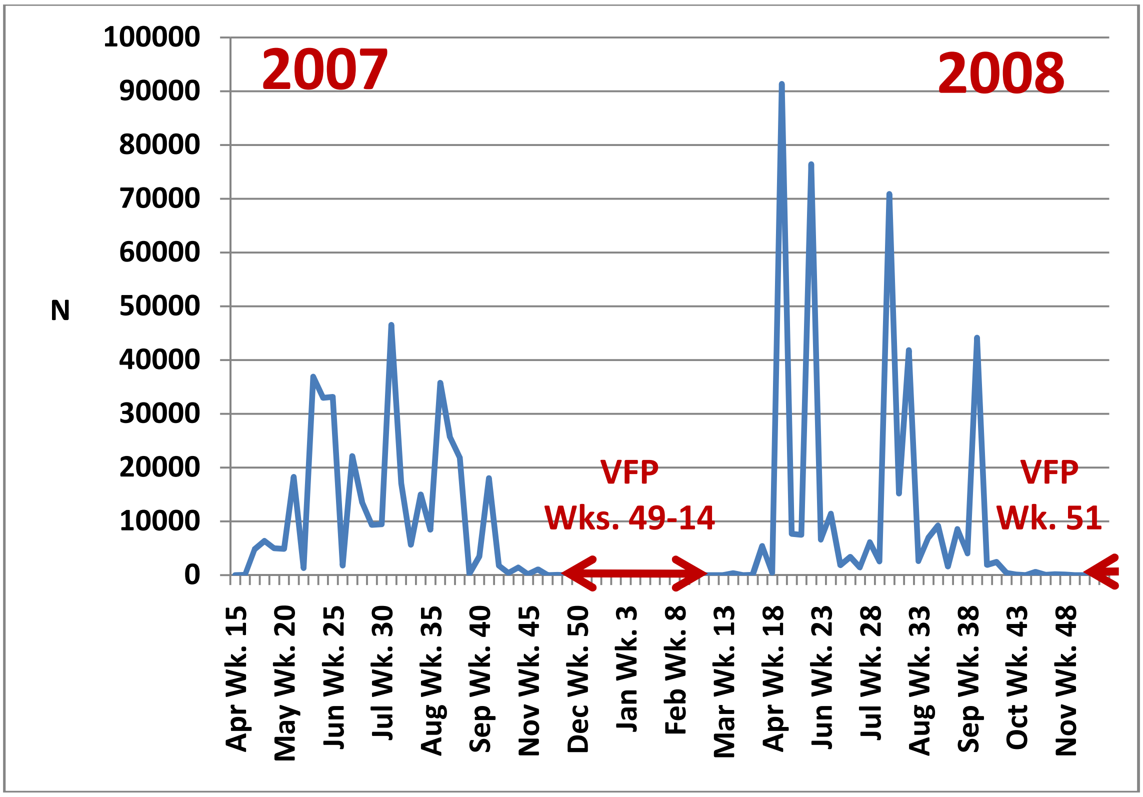


Fig. 16. Seasonal abundance of *Culicoides* with the vector free period (VFP) highlighted.

OVERVIEW AND RECOMMENDATIONS FOR 2009 SURVEY

The work load associated with the 2007 and 2008 BTV Vector Surveillance Programmes was considerable. In addition to the significant effort and costs, associated with sampling the 34 randomly selected sites, the laboratory processes and data analysis proved much more time consuming than initially anticipated. This was particularly evident in respect of: initial sample sorting/removal of other unwanted insects; *Culicoides* counts for very large samples and the demanding taxonomic work involved in analyses of index site samples. As a consequence, a major backlog in sample processing occurred in respect of the 2007 survey which had to be dealt with during the early part of 2008. In Fig. 17 the locations of the sites selected for 2009 are indicated. They include 7 of the former 10 index sites plus 3 alternates selected from the former non-index sites. The latter were selected to replace index sites with low *Culicoides* activity to ensure a large and representative *Culicoides* assemblage which will be sufficient to define overall seasonality and vector free period limits for 2009.

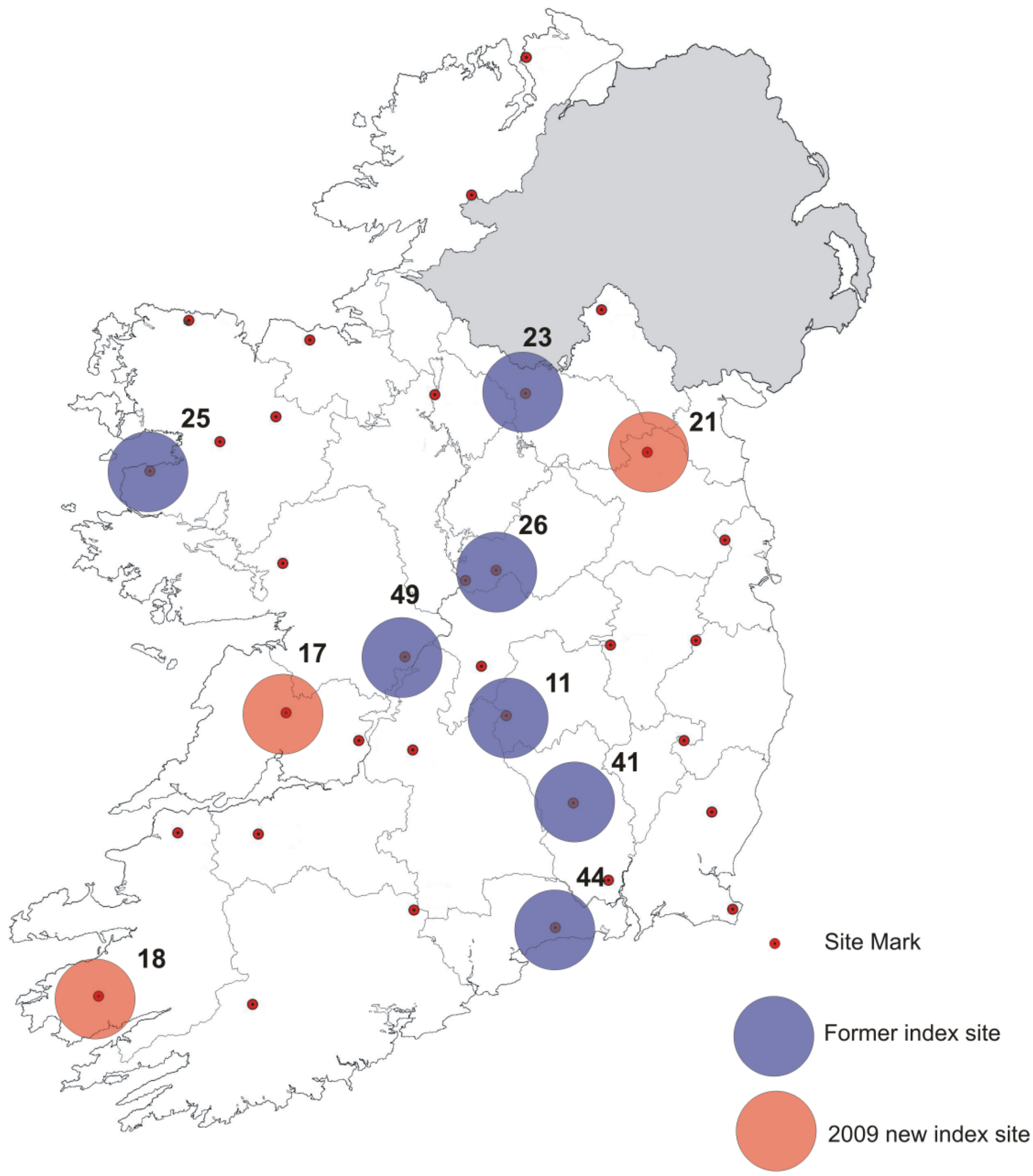


Fig. 17. Map of Ireland illustrating the 10 sampling sites selected for 2009.

To increase efficiency of laboratory work an internationally accepted sub-sampling protocol was adopted in 2008 and this enabled the analysis of the years' field sampling collection to be concluded more efficiently. There is thus no back log in sample processing at this stage. However, many of the research objectives proposed for 2007-2009 have not been addressed. Thus for 2009, as initially described in 2006/2007, it is proposed that a new field sampling programme be adopted. This will involve sampling 10 sites weekly over the full season and replicate traps will be used at some of these sites to address various research topics. Among the research topics proposed are: comparison of field and farm yard *Culicoides* populations; differences between immediately adjacent habitats e.g. pasture, wetland, woodland; host selection and diel activity patterns.

The number of sampling sites required to provide an adequate overall perspective on *Culicoides* population abundances in Ireland depends on the criteria used in site selections. This is illustrated (Fig. 18) by cumulative abundance curves describing cumulative addition of midge count data of sites to the series *Culicoides* total in decreasing order (blue curve) or increasing order (red curve) of 2008 total abundances. If sites are selected randomly from among the 34 sites, different results (green curve) can be obtained if data from particularly *Culicoides* rich sites (e.g. 23) are added at contrasting times to the cumulative abundance. Accordingly, it was decided that for 2009 it would be best to subjectively select some *Culicoides* rich sites as replacements for poor index sites in the list of 10 sites to be surveyed.

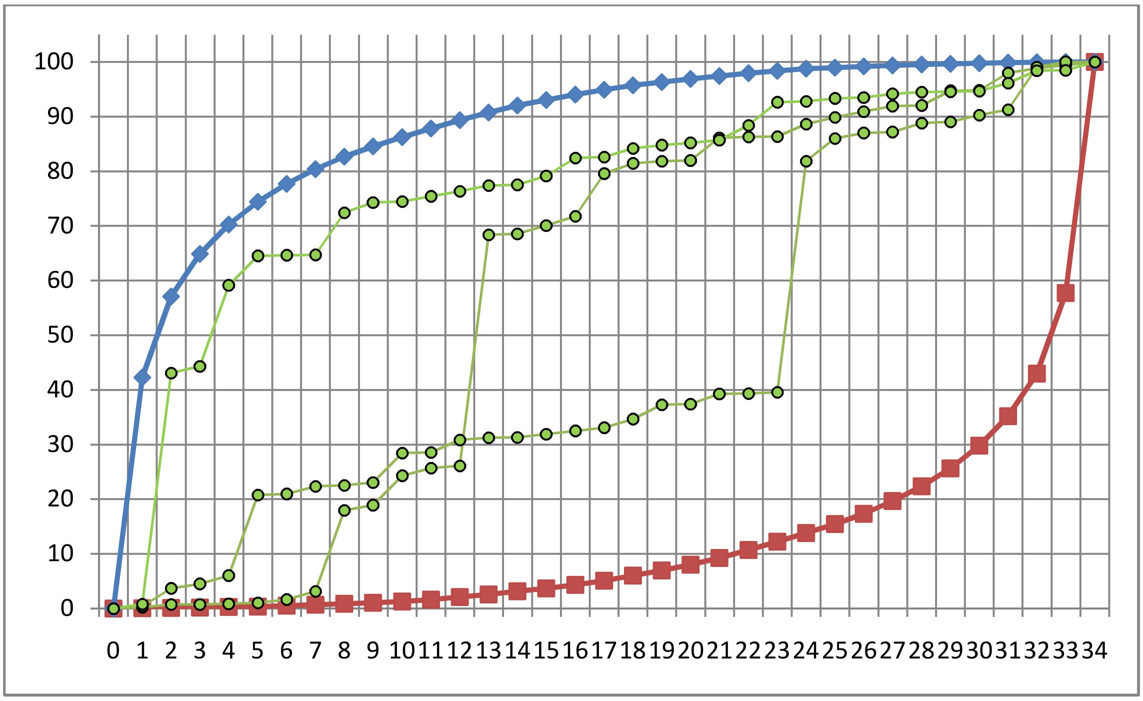


Fig. 18. Cumulative abundance curves based on inverse abundance series (blue), increasing abundance series (red), and three random site sequence selections (green).

Information on farmyard and farm shed *Culicoides* populations is needed and the 2009 programme should seek to address this research topic. Studies in northern France, during the 2006 BTV outbreak showed that relatively high numbers of *C. obsoletus* complex midges occurred indoors. They were considered to be more likely to feed in such locations (endophagic) than was the *C. pulicaris* group midges which typically fed out of doors (exophagic) and the endophagy of some species was thought to be linked to low external temperatures (Baldet *et al*., 2008). The importance of considering the inclination of *Culicoides* to enter farm dwellings (endophily) which has been previously noted in South Africa, was also highlighted by Meiswinkel *et al*., (2008) in respect of the initial outbreaks of BTV in the Netherlands.

Likewise, the spatial distribution of *Culicoides* among the various types of habitat in landscapes is increasingly being researched. For example, Meiswinkel *et al.* (2008) noted that almost 50% of the *Culicoides* captured in deciduous forest near infected Dutch farms contained gravid females of *C. obsoletus* and *C. scoticus*. This was considered to indicate that a significant proportion – after first feeding on cattle both inside and outside sheds situated nearby – moved into the forest subsequently to complete blood meal digestion and oogenesis (Meiswinkel *et al*., 2008). Tracking of *Culicoides* local movements and analysis of their diel cycles of flight and feeding activity is also likely to provide a better understanding of the vector-host interactions that are critical to disease transmission. In the 2009 programme this sort of research should be initiated. Furthermore, since very little information is available on *Culicoides* host selection in Ireland, novel trapping approaches to this topic need to be developed. The pioneering study on *Culicoides* feeding behaviour by Townley *et al.* (1998) in Ireland is still widely cited in publications on BTV vector ecology. Use of alternatives to the standard UV light trap protocols, which are well suited to the EU protocols for BTV vector surveillance surveys, will also be helpful in providing better knowledge of rare species, day flying species, etc.

A better knowledge of species, larval habitats, activity patterns, etc. may permit control of BTV transmission by reduction of possible *Culicoides* bites. In general, because the wide geographical distribution and abundances of potential vector species/species in Ireland, biocidal control measures are unlikely to be of much value. However, in special circumstances such as for animals in transit or in quarantine or for specially protected high-value animals, use of insecticidal treatments such as those reviewed in recent publications (Liebisch and Liebisch, 2008, Mehlhorn *et al*. 2008 and Schmal *et al.*, 2008) could be effective. If such actions need to be initiated in Ireland alternatives to the light trap approach will be needed for monitoring their success rates.

Understanding the population dynamics of potential *Culicoides* vector species is also important in respect of vaccination programmes (Savini *et al*., 2008). Increasing knowledge of the molecular epidemiology of bluetongue virus in Europe has not provided any clear understanding of the route or dispersal mechanisms that lead to the outbreak of BTV-8 in northern Europe (Saegerman *et al*., 2008). Likewise, though there have been significant improvements in knowledge of the vector competence of indigenous northern European *Culicoides* species and of the vector capacity of their populations, there are still major gaps in our understanding of the factors that may limit BTV transmission under Irish environmental conditions. For example, it is not yet clear why in contrast to 2007, there were no outbreaks of bluetongue in the UK in 2008 (Burgin *et al*., 2009). However, though evidence of transplacental and contact transmission of bluetongue virus (BTV-8) was obtained following importation of pregnant heifers from the Netherlands to Northern Ireland (Menzies *et al*., 2008) it is clear that for a serious outbreak to occur there will have to be involvement of vector

competent *Culicoides* and that environmental conditions (temperature, etc.) would have to be favourable to BTV transmission. Though transmission models are being developed (e.g. Gubbins *et al*., 2008) these need to be tested by reference to natural environmental conditions in affected areas and that may well be too late to prevent outbreaks in Ireland. There is still not an adequate understanding of the overwintering by BTV in ruminant populations (Wilson *et al*., 2008). Improved vector ecology research may be critical to solving such problems. There is increasing evidence that light trapping surveillance is not providing an adequate basis for investigation of BTV transmission phenomena, as evidenced by recent studies that suggest that important vectors are underestimated (Carpenter *et al.*, 2008).

The epidemiology of BTV in Europe and elsewhere in the world has been increasingly linked to climate change. It is known, for example, that the time taken for BTV to spread within a vector from it's gut to it's salivary glands can vary from a few days to several weeks and that this is partly temperature dependant (Wilson and Mellor, 2008). Consequently, predicted changes in Ireland's climate will mean that the potential for BTV transmission will increase in the coming decades.

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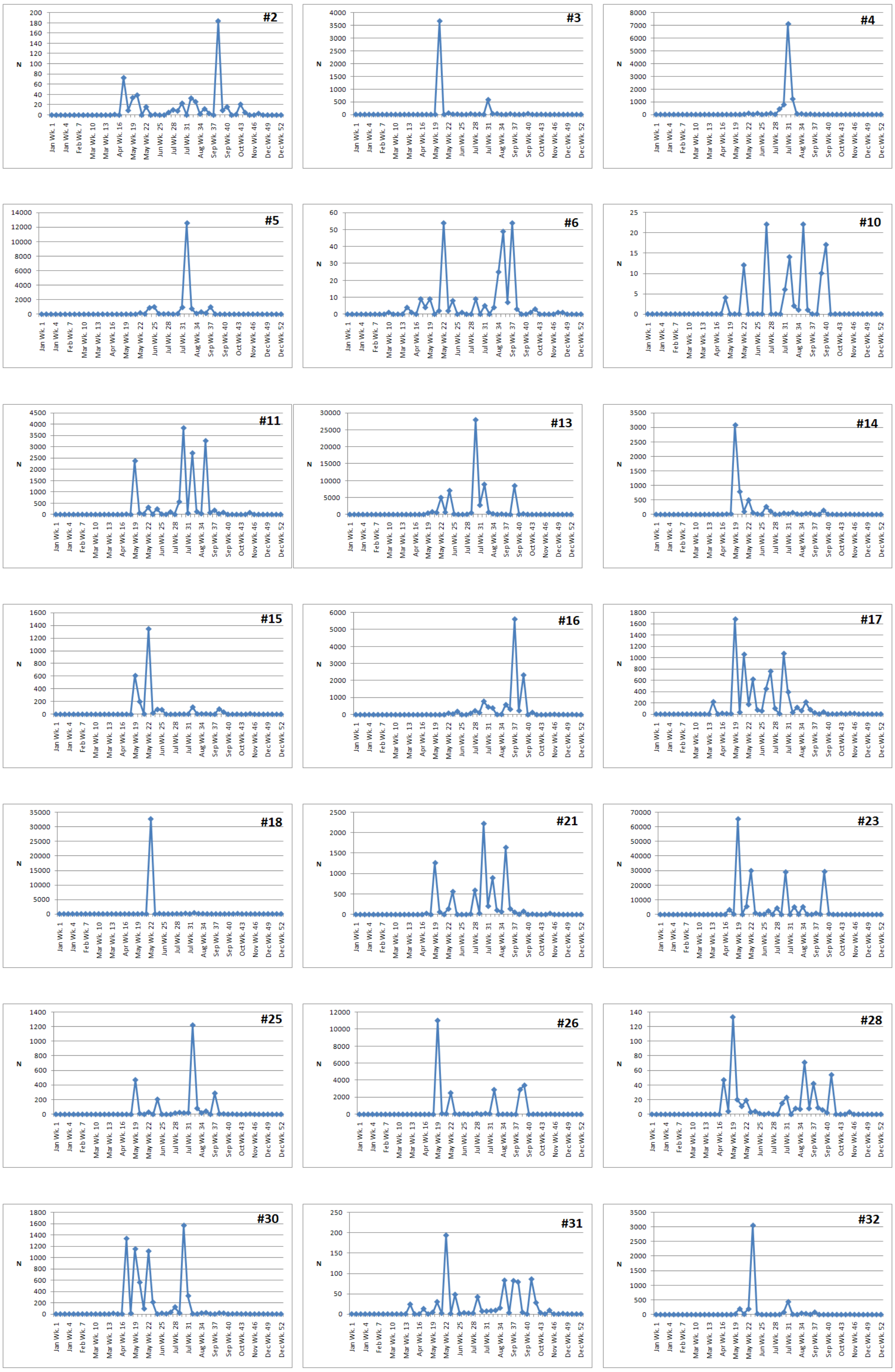
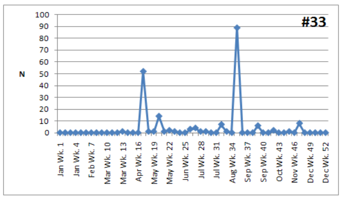
Appendix I

Weekly variations in *Culicoides* abundance at 34 sites in 2008

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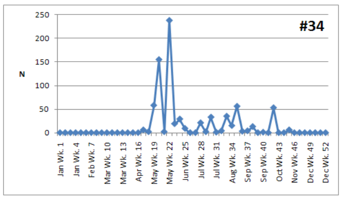
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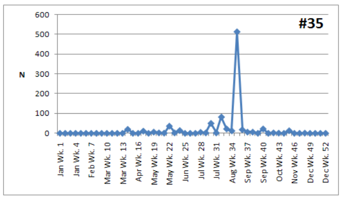
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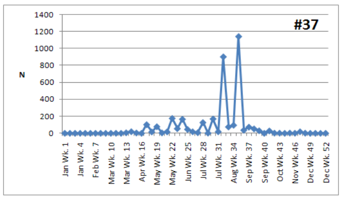
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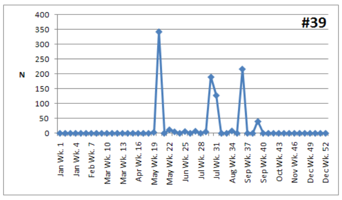
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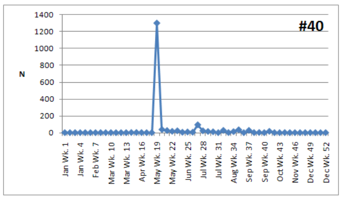
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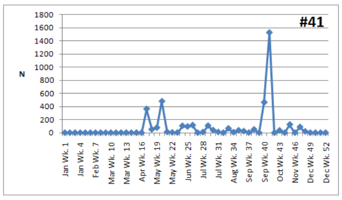
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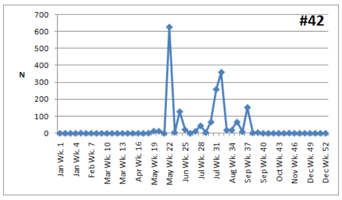
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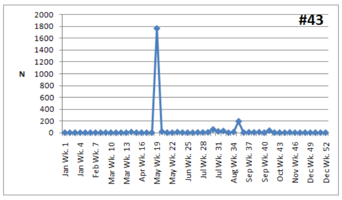
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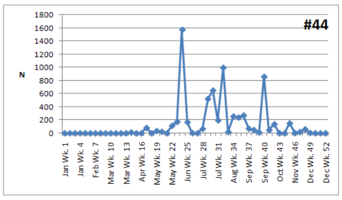
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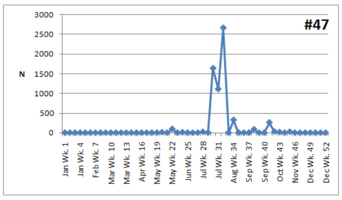
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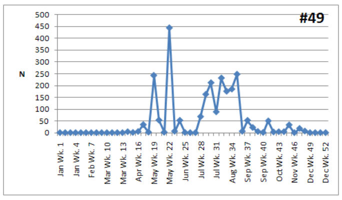
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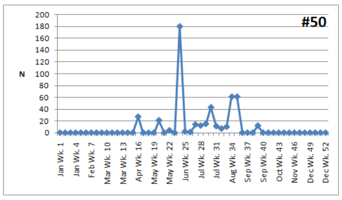
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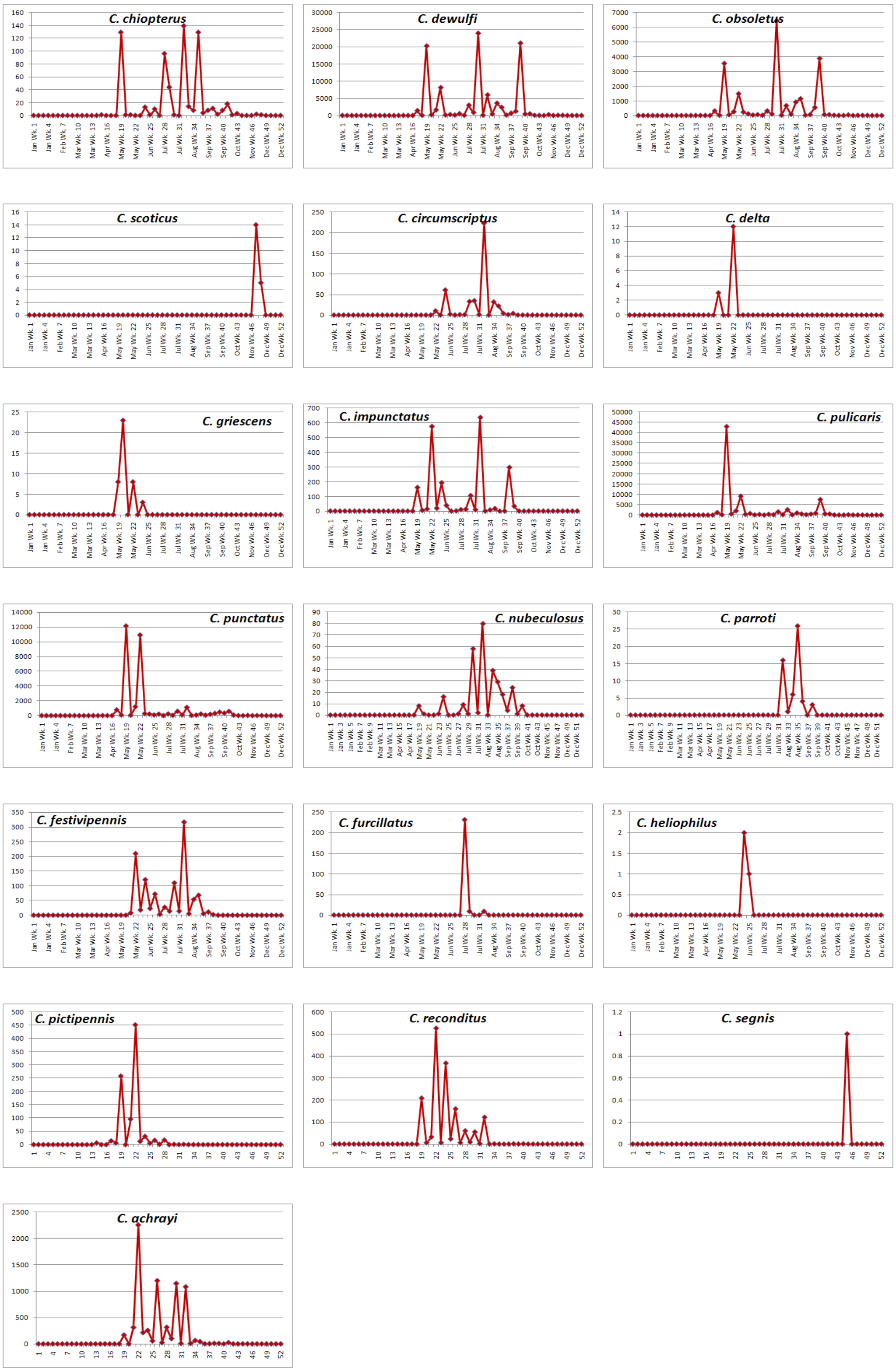
Appendix II

Weekly variations in counts for 19 species of *Culicoides* recorded at 10 index sites (combined data) in 2008



Appendix III

Percentage taxonomic composition of the *Culicoides* species/species complex assemblages sampled from the 10 index sites in 2008.