BTV Vector Surveillance 2007-2008
1st Annual Technical Report

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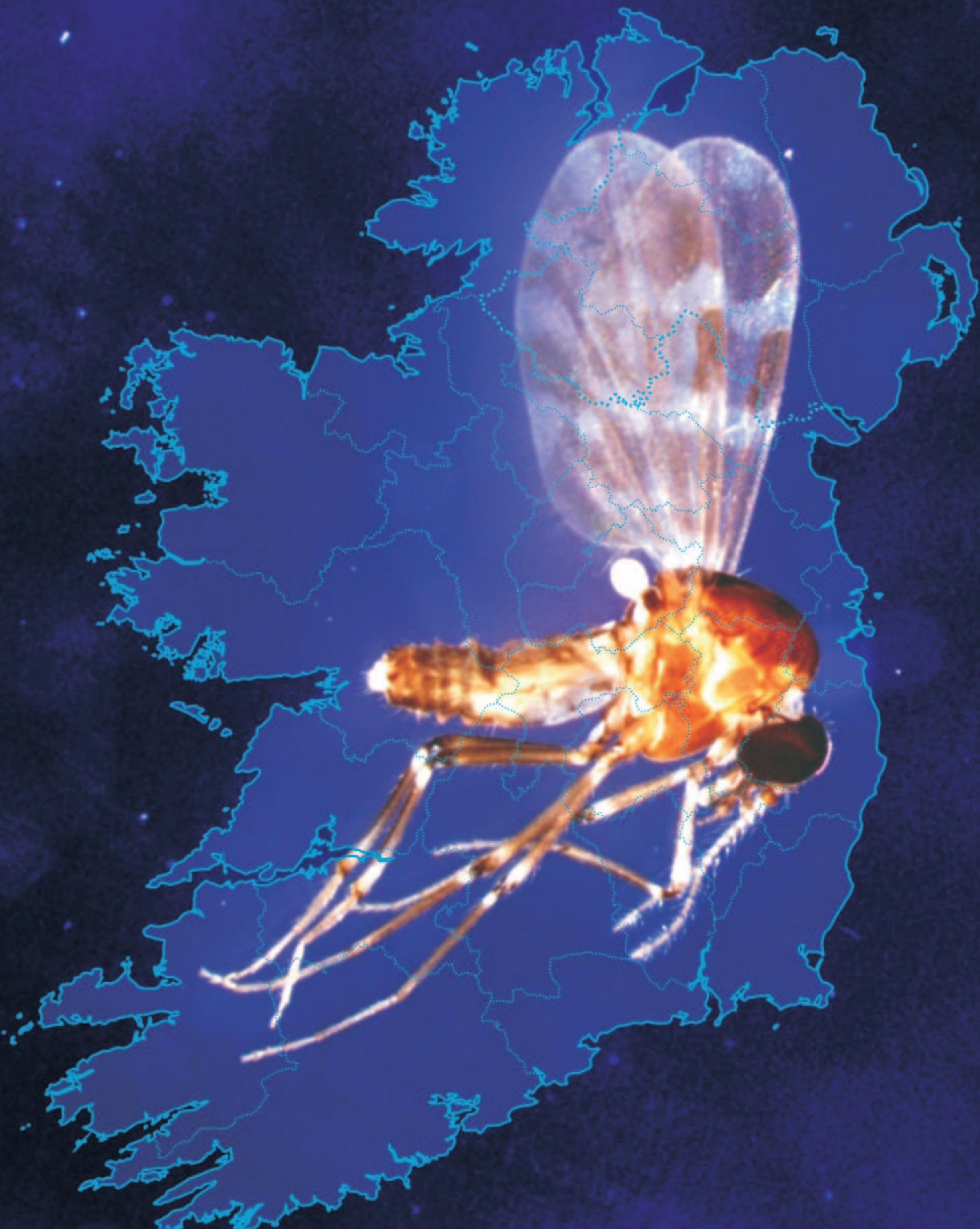

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EXECUTIVE SUMMARY

- A national BTV vector surveillance programme was initiated in Ireland in 2007, using entomological sampling protocols adopted in other EU member states, with a view to providing better knowledge of the Irish *Culicoides* (biting midge) species (especially BTV vector forms), to establish their geographical distribution in the country and to record their seasonal and between year population dynamics. This was part of a series of measures that were intended to assist in having a clearer understanding of the potential consequences and risks to Irish agriculture in the event of there being an outbreak of bluetongue disease in the country.

- A series of 34 randomly distributed farmland sites have been sampled, using specially designed UV light traps, on a weekly basis during 2007 by DAFF from the local District Veterinary Offices and with the full co-operation of the land-owners. Environmental data, logged during sampling, and preserved samples of the biting midges captured are sent to a dedicated Vector Ecology Unit at the National University of Ireland, Galway, for analysis.

- The sampling programme has been effective, with over 85% of potential sampling events resulting in *Culicoides* samples and / or relevant field data. This has involved weekly samples containing up to 21,116 specimens of midges per site, plus large numbers of other small flying insects (mostly Diptera and Hymenoptera), being sent to the university entomologists for sorting and for taxonomic species identifications.

- The combined samples obtained in 2007 included nearly half a million *Culicoides* and of these over 90,000 were identified to species or species complex level. A series of 10 randomly designated Index Sites, were examined in greatest detail and the samples from these sites were subjected to full taxonomic analysis, with all their *Culicoides* being identified to species / species complex level. Selected samples from all other sites were similarly examined. Other samples were analysed in less detail, with total numbers of *Culicoides* per site being recorded on a weekly basis to provide a better national perspective on questions relating to seasonality of midge activity and for defining vector free periods.

- Although a broad seasonal trend was evident in the combined site abundance data from April 2007 to November 2007, the *Culicoides* abundances exhibited large spatial and temporal variability. Temperature, sunshine and wind seem to have been the chief determinants of the variation in *Culicoides* abundance.

- Numbers of *Culicoides* trapped were positively correlated with numbers of species present and with species diversity indices. Likewise numbers of *Culicoides* trapped, species richness (number of species present) and species diversity were strongly positively related to temperature and sunshine and strongly inversely related to wind speed and wind gusts.

- The variations in the species assemblages of *Culicoides* collected were analysed and it was shown that the 10 index sites were collectively representative, in terms of both overall midge abundances and of the species composition, of the catches of the full set of 34 sampling sites analysed in 2007.

- The relative abundances of the 21 species / species complexes recorded in 2007 varied greatly with the following being the most abundant (as indicated by their percentage representation in the combined index site collections) *C. dewulfi* (35%), *C. impunctatus* (17%), *C. obsoletus* (15%), *C. punctatus* (12%), *C. pulicaris* (10%) and *C. chiopterus* (0.6%).

- Species considered to be potential vectors of BTV included *C. obsoletus, C. chiopterus, C. dewulfi, C. scoticus, C. pulicaris, C. punctatus, C. newsteadi, C. griescens* and *C. impunctatus* and these made up 90% of the *Culicoides* identified to species or species complex level for the 10 index sites. The large numbers of potential vectors and their widespread distribution in the country highlights the problems that could arise if an outbreak of BTV were to occur in Ireland.

- The potential BTV vector species also seem to have a long activity season in Ireland, especially when data for all such species are combined. Initial results suggest that the vector free period in Ireland extends from mid November to end of March. However, climatic variation between years and occurrence of local *Culicoides* activity cycles associated with particular micro-habitat conditions, means that for disease control purposes the vector free period may have to be defined more conservatively than the 2007 data suggests.

- Improved trapping and sample processing protocols are proposed for the 2008-2009 BTV vector surveillance programme and it is suggested that further research on the population ecology of Irish *Culicoides* is needed.

- The extensive records in the NUI, Galway *Culicoides* database, and other data available from meteorological stations and farm records, could in future be used to develop mathematical models for better analyses of *Culicoides* population dynamics and of their activities (biting, flight, swarming, mating, dispersal, etc.). Improved knowledge of the biology of Culicoides may be important in control of BTV disease and of other livestock diseases for which these insects can act as vectors.

INTRODUCTION

This report, which is being submitted to the Department of Agriculture Fisheries and Food (DAFF), is the 1st Annual Technical Report from the Vector Ecology Unit that is located at National University of Ireland, Galway. The main work programme of the Vector Ecology Unit is funded by the Department of Agriculture and Food. This involves a detailed national survey of the composition and seasonal dynamics of species assemblages of biting midges (*Culicoides* species), which may potentially be involved in transmission of bluetongue viral disease (BTV) should there be an outbreak of the disease in Ireland.

<u>The report contains:</u>

1. A short review section that is intended to provide a general introduction to the biology of *Culicoides* and to their role as vectors of bluetongue disease.

2. Details of protocols adopted for the 2007 Irish BTV entomological surveillance programme.

3. Summaries of the results obtained for the period mid-April 2007 to end of December 2007.

4. Details of technical improvements that can be made to the BTV vector surveillance programme for 2008 and a short discussion on research needed to provide a better understanding of the population ecology of Ireland's *Culicoides* species.

THE BIOLOGY OF *CULICOIDES* AND THEIR ROLE AS VECTORS OF BLUETONGUE DISEASE

Biting midges (Diptera: Ceratopogonidae), belonging to the genus *Culicoides*, are a generally under-researched group of insects in Ireland. Some species are familiar as transient ecto-parasitic biters of man and domestic livestock and are generally regarded as nuisance insects that can limit out-door leisure activities, farm work etc. during periods of peak midge abundance. A total of 29 species were included in the most recent check list of Irish *Culicoides* by Ashe *et al.* (1998) and some data are available on the distribution and relative abundances of species as a result of some local and limited scale national surveys undertaken over the past 120 years (Haliday, 1833, Kelly & Walton, 1977, Townley, 1981, Townley *et al*., 1984, Blackith *et al.,* 1991, Geraghty, 2004, McCarthy & Geraghty, 2005). British review papers on the species occurring in these islands, such as those of Campbell and Pelham-Clinton (1960) and Boorman (1986) also give some additional locality records for species recorded in Ireland. Likewise, the research undertaken in Britain on the ecology of common species such as *Culicoides impunctatus*, referred to as the Highland Midge in Scotland, has through popular accounts (e.g. Hendry 2003) and through sales promotional activities for equipment designed to reduce the impact of midges on humans (e.g. < www.midge-free-zone.co.uk>) increased public awareness of the need for better information on this aspect of Ireland's insect biodiversity. Studies on the condition referred to as "sweet itch", have also contributed to an awareness of damaging effects of *Culicoides* bites on horses in Ireland (Mellor and McCaig, 1974, Baker and Quin, 1978, and Anderson *et al.* 1991) and commercial promotion of products for prevention and cure of the condition (e.g. www.sweet-itch.co.uk) serve to highlight the pathology of the "sweet itch" or SSRD (Summer Seasonal Recurrent Dermatitis) that results from hypersensitivity of horses to *Culicoides* bites (Anderson et al., 1991). However, it is the potential role that *Culicoides* may play in transmission of animal diseases, especially Bluetongue Virus (BTV) disease of ruminants that has added urgency to the tasks associated with establishment of national databases on the distribution and population dynamics of Ireland's *Culicoides* species assemblages.

Biting midges (*Culicoides* species) are tiny dipteran flies that belong to the Family Ceratopogonidae. The family has at least 5,476 described species, and according to Tothova (2006), 588 of these occur in Europe. However, only representatives of four genera bite man and domestic animals (Mullen, 2002). However, the best known of these genera is *Culicoides* and this has over 1200 described species which are widely distributed around the world (Mullen, 2002). The life-cycles of many species have been studied, though most knowledge relates to the adult flying stage. The larvae, typically 2-5mm long are simple in form. They are apodous, lacking both thoracic and abdominal appendages and they are also typically apneustic. Thus, lacking open spiracles, they rely mostly on cutaneous respiration. The larvae live in a variety of aquatic or terrestrial habitats and so they may be found in marginal areas of lakes, rivers, and ditches, as well as in wet soils, peat or leaf litter. Some are specialised forms that live in specific micro-habitats such as in dung (e.g. cow pats) or in water filled tree holes. *Culicoides* larvae exploit a variety of food items and exhibit a variety of feeding behaviours. Though many feed on detritus and associated micro-organisms, others are predatory and feed on small soil invertebrates such as free living nematodes. There are typically four larval instars, with the 4^{th} instar being the most long lived stage and many *Culicoides* over-winter as larvae. Thus many species spend 7-8 months of the year as larvae. Larval populations may often be restricted to the upper layers of the wet soil or peat but

vertical movements have been recorded in 24 studies. Larval population densities and survival rates can vary greatly in response to environmental factors and variation in rainfall, soil moisture and soil temperature can thus affect the numbers of adults emerging from particular habitats in different seasons or years. The pupal stage, often with respiratory tubes that are hydrofugic, is generally of short duration. After emergence from the pupal stage, the flying adults may or may not assemble in swarms for mating purposes but such phenomena have not been extensively researched. The adult females are generally biting forms, requiring a blood meal for egg development to occur. However, in some cases they may be autogenic (i.e. able to develop eggs using nutrients retained from the larval phase or using alternative food sources such as nectar). In the case of the well studied Highland Midges (*Culicoides impunctatus*) of Scotland, it has been shown that the 1st gonotrophic cycle of females is regularly autogenic, with relatively small egg batches being involved. Subsequent egg laying requires the female to obtain a blood meal (Hendry, 2003). Autogeny, which may be typical of many lesser known Irish species, can enable significant midge populations to survive in areas such as upland moorland habitats where potential mammalian or avian hosts are sparsely distributed. Some *Culicoides* species are univoltine, having a single annual generation, but most appear to be multivoltine and 2-3 generations per year is common. The occurrence of overlapping generations, and a general lack of quantitative information on individual survival rates and on local dispersal patterns, frequently makes interpretation of seasonal variation in adult midge abundance difficult.

In addition to being recognised as the vectors of bluetongue viral disease of ruminants, *Culicoides* are well known in both medical and veterinary contexts as annoying pests, whose irritable biting habits may lead also to secondary infections or hypersensitivity reactions in man and domestic animals in many parts of the world. They act as vectors for viral diseases such as Oropouche Fever in humans, Epizootic hemorrhagic disease of ruminants, African horse sickness of equines and many other arboviruses (Mellor *et al*., 2000, Mullen, 2002). They also transmit various protozoan pathogens, especially parasites of birds such as *Leucocytozoon*, *Haemoproteus* and *Hepatocystus.* Infection of poultry and of exploited game bird populations (Mullens, 2002, Mullens *et al.* 2006) can have significant economic impacts, though this does not seem to be the case in Ireland. They can also act as vectors of filarial nematode infections, such as tropical species of *Mansonella* in humans and *Onchocerca* in cattle, buffalo and horses. *Onchocerca cervicalis* is the most widely distributed nematode transmitted by *Culicoides* to domestic animals, with horses being its only known host, and this condition is known to occur in Ireland and Britain, as well as elsewhere (McCall *et al.*, 1993, Mullen, 2002).

Bluetongue viral disease is endemic in America, Australia, Asia and Africa. However, by 1999-2000 it had spread into southern Europe (Spain, France, Italy and Greece) and the introduction of the disease to Europe was generally thought to be linked to the spread of *Culicoides imicola* from Africa. The northward extension was thought to be linked to global warming. However, research soon established that indigenous biting midges, in both the *Culicoides obsoletus* and *Culicoides pulicaris* species complexes were involved in BTV disease transmission in southern Europe (Caracappa *et al.,* 2003). This in turn lead to concerns that novel vectors such as these, currently with much more northerly ranges than the established African vector (*C. imicola*) might achieve over several decades of predicted global climate change. As *Culicoides* taxonomy is a particularly difficult topic, due to their small size and the occurrence of morphologically indistinguishable sibling species in widely distributed species complexes, there has been a general reluctance among European entomologists to

become involved in regional surveys of these insects. Limited resources and time constraints, affecting the small number of skilled personnel, resulted in significant problems in establishment of effective vector surveillance and monitoring programmes in most affected European countries. The need for development of new protocols, mainly involving modern molecular methodologies rather than classical morphological taxonomy, was recognised and a series of reports and scientific papers illustrating the potential of such developments have been published in the past decade (e.g. Gomulski *et al*., 2004, 2005, 2006, Pages *et al*., 2005, Perrin *et al*., 2006, Tothova, 2006, Mathieu *et al*., 2007, Nolan *et al.*, 2007). However, most national programmes still rely heavily on the classical taxonomic survey approaches to BTV vector monitoring and surveillance (Anon. 2006, Rawlings *et al*., 2003).

The extensive data being compiled, on *Culicoides* species abundances and distributional patterns, in Europe and elsewhere during regional and national surveys have been used to varying extents for analyses of roles of environmental abiotic factors in the ecology of *Culicoides* (Conte *et al.,* 2007). Use of GIS facilities, with meteorological and information from other national databases, and development of predictive models that may help in development of better BTV control strategies has been attempted in respect of both the southern European BTV epidemics and the more recent outbreaks elsewhere in Europe (Wittman *et al.,* 2001, Capella *et al*., 2003, Tatem *et al*. 2003, Purse *et al.* 2004, Racloz *et al.* 2007). This approach may also be possible in Ireland, or in a wider north-western European context. Likewise, models developed for investigation of dispersal of *Culicoides* in other parts of the world may be applicable to Irish data (Bishop *et al.,* 2000). Though *Culicoides* are poor flyers, they can be transported by prevailing winds as far as 700km (Mellor *et al*., 2000). Thus they can colonise off-shore islands (Kelly and Walton, 1977) and windblown *Culicoides imicola* are thought to have brought BTV from Africa to Sardinia (Pili *et al.*, 2006). This method of dispersal also seems most likely to have been involved in the spread of BTV from continental Europe to Britain in 2007. The capacity of *Culicoides* to survive such dispersal over marine barriers is consequently of considerable concern in Ireland.

The outbreak of BTV (serotype-8) disease in northern European countries (Belgium, Netherlands, Luxemburg, N.E. France, Germany and Poland) in 2006 and the perceived risk that the disease would soon spread to other countries including Britain and Ireland, was a matter of immediate national concern in this country. It was recognised that information on the geographical distribution of the *Culicoides* present in Ireland; their relative abundances; their seasonal patterns in biting activity; their responses to environmental variables, their host specificities and their larval habitat preferences could facilitate BTV risk assessments and might assist in development of control strategies. In response to a request from the Department of Agriculture and Food, Dublin, the Department of Zoology at the National University, Galway established a Vector Ecology Unit in September 2006. This laboratory aimed to begin systematically surveying Ireland's *Culicoides* and to assist in the development of a national BTV vector entomological surveillance programme. In 2007, a three year collaborative programme, involving the Department of Agriculture, the National University of Ireland, Galway and CVERA, in University College Dublin, was initiated. The importance of the 2007 work programme, and the allied serological surveillance that was initiated by the Department of Agriculture and Food in 2006, was highlighted by the subsequent outbreak of BTV in England in August 2007.

THE 2007 *CULICOIDES* SURVEY

Trapping programme and site selection

Consultations with colleagues, discussions with the project steering group and reviews of alternative sampling strategies used in similar surveys, were undertaken in the planning stage of the 2007 *Culicoides* surveillance programme. It was decided that the sampling programme should, in addition to providing data on the distribution and relative abundances of the species of *Culicoides* in Ireland, additionally aim to involve as many District Veterinary Offices (DVO) areas as was reasonably possible in order to establish a comprehensive network of experienced trapping teams. Thus, a training element would be part of the initial year's surveillance and, in the event of there being a need for more intensive surveillance or monitoring in the event of a BTV outbreak in Ireland, these personnel could assist in later years through both active trapping and participation in training programmes for additional personnel that may be required. Accordingly, a wide geographical coverage was considered desirable, rather than focused studies in particular parts of the country or than a survey along pre-selected environmental gradients. It was decided that the sampling would be restricted to farmland, with identifiable herds / land owners and that selection of sites would be done on a random basis. A series of 50 sites were randomly selected, using GIS facilities at CVERA in UCD and with assistance of Mr Guy McGrath, and by reference to available databases on agricultural holdings, this number was then reduced to 35 by exclusion of non-farm sites (urban areas, lake waters, and areas with no recorded herds). All sites were visited, with local DVO staff and support from Mr James O'Keeffe, and in most cases the land-owner's co-operation had previously been secured by the local DVO staff. However, on one occasion a site in Co. Roscommon had to be dropped from the list of potential trapping sites because the lease held by the herd owner was not for the full season and access issues might arise in future. Thus a total of 34 trapping sites remained available for sampling and these were widely distributed and deemed to be well representative of the landscape and farming activities in Ireland. In Fig. 1 the locations and initial site numbers assigned in the random selection process are indicated. These site numbers were retained and used in the 2007 survey on trap record forms, specimen samples, laboratory notes and in the project database.

Following 35 site visits (T.K. Mc Carthy, local DVO staff) a series of specific trap deployment locations were selected as close as possible to the sites indicated by the random selection process. Ordnance Survey maps and aerial photography images of farms, with random points indicated, were especially helpful in discussions with farmers and in selection of effective trapping locations. Minor deviations from the pre-selected points were agreed, to enable traps to be deployed using existing fence poles and tree branches and in a small number of cases special trap deployment structures were constructed from wood or metal, to allow the traps to be deployed in open field locations as were appropriate in respect of the random sampling objectives. During the 2007 trapping season, few difficulties were experienced in respect of the sites and specific trap deployment locations and the success of the trapping programme, described below, reflects in part this initial fieldwork and sampling programme design.

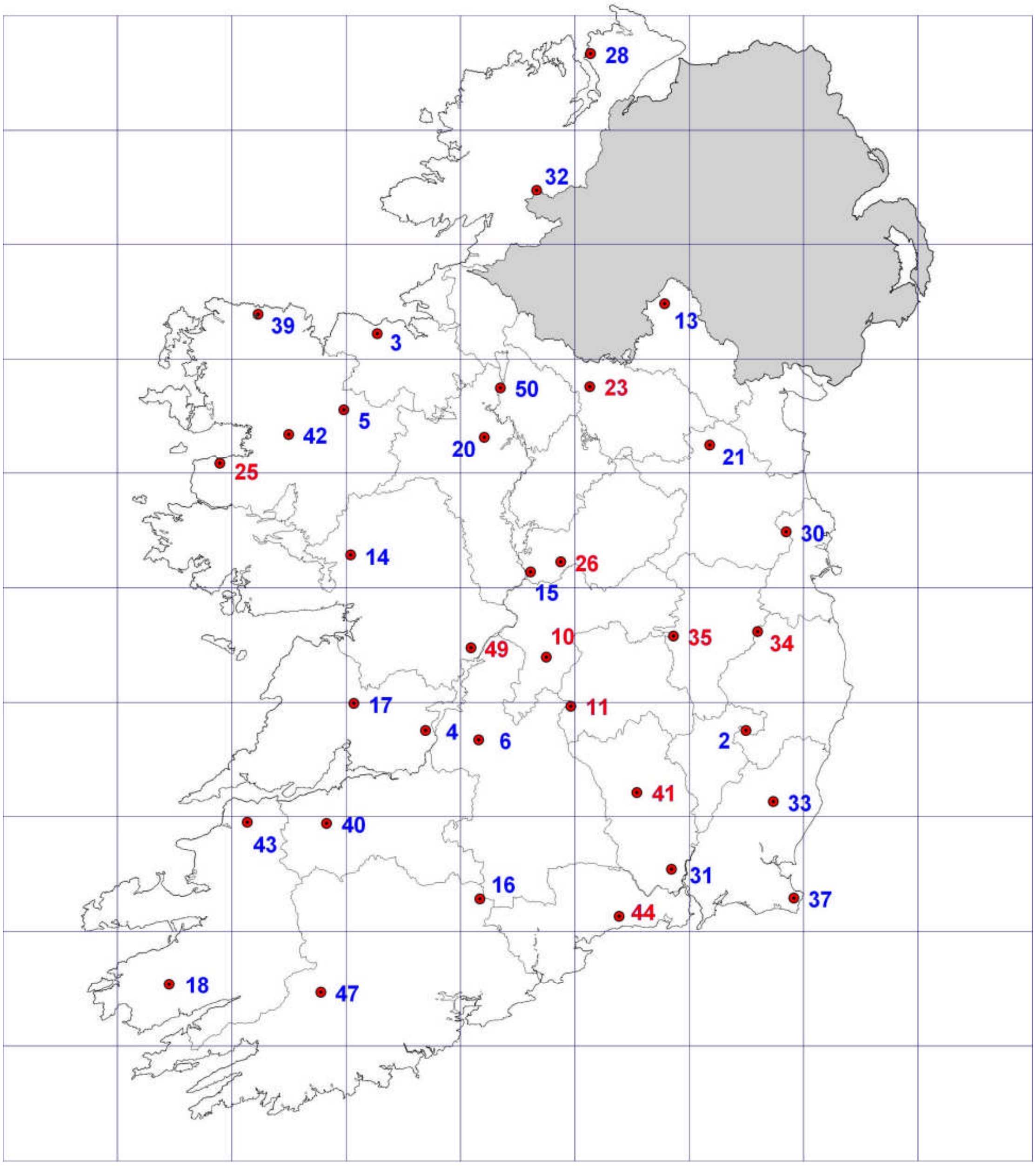

Fig. 1. Location of the 34 sampling sites (with the 10 index sites highlighted in red font).

Trap design

A variety of trap types were used in previous work on *Culicoides* population ecology in Ireland, and by researchers in other countries, and experience gained in 2005 suggested that the best approach to surveying Irish species assemblages of biting midges would involve use of UV light attractant traps. Alternative traps, such as those involving use of CO2 or octenol as attractant chemicals, were found previously to be very species selective or attractive only to female midges. To conform with trapping programmes initiated elsewhere in Europe in BTV vector surveillance / monitoring programmes, it was decided that the system to be routinely used in the 2007 entomological surveillance programme would be the trap type referred to as the South African black light trap. These traps have been used for many years in South African *Culicoides* surveys and their efficiency has been tested under field conditions (Venter *et al.* 1997, Venter & Hermaneides, 2006). One example of this type of trap, on loan from the Natural History Museum London, was used as a model for construction of 40 traps. These were constructed in NUI, Galway workshops and fitted with UV light tubes and Netlon screens. In contrast to traps used in South Africa and because of the decision to kill trapped midges by freezing, a water filled container was not attached to the base of the trap nets in the Irish *Culicoides* sampling programme. Alternative trap types and trapping protocols will be used in some linked research activities. An example of a SA type light trap, being deployed at an exposed area in west Kerry (Site 18) is illustrated in Fig. 2 and the associated cables, 12v battery in a white lidded bucket container can be seen. Trap kits supplied to DVO staff included, battery chargers as well as containers etc. needed for sample retention and transport to the laboratory. The traps were equipped with Nytal mesh screens to exclude moths and other by-catch insects and fine mesh nylon collecting bags for retention of midge samples. The batteries were sealed lead acid (Horizon AM 12 V, 20Ah) type. The traps employed two tubular UV light units (Phillips TL8W actinic BL) and were equipped with 12 V, 0.25A, DC brushless fan motors (Bi-Sonic Technology corp. Model SP 1202512m) to blow the captured midges into the collecting bag. The battery is charged by a “Street Wise” fully automatic 3.5 amp charger.

It is hoped to extend the scope of the survey in future, by compiling more extensive distributional records. Likewise, it is hoped to undertake some local intensive sampling to obtain better information on diel (24 hr) activity cycles, emergence rates and to detect species that may exhibit light avoidance behaviour. For these research objectives, alternative sampling methods described by Service (1993) and other authors will be adopted. The use of car nets, similar to those used by Tothova *et al.* (2005) in biomonitoring Ceratopogonidae, will be evaluated in summer 2008

Fig. 2. DVO Staff person setting trap.

Sampling protocols and field records

The *Culicoides* trapping protocols, detailed in earlier documentation, involved deployment of light traps at the selected trapping sites / locations on a weekly basis from April 2007 onwards. This usually took place on the Tuesday / Wednesday of each week and traps were set overnight, being switched on at about 5.00 pm and emptied at about 9.00am the following morning. The trap operators carefully removed the trap bags, with all the insects within, and brought them back to the DVO where they then chilled them in a freezer or fridge ice compartment, to immobilise them and facilitate their transfer to containers (usually 75 ml) containing ethanol preservative. Sample containers were then labelled, with site and date details, and posted in padded envelopes to the NUI, Galway BTV vector units laboratory for analysis. Trapping teams also supplied, on special forms, details of the weather conditions at the start and finish of each trapping event. They also provided details of trap setting / emptying times and of observations they made on the presence of livestock and potential wildlife hosts for midges. They were also asked to comment on any midge biting activity they experienced and on other field observations they wished to report.

The success of the field programme, as reflected in trapping crew performance is evident from the high proportion of trapping events for which either samples / forms were received at NUI, Galway. The progress of the survey in this regard can be noted in Table 1 in respect of the 38 weeks of the surveillance programme, which included a 2 week start-up phase when some crews voluntarily began trapping. In the following weeks documented in Table 1 a total of 1,100 returns (85%) out of a possible maximum of 1,292 were made by field crews and this resulted in receipt of 981 insect samples i.e. 76% of the total 1292 possible. However, when the two start up weeks, week 52 and the 7 sites which performed particularly badly with regard to returns are excluded the return percentage of 85% increases to 96%. A full (100%) response rate was recorded for 11 of the 34 sites being surveyed. The weekly response rate has not fallen below 85% from April to December and by comparison with other comparable surveys this is considered to be exceptionally good trapping crew performance. Environmental conditions have not been as favourable to such fieldwork in 2007 as in recent years and continued efficient trapping is anticipated in the coming months. Gaps in the data series for some sites are largely accounted for by trap failure, initial difficulties with battery chargers or other minor technical problems.

Some difficulties were encountered in interpretation of handwritten field records and occasional errors in recording environmental conditions. Improvements to forms proposed for 2008 may help ensure better data and deployment of data loggers, (which were secured too late to be of great value for 2007), should also result in better environmental data in future. Observations on wildlife by trap operators do not appear to give a comprehensive summary of the host populations that may be available to *Culicoides* species at the various sites and site visits by research team members appear to be needed to provide a better ecological framework within which differences in *Culicoides* assemblages and their population trends may be analysed.

Table 1. A record of trapping results and/or samples received by the NUIG laboratory from the 34 trapping sites during 9th April (week 15) to 25th December (week 52) 2007 (0=No completed forms or samples received; 1=either or both forms/samples received)

Site

Week 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	
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	15
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	16
17	1	0	1	1	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	17
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
19	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	19
20	1	1	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	20
21	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	21
22	1	1	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	22
23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	23
24	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	1	1	1	1	1	1	1	24
25	1	0	0	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	25
26	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	26
27	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	27
28	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	28
29	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	29
30	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	1	30
31	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	1	1	1	31
32	1	1	1	1	0	0	1	0	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	32
33	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	33
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
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
36	1	1	1	1	1	0	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	36
37	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	37
38	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	38
39	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	39
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
41	1	1	1	1	1	0	1	1	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	41
42	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	1	1	1	42
43	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	43
44	1	1	1	1	1	0	1	1	1	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	44
45	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	0	1	1	1	0	1	1	1	1	1	1	0	1	1	45
46	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	46
47	1	1	1	1	1	0	1	1	1	0	1	1	1	1	0	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	47
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	0	1	1	48
49	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	49
50	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	50
51	1	0	1	1	1	0	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	0	1	1	51
52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	52

Transport and initial laboratory processing of samples

The posting of samples from DVO's to the BTV vector ecology team in NUI, Galway presented a number of problems and a higher (8.5%) than expected proportion were damaged in the post. In general the specimens could be retrieved (100%) and included in the laboratory analyses. However, in several cases the labelling on the containers was obscured if written using a ball-point pen rather than a recommended pencil. Also, placing the field record forms with the samples and sometimes along with them in sealed plastic bags, postal damage resulted in some loss of environmental and trap operational data.

All samples received at the laboratory are logged electronically on arrival, the contents and 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, which are retained in a separate archival system for possible identification in future, include a variety of other Diptera families as well as small Hymenoptera and representatives of several other insect orders. This process has proven to be extremely time consuming and may need to be reviewed.

A total of 220,347 *Culicoides* have been counted in the 2007 samples examined to date and 91,453 have been identified to species level. It is estimated that, including samples counted by sub-sampling, the total number of *Culicoides* trapped during 2007 was approximately half a million. 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. 42 samples have been sub-sampled based on sub-

sampling methods developed by Van Ark & Meiswinkel, (1992). Of these 42 samples, 23 were counted only and 19 samples were identified using the above-mentioned sub-sampling method. 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.

Database management

A dedicated BTV vector database was created using Microsoft Access© 2000 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.

Species recorded

A checklist of the 29 species of Culicoides recorded from Ireland is presented in Table 2, in which the 21 species recorded in the 2007 surveillance programme are highlighted. Of the 21 species recorded so far, 3 species (*C. stigma, C. heliophilus and C. parroti*) have yet to be confirmed. Considerable variation in species richness (number of species) present in individual samples has been observed, reflecting both within site and between site variation. Likewise, the relationship of observed species richness to sample size, numbers of samples analysed per site, and seasonal patterns will need to be examined. The number of species varied from site to site ranging from 4 to 14 (mean 10).

Table 2. Checklist of Irish *Culicoides* species with those recorded in the 2007 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)	***achrayl* KETTLE & LAWSON***
	fascipennis* (STAEGER)
	pallidicornis KIEFFER

Taxonomic problems

The complex and difficult taxonomy of *Culicoides* midges is well known and this has been a major limitation in researching these species. Molecular taxonomy has provided additional information in the past decade but the identification of species, or even species complexes, is still a difficult and at times a seemingly impossible task. Training of student summer research assistants and the longer term new members of the team has greatly delayed the progress of the 2007 surveillance programme. Such problems will continue to be a feature of future years work. In 2007 a visit by Dr R. Meiswinkle, a leading *Culicoides* taxonomist, was of great value in this regard. Subsequent to his visit it proved necessary to re-examine a significant number of samples and this also delayed sample processing. However, the main constraints on taxonomic work is the large sample size typical of the SA light trap survey and the difficulty in distinguishing certain species. Within the Obsoletus Complex there is difficulty in identifying *C. obsoletus, C. scoticus* and female *C. chiopterus*. High quality digital photography, especially of wing patterns is proving helpful and also this means that electronic records of features such as wing patterns used in species identifications is possible (Fig. 3).

Fig 3. *C. pulicaris* (site 16) and *C. festivipennis* (site 21).

Relative abundances of species

The relative abundances of the 21 species so far identified have been observed to vary greatly both between sites and between weeks at particular sites. Dominance of samples by particular species was a regular feature in the 2007 survey results, though the species in question often seemed to vary in rather unpredictable patterns.

Overall abundance of *Culicoides* at 19 study sites, illustrated in Fig. 4, highlights the very high temporal variability in midge numbers over the study period (weeks 17–52 of 2007). A broad seasonal trend is evident, with low numbers of midges in spring/early summer (weeks 17–20, <5000) and autumn/early winter (weeks 39–48, <5000) and no midges recorded after week 49. The greatest densities of *Culicoides* occurred from weeks 23–38 (5/06/07–18/09/07).

During this growing season, however, *Culicoides* numbers varied greatly from week to week. A prominent feature of the data in Fig. 4 is the sudden peaks and troughs that occurred in midge numbers. Notable sudden drops in midge densities occurred on weeks 22, 26, 29 and 33. Peaks densities (>15,000) were recorded on weeks 21, 24, 25, 27 (late-May and June) and later on in the growing season on weeks 36, 37, 38 (September). Fig. 4 also compares the patterns of *Culicoides* abundance at the 10 index sites with that at the additional 9 non-index sites for which all weekly counts are completed. It is evident that patterns of *Culicoides* abundances at the two sets of sites were very similar, showing the same broad seasonal trend interspersed with sudden peaks and drops in midge numbers.

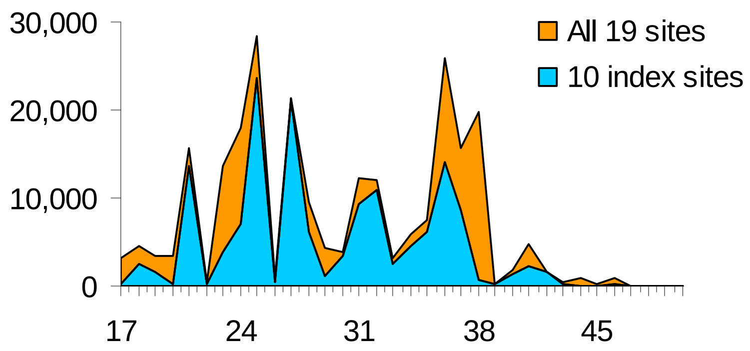

Fig. 4. Temporal variation in overall *Culicoides* abundance (April–Dec 2007). Data shown is the cumulative of the 19 study sites for which data analysis is completed.

Temporal variability in overall *Culicoides* abundance, illustrated in Fig. 4, is explained by the highly variable patterns of midge abundance at individual study sites Fig. 5. In examining midge catches on a site-by-site basis, it is evident that midge numbers showed both high spatial and high temporal heterogeneity. Peaks in abundances did not coincide between sites but occurred in a sporadic manner. Many sites exhibited numerous large peaks in *Culicoides* abundance dispersed throughout the growing season (e.g. sites 2, 15, 25, 26, 35, 44), while other sites recorded just a single large, sudden spike in midge numbers (e.g. sites 4, 5, 10 and 34).

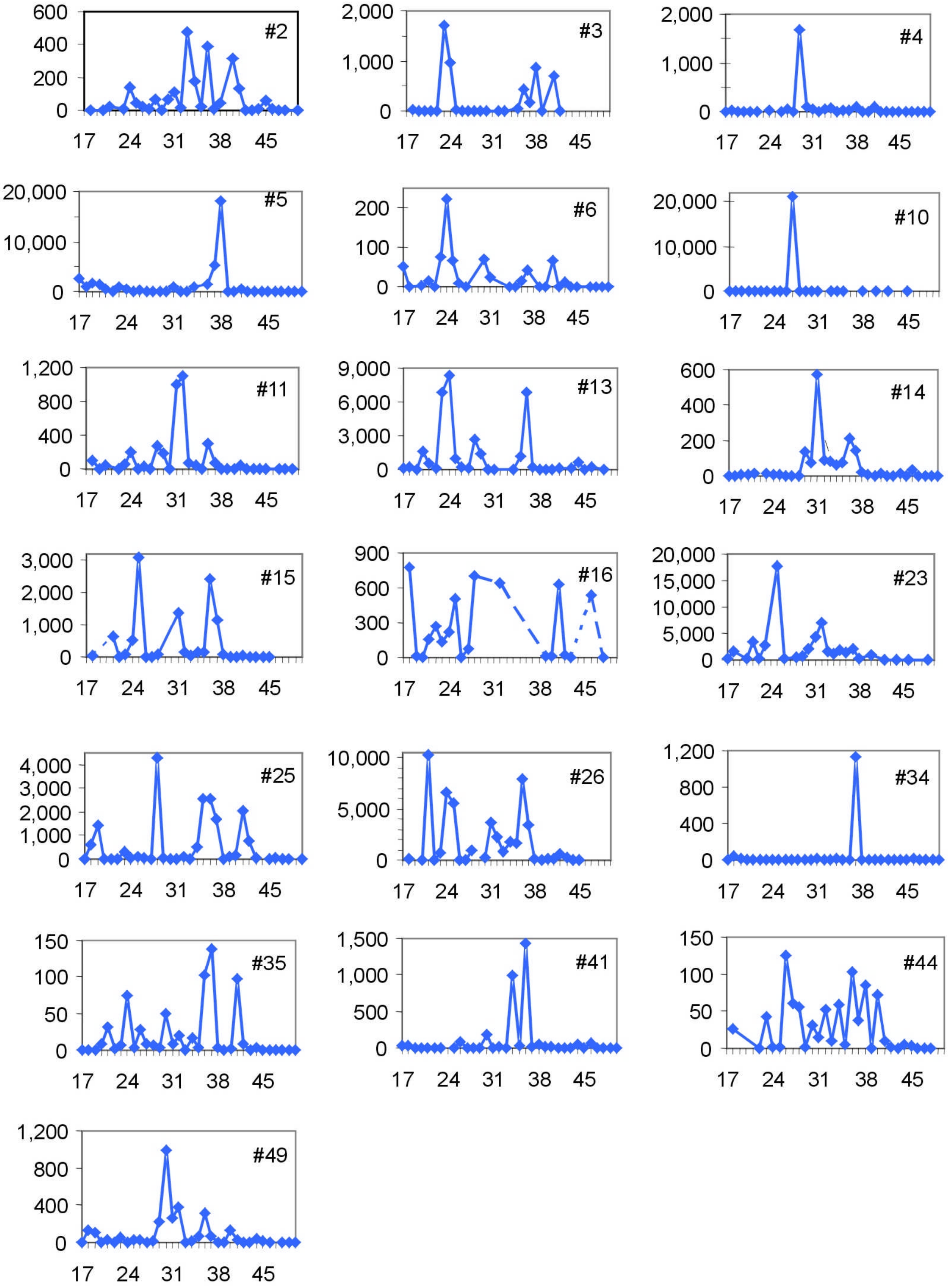

Fig. 5. Temporal variation in *Culicoides* abundance at the 19 sampling sites for which quantitative data analysis is complete (broken lines indicates dates for which no samples were received).

As a consequence of the large inter-site variability in midge distribution, total *Culicoides* numbers at individual sites likewise varied greatly (Table 3). Of the 19 sites for which quantitative analysis is completed, three sites can be classed as 'low density' sites, having < 1,000 midges (sites 6, 35 and 44). Some 8 sites recoded 'moderate densities' (1,000–10,000), 2 sites had 'high densities' (10,000–20,000) and 5 sites had 'very high densities' (>20,000).

Table 3. Total *Culicoides* numbers for 19 sites.

Site	N
#2	2,141
#3	4,924
#4	2,360
#5	36,315
#6	683
#10♦	21,199
#11♦	3,501
#13	32,308
#14	1,584
#15	10,059
#16	4,709
#23♦	49,976
#25♦	17,499
#26♦	50,054
#34♦	1,275
#35♦	615
#41♦	3,048
#44♦	801
#49♦	2,903

♦ Index sites

Examining the geographical distribution of total *Culicoides* abundances at the various sampling sites (Fig. 6), no striking regional pattern in midge densities is evident. The lack of a regional pattern implies that the localised characteristics of individual study sites are a greater determinant of midge abundance than broad regional characteristics.

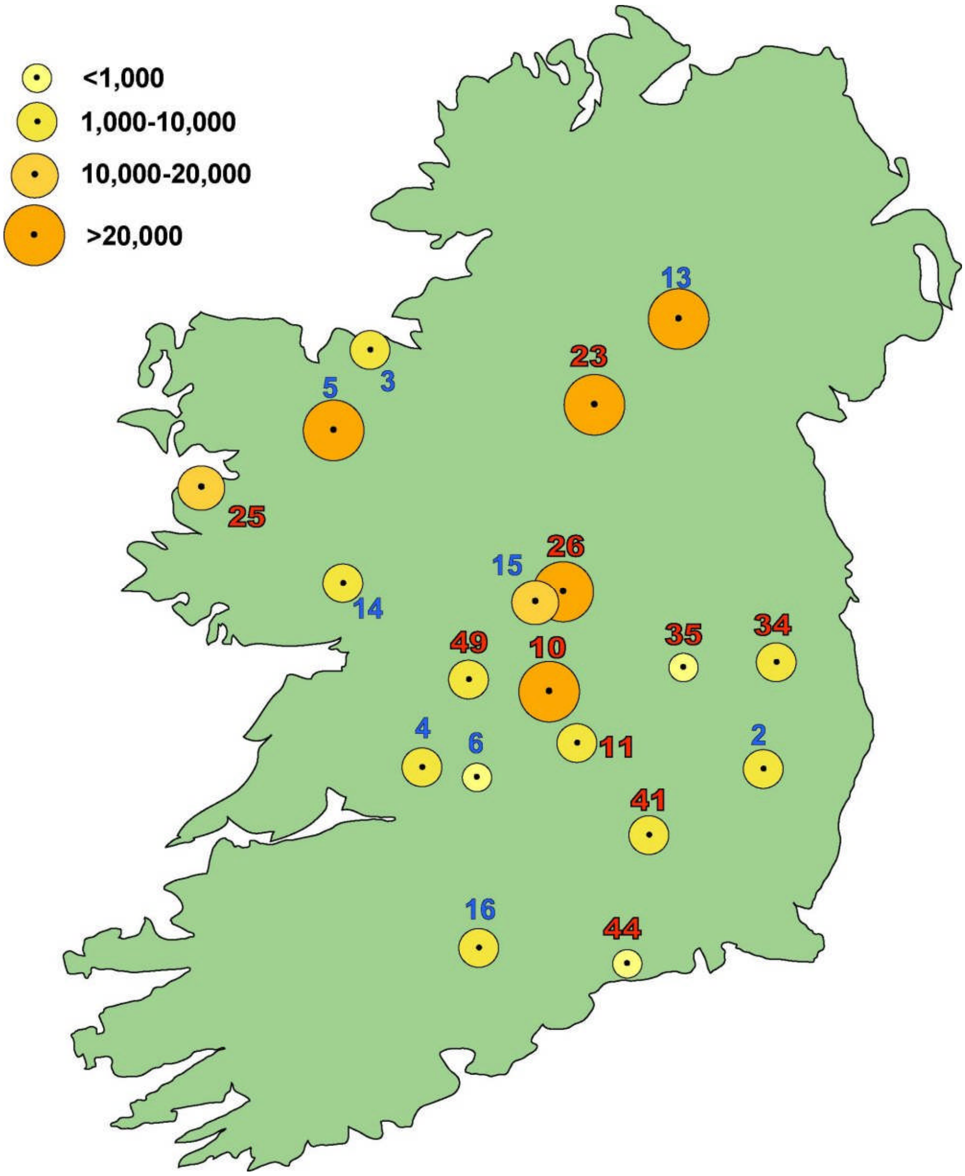

Fig. 6. Total *Culicoides* abundance at the 19 study sites.

To interpret and explain patterns of *Culicoides* abundance, key environmental variables, such as weather conditions, must be considered. Fig. 7 displays the weather scores for precipitation, cloud cover and wind speed which were recorded by trappers in the field over the course of the study period. Data are shown for days on which traps were both set and removed.

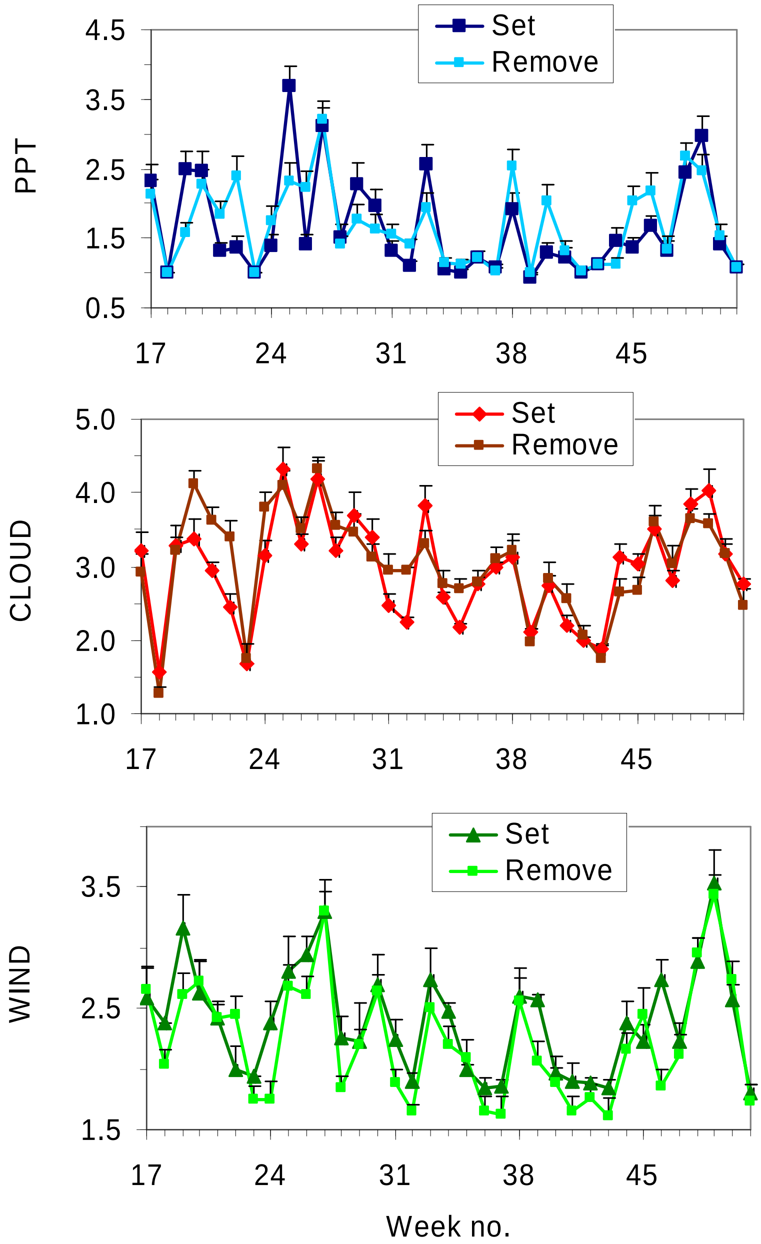

Fig. 7. Weather score data for sampling period (weeks 17-52, 2007). Data shown are the average scores of all 34 sites for days on which traps were set and removed; error bars show the standard error

Temporal weather patterns are evident in Fig. 7, so that the data on ‘set’ and ‘remove’ days are very similar for all three variables. It is also apparent that there was very good agreement between weekly weather scores from the different sites for all three weather variables, as evidenced by the small error bars in Fig. 7.

The weather score data recorded by trappers in the field is compared with daily Met Éireann data for Shannon in Fig. 8.

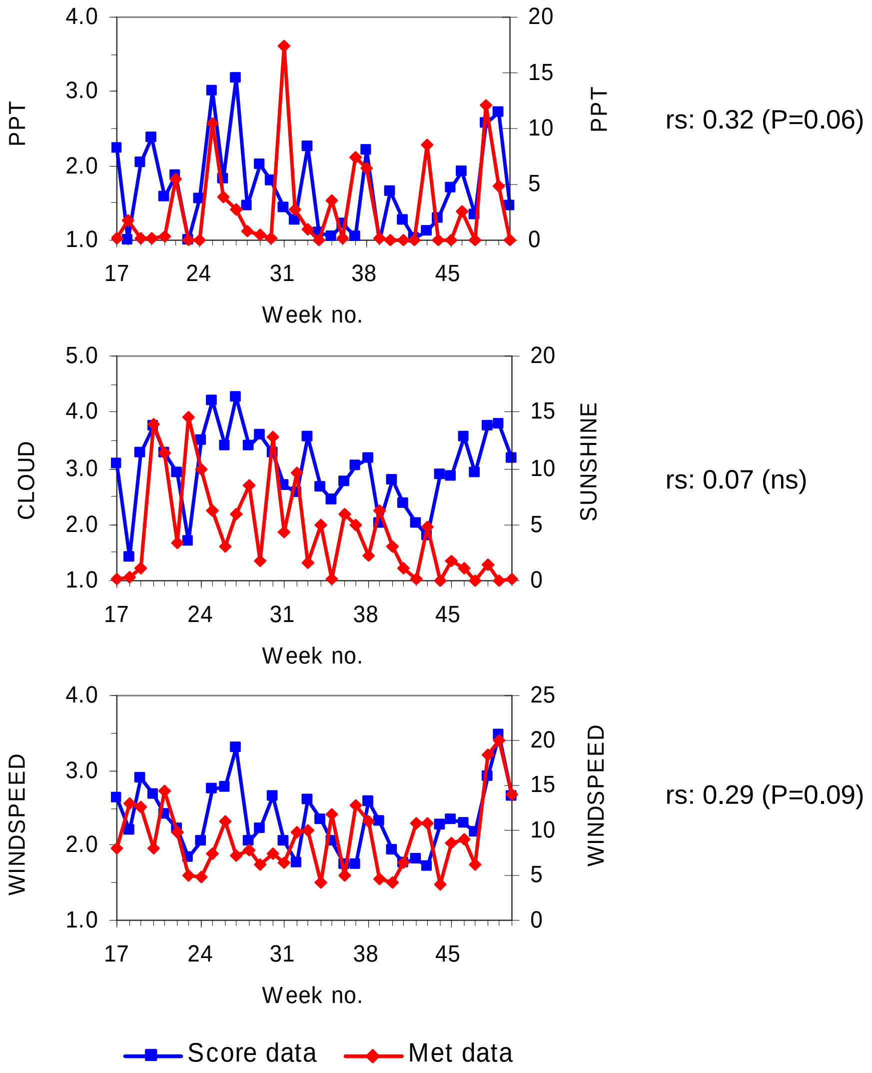

Fig. 8. Comparison of score data (from site reports) and daily Met Éireann data for Shannon.

In general, the weather score data recorded in the field correlated only moderately well with the daily Met Éireann data for Shannon (Fig. 8 and Table 4). It is highly probable that the weakly significant ($P<0.1$) correlation between the two rainfall datasets reflects the highly localised and sporadic nature of rainfall events in Ireland, whereas the Met Éireann data presented are for one site only (Shannon). Bias may also have been introduced into the score data by trapper behaviour, selectively determining the weather conditions in which records were taken. A more complete analysis involving data from all Irish met stations will be undertaken when the full entomological data set is available (i.e. all 34 sites fully counted).

Table 4. Correlation matrix showing the relationship between weather score data and daily Met Éireann data for Shannon

	SCORE PPT	SCORE CLOUD	SCORE WIND	MET RAIN	MET MAX TEMP	MET MIN TEMP	MET SUNSHINE	MET WIND GUST	MET WIND SPEED	MET GRASS MIN T
SCORE PPT	1.000									
	.									
SCORE CLOUD	.877(***)	1.000								
	.000	.								
SCORE WIND	.759(***)	.708(***)	1.000							
	.000	.000	.							
MET RAIN	.322(*)	.291(*)	.169	1.000						
	.064	.095	.340	.						
MET MAX TEMP	-.266	-.174	-.361(**)	-.004	1.000					
	.129	.324	.036	.981	.					
MET MIN TEMP	-.328(*)	-.347(**)	-.378(**)	.103	.618(**)	1.000				
	.058	.045	.027	.562	.000	.				
MET SUNSHINE	-.058	.074	-.103	.081	.608(***)	.018	1.000			
	.744	.677	.561	.650	.000	.922	.			
MET WIND GUST	.199	.197	.205	.363(**)	-.353(**)	-.412(**)	-.052	1.000		
	.259	.265	.245	.035	.041	.016	.768	.		
MET WIND SPEED	.212	.194	.292(*)	.501(***)	-.451(***)	-.163	-.288(*)	.500(***)	1.000	
	.229	.272	.094	.003	.007	.357	.099	.003	.	
MET GRASS MIN T	-.362(**)	-.390(**)	-.368(**)	.168	.541(***)	.911(***)	-.015	-.423(**)	-.157	1.000
	.036	.023	.032	.343	.001	.000	.932	.013	.375	.

*** Correlation is significant at the 0.01 level, ** Correlation is significant at the 0.05 level, * Correlation is significant at the 0.1 level (all 2-tailed)

The score data for cloud cover correlated moderately well with the Met Éireann sunshine data during the early and middle parts of the sampling period, but poorly later on. This trend reflects the important influence of daylight hours on the Met Éireann sunshine data, an element which does not affect the cloud cover score data.

Finally, the wind speed datasets correlated reasonably well over parts of the sampling period, while at other times peaks in wind speed occurred in just one of the datasets (overall, P<0.1). Again, this trend may reflect the variability of wind speeds throughout Ireland and the fact that the Met Éireann data considered here are for Shannon only.

It is noteworthy that the score data for precipitation, cloud cover and wind speed all correlated highly significantly (P<0.001), again highlighting the consistency between the estimates of different trappers in the field As would be expected, the Met Éireann data also show very strong correlations amongst several variables, such as maximum temperature and sunshine (P<0.001) and rainfall and wind speed (P<0.01).

Table 5 examines the influence of the various weather variables and altitude on *Culicoides* abundance at both the 19 sites for which quantitative data analysis are completed and the 10 index sites.

Table 5. Correlation Matrix showing relationship between *Culicoides* abundance and environmental variables. *Culicoides* abundance is log-transformed abundance at 19 sites and 10 index sites. Meteorological variables are Shannon Met data. Correlations coefficients = Spearman coefficients (rs)

	LOG N (19 sites)	LOG N (index sites)
LOG N (index)	.908(***)	
	P=0.000	
SCORE PPT	-.079	-.173
	ns	ns
SCORE CLOUD	.039	-.035
	ns	ns
SCORE WIND	-.208	-.258
	ns	ns
MET RAIN	.070	.131
	ns	ns
MET MAX TEMP	.560(***)	.550(***)
	P=0.001	P=0.001
MET MIN TEMP	.353(**)	.410(**)
	P=0.044	P=0.018
MET SUNSHINE	.659(***)	.571(***)
	P=0.000	P=0.001
MET WIND GUST	-.300(*)	-.252
	P=0.090	ns
MET WIND SPEED	-.350(**)	-.350(**)
	P=0.046	P=0.046
MET GRASS MIN T	.342(*)	.386(**)
	P=0.051	P=0.027
ALTITUDE	-.140	-.012
	ns	ns

*** Correlation is significant at the 0.01 level,
** Correlation is significant at the 0.05 level,
* Correlation is significant at the 0.1 level (all 2-tailed)

The strong correlation (P<0.001) between midge abundances at the 10 index sites and the 19 sites reaffirms that the abundance data for the index sites are statistically representative of the overall dataset. In terms of the primary environmental determinants of midge abundance in Ireland, the data in Table 5 indicate that maximum air temperature and sunshine are very important (P<0.01), minimum air temperature and wind speed are moderately important (P<0.05) and wind gusts and minimum grass temperature are of somewhat importance (P<0.1) (all Met Éireann data for Shannon). The strong relationships observed are explained to a large extent by the seasonality of midge abundance, with high numbers being associated with the warmer parts of the year. The significant inverse relationships between midge abundance and both wind speed and wind gusts support expectations based on published studies and personal observations relating midge activity to times of low wind. No significant relationships were found between the weather score data for any of the three variables and midge abundance, indicating that the Met Éireann data is a superior indicator of trends in *Culicoides* abundance. Finally, no relationship was found between the altitude of individual study sites and their overall *Culicoides* abundance. The altitude of the sites ranged from 25-365m.

Culicoides composition

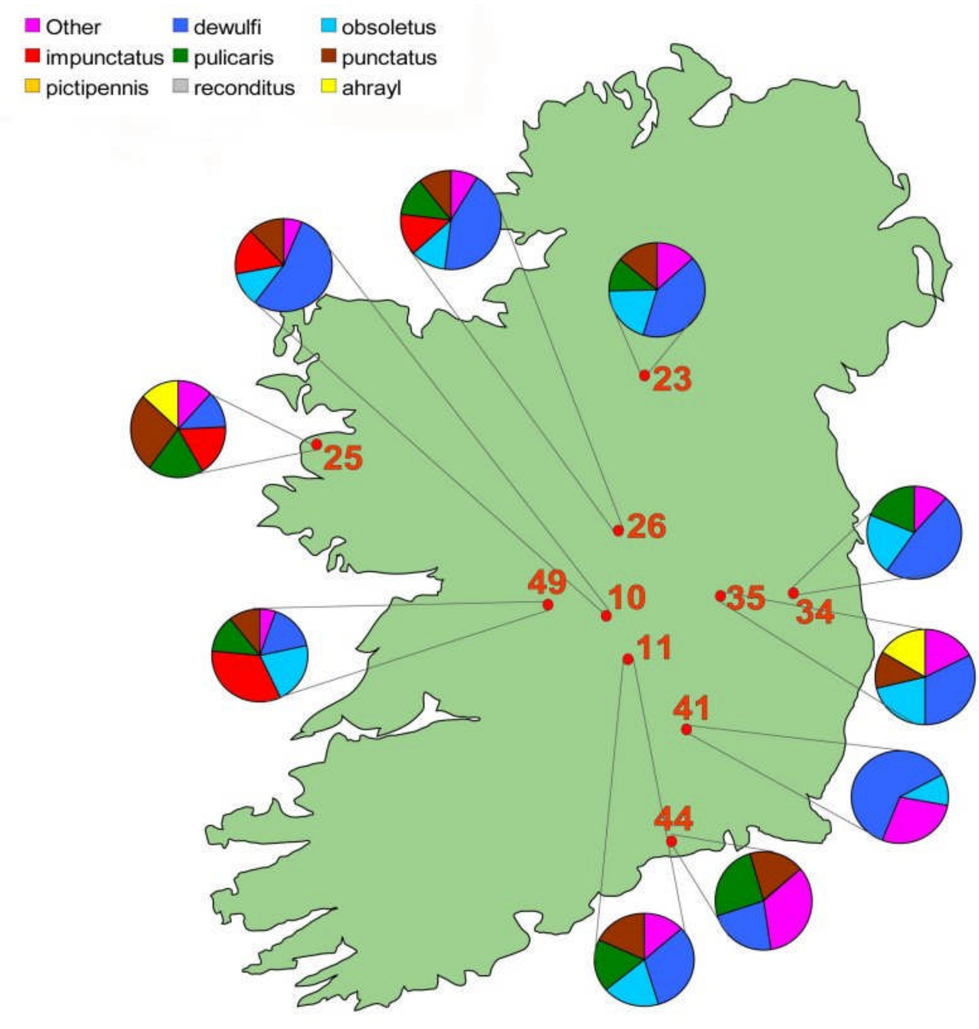

Fig. 9. Dominant *Culicoides* species at the 10 index sites.

A total of seven *Culicoides* species were present at all 10 index sites: *C. dewulfi*, *C. obsoletus*, *C. pulicaris*, *C. punctatus*, *C. festivipennis*, *C. reconditus*, and *C. achrayl*. (Table 6). Site 44 had the highest species richness of the index sites, recording 15 species of *Culicoides*. The sites with the lowest species richness were sites 10 and 34. Rare species, recorded at just a single or two sites, comprised *C. delta*, *C. parroti*, *C. stigma*, *C. duddingstoni* and *C. heliophilus*.

Table 6. Species richness (R), species diversity (D) and relative abundance of species at the 10 index sites (* = 1–99, ** = 100–999, *** = 1,000–9,999, **** = 9,999–10,000, ***** = 10,001-20,000).

	#10	#11	#23	#25	#26	#34	#35	#41	#44	#49	TOTAL
chiopterus	*	**	***	**	***	**		***	*	*	9
dewulfi	*****	****	****	****	****	***	***	****	***	***	10
obsoletus	****	***	****	****	****	***	***	***	**	***	10
circumscriptus		*		*	*			***	**		5
delta		*					*				2
grisescens				**	*		*		*	*	5
impunctatus	****	*	**	****	****	*	*		*	***	9
pulicaris	***	***	****	****	****	***	**	***	***	***	10
punctatus	****	***	****	****	****	***	**	***	***	***	10
nubeculosus					***			**	**		3
parroti		*							*		2
stigma								*			1
duddingstoni			*								1
festivipennis	*	*	****	***	***	*	**	*	**	**	10
heliophilus				*							1
pictipennis	*	*	***	**	***	*	*		*	*	9
reconditus	**	***	***	**	***	*	**	*	**	**	10
achrayl	***	***	****	****	****	**	***	***	**	***	10
unknown		*							*		2
Spp. R	10	14	11	13	13	10	11	11	15	11	
Spp. D[1]	0.747	0.657	0.70	0.631	0.663	0.790	0.681	0.645	0.703	0.691	

[1]D = Simpson's Diversity Index, where 1 = maximum diversity and 0 = no diversity.

Fig. 10 compares the temporal variation in *Culicoides* abundance at the 10 index sites with variations in species diversity (Simpson's Index). It is evident that slumps in *Culicoides* numbers over the course of the study period were associated with sharp declines in species diversity. Likewise, high *Culicoides* abundances were accompanied by high levels of species diversity.

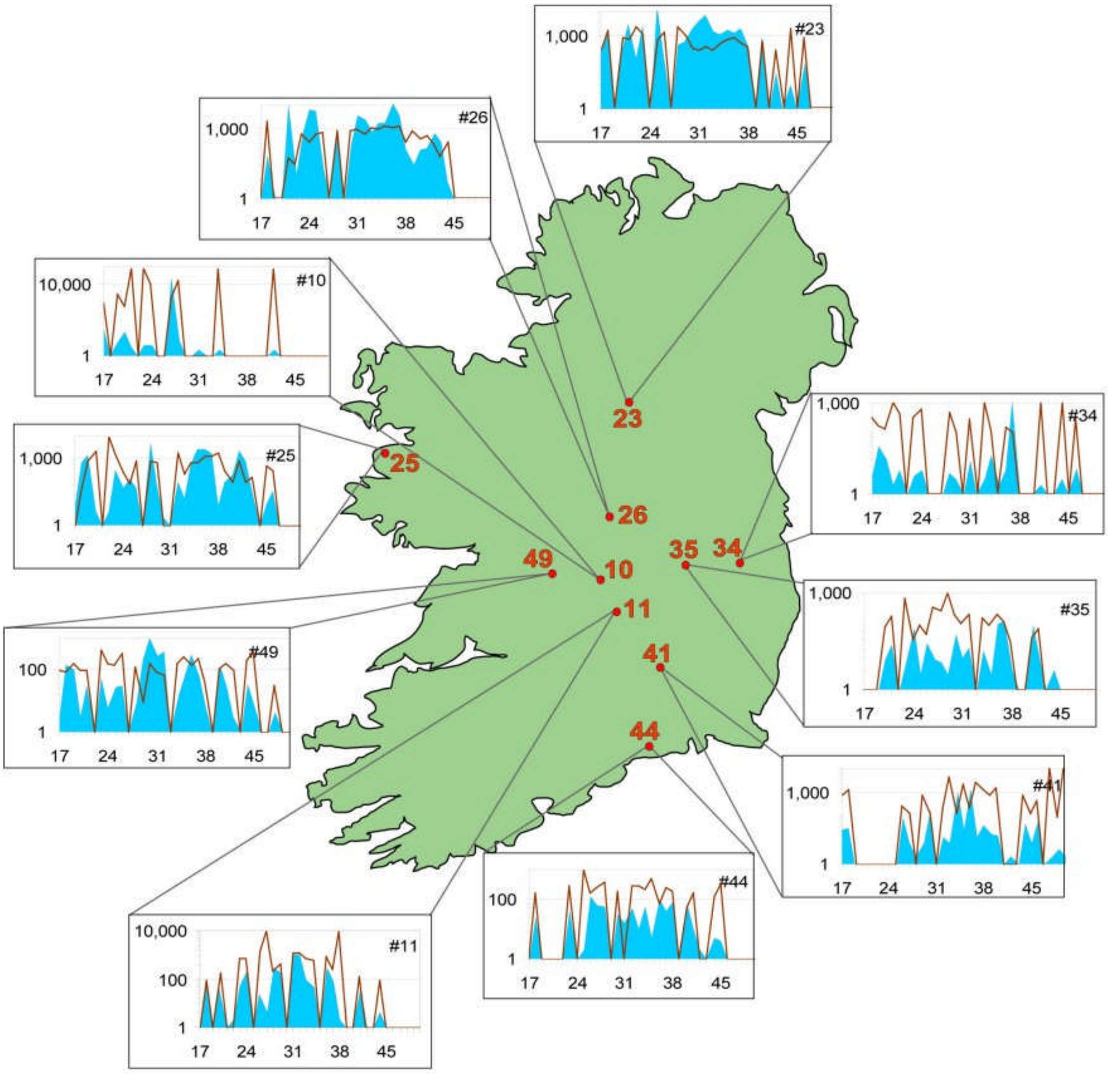

Fig. 10. Temporal variation in *Culicoides* abundance (log-transformed) and diversity (Simpson's D, shown as brown line) at the 10 index sites during 2007 (weeks 17-50).

The influence of environmental variables on *Culicoides* species richness and species diversity at the 10 index sites is illustrated in Table 7. It is evident that *Culicoides* abundance correlated positively with species richness and species diversity. Like *Culicoides* abundance, species richness and species diversity were strongly positively related to temperature and sunshine, and strongly inversely related to wind speed and wind gusts. Altitude affected only *Culicoides* species richness.

Table 7. Influence of weather variables on species richness and species diversity at the 10 index sites.

	Spp. R (index)	Spp. D (index)
Log N (19 sites)	.650(***)	.366(**)
	P=0.000	P=0.039
Log N (index)	.690(***)	.325(*)
	P=0.000	P=0.069
SCORE PPT	-.407(**)	.078
	P=0.019	ns
SCORE CLOUD	-.264	.232
	ns	ns
SCORE WIND	-.274	.167
	.ns	ns
MET RAIN	-.048	-.0.65
	ns	ns
MET MAX T	.660(***)	.161
	P=0.000	ns
MET MIN T	.434(**)	.002
	P=0.012	ns
MET SUNSHINE	.597(***)	.428(**)
	P=0.000	P=0.015
MET WIND GUST	-.449(***)	.135
	P=0.009	ns
MET WIND SPEED	-.391(**)	.083
	P=0.025	ns
MET GRASS MIN T	.322	-.010
	P=0.068(*)	ns
ALTITUDE	-.384(*)	-.262
	P=0.100	ns

*** Correlation is significant at the 0.01 level,
** Correlation is significant at the 0.05 level,
* Correlation is significant at the 0.1 level (All 2-tailed).

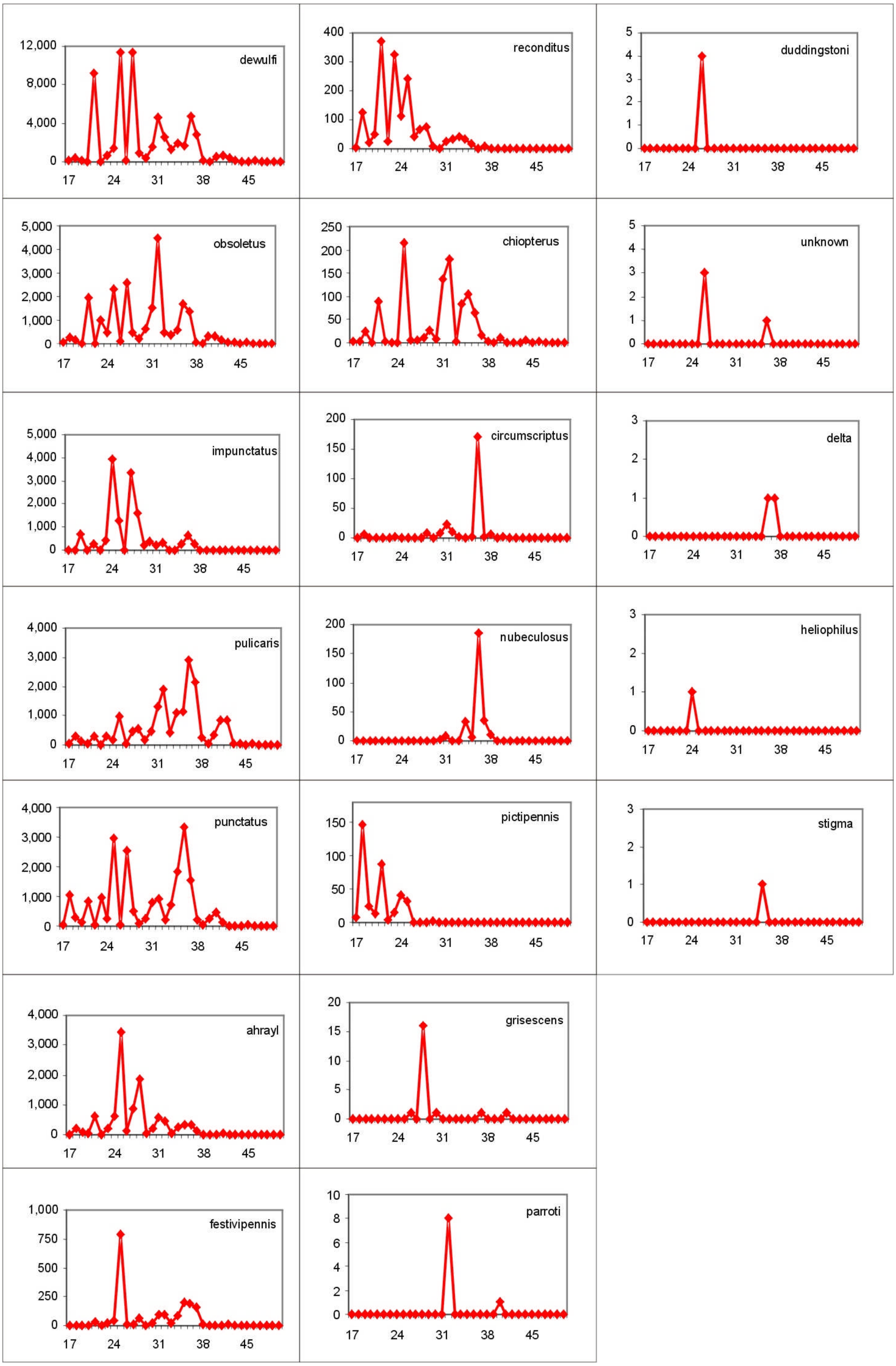

Fig. 11 illustrates the temporal variation in abundance of the 19 different *Culicoides* species recorded at the 10 index sites.

It is evident from Figure 3.7 that individual species showed very different patterns of abundance. Certain species showed peaks early on in the growing season, such as *C. pictipennis* (weeks 18–25), *C. reconditus* (weeks 18–28), *C. dewulfi* (weeks 21–37) and *C. achrayl* (weeks 21–36). Other species showed peak abundances later on in the growing season, such as C. pulicaris (weeks 25–42). Some species exhibited a single peak in abundance, notably *C. circumscriptus* and *C. nubeculosus*, as well as many of the rarer species such as *C. grisescens* and *C. parroti*.

Composition of Species Assemblages

Species identifications or determinations to species complex level were undertaken for all available samples in respect of the 10 index sites (Fig. 12) randomly selected for full taxonomic analyses. In addition, and with a view to providing an overview of the variation in species composition in the catches being obtained at the full compliment (N=34) of sites, collections from selected samples were fully analysed for all sites. In all, this supplementary taxonomic analysis involved 407 samples. The taxonomic analysis involved direct identifications of 229,536 specimens to species/species complex level. 148,052 of these were the specimens from the 10 index sites. The additional 81,484 represented the combined numbers for supplementary analysis. In 2007, 42 samples were sub-sampled with approximately one eighth (13,182) of the total (105,456) being actually identified microscopically. The results of the studies on species composition of the two sets of samples were compared by reference to the relative numbers of the various species present in the respective sample series. In Fig. 12 these data are summarised and the data are presented, following log transformation, in decreasing order of abundance. Linear and polynomial regression lines are also shown, which are very highly significant in all cases, to highlight the similarity of the cumulative results for the 10 index sites relative to the full data set for all 34 sites. These analyses illustrate how representative the index sites are of the overall national survey and they help justify the particular attention being given to the results for the index sites. In Fig. 13 the relationship between species abundance and geographical distribution of Irish *Culicoides* are illustrated. As can be seen abundance levels (log transformed data) for the 19 species involved correlate closely with the number of sampling sites in which they were recorded during 2007. Some minor exceptions e.g. *C. nubeculosus* and *C. circumscriptus* may be attributable to the specificity of their habitat requirements. The species that were rarely recorded during the 2007 BTV vector survey appear to be generally rare in Ireland and some may require specialised sampling protocols to determine their distributional and abundance patterns.

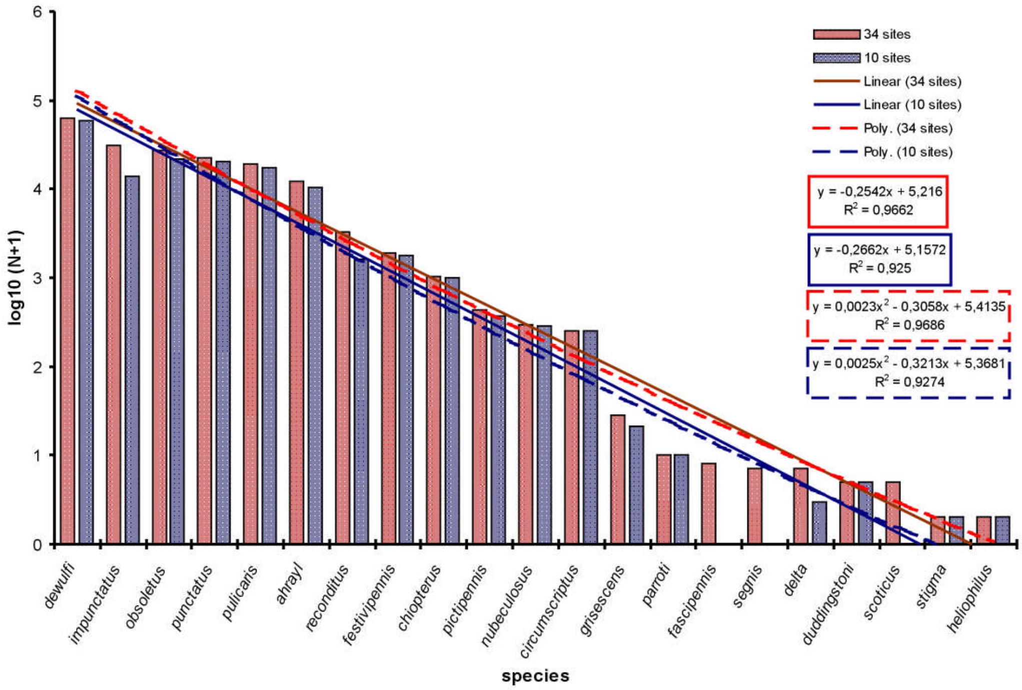

Fig. 12. Species composition for 34 sites versus 10 index sites.

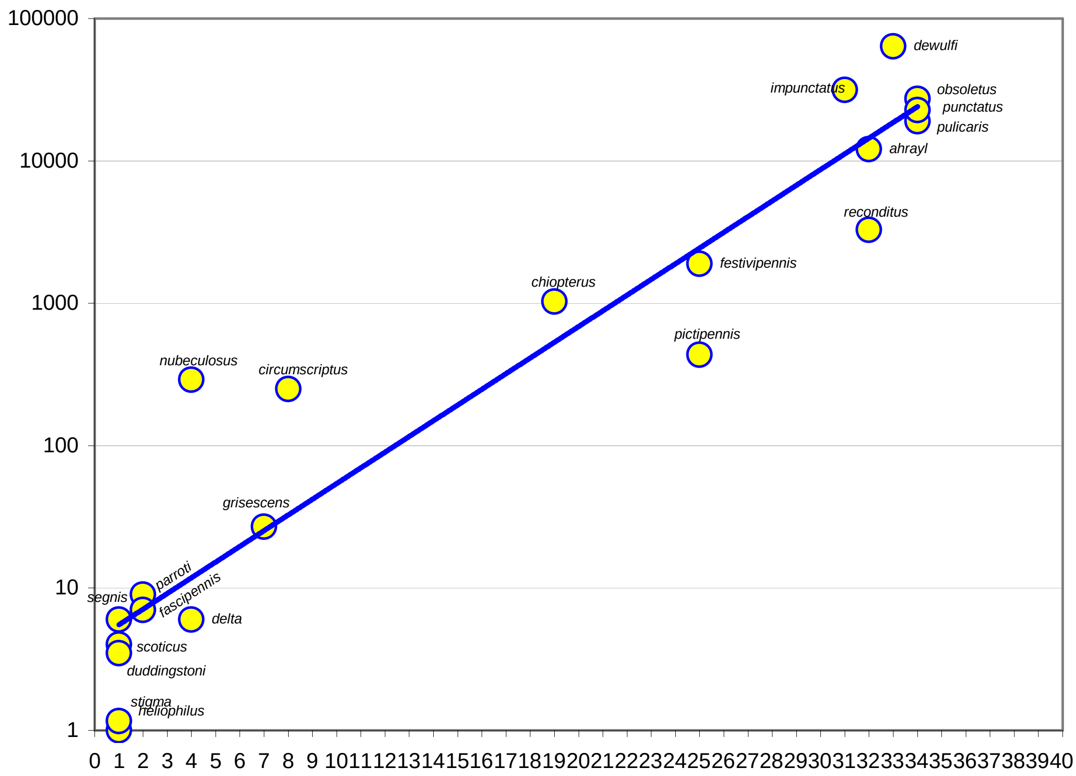

Fig. 13. Illustration of the relationship between species abundance and geographical distribution of Irish *Culicoides*.

To explore the relationships between species as indicated by the composition of the species assemblages at index sites (all data for weeks 17-50 combined) a community matrix was subjected to multivariate analyses. This involved data for the 12 most abundant species only. Initially, cluster analysis (Fig. 14 and Fig. 15) was undertaken using log-transformed data. Though clear groupings of species were suggested by the cluster analysis, it soon became apparent that this largely reflected the abundance/distribution gradients illustrated in Fig. 13. Likewise, the cluster analysis of the sites seemed mostly to reflect a similar trend in overall midge abundance in the site series. Principal Component Analysis (PCA) was also not particularly informative as a means of identifying ecologically meaningful groupings of either sites or species. The results Fig. 16 showed that the first 2 axes (Factor 1-Factor 2) accounted for a very high proportion of the variation (80.51%). However, axis 1 was strongly correlated with abundance levels for the species and sites. A more comprehensive analysis of these data, together with quantitative data on species composition from other sites, will be undertaken at a later date and this may provide better insights into the community ecology of Irish *Culicoides*.

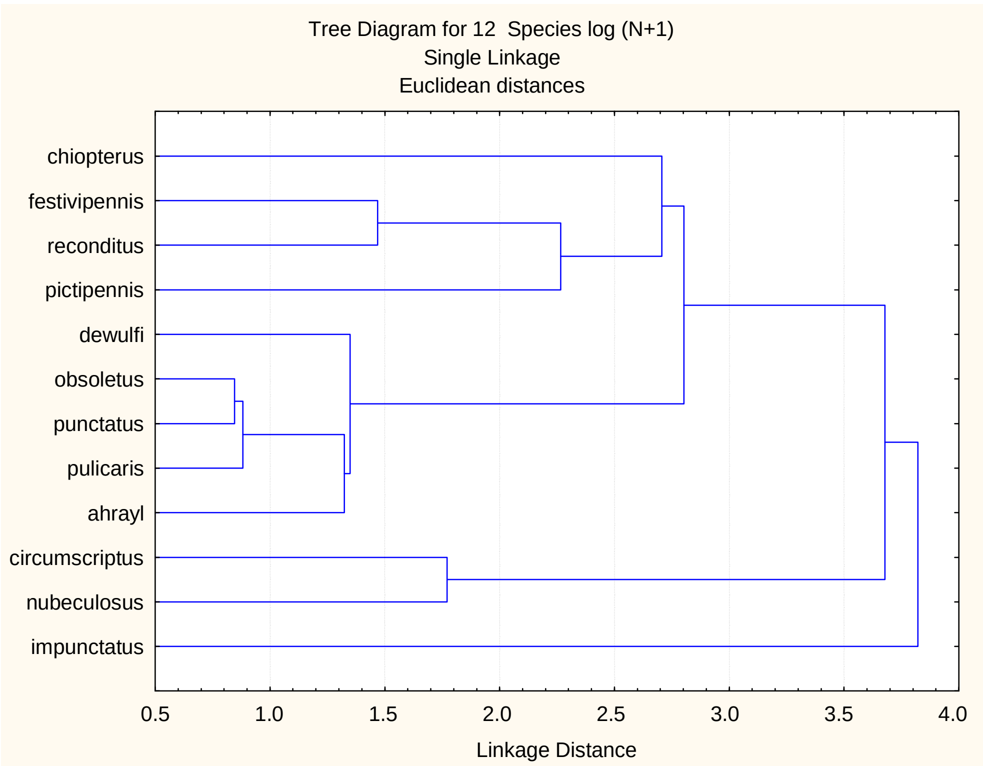

Fig. 14. Cluster analysis illustrating grouping of species.

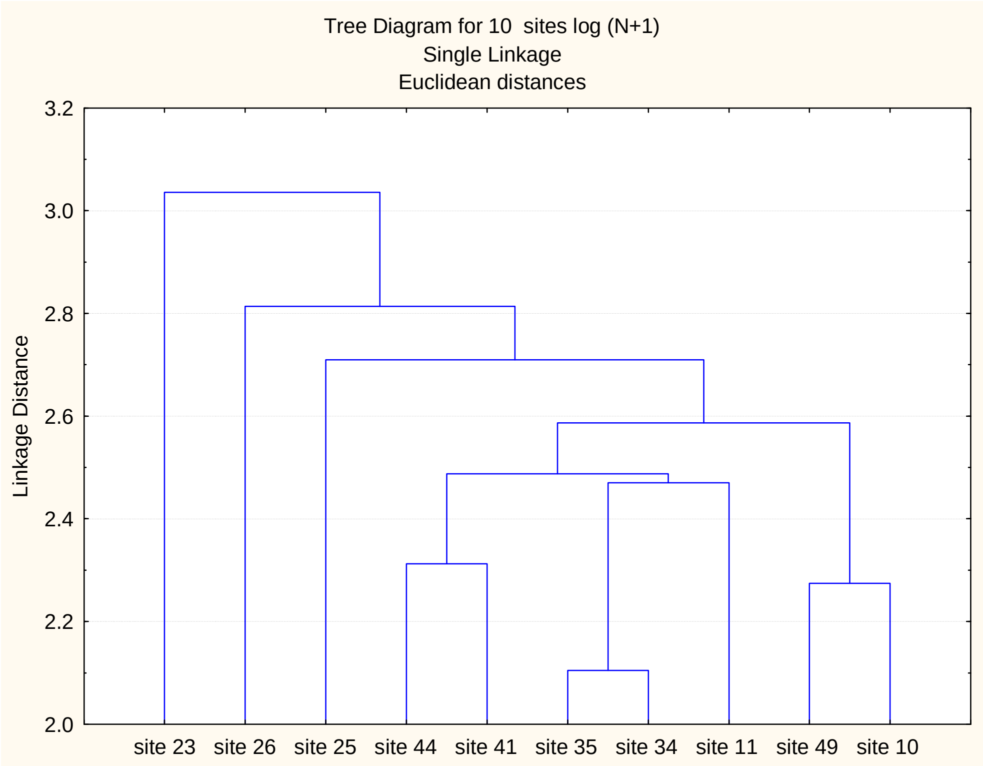

Fig. 15. Cluster analysis illustrating grouping of sites.

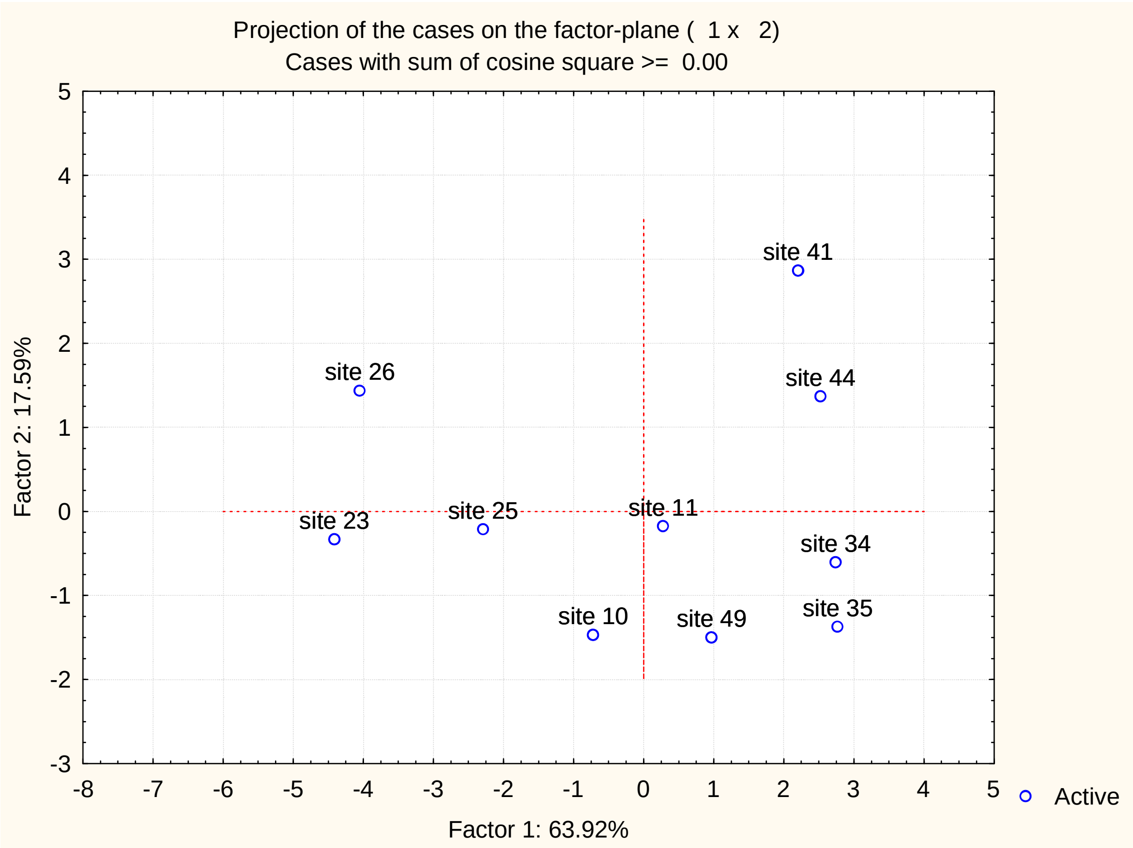

Fig. 16. PCA illustrating grouping of species by site.

Potential BTV Vectors in Ireland

The percentage composition of the catches from the 10 index sites for all weeks in 2007, are illustrated as a pie chart in Fig. 17. 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* and *C. chiopterus* comprised more than half of the total catch. *C. dewulfi* was the dominant species in the Obsoletus Complex and it comprised 40% of the total index site samples. 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 of these potential vector species is a matter of concern in Ireland. Likewise, in the case of the Pulicaris Complex also known to have vector potential, they represented a very high proportion of the index site samples, 35% 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 90% of the total samples. In addition to the index sites full taxonomic analyses were undertaken for selected samples for all 34 sampling sites. The percentage composition of these samples when combined is almost identical to the data presented below (Fig. 17) for the index sites and thus it can be concluded that in 2007 potential BTV vector species dominated the *Culicoides* species assemblages throughout Ireland. Whether these results are typical for Ireland remains to be established, as it is possible that weather conditions in spring and early summer of 2006 created an environment in which species such as *C. dewulfi* were unusually abundant. The results of the 2008 BTV vector surveys may, if weather conditions are different, help provide a better understanding of the role of environmental factors as determinants of the population dynamics of Irish *Culicoides.*

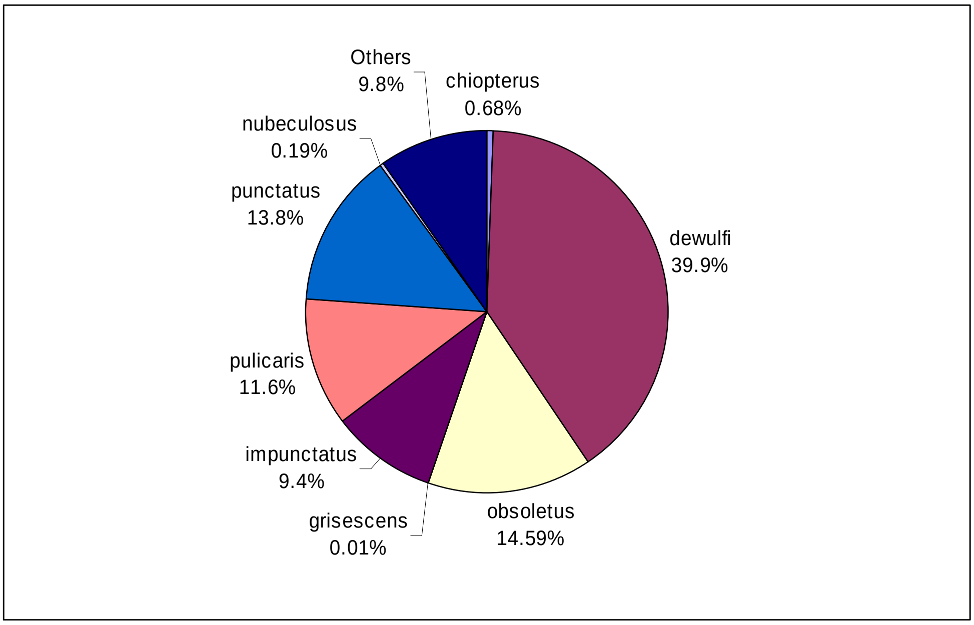

Fig. 17. The percentage composition of the combined catches at the 10 index sites during 2007.

Fig. 18 illustrates the seasonal variation in abundance for the more abundant species regarded as being potential BTV vectors in Ireland. As can be seen the combined data indicate that potential vectors were abundant over an extended season ranging from April to December in 2007. The peak abundances coincided with warm >10˚C when vectorial capacity for BTV is highest in *Culicoides* species.

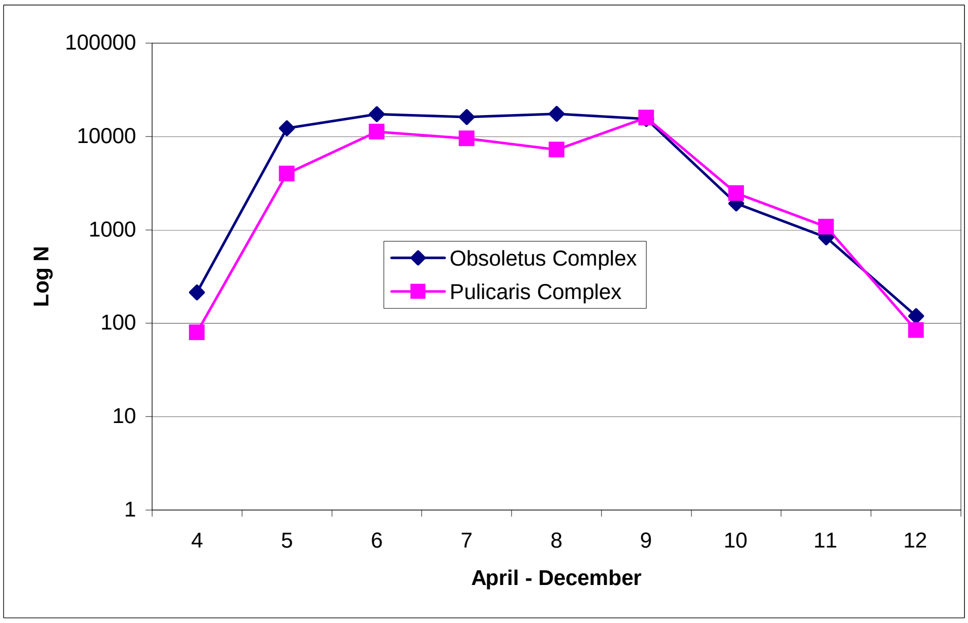

Fig. 18. Seasonal variation in abundance for potential vector complexes.

IMPROVED SURVEILLANCE PROTOCOLS AND RESEARCH

- The experience gained during the initial 2007 entomological surveillance programme suggests that, while the field sampling programme has generally been very successful, some improvements to trapping protocols, sample processing and data recording can be recommended for future BTV vector surveys. Field records of environmental conditions on trapping dates and of host availability might be improved by changes to forms and better instruction leaflets. It may also be possible to have field data transmitted electronically from participating DVO's to the Vector Ecology Unit in NUI, Galway. However, continued use of traditional paper records is considered appropriate. The direct association of field records with *Culicoides* samples sent for analysis is also considered appropriate so that occasional human errors in labelling of samples can be corrected. More robust containers, better packaging arrangements and other minor improvements have been proposed for 2008. More effective communication of trap faults, replacement parts / other materials needed by field crews and other problems experienced by trapping crews is also needed. This can be achieved in future by direct email contact with NUIG technical staff, by use of new forms (rather than comments on trap record forms) and by weekly feedback on field survey progress from the laboratory to appropriate DAFF personnel.

- Some significant changes are also proposed for future laboratory processing of *Culicoides* samples. It is now clear that in other European BTV vector surveillance and monitoring programmes, that both the reported total counts and details on individual species / species complexes are largely based on sub-sampling procedures (Van ark & Meiswinkel, 1992), which greatly reduce the taxonomic work load and allow for more timely reporting of seasonal trends etc. Though such protocols have been adopted elsewhere, some reservations can be expressed as sub-sampling of this kind can lead to difficulties later during statistical analyses. The methodology adopted will need to be critically evaluated. Likewise, the need to focus more on 10 index sites, as originally proposed, became increasingly apparent as a back-log of samples accumulated rapidly during peak *Culicoides* abundance/activity in summer months of 2007.

- Improved taxonomic training, through travel to other laboratories and use of museum based reference collections, will have to be a feature of future *Culicoides* surveys. This can also be facilitated by research visits by taxonomists from other countries. However, as is becoming increasingly evident in other European countries, specialist taxonomists are rarely available for such work nowadays and increased reliance on use of modern molecular methods (e.g. Nolan *et al*., 2007) could be an effective alternative in routine sample analyses as well as providing support for conventional taxonomy. Such methods may also allow for analyses of larval populations, without recourse to time-consuming rearing experiments or deployment of emergence traps.

- Research on life-cycles, larval ecology, activity cycles and local effects of wind / rain is also needed in order that the extensive data being generated by the surveillance programme can be more effectively interpreted. The time consuming full sample processing strategy adopted in 2007 allowed little time

for such research. Deployment of data loggers and analyses involving meteorological data will be important in interpretation of sampling results and the accuracy of field observations on such environmental conditions will need to be evaluated. However, we also need better information on host preferences of different species, factors affecting biting rates, diel activity cycles and other aspects of *Culicoides* biology. Novel trapping techniques and experimental manipulations of midge environments may be possible by application of studies on the responses of *Culicoides* species to olfactory stimuli, like volatile plant defence secretions, host animal odours, pheromones and other attractant / repellent substances (e.g. Blackwell *et al*., 1996, Stuart and Stuart, 1998, Hendry, 2003). Likewise, it will become increasingly important to assess information on the vector competence of the abundant potential vector species now being sampled throughout Ireland. Vector competence is known to vary within species as well as between species. Furthermore, since low environmental temperature can play a major role in limiting the vectorial capacity of potential vector species, it will also be important to have better information on the thermal ecology of Irish *Culicoides* and to relate this to experimental data on bluetongue virus transmission rates in different temperature regimes (e.g. Wittmann *et al*., 2002, Paweska *et al.,* 2002). Such information is needed before the vectorial capacity of particular Irish *Culicoides* species populations can be evaluated and before full epidemiological analyses can begin (Birley and Boorman, 1982, Reisen, 2002). The vector surveillance programme now being undertaken is a timely, appropriate and important action but it needs to be supported by more fundamental research if it is to deliver more than the primary objectives that led to its establishment in 2006 / 2007.

- Support, perhaps involving a part-time post-doctoral researcher at critical times in annual work programmes is being considered, as a means of improving project management. Likewise, some of the field work such as site characterising floral / faunal surveys and additional training courses for trapping crews in DVO's not currently involved in the *Culicoides* surveillance programme could benefit from post-doctoral level support. Future modelling of *Culicoides* population dynamics and of potential BTV disease outbreaks and development of links to molecular taxonomic studies abroad are also activities that will require involvement of experienced research personnel. A review of project staffing requirements is in progress and the outcome will, to some extent, be determined by the availability of suitable post-graduate students and research assistants in the coming year.

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