National BTV Vector Surveillance Programme 2007-2009

Annual Report 2009/2010

T.K. McCarthy, A. Bateman, D. Nowak
and A. Lawless

*Vector Ecology Unit, School of Natural Sciences
and Martin Ryan Institute,
National University of Ireland, Galway,*

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Table of Contents

EXECUTIVE SUMMARY

- The 2009 BTV Vector Surveillance Programme involved weekly field sampling over the complete year at 10 sites previously investigated from April to December 2007 and over the entire year of 2008.
- Protocols developed in 2007 and 2008 resulted in efficient 2009 field sampling and collection of relevant environmental data. Likewise, specimen transport and laboratory entomological species determinations presented few problems in 2009.. *Culicoides* samples were obtained on 92% of the total scheduled sampling events (520) and sampling gaps were partly attributable to extreme weather events.
- It is estimated that 167,921 *Culicoides* were sampled in the 10 sites and full taxonomic determinations of the 25 species/species complexes present were made.
- The duration of the 2009 *Culicoides* activity season, April (week 17) to December (week 50) was similar in those recorded in 2007 and 2008.
- Differences in mean monthly *Culicoides* abundances were noted, when comparisons were made between the results for the three years (2007-2009) in which *Culicoides* assemblages were sampled. These reflected differences in meteorological conditions during the three year study period. Cold, wet, windy conditions were associated with reduced *Culicoides* catches.
- The majority (83%) of the *Culicoides* specimens sampled in 2009 can be regarded as potential BTV vectors. Compared to 2008 and 2007, midges in the *C. pulicaris* complex were proportionally more abundant in 2009 than were those in the *C. obsoletus* complex.
- Differences in results obtained in 2007, 2008 and 2009 environmental conditions were reflected in the overall annual population dynamics of *Culicoides*. However, as in previous years, considerable week to week variations in total *Culicoides* counts and activities of particular species/species complexes occurred in 2009. These were also influenced by changes in weather conditions (wind speed, rainfall, etc.).
- The 8 most abundant species/species complexes recorded in 2009 (*C. pulicaris*, *C. dewulfi, C. punctatus*, *C. achrayl, C. newsteadi, C. obsoletus, C. reconditus/segnis and C. nubeculosus*) were very similar to those recorded 2007 and 2008. However, there were some differences such as *C. newsteadi* which was recorded for the first time in 2009.
- The difference in the BTV vector-free period between years was relatively minor and this period (early December-late April) may provide opportunities for livestock movements that would otherwise involve risk of BTV transmission.
- The need to archive data and *Culicoides* specimens, so that future BTV vector surveillance and monitoring activities are facilitated, is recognised. Concern is expressed about potential loss of taxonomic expertise, which will be essential in the event of a BTV outbreak when a BTV modified vector monitoring programme may have to be initiated at short notice.

INTRODUCTION

The threat of bluetongue disease in Irish livestock is a matter of concern. Bluetongue is an infectious, non-contagious, arthropod borne disease affecting ruminants. The natural host range is strictly limited to ruminants, though sero-conversions without disease have been reported in carnivores (Alexander, C. *et al.*, 1994). The causative agent is Bluetongue Virus (BTV) which 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 throughout the warmer regions of the world.

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 be 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). The change in disease status in Great Britain to BTV-8 Low Risk Zone in June 2010 reflects the improving BTV status there, following no virus circulation for two years. However, the epidemiology of BTV in north-western Europe is still an area of active research and, apart from uncertainties concerning weather, many aspects of vector ecology require additional research before accurate forecasting of potential BTV outbreaks can be provided.

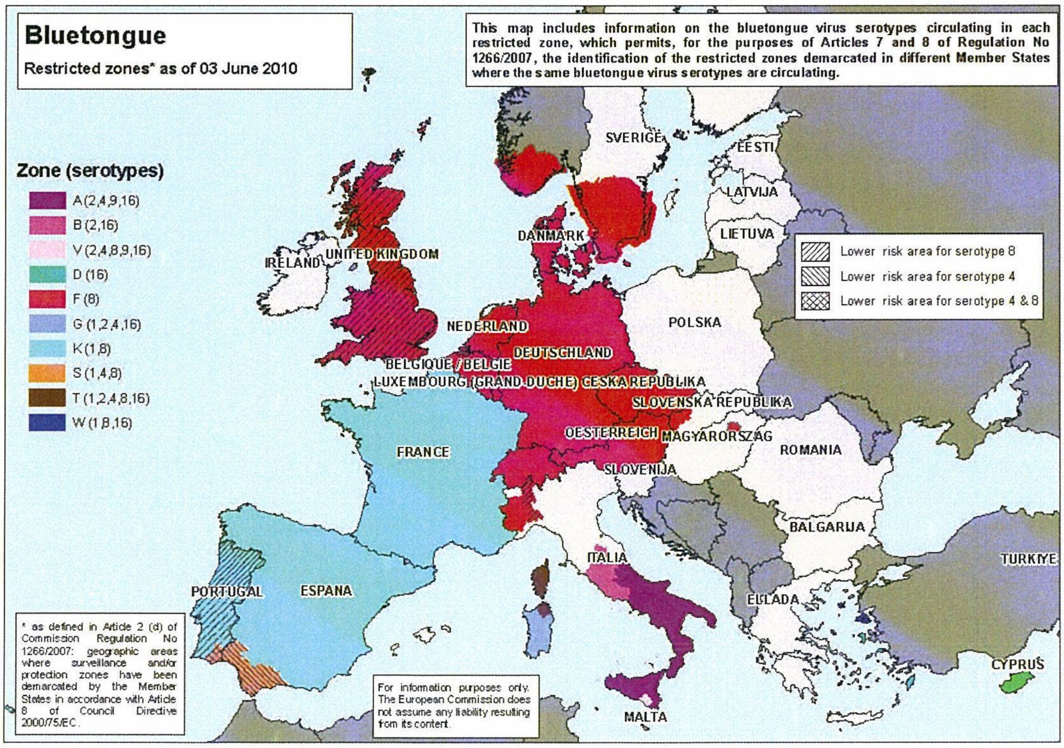

Figure 1 - Map illustrating the current spread of Bluetongue serotypes across Europe.
(http://ec.europa.eu/food/animal/diseases/controlmeasures/bt_restrictedzones-map.jpg)

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. 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 and to be completed by December 2009. However, because of the need to clearly establish the duration and timing of the vector-free period, it was decided to continue the midge sampling programme till the end of April 2010. This third annual report describes the results of the BTV vector surveillance work undertaken in 2009 together with the results for 2010. Thus this represents the final report on the NUI, Galway contribution to the National BTV vector surveillance programme. Previous reports (McCarthy *et al.* 2008 & 2009) described the results of the 2007-2008 work and additional interim reports (2007 and 2008) have given mid-season reviews of the sampling programmes. As in 2007-2008, 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 at the Vector Ecology Unit's laboratory in NUI, Galway.

This report presents the results of weekly sampling of *Culicoides* at 10 sites, distributed around the country, at which total midge abundance and species/species complex composition of the midge assemblages were recorded. A comparison is made with the results obtained in 2007-2008 and the need for data and sample archiving is outlined.

RESULTS

Sample sites

Sampling was undertaken on a weekly basis at 10 sites during 2009. The sites comprised seven of the 10 index sites used in the 2008 BTV vector survey, together with three selected from the original full set of 34 randomly selected sites. The three sites in question were selected, using previous years *Culicoides* data so as to ensure that the 10 sites being surveyed in 2009 were likely to give good results (i.e. in respect of sample size and species diversity). The distribution of the 2009 sites is shown in Fig. 2.

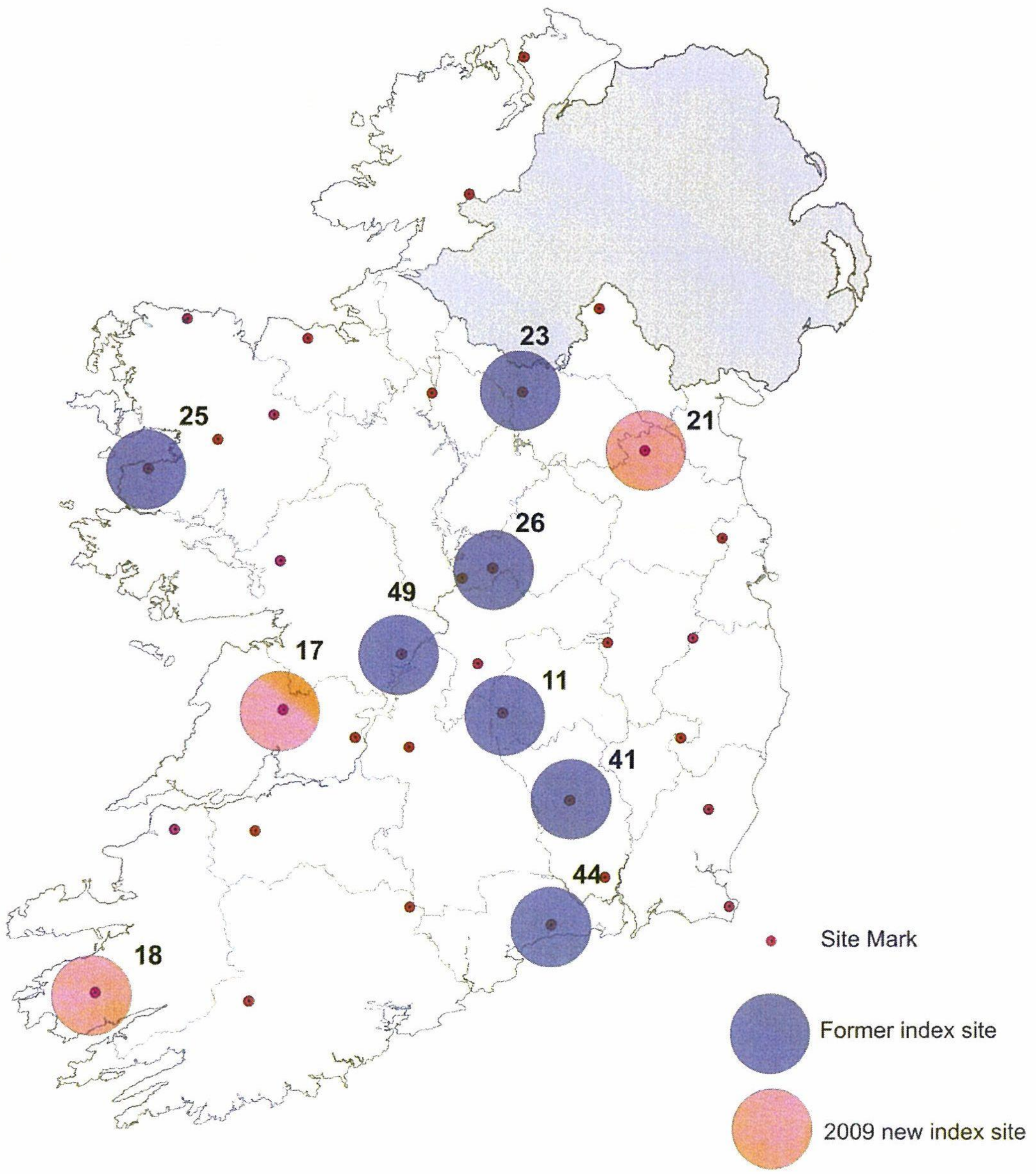

Figure 2 - Location of the 10 sampling sites at which weekly *Culicoides* collections were made in 2009.

Trapping programme

In Table 1 a summary of the trapping activities at each site is presented for the period of January to the end of December 2009. This documents the weekly records of trapping conditions and insect samples which have been sent to the Vector Ecology Unit in NUI, Galway from the 10 sampling sites. As can be seen (Table 1) the response rate from the DVO trapping staff was generally very good for most locations but poor response rates were noted for certain sites (11, 26) at the start of the year and these seem to have largely been attributable to unresolved technical problems with traps. Likewise, one site (23) did not operate effectively towards the end of the year. The good overall activity rate reflected improvements in record keeping, and more effective communication protocols introduced in 2008.

Field sampling protocols

The standard UV light traps used in the 2007 and 2008 surveys were deployed in 2009/2010 at the 10 sampling sites (Fig. 2). Improvements to trap maintenance and spare parts supply lines initially implemented in 2008 contributed to the overall success of the 2009 field sampling programme (Table 1). Data loggers (Tiny Tag) recording both temperature and relative humidity were positioned close to the traps at the 10 sites. Improvements, initially made in 2008, to the packaging and transportation of samples to the Vector Ecology Unit laboratory resulted in no loss of samples during 2009/2010. However, occasionally samples still arrived in poor condition. It is not clear why this occurred but it may have been due to rough handling of dry specimens before they were placed in preservative for transport to the lab. Loss of wings, legs and damage to the midge morphology made accurate species identifications difficult or impossible in such damaged samples.

Samples and environmental data received

A total of 477 returns (i.e. insect samples and/or weekly field reports) were received by the Vector Ecology Unit in 2009. This represented 92% of a possible maximum of 520 (i.e. 10 x 52 weeks). However, when weeks 1 and 52 in which trap operator returns were particularly bad, are excluded the overall percentage of returns was 95%. The good response rate by field crews in 2009 resulted in receipt of 330 insect samples. No sites had a full (100%) response rate in 2009, however, if weeks 1 and 52 (Christmas holidays) are excluded, 5 sites had a full response rate. The weekly response rate has not fallen below 80% 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 2009 trapping programme response rate are presented in Table 1. The extension of the sampling programme in 2010 included 17 weeks trapping at all 10 sites, for which an overall highly satisfactory 97.6% response rate was obtained, and this was terminated when the vector free period for Ireland was shown to have ended at several sites.

Table 1- A record of trapping results and/or samples received by the NUIG laboratory from the 10 trapping sites during 2009 (0=No completed forms or samples received; 1=either or both forms/samples received).

Week No. \ Site No.	11	17	18	21	23	25	26	41	44	49	Week No.	%
1	0	0	0	1	0	0	0	0	1	0	1	20.00
2	0	1	1	1	0	1	0	1	1	1	2	70.00
3	0	1	1	1	1	1	0	1	1	1	3	80.00
4	0	1	0	1	1	1	0	1	1	1	4	70.00
5	0	1	1	1	1	1	0	1	1	1	5	80.00
6	0	1	1	1	1	1	0	1	1	1	6	80.00
7	0	1	1	1	1	1	0	1	1	1	7	80.00
8	0	1	1	1	1	1	0	1	1	1	8	80.00
9	0	1	1	1	1	1	1	1	1	1	9	90.00
10	1	1	1	1	1	1	0	1	1	1	10	90.00
11	1	1	1	1	1	1	1	1	1	1	11	100.00
12	1	1	1	1	1	1	1	1	1	1	12	100.00
13	1	1	1	1	1	1	1	1	1	1	13	100.00
14	1	1	1	1	1	1	1	1	1	1	14	100.00
15	1	1	1	1	1	1	1	1	1	1	15	100.00
16	1	1	1	1	1	1	1	1	1	1	16	100.00
17	1	1	1	1	1	1	1	1	1	1	17	100.00
18	1	1	1	1	1	1	1	1	1	1	18	100.00
19	1	1	1	1	1	1	1	1	1	1	19	100.00
20	1	1	1	1	1	1	1	1	1	1	20	100.00
21	1	1	1	1	1	1	1	1	1	1	21	100.00
22	1	1	1	1	1	1	1	1	1	1	22	100.00
23	1	1	1	1	1	1	1	1	1	1	23	100.00
24	1	1	1	1	1	1	1	1	1	1	24	100.00
25	1	1	1	1	1	1	1	1	1	1	25	100.00
26	1	1	1	1	1	1	1	1	1	1	26	100.00
27	1	1	1	1	1	1	1	1	1	1	27	100.00
28	1	1	1	1	1	1	1	1	0	1	28	90.00
29	1	1	1	1	1	1	1	1	1	1	29	100.00
30	1	1	1	1	1	1	1	1	1	1	30	100.00
31	1	1	1	1	1	1	1	1	1	1	31	100.00
32	1	1	1	1	1	1	1	1	1	1	32	100.00
33	1	1	1	1	1	1	1	1	1	1	33	100.00
34	1	1	1	1	1	1	1	1	1	1	34	100.00
35	1	1	1	1	1	1	1	1	1	1	35	100.00
36	1	1	1	1	1	1	1	1	1	1	36	100.00
37	1	1	1	1	1	1	1	1	1	1	37	100.00
38	1	1	1	1	1	1	1	1	1	1	38	100.00
39	1	1	1	1	1	1	1	1	1	1	39	100.00
40	1	1	1	1	1	1	1	1	1	1	40	100.00
41	1	1	1	1	1	1	1	1	1	1	41	100.00
42	1	1	1	1	1	1	1	1	1	1	42	100.00
43	1	1	1	1	1	1	1	1	0	1	43	90.00
44	1	1	1	1	1	1	1	1	1	1	44	100.00
45	1	1	1	1	1	1	1	1	1	1	45	100.00
46	1	1	1	1	1	1	1	1	1	1	46	100.00
47	1	1	1	1	1	1	1	1	1	1	47	100.00
48	0	1	1	1	1	1	1	1	0	1	48	80.00
49	1	1	1	1	0	1	1	1	1	1	49	90.00
50	1	1	1	1	0	1	1	1	1	1	50	90.00
51	1	1	1	1	0	1	1	1	1	1	51	90.00
52	0	0	0	0	0	0	0	0	0	0	52	0.00

Laboratory processing of samples

Insect samples received at the Vector Ecology Unit laboratory in 2009 were logged electronically on arrival. The contents and field report forms were examined and stored and all records were 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 were 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 2009 was approximately 167,921. 60 samples were processed using sub-sampling methods developed by Van Ark & Meiswinkel, (1992). All 60 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.

Culicoides counting protocols

The sub-sampling protocol, adopted for completion of the 2007 sample processing and for the full 2008 survey, which was used for the 2009 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 into 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 in 2009 involved full taxonomic analyses for all 10 sites/weeks. The process was 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 was fully processed the internal report information was input into the database and hard copies of the internal reports were attached to the relevant field reports for systematic filing in the Vector Ecology Unit archive. Errors in reports or differences associated with sub-sampling protocols were signalled by cross-referencing between abundance data-sets and cumulative numbers generated from individual species counts. This and other quality control measures contributed to the overall efficiency of the laboratory sample processing operations.

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 27 species recorded in the 2007-2009 surveillance programmes are highlighted. 5 species not previously recorded in 2007-2008 were recorded in 2009 and these were: *C. newsteadi, C. riethi, C. kibunensis, C. vexans* and *C. pallidicornis*. Of the 27 species recorded, some species (*C. stigma, C. heliophilus, C. parroti, C. furcillatus C. newsteadi, C. riethi, C kibunensis, C. vexans* and *C. pallidicornis*) are provisionally listed pending consultation with international experts. As in 2007 and 2008, considerable variation in species richness (number of species) present in individual samples was observed in 2009, reflecting both within-site and between-site variations. The number of species varied in 2009 from site to site ranging from 14 to 20 (mean 17). 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, 2008 and 2009

In Fig. 3 the results for 2007, 2008 and 2009 are compared, based on full season data sets of 10 sites selected for 2009. However, because of the late start of sampling in the 2007 season, the number of zero counts appears to be much lower in the 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 at the 10 sites varied from 0 to 28,592. The mean weekly count ($\bar{x}$±S.E.) per trap was 678.19±130.89 (N=327). When comparable data for the same period (weeks 17 to 51 inclusive) in 2008 are combined, weekly counts varies from 0 to 65,536 value ($\bar{x}$±S.E.) was 602.12±190.62 (N=474). Comparable data for the 10 sites in 2009 when combined the weekly count varies from 0 to 21,600 ($\bar{x}$±S.E) was 363.47±68.31 (N=462). Comparison of frequency distributions of weekly counts for 2007, 2008 and 2009 showed that 2007 was significantly different from 2008 and 2009 (Mann-Whitney U test, 2007 V 2008 U=715.5; P≤0.05; 2007 V 2009 U=740.5;P≤0.05; 2008 V 2009 U=1298.5; P≤0.05) while 2008 and 2009 were not significantly different. The complete frequency distributions of weekly counts for 2008 and 2009 (i.e. 34 sites in 2008 and 10 sites in 2009) were compared and there was no statistical difference between the two years (Mann-Whitney U test, U=1214.5; P≤0.05;).

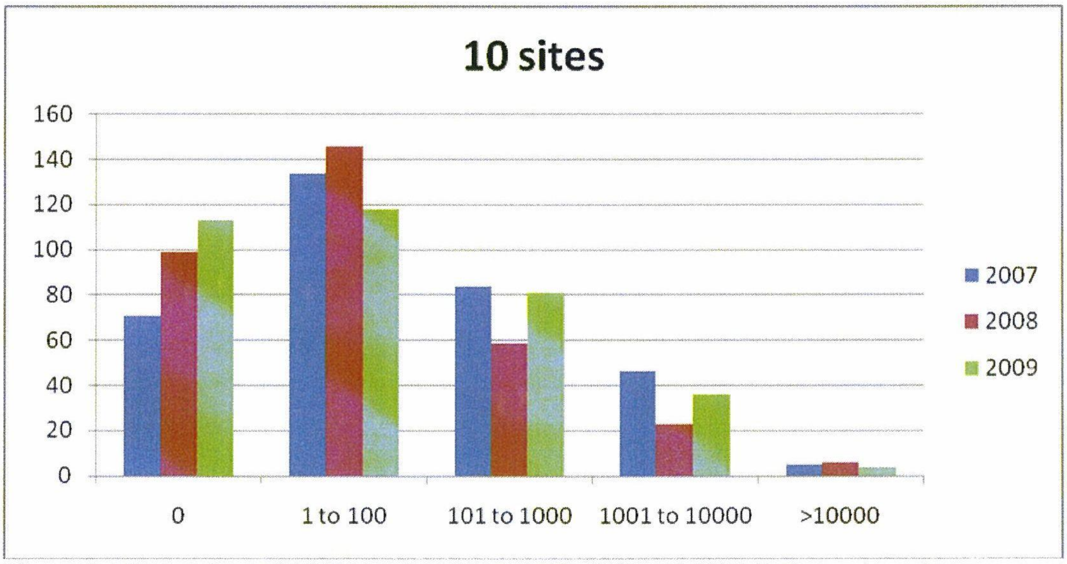

Figure 3 - Comparison of 2007 (April to December), 2008 and 2009 (full years) weekly sample sizes from 10 sites.

Comparison of sites 2007, 2008 and 2009

Table 3 - Total *Culicoides* abundance and species per site for the 10 sites sampled in 2009.

Site No.	Abundance	Species per site
11	21,780	18
17	7,301	17
18	2,409	14
21	35,693	20
23	50,686	18
25	1,014	14
26	19,768	18
41	3,248	16
44	19,512	19
49	6,510	18

In Table 3 the cumulative weekly total *Culicoides* counts of 10 sampling sites in 2009 are presented and as can be seen, the values varied from 1,014 to 50,686 ($\bar{x}$±S.E.= 16,792.1±5,144.84). The results can be compared with those obtained in 2007 and 2008. In 2007, site totals varied from 801 to 56,270 ($\bar{x}$±S.E.= 22,176.8±7,160.51) and in 2008, site totals varied from 2,416 to 183,199 ($\bar{x}$±S.E.= 28,540.4±17,477.01). The results are also presented as percentage frequency distributions, in Fig. 4. As can be seen, in 2007, the midge catches for two sites (17, 21) each represented more than 15% of the cumulative annual total, and two exceeded 20% (23 and 26). In contrast, in 2008, only two sites (18 and 23) had catches exceeding 10% of the total, but in the case of one of these (23) the catch was 64.19% of the total number of midges recorded. In 2009, three sites exceeded 10% (11, 26 and 44) and 2 exceeded 20% (21 and 23). Site 23 had the highest cumulative *Culicoides* abundance values in all three years.

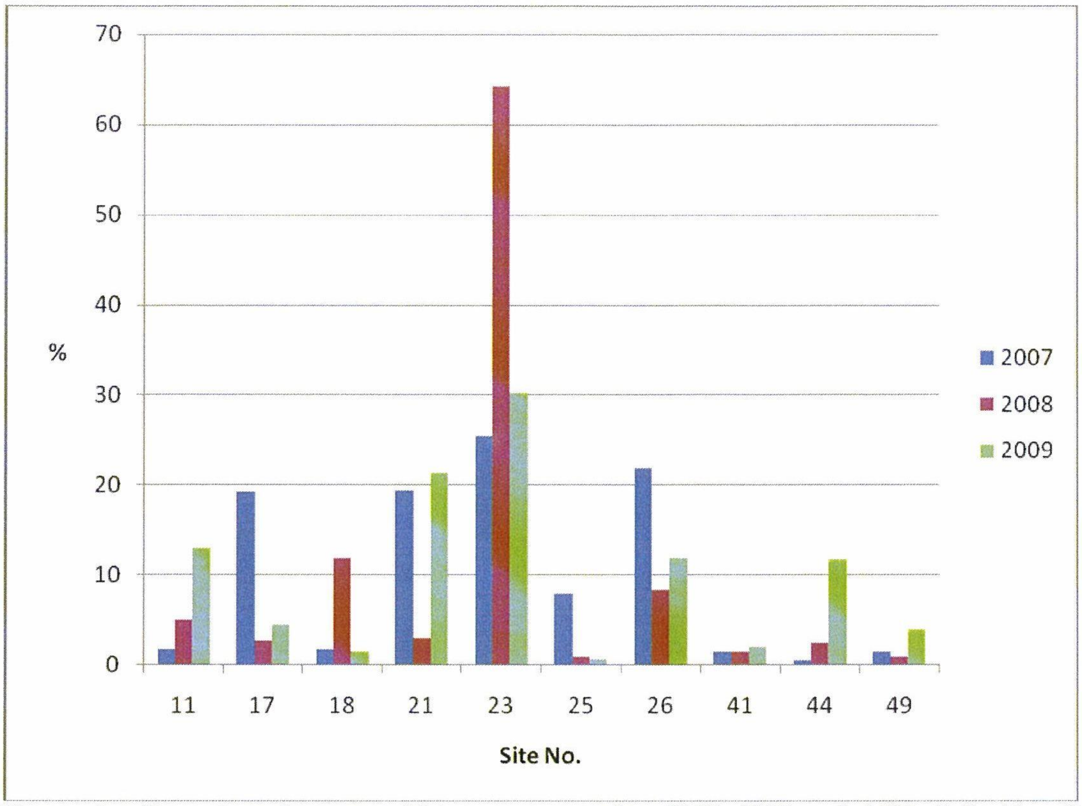

Figure 4 - Percentage frequency histogram comparing 2007, 2008 and 2009 *Culicoides* site abundances.

Comparison of the cumulative weekly counts for these sites in respect of the three years showed that the data sets were not significantly different. Likewise, examination of scatter plots comparing data on total counts per site in 2007, 2008 and 2009 (Fig. 5) and results of non-parametric correlation analyses showed that considerable between-year variation in abundances was observed for many sites. A significant correlation had been recorded when 2007 and 2008 data were compared (Fig. 5). However, no such relationship could be observed when the 2009 data were compared with those for 2007 or with 2008.

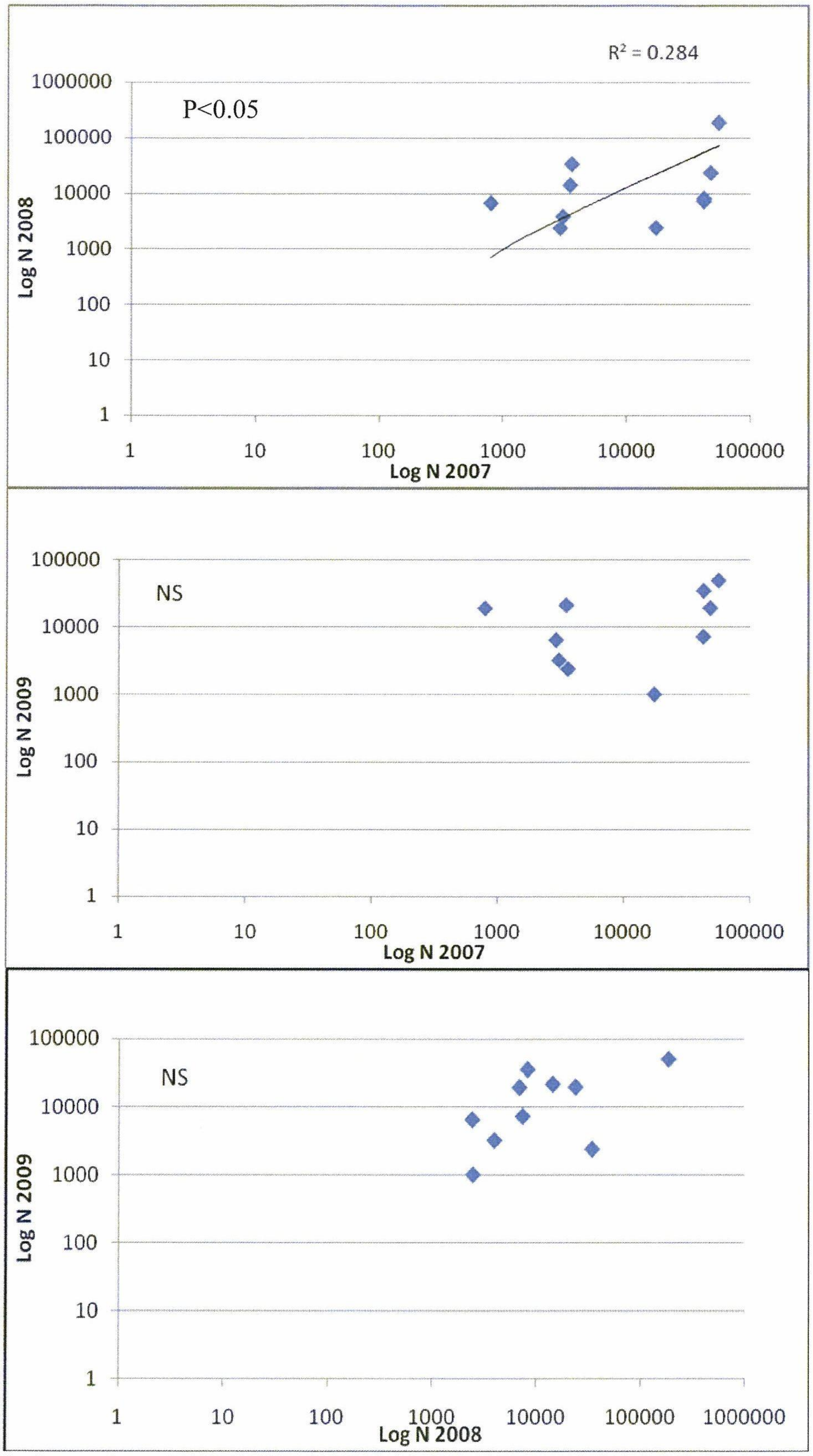

Figure 5 - Comparison of 2007, 2008 and 2009 total *Culicoides* abundances at sites (combined site catches for year).

In Fig. 6 the distribution of sites and their different *Culicoides* abundance levels (1,000-15,000, 15,000-30,000 and >30,000) are indicated on a map of Ireland and it can be seen that the sites with the highest midge (>30,000) numbers were located in the northern half of the country. Sites with intermediate abundance are represented in the central axis of the island and low abundance values were widely distributed around the country and no clear pattern can be discerned.

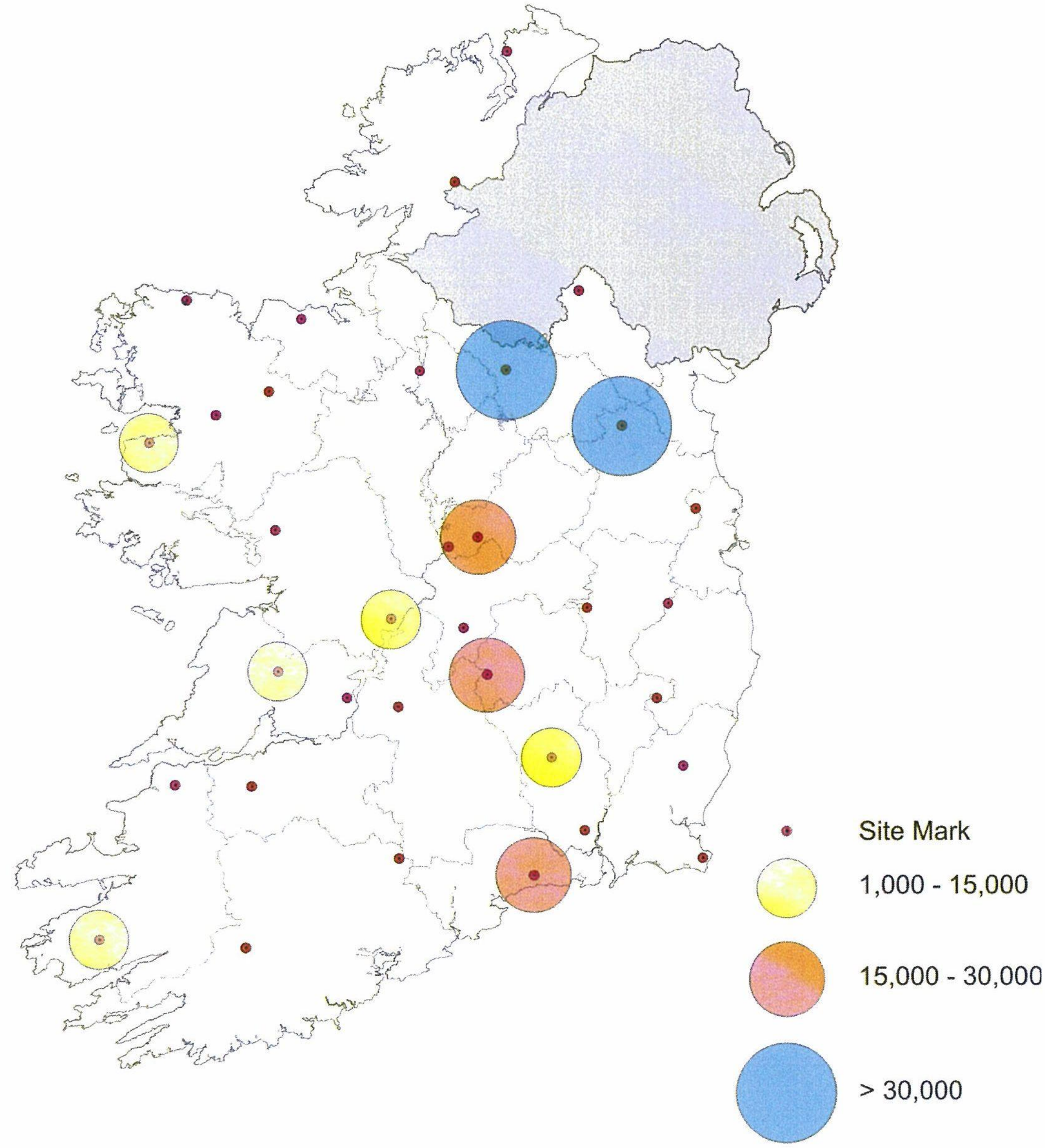

Figure 6 - Map of Ireland showing distributions of *Culicoides* sampling sites with different abundance levels (2009 annual totals).

Seasonal variation in Culicoides abundance

In Fig. 7 the seasonal variation in weekly *Culicoides* counts (all 10 sites) are illustrated for the 2007, 2008 and 2009 surveys. As can be seen, the periods of midge activity were broadly similar. Surveillance began a little later (mid-April) in 2007, whereas in 2008 and 2009, full year sampling programmes were 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) illustrate 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 2009 and 2008, whereas this occurred twice in 2007. In 2008, two site peaks represented more than 20% of the total *Culicoides* sampled that year but no such proportionally high peak was observed in 2007 or 2009. The within and between-year variation in weekly total counts illustrate the need for good time series for analyses of *Culicoides* population dynamics. Weekly variations in total *Culicoides* numbers recorded at each of the 10 sampling sites are illustrated in Appendix I, where the considerable between-site variations in 2009 can be clearly observed. Likewise, the weekly total counts for each of the 25 taxa (species or species complexes) recorded in 2009 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 and 2008 concerning between-site and between-taxa variations in weekly abundances.

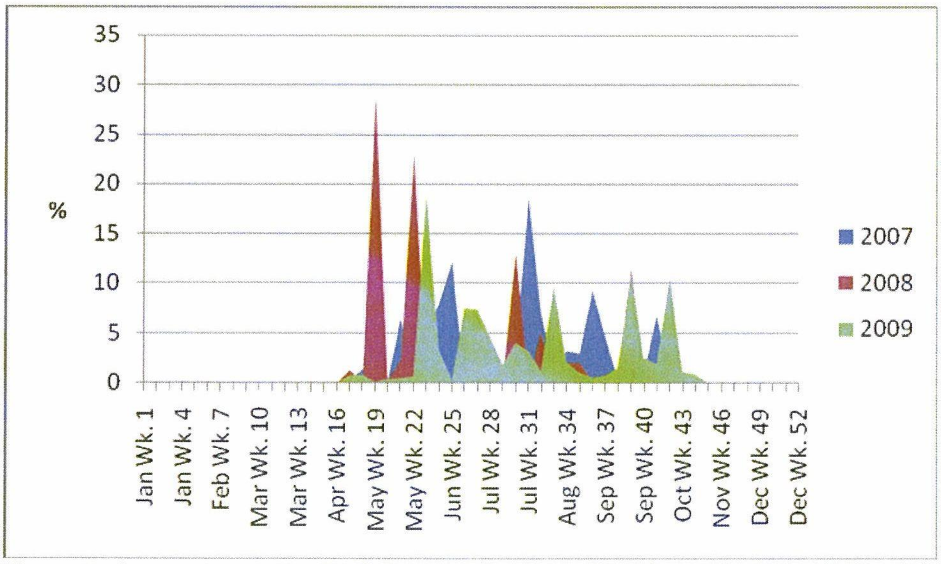

Figure 7 - Seasonal variation in *Culicoides* abundance for 10 sites in 2007, 2008 and 2009.

Differences between sites can be considerable both in terms of numbers of different species/species complexes and overall midge abundance. Major peaks in abundance, such as was noted in 2008 at site 23 where a total (estimated) *Culicoides* count of 66,536 was recorded, can make interpretation of overall seasonal patterns (using combined site data) difficult. Likewise, irregular 2009 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, 2008 and 2009 with respect to the relative abundances of the various taxa was made using log-transformed abundances. The results are illustrated in Fig. 8 (N>234) in which it can be seen that the rank order of the numerically dominant species in the combined weekly catch at the 10 sites were broadly similar in all three 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. In 2009, a series of rarer species were relatively better represented in the samples (e.g. *C circumscriptus, C. segnis, C. nubeculosus, C. newsteadi, C. furcillatus* and *C. pallidicornis*).

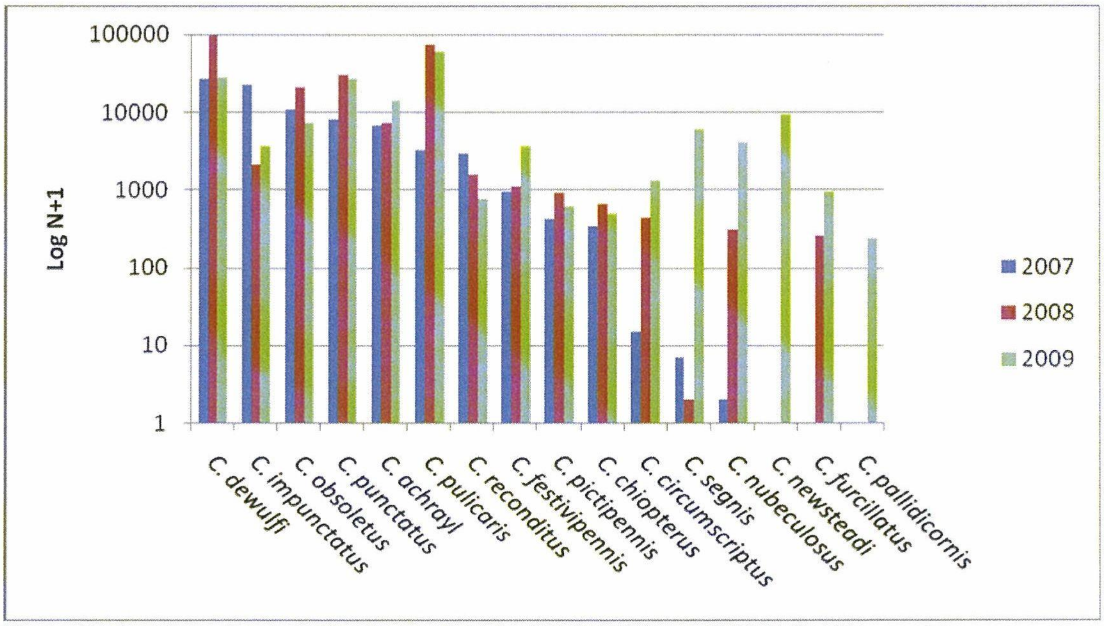

Figure 8 - Log-transformed abundance data for 2007, 2008 and 2009 *Culicoides* species.

In Fig. 9 the relationship between overall abundances of the 25 taxa recorded in 2009 and their estimated geographical range in Ireland (i.e. numbers of 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 surveyed sites.

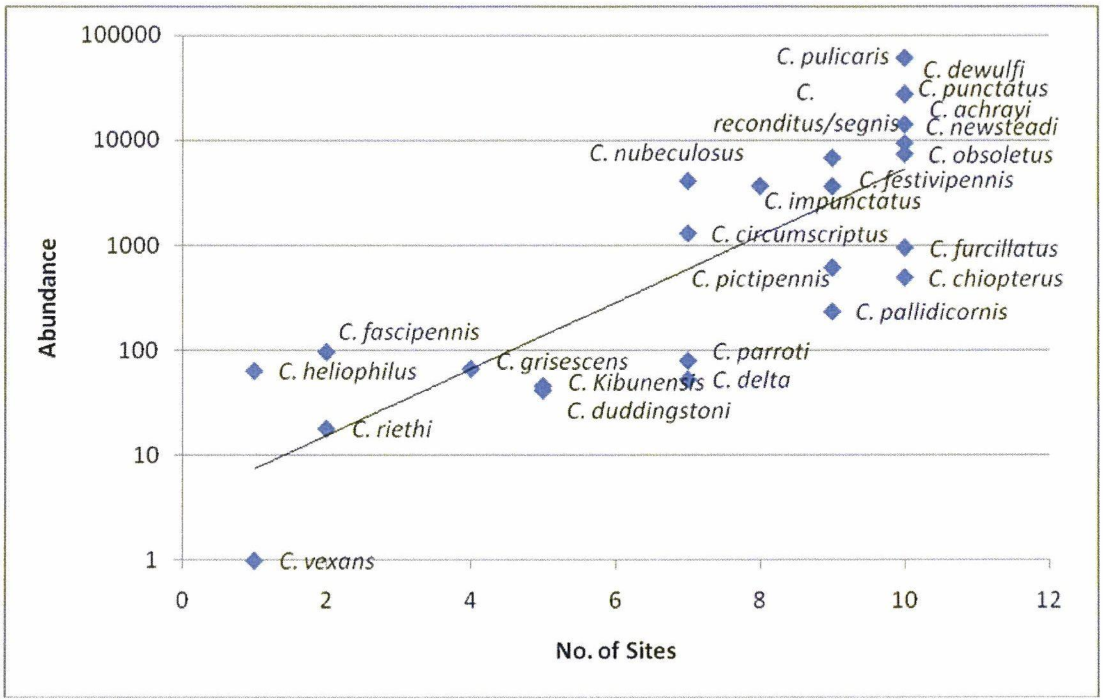

Figure 9 - The relationship between species abundance and geographical distribution of Irish *Culicoides* observed in the 2009 survey.

Species richness

The total number of species/species complexes record in 2009 was 25. This can be compared with the results obtained in 2007 and 2008, when 21 and 19 taxa were involved respectively. The main differences noted involved 3 rare species recorded only in 2007 and one additional rare species encountered in 2008. A further 5 species were recorded in 2009, 4 of which are rare (*C. riethi, C. kibunensis, C. vexans* and *C. pallidicornis*.). *C. newsteadi* was also recorded for the first time in 2009 and was the fifth most abundant species. The most probable reason that this species was not recorded previously is that it is very difficult to distinguish it from *C. pulicaris*. These differences are not considered to be of any great significance with respect to the BTV Surveillance Programme objectives. The fact that the remaining 2 species in the Irish check list (N=29) have not been detected 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 20).

Composition of species assemblages

In 2009, full identifications, subject to certain taxonomic constraints detailed in 2007, were made in respect of the 10 sites sampled. A proportion (90.5%) of the total number of weekly site samples (N=330) had to be analysed using sub-sampling protocols. However, the total number of actual species identifications was still very high (N=34,873) and the process was very time consuming. The direct counts and estimated counts for the sub-sampled *Culicoides* samples collectively represented an estimated 167,921 midge specimens.

The overall percentage composition of the sampled *Culicoides* catch of 2009 is illustrated in Fig. 10. The relative abundance of species/species complexes is also similarly illustrated for each of the 10 sites, Appendix III.

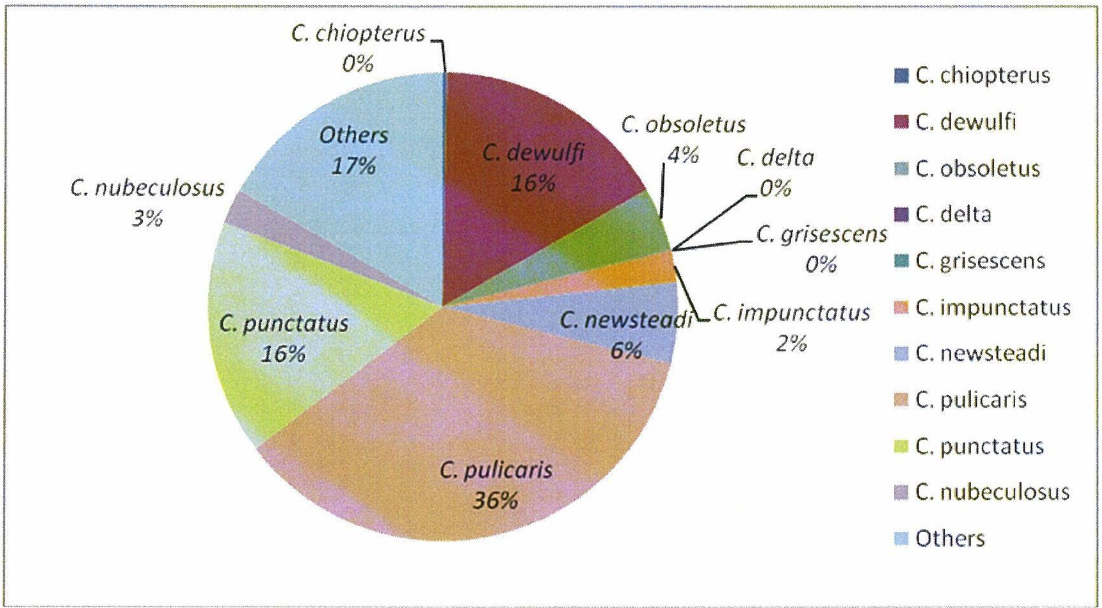

Figure 10 - The percentage composition of the combined catches at the 10 sites during 2009, with respect to the more abundant species.

Environmental conditions

Met Éireann data have been used in some 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 2009 is shown in Fig. 11. These data indicate that fluctuations occur 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.

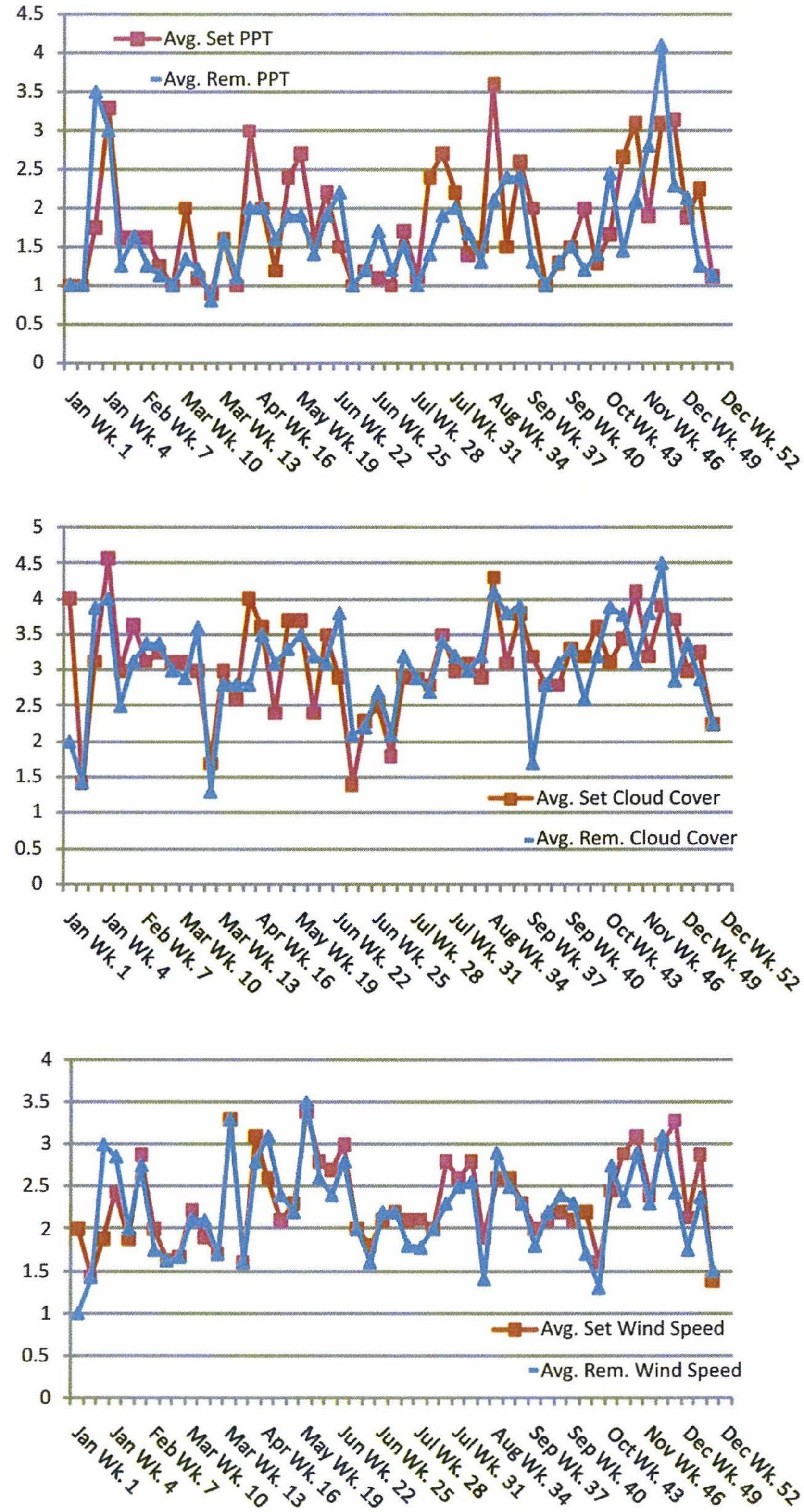

Figure 11 - DVO personnel weather score data for 2009. Data shown are the average scores of all 10 sites for days on which traps were set and removed.

The environmental conditions experienced in 2007, 2008 and 2009 differed in several important respects. The average values for 8 Met Éireann recording sites were used, namely Ballyhaise, Belmullet, Gurteen, Johnstown Castle, Mullingar, Oak Park, Shannon and Valentia. Differences between the three years can be seen in Fig. 12 in which Met Éireann monthly averages for air temperature, precipitation and wind are summarised. While seasonal variations in temperature were broadly similar during Spring, Summer and Autumn, the Winter conditions varied considerably. January 2007 and the 2007/08 Winter were considerably milder than Winter 2008/09. December 2009 was the coldest month sampled. Greater between-year variation in seasonality of rainfall patterns were recorded, and the latter months of 2009 were particularly wet. In the case of wind records, the difference between years was not so extreme.

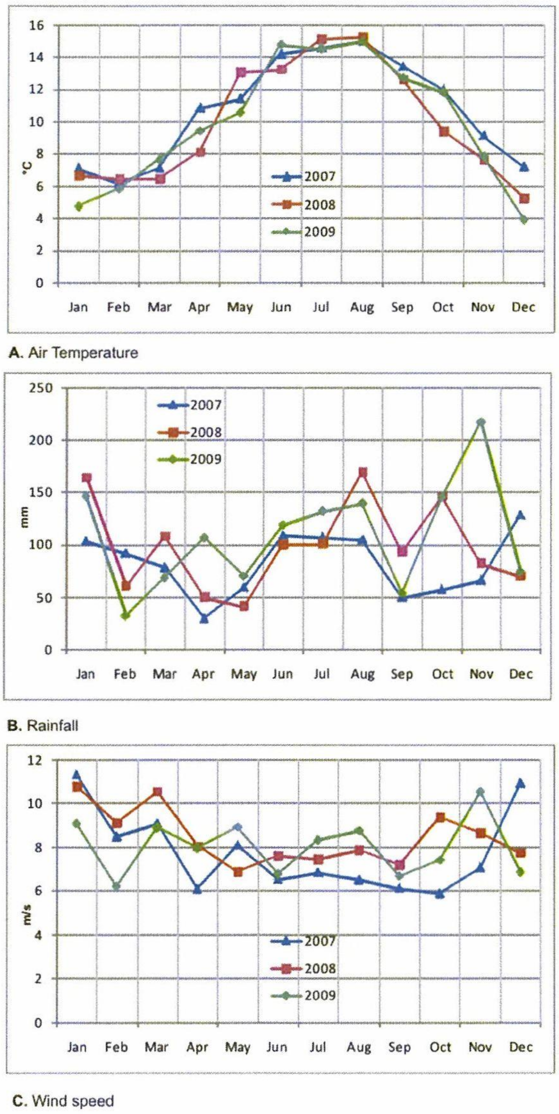

Figure 12 - Met Éireann monthly mean data for (A) air temperature, (B) rainfall, (C) wind speed for 2007, 2008 and 2009.

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 and 2009. 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 three years. However, in May 2009, the *Culicoides* abundance was less than in 2007 and 2008. This can be interpreted with respect to the meteorological summaries above (Fig. 12) in which it can be noted that this month was cooler, wetter and more windy in 2009. In June 2009, the mean monthly *Culicoides* abundance increased sharply and it can be seen in Fig. 12 that in June 2009 the wind speed was lower, rainfall was similar to 2007 and 2008 and it was slightly warmer than the previous 2 years. The overall similarity of the seasonal patterns of *Culicoides* abundance in the three years investigated can be seen in Fig 13. In Fig. 14 the relative contribution made by a single site (23) to the overall seasonal pattern in 2009 can be observed.

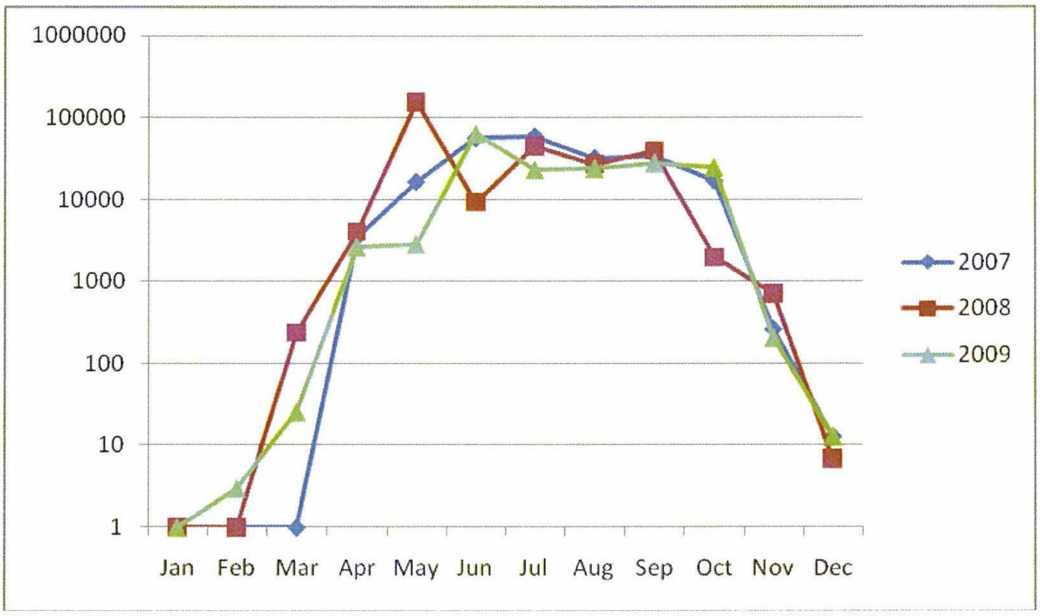

Figure 13 - *Culicoides* log transformed abundance for 2007, 2008 and 2009 (2007 survey started in April).

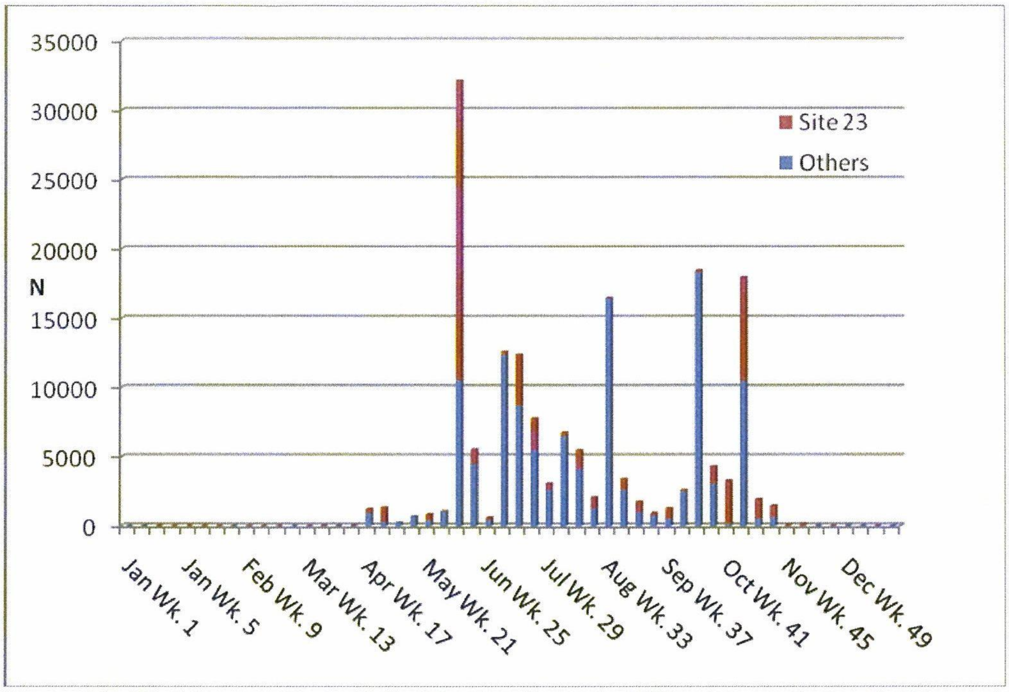

Figure 14 - Weekly variation in *Culicoides* abundance in 2009, with results for site 23 compared with those for the other sites combined.

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 approximately 21% of the total catch. *C. dewulfi* was the dominant species in the *Obsoletus* complex and it comprised 16% of the total samples in 2009. 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 high numbers of these potential vector species in 2009 is a matter of concern. Likewise, the *Pulicaris* complex, represented by *C. pulicaris, C. punctatus, C. newsteadi, C. impunctatus, C. delta, C. grisescens* and *C. nubeculosus*, which is also known to have vector potential, represented a very high proportion of the total *Culicoides* samples (62%) in 2009. The 3 main species in 2009, *C. pulicaris, C. punctatus* and *C. newsteadi,* 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 83% of the total samples. In Fig. 15 the seasonal variation in abundance of the two major vector complexes (*Obsoletus* complex and *Pulicaris* complex) are illustrated for 2007, 2008 and 2009. 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. However, in 2009 the *Pulicaris* complex numbers were notably higher from March to October, with the exception of July, where the two complexes had similar numbers. The two most abundant species recorded in 2009 were *C. pulicaris* (N=59,989) and *C. dewulfi* (N=27,441) - both of which are important BTV vectors.

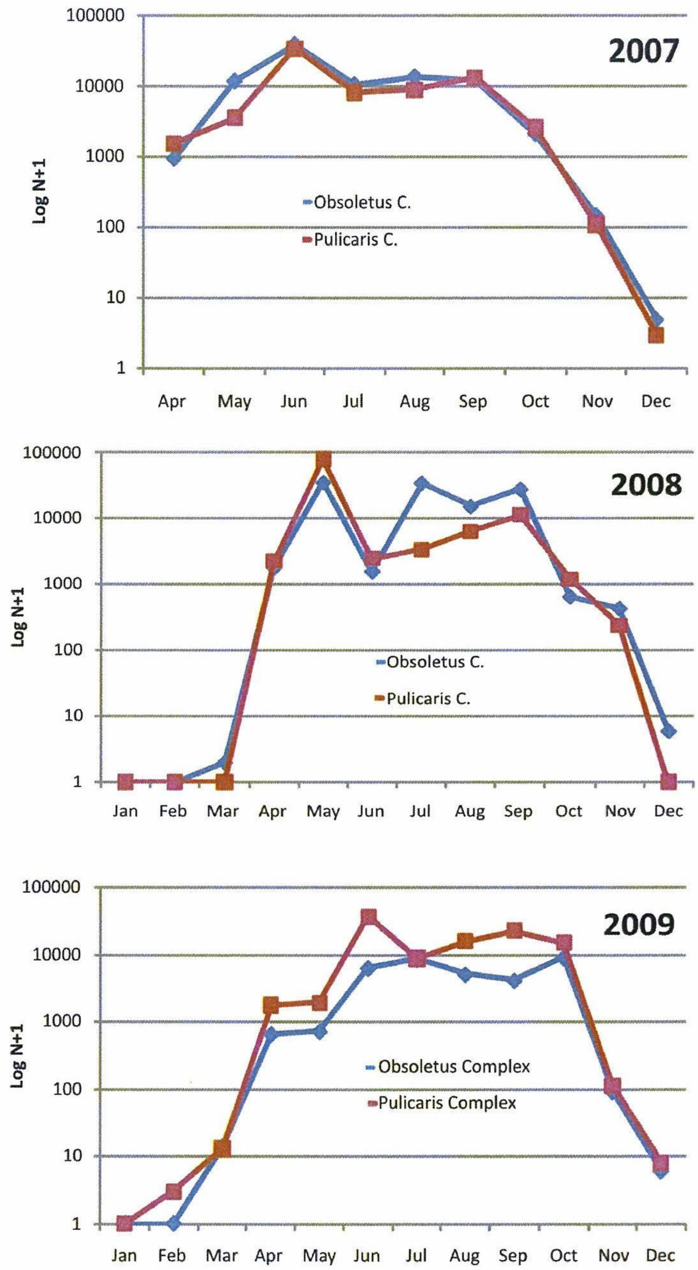

Figure 15 - Seasonal variation in abundance for potential vector complexes for 2007, 2008 and 2009.

Vector free period

The BTV vector free period (VFP) is defined in accordance with the criteria outlined in paragraph 2(c) of Annex V of Commission Regulation (EC) 1266/2007 in the Official Journal of the European Union. This defines 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 were met, i.e. less than 5 parous females per trap, the VFP was deemed to be in effect. The 2008/2009 VFP (Fig. 16) commenced in the third week of December (week 51) when only 2 *Culicoides* were trapped at site 16 (Cork). In the previous week, a total of 15 *Culicoides* were trapped. However, only 1 site trapped more than 5 *Culicoides*. Site 16 (Cork) trapped 14 *Culicoides* and this sample contained 5 parous females. *Culicoides* activity stopped completely in January 2009. The VFP ended in the third week of April 2009 (week 17), when 5 sites submitted samples with 5 or more parous females. The largest sample was received from site 11 (Laois), and contained 552 *Culicoides* specimens. The 2009/2010 VFP commenced in mid-December (week 50) when a total of 3 *Culicoides* were trapped at sites 26 (Westmeath) and 41 (Kilkenny). In the previous week, a total of 9 *Culicoides* were trapped from 3 sites however, only site 44 (Waterford) contained more than 5 parous females. Midge activity ceased completely in late December 2009 (week 51). The 2009/2010 VFP ended in late April (week 17) when a total of 2,488 *Culicoides* were recorded from 6 sites, with the sample totals ranging from 1 to 1,456. There was some activity in the weeks leading up to week 17 but the samples did not contain the minimum requirement of 5 parous females. A total of 39 *Culicoides* were trapped during the 2009-2010 VFP none of which were parous females.

The observations made on the start and finish dates of *Culicoides* activity during the present 2007-2010 study period did not suggest that meteorological data can be used to accurately define the VFP. Therefore it is recommended that, pending additional information, it is best to operationally define the VFP by reference to the earliest start (week 49) and earliest end (week 13) recorded during the 2007-2010 surveys.

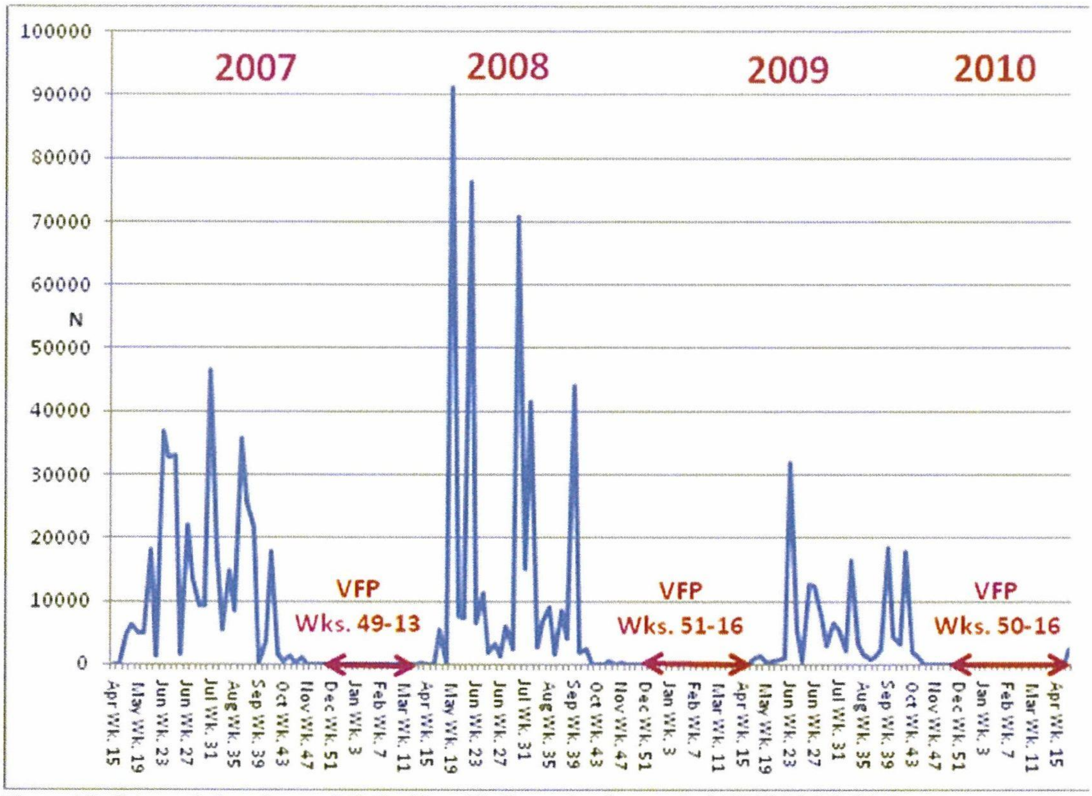

Figure 16 - Seasonal abundance of *Culicoides* with the vector free period (VFP) highlighted.

OVERVIEW AND RECOMMENDATIONS

The completion of the BTV vector surveillance programme, for which results are presented in this and earlier reports, provides an opportunity to review the status of our knowledge of *Culicoides* ecology and the potential BTV vectors present in Ireland. The work involved the first comprehensive survey of these insects in Ireland and the results reflect the scale and intensity of sampling. In addition, particular attention was paid to taxonomic determinations and to the establishment of a detailed database, which can be accessed in the future by other *Culicoides* researchers. The results give a good understanding of the composition of the *Culicoides* species assemblages present in Ireland, with 27 of the recorded Irish *Culicoides* species having been sampled. Likewise, the ecological diversity and geographical distribution of the sites sampled were very representative of the Irish landscape. Thus, the results provide a good platform for future research and a basis for establishment of a BTV vector monitoring programme in the event of the disease occurring in Ireland.

The 2007-2010 BTV vector surveillance work programme was both extensive (34 sites in 2007-8, 10 sites in 2009-2010) and intensive (weekly *Culicoides* sampling, environmental data compilation). The total number of *Culicoides* sampled (N=1,003,882) and fully identified (N=488,143), together with other laboratory work, will enable ongoing studies that may permit effective mathematical modelling of *Culicoides* population dynamics in Ireland. The results of the 2007-2010 work programme showed that potential BTV vectors are abundant and widely distributed in Ireland. Likewise, the results showed that the vector-free period was limited to December to late March/early April. Between-site variation in both species composition and dynamics of *Culicoides* assemblages has been documented. Though between-week variation in *Culicoides* abundances could often be related to weather conditions, the overall seasonality patterns were remarkably similar in the 3 years of the surveillance programme.

The completion of the 2007-2010 BTV surveillance programme requires arrangements to be made concerning the archiving of data, specimens and unpublished analyses of research results. In the event of an outbreak of BTV in Ireland in the future a modified vector monitoring programme may be required. Therefore, the manner in which the current programme is wound-down will be important. At present, the database, with paper and digital records is located in NUI, Galway. Likewise, *Culicoides* collections and other insect specimens sampled during 2007-2010 are stored in the former BTV Vector Ecology Laboratory and university stores. The future location of these, especially if there is no immediate threat of a BTV outbreak, is under consideration.

The expertise generated during the 2007-2010 programme was considerable, involving project management, field sampling, *Culicoides* taxonomy, data analyses, etc, and the need to ensure that such knowledge and skills are not lost may be important in the coming years. Though field sampling protocols and equipment are now fairly standard, concern must be expressed about future laboratory based aspects of BTV vector surveillance/monitoring in Ireland. Taxonomic training opportunities, which were available in 2006/2007 and which facilitated rapid start up of the 2007 work programme, may be much more difficult to secure in the future.

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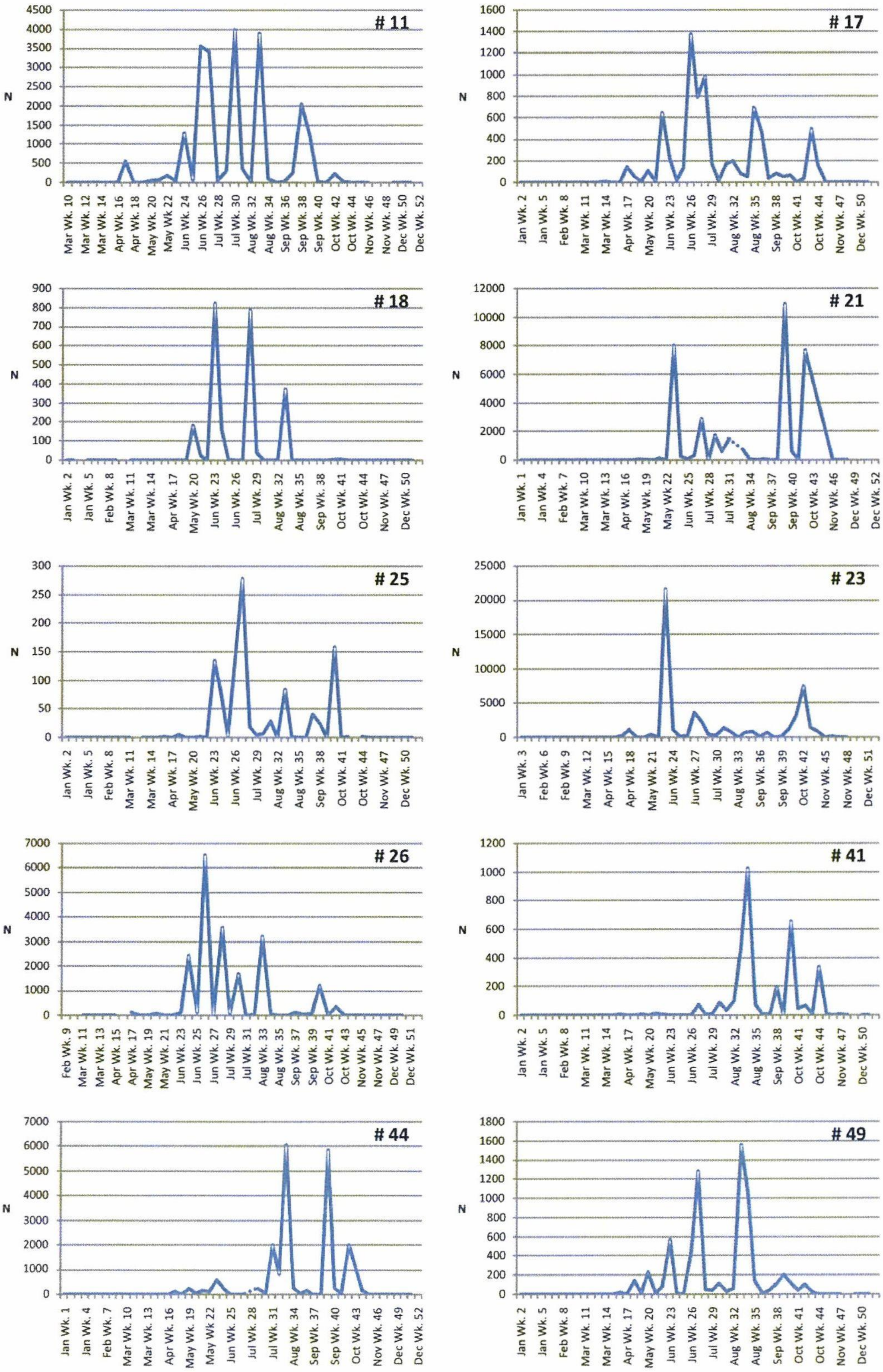
APPENDICES

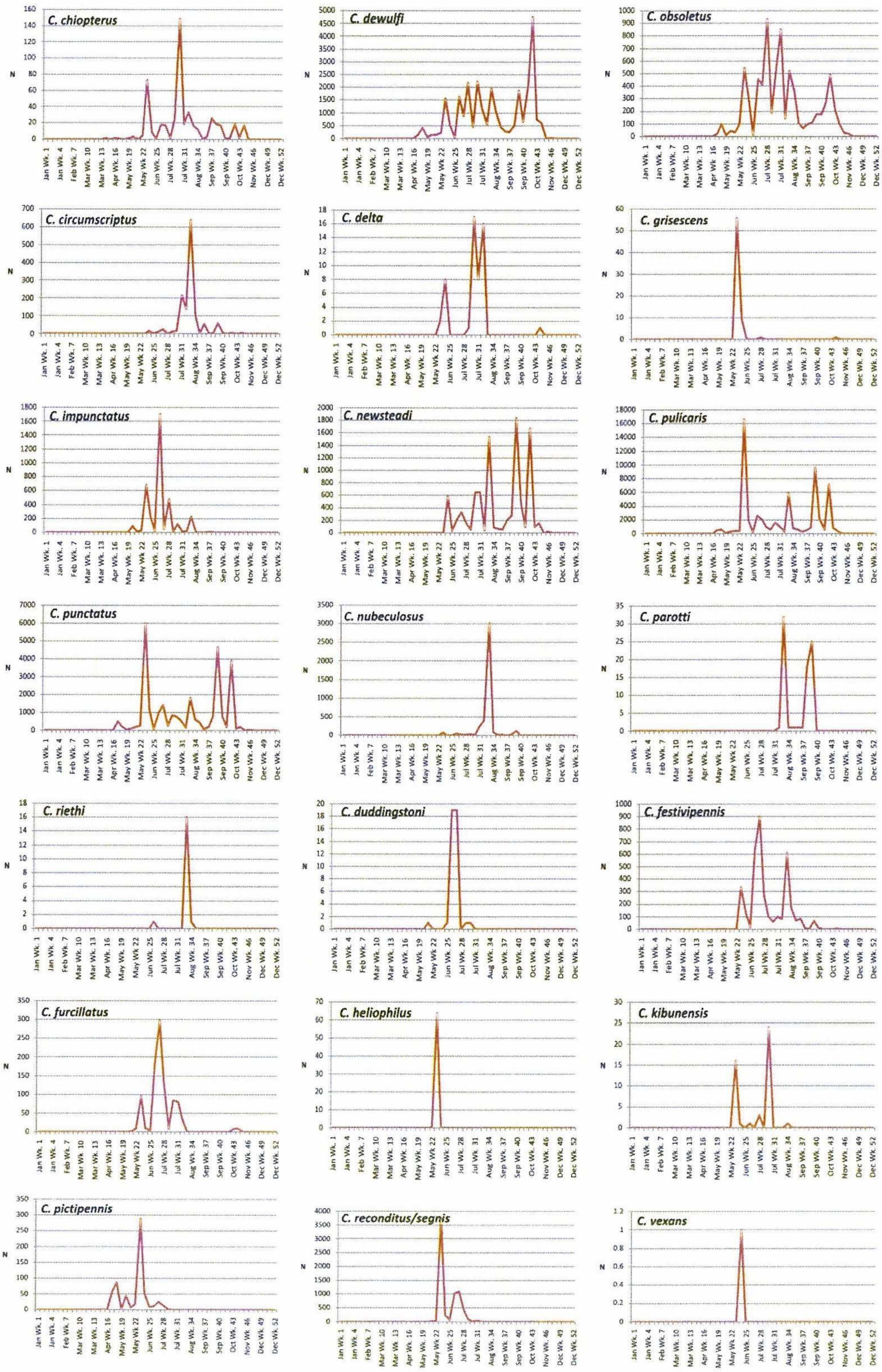
Appendix I

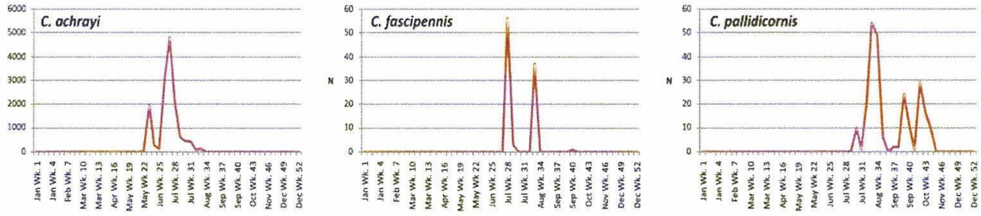
Weekly variations in *Culicoides* abundance at 10 sites in 2009.



Appendix II

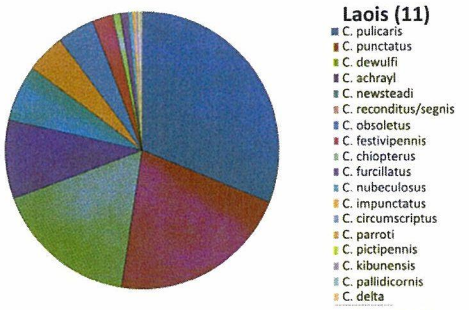
Weekly variations in counts for 25 species of *Culicoides* recorded at 10 sites (combined data) in 2009.

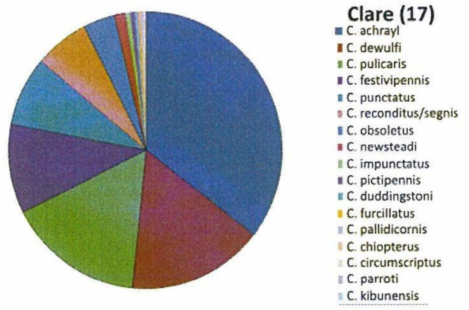


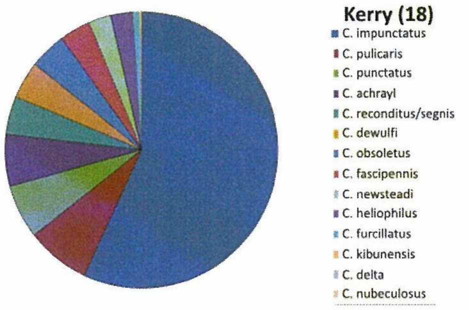

C. achrayi
C. fascipennis
C. pallidicornis
N

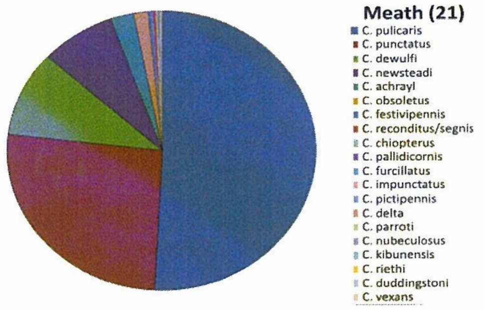
Appendix III

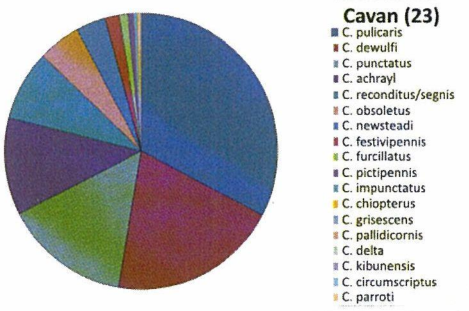
Taxonomic composition of the *Culicoides* species/species complex assemblages sampled from the 10 sites in 2009.

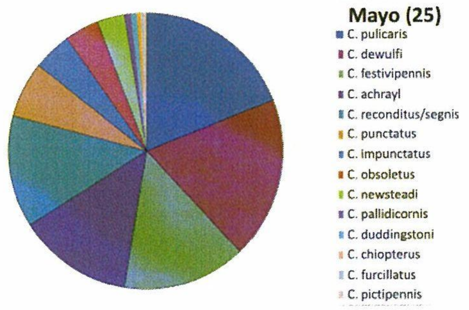


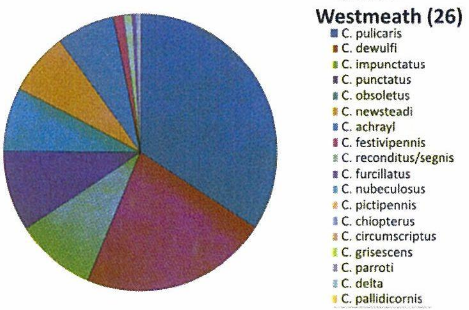


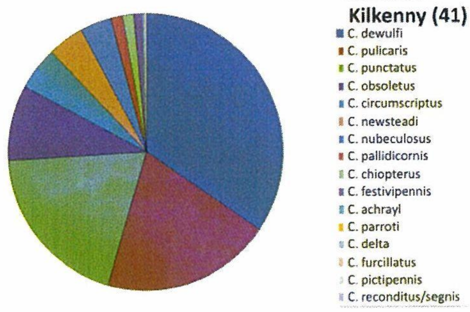


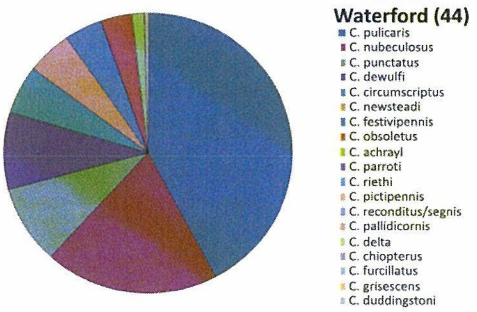


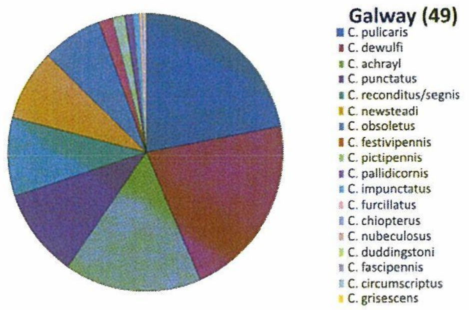






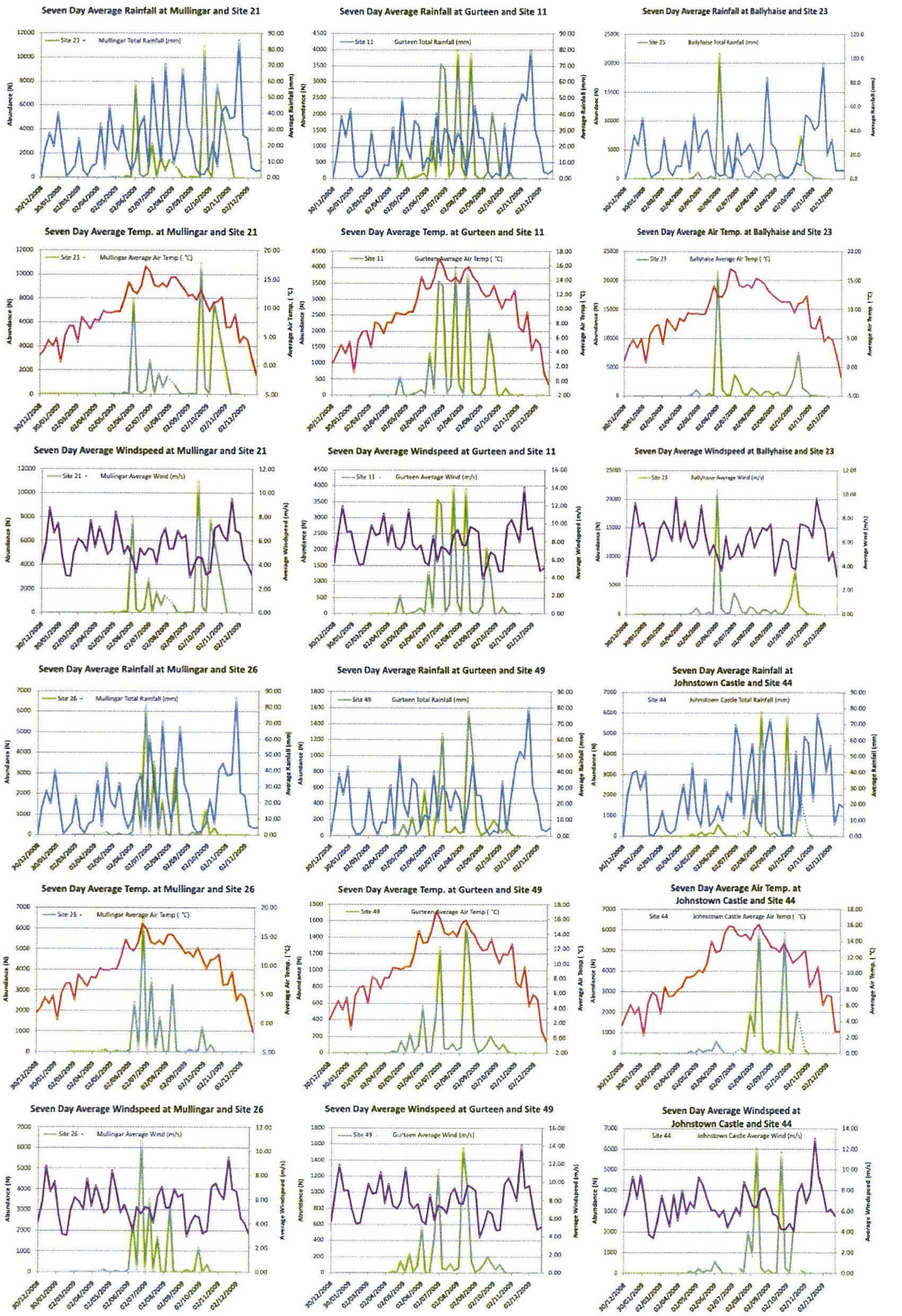


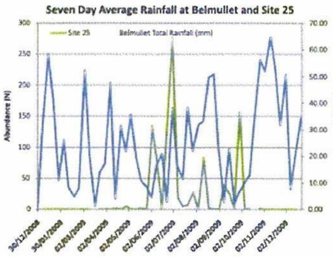


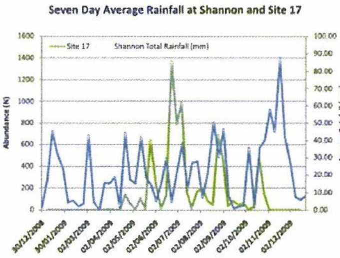


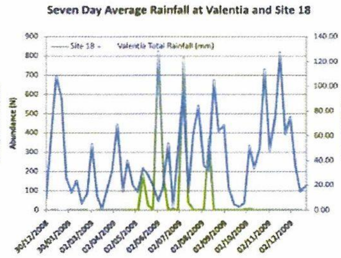
Appendix IV

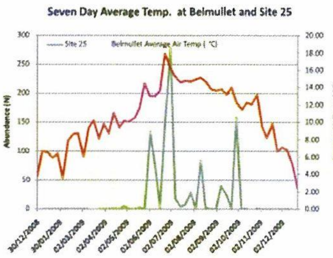
Met Éireann weekly mean data for rainfall, air temperature, wind speed and cloud cover (where available).

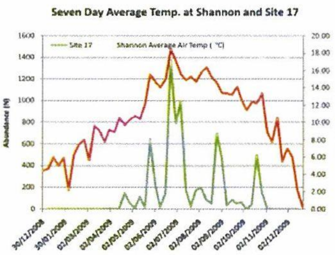


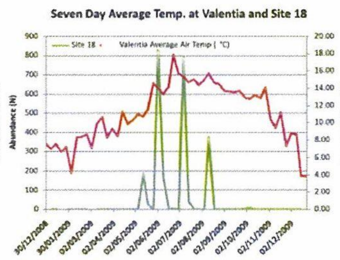

Seven Day Average Rainfall at Belmullet and Site 25

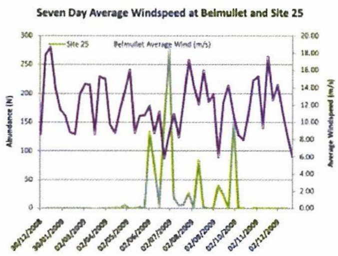

Seven Day Average Rainfall at Shannon and Site 17

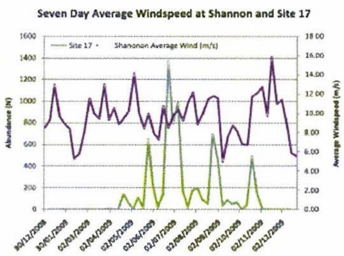

Seven Day Average Rainfall at Valentia and Site 18

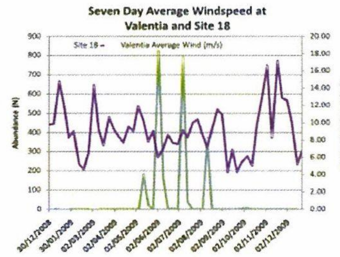

Seven Day Average Temp. at Belmullet and Site 25

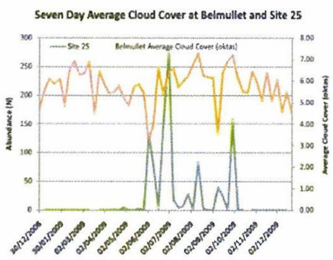

Seven Day Average Temp. at Shannon and Site 17

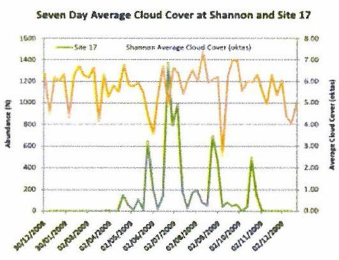

Seven Day Average Temp. at Valentia and Site 18

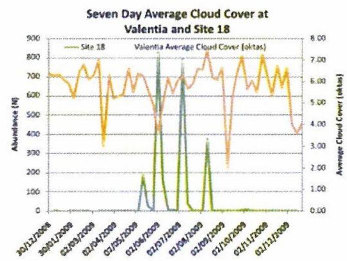

Seven Day Average Windspeed at Belmullet and Site 25

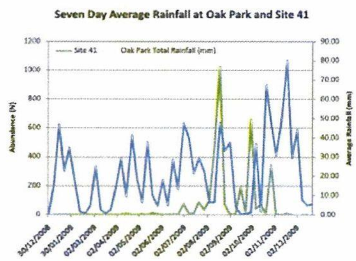

Seven Day Average Windspeed at Shannon and Site 17

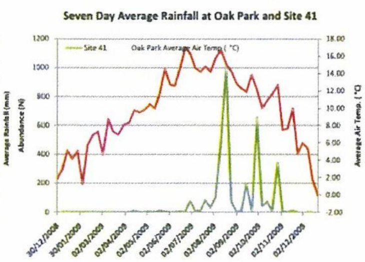

Seven Day Average Windspeed at
Valentia and Site 18

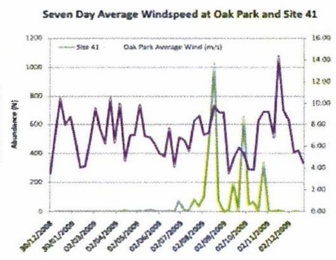

Seven Day Average Cloud Cover at Belmullet and Site 25


Seven Day Average Cloud Cover at Shannon and Site 17


Seven Day Average Cloud Cover at
Valentia and Site 18

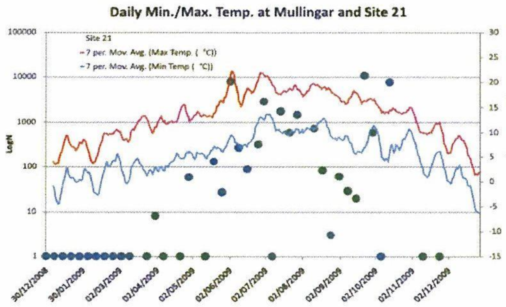

Seven Day Average Rainfall at Oak Park and Site 41

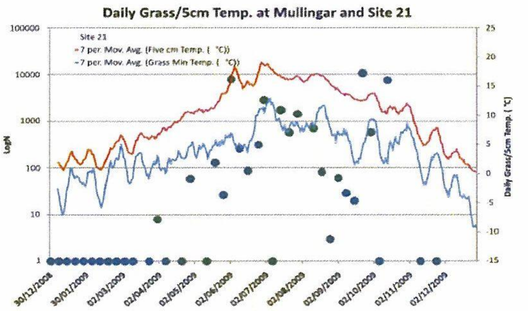

Seven Day Average Rainfall at Oak Park and Site 41

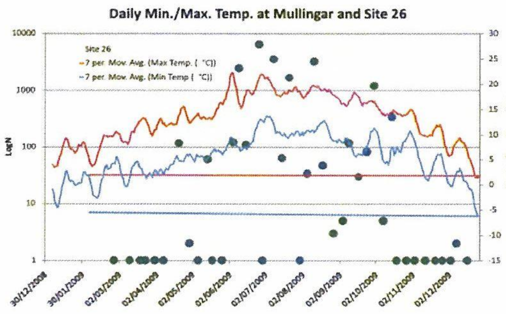

Seven Day Average Windspeed at Oak Park and Site 41

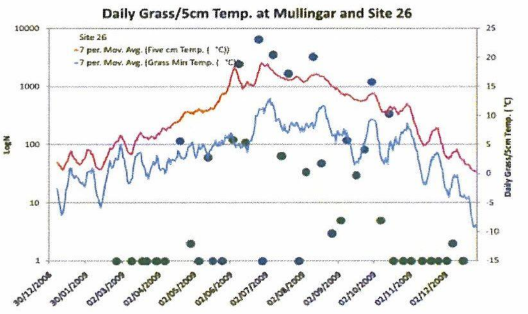
Appendix V

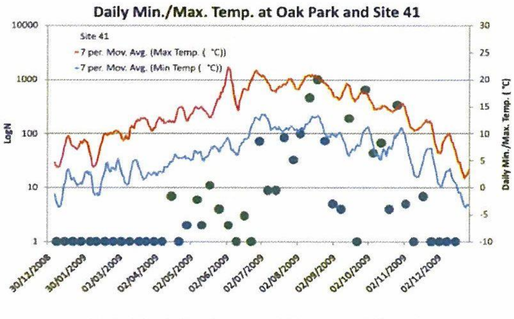
Met Éireann daily mean data for min./max.temperature grass temperature and 5cm soil temperature.

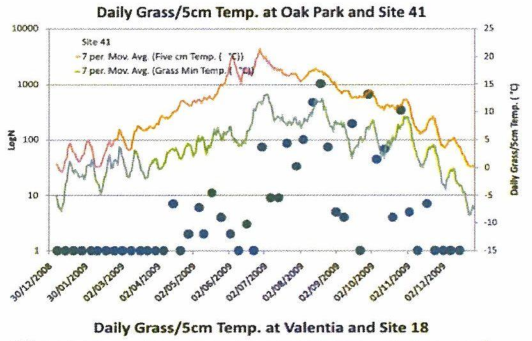


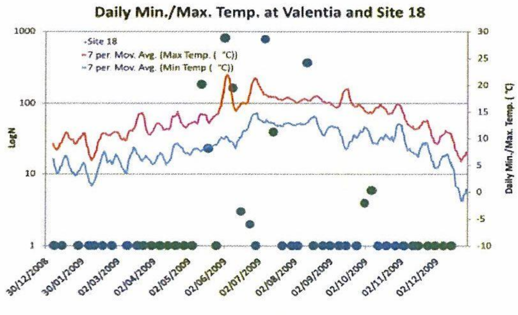


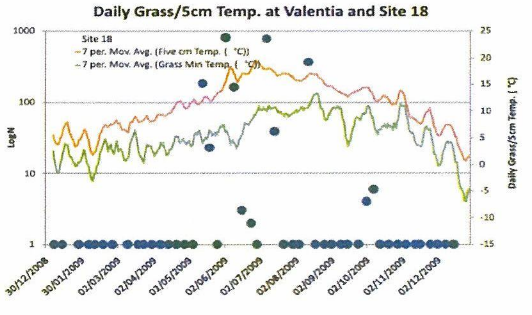


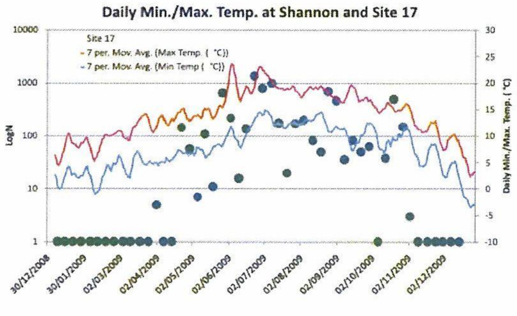


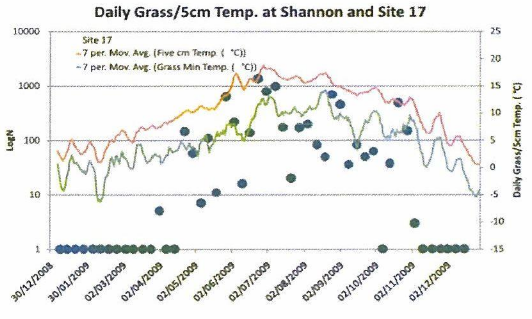




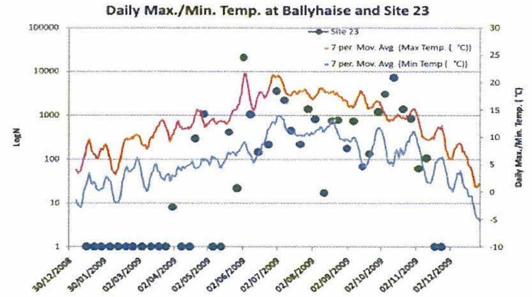




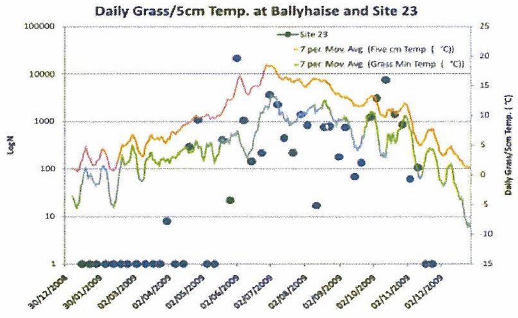




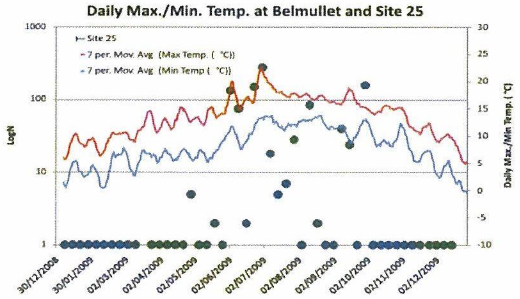
Daily Max./Min. Temp. at Ballyhaise and Site 23



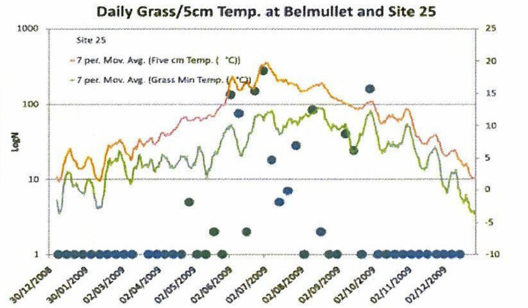
Daily Grass/5cm Temp. at Ballyhaise and Site 23



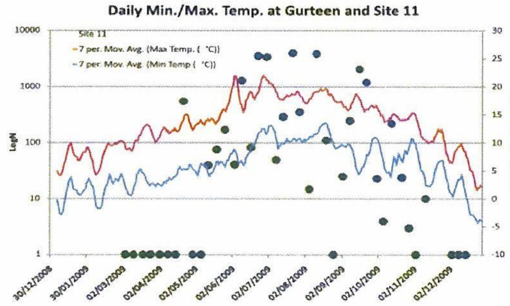
Daily Max./Min. Temp. at Belmullet and Site 25



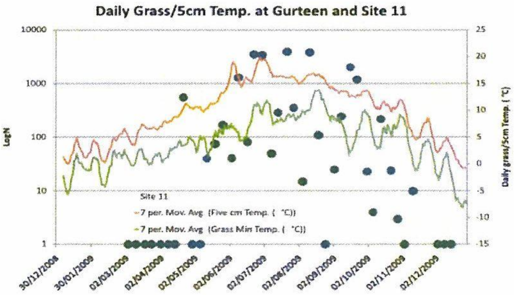
Daily Grass/5cm Temp. at Belmullet and Site 25



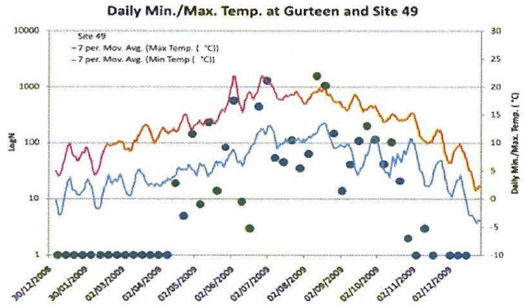
Daily Min./Max. Temp. at Gurteen and Site 11



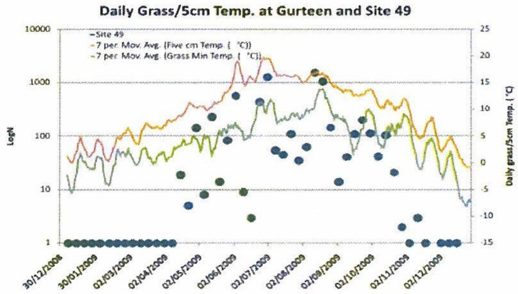
Daily Grass/5cm Temp. at Gurteen and Site 11



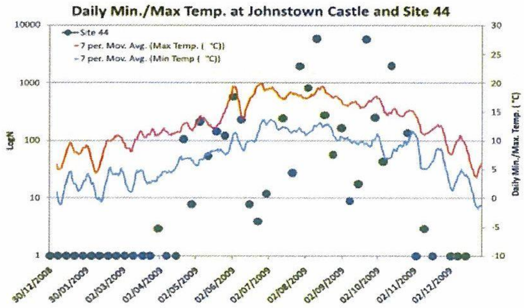
Daily Min./Max. Temp. at Gurteen and Site 49



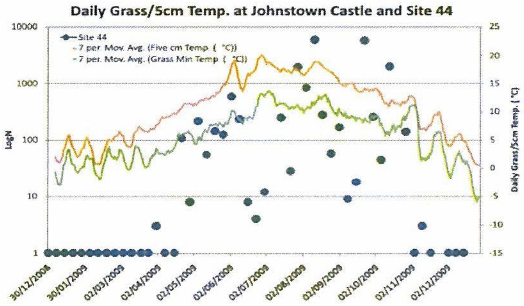
Daily Grass/5cm Temp. at Gurteen and Site 49



Daily Min./Max Temp. at Johnstown Castle and Site 44

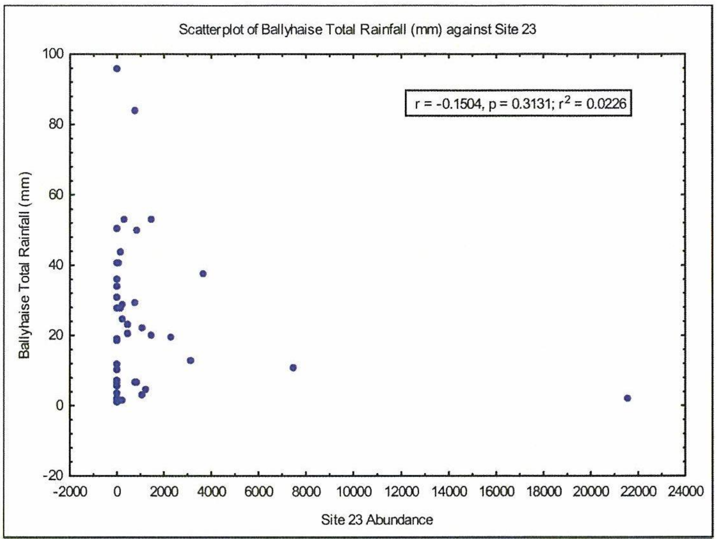


Daily Grass/5cm Temp. at Johnstown Castle and Site 44

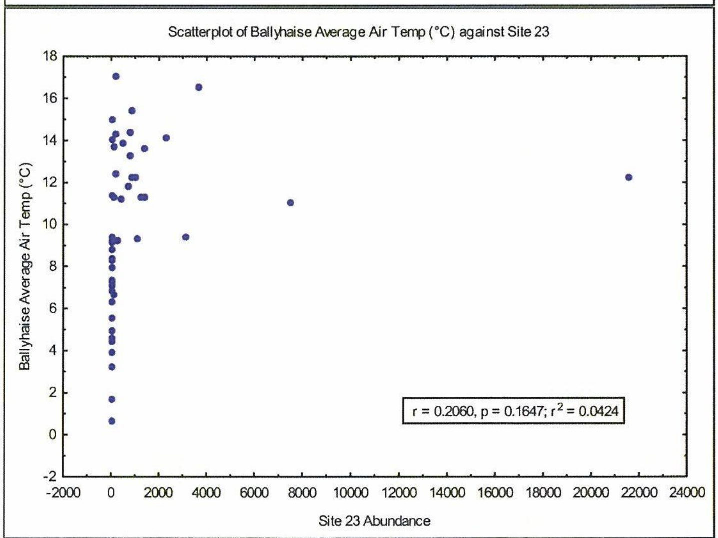


Appendix VI

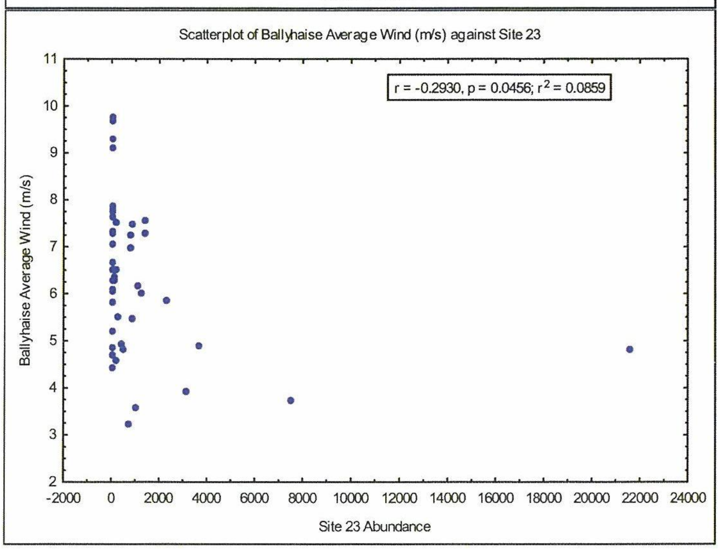
Spearman Rank Correlations between site and corresponding weather station parameters.



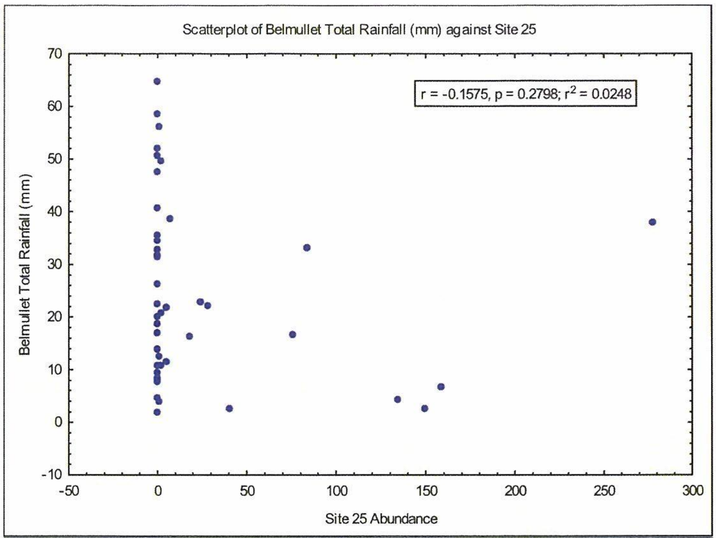
Scatterplot of Ballyhaise Total Rainfall (mm) against Site 23

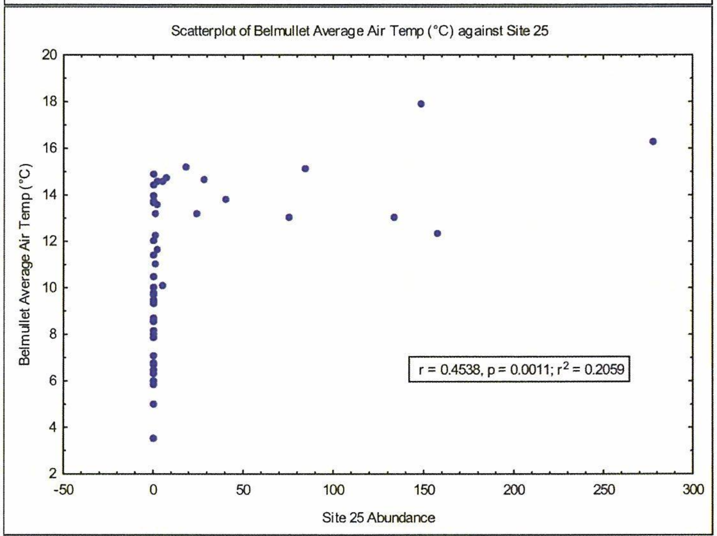


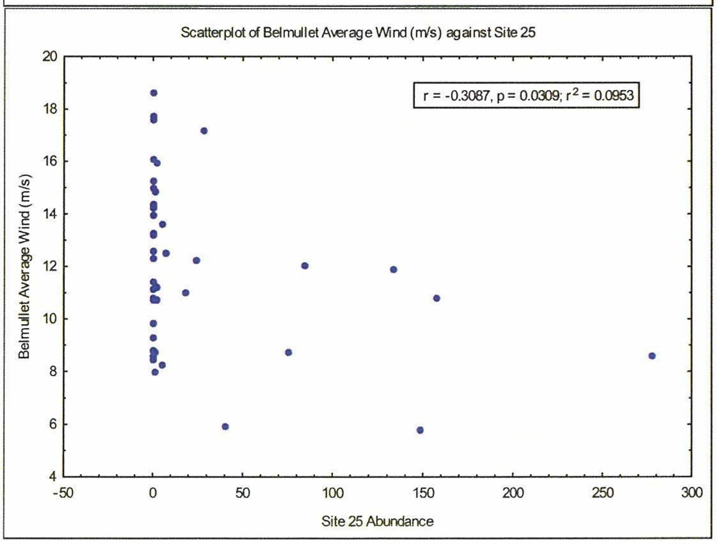
Scatterplot of Ballyhaise Average Air Temp (°C) against Site 23

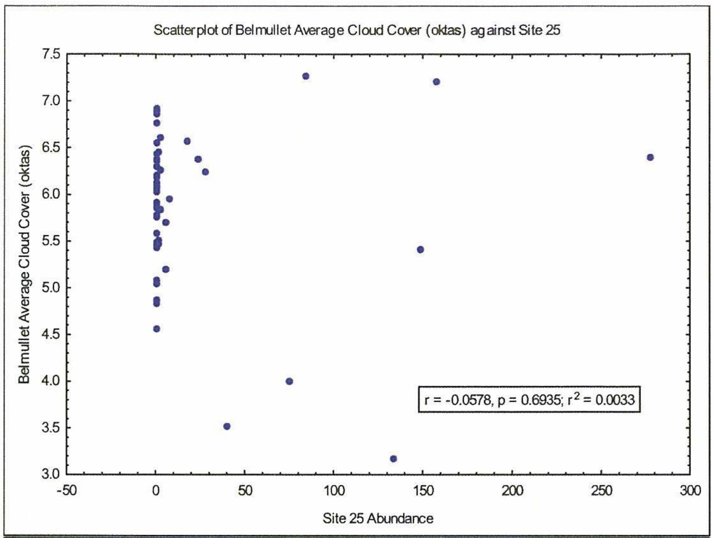


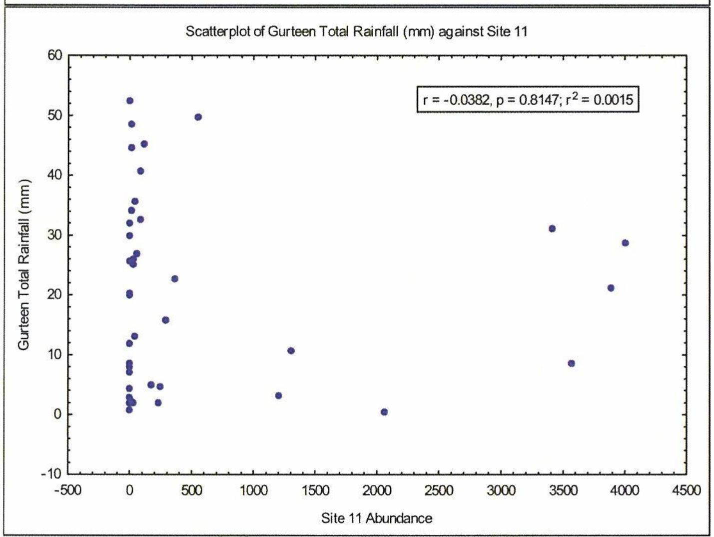
Scatterplot of Ballyhaise Average Wind (m/s) against Site 23

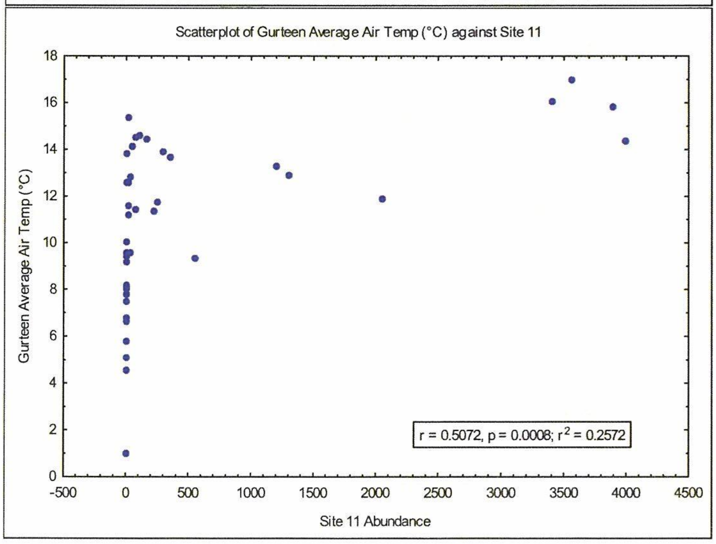

Scatterplot of Belmullet Total Rainfall (mm) against Site 25
r = -0.1575, p = 0.2798; r² = 0.0248
Belmullet Total Rainfall (mm)
Site 25 Abundance
70
60
50
40
30
20
10
0
-10
-50
0
50
100
150
200
250
300

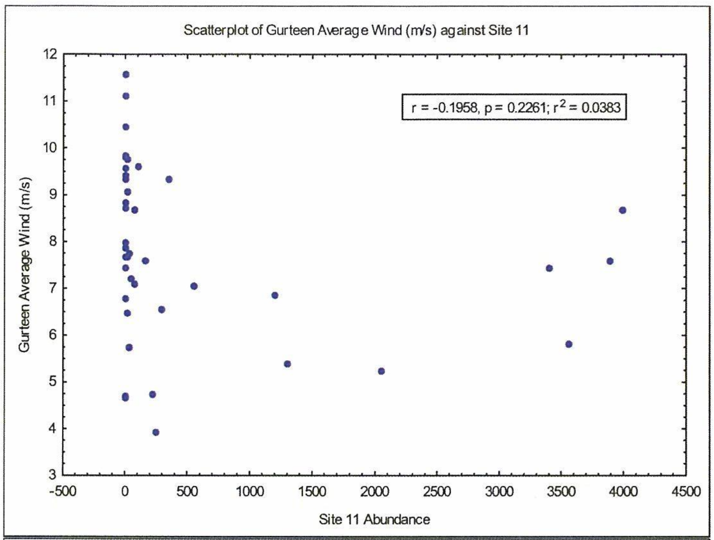

Scatterplot of Belmullet Average Air Temp (°C) against Site 25
r = 0.4538, p = 0.0011; r² = 0.2059
Belmullet Average Air Temp (°C)
Site 25 Abundance
20
18
16
14
12
10
8
6
4
2
-50
0
50
100
150
200
250
300


Scatterplot of Belmullet Average Wind (m/s) against Site 25
r = -0.3087, p = 0.0309; r² = 0.0953
Belmullet Average Wind (m/s)
Site 25 Abundance
20
18
16
14
12
10
8
6
4
-50
0
50
100
150
200
250
300

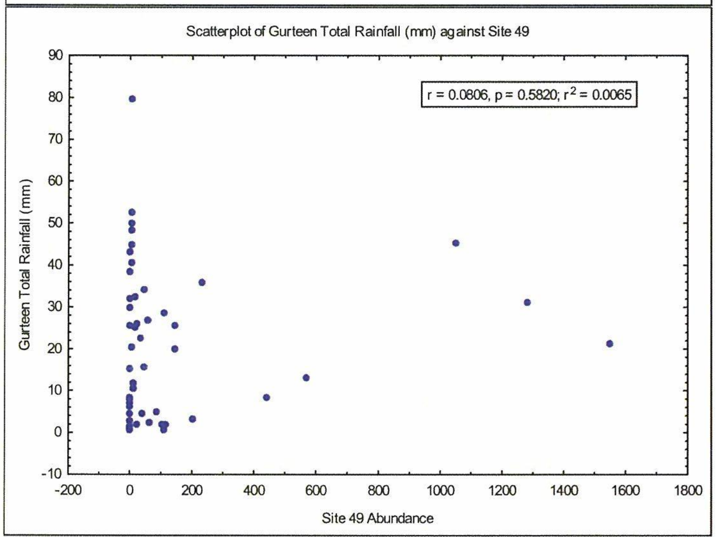

Scatterplot of Belmullet Average Cloud Cover (oktas) against Site 25
Belmullet Average Cloud Cover (oktas)
Site 25 Abundance
r = -0.0578, p = 0.6935; r2 = 0.0033


Scatterplot of Gurteen Total Rainfall (mm) against Site 11
Gurteen Total Rainfall (mm)
Site 11 Abundance
r = -0.0382, p = 0.8147; r2 = 0.0015

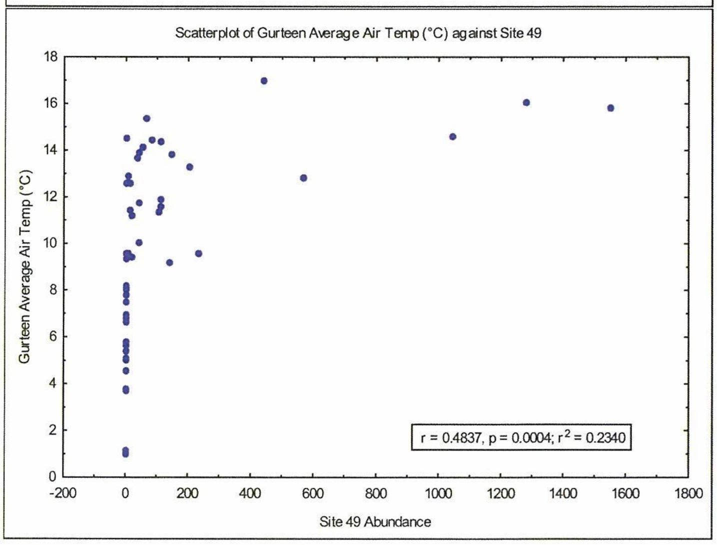

Scatterplot of Gurteen Average Air Temp (°C) against Site 11
Gurteen Average Air Temp (°C)
Site 11 Abundance
r = 0.5072, p = 0.0008; r2 = 0.2572



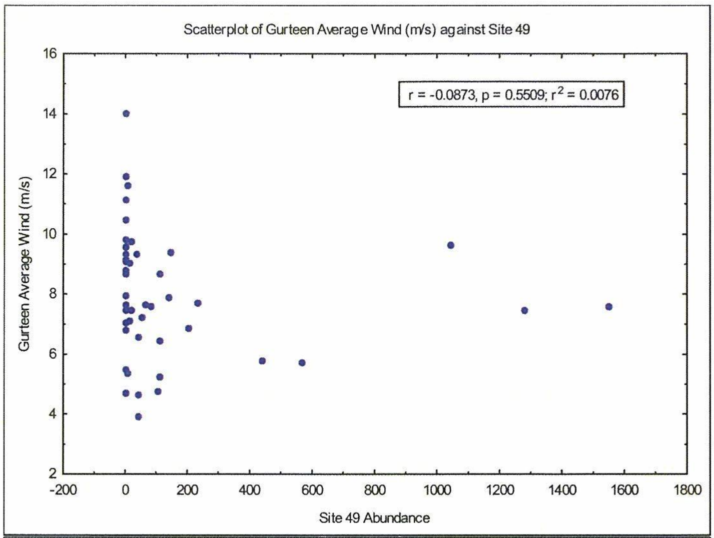
Scatterplot of Gurteen Average Wind (m/s) against Site 11
r = -0.1958, p = 0.2261; r2 = 0.0383
Gurteen Average Wind (m/s)
Site 11 Abundance

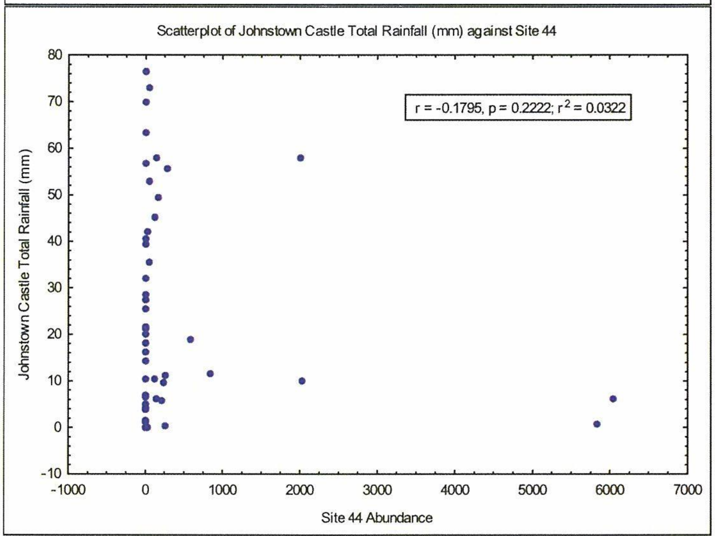


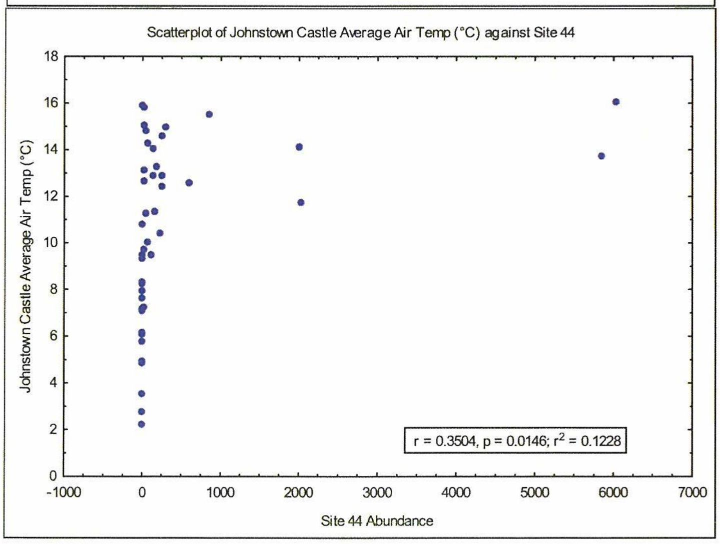
Scatterplot of Gurteen Total Rainfall (mm) against Site 49
r = 0.0806, p = 0.5820; r2 = 0.0065
Gurteen Total Rainfall (mm)
Site 49 Abundance

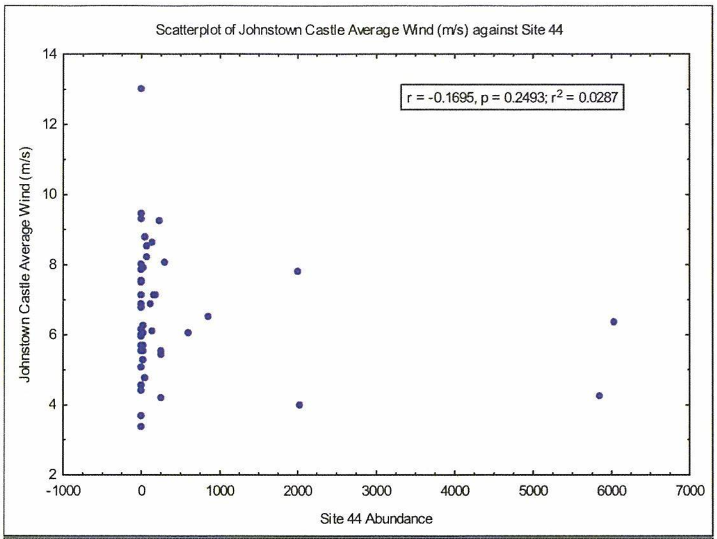


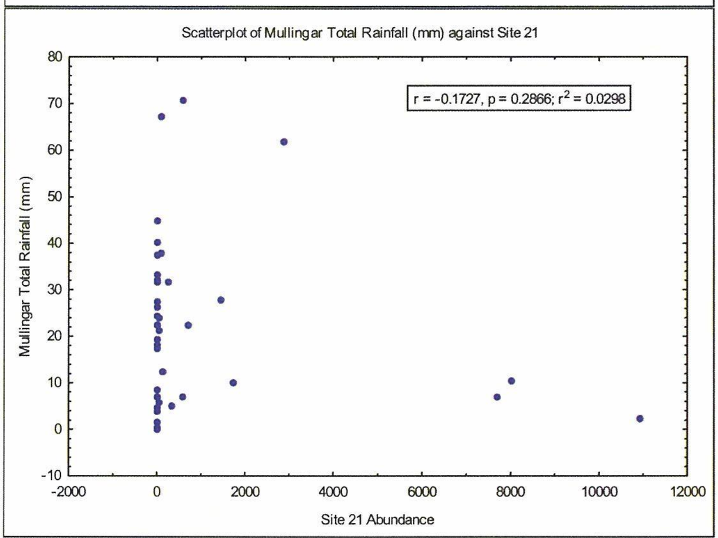
Scatterplot of Gurteen Average Air Temp (°C) against Site 49
r = 0.4837, p = 0.0004; r2 = 0.2340
Gurteen Average Air Temp (°C)
Site 49 Abundance

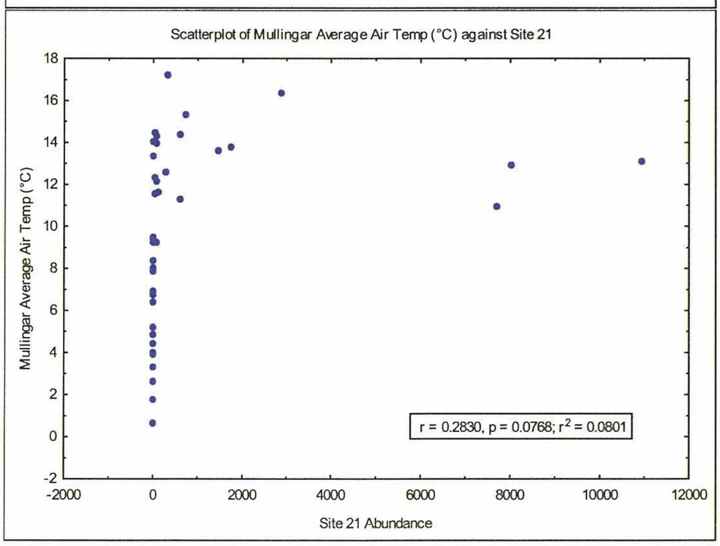

Scatterplot of Gurteen Average Wind (m/s) against Site 49
r = -0.0873, p = 0.5509; r² = 0.0076
Gurteen Average Wind (m/s)
Site 49 Abundance
16
14
12
10
8
6
4
2
-200
0
200
400
600
800
1000
1200
1400
1600
1800

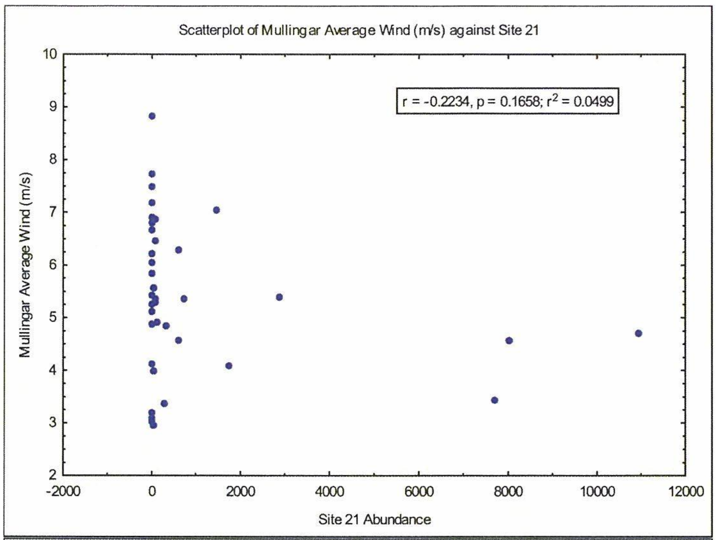

Scatterplot of Johnstown Castle Total Rainfall (mm) against Site 44
r = -0.1795, p = 0.2222; r² = 0.0322
Johnstown Castle Total Rainfall (mm)
Site 44 Abundance
80
70
60
50
40
30
20
10
0
-10
-1000
0
1000
2000
3000
4000
5000
6000
7000

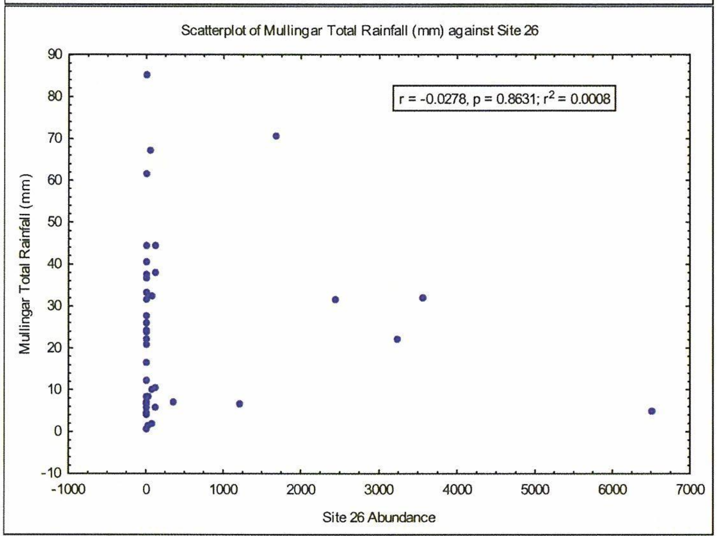

Scatterplot of Johnstown Castle Average Air Temp (°C) against Site 44
r = 0.3504, p = 0.0146; r² = 0.1228
Johnstown Castle Average Air Temp (°C)
Site 44 Abundance
18
16
14
12
10
8
6
4
2
0
-1000
0
1000
2000
3000
4000
5000
6000
7000

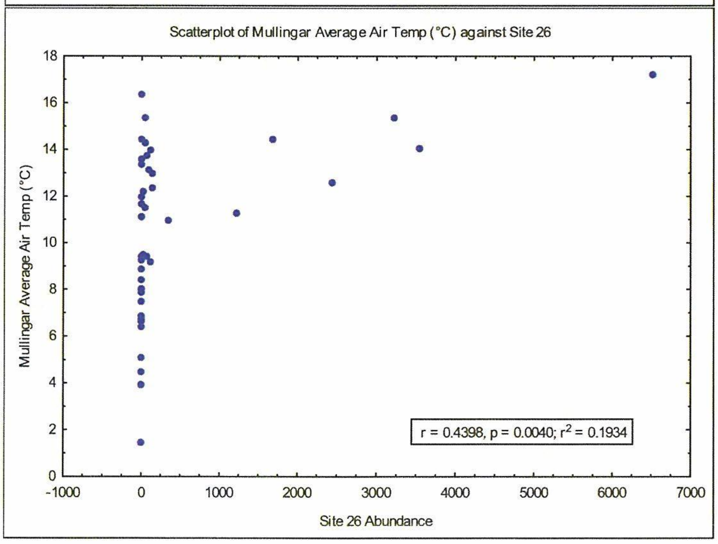

Scatterplot of Johnstown Castle Average Wind (m/s) against Site 44
Johnstown Castle Average Wind (m/s)
Site 44 Abundance
r = -0.1695, p = 0.2493; r2 = 0.0287
14
12
10
8
6
4
2
-1000
0
1000
2000
3000
4000
5000
6000
7000

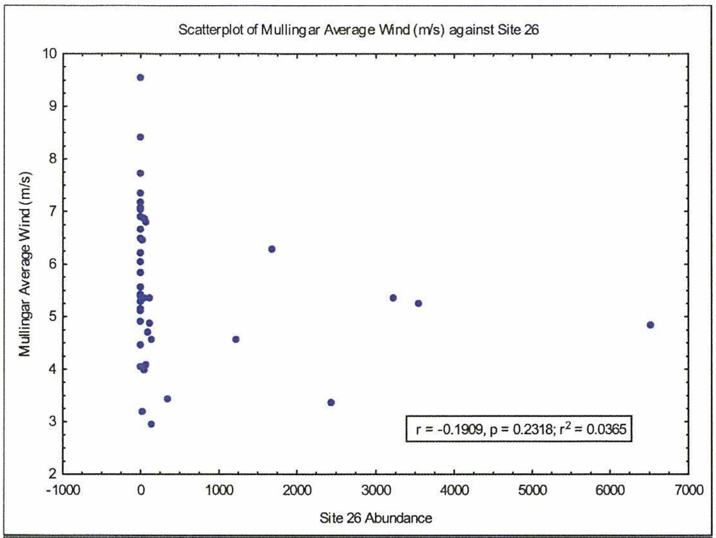

Scatterplot of Mullingar Total Rainfall (mm) against Site 21
Mullingar Total Rainfall (mm)
Site 21 Abundance
r = -0.1727, p = 0.2866; r2 = 0.0298
80
70
60
50
40
30
20
10
0
-10
-2000
0
2000
4000
6000
8000
10000
12000

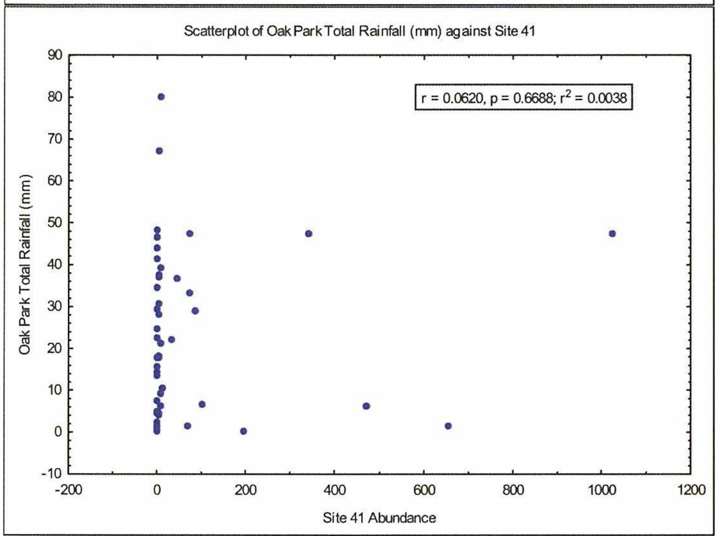

Scatterplot of Mullingar Average Air Temp (°C) against Site 21
Mullingar Average Air Temp (°C)
Site 21 Abundance
r = 0.2830, p = 0.0768; r2 = 0.0801
18
16
14
12
10
8
6
4
2
0
-2
-2000
0
2000
4000
6000
8000
10000
12000

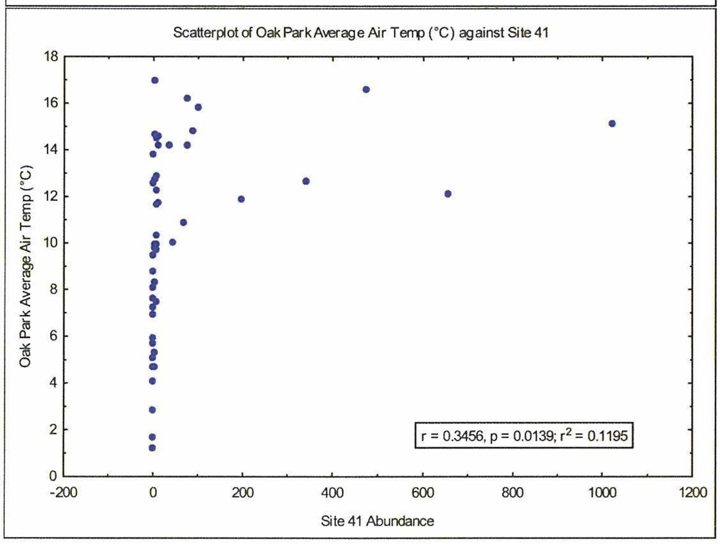

Scatterplot of Mullingar Average Wind (m/s) against Site 21
r = -0.2234, p = 0.1658; r2 = 0.0499
Mullingar Average Wind (m/s)
Site 21 Abundance
10
9
8
7
6
5
4
3
2
-2000
0
2000
4000
6000
8000
10000
12000

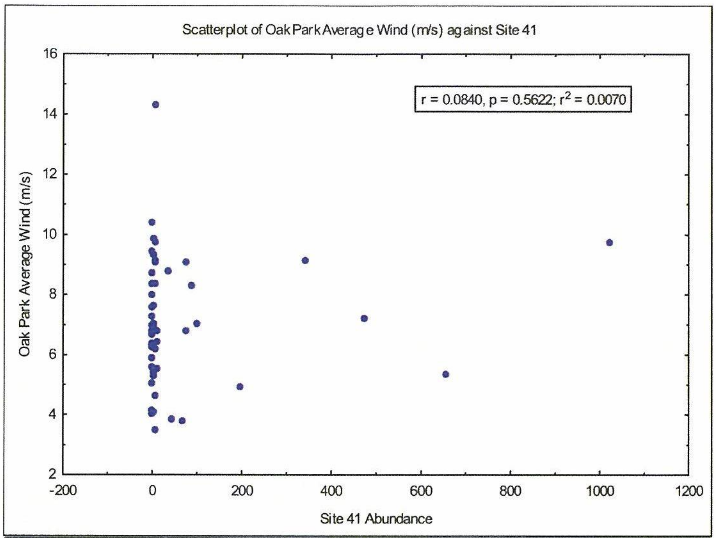

Scatterplot of Mullingar Total Rainfall (mm) against Site 26
r = -0.0278, p = 0.8631; r2 = 0.0008
Mullingar Total Rainfall (mm)
Site 26 Abundance
90
80
70
60
50
40
30
20
10
0
-10
-1000
0
1000
2000
3000
4000
5000
6000
7000

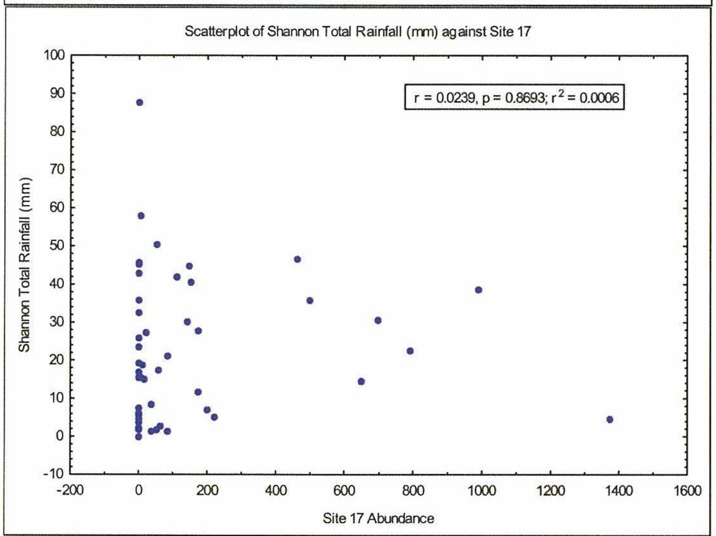

Scatterplot of Mullingar Average Air Temp (°C) against Site 26
r = 0.4398, p = 0.0040; r2 = 0.1934
Mullingar Average Air Temp (°C)
Site 26 Abundance
18
16
14
12
10
8
6
4
2
0
-1000
0
1000
2000
3000
4000
5000
6000
7000

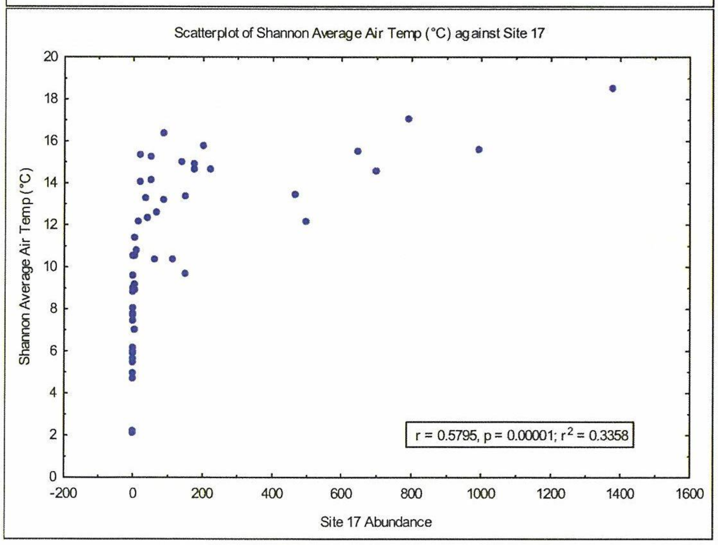

Scatterplot of Mullingar Average Wind (m/s) against Site 26
Mullingar Average Wind (m/s)
Site 26 Abundance
r = -0.1909, p = 0.2318; r2 = 0.0365

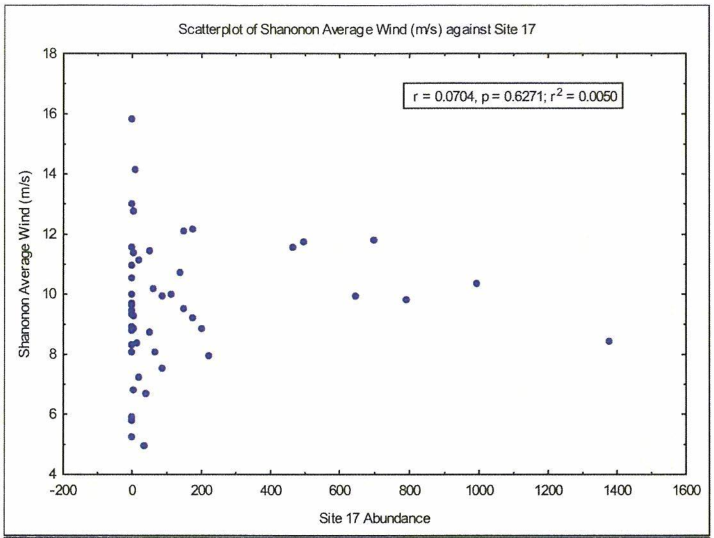

Scatterplot of Oak Park Total Rainfall (mm) against Site 41
Oak Park Total Rainfall (mm)
Site 41 Abundance
r = 0.0620, p = 0.6688; r2 = 0.0038

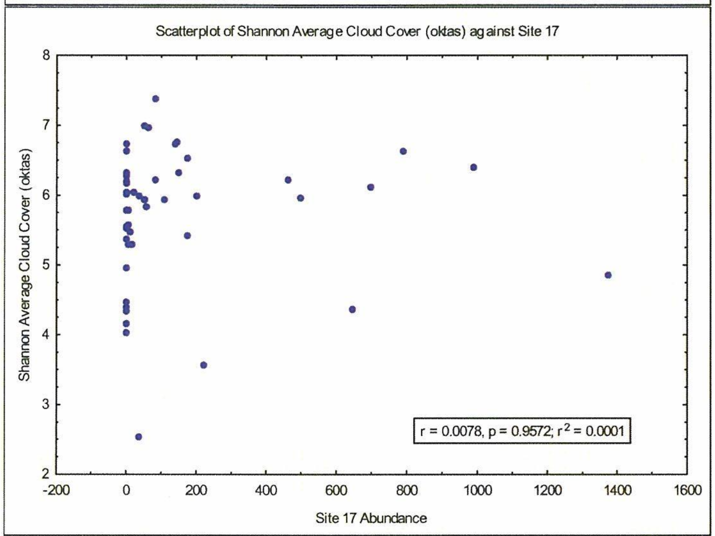

Scatterplot of Oak Park Average Air Temp (°C) against Site 41
Oak Park Average Air Temp (°C)
Site 41 Abundance
r = 0.3456, p = 0.0139; r2 = 0.1195

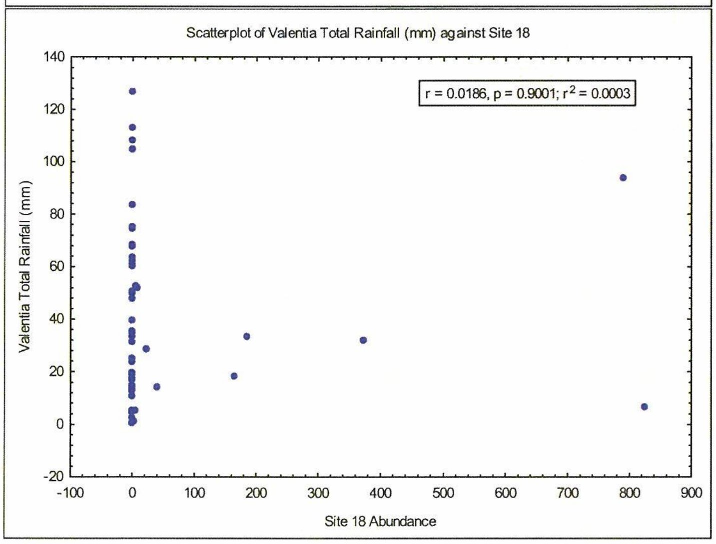

Scatterplot of Oak Park Average Wind (m/s) against Site 41
r = 0.0840, p = 0.5622; r² = 0.0070
Oak Park Average Wind (m/s)
Site 41 Abundance

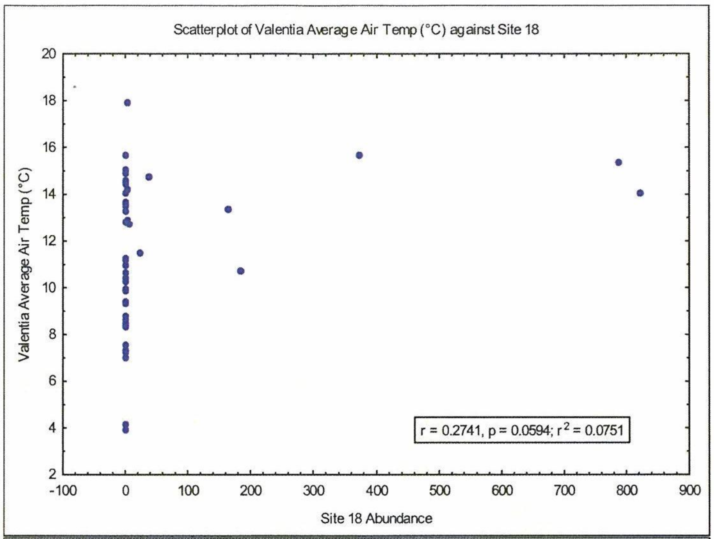

Scatterplot of Shannon Total Rainfall (mm) against Site 17
r = 0.0239, p = 0.8693; r² = 0.0006
Shannon Total Rainfall (mm)
Site 17 Abundance

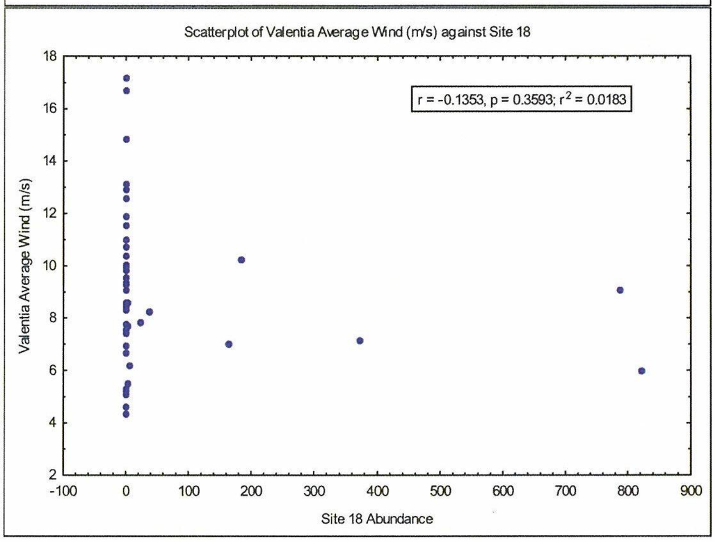

Scatterplot of Shannon Average Air Temp (°C) against Site 17
r = 0.5795, p = 0.00001; r² = 0.3358
Shannon Average Air Temp (°C)
Site 17 Abundance


Scatterplot of Shanonon Average Wind (m/s) against Site 17
r = 0.0704, p = 0.6271; r² = 0.0050
Shanonon Average Wind (m/s)
Site 17 Abundance


Scatterplot of Shannon Average Cloud Cover (oktas) against Site 17
r = 0.0078, p = 0.9572; r² = 0.0001
Shannon Average Cloud Cover (oktas)
Site 17 Abundance


Scatterplot of Valentia Total Rainfall (mm) against Site 18
r = 0.0186, p = 0.9001; r² = 0.0003
Valentia Total Rainfall (mm)
Site 18 Abundance


Scatterplot of Valentia Average Air Temp (°C) against Site 18
Valentia Average Air Temp (°C)
Site 18 Abundance
r = 0.2741, p = 0.0594; r2 = 0.0751
20
18
16
14
12
10
8
6
4
2
-100
0
100
200
300
400
500
600
700
800
900


Scatterplot of Valentia Average Wind (m/s) against Site 18
Valentia Average Wind (m/s)
Site 18 Abundance
r = -0.1353, p = 0.3593; r2 = 0.0183
18
16
14
12
10
8
6
4
2
-100
0
100
200
300
400
500
600
700
800
900