UNIVERSITY OF LIVERPOOL

Quantitative Analysis of the Ecology and Feeding Behaviour of *Aedes detritus*

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy

by Aislinn Rhiannon Currie-Jordan

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Dedication

I would like to dedicate this thesis to my Grandad, Dr Anthony Jordan.

I am forever inspired by you.

Acknowledgements

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Abstract

The United Kingdom is currently at risk from mosquito-borne viruses with several native mosquito species capable of virus transmission. Ongoing surveillance efforts are important to provide a detailed understanding of the ecology and distribution of mosquito species within the UK. This thesis aimed to explore the entomological aspects of the risk posed to the UK from mosquito-borne viruses.

The seasonality and distribution of mosquito species along the Dee estuary was assessed during a three year longitudinal study. Twelve different species were collected with clear seasonal trends in mosquito abundance identified. *Aedes detritus* and *Anopheles claviger* were the most abundant species.

The ecology of the immature stages of *Aedes detritus* was further explored with reference to the impact of tidal flooding on this species. Seasonal trends in abundance were identified with the number of mosquito larvae greatly reduced in the summer months as a result of breeding site desiccation. A drone was successfully used to produce high resolution maps of these breeding sites. These maps show how mosquito breeding sites, on the Dee estuary salt marsh, change over the course of a year.

Field studies were undertaken to compare methods of sampling anthropophilic mosquitoes in the UK. Several trapping methods were identified as having the potential to be used in place of human landing catches. This is especially important considering the potential for future virus transmission in the UK.

Given the likelihood of arbovirus introduction, it is important that we understand which of our native mosquito species may act as disease vectors in the future. To this end, the vector competence of *Aedes detritus* to Japanese encephalitis virus was assessed under different temperature conditions.

Collectively, this thesis provides further insight into the ecology, feeding behaviour and vector competence of UK mosquito species. It is important that surveillance within the UK continues to mitigate the risk posed from mosquito-borne viruses and invasive mosquito species.

List of Abbreviations

Ae.	*Aedes*
AIC	Akaike Information Criterion
An.	*Anopheles*
APHA	Animal and Plant Health Agency
Bti	*Bacillus thuringiensis israelensis*
BTV	Blue Tongue Virus
C.	*Culicoides*
CDC	Centers for Disease control and Prevention (USA)
cDNA	Complementary deoxyribonucleic acid
CHIKV	Chikungunya Virus
CI	Confidence Interval
CO_2	Carbon dioxide
Cq.	*Coquillettidia*
Cs.	*Culiseta*
Cx.	*Culex*
DENV	Dengue Virus
DNA	deoxyribonucleic acid
DTR	Diurnal Temperature Range
ECDC	European Centre for Disease Prevention and Control
E-Grid	Electric Grid
EIP	Extrinsic Incubation Period
ELISA	Enzyme Linked Immunosorbent Assay
ERTS	Earth Resources Technology Satellite
GLMM	Generalised Linear Mixed Model
GPS	Global Positioning System
h	Hour
HDT	Host Decoy Trap
HLC	Human Landing Catch
Hz	Hertz
JEV	Japanese Encephalitis Virus
kHz	Kilo Hertz
km	Kilometre
kV	Kilo Volt
L	Litre
LA	Local Authority
LITE	Liverpool Insect Testing Establishment
LSM	Larval Source Management
LSTM	Liverpool School of Tropical Medicine

m	Meter
m/s	Meters per Second
µl	Microlitre
MET	Mosquito Electrocuting Trap
mJ	Millijoule
ml	millilitre
MRS	Mosquito Reporting Scheme
ms	Millisecond
nM	Nanomolar
NOC	National Oceanography Centre
^{o}C	degrees Celsius
OMWM	Open Marsh Water Management
P.	*Plasmodium*
PCR	Polymerase Chain Reaction
pfu	Plaque Forming Units
PHE	Public Health England
p-value	Probability Value
rcf	Relative Centrifugal Force
RLGC	Royal Liverpool Golf Course
RNA	Ribonucleic acid
RRV	Ross River Virus
RSPB	Royal Society for the Protection of Birds
RVF	Rift Valley Fever
s.l.	sensu lato
SE	Standard Error
SINV	Sindbis Virus
SSSI	Site of Special Scientific Interest
TAHV	Tahyna Virus
UAVs	Unmanned Aerial Vehicles
UK	United Kingdom
USUV	Usutu Virus
VEEV	Venezuelan Equine Encephalitis Virus
WNV	West Nile Virus
ZIKV	Zika Virus

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Chapter 1 - General Introduction

1.1 Introduction to Mosquitoes in the United Kingdom

There are currently 36 species of mosquito that have been reported in the United Kingdom (UK) (Table 1.1) (Medlock and Vaux, 2009, Medlock and Snow, 2008). These species can be split across six different genera: *Aedes* (17), *Anopheles* (6), *Conquillettidia* (1), *Culex* (4), *Culiseta* (7) and *Orthopodomyia* (1) (Table 1.1). The most recent additions to the list of UK species include: *Aedes geminus* (*Ae. geminus*)*, Aedes nigrinus* (*Ae. nigrinus*)*, Culex modestus* (*Cx. modestus*) and *Aedes albopictus* (*Ae. albopictus)* (Medlock *et al.,* 2017, Medlock and Vaux, 2009, Golding *et al.,* 2012, Harbach *et al.,* 2017) (Table 1.1). *Ae. geminus* was included in the list of British mosquitoes following the examination of several museum specimens, previously identified as *Aedes cinereus* (*Ae. cinereus*) (Medlock and Vaux, 2009). *Ae. geminus* was only identified as a separate species to *Ae. cinereus* in the 1970s meaning that, prior to this date, specimens may have been mis-identified (Medlock and Vaux, 2009). In 2010 an established population of *Cx. modestus* was found in the marshland of north Kent (Golding *et al.,* 2012). *Ae. nigrinus* larvae were collected in 2016 in southern England (Harbach *et al.,* 2017). This species is morphologically similar to *Aedes sticticus* (*Ae. sticticus*) and it is thought that previous *Ae. nigrinus* samples may have been mis-identified (Harbach *et al.,* 2017). *Ae. albopictus* eggs have been collected in oviposition traps in southern England over the last three years although adults of this species have not been caught (Medlock *et al.,* 2018, Medlock *et al.,* 2017).

Freely available information from the National Biodiversity Network Gateway can be used to create distribution map of all species of mosquito found in the UK correct to 10 km (Figure 1.1). The maps demonstrate the widespread distribution of mosquitoes in the UK although with ongoing research, these maps are constantly being updated. Table 1.1 gives an overview of the habitat, seasonality and voltinism of the mosquitoes present in the UK.

UK populations of mosquito have specific seasonal activities (Table 1.1). For univoltine species, adult numbers have a single peak in the spring and then decline

throughout the summer months. Larvae of these species are reported to be found all year round (Table 1.1). Several peaks in mosquito abundance are observed for the multivoltine mosquito species (Medlock and Vaux, 2015b). Mosquitoes are known to exploit a range of breeding sites including temporary pools and ditches, salt marsh and tree-holes (Medlock *et al.,* 2012a). In urban areas there are several species of mosquito that utilise containers, such as water butts in gardens, as a breeding site (Cranston *et al.,* 1987). A study into container breeding mosquitoes in southern England reports that one species, *Culex pipiens* (*Cx. pipiens*) was highly abundant in water containers in urban compared to rural gardens (Townroe and Callaghan, 2014). A nationwide mosquito surveillance programme run by Public Health England (PHE) is currently ongoing providing detailed information on the seasonality and distribution of mosquitoes in the UK.

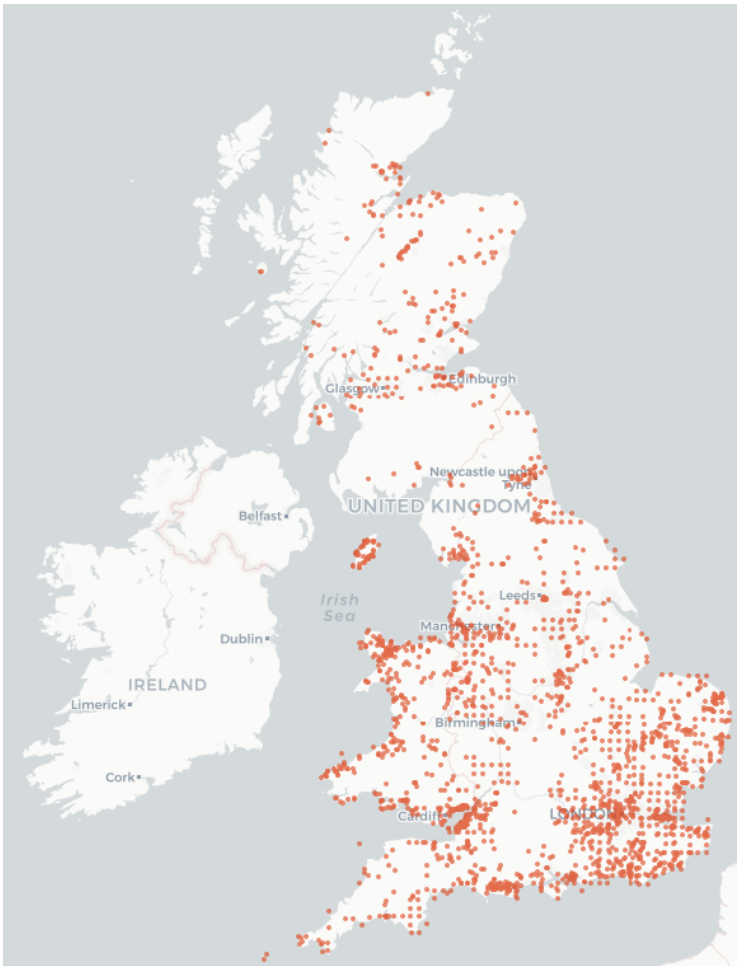

Figure 1.1: Map of the distribution of all species of mosquito found in the United Kingdom (National Biodiversity Atlas, 2019).

Table 1.1: Summary of the 36 species of mosquito found in the United Kingdom along with their habitat and seasonality. Those marked with an * are rare in the UK therefore some information in unknown (Medlock *et al.,* 2005, Medlock *et al.,* 2012a, Medlock, 2014, Vaux *et al.,* 2015, Cranston *et al.,* 1987, Bessell *et al.,* 2014, Medlock *et al.,* 2007, Service, 1971a, Medlock and Vaux, 2015b, Gillooly and Dodson, 2000, Medlock and Vaux, 2009, Medlock *et al.,* 2017, Harbach *et al.,* 2017, Golding *et al.,* 2012, Yates, 1979).

Species	**Habitat**	**Annual Generations**	**Seasonality of Larvae**	**Seasonality of Adults**
Aedes albopictus (Skuse, 1895)	Artificial containers	Multivoltine	*	*
Aedes annulipes (Meigen 1830)	Woodland	Univoltine	November-April	April-September
Aedes cantans (Meigen 1818)	Woodland	Univoltine	January-July	April-October
Aedes caspius (Pallas 1771)	Brackish water	Multivoltine	April-October	April-October
Aedes cinereus (Meigen 1818)	Reed beds and flooded grasslands	Univoltine	April-June	June-September
Aedes communis (DeGeer 1776)	Shaded pools	*	*	*
Aedes detritus (Haliday 1833)	Brackish water	Multivoltine	January-December	March-November
Aedes dorsalis (Meigen 1830)	Salt marsh	Multivoltine	*	May-September
Aedes flavescens (Muller 1764)	Salt marsh	Univoltine	*	May-July
Aedes geminus (Peus, 1970)	Fresh water, floodwater	Univoltine	April-June	June-September
Aedes geniculatus (Olivier 1791)	Tree holes	Multivoltine	*	May-September
Aedes leucomelas (Meigen 1804)	Fresh water pools and ditches	*	*	*

Aedes nigrinus (Eckstein, 1918)	Floodwater	Univoltine	May	September
Aedes punctor (Kirby 1837)	Woodland	Univoltine	November-August	May-October
Aedes rusticus (Rossi 1790)	Woodland	Univoltine	*	*
Aedes sticticus (Meigen 1838)	Temporary water collections	Multivoltine	*	*
Aedes vexans (Meigen 1830)	Flooded grasslands	Multivoltine	*	*
Anopheles algeriensis (Theobald 1903)	Marshland	*	*	*
Anopheles atroparvus (van Thiel 1927)	Brackish water	Multivoltine	May-September	March-October
Anopheles claviger (Meigen 1804)	Fens, ditches and ponds	Multivoltine	January-December	March-November
Anopheles daciae (Linton, Nicolescu, Harbach 2004)	Permanent Waters	Multivoltine	May-September	March-October
Anopheles messeae (Falleroni 1926)	Fens, ditches and ponds	Multivoltine	May-September	March-October
Anopheles plumbeus Stephens 1828)	Tree holes	Bivoltine	January-December	April-October
Conquillettidia richiardii (Ficalbi 1889)	Fens, ditches and ponds	Univoltine	January-December	June-September
Culex europaeus	Small, permanent collections of ground water	*	*	*
Culex modestus (Ficalbi 1889)	Marsh and ditches	*	January-December	January-December
Culex pipiens s.s	Permanent Waters	Multivoltine	April-November	May-September
Culex torrentium (Martini 1889)	Permanent waterbodies	Multivoltine	April-November	April-November

Culiseta alakaensis (Ludlow 1906)	*	*	*	March-October
Culiseta annulata (Schrank 1776)	Fens, ditches and ponds. Containers in urban environments	Multivoltine	January-December	All year round
Culiseta fumipennis (Stephens 1825)	Open water bodies	Univoltine	*	*
Culiseta litorea (Shute 1928)	Coastal pools	Univoltine	January-December	May-September
Culiseta longiareolata (Macquart 1838)	*	*	*	*
Culiseta morsitans (Theobald 1901)	Fens, ditches and ponds	Univoltine	September-June	May-October
Culiseta subochrea (Edwards 1921)	Fens, ditches and ponds	*	*	*
Orthopodomyia pulcripalpis (Rondani 1872)	Tree holes	*	*	June-October

1.2 Feeding Preferences and Host Seeking Behaviour of UK Mosquito Species

It is important to understand the range of hosts that mosquitoes take a blood meal from when looking to understand the ecology of mosquito populations. Information on host preference provides an insight into identifying potential vectors of both human and animal diseases which, in turn, can identify bridge vectors of zoonotic diseases (Takken and Verhulst, 2013, Brugman *et al.,* 2015). Molecular methods can be used to determine host preference through analysis of blood meals from wild caught mosquitoes (Takken and Verhulst, 2013). However, collection of wild blood fed mosquitoes can prove difficult. Additionally, several other factors can have an impact on mosquito host preference including both genetic and environmental factors such as seasonality and host abundance (Takken and Verhulst, 2013). The known host preferences of the UK mosquitoes are summarised in Table 1.2.

Molecular methods have been used to determine the host preference of several species of mosquito in the UK. Historically, the precipitin and enzyme-linked immunosorbent assay (ELISA) tests were used to identify blood meal origin. (Service, 1969) used precipitin tests to determine host preference of 12 different species of mosquito. In the 1980s ELISA was used to identify the origin of blood meals from *Aedes cantans* (*Ae. cantans*) and *Ae. punctor* (*Ae. punctor*) (Renshaw *et al.,* 1994, Service *et al.,* 1986). More recently, PCR-sequencing has been used to determine blood meal origin from blood fed mosquitoes collected in Kent (Brugman *et al.,* 2017a). Blood meal origin was successfully determined in 72% of the 964 mosquitoes tested with five mammalian and fourteen bird host species identified from nine different mosquito species (Brugman *et al.,* 2017a). Importantly, this study identified that several species of migratory bird are fed on by *Cx. pipiens* and *Cx. modestus* highlighting the potential risk of arbovirus introduction into the UK mosquito populations (Brugman *et al.,* 2017a).

Observational studies, directly visualising mosquitoes feeding from a host, can also be used as a measure of host preference. Human landing catches (HLC) are the gold standard when looking to determine which species of mosquitoes are feeding on humans (Briët *et al.,* 2015). HLCs conducted in Dorset reported human feeding from six different mosquito species (Service, 1971a). More recently, HLC studies were conducted in southern England with 15 different species/species groups collected (Brugman *et al.,*

2017b). It is also possible to use other vertebrate hosts in observational studies, although this is far less common. Several studies report mosquito species that are attracted to and feed from rabbits (Service, 1971b, Brugman *et al.,* 2015, Muirhead-Thomson, 1956a, Muirhead-Thomson, 1956b). Further to this, there is evidence that mosquitoes may be involved in the transmission of myxomatosis virus in the UK (Brugman *et al.,* 2015). In 2013, an investigation using bird-baited (chicken) traps was undertaken in southern England (Brugman *et al.,* 2018b). *Cx. pipiens sl/Cx. torrentium*, *Cx. modestus* and *Coquillettidia richiardii* (*Cq. richiardii*) were collected during this study providing further information of host seeking behaviour of these mosquito species (Brugman *et al.,* 2018b). In 2016, three species of mosquito (*Aedes detritus* (*Ae. detritus*)*, Culiseta annulata* (*Cs. annulata*) and *Anopheles claviger* (*An. claviger*)), were all found to have fed from horses during a study exploring the impact of repellents to reduce the numbers of mosquitoes biting horses (Chapman, 2017).

Table 1.2: Summary of the 36 species of mosquito found in the United Kingdom along with their host preference. Those marked with an * are rare therefore some information in unknown (Medlock *et al.,* 2005, Medlock *et al.,* 2012a, Medlock, 2014, Vaux *et al.,* 2015, Cranston *et al.,* 1987, Bessell *et al.,* 2014, Medlock *et al.,* 2007, Service, 1971a, Medlock and Vaux, 2015b, Gillooly and Dodson, 2000, Golding *et al.,* 2012, Brugman *et al.,* 2017a, Renshaw *et al.,* 1994, Brugman *et al.,* 2017b, Chapman, 2017).

Species	**Host Preference**
Aedes albopictus	Wide range of hosts in Europe
Aedes annulipes	Humans and domestic animals
Aedes cantans	Bovids, humans and birds
Aedes caspius	Humans and birds
Aedes cinereus	Bovids, humans and birds
Aedes communis	Humans and birds infrequently
Aedes detritus	Humans, bovids, horses and birds
Aedes dorsalis	Humans and birds infrequently
Aedes flavescens	Humans and other mammals

Aedes geminus	Humans, bovids and birds
Aedes geniculatus	Humans and birds infrequently
Aedes leucomelas	*
Aedes nigrinus	*
Aedes punctor	Humans and birds infrequently
Aedes rusticus	Humans and birds infrequently
Aedes sticticus	Humans
Aedes vexans	Birds and Humans
Anopheles algeriensis	Humans and birds infrequently
Anopheles atroparvus	Animals more than humans
Anopheles claviger	Humans, bovids, horses and birds
Anopheles daciae	*
Anopheles messeae	Humans and birds infrequently
Anopheles plumbeus	Humans and birds
Conquillettidia richiardii	Humans, bovids and birds
Culex europaeus	Birds and Humans
Culex modestus	Humans, horses and birds
Culex pipiens s.s	Humans, Birds
Culex torrentium	Birds
Culiseta alakaensis	*
Culiseta annulata	Humans, bovids, rabbits, horses and birds
Culiseta fumipennis	Birds
Culiseta litorea	Humans, birds and mammals infrequently
Culiseta longiareolata	*
Culiseta morsitans	Humans, birds
Culiseta subochrea	Humans and domestic animals
Orthopodomyia pulcripalpis	Birds

1.3 Mosquito Nuisance Biting and Control in the UK

There are several species of mosquito in the UK that will feed on humans (Table 1.2) meaning that there are several species that are considered nuisance biters. There are four publications on mosquito nuisance biting in the UK. The first in 1969-1970 (Service, 1970), then in 1985-1986 (Snow, 1986), again in 1996 (Snow, 1996) and finally in 2009 (Medlock *et al.,* 2012a). The most recent survey was conducted across all 347 local authorities in the UK (Medlock *et al.,* 2012a). A total of 221 responses were gathered with 57 local authorities reporting mosquito nuisance data during the last ten years (Medlock *et al.,* 2012a). *Cs. annulata, Ae. detritus, Cx pipiens s.l., Ae. cantans* and *Anopheles. maculipennis s.l.* (*An. maculipennis*) were the main species involved in nuisance biting with seven other species also reported (Medlock *et al.,* 2012a). Eleven of the local authorities reported mosquito control; usually in areas that have a long history of nuisance biting including at sewage works in London and salt marsh habitats in Cheshire (Medlock *et al.,* 2012a). Control is often focused on treating the larval habitats with *Bacillus thuringiensis israelensis* (Bti) (Medlock *et al.,* 2012a). *Ae. detritus* is the main nuisance species in the salt marsh habitats of Cheshire and control with *Bti* took place in this area annually from 1986 (Clarkson and Setzkorn, 2011). This salt marsh habitat is a Site of Special Scientific Interest (SSSI) making mosquito control in this protected habitat somewhat challenging and larval control has now ceased in this area.

1.4 The Risk to the UK from Emerging and Remerging Vector-Borne Diseases

Mosquito-borne pathogens, particularly viruses, pose an ever-increasing threat to human health. Whilst historically associated with the tropics, there have been recent outbreaks of disease as a result of mosquito-borne viruses in temperate latitudes (Medlock *et al.,* 2005). The large outbreak of West Nile virus (WNV) in America in 1999, which spread to 47 states resulting in over 16,000 cases, raised the profile of arbovirus transmission in temperate regions (Medlock *et al.,* 2005). Additionally, the spread of *Culicoides*-borne viruses affecting livestock throughout Europe has also increased awareness of vector borne diseases. For example, in 2006 there was an outbreak of Bluetongue virus (BTV) in northern Europe, including in the UK, transmitted by indigenous *Culicoides* species from the *C. obsoletus* and *C. pulicaris* groups (Guis *et al.,*

2012, Mehlhorn *et al.,* 2007, Kiehl *et al.,* 2009). As a result of the outbreak, there are strict rules on the movement of potentially infected livestock which, in turn, has a knock-on economic impact on the farming industry (Carpenter *et al.,* 2009). Following on from the emergence of BTV, there was an outbreak of another midge-borne virus, Schmallenberg, in 2011. Schmallenberg disease is characterised by a fever and a reduction in milk yield in livestock, cattle in particular (Doceul *et al.,* 2013, Beer *et al.,* 2012). In pregnant animals, high rates of abortion and foetal abnormality also occur (Beer *et al.,* 2012). Schmallenberg was first reported in Germany in 2011 with the virus first detected in UK livestock populations in January 2012 (Doceul *et al.,* 2013). The virus is also transmitted largely by species from the *C. obsoletus* group (Regge *et al.,* 2012, Doceul *et al.,* 2013).

There are currently no mosquito-borne human diseases transmitted in the UK although increasing instances of disease outbreaks in Europe, including malaria and several arboviruses, means we have a heightened awareness of the risk these pathogens pose to the UK (Medlock *et al.,* 2018). The emergence and re-emergence of vector-borne diseases in Europe has highlighted the need to re-evaluate the ability of UK mosquito and midge species to transmit diseases of public health importance.

1.4.1 Malaria

Mosquitoes are well documented vectors of malaria in the tropics but they also used to transmit malaria in most European countries, including the UK (Zhao *et al.,* 2016, Dobson, 1980). Throughout the 20th century, the range of malaria transmission decreased and by 1975 Europe was declared free of endemic malaria transmission (Zhao *et al.,* 2016). There were several factors that led to the elimination of malaria from Europe including both natural and human driven factors (Schaffner *et al.,* 2012, Zhao *et al.,* 2016, Kuhn *et al.,* 2003). In France, the decline is thought to be related to a shift in the *Anopheles* vectors from feeding largely on humans to livestock, whilst changes in land use and improved socioeconomic conditions are thought to be responsible for the reduction of malaria incidences in the UK (Hackett and Missiroli, 1931, Dobson, 1980, Lindsay *et al.,* 2010a). Vector control programmes, including elimination of breeding sites, also

contributed to the elimination of malaria from Europe, particularly in the Mediterranean countries (Schaffner *et al.,* 2012).

The primary vectors of the malaria parasite *Plasmodium vivax* (*P. vivax*) in Europe belonged to the *An. maculipennis* complex, specifically *Anopheles atroparvus* (*An. atroparvus*) and *Anophles labranchiae* (*An. labranchiae*)*,* along with several other minor vector species (Schaffner *et al.,* 2012). Several of the minor vector species are found in the UK as well as mainland Europe: *An. messeae* (*An. messeae*)*, An. claviger, An. algeriensis* (*An. algeriensis*) and *An. plumbeus* (*An. plumbeus*) (Schaffner *et al.,* 2012). Models developed to determine the likelihood of *P. vivax* malaria returning to the UK concluded that, despite future temperatures being favourable for transmission, low biting rates means that there is a low risk of future malaria transmission in the UK (Lindsay *et al.,* 2010b).

1.4.2 West Nile Virus

WNV is a flavivirus, family Flaviviridae, and is a member of the Japanese encephalitis serocomplex (De Madrid and Porterfield, 1974, Chancey *et al.,* 2015). The virus was first isolated from the blood of a Ugandan woman in 1937 and has since spread across the globe facilitated by the spread of the mosquito vector (Hayes and Gubler, 2006). The virus is transmitted by mosquitoes and can infect birds, humans and horses (Campbell *et al.,* 2002). The transmission cycle of WNV is summarised in Figure 1.2.

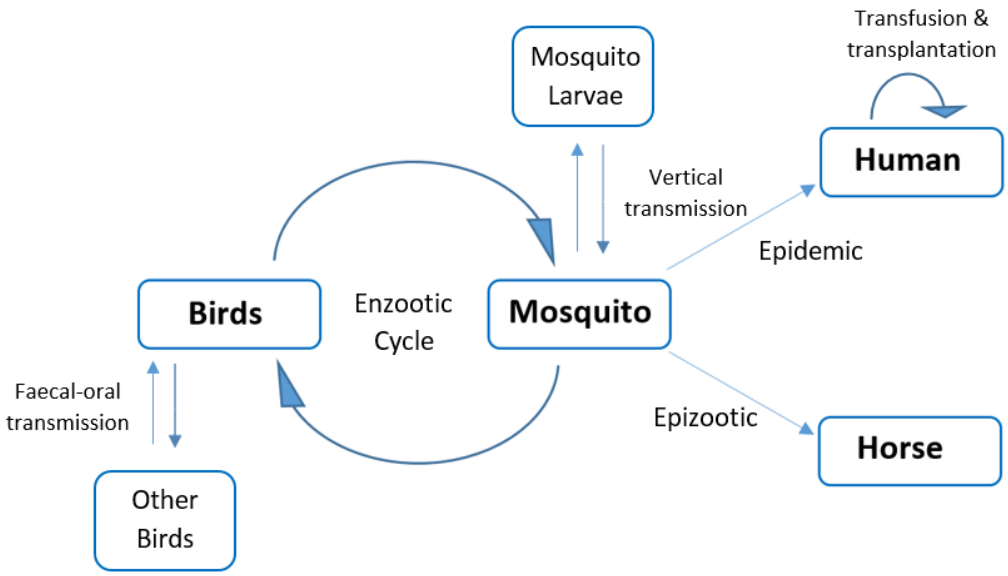

Figure 1.2: Transmission cycle of West Nile Virus. Adapted from (Chancey *et al.,* 2015).

WNV is maintained in the wild in an enzootic cycle between birds and mosquitoes with vertebrates, including humans, acting as dead-end hosts (Chancey *et al.,* 2015, Rizzoli *et al.,* 2015a, Petersen *et al.,* 2013). *Culex* mosquitoes are the main vectors of WNV with *Cx. pipiens* considered one of the main vectors in Europe (Hayes *et al.,* 2005). Birds are an important WNV host as they develop the high level of viremia required for successful mosquito infection (Van Der Meulen *et al.,* 2005). Migrating birds play an important role in translocating WNV and a greater understanding of the spread of WNV through bird populations is important (Rizzoli *et al.,* 2015b, Hagman *et al.,* 2014). It is thought that approximately 50% of the UKs bird population are migratory increasing the potential for WNV to reach the UK (Royal Society for the Protection of Birds, 2016). Migratory birds returning to the UK, after overwintering in WNV endemic African countries, could potentially bring this virus back to the UK (Brugman *et al.,* 2013). A 2003 study reported detection of WNV, Usutu virus and Sindbis virus antibodies in wild bird populations in the UK (Buckley *et al.,* 2003). However, a more recent study found no evidence of WNV in UK bird populations during a five year survey (Phipps *et al.,* 2008). Further to this, no mosquitoes have been found positive for WNV in the UK despite there being several species of mosquito that feed on both birds and humans and therefore may act as bridging vectors (Medlock *et al.,* 2005, Brugman *et al.,* 2013, Higgs *et al.,* 2004).

Additionally, there have been no reports of WNV in the UK equine populations or human cases in the UK (Brugman *et al.,* 2013).

In 1999 WNV was detected for the first time in North America and has since spread rapidly to over 47 states with more than 50,000 cases and 2,330 deaths reported (Hayes and Gubler, 2006, Lanciotti *et al.,* 1999, Medlock *et al.,* 2005, Murray *et al.,* 2010, Cdc, 2019). WNV outbreaks have also occurred in Europe with more than 2000 cases reported in southern Europe in 2018 Figure 1.3 (ECDC, 2018e). The rapid spread of WNV in temperate regions means we need to remain vigilant to the potential arrival of this virus into the UK.

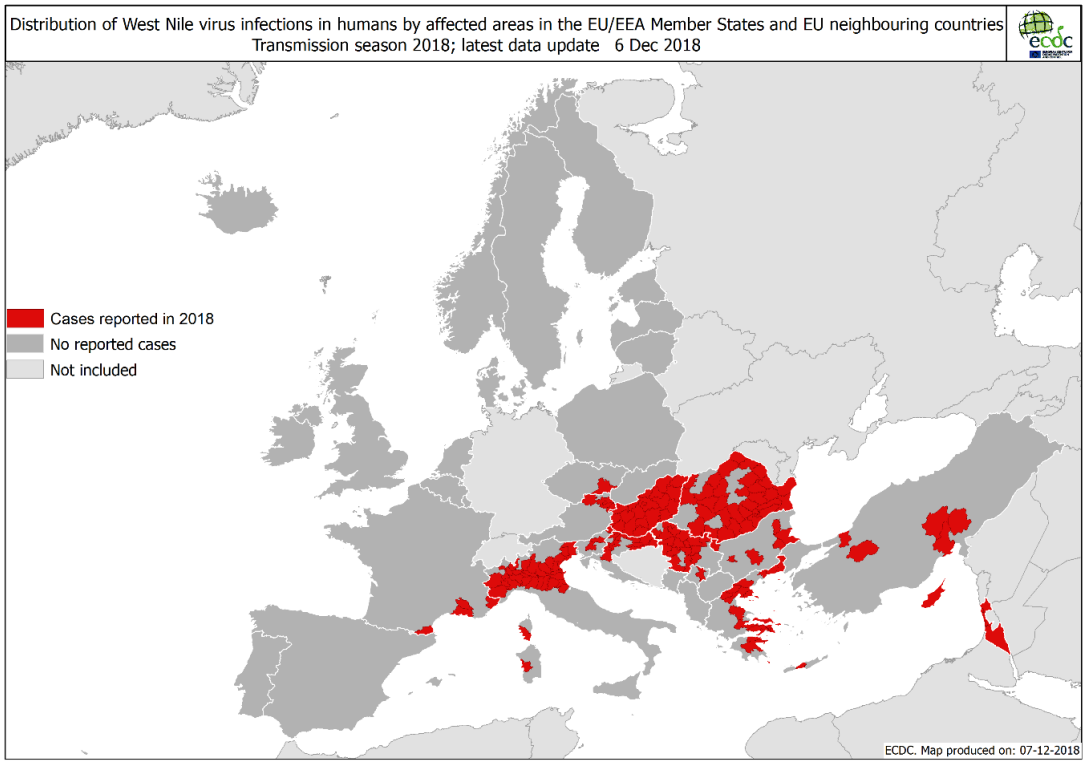

Figure 1.3: Distribution of WNV in Europe (ECDC, 2018g).

1.4.3 Usutu Virus

Usutu Virus (USUV) is a flavivirus predominantly transmitted by mosquitoes in the *Cx. pipiens* complex (Vazquez *et al.,* 2011). USUV was first detected in Europe in 1996 when sampling dead blackbirds (*Turdus merula)* in Italy (Mani *et al.,* 1998). It was later found in Austria in 2001 where the virus was responsible for the death of several birds

(Weissenböck *et al.,* 2003). Since 2001, the virus has spread to several other northern European countries including the Netherlands in 2016 (Rijks *et al.,* 2016). USUV antibodies were detected in wild bird populations in the UK in 2003 (Buckley *et al.,* 2003) although no further studies have found evidence of this (Phipps *et al.,* 2008). USUV has been associated with human disease in immunocompromised patients who exhibited neurological symptoms (Cavrini *et al.,* 2009, Pecorari *et al.,* 2009). A 2018 study evaluating the vector competence of British *Cx. pipiens* mosquitoes found a single positive mosquito and, although not conclusive, this study demonstrates the potential for these mosquitoes to act as USUV vectors in the UK (Hernández-Triana *et al.,* 2018).

1.4.4 Sindbis Virus

First detected in Egypt in 1952, Sindbis virus (SINV) is an alphavirus transmitted primarily by *Culex* mosquitoes, including *Cx. pipiens* and *Culex torrentius* (*Cx. torrentium*) (Taylor *et al.,* 1955, Brugman *et al.,* 2018a, Medlock *et al.,* 2007). The virus causes fever and myalgia in humans, although no human deaths have been reported (Hubálek, 2008). Disease outbreaks occur approximately every seven years in northern Europe (Hubálek, 2008). Several of the SINV vectors are present in the UK with the enzootic vectors *Cx. torrentium* and *Culiseta morsitans* (*Cs. morsitans*) both widespread throughout the country (Medlock *et al.,* 2007).

1.4.5 Tahyna Virus

Tahyna virus (TAHV) is an orthobunya virus originally isolated in 1958 in present day Slovakia (Bardos and Danielova, 1959). Culicine mosquitoes are the vectors of TAHV including *Aedes vexans* (*Ae. vexans*) and *Cx. pipiens* (Hubálek, 2008). The disease occurs in several European countries although many cases of disease remain undiagnosed (Hubálek, 2008).

1.4.6 Chikungunya Virus

Chikungunya virus (CHIKV) is an alphavirus first isolated in Tanzania in 1953 (Ross, 1956, Tsetsarkin *et al.,* 2007). The virus was restricted to parts of Africa and Asia until its spread to Islands in the Indian Ocean in 2005 (Akiner *et al.,* 2016, Cauchemez *et al.,* 2014). The

2005 outbreak occurred on Reunion Island with *Ae. albopictus* implicated as the vector responsible for disease transmission (Reiter *et al.,* 2006, Tsetsarkin *et al.,* 2007). A mutation in the envelope protein gene (E1-A226V) was found to significantly increase CHIKV infection rates in *Ae. albopictus* thus allowing a large outbreak of CHIKV to occur in an area where the usual mosquito vector, *Ae. aegypti,* was present in low numbers (Tsetsarkin *et al.,* 2007). Given the extensive global distribution of *Ae. albopictus,* this mutation means that CHIKV has the potential to continue to spreading with more disease outbreaks occurring in Europe and the Americas (Tsetsarkin *et al.,* 2007). In 2007 CHIKV was detected in Northern Italy (Rezza *et al.,* 2007). Since this outbreak there have been further reports of autochthonous CHIKV transmission in Italy and France (Calba *et al.,* 2017, Venturi *et al.,* 2017).

1.4.7 Dengue Virus

Dengue virus (DENV) is a flavivirus with four distinct serotypes (DENV 1-4). There have been sporadic cases of DENV reported in Europe since 2010 following an outbreak on Madeira Island (Akiner *et al.,* 2016). Autochthonous DENV transmission was reported in France in both 2014 and 2015 (Akiner *et al.,* 2016). In 2018, there was further autochthonous transmission in both France and Spain with six and three cases being reported respectively (ECDC, 2018f). Virus introduction in both France and Spain is thought to have occurred through viraemic travellers entering with the spread then facilitated by local populations of *Ae. albopictus* (ECDC, 2018f).

In 2018 there was a large DENV outbreak on the French island of Réunion with 6,763 cases recorded during the year (Pascalis *et al.,* 2019). This outbreak posed a risk to continental Europe with the potential for increased numbers of infected travellers importing the virus onto the mainland (ECDC, 2018d).

Although the *Aedes* mosquitoes responsible for the transmission of DENV are not currently established within the UK, there is the potential for these mosquitoes to become established in the future (Medlock *et al.,* 2018, Metelmann *et al.,* 2019).

1.4.8 Japanese Encephalitis Virus

Japanese encephalitis virus (JEV) is not currently transmitted in Europe, although one preliminary study has detected viral RNA in *Cx. pipiens* mosquitoes from Northern Italy (Ravanini *et al.,* 2012). Two species of UK mosquito, *Cx pipiens* and *Ae. vexans* are known vectors of JEV and several more UK species are competent vectors in the laboratory (Pearce *et al.,* 2018, Mackenzie-Impoinvil *et al.,* 2015, Chapman, 2017).

1.5 Vector Competence of UK Mosquito Species

Vector competence is a measure of a mosquito's ability to become infected with, replicate and transmit a virus (Kramer and Ebel, 2003). There are several factors that can influence a mosquito's ability to be considered a competent disease vector including: the extrinsic incubation period (EIP) and temperature (Mackenzie-Impoinvil *et al.,* 2015). The EIP is the time period between ingestion of a pathogen during a blood meal and the ability for further transmission of that pathogen (Higgs and Beaty, 2005). This is a temperature dependent process with the shorter EIP occurring at higher temperatures (Mackenzie-Impoinvil *et al.,* 2015, Hardy *et al.,* 1983, Chamberlain and Sudia, 1961). It is possible to conduct laboratory studies to determine whether a given mosquito species is a competent disease vector at a specific temperature.

Laboratory research into the vector competence of several British mosquito species has been carried out and further studies continue to be conducted (Blagrove *et al.,* 2016, Hernández-Triana *et al.,* 2018, Mackenzie-Impoinvil *et al.,* 2015, Lumley *et al.,* 2018, Chapman, 2017). The vector competence of UK mosquito species is discussed further in Chapter 5 of this thesis. The results of these studies suggest that there are several species of mosquito found in the UK that have the potential to act as vectors for a range of arboviruses should they emerge in the UK.

1.6 The Risk of Invasive Mosquito Species to Europe and the UK

The increase in cases of vector borne disease globally has been attributed, in part, to climate change and globalisation; including human and animal movement (Robin *et al.,* 2014). Increased global travel and trade has contributed to the spread of mosquito

species around the globe. There are several invasive mosquito species found in Europe which may act as potential disease vectors: *Ae. albopictus, Aedes aegypti* (*Ae. aegypti*), *Aedes japonicus* (*Ae. japonicus*), *Aedes koreicus* (*Ae. koreicus*) and *Aedes atropalpus* (*Ae. atropalpus*) (Vaux and Medlock, 2015). PHE have ongoing mosquito surveillance at used tyre distributors and 37 sea and airports across the UK (Medlock *et al.,* 2018). Whilst there are currently no established populations of these vectors within the UK, ongoing surveillance and risk assessments are increasingly important (Medlock *et al.,* 2018, Medlock *et al.,* 2017). This is especially true in light of the detection of *Ae. albopictus* eggs in Kent each summer for the past three years (2016-2018) (Medlock *et al.,* 2018, Medlock *et al.,* 2017).

1.6.1 *Aedes albopictus*

Ae. albopictus, also known as the Asian Tiger mosquito, has been reported in 25 European countries, including parts of northern Europe Figure 1.4 (Vaux and Medlock, 2015, Medlock *et al.,* 2018). The spread of this species throughout Europe and other parts of the globe is attributed, in part, to the used tyre and lucky bamboo trades (Vaux and Medlock, 2015). *Ae. albopictus* is able to produce eggs capable of withstanding desiccation allowing them to survive transportation around the globe (Medlock *et al.,* 2012b). The eggs of *Ae. albopictus* are able to overwinter in temperate regions as a result of photoperiodic egg diapause allowing the species to become established in colder climates (Medlock *et al.,* 2006).

Ae. albopictus was first detected in Belgium in 2000 at a used tyre centre and has since been found in lucky bamboo transported into Belgium from China (Schaffner *et al.,* 2004, Hendy and Madder, 2014). Additional surveillance programmes have also found this species as far north as Normandy and although now eliminated from these foci, reintroduction is a potential risk (Medlock *et al.,* 2006). This mosquito poses a major biting nuisance and is a competent vector for several arboviruses (Medlock *et al.,* 2015). For example, *Ae. albopictus* was the vector implicated with the transmission of CHIKV in Italy in 2007 (Medlock *et al.,* 2012a).

Models have demonstrated that the climate and habitat in the UK would allow for the establishment of *Ae. albopictus* if this species were to arrive (Medlock *et al.,* 2006, Metelmann *et al.,* 2019). If *Ae. albopictus* was imported to the UK in the tyre trade then it is likely that the internal UK tyre trade would allow for further spread of this species across the UK (Medlock *et al.,* 2006).

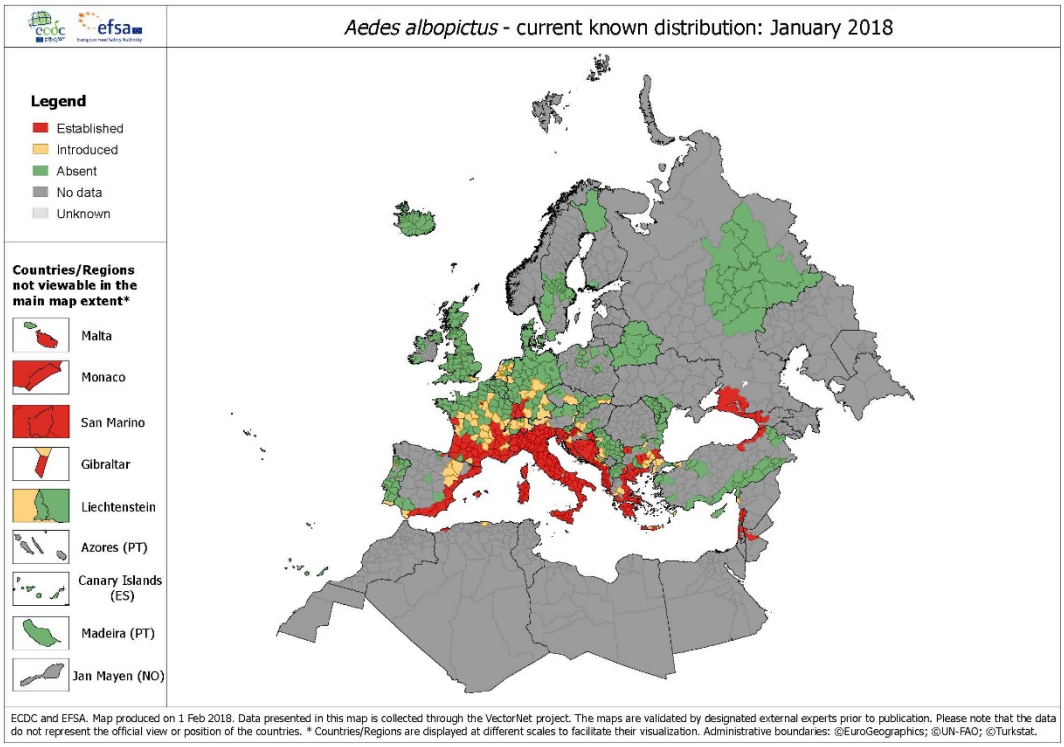

Figure 1.4: Map of the distribution of *Aedes albopictus* in Europe (ECDC, 2018b).

Ae. albopictus has continued to spread through Europe in vehicles including introduction into the UK (Eritja *et al.,* 2017). The detection of *Ae. albopictus* eggs in the UK have all occurred in truck stops in southern England (Medlock *et al.,* 2018, Medlock *et al.,* 2017).

1.6.2 *Aedes aegypti*

Globally, *Ae. aegypti* is considered to be one of the most invasive mosquito species and is found on all continents (Medlock *et al.,* 2018, Kraemer *et al.,* 2015). This species is a vector of many tropical arboviruses including both DENV and yellow fever (Medlock *et al.,* 2012a, Vaux and Medlock, 2015). *Ae. aegypti* has historically been established in southern Europe where it was responsible for outbreaks of yellow fever virus and DENV

(Medlock *et al.,* 2012a). During the 1920s, *Ae. aegypti* is thought to have been responsible for more than one million cases of dengue fever in Greece (Rosen, 1986). In 2005, *Ae. aegypti* was reported in Madeira (Margarita *et al.,* 2006). Subsequently, in 2012, over 2000 cases of DENV were reported in Madeira (Akiner *et al.,* 2016, Lourenço and Recker, 2014). *Ae. aegypti* has also been found in Italy and as far north as the Netherlands; although its spread in the Netherlands appears to be limited (Medlock *et al.,* 2012a). The distribution of *Ae. aegypti,* correct as of January 2018 is shown in Figure 1.5.

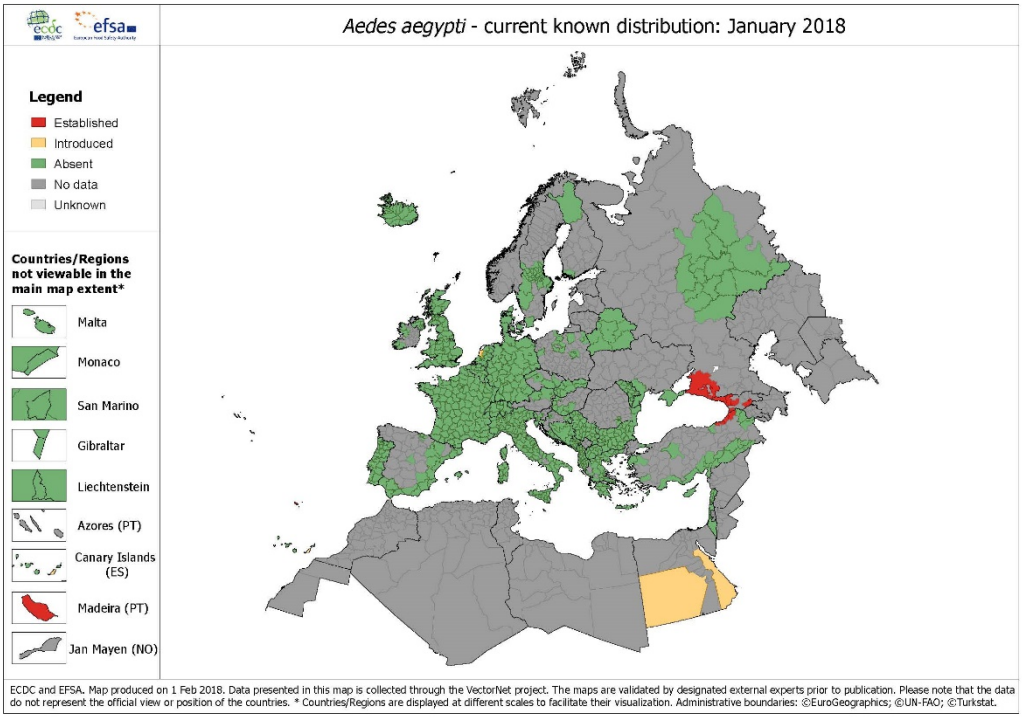

Figure 1.5: Map of the distribution of *Aedes aegypti* in Europe (ECDC, 2018a).

There have been three reported cases of *Ae. aegypti* found in the UK: Swansea in 1865; Essex in 1919 and Merseyside in 2014 (Dallimore *et al.,* 2017, Meers, 1986, Medlock *et al.,* 2018). However, these populations did not persist and *Ae. aegypti* is still absent in the UK (Medlock *et al.,* 2018). The climate in the UK is not considered suitable for the survival of *Ae. aegypti* with predictions stating an average temperature of greater than 15^{o}C being required for this mosquito species to survive (Medlock *et al.,* 2018).

1.6.3 *Aedes japonicus*

Larvae of *Ae. japonicus* were detected in France in 2000 although the population was successfully eliminated (Schaffner *et al.,* 2003). Investigations in 2008, following a series of complaints, found a well-established population of *Ae. japonicus* across a large area of Switzerland and part of Germany (Schaffner *et al.,* 2009). It has since spread further into Europe (Figure 1.6).

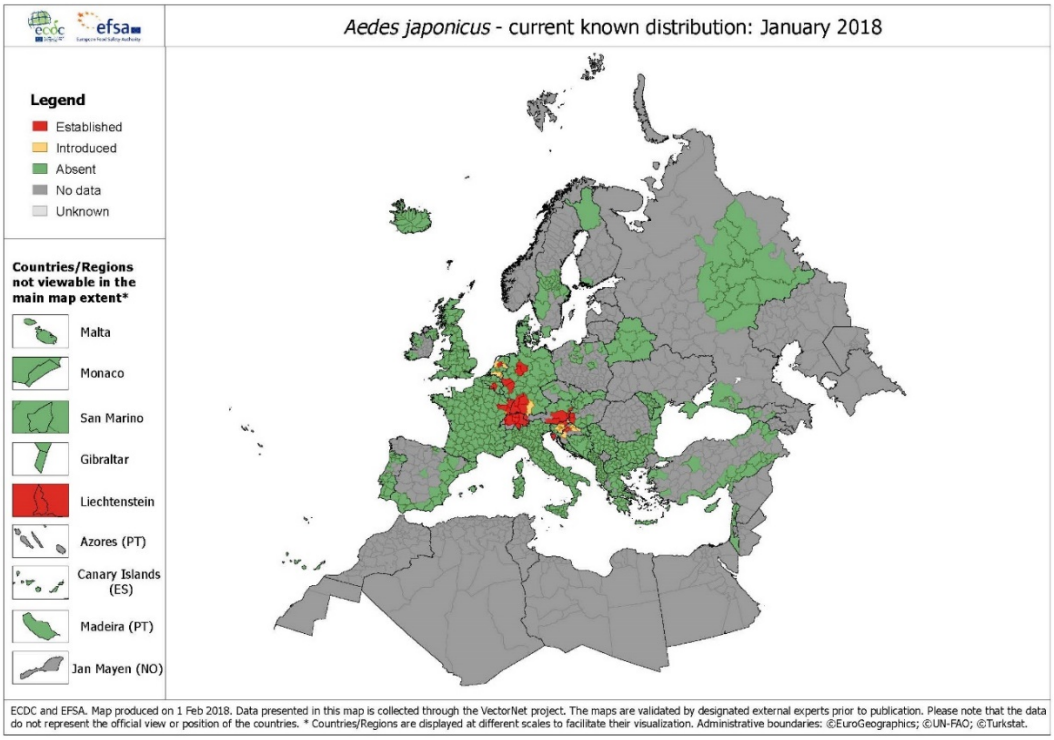

Figure 1.6: The distribution of *Ae. japonicus in* Europe (ECDC, 2018c).

Both active and passive surveillance systems are in place in the UK to detect invasive mosquito species which could potentially reach the UK and act as a disease vectors (Vaux and Medlock, 2015). For example the Mosquito Reporting Scheme (MRS) was established in 2005 by PHE to collect data on Culicidae in the UK (Kampen *et al.,* 2015). In 2014 these surveillance efforts were increased to include the highways with several truck stops, and service stations being targeted (Medlock *et al.,* 2018). These surveillance programmes are especially important to ensure that invasive species are detected early to allow for appropriate control strategies to be put in place as soon as possible.

1.7 The Risk of Future Disease Transmission in the UK

Increased incidences of disease transmission, coupled with the spread of invasive mosquito species in Europe, mean that it is important that we understand the potential risk of future disease transmission in the UK. In order for a mosquito species to be considered a potential arbovirus vector, a detailed understanding of its ecology and distribution is first required (Medlock *et al.,* 2007). Research into some of the potential UK mosquito vectors, such as *Cx. modestus,* a known WNV vector in Europe*,* are ongoing (Vaux *et al.,* 2015, Golding *et al.,* 2012). However, less is known about some of the other potential vector species in the UK. For example, in a 2005 review *Ae. detritus* was highlighted as a potential bridge vector of WNV due to the fact that it feeds on both birds and humans (Medlock *et al.,* 2005). Further to this, *Ae. detritus* has been shown to be a competent laboratory vector of several arboviruses including WNV and JEV (Blagrove *et al.,* 2016, Mackenzie-Impoinvil *et al.,* 2015). *Ae. detritus* is a highly abundant mosquito species in low-lying coastal regions of the UK where it acts as a prolific nuisance biter (Rees and Snow, 1996, Service, 1968, Medlock *et al.,* 2012a). Along the Dee estuary in the northwest of England, *Ae. detritus* is found in high numbers due to an abundance of suitable salt marsh habitat where it acts as a prolific nuisance biter (Clarkson and Setzkorn, 2011). Preliminary research into the ecology of this mosquito species has been conducted (Clarkson and Setzkorn, 2011, Service, 1968). Given the potential for *Ae. detritus* to act as a disease vector, and its abundance along the Dee estuary, it is important that we improve our understanding of the ecology and feeding behaviour of this species.

1.8 PhD Aims and Objectives

The overall aim of this thesis is to quantify four important components of the risk posed to the United Kingdom by mosquito-borne pathogens. Each component is the subject of a thesis chapter as summarised:

Chapter 2 – Seasonal Dynamics of Mosquitoes Along the Dee Estuary: A Three Year Longitudinal Study

Describes a three-year longitudinal survey with the aim of ascertaining data on the seasonal abundance and distribution of adult *Ae. detritus* and other sympatric mosquito species along a 18 km transect of the Wirral peninsular. The potential ecological drivers underlying changes in abundance data will be quantified.

Chapter 3 – The Ecology of the Immature Stages of Aedes detritus on the Dee Estuary Salt Marsh

Following an analysis of the dynamics of adult mosquitoes, Chapter 3 analyses the impact of tidal flooding on the abundance and distribution of *Ae. detritus* larvae in a key habitat. The chapter combines sampling of larval stages from aquatic habitats with the use of a drone to collect remotely-sensed data and produce maps of changes in the extent of larval breeding sites.

Chapter 4 – Quantifying the Biting Rates of Aedes detritus and Other Mosquito Species on Humans.

Most studies of mosquito abundance use various designs of trap to catch mosquitoes but the relationship between the catch from a trap and the numbers of mosquitoes biting a human are largely unknown. This chapter quantifies the relationship between a human landing catch (HLC) and a mosquito magnet, a widely used design of mosquito trap, and goes on to assess the performance of existing and novel designs of trap which could be used to assess biting risk in the UK.

Chapter 5 – Vector Competence of Aedes detritus to Japanese Encephalitis Virus

Previous studies of the effect of temperature on the development of viruses in UK species of mosquito have used a constant temperature. In nature, temperature fluctuates diurnally and seasonally. This chapter aims to determine the impact of daily

temperature fluctuations on the vector competence of *Ae. detritus* to Japanese encephalitis virus.

Chapter 6 – General Discussion

This chapter combines the new information produced in Chapters 2-5 to assess the potential risk posed to the UK from pathogenic viruses and discusses how this risk might be monitored and managed.

Chapter 2 - Seasonal Dynamics of Mosquitoes Along the Dee Estuary: A Three-Year Longitudinal Study

2.1 Abstract

Given the risk currently facing the UK from mosquito-borne pathogens and invasive mosquito species, it is essential that we have a detailed understanding of our native mosquito ecology. There are several species of mosquito in the UK that can act as vectors for arboviruses and improved understanding of the ecology of these species can help inform future control strategies. Studies described in this chapter aimed to quantify the seasonal dynamics of adult mosquitoes on the Dee estuary over a three-year sampling period.

Field studies were conducted over three years (2016-2018) with ~34 weeks of sampling each year. Adult mosquitoes were collected using Mosquito Magnets at six sites along an 18 km transect of the Dee estuary. Simultaneously, environmental data, including temperature, rainfall, windspeed, humidity and tide height were collected. Over 12,000 mosquitoes of 12 different species were collected. A clear seasonal pattern can be seen across the three years with mosquito abundance peaking both in the Spring and Autumn across all sample sites. *Ae. detritus and An. claviger* were the main species collected representing 52.7% and 31.7% of the total catch respectively.

There are two peaks in the numbers of *Ae. detritus* recorded annually. The first peak in abundance occurs in May and the second, much larger peak, in October. Over the three years of sampling, both peaks in abundance have occurred within one week of each other. Similarly, a large peak in the numbers of *An. claviger* have been recorded in one sample site across the three years. This peak occurred each year within a window of three weeks. Generalised linear mixed models were applied to the data along with meteorological data to explore the ecological drivers of mosquito abundance over the three years. The models show little correlation between mosquito catch and the seasonal fluctuations in meteorological variables. Rather, seasonal mosquito abundance appears to be highly correlated with certain weeks of the year.

The abundance of mosquitoes on the Wirral appears to be highly predictable and is unaffected by seasonal changes in meteorological variables. It is possible to predict the peak in abundance of several mosquito species based solely on the week of the year. This study has provided a detailed insight into the ecology of several species of mosquito, some of which may have the potential to act as disease vectors in the future.

2.2 Introduction

The research conducted into British mosquitoes has been summarised in the opening chapter of this thesis. The 1999 outbreak of WNV in America sparked awareness of the threat of vector borne diseases in temperate regions of the world (Lanciotti *et al.,* 1999). This outbreak, coupled with changes in global temperature, travel and environments means that increasing amounts of research is now ongoing into mosquito populations around the world. In the UK the Medical Entomology group in PHE has partnered with the Animal and Plant Health Agency (APHA) to conduct both active and passive surveillance of disease vectors in the UK as well as conducting research into these disease vectors (Medlock *et al.,* 2018). There are several species of mosquito found in the UK that have the ability to transmit arboviruses (Medlock *et al.,* 2018, Medlock *et al.,* 2005). A better understanding of the seasonal activity of these mosquitoes and the drivers that affect this seasonality is important for informing future vector control strategies. Although new research is accumulating, there are few studies that have explored the ecology of mosquitoes in the North West of England.

2.2.1 Mosquito Research in the North West of England

In Cranston's 1987 key to British mosquitoes there are four reports of mosquito studies in North West England (Cranston *et al.,* 1987). The next studies into North West mosquito populations were conducted in the late 1980s and early 1990s in Ness Woods. These studies analysed the ecology of adult and larval stages of *Aedes cantans* (*Ae. cantans*) (Renshaw *et al.,* 1994, Renshaw *et al.,* 1995). ELISA results of the mosquito blood meals showed a host preference for sheep and cows while mark-release-recapture studies found a large population of *Ae. cantans* in this area (Renshaw *et al.,* 1994). The larval studies found that *Ae. cantans* larvae are aggregated due to the heterogeneity of the larval habitats in Ness Woods (Renshaw *et al.,* 1995).

Local authorities (LAs) became interested in the mosquito populations on the Wirral in the 1980s following a succession of complaints about nuisance biting (Clarkson and Setzkorn, 2011). The mosquitoes responsible for the nuisance biting were identified as *Ae. detritus.* Efforts to control the mosquito population utilising *Bti* were put into place

and spraying of the mosquito breeding sites took place annually from 1986 (except for 2007) but has since ceased (Clarkson and Setzkorn, 2011). The LA has also made attempts to manipulate the habitat to reduce the number of mosquitoes by digging out small pools and creating larger habitats and trenches (M. Clarkson, Personal communication, 2016). In 2009, a survey of nuisance biting reports in different LAs in the UK was conducted (Medlock *et al.,* 2012a). Ten LAs in the North West reported nuisance biting with four of these LAs identifying the nuisance biting mosquitoes as: *Cs. annulata, Cx. pipiens* s.l., *An. maculipennis* s.l, *Ae. punctor, Ae.* cantans and *Ae. detritus* (Medlock *et al.,* 2012a). Three of the LAs in the North West had engaged mosquito control measures with a further one providing advice to local residents (Medlock *et al.,* 2012a).

Given that *Ae. detritus* is a significant nuisance biter on the Wirral peninsular, a preliminary study into its ecology in this area has been conducted (Clarkson and Setzkorn, 2011). The study found *Ae. detritus* to be present eleven months of the year, with a peak in abundance recorded in September (Clarkson and Setzkorn, 2011). The ecology of *Ae. detritus* means that the eggs require multiple immersions from the tides or precipitation to hatch (Service, 1968). The seasonal high tides in Spring and Autumn have been linked to emergence and peaks in the numbers of *Ae. detritus* (Clarkson and Setzkorn, 2011).

In 2014 a male *Ae. aegypti* mosquito was found approximately 6 km north of Liverpool by sweep netting (Dallimore *et al.,* 2017). Further research was conducted in this area and while no other *Ae. aegypti* mosquitoes were found, a further six species of mosquito were identified with *Cx. pipiens* being the most abundant (Dallimore *et al.,* 2017).

A 2015 study looking at adult mosquitoes trapped at equine premises, reports high numbers of *Ae. detritus* and *Cs. annulata*, along with lower numbers of other species, at several sites around the North West (Chapman *et al.,* 2016, Chapman, 2017).

Mosquito populations from the North West of England have also been used in several laboratory vector competence studies. The *Ae. detritus* mosquitoes collected from the Dee salt marsh have been shown to be competent for several tropical viruses under laboratory settings (Blagrove *et al.,* 2016, Mackenzie-Impoinvil *et al.,* 2015, Lumley *et al.,*

2018). Additionally, insecticide resistance assays have shown that *Ae. detritus* is resistant to the insecticide bendiocarb (Brown *et al.,* 2018).

Given the detection of *Ae. albopictus* in southern England, ongoing surveillance to detect invasive mosquitoes in the UK is especially important (Medlock *et al.,* 2018, Medlock *et al.,* 2017). Improved knowledge of the distribution and abundance of British mosquito species will improve the UK's readiness to monitor and manage arboviruses that pose a risk to human and animal health.

2.3 Aim and Objectives

This chapter describes a three-year longitudinal survey with the aim of ascertaining data on the seasonal abundance and distribution of adult *Ae. detritus* and other sympatric mosquito species along an 18 km transect of the Wirral peninsular.

The objectives of this chapter were to:

1. Quantify fluctuations in the seasonality and abundance of adult mosquitoes along an 18 km transect of the Dee estuary encompassing estuarine, grassland and mixed-woodland habitats.
2. Quantify the potential ecological drivers underlying changes in mosquito abundance.

2.4 Materials and Methods

2.4.1 Selection of Sample Sites

Six sample sites, along an 18 km stretch of the Dee Estuary, were recruited to the study in early 2016

Figure 2.1). Ethical approval was obtained prior to the start of the study from the Liverpool School of Tropical Medicine (LSTM) research ethics committee, reference number 16-012 (Appendix A). Sites for the study were selected on the basis of being easily accessible to researchers but with restricted public access to ensure that members of the public did not come into contact with the mosquito traps. Informed consent was obtained from each of the sites prior to any work commencing. At each of the six sites, two traps were operated at locations 50 m apart, unless reported otherwise. In some instances, more than one location was required to ensure traps were sufficiently spaced apart (>50 m).

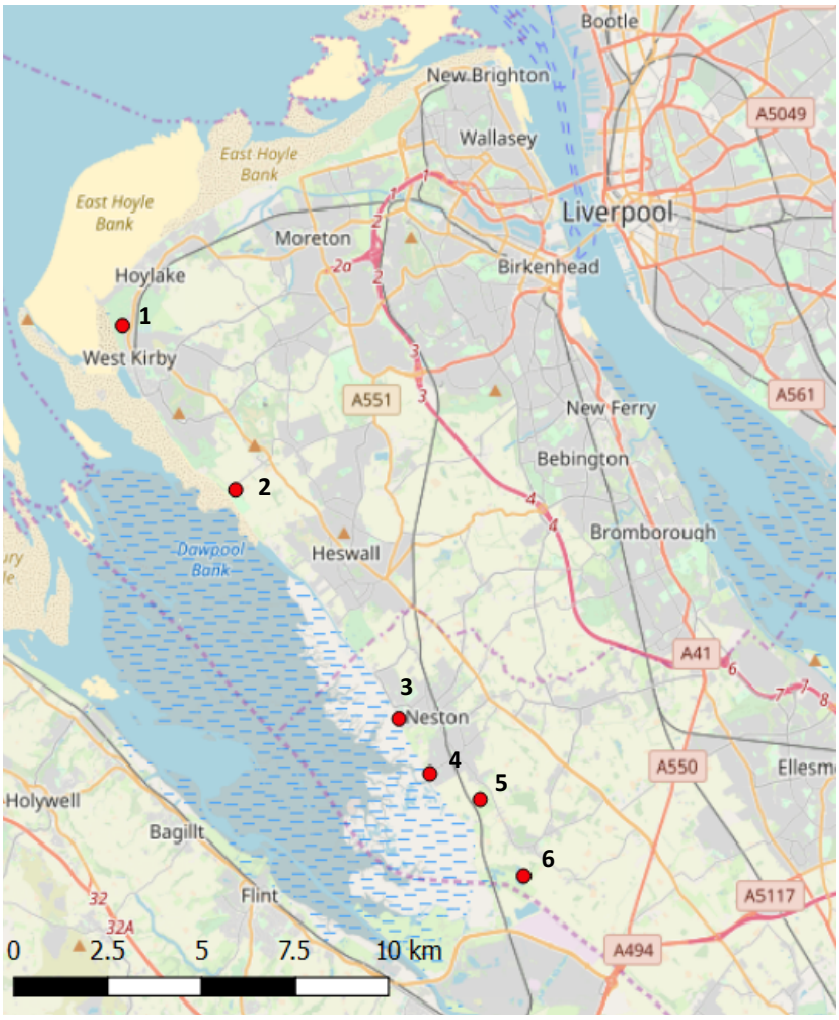

Figure 2.1: Map showing the six main sample sites along the Dee Estuary. Basemap: © OpenStreetMap contributors, CC-BY-SA (https://www.openstreetmap.org/copyright). 1: RLGC, 2: Thursaston, 3: Parkgate, 4: Little Neston, 5: Ness Gardens, 6: Burton Mere.

2.4.2 Trap Running and Operation

Mosquito Magnet traps (Independence Model), (Midge Free Zone, Argyll, UK) along with a propane tank were used to trap adult mosquitoes. The traps were operated continuously for three nights every week between 10:00 hours on Friday and 16:00 hours on Monday. Batteries were removed and freshly charged each weekend of sampling. Propane gas bottles were changed as necessary. Traps were baited with 1-octen-3-ol (0.5 mg/h) dispensed from sachets provided for use with the Mosquito Magnets (Midge Free Zone, Argyll, UK). The sachets were changed for new ones every four weeks. In 2016 the experiment ran for 33 weeks (8th April to 21st November). In 2017 the experiment ran for 36 weeks (17th March to 20th November). In 2018 the experiment ran for 38 weeks (9th March to 26th November). Mosquito collections ended following two successive weeks of zero catch.

2.4.3 Description of Sample Sites

Site 1: Royal Liverpool Golf Course (RLGC)
Two mosquito traps were placed at the RLGC (53°22'50.79"N, 3°11'22.13"W). Both traps were located away from members of the public. One trap was located near some outbuildings while the other was placed along a hedge line. The traps were located approximately 300 m from the Dee estuary and were 70 m apart from each other.

Site 2: Wirral Country Park Visitors Centre, Thurstaston
Two traps were placed, away from the general public, within the Wirral Country Park (53°20'33.93"N, 3° 8'37.46"W). One trap was located near some outbuildings while the other was placed within a small wooded area near a large pond. The traps were approximately 300 m from the Dee estuary and were 110 m apart.

Site 3: Private Garden, Parkgate
One trap was placed within a private garden in the village of Parkgate (53°17'23.96"N, 3°4'43.12"W). No suitable volunteers for the placement of the second trap was recruited. The trap was placed approximately 15 m from the salt marsh.

Site 4: Harp Inn Pub, Little Neston and Private Garden, Little Neston
Two traps were placed within the village of little Neston (53°16'37.14"N, 3° 3'37.82"W). The first was placed in the beer garden of a local pub, approximately 15 m from the marsh. The second, 400 m away in a private garden.

Site 5: Ness Gardens
Two traps were placed within Ness Gardens (53°16'19.69"N, 3° 2'35.43"W). One was located near some offices in the main gardens and the other was located within the garden's weather station area. The traps were approximately 750 m from the salt marsh and were located 200 m apart.

Site 6: Burton Mere RSPB Reserve
One trap was placed at Burton Mere RSPB reserve (53°15'13.93"N, 3° 1'44.28"W). The trap was placed within a dense wooded area close to the main hide and reedbeds. The trap was placed approximately 100 m from the salt marsh.

2.4.4 Mosquito Identification

The collection net from the mosquito trap was carefully removed ensuring no mosquitoes escaped. The nets were then returned to the laboratory and placed in a freezer. After 24 h the nets were removed and any mosquitoes counted and identified to species level using the keys of Cranston and Snow (Cranston *et al.,* 1987, Snow, 1990). If mosquitoes were too damaged for identification these have been recorded as unidentified. If male mosquitoes were collected these were also identified to species level but were not included in any subsequent analysis.

No attempt was made to separate *Ae. cantans* and *Aedes annulipes* (*Ae. annulipes*) (hereafter *Ae. cantans/annulipes*), *Aedes cinereus* (*Ae. cinereus*) and *Aedes geminus* (*Ae. geminus*) or *Cx. pipiens* s.l (hereafter *Cx pipiens/torrentium*). Morphological keys are unable to separate species within the *Cx. pipiens* group (Medlock and Vaux, 2009, Hesson *et al.,* 2014).

2.4.5 Meteorological Data

In each of the six sampling sites, a TinyTag View2 data logger (Gemini Data Loggers Ltd., West Sussex, UK) was attached to a trap to record the temperature and relative humidity. The data loggers were programmed to recorded temperature and relative humidity at 10 minute intervals and were kept out of direct sunlight to prevent insolation. From 2017 onwards, Stevenson screens were used to protect the data loggers from radiant heat and precipitation.

Meteorological data was also obtained from a weather station located within Ness Gardens operated by the National Centre for Atmospheric Science (Lat: 53^{o}16.20'N, Long: 003^{o}03.00'W). This weather station was used to provided information on: temperature, humidity, rainfall and windspeed. Inconsistencies with the windspeed data recorded in 2017 mean that this data is not included in subsequent data analyses.

2.4.6 Tidal Data

Data on the daily high and low tides along the Dee estuary salt marsh was provided by the National Oceanography Centre (NOC) in Liverpool. The tide height was measured twice daily at Hilbre Island.

2.4.7 Data analysis

Diversity and evenness indices were calculated for each of the sampling locations both as individual years and collectively. Simpson's diversity index (1-D) was used to assess diversity and the Shannon-Weiner index (H) was used to assess species dominance.

A generalised linear mixed model (GLMM) was fitted to all data sets using R, version 3.3.1., in the 'glmmADMB' package (Appendix B). The initial maximal model included two random effects (trap location and year) and several fixed effects (temperature (^{o}C), windspeed (m/s), rainfall (mm), relative humidity (%)). When considering all species combined and *Ae. detritus* alone, tide height (m) was also included as a fixed effect. Subsequently, the fixed effects were also read into the model as factors (low, mid and high ranges). Deletion testing (using the drop1 function in R) was used to obtain the minimal adequate model. The minimal adequate model included only variables with significant ($P<0.05$) effects on catch.

2.5 Results

2.5.1 Summary of the Three Years

2016

Mosquito Magnets were run for 72 hours over 33 weeks from 8th April to 21st November 2016 (weeks 14-46) at six different sample sites. A total of 4,613 adults were collected from 12 different species (Table 2.1). A total of nine mosquitoes were unidentifiable due to damage and these were then excluded from any subsequent analyses. The total of identified mosquitoes was 4,604. The most abundant species were *Ae. detritus* (2,319, 50.4%), *An. claviger* (1,473, 32.0%), *Ae. rusticus* (319, 6.9%), *Cs. annulata* (167, 3.6%) and *Ae. cantans/annulipes* (114, 2.5%). Other species, comprising *Ae. caspius, Ae. cinereus, Ae. flavescens, Ae. geniculatus, An. plumbeus, Cs. morsitans* and *Cx. pipiens/torrentium,* made up the remaining 4.6% of the total catch (212).

RLGC: A total of 257 mosquitoes from eight different species were collected at this site over 31 weeks sampling (Table 2.1). *Cs. annulata* and *Ae. detritus* were the main species of mosquito collected at this site making up 35.8% and 30.7% of the total catch respectively.

Thurstaston: A total of only four mosquitoes from two different species were collected at this site over 30 weeks of sampling (Table 2.1). Given the extremely low catch at site sampling ceased at the end of 2016. Any subsequent analysis does not include the mosquitoes caught in this location.

Parkgate: A total of 1,266 mosquitoes from eight different species were collected at this site over 28 weeks sampling (Table 2.1). *Ae. detritus* was the main mosquito species in this location making up 85.6% of the total catch.

Little Neston: A total of 315 mosquitoes from seven different species were collected at this site across 33 weeks of sampling (Table 2.1). The main species of mosquito caught in this location was *Ae. detritus* making up 70.2% of the total catch.

Ness Gardens: A total of 904 mosquitoes from eight different species were collected at this site over 31 weeks sampling (Table 2.1). There were two main species collected: *Ae. detritus* and *Ae. rusticus* making up 66.8% and 21.5% of the total catch respectively.

Burton Mere: A total of 1,858 mosquitoes from 12 different species were collected at this site over 30 weeks sampling (Table 2.1). *An. claviger* was the main species at this location making up 69.9% of the total catch, followed by *Ae. detritus* at 17.8% of the total catch.

Table 2.1: Summary of trap data from each of the six sample sites in 2016. Catches over 100 are highlighted bold.

Sample Site	**RLGC**	**Thurstaston**	**Parkgate**	**Little Neston**	**Ness Gardens**	**Burton Mere**
Survey weeks	31	30	28	33	31	30
Number of species	9	2	8	7	8	12
Number of mosquitoes	257	4	1,266	315	904	1,858
Species						
Aedes caspius	0	0	37	4	18	3
Aedes cantans/annulipes	4	0	0	0	0	**110**
Aedes cinereus	0	0	0	0	0	2
Aedes detritus	79	1	**1,084**	**221**	**604**	**330**
Aedes flavescens	17	0	0	0	0	1
Aedes geniculatus	1	0	3	0	10	19
Aedes rusticus	1	0	62	58	**194**	4
Anopheles claviger	51	3	47	18	55	**1,299**
Anopheles plumbeus	11	0	10	3	3	2
Culiseta annulata	92	0	19	5	12	39
Culiseta morsitans	0	0	0	0	0	3
Culex pipiens/torrentium	1	0	4	6	8	46

At both Parkgate and Burton Mere, the diversity indices and Evenness values were all low. This was due to the dominance of certain species (*Ae. detritus* and *An. claviger*) (Table 2.2). The greatest mosquito diversity was observed at the RLGC as shown by the higher diversity values in this location. There was no clearly dominant species collected at RLGC. The values obtained for both the Ness Gardens and Little Neston sites are very

similar; there is high mosquito diversity in these locations but also very high numbers of just one species, *Ae. detritus*.

Table 2.2: Diversity indices and evenness values for each of the sample sites.

Location	Simpson's Diversity Index	Shannon Weiner Index	Evenness
RLGC	0.73	1.49	0.68
Parkgate	0.26	0.64	0.31
Little Neston	0.47	0.96	0.50
Ness Gardens	0.50	1.02	0.49
Burton Mere	0.48	1.00	0.40

2017

Mosquito Magnets were run for 72 hours over 36 weeks from 17th March to 20th November 2017 (weeks 11-46) at the five different sample sites. A total of 4,052 adults were collected from 12 different species (Table 2.3). A total of 11 mosquitoes were unidentifiable due to damage and these were then excluded from any subsequent analysis. The total of identified mosquitoes was 4,041. The most abundant species were *Ae. detritus* (2,291, 56.5%), *An. claviger* (1,211, 29.9%), *Ae. rusticus* (186, 4.6%), *Cs. annulata* (164, 4.0%) and *Ae. cantans/annulipes* (53, 1.3%). Other species, *Ae. caspius, Ae. cinereus, Ae. flavescens, Ae. geniculatus, An. plumbeus, Cs. morsitans* and *Cx. pipiens/torrentium* made up the remaining 3.4% of the total catch (136).

RLGC: A total of 261 mosquitoes from seven different species were collected at this site over 35 weeks sampling (Table 2.3). *An. claviger, Cs. annulata* and *Ae. detritus* were the main species collected at this site making up 39.1%, 28.7% and 23.0% of the total catch respectively.

Parkgate: A total of 847 mosquitoes from eight different species were collected at this site over 29 weeks sampling (Table 2.3). *Ae. detritus* and *Ae. rusticus* were the main

mosquito species in this location making up 75.8% and 13.8% of the total catch respectively.

Little Neston: A total of 93 mosquitoes from seven different species across 32 weeks sampling were collected in Little Neston (Table 2.3). The main species of mosquito caught in this location was *Ae. detritus* making up 72.0% of the total catch.

Ness Gardens: A total of 1,659 mosquitoes from nine different species were collected at this site over 36 weeks sampling (Table 2.3). There were two main species collected at this site: *Ae. detritus* and *An. claviger* making up 83.9% and 8.2% of the total catch respectively.

Burton Mere: A total of 1,181 mosquitoes from ten different species were collected at this site over 36 weeks sampling (Table 2.3). *An. claviger* was the main species making up 78.7% of the total catch, followed by *Ae. detritus* at 11.0% of the total catch.

Table 2.3: Summary of the catch data from the five sample sites in 2017. Catches over 100 are highlighted bold.

Sample Site	**RLGC**	**Parkgate**	**Little Neston**	**Ness Gardens**	**Burton Mere**
Survey weeks	35	29	32	36	36
Number of species	7	8	7	9	10
Number of mosquitoes	261	847	93	1,659	1,181
Species					
Aedes caspius	0	29	1	13	7
Aedes cantans/annulipes	0	0	0	3	50
Aedes cinereus	0	0	0	0	4
Aedes detritus	60	**642**	67	**1,392**	**130**
Aedes flavescens	9	0	0	0	0
Aedes geniculatus	0	0	0	10	14
Aedes rusticus	0	**117**	3	64	2
Anopheles claviger	**102**	28	15	**136**	**930**
Anopheles plumbeus	8	1	1	4	20
Culiseta annulata	75	27	4	35	23
Culiseta morsitans	1	1	0	0	0
Culex pipiens/torrentium	6	2	2	2	1

At both Ness Gardens and Burton Mere, the diversity indices and Evenness values were low as a result of one species dominating the total catch in this location (*Ae. detritus* and *An. claviger* respectively) (Table 2.4). RLGC was the most diverse site with no one species dominating the total catch.

Table 2.4: Diversity indices and evenness values for each of the sample sites.

Location	Simpsons Diversity Index	Shannon Weiner Index	Evenness
RLGC	0.71	1.39	0.63
Parkgate	0.48	1.02	0.46
Little Neston	0.45	0.96	0.49
Ness Gardens	0.29	0.66	0.30
Burton Mere	0.37	0.83	0.36

2018

Mosquito Magnets were run for 72 hours over 38 weeks from 9th March to 26th November 2018 (weeks 10-47) at the five different sample sites. A total of 4,181 adults were collected from 11 different species (Table 2.5). A total of seven mosquitoes were unidentifiable due to damage and these were then excluded from any subsequent analysis. The total of identified mosquitoes was 4,174. The most abundant species were *Ae. detritus* (2,144, 51.4%), *An. claviger* (1,380, 33.1%), *Ae. rusticus* (248, 5.9%), *Cs. annulata* (131, 3.1%) and *Ae. cantans/annulipes* (119, 2.9%). Other species, *Ae. caspius, Ae. cinereus, Ae. flavescens, Ae. geniculatus, An. plumbeus* and *Cx. pipiens/torrentium* made up the remaining 3.6% of the total catch (152).

RLGC: A total of 160 mosquitoes from six different species were collected at this site over 38 weeks sampling (Table 2.5). *Ae. detritus, An. claviger* and *Cs. annulata* were the main collected at this site making up 38.8%, 26.9% and 23.1% of the total catch respectively.

Parkgate: A total of 652 mosquitoes from nine different species were collected at this site over 38 weeks sampling (Table 2.5). *Ae. detritus, Ae. rusticus* and *An. claviger* were

the main mosquito species in this location making up 79.7%, 12.4% and 11.8% of the total catch respectively.

Little Neston: A total of 118 mosquitoes from six different species across 38 weeks sampling were collected at this sample site (Table 2.5). The main species caught in this location were *Ae. detritus* and *Ae. rusticus* making up 44.1% and 28.8% of the total catch respectively.

Ness Gardens: A total of 1,897 mosquitoes from ten different species were collected at this site over 38 weeks sampling (Table 2.5). There were three main species collected at this site: *Ae. detritus*, *An. claviger* and *Ae. rusticus* making up 79.7%, 9.0% and 7.0% of the total catch respectively.

Burton Mere: A total of 1,347 mosquitoes from nine different species were collected at this site over 38 weeks sampling (Table 2.5). *An. claviger* was the main species making up 79.2% of the total catch, followed by *Ae. cantans/annulipes* at 8.5% of the total catch.

Table 2.5: Summary of the catch data from the five sample sites in 2018. Catches over 100 are highlighted bold.

Sample Site	**RLGC**	**Parkgate**	**Little Neston**	**Ness Gardens**	**Burton Mere**
Survey weeks	38	38	38	38	38
Number of species	6	9	6	10	9
Number of mosquitoes	160	652	118	1,897	1,347
Species					
Aedes caspius	2	4	1	12	10
Aedes cantans/annulipes	0	0	0	4	**115**
Aedes cinereus	0	0	0	4	9
Aedes detritus	62	**452**	52	**1,512**	66
Aedes flavescens	12	1	0	0	0
Aedes geniculatus	0	3	2	12	17
Aedes rusticus	0	81	34	**133**	0
Anopheles claviger	43	77	22	**171**	**1,067**
Anopheles plumbeus	4	4	0	3	19
Culiseta annulata	37	28	7	41	18
Culex pipiens/torrentium	0	2	0	5	26

The diversity indices and evenness values at Ness Gardens, Parkgate and Burton Mere were both low as *Ae. detritus* and *An. claviger* dominated the mosquito catches in these locations (Table 2.6). RLGC had the greatest diversity of all the sites with the highest diversity indices.

Table 2.6: Diversity indices and evenness values for each of the sample sites.

Location	Simpsons Diversity Index	Shannon Weiner Index	Evenness
RLGC	0.72	1.4	0.78
Parkgate	0.49	1.02	0.46
Little Neston	0.69	1.3	0.73
Ness Gardens	0.35	0.78	0.34
Burton Mere	0.36	0.86	0.39

2.5.2 Three Years Combined

A total of 12,846 mosquitoes were collected during the three years of sampling. Overall, Burton Mere and Ness Gardens were the most prolific sample sites, followed by Parkgate. Annual trends in the seasonal activity of mosquitoes can be seen across each of the five sample sites (Figure 2.2). Across all sites there are two peaks in mosquito density. The first occurs each year between the last week in May and the first week in June (~weeks 20 and 22). Although seen across all sites, this peak is especially pronounced in Burton Mere where the mosquito catch increases to several hundred per week (**Figure *2.2***). The greatest catch at Burton Mere was in 2016 with over 450 mosquitoes being collected in one weeks sampling, over double the catch seen during this week in both 2017 and 2018. After the first peak in numbers, the mosquito catch drops for approximately ten weeks during the summer, July-September, (~weeks 28-38). In 2018, this drop in mosquito abundance lasted two weeks longer than both 2016 and 2017 beginning in June (weeks 25-28). The second peak in mosquito abundance occurs each year in the first week in October (week 40-41). This peak is greatest in both Parkgate and Ness Gardens. In 2016 the peak was greatest in Parkgate with 212 mosquitoes being

collected in one week. In both 2017 and 2018 the peak was highest in Ness Gardens with 468 and 334 collected in one weeks sampling respectively. Across all three years, mosquito catch as dropped to zero by the end of November (~week 46).

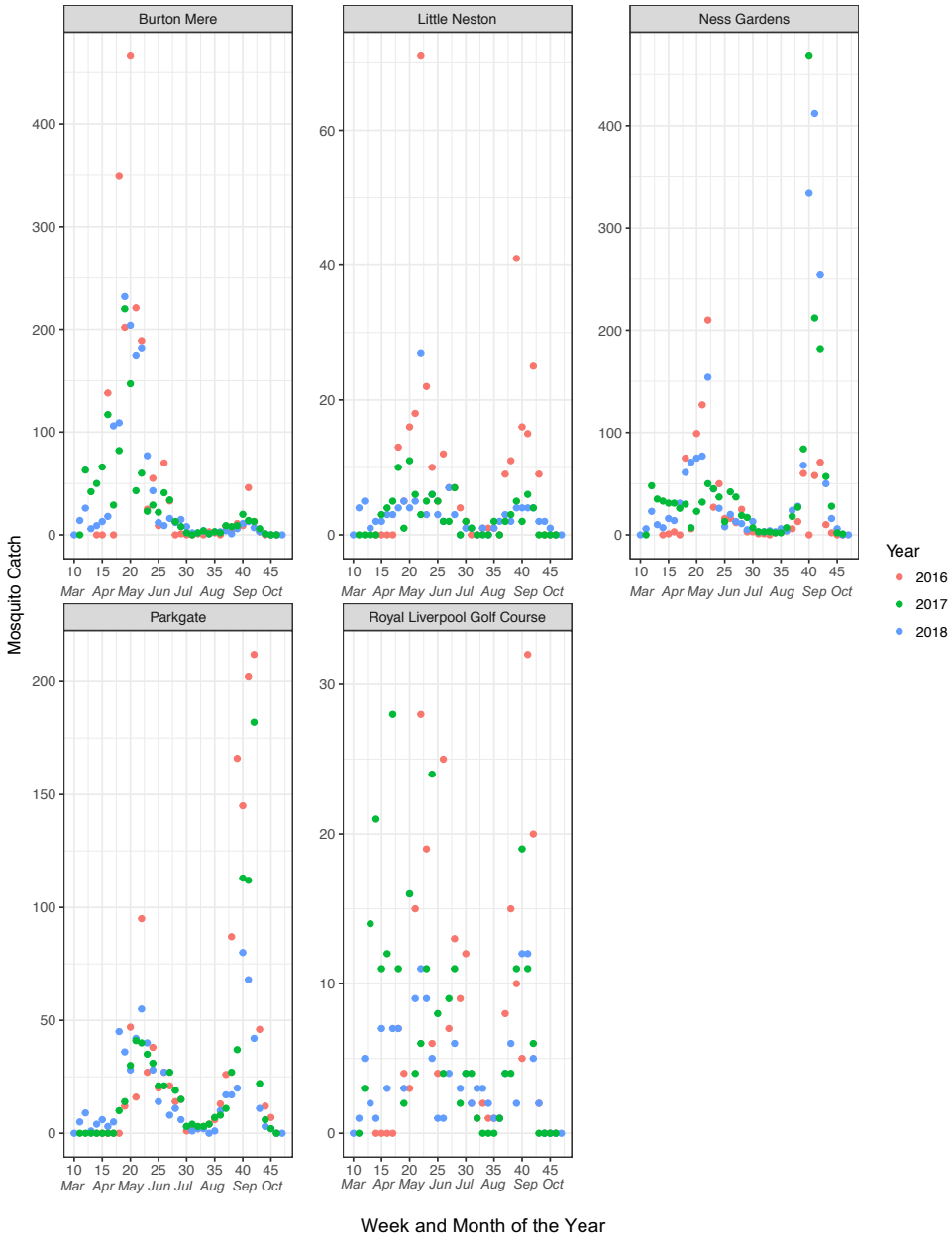

Figure 2.2: Total mosquito catch at each of the sample sites over the three years of sampling.

Ae. detritus

Ae. detritus is present in the first peak in mosquito numbers in May each year across all of the sample sites (Figure 2.3). Mosquito densities are greatest in Parkgate and Ness Gardens. The greatest numbers of *Ae. detritus* (210) was recorded in the first week in

June in Ness Gardens in 2016 (week 22). The same peak is also present in both 2017 and 2018 although the overall numbers of *Ae. detritus* is lower (maximum of 154). *Ae. detritus* abundance remains low for approximately fourteen weeks between the second week in June and the second week in September (weeks 23-36) (**Figure *2.2***). A larger second peak was also seen for this species (**Figure *2.3***). This peak is seen across all sample sites although the greatest numbers are consistently collected at both Ness Gardens and Parkgate with several hundred mosquitoes caught over several weeks of sampling in October (week 40-42). In 2016 the greatest numbers of *Ae. detritus* was caught in Parkgate: 212 mosquitoes in mid-October (week 42). In both 2017 and 2018 the greatest peak was recorded in Ness Gardens (week 40, 5/6th October) with 466 and 380 mosquitoes collected respectively. The peak in *Ae. detritus* is also seen at Burton Mere, Little Neston and RLGC although at much lower densities (maximum catch 124 at Burton Mere in 2016).

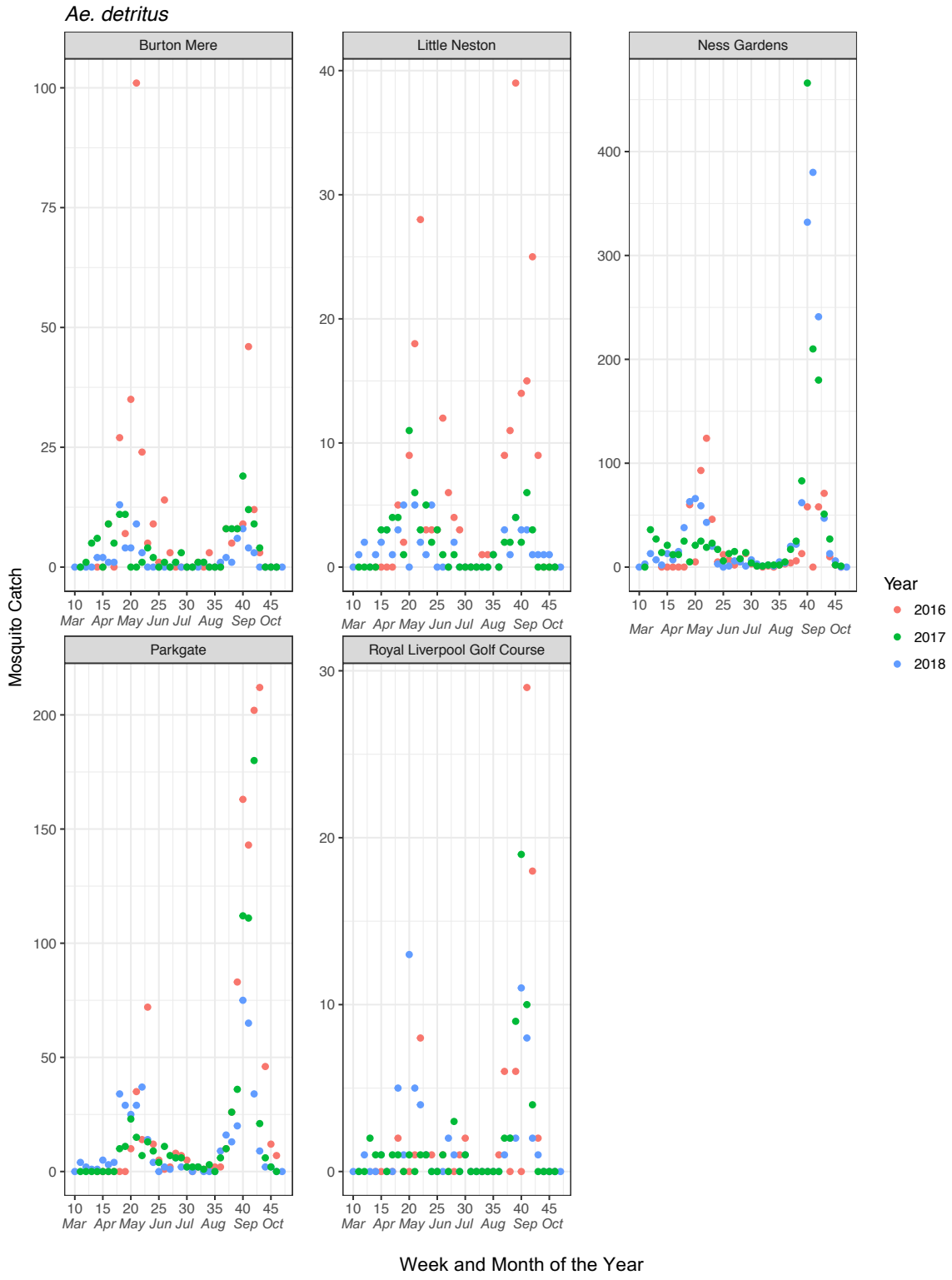

Figure 2.3: *Ae. detritus* catch at each of the sample sites over the three years of sampling.

An. claviger

An. claviger was collected at all sample sites, although Burton Mere was by far the most prolific sample site for this species (Figure 2.4). There is a clear peak in the abundance of *An. claviger* at Burton Mere with several hundred mosquitoes being collected over six weeks of sampling between April and May (weeks 16-21). The greatest number of *An.* claviger (407) was collected in 2016 between the 22nd and 25th April (week 16) (Figure 2.4). The peak in numbers in both 2017 and 2018 occurred in the second week in May (week 19) with a total of 185 and 219 mosquitoes collected respectively. No clear peaks in *An. claviger* abundance can been seen across the other sample sites (Figure 2.4). *An. claviger* is collected at the remaining sample sites between April-November (weeks 11-44) over the three years, all be it at low densities.

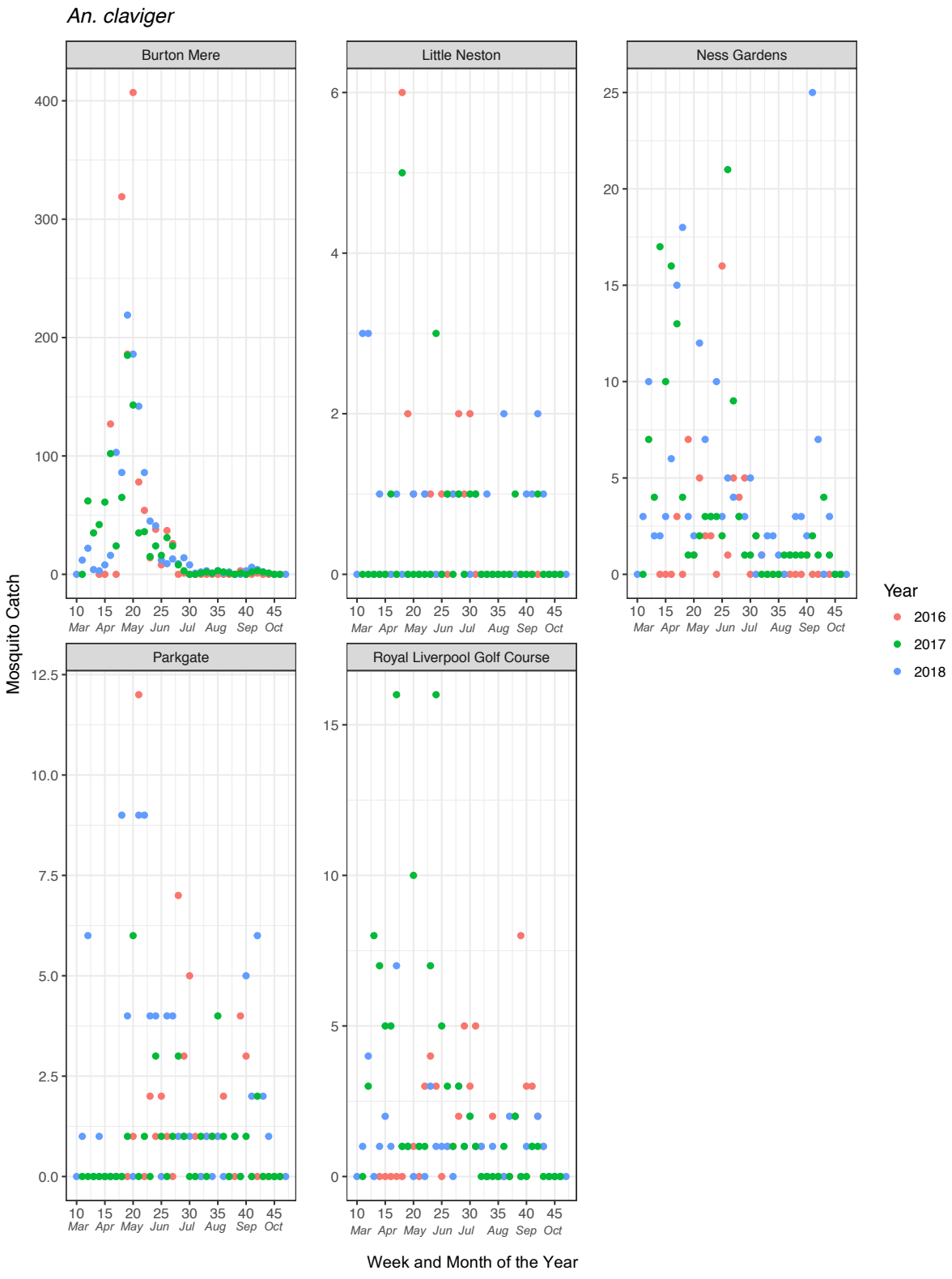

Figure 2.4: *An. claviger* at each of the sample sites over the three years of sampling.

Cs. annulata

Cs. annulata was collected at all of the sample sites with no clear peaks or drops in mosquito abundance (Figure 2.5). *Cs. annulata* was collected between the 1st April and the 31st October (weeks 13-43).

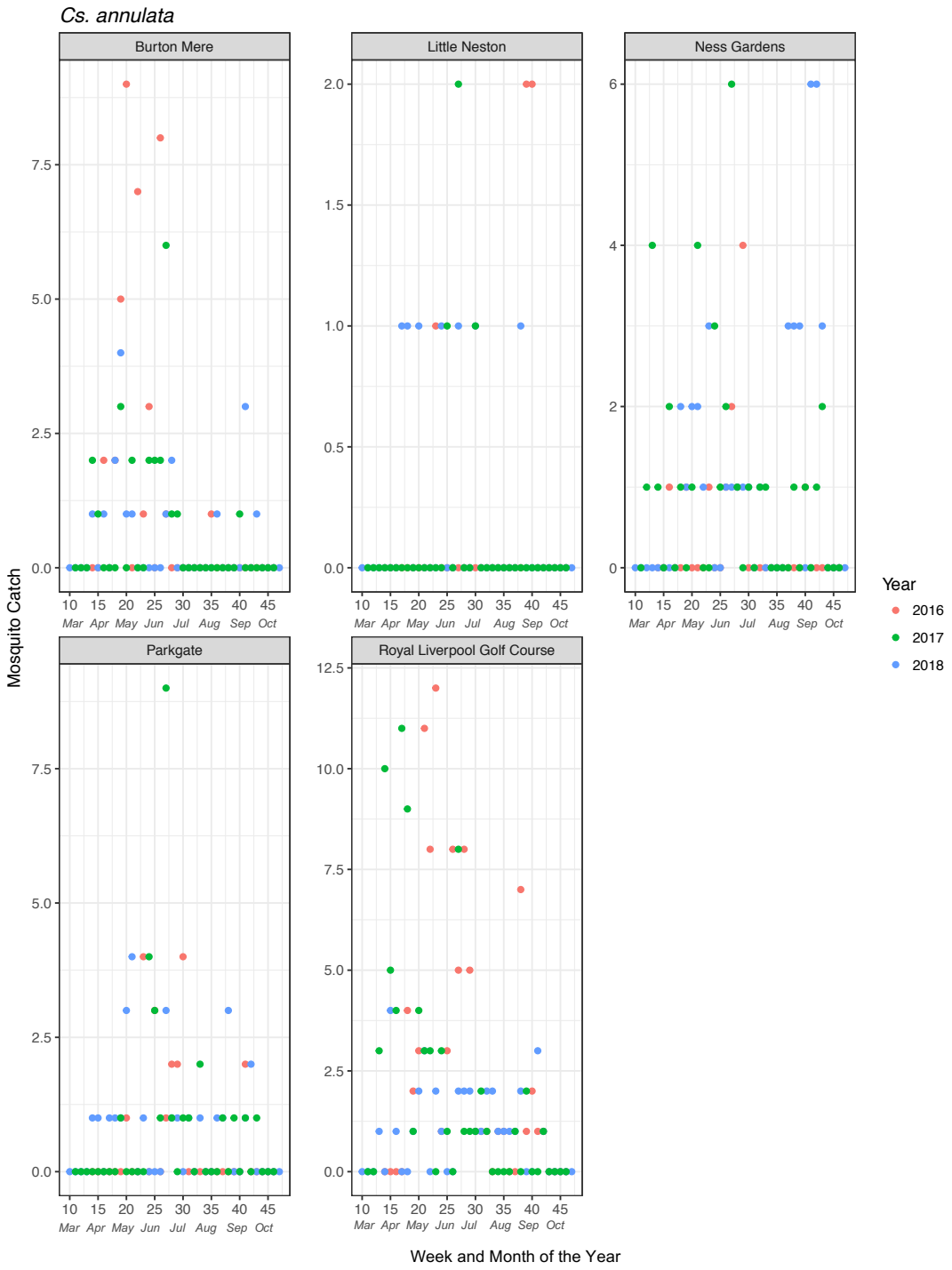

Figure 2.5: *Cs. annulata* catch at each of the sample sites over the three years of sampling.

Ae. cantans/annulipes

Ae. cantans/annulpies was only collected at three of the five samples sites across the three years of sampling (Figure 2.6). Very low numbers were caught in Ness Gardens and the RLGC in across all three year of sampling with a maximum catch of four mosquitoes at these sites. Higher numbers of *Ae. cantans/annulipes* were collected at Burton Mere (Figure 2.6). All mosquitoes were collected during a two-month sampling period between the beginning of May and the end of June (weeks 18-26). The peak in *Ae. cantans/annulipes* abundance occurred in the first week of June across each of the three years with a total of 85, 23 and 72 collected each year respectively. No mosquitoes were collected after the end of June in any year.

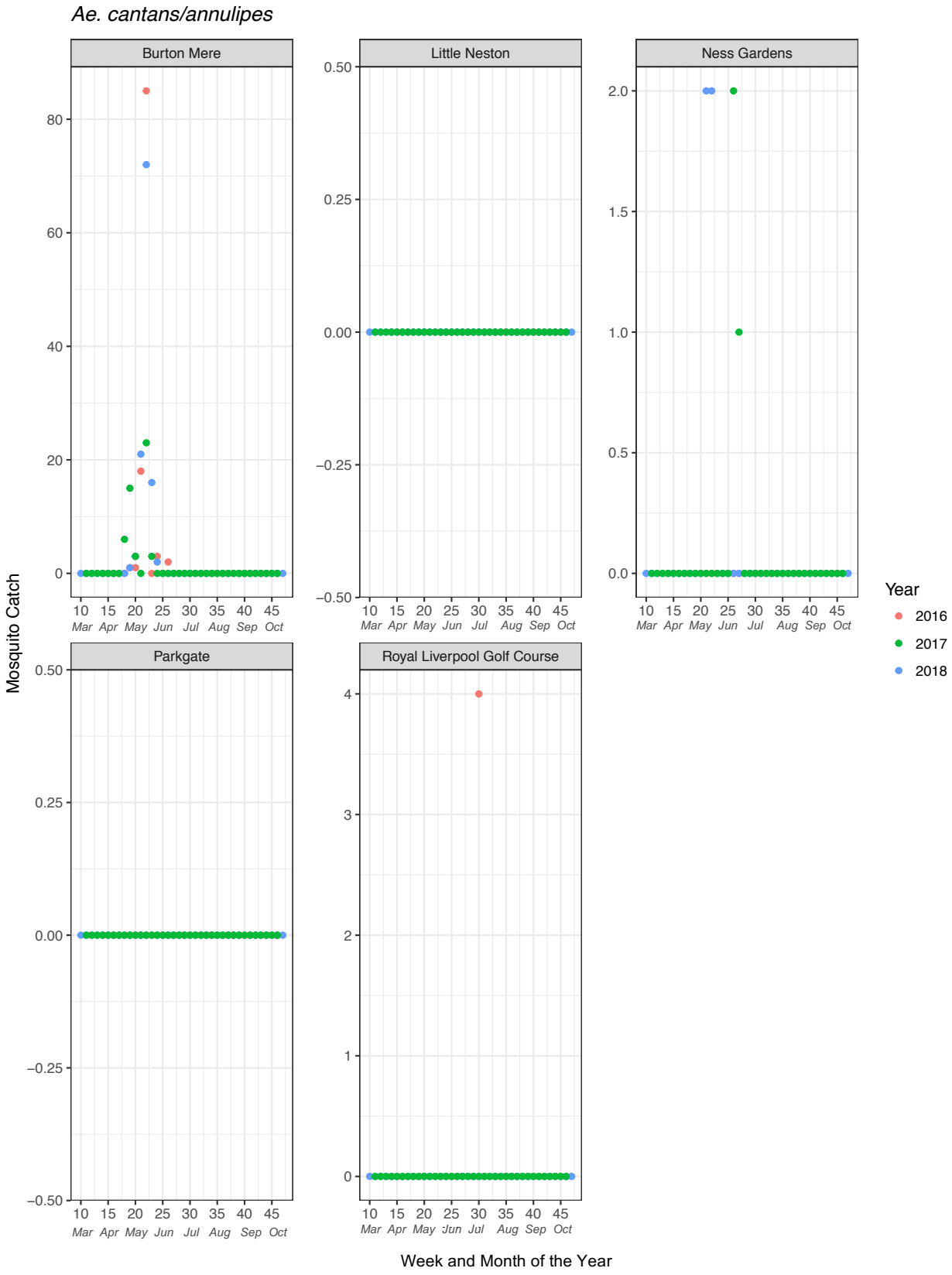

Figure 2.6: *Ae. cantans/annulipes* catch at each of the sample sites over the three years of sampling

Ae. rusticus

Ae. rusticus was collected at all sample sites although at Burton Mere and the RLGC the numbers were very low (Figure 2.7). The peak in *Ae. rusticus* abundance occurs between May and June across all sample sites. The maximum catch of more than 150 mosquitoes was recorded in Ness Gardens in 2016 during the first week in June (week 22). After the 25th July, no *Ae. rusticus* were collected in any of the sites over the three years of sampling.

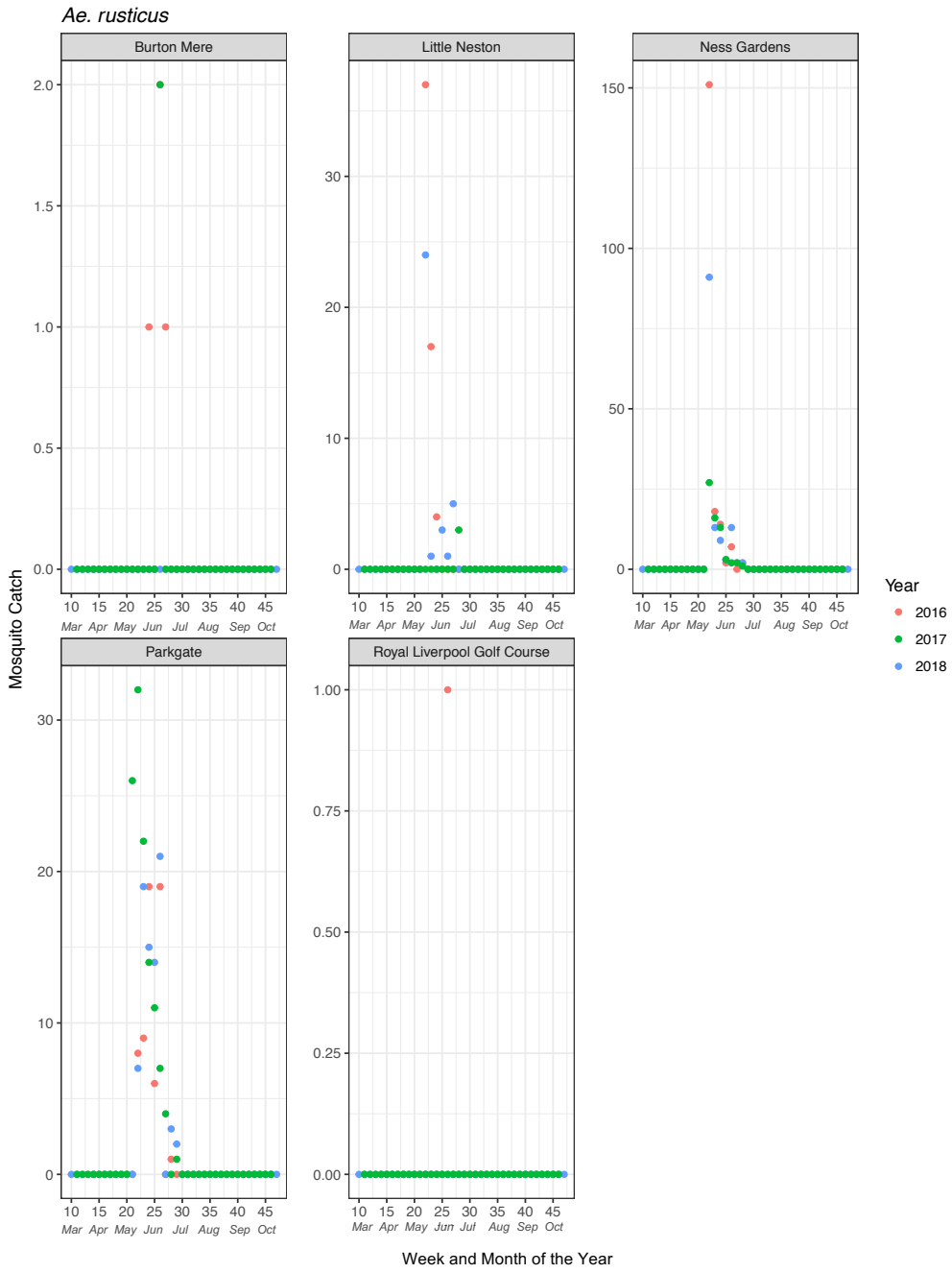

Figure 2.7: *Ae. rusticus* catch at each of the sample sites over the three years of sampling.

2.5.3 Description of Seasonality

To further explore patterns in seasonality, mosquito catch across all sites was combined and compared over three years (Figure 2.8). For the total mosquito catch, and for each of the five main species, very similar patterns in seasonal abundance can be seen (Figure 2.8).

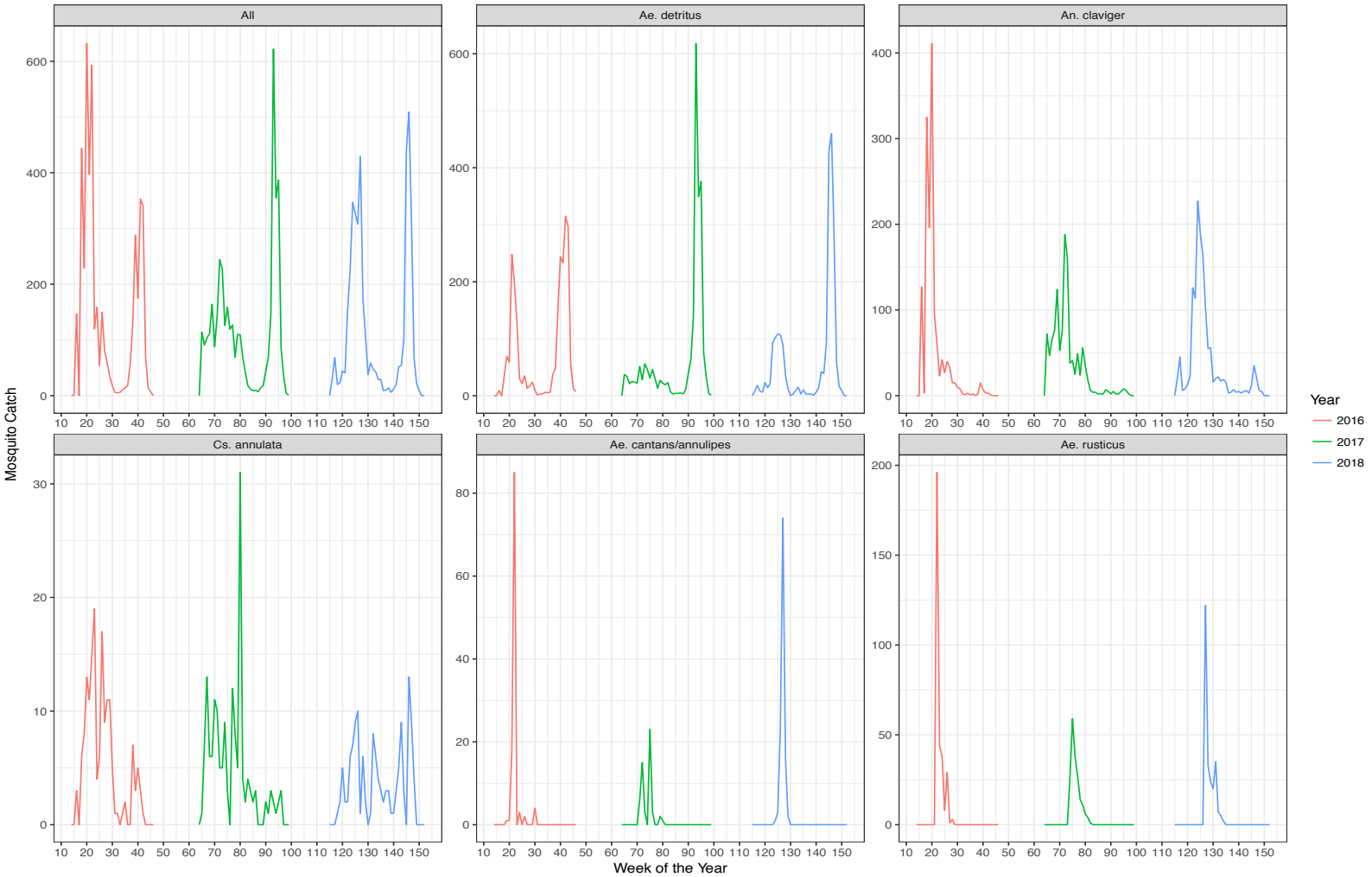

Figure 2.8: Total catch from all species combined and the five main species separately across each of the three years of sampling.

Total catch

Two clear peaks in total abundance can be seen across each of the three years (Figure 2.8). The first peak in abundance is greater in 2016 compared to both 2017 and 2018 when the peak in abundance occurs later in the year. During the summer mosquito catch drops to low levels until the second peak in abundance is seen. In 2017 the autumn peak in abundance is larger than in both 2016 and 2018.

Ae. detritus

Two clear peaks in the abundance of *Ae. detritus* can be seen across the three years of sampling (Figure 2.8). The first peak occurs between May and June each year. In 2016 this peak was much larger than in the two subsequent years with several weeks of mosquito catches over 100 per week. This spring peak in abundance is still observed in both 2017 and 2018 although it is less pronounced. Over all three years of sampling the peak in mosquito numbers takes place in autumn (between September and October). In 2016 there is less of a difference between the two peaks in abundance with a much greater difference observed in both 2017 and 2018. 2017 sees the largest number of *Ae. detritus* caught during this time with a total of over 600 mosquitoes caught in one week of sampling. The numbers of *Ae. detritus* collected across all sample sites drops in the summer months.

An. claviger

There is one large peak in the abundance of *An. claviger* which occurs every year between May and June (Figure 2.8). The majority of *An. claviger* are caught at Burton Mere, although they are caught in all of the sampling locations (Figure 2.4). The peak in abundance of *An. claviger* occurs in 2016 with over 400 mosquitoes caught during the spring peak in abundance. In both 2017 and 2018 the peak abundance is approximately half of the peak in 2016. The catches of *An. claviger* persist at low levels into October/November over all three years of sampling.

Cs. annulata

In both 2016 and 2018 there are no distinct peaks in the abundance of *Cs. annulata* (Figure 2.8). Mosquito catch does drop in the summer but not as clearly as some of the other multivoltine species (*Ae. detritus* and *An. claviger).* In 2017 there is a large spike in abundance where the total catch peaks at over 30 mosquitoes in one weeks sampling. Catches of *Cs. annulata* continue throughout the entire sampling period over the three years.

Ae. cantans/annulipes

Annually there is one peak in the abundance of *Ae. cantans/annulipes* which occurs between May and June (Figure 2.8). Abundance is greatest in 2016 with the majority of mosquitoes being collected at Burton Mere (Figure 2.5). The lowest numbers of *Ae. cantans/annulipes* are caught in 2017. No mosquitoes are caught after the end of June across all three years.

Ae. rusticus

There is one peak in the abundance of *Ae. rusticus* across each of the three years of sampling (Figure 2.8). The peak occurs between May and June each year after which the mosquito catch drops to zero for the remainder of the years sampling.

2.5.4 Consideration of the Environmental Variables

The meteorological data collected using the TinyTag in Ness Gardens during the 2016 sampling period was compared to the weather station in Ness Gardens (Figure 2.9 and Figure 2.10).

The average weekly temperature from both the TingTag and the weather station follow a similar pattern over the year (Figure 2.9). However, overall the TinyTag is recording a higher average temperature by +1.3^{o}C. The greatest disparity between the two recordings takes placed at the end of October (week 43) with the TinyTag recording the average temperature as 2.3^{o}C higher than the weather station.

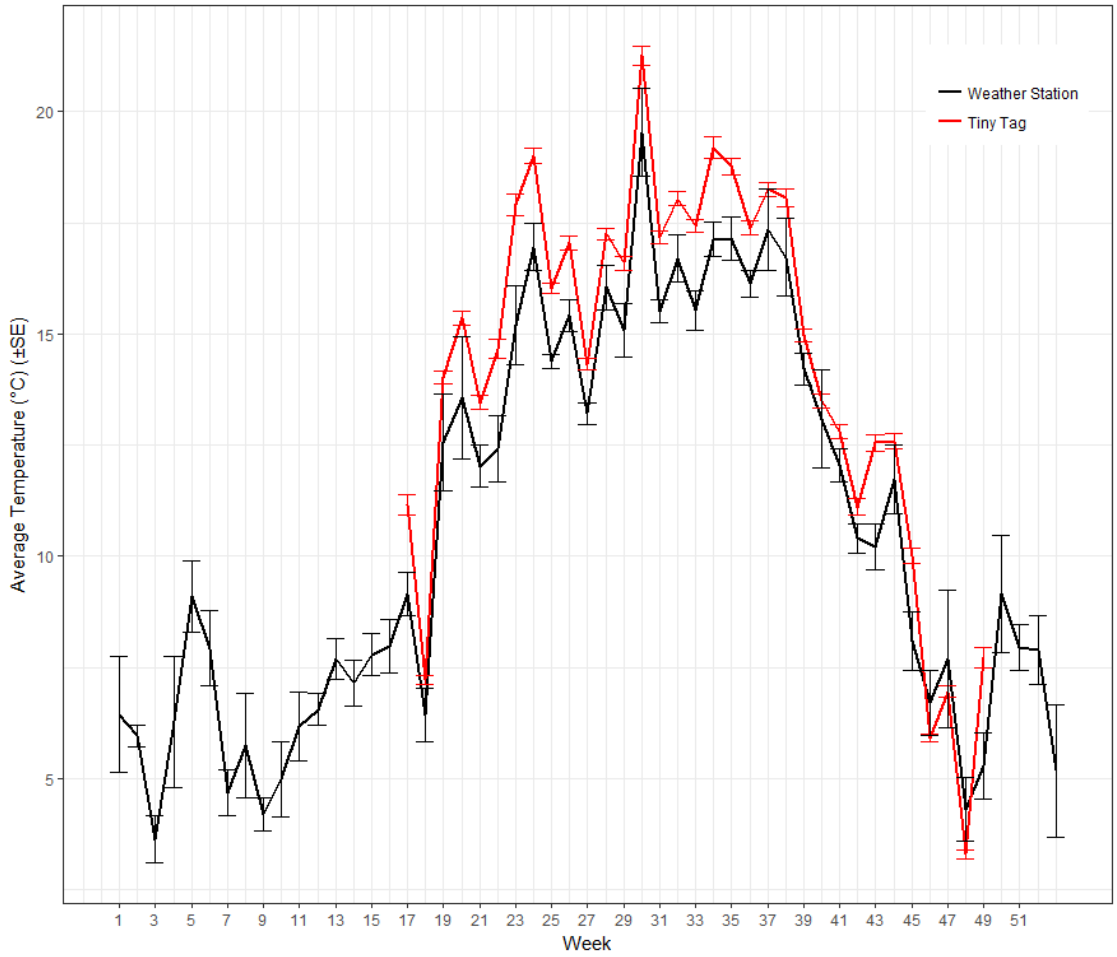

Figure 2.9: A comparison of the TinyTag and weather station temperature data recorded in Ness Gardens in 2016.

Again, a similar trend in the average weekly humidity from the TingTag and the weather station is observed (Figure 2.10). However, in some instances there is a large difference between recordings from the two methods. For example, over the last three weeks in November the TinyTag recorded an average humidity approximately 10% higher than that of the weather station (Figure 2.10).

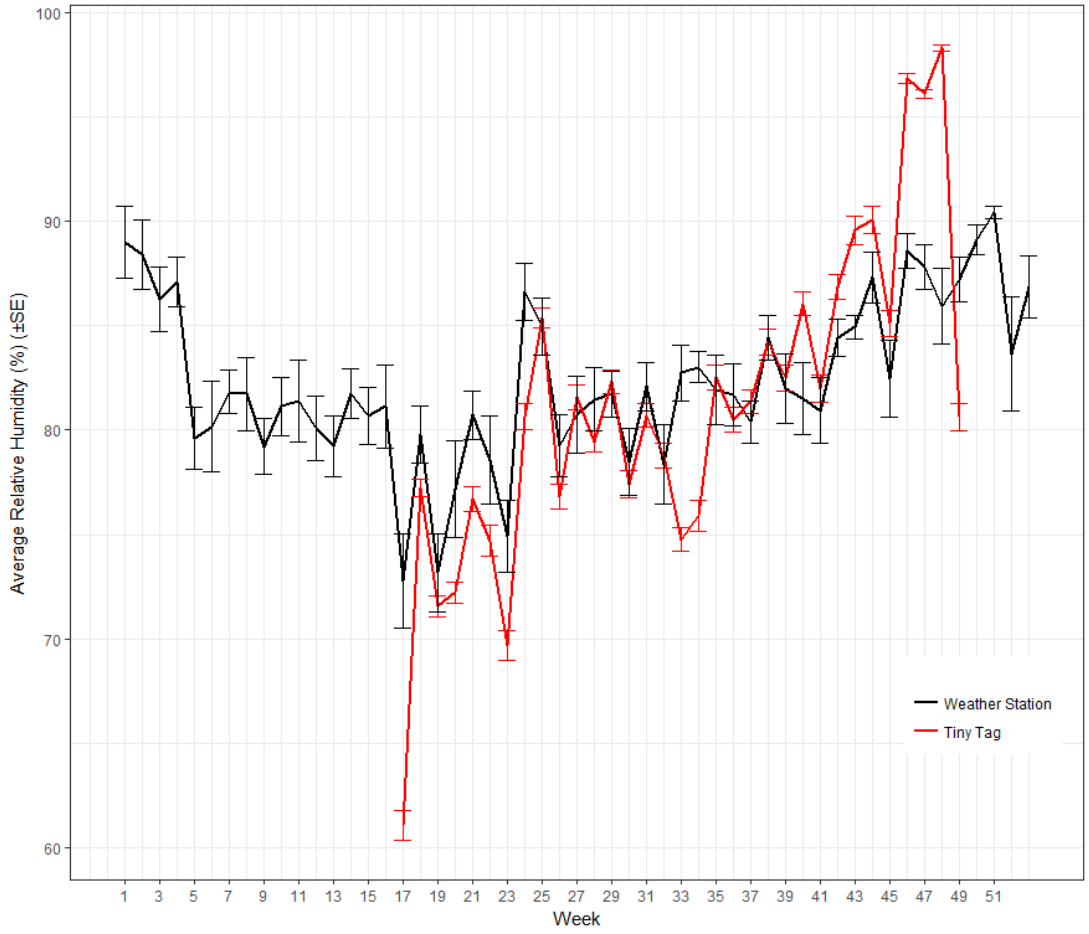

Figure 2.10: A comparison of the TinyTag and weather station humidity data recorded in Ness Gardens in 2016.

Given that both the temperature and humidity recordings from the TinyTag and weather station follow a similar trend, the measurements from the weather station only will be used in subsequent analyses.

2.5.5 Environmental Variables over the Three Years

Overall, the temperature follows a similar pattern over the three years (Figure 2.11). The temperature in 2017 was lower in the summer months (between May and August) compared to both 2016 and 2018. In 2018 there was a cold spell at the end of February and beginning of March with snowfall across all of the sample sites. This was a much colder start to the years mosquito sampling than experienced in both 2016 and 2017. Again in 2018, it was warmer than the previous two years of sampling from May to August (Figure 2.11). With the exception of a noticeable drop in humidity at the end of June in 2018, humidity remained fairly constant over the three years of sampling (Figure 2.11). There were inconsistencies with the windspeed data collected during 2017. For this reason, windspeed data for 2017 is omitted from subsequent data analysis and discussions (Figure 2.11). The average weekly windspeed was greatest in 2016 at the start of the year, peaking at ~5 m/s in the first week in February (Figure 2.11). Average windspeed drops from the beginning of March over the three years and remains at <3 m/s for the rest of the year. Overall the patterns between 2016 and 2018 are similar.

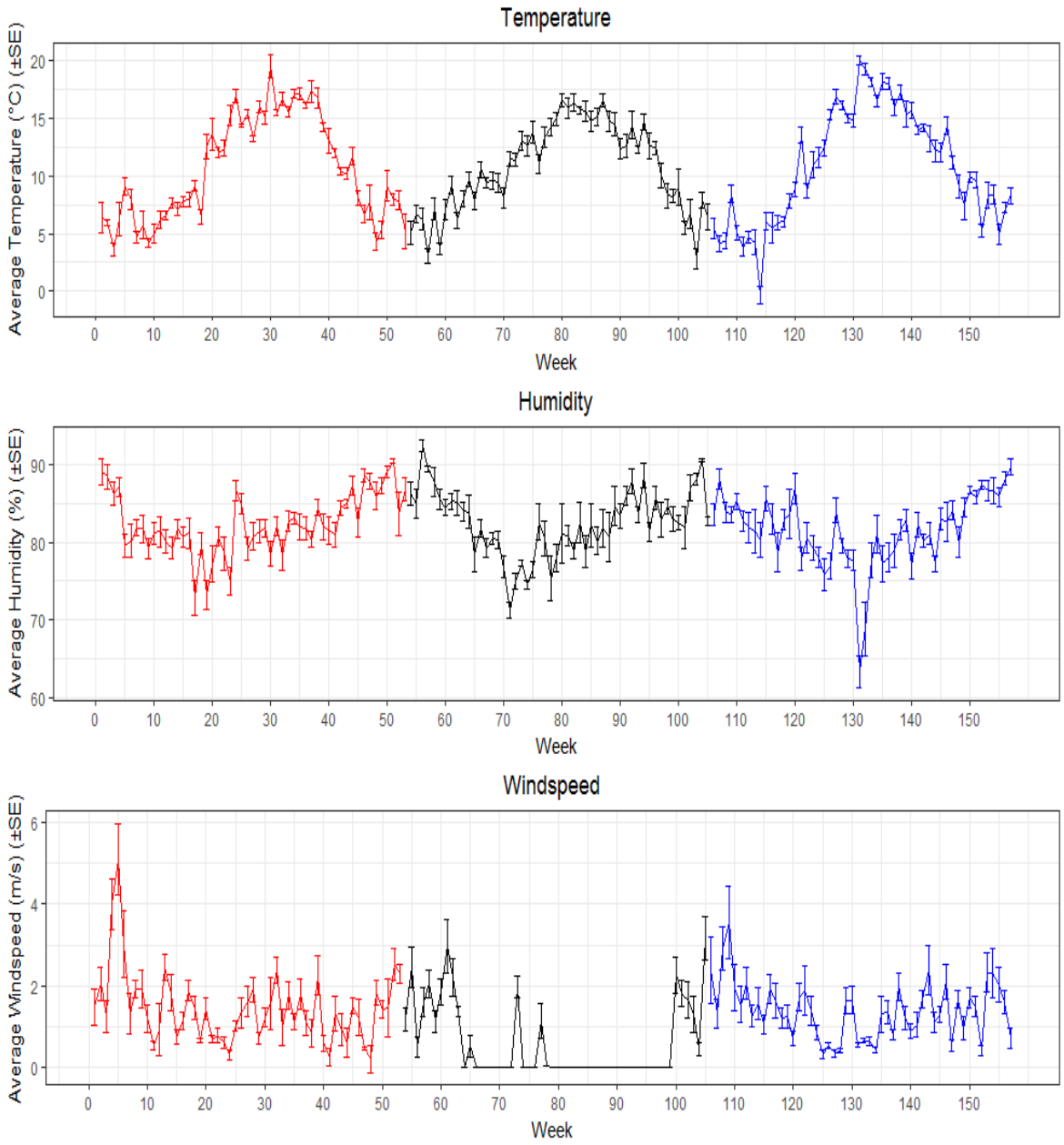

Figure 2.11: Average weekly temperature, humidity and windspeed ±SE over the three years of sampling. 2016 is shown in red, 2017 in black and 2018 in blue.

Rainfall follows a similar pattern across each of the three years of sampling with a higher average rainfall at both beginning and end of the year (Figure 2.12). Over all three years, rainfall drops between May and September. In 2018 the average weekly rainfall from September (week 35) is greater than that of the previous two years (Figure 2.12). Across the three years of sampling the average weekly high tides follow a similar trend (Figure 2.12). In both 2017 and 2018 the average high tides are larger

at the beginning of the year, peaking between January and March. In 2016, the average high tides are larger at the end of the year, peaking in October.

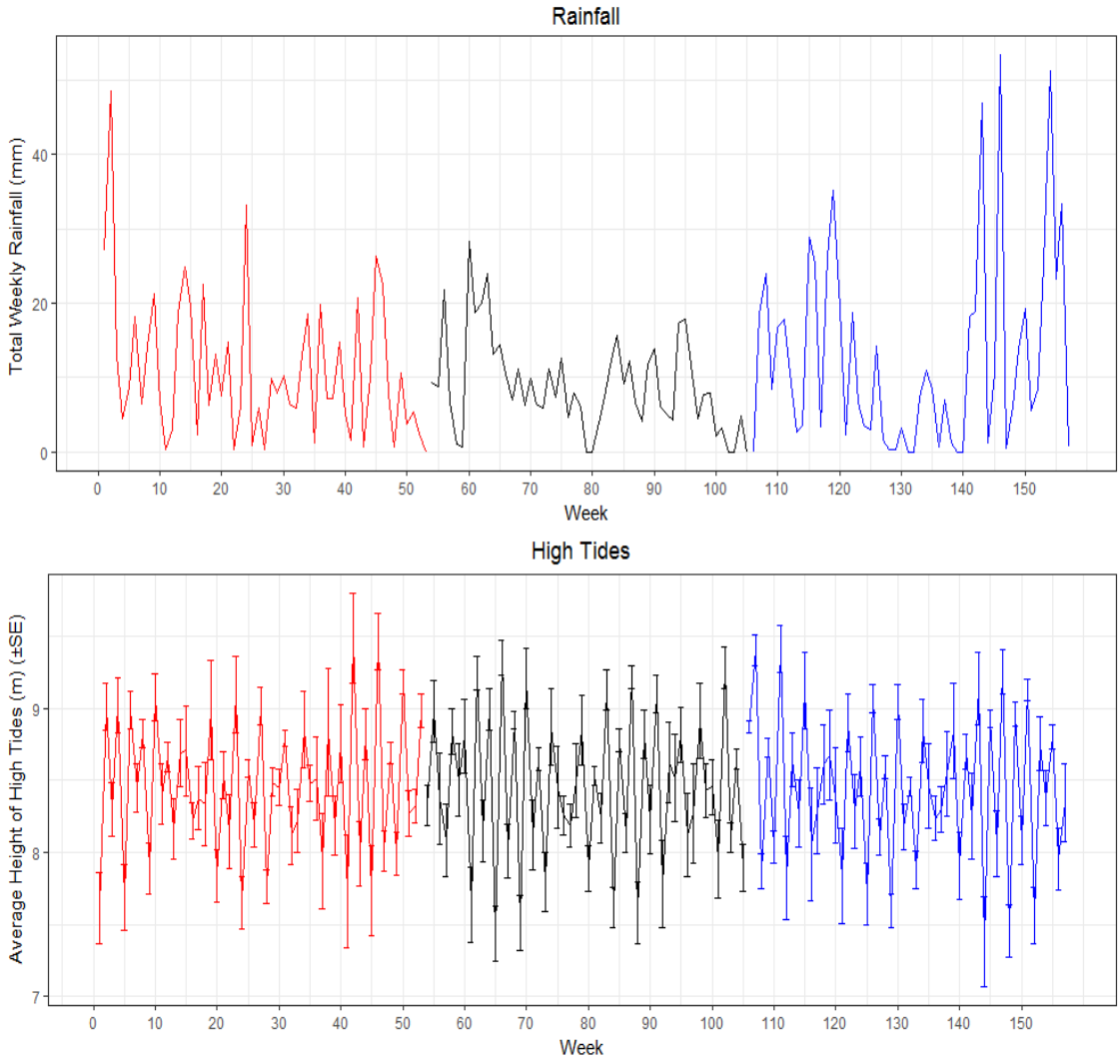

Figure 2.12: Total weekly rainfall and average weekly high tides ±SE over the three years of sampling. 2016 is shown in red, 2017 in black and 2018 in blue.

2.5.6 The Effect of Environmental Variables on Mosquito Catch

To explore the impact of the various environmental variables on mosquito abundance the average mosquito catch across all sample sites was plotted against the environmental variable in question (Figure 2.13, Figure 2.14, Figure 2.15, Figure 2.16 and Figure 2.17). This was done for all species combined as well as for the five main species in turn.

Temperature

For all mosquitoes collected over the three years there appears to be a threshold temperature, below which mosquito catch remains at zero (Figure 2.13). When considering all species combined, this threshold appears to be around 5^{o}C. For both *Ae. cantans/annulipes* and *Ae. rusticus* the threshold temperature is much greater, approximately 11^{o}C (Figure 2.13). Similarly, there is a threshold temperature, above which mosquito catch begins to decline. Considering all species combined this threshold appears to be approximately 14^{o}C. For *Ae. detritus* the decline in mosquito abundance after a threshold temperature (approximately 12^{o}C) is especially clear. The catch of *Ae. detritus* is lower during the warmer weeks of the year across all three years of sampling. The catches of *An. claviger* and *Cs. annulata* seem more resilient to the increased temperatures with less of a noticeable decline in the abundance of these species during warmer periods (Figure 2.13).

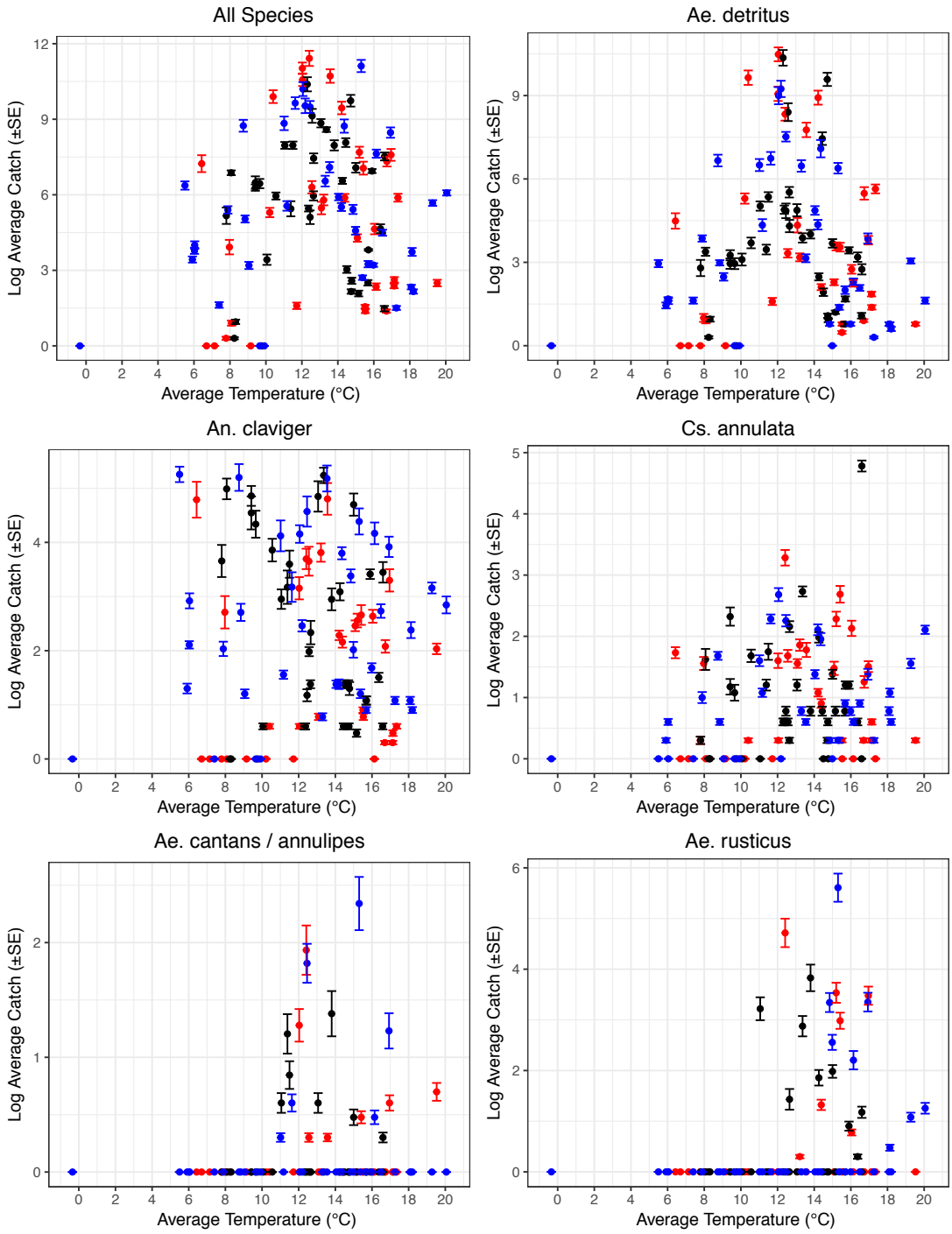

Figure 2.13: The average weekly catch across all sample sites plotted against average weekly temperature. 2016 data is shown in red, 2017 in black and 2018 in blue.

Humidity

A humidity between 75% and 85% appears to be optimum for the peaks in mosquito abundance (Figure 2.14). Considering all species combined, the majority of mosquitoes are caught between these two values. However, a drop in mosquito

abundance is not observed at humidity readings higher or lower than these values. Given the coastal location of the present study, humidity remains relatively constant throughout the three years of sampling and is therefore unlikely to have a noticeable impact on mosquito abundance.

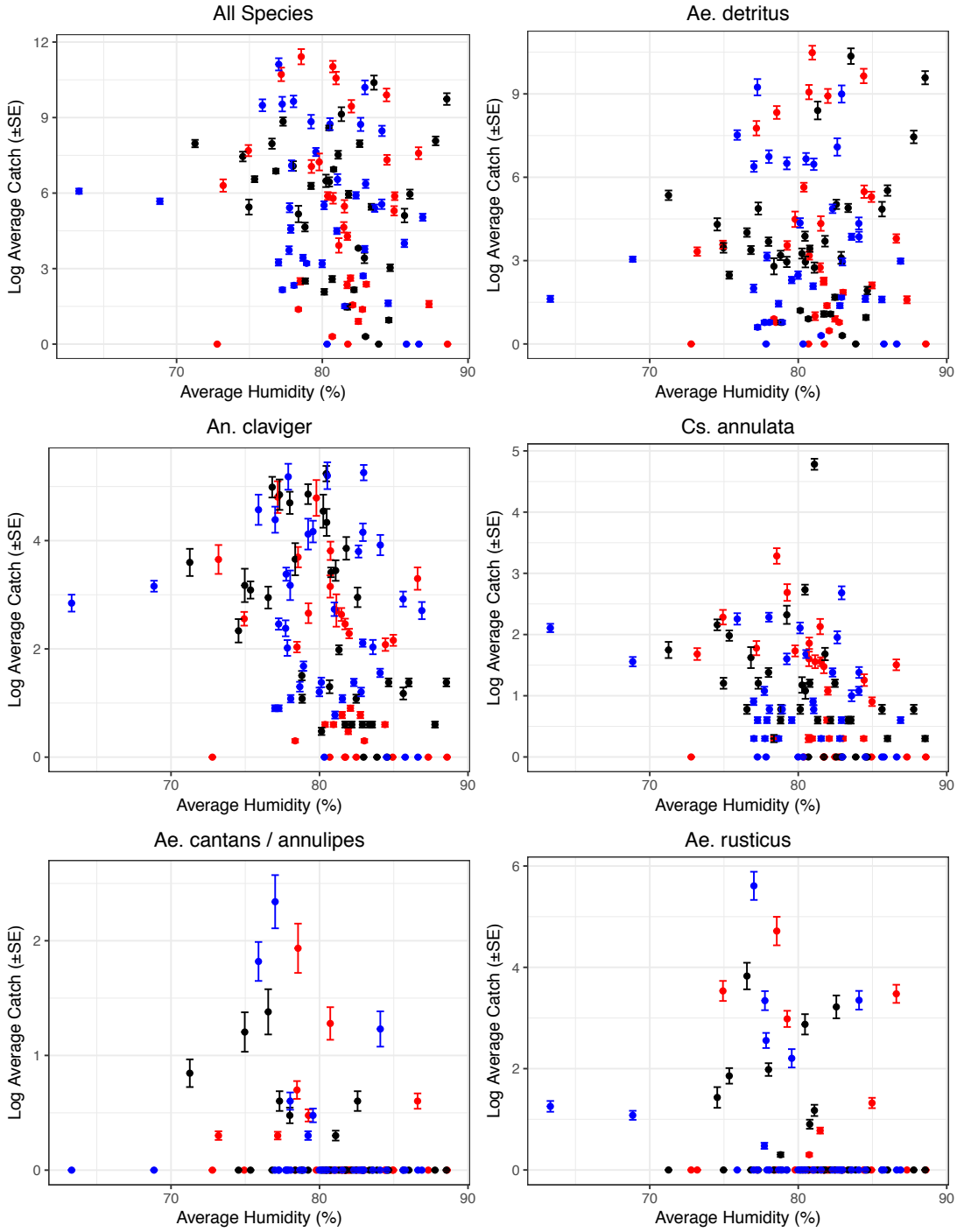

Figure 2.14: The average weekly catch across all sample sites plotted against average weekly humidity. 2016 data is shown in red, 2017 in black and 2018 in blue.

Rainfall

There is not a clear link between rainfall and mosquito catch (Figure 2.15). Good mosquito numbers are collected across the range of rainfall measurements.

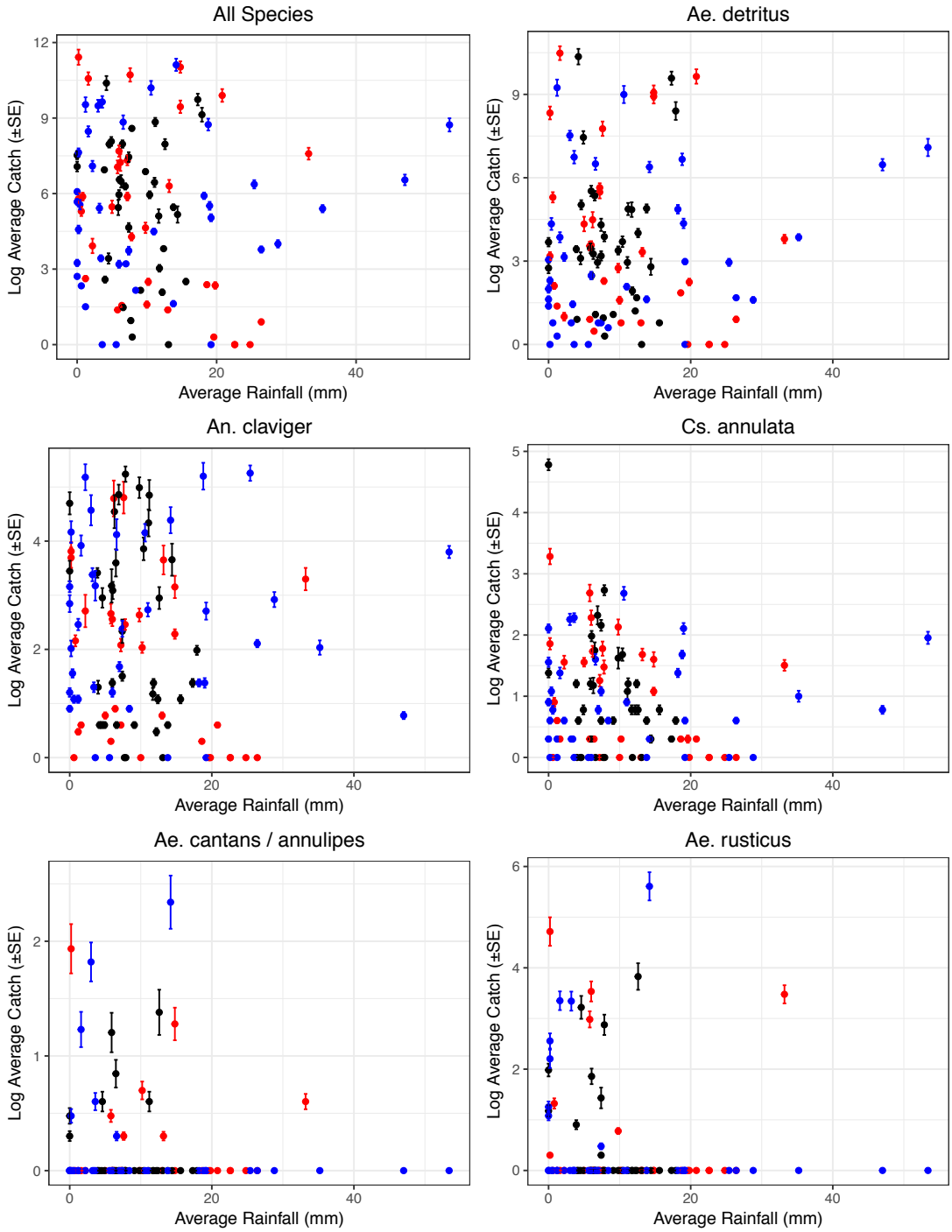

Figure 2.15: The average weekly catch across all sample sites plotted against weekly rainfall. 2016 data is shown in red, 2017 in black and 2018 in blue.

Windspeed

There does not appear to be an impact of windspeed on mosquito abundance (Figure 2.16). Equally high numbers of mosquitoes are caught across the entire range of windspeeds recorded over the three years of this study.

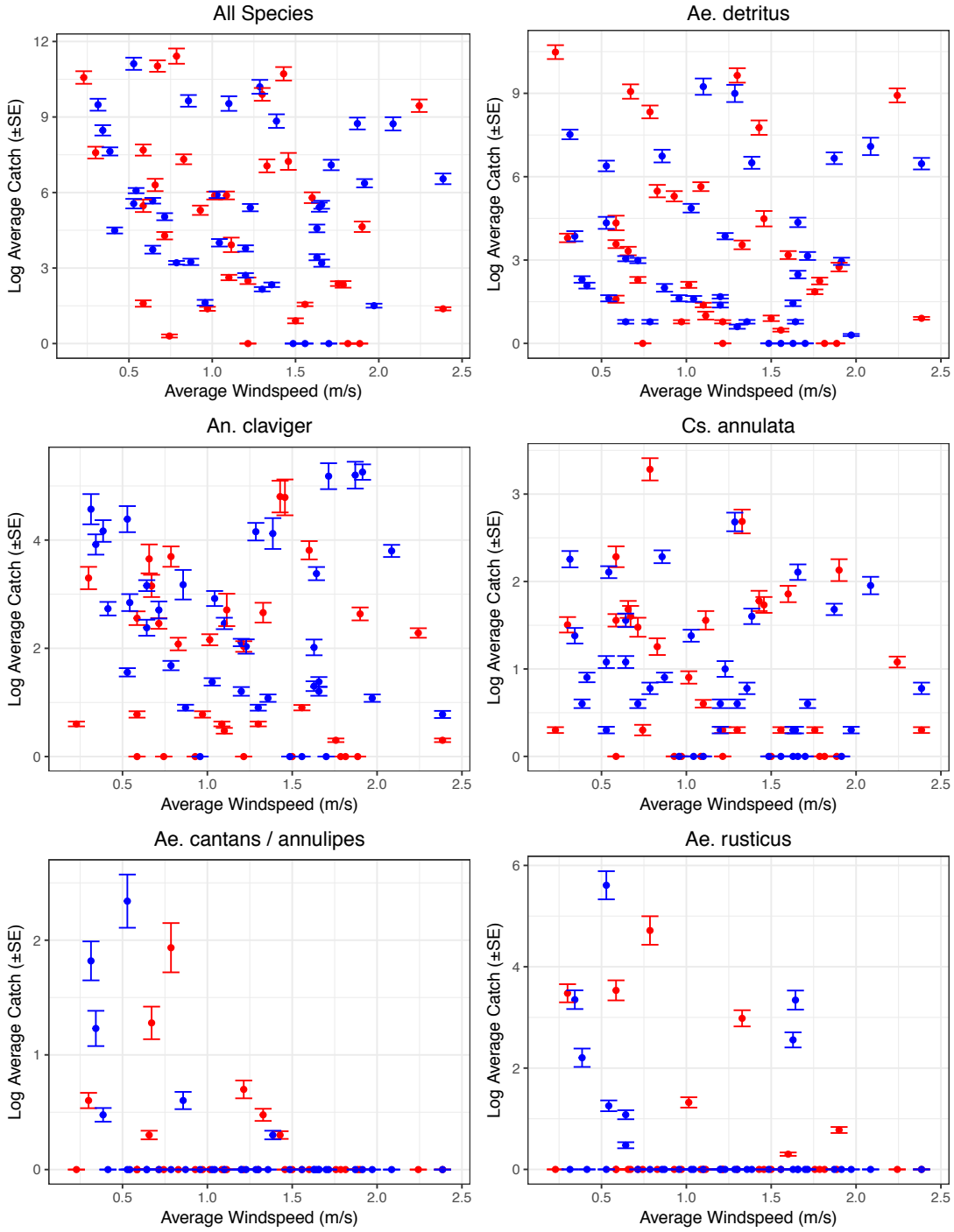

Figure 2.16: The average weekly catch across all sample sites plotted against average weekly windspeed. 2016 data and 2018 in blue. The 2017 windspeed data has been omitted.

Tide height

Tide height does not appear to be an important ecological driver behind mosquito abundance (Figure 2.17). Mosquito abundance is constant across the range of tide heights recorded over the three years of this study.

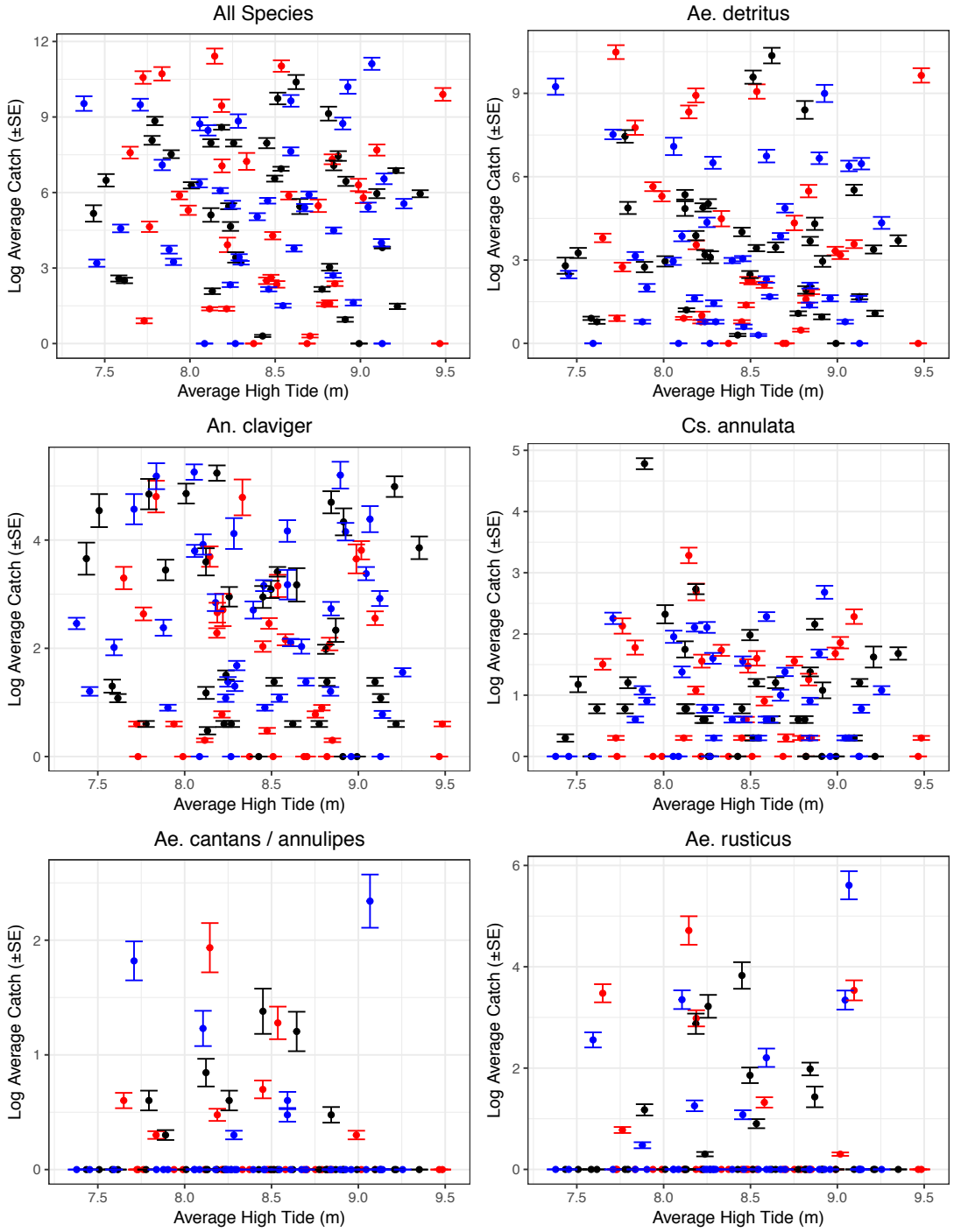

Figure 2.17: The average weekly catch across all sample sites plotted against average weekly high tides. 2016 data is shown in red, 2017 in black and 2018 in blue.

2.5.7 Generalized Linear Mixed Models

The three years of data were combined and GLMMs fitted to consider the effects over the entire sampling period. This was first done for the total mosquito catch before being completed for each of the main five species in turn.

A Poisson model was first fitted to the data followed by a negative binomial model. In all instances, the negative binomial model provided a better fit to the data as determined by comparison of the Akaike information criterion (AIC) values. To ascertain the maximal model, the fixed effects were first read in as continuous variables and then later as factors. The minimally adequate model contains a mixture of factors and continuous variables.

When considering the analysis for all species combined and *Ae. detritus* alone, a range of different tidal variables were explored to determine whether or not these impacted on mosquito abundance. Firstly, a lag period of up to two months was applied to the tidal data to explore the impact of historical tide height. Secondly, the impact of the equinoctial tides, in the spring and autumn, was assessed to determine whether or not these larger tides had an important impact on mosquito abundance. However, under these different scenarios, tide continued to not be a significant predictor of mosquito catch and was therefore omitted from the models.

Total mosquito catch

Considering all species combined, both temperature and humidity had a significant effect on mosquito catch (Table 2.7). Significantly more mosquitoes are caught within the 'mid' temperature range (12.03^{o}C-15.16^{o}C).

Table 2.7: Regression coefficients with 95% confidence intervals and standard error for the minimal adequate model for all species.

	Estimate (95% CI)	Standard Error
(Intercept)	4.5222 (1.783; 7.262) **	1.398
Humidity	-0.043 (-0.073; -0.013) **	0.015
Temperature – Low	0.449 (0.167; 0.731) **	0.144
Temperature – Medium	1.349 (1.089; 1.609) ***	0.133

***p<0.001, **p<0.01

Ae. detritus

Temperature is an important predictor in the catches of *Ae. detritus* (Table 2.8). Significantly more mosquitoes are caught in the 'low' and 'mid' (12.03^{o}C-15.16^{o}C) temperature ranges. Mosquito catch begins to drop when the temperature exceeds 15.16^{o}C.

Table 2.8: Regression coefficients with 95% confidence intervals and standard error for the minimal adequate model for *Ae. detritus.*

	Estimate (95% CI)	Standard Error
(Intercept)	-0.362 (-1.979; 1.256)	0.825
Temperature – Low	0.642 (0.281; 1.003) ***	0.184
Temperature – Medium	1.863 (1.529; 2.197) ***	0.170

***p<0.001

An. claviger

Both temperature and humidity in the 'low' and 'mid' ranges were found to be significant predictors of the catch of *An. claviger* (Table 2.9). Temperature (12.03^{o}C-15.16^{o}C) and humidity (79.3%-82.6%) values within these ranges have a positive impact on the number of *An. claviger* mosquitoes collected.

Table 2.9: Regression coefficients with 95% confidence intervals and standard error for the minimal adequate model for *An. claviger.*

	Estimate (95% CI)	Standard Error
(Intercept)	-0.926 (-2.118; 0.265)	0.608
Temperature – Low	0.535 (0.187; 0.883) **	0.178
Temperature – Medium	0.555 (0.216; 0.893) **	0.173
Humidity – Low	1.099 (0.771; 1.428) ***	0.168
Humidity – Medium	0.622 (0.285; 0.959) ***	0.172

***p<0.001, **p<0.01

Cs. annulata

Humidity was found to have a significant impact on the catches of *Cs. annulata* in both the 'low' (<79.3%) and 'mid' range (79.3%-82.6%) (Table 2.10). Temperature with the 'mid' range also had a significant impact on the numbers of *Cs. annulata* collected.

Table 2.10: Regression coefficients with 95% confidence intervals and standard error for the minimal adequate model for *Cs. annulata.*

	Estimate (95% CI)	Standard Error
(Intercept)	-2.106 (-3.159; -1.053) ***	0.537
Humidity – Low	1.083 (0.706; 1.460) ***	0.193
Humidity – Medium	1.054 (0.672; 1.437) ***	0.195
Temperature – Medium	0.424 (0.072; 0.776) *	0.180

***p<0.001, *p<0.05

Ae. cantans/annulipes

It was not possible to fit the GLMM to the catch data for *Ae. cantans/annulipes* due to the small sample size.

Ae. rusticus

Only humidity was found to be a significant predictor of the catch of *Ae. rusticus* (Table 2.11). When the humidity readings were within the 'mid' humidity range (77.8% and 80.7%) this was found to have a positive impact on the catches of *Ae. rusticus*.

Table 2.11: Regression coefficients with 95% confidence intervals and standard error for the minimal adequate model for *Ae. rusticus.*

	Estimate (95% CI)	Standard Error
(Intercept)	-1.931 (-4.418; 0.556)	1.269
Humidity – Medium	1.751 (0.857; 2.646) ***	0.457

***p<0.001

2.6 Discussion

2.6.1 General Summary

The sample site at the RLGC was located at the top end of the 18 km transect within the grounds of a golf course in a largely residential area. No easily accessible mosquito breeding sites were located in close proximity to the mosquito traps: these were likely to be present in private residences nearby. *Cs. annulata* and *Ae. detritus* were the most abundant species collected at the RLCG (30.1% and 29.6% respectively). Over all years of sampling, the highest diversity index scores were obtained from this sample site.

Extremely low numbers of mosquitoes were collected at Thurstaston during the 2016 sampling period. This sample site was a visitor's centre located at the top of cliffs overlooking the Dee estuary. It is not entirely clear why mosquito sampling was poor in this location. A survey of the surrounding area located several potential mosquito breeding sites (one small permanent pond and several artificial containers). However, the pond contained several potential predators and mosquito larvae were never found. No mosquito larvae were ever found in any of the artificial containers. Due to the time-consuming nature of the study sampling therefore ceased at this location at the end of the 2016 period.

Sampling in Parkgate took place in a private residence in very close proximity to the Dee estuary salt marsh. Larval sampling on the salt marsh found several breeding sites containing high numbers of *Ae. detritus. Ae. detritus* made up 78.8% of the total mosquito catch in Parkgate over the three years of sampling. Two peaks in the abundance of *Ae. detritus* were seen in this sample site over the three years of sampling. The first peak occurs between May and June and the second in October. A peak in the abundance of *Ae. rusticus* is also seen each year between May and June in Parkgate.

Two mosquito traps were located within the village of Little Neston. The first was in very close proximity to Dee estuary salt marsh were *Ae. detritus* breeds. The second sample site was slightly further from the salt marsh within a housing estate. Over the three years of sampling the lowest number of mosquitoes (total of 526) were caught

in Little Neston compared to all other sample sites. Annual catch was lowest in 2017 when only 93 mosquitoes were collected. *Ae. detritus* was the most abundant species making up 64.6% of the total catch followed by *Ae. rusticus* (18.1% of total). A maximum of seven different species were collected in Little Neston although the diversity index scores are low given the dominance of *Ae. detritus.*

Ness Gardens is the botanical gardens of the University of Liverpool spanning more than 60 acres comprised of a mixture of grassland and woodland. The bottom end of the garden's backs onto the Dee estuary salt marsh. Upon examination, several containers within the gardens were found to be productive mosquito breeding sites. Additionally, pools on the salt marsh were positive for *Ae. detritus* larvae. Ness Gardens was the most prolific sample site over the three-year study. *Ae. detritus* was the most abundant species collected making up 78.7% of the total catch. *Ae. rusticus* and *An. claviger* were also collected in high numbers (8.8% and 8.1% respectively). A maximum of ten species were collected in Ness Gardens over the three years. Two clear peaks in mosquito abundance are seen in Ness Gardens over the three years of sampling. These peaks consist mainly of *Ae. detritus* and occur between May-June and October-November. There is also a large peak in the density of *Ae. rusticus* in Ness Gardens

Burton Mere was a densely wooded sample site that backed onto the Dee estuary salt marsh. The ability to search for productive mosquito breeding sites was limited at this site in order to prevent disruption to the bird population. Burton Mere was the most prolific site for adult mosquito numbers in 2016 and was the second most prolific site in both 2017 and 2018. *An. claviger* was the most abundant species of mosquito collected with more than 70% of the total mosquito catch at this site being identified as *An. claviger.* Each year, a large peak in the abundance of *An. claviger* was seen at this site towards the end of April and beginning of May. *Ae. detritus* was also caught in high numbers in this location. Burton Mere was the only sample site where good numbers of *Ae. cantans/annulipes* were caught. Burton Mere was consistently one of the more diverse sample sites with a total of 12 different species collected over the three years. The lower diversity index scores at this site reflect the fact that *An. claviger* was highly dominant in this sampling location.

2.6.2 Summary of Mosquitoes

Ae. detritus

Ae. detritus was the most abundant mosquito species collected over the three years of sampling with two peaks in the mosquitoes density each year. *Ae. detritus* is a multivoltine mosquito species found in low-lying coastal areas breeding on salt marsh which experiences tidal flooding (Rees and Snow, 1996, Cranston *et al.,* 1987, Service, 1968). Given the large expanse of salt marsh on the Dee estuary, this is an important habitat for *Ae. detritus* (Clarkson and Setzkorn, 2011, Huckle *et al.,* 2004). The eggs of *Ae. detritus* hatch following immersion from the tide or precipitation (Service, 1968). Eggs that hatch during the winter months develop to the 4th larval instar stage to overwinter before pupation in March (Service, 1968). Larvae can be found all year round (Medlock *et al.,* 2005). Sampling to find *Ae. detritus* larvae was successful in several locations in this study. Adults emerge from March until November and can be widely dispersed. A previous Wirral based study reports adult catches more than 2 km from the salt marsh breeding sites (Clarkson and Setzkorn, 2011). *Ae. detritus* was the most abundant species of mosquito collected during this study. Annually, two clear peaks in the abundance of *Ae. detritus* were observed. The first between May and June and the second, much larger peak, between September and October. The unique ecology of *Ae. detritus,* which requires periodic tidal flooding of its breeding sites, is further explored in Chapter 3 of this thesis.

Ae. detritus is reported to bite humans, other mammals and birds outdoors (Medlock *et al.,* 2005, Service, 1971a). The Dee estuary is an SSSI supporting a huge array of birdlife and is especially important for waders and wildfowl (Natural England, 1998). Due to the fact that *Ae. detritus* will feed on both birds and humans, it has been highlighted as a potential bridge species for WNV. Several vector competence studies exploring the ability of *Ae. detritus* to transmit a range of arbovirus infections have been carried out (Blagrove *et al.,* 2016, Mackenzie-Impoinvil *et al.,* 2015, Hernández-Triana *et al.,* 2018). *Ae. detritus* has been shown to be competent for WNV, JEV and Rift Valley Fever virus (RVF) (Blagrove *et al.,* 2016, Mackenzie-Impoinvil *et al.,* 2015, Lumley *et al.,* 2018). The potential role of *Ae. detritus* in arbovirus transmission is further explored in Chapter 5 of this thesis.

An. claviger

An. claviger is a multivoltine species with previous reports of adult activity between March and November and larvae all months of the year (Cranston *et al.,* 1987). *An. claviger* was the second most abundant mosquito species found during the three years of sampling. There is a large peak in the density of this species in May although the numbers persist throughout the year. The peak in abundance of *An. claviger* has occurred within a one-week window over the last three years.

An. claviger preferentially breeds in densely vegetated ditches of which there were many in this sample site. (Medlock and Vaux, 2015a). However, ability to sample the larval population at Burton Mere was limited. Although some pools and ditches were available for sampling, no mosquito larvae were ever found during the three-year sampling period. *An. claviger* has been reported to bite several different mammalian species, including humans, and birds (Cranston *et al.,* 1987, Medlock *et al.,* 2005).

Cs. annulata

Cs. annulata is a multivoltine mosquito species which exploits a range of different breeding sites in both urban (artificial containers) and rural areas (ditches and ponds) (Cranston *et al.,* 1987, Snow, 1990). *Cs. annulata* is widespread within the UK where it is a prolific nuisance biter with adults found all year round (Medlock *et al.,* 2012a, Medlock *et al.,* 2005). This species has the longest period of activity of all the UK mosquito species which is reflected by the long season of positive *Cs. annulata* catches observed during this study. *Cs. annulata* will bite both humans and birds and has therefore been considered as a potential WNV vector (Medlock *et al.,* 2005). In the laboratory UK *Cs. annulata* has been shown to be a competent vector for WNV (M. Blagrove, personal communication, 2019).

Ae. cantans/annulipes

Given that morphologically *Ae. cantans* and *Ae. annulipes* are very similar no attempt to distinguish these two species was made (Snow, 1990). Both species are univoltine with adults typically found between May and October (Snow, 1990). The peak in abundance of these species occurs at the beginning of June over the three-year

sampling period. Both species are known nuisance biters with *Ae. cantans* previously reported as a nuisance biter on the Wirral (Medlock and Vaux, 2015a, Medlock *et al.,* 2012a). *Ae. cantans* is a known WNV vector in other parts of Europe (Labuda *et al.,* 1974, Hubálek and Halouzka, 1999).

Ae. rusticus

Ae. rusticus is a univoltine species which breeds in temporary freshwater pools often in woodland habitats (Cranston *et al.,* 1987). Adults are known to bite humans with several reports of nuisance biting from this species (Medlock *et al.,* 2012a). Vector competence studies have demonstrated that UK populations of *Ae. rusticus* are not competent vectors for Rift Valley fever virus (Lumley *et al.,* 2018). A clear seasonal trend in the abundance of *Ae. rusticus* was seen in this study with the peak in activity occurring between May and June.

Other Species of Interest

Ae. caspius is another multivoltine mosquito species that breeds in brackish water (Cranston *et al.,* 1987, Snow, 1990). Despite the large expanse of salt marsh in this study very few *Ae. caspius* mosquitoes were collected: a total of 141 over the three years of sampling. Both the adults and larvae are typically found between April and October (Cranston *et al.,* 1987, Snow, 1990). Adults will feed on both birds and humans meaning this species has also been highlighted as a potential vector of WNV (Medlock *et al.,* 2005). *Ae. caspius* has also been shown to be a competent vector for RVF (Turell *et al.,* 1996).

Cx. pipiens s.l. is a multivoltine mosquito species with the adults found between May and September. Adults will prolifically bite both birds and humans (Snow, 1990). *Cx pipiens s.l.* is an important vector of WNV in Europe, acting a s a bridge species between birds and humans (Hubálek and Halouzka, 1999). *Cx. pipiens s.l.* is also the vector of Usutu and Sindbis viruses in Europe (Vazquez *et al.,* 2011, Ashraf *et al.,* 2015, Taylor *et al.,* 1955, Medlock *et al.,* 2007). Studies have also shown that UK populations of *Cx. pipiens* are competent vectors of RVF and USUV in the laboratory

(Lumley *et al.,* 2018, Hernández-Triana *et al.,* 2018). High densities of *Cx. pipiens s.l.* have previously been collected in this area of the Wirral (Clarkson and Setzkorn, 2011). However, low numbers of this species were collected during this study (111 in total over the three years). One study in the UK found that CDC light traps collected *Cx. pipiens* in greater numbers compared to Mosquito Magnets (Hutchinson *et al.,* 2007). In order to catch greater numbers of *Cx. pipiens* a different mosquito trap could be used.

Overall, the total number of mosquitoes collected during the summer months declines. However, the greatest diversity of species is collected during this time of the year.

2.6.3 Ecological Drivers Underlying Changes in Mosquito Abundance

In order to explore the ecological drivers behind mosquito abundance, mosquito catch was first compared to the various meteorological observations. From these plots it was clear that only temperature had an impact on mosquito catch, noticeable for *Ae. detritus, Ae. cantans/annulipes* and *Ae. rusticus.* The impact of temperature was less clear for *An. claviger* and *Cs. annulata.* Whilst the overall number of mosquitoes is lower during the summer months, there is more diversity with a greater number of mosquito species recorded during this time.

The salt marsh breeding site of *Ae. detritus* is prone to desiccation in the summer months (this phenomenon is further explored in Chapter 3 of this thesis). This being said, as the temperature increases the breeding sites of this mosquito begin to disappear. The eggs of *Ae. detritus* require immersion from the tide or precipitation to hatch (Service, 1968). Eggs that are not flooded, do not hatch and are reported to be able to survive for over a year (Marshall, 1938). This drying of the *Ae. detritus* breeding sites could explain why the numbers of this species decline during the summer months when the temperature is high. The second peak in numbers occurs once the breeding sites have flooded once again and the eggs begin to hatch. Other studies exploring the ecology of *Ae. detritus* mosquito populations in mainland

Europe also report low numbers of mosquitoes during the warmer summer months (Veronesi *et al.,* 2012).

Both *An. claviger* and *Cs. annulata* exploit ditches for their breeding sites (*Cs. annulata* also in containers in urban areas). These breeding sites are less prone to desiccation during the summer months meaning they remain as active breeding sites throughout the year. This could explain why increases in temperature do not appear impact so greatly on the catches of these two species.

Subsequently, GLMMs were fitted to the data. This was done for the total mosquito catch as well as for the five main species caught during sampling. The results of the models suggest that both humidity and temperature are significant predictors of mosquito catch. However, the effects of these variables are very small. Rather, week of the year appears to be the main driver of mosquito abundance. However, it must be noted that week of the year will be strongly correlated with other factors, such as photoperiodism and day length. There are other species of insect within the UK that show strong seasonal peaks in abundance. For example, Mayfly abundance is highly seasonal with huge peaks in abundance occurring within the same weeks each year (Brittain, 1982, Harper and Peckarsky, 2006).

Previous studies exploring the impact of different meteorological variables on mosquito abundance in mainland Europe have found that these variables are important predictors of mosquito abundance (Roiz *et al.,* 2014). For example, a positive relationship between accumulated temperatures (1-4 weeks before sampling) was reported for *Cx. modestus* whilst a negative relationship between the same accumulated temperatures was reported for *Cx. pipiens and Ae. detritus* as high temperature was thought to impact on the larval stages of these mosquitoes (Roiz *et al.,* 2014).

The present study provides a detailed account of the seasonality and abundance of mosquito species along the Dee estuary. Ongoing mosquito surveillance studies being conducted by PHE need to continue to ensure that we have a detailed understanding of the ecology of UK mosquito populations. This is especially important in high risk regions of the UK such as southern England.

2.7 Conclusions

It appears that the abundance of mosquitoes along the Dee estuary is highly predictable and is unaffected by seasonal changes in meteorological variables. The data generated during this study shows that week of the year appears to be the key driver behind peaks in mosquito abundance being recorded.

Several of the mosquito species in this study act as nuisance biters to the local community. The results of the present study could be used to inform local authorities on when to expect peaks in nuisance biting reports. This would enable appropriate strategies to be put into place to deal with the mosquito populations. Further to this, in the event of arbovirus transmission in the UK, it would be possible to predict when key mosquito species, such as *Ae. detritus,* will reach their peak of activity regardless of climatic conditions. This is especially helpful for designing future control strategies. Given that the UK is currently at risk from invasive mosquito species, such as *Ae. albopictus,* or arboviruses, such as WNV, it is especially important to have a detailed knowledge of the abundance of mosquitoes across the entirety of the UK. This study provides a detailed account of the presence and seasonality of mosquitoes along the Dee estuary.

Chapter 3 - The Ecology of the Immature Stages of *Aedes Detritus* on the Dee Estuary Salt Marsh

3.1 Abstract

Ae. detritus is a highly abundant mosquito species along the Dee estuary salt marsh where it is a prolific nuisance biter. Previous studies into the larvae of *Ae. detritus* have shown that periodic tidal flooding plays an important role in the ecology of this mosquito species. This study aimed to explore the impact of tidal flooding on the ecology of *Ae. detritus* along the Dee estuary salt marsh.

Sentinel pools were monitored for *Ae. detritus* larvae over a three-year period. Estimates of larval abundance were obtained through larval sampling carried out fortnightly in 2016 and weekly in 2017 and 2018. A range of meteorological variables and information on local tide heights were also gathered. Over 27,500 larvae were collected during the three years of sampling. Two clear peaks in the abundance of *Ae. detritus* larvae in the breeding sites were observed. The first occurs in early May and the second, much larger, peak occurs in September/October. Analysis of the relationship between meteorological variables and abundance of larvae showed that both temperature and humidity are important predictors of the abundance of *Ae. detritus* larvae. Above a certain temperature threshold (approximately 16^{o}C), the average number of mosquito larvae present in the pools begins to decrease. During the summer months, the breeding sites are beginning to dry out with several pools disappearing completely. Once the pools are filled again by seasonal high tides in September, the numbers of *Ae. detritus* larvae begin to increase again.

A drone was used to produce high resolution maps of *Ae. detritus* breeding sites along the Dee estuary. Maps were produced every two weeks to provide a detailed picture of how this habitat changes over the course of a year. The impact of increasing temperature and desiccation on the number of mosquito larvae in these pools can be clearly seen from these maps. The number of suitable mosquito breeding sites reduces in warmer periods. Additionally, it is possible to visualise how tidal flooding replenishes these pools and thus the number of suitable breeding sites increases again.

3.2 Introduction

3.2.1 The Dee Estuary

The Dee is a macrotidal estuary, approximately 30 km long, running between England and Wales (Moore *et al.,* 2009) (Figure 3.1). The main channel runs close to the Welsh shore with large areas of salt marsh spanning the English shoreline (Moore *et al.,* 2009). It is estimated that there is over 2000 ha of salt marsh on the estuary (Huckle *et al.,* 2004). At Parkgate the salt marsh extends to 3 km out into the estuary (Huckle *et al.,* 2004). The area is an SSSI managed by the Royal Society for the Protection of Birds (RSPB) and is an extremely important habitat for several species of wildfowl and waders (Clarkson and Setzkorn, 2011, Huckle *et al.,* 2004).

Figure 3.1: Map of the United Kingdom with a close up of the Dee estuary. Basemap: © OpenStreetMap contributors, CC-BY-SA (https://www.openstreetmap.org/copyright).

There has long been a problem of nuisance mosquito biting along the Dee estuary, particularly focused around Neston and Parkgate (Clarkson and Setzkorn, 2011, Medlock *et al.,* 2012a). The local authorities in this area have made previous attempts to control the mosquito population breeding on the salt marsh. Control has largely

focused on treatment of breeding sites with *Bti*, a biological control agent that kills mosquito larvae (Clarkson and Setzkorn, 2011, Melo *et al.,* 2016). Despite control efforts, nuisance biting continued to be reported and control efforts have since ceased. The mosquito responsible for the majority of nuisance reports was identified to be *Ae. detritus* (Clarkson and Setzkorn, 2011, Medlock *et al.,* 2012a).

3.2.2 Salt Marsh Mosquitoes and Tidal Flooding

Globally, salt marshes are important breeding sites for several species of mosquito. These salt marsh habitats are classified as either low or high marsh (Rey *et al.,* 2012). Low salt marsh is impacted by the daily tide systems whilst high salt marsh is only flooded by seasonal high tides (Rey *et al.,* 2012). In terms of mosquito ecology, low salt marsh does not make a suitable breeding site; daily flooding flushes mosquito larvae from pools and can introduce predators (Rey *et al.,* 2012). High marsh, where pools are flooded periodically, can be prolific breeding sites although these pools can be at risk from desiccation during dry periods (Saintilan, 2009, Russell, 1998).

Open Marsh Water Management (OMWM) is a method of habitat manipulation to help control mosquito populations within salt marsh habitats (Meredith *et al.,* 1985, Medlock and Vaux, 2014). For example, in Australia, control of the Ross River virus (RRV) vector *Aedes vigilax* focuses on OMWM through the creation of 'runnels' to connect isolated pools in the marsh to the main tidal source (Saintilan, 2009). This OMWM increases the rate at which pools are flushed by high tides and allows for the introduction of predators both of which impact on mosquito density (Saintilan, 2009).

Within the UK, there are several species of mosquito for which salt marshes are important habitats (Medlock *et al.,* 2012a, Medlock and Vaux, 2014). Studies into the ecology of one such species, *Ae. detritus,* have previously been conducted with particular reference to the impact of tidal flooding within these habitats (Service, 1968, Clarkson and Setzkorn, 2011, Clarkson *et al.,* 2016).

3.2.3 Ecology of *Aedes detritus*

Ae. detritus is a multivoltine mosquito species found in low-lying coastal areas (Rees and Snow, 1996, Cranston *et al.,* 1987). The oviposition sites of *Ae. detritus* are on exposed muddy areas of salt marsh which are prone to flooding by tides and precipitation (Service, 1968, Medlock *et al.,* 2005). Given the large expanse of salt marsh on the Dee estuary this is an important habitat for *Ae. detritus* (Clarkson and Setzkorn, 2011, Huckle *et al.,* 2004).

The eggs of *Ae. detritus* hatch following immersion from the tide or precipitation, even during the winter months (Service, 1968). Eggs that hatch during the winter months develop to the 4th larval instar stage to overwinter before pupation in March (Service, 1968). Larvae can be found in pools all year round (Medlock *et al.,* 2005). Laboratory studies have demonstrated the importance of tidal flooding to this mosquito species: the greatest number of eggs hatch following the second to fifth consecutive soaking (Service, 1968). Eggs that are not flooded, and therefore do not hatch, can survive for over a year (Marshall, 1938). The distribution of *Ae. detritus* breeding sites is limited by the tides: locations that are regularly flushed by the tides do not make suitable breeding sites as the populations cannot become established (Service, 1968). Only those breeding sites that are protected from all but the very highest of tides allow populations of *Ae. detritus* to establish (Service, 1968, Edwards *et al.,* 1939). This has previously been observed on Brownsea Island in southern England, where breeding sites that were only flooded periodically by the tides were found to contain *Ae. detritus* larvae (Service, 1968).

Adults emerge from March until November and prolifically bite both humans and birds outdoors (Medlock *et al.,* 2005). A historical study looking at the feeding behaviour of UK mosquito species demonstrated that the majority of bloodmeals from *Ae. detritus* were from bovids (51%) followed by humans (32%) and birds (4%) (Service, 1971a). Adult catches can be rare when not located near to the salt marsh breeding sites (Service, 1968). Due to the fact that *Ae. detritus* feed on both birds and humans it has been highlighted as a potential bridge species for WNV transmission (Medlock *et al.,* 2005).

Several vector competence studies exploring the ability of *Ae. detritus* to transmit a range of arbovirus infections have been carried out (Blagrove *et al.,* 2016, Mackenzie-Impoinvil *et al.,* 2015). *Ae. detritus* has been shown to be a competent laboratory vector of WNV, JEV and RVF (Blagrove *et al.,* 2016, Mackenzie-Impoinvil *et al.,* 2015, Lumley *et al.,* 2018).

3.2.4 Why Map Mosquito Breeding Sites?

Successful mapping of mosquito breeding sites can be extremely beneficial. For example, in malaria endemic areas, where targeted vector control strategies are implemented, maps can identify productive breeding sites and assist with logistics and planning of control strategies (Ahmad *et al.,* 2011, Hardy *et al.,* 2017, Fillinger *et al.,* 2009). Maps can be used to help target key breeding sites to reduce costs of control interventions (Kenyeres *et al.,* 2017). Additionally, changes in the habitat over time, such as differences in the mosquito populations between the wet and dry seasons (Govoetchan *et al.,* 2014, Charlwood *et al.,* 2000), can be analysed.

3.2.5 Methods of Mapping Mosquito Breeding Sites

It is possible to map mosquito breeding sites on foot. However, this can be extremely time consuming and expensive whilst also encountering problems with land access and inconsistent or incomplete maps being produced (Hardy *et al.,* 2017). There are however several alternative approaches using remotely sensed data that can be used to help produce habitat maps.

Satellite imagery

Satellite imagery has long been used to map mosquito breeding sites and other factors related to malaria transmission (Hardy *et al.,* 2017, Bøgh *et al.,* 2007, Dambach *et al.,* 2012). In 1972 the Earth Resources Technology Satellite (ERTS-1) (since renamed Landsat 1) was launched to gather images of the earth's surface (Wulder *et al.,* 2012). Since 1972 a further seven satellites have been launched and a repository of millions of images collated (Woodcock *et al.,* 2008). These satellites provide medium resolution (30-50 m) images every 16 days (Hardy *et al.,* 2017,

Kalluri *et al.,* 2007). There are several studies which have successfully utilised Landsat imagery to map mosquito breeding sites (Bøgh *et al.,* 2007, Beck *et al.,* 1997, Zou *et al.,* 2006). However, high resolution imagery is often required to produce the most accurate maps (Koh and Wich, 2012). Other satellites, such as IKONOS and SPOT, provide higher resolution imagery (1 m and 10 m respectively) and have a spatial resolution of 3 days and 26 days respectively (Kalluri *et al.,* 2007, Lejot *et al.,* 2007).

There are several limiting factors to use of satellite images for producing maps of mosquito breeding sites. For example, until 2008, Landsat images were available for approximately $800 making this an expensive option for habitat mapping (Wulder *et al.,* 2012). However, Landsat images have since become open source , leading to their usage increasing greatly (Wulder *et al.,* 2012, Woodcock *et al.,* 2008). Vegetation and climatic conditions can also be limiting factors to the quality of map produced (Carrasco-Escobar *et al.,* 2019). This can be particularly troublesome in the rainy season when cloud cover limits the habitats that can be visualised from the satellite images (Carrasco-Escobar *et al.,* 2019). Additionally, infrequent revisits means that satellite images can be many months or even years out of date which is a hindrance when mapping ever changing breeding site habitats (Hardy *et al.,* 2017).

Drone Imagery

Drones or unmanned aerial vehicles (UAVs) are aircrafts controlled by computers or humans on the ground (Coeckelbergh, 2013). Drones have long been used in the agricultural sector, and this technology is now being applied to ecological studies as awareness of their capabilities are being realised (Fornace *et al.,* 2014). Additional sensors and cameras can be combined with the standard drone set-up to provide more detailed data collection (Sanfourche *et al.,* 2015).

Within the world of mosquito ecology, drones have successfully been used to map environmental risks for malaria transmission and mosquito breeding sites to assist with control interventions (Hardy *et al.,* 2017, Fornace *et al.,* 2014, Carrasco-Escobar *et al.,* 2019).

The first of these studies was conducted in 2014 and used drones to map environmental risk factors associated with malaria transmission (Fornace *et al.,* 2014). The study successfully used drones to collect real time data to study environmental risks over a small area (Fornace *et al.,* 2014). In 2016, a second study successfully used drones to map water bodies associated with malaria transmission in Tanzania (Hardy *et al.,* 2017). This proof of concept demonstrated that drones can play an important role in planning vector control programmes (Hardy *et al.,* 2017). Similarly, a 2019 study used drones to map larval habitats in Peru (Carrasco-Escobar *et al.,* 2019). The results of this work demonstrated the ability of drones to map breeding sites whilst techniques to accurately classify pools which were and were not colonised by mosquito larvae were developed (Carrasco-Escobar *et al.,* 2019).

A significant advantage of drones is their ability to be flexible with data collection whilst still providing high quality, low cost imagery (Hardy *et al.,* 2017, Fornace *et al.,* 2014). Multiple flights over the same area can be conducted providing a detailed picture of how an environment changes over time (Hardy *et al.,* 2017). Drones offer a low cost solution to data collection with complete set-ups available from approximately £800, although this cost is constantly reducing (Hardy *et al.,* 2017, Carrasco-Escobar *et al.,* 2019). Given their flexibility, drones can be used in areas where it is too difficult, costly or dangerous for traditional survey methods to be applied (Fornace *et al.,* 2014). Finally, drones are also able to cover large areas in an extremely small time frame compared to traditional on-foot survey methods (Carrasco-Escobar *et al.,* 2019)

However, there are also limitations to using drones for mapping mosquito breeding sites. Firstly, local laws and rulings on drone use in a sampling area need to be adhered to. For example, in the UK drones must not be flown above 120 m and should be kept in line of sight at all times (Civil Aviation Authority, 2019). Weather can also be a limiting factor with drones unable to fly in rainy or high wind conditions (Hardy *et al.,* 2017). Further to this, shadows caused by bright sunshine might limit the ability of breeding sites being identified during subsequent image processing (Hardy *et al.,* 2017). Finally, identification of some water bodies can prove difficult particularly, if they are densely vegetated or are the same colour as surrounding

ground (Hardy *et al.,* 2017). To overcome these issues, it is possible to use additional sensors to collect further multispectral images (such as Near Infrared) (Carrasco-Escobar *et al.,* 2019).

3.3 Aim and Objectives

This chapter aims to determine how tidal flooding on the Dee estuary impacts the larval population of *Ae. detritus.* In order to achieve this aim, the following four objectives were set:

The objectives of this chapter were to:

1. To record the larval abundance of *Ae. detritus* in a series of sentinel pools on the salt marsh of the Dee Estuary.
2. To use a drone to produce maps of the mosquito habitat.
3. To use the produced maps to make predictions about which pools do and do not contain mosquito larvae at different time points during the year.
4. To determine the impact of tidal flooding on the number of *Ae. detritus* larvae in this salt marsh habitat.

3.4 Materials and Methods

3.4.1 Larval Sampling

2016

An area of salt marsh within a 150 m radius of a Mosquito Magnet trap located at (53°16'38.25"N, 3° 3'58.84"W) was selected for larval sampling. The GPS coordinates of all pools within this area were taken. Ten of these pools were randomly selected to be visited every two weeks (Figure 3.2). The perimeter of the pool was walked (heel-toe) and dips, using a 250 ml dipper, taken every three steps. On each visit the perimeter of the pool was estimated (number of heel-toe steps multiplied by shoe size in cm (25.5 cm)). If mosquito larvae or pupae were present, the dip was poured into a white tray and the number and life stage recorded. The sample was then returned to the pool, the water allowed to settle, and the sampling continued. If predators were observed within the pool this was also noted. Larval sampling took place from May to November 2016.

Figure 3.2: Map of the 2016 larval sampling area. Mosquito magnet trap is shown in red, while the pools for sampling are shown in blue. Basemap: © OpenStreetMap contributors, CC-BY-SA (https://www.openstreetmap.org/copyright).

2017 and 2018

Sampling area was extended to include pools up to 250 m from the Mosquito Magnet located at (GPS 53°16'38.25"N, 3° 3'58.84"W). The GPS coordinates of all pools were taken and 50 randomly selected (using a random number generator) for sampling each week (Figure 3.3). The same sampling protocol as 2016 was followed. Larval sampling took place from March-November in both 2017 and 2018.

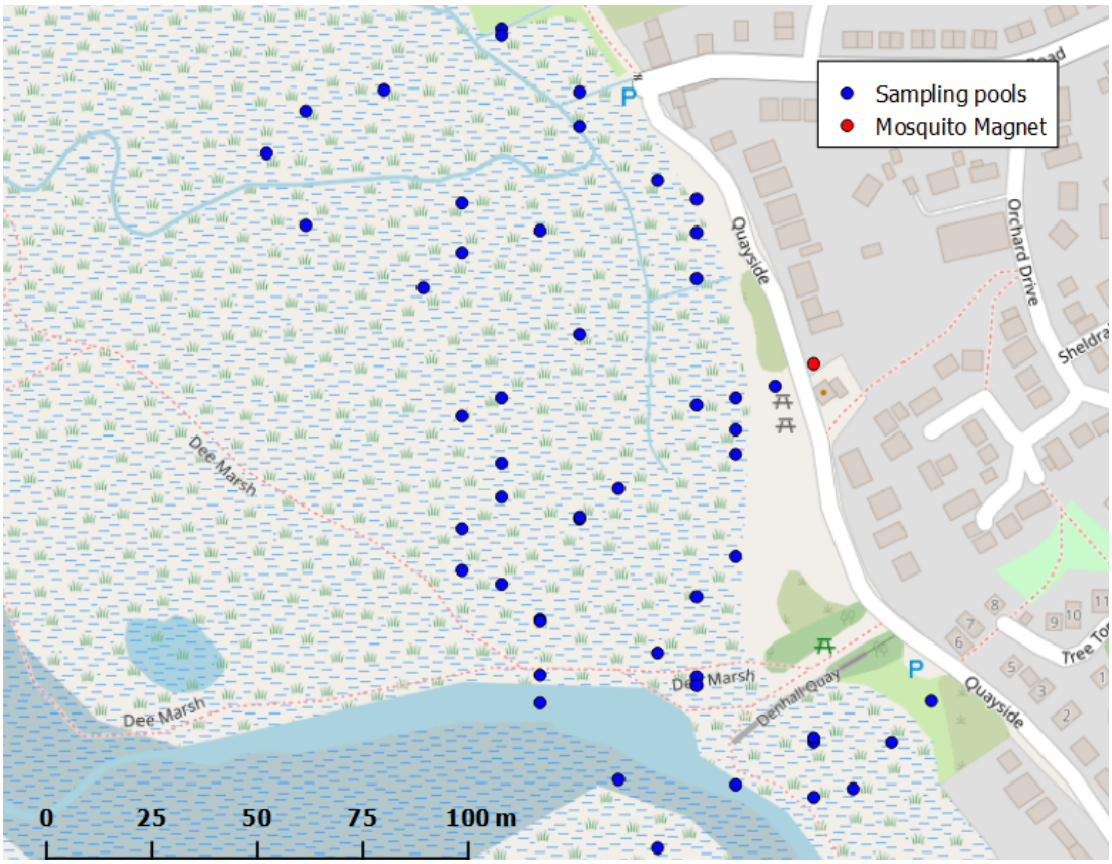

Figure 3.3: 2017 and 2018 larval sampling area. Basemap: © OpenStreetMap contributors, CC-BY-SA (https://www.openstreetmap.org/copyright).

3.4.2 Larval Identification

Not all larvae from each pool were identified during each sampling period. However, some larvae were removed at different sampling intervals and identified morphologically to genus level (Cranston *et al.,* 1987). In both 2017 and 2018, a subset of larvae were reared to adults (2,504 and 3,197 in each year respectively); these were then identified morphologically to species level (Cranston *et al.,* 1987, Snow, 1990).

3.4.3 Climate Data

Meteorological data was obtained from a weather station operated by the National Centre for Atmospheric Science (Lat: 53°16.20'N, Long: 003°03.00'W). This weather station provided information on: temperature, humidity, rainfall and windspeed.

The meteorological data generated across the three years has previously been described in Chapter 2. Therefore, the data is only used for modelling purposes in this chapter.

3.4.4 Tidal Data

Data on the daily high and low tides along the Dee estuary salt marsh was provided by NOC in Liverpool. The tide height was measured twice daily at Hilbre Island. The tidal data generated across the three years has previously been described in Chapter 2. Therefore, the data is only used for modelling purposes in this Chapter.

3.4.5 Data Analysis

A GLMM was fitted to all data sets using R, version 3.3.1., in the 'glmmADMB' package (Appendix B). Pool ID and sampling week were included in the model as random effects and environmental variables were fixed effects. Larval catch was the response variable. The AIC values were used to assess the goodness-of-fit of each model. Final models were generated following a series of stepwise deletions of non-significant factors dependent on the change in AIC.

3.4.6 Drone Flights

A DJI Phantom 4 Pro drone was used for all flights (DJI, Shenzhen, China: http://www.dji.com). This drone is fitted with a DJI 4K, 20 Megapixel camera. Flights were pre-planned using the Pix4D application. An overlap of at least 70% between images was applied to ensured that a high quality orthomosaic was produced. Once programmed, the flight path was flown automatically with flight times lasting approximately 30 minutes. Flights took place on a fortnightly basis between March and November 2018. The total area covered was approximately 41.1 ha.

3.4.7 Creation of an Orthomosaic

The images captured during each flight were imported into AgiSoft Photoscan Pro (http://www.agisoft.com) for processing. The workflow outlined in Figure 3.4 was followed in order to generate an orthomosaic. Full details of the methodology can be found in Appendix C.

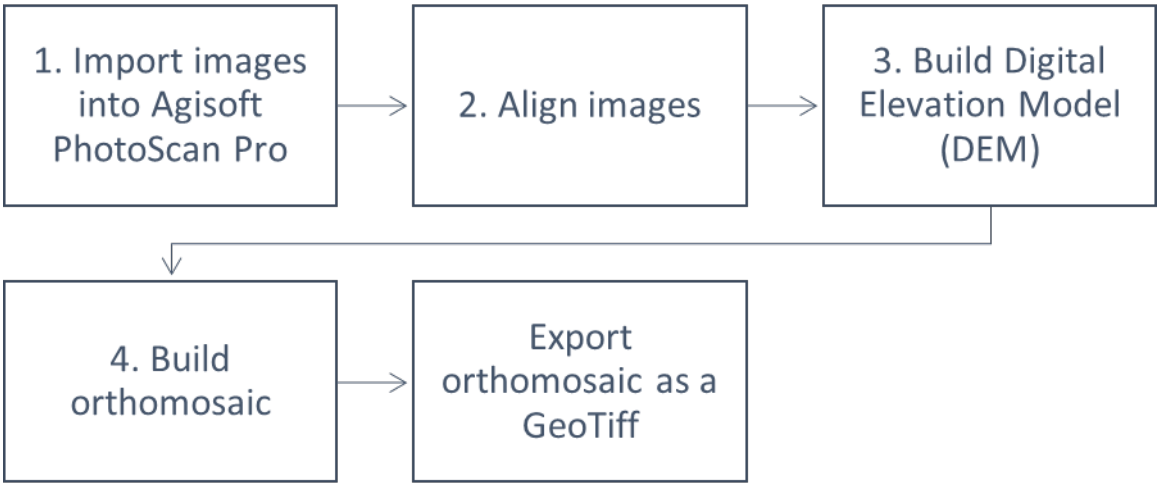

Figure 3.4: Workflow for creating an orthomosaic in Agisoft PhotoScan Pro.

3.4.8 Image Classification

Once generated, the orthomosaic was opened in QGIS (https://www.qgis.org) for image classification. Two additional plug-ins were required for this process: 'semi-automatic classification' and 'value tool'. The workflow described in Figure 3.5 was used to classify the waterbodies from the orthomosaic into different categories. Full details of the methodology can be found in Appendix D. Waterbodies were defined as either large (perimeter >50 m) or small for classification. To determine how the dynamics of the breeding sites on the salt marsh changed over time, the output from post classification reports were compared.

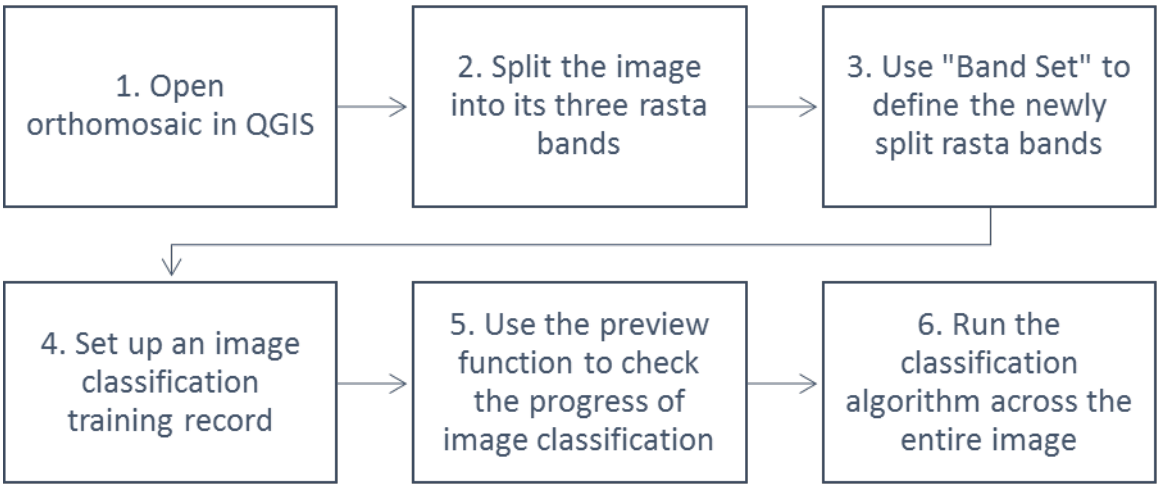

Figure 3.5: Workflow for image classification in QGIS.

3.5 Results

3.5.1 Larval Sampling

2016

Larval sampling took place every two weeks between 2nd May and 14th November 2016. A total of 3,802 larvae were collected during across 15 timepoints (Figure 3.6). All larvae that underwent morphological identification were identified as *Ae. detritus.*

Larval numbers increased from the start of the sampling period to reach a first peak in density at the end of May (week 22) (Figure 3.6). The number of mosquito larvae in the pools then decrease resulting in two consecutive catches of zero larvae across all ten pools in July (weeks 28 and 30). A second, much larger peak in larval density occurs on 19th September (week 38). Mosquito larvae numbers then decrease again for the remainder of the sampling period.

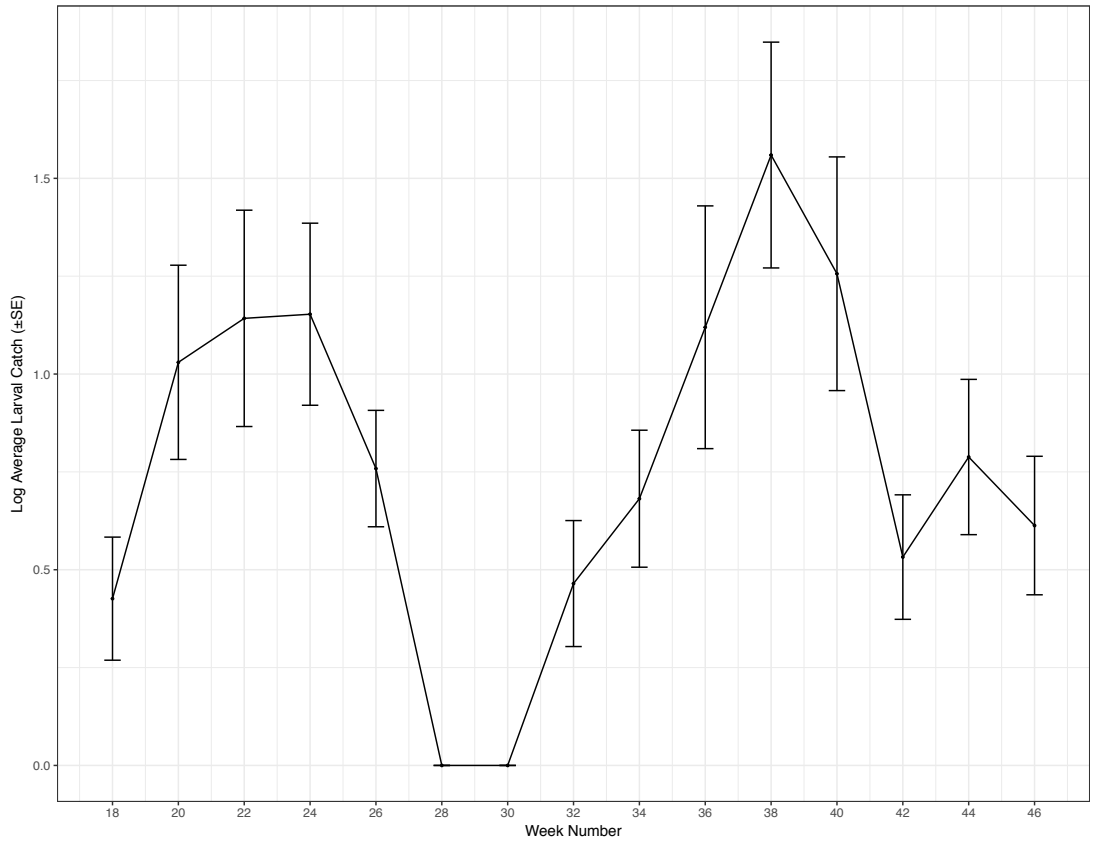

Figure 3.6: Log_{10} of the average fortnightly larval catches (±SE) from all ten sampling pools combined.

There were clear heterogeneities within the pools across the years sampling (Figure 3.7). No mosquito larvae were ever collected from pools 6 and 10. Pools 1,2 3 and 7 were the most prolific breeding sites over the year.

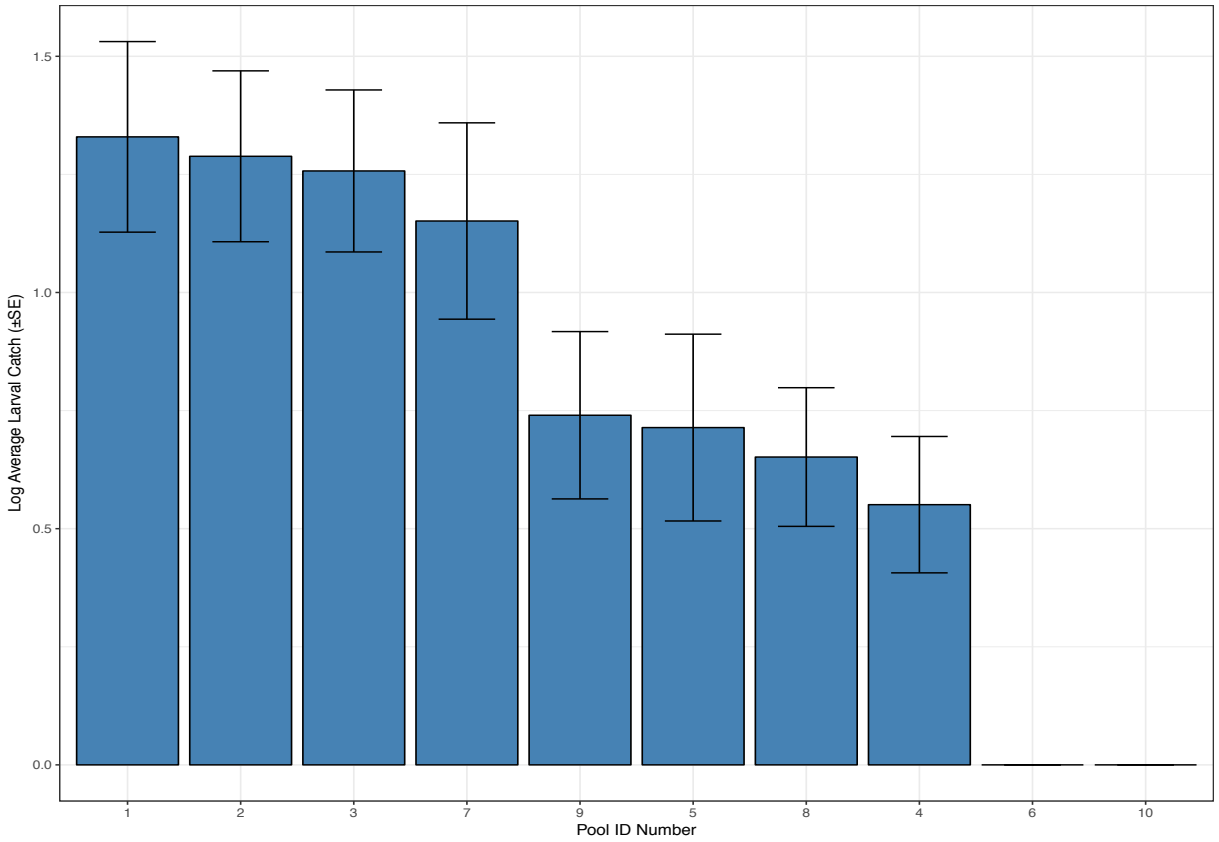

Figure 3.7: Log_{10} of the average catch (±SE) from each of the ten pools across the 2016 sampling period.

An inverse relationship between pool size and number of larvae present was seen across the pools sampled in 2016 (Figure 3.8). The smaller pools contained more mosquito larvae compared to the larger pools. However, a linear model showed that this relationship was not significant P>0.05.

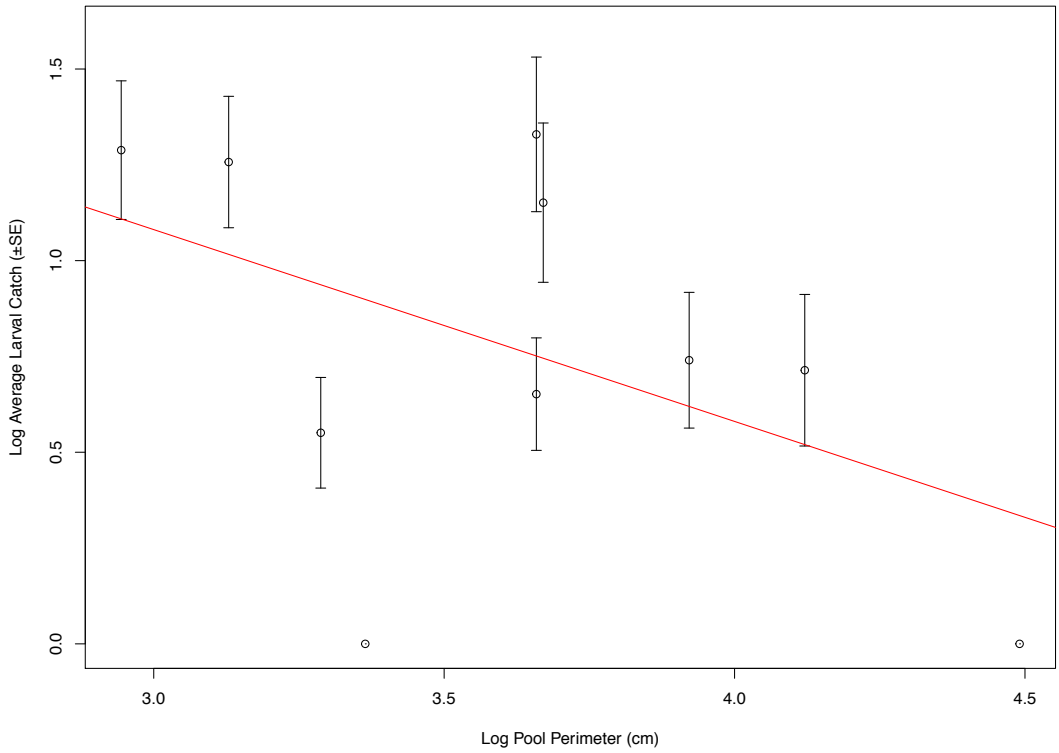

Figure 3.8: Log_{10} of the average larval catch vs. size of the pool (estimate of the perimeter in cm).

2017

Weekly larval sampling took place over 34 weeks between 3rd April and 20th November. A total of 12,105 larvae were collected (Figure 3.9). A small number of larvae that underwent identification were found to be *Cs. annulata* (n=34/2,504).

Two peaks in larval abundance can be seen. The first occurred at the end of May (week 22). During the summer months the average number of mosquito larvae drops to low numbers with several weeks of zero catches across all fifty pools. The second, larger peak, in abundance occurred at the beginning of September.

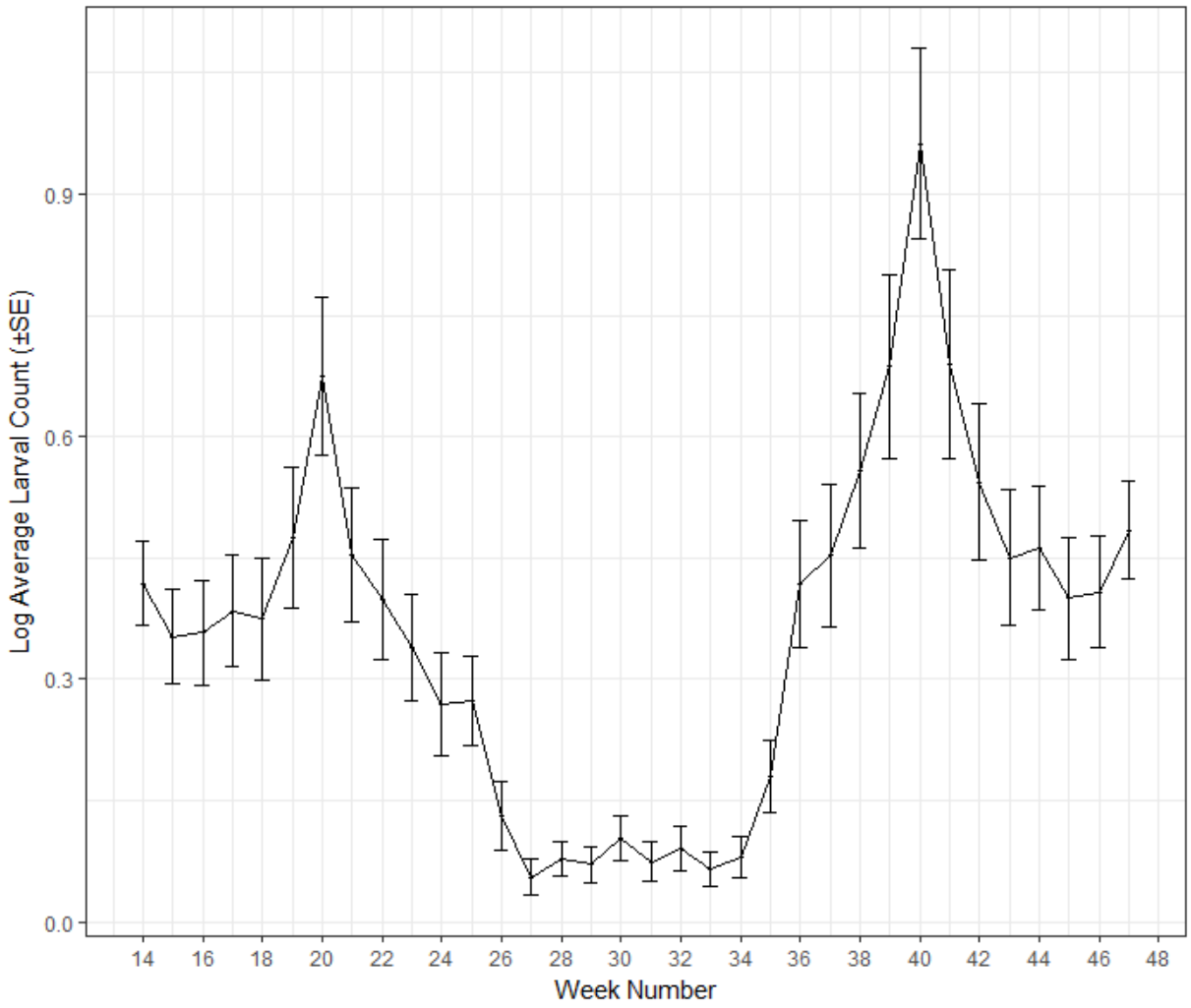

Figure 3.9: Log_{10} of the average weekly larval catches (±SE) from all fifty sampling pools in 2017.

Sampling was varied across the fifty pools (Figure 3.10). Several pools contained no mosquito larvae for the entire sampling period, whilst others had weeks with catches of over 1,000 larvae.

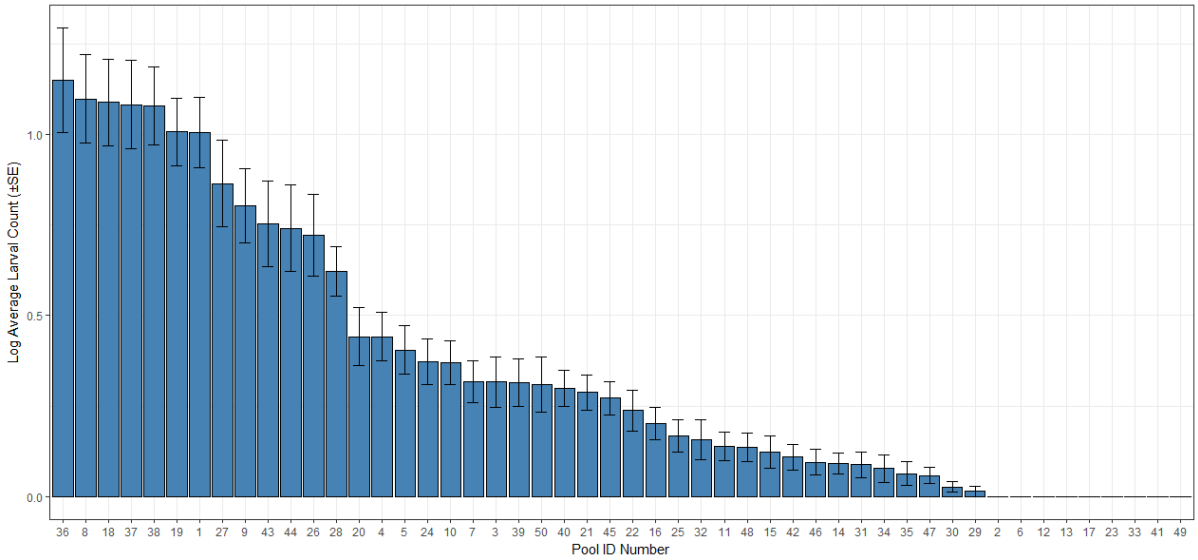

Figure 3.10: Log_{10} of the average total catch (±SE) from each of the fifty pools across the 2017 sampling period.

Once again, there was an inverse relationship between pool size and the number of mosquito larvae collected in that pool. The highest larval catches came from the smaller breeding sites (Figure 3.11). Results of a linear model show that this relationship is significant $P<0.001$.

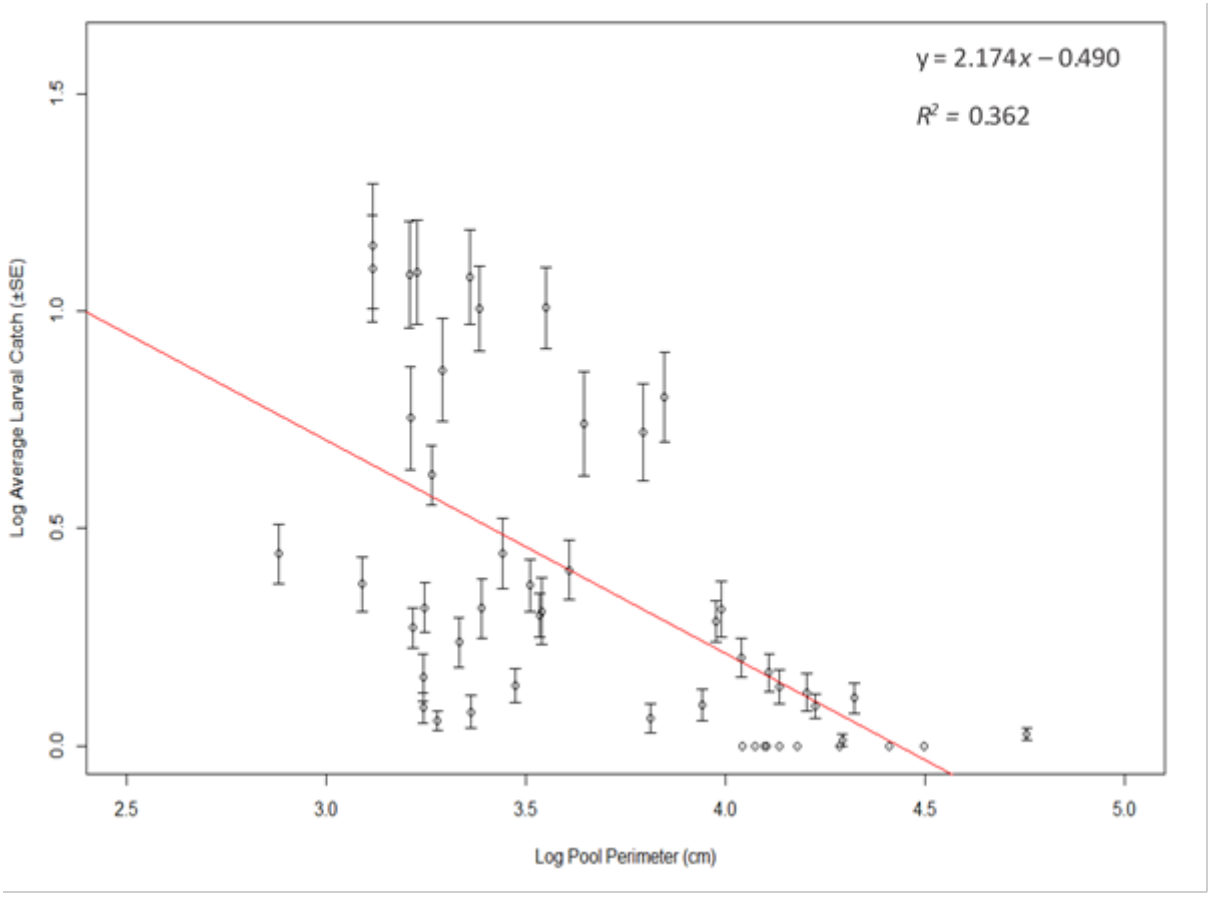

Figure 3.11: Log_{10} of the average larval catch vs. size of the pool (estimated of the perimeter in cm) for the 50 different pools sampled in 2017.

2018

Weekly larval sampling took place over 38 weeks between 12th March and 26th November. A total of 11,950 larvae were collected (Figure 3.12). All larvae that underwent identification were identified as *Ae. detritus.*

There were two clear peaks in larval abundance observed in 2018 (Figure 3.12). The first occurs in mid-April. During the summer months, the average number of larvae drops to very low numbers with several weeks of zero catches across all pools. The second, larger peak, in abundance occurs in mid-September.

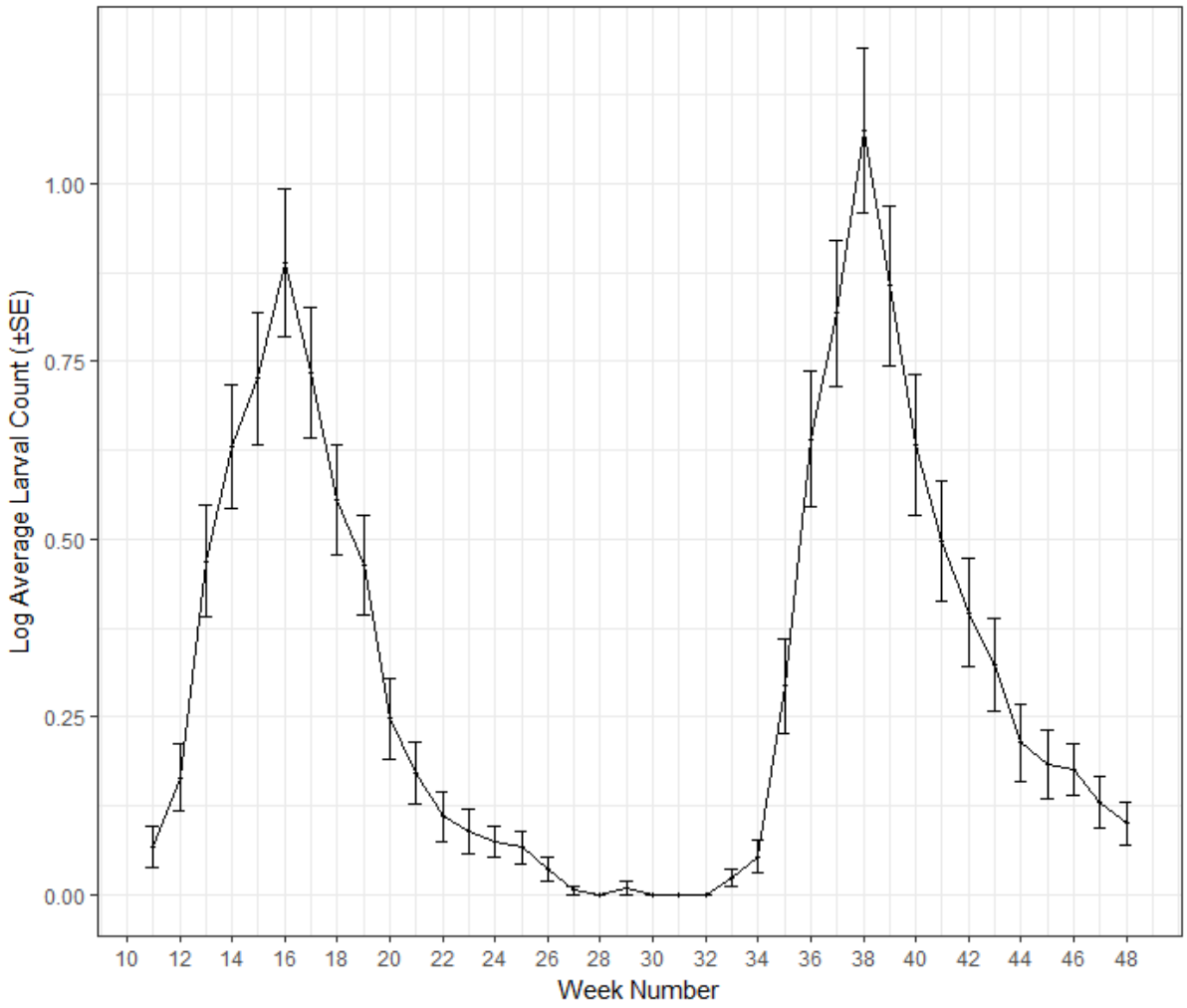

Figure 3.12: Log_{10} of the average weekly larval catches (±SE) from all fifty sampling pools in 2018.

As previously seen, there were clear differences between the sampling pools with several pools containing no mosquito larvae throughout the entire sampling period (Figure 3.13).

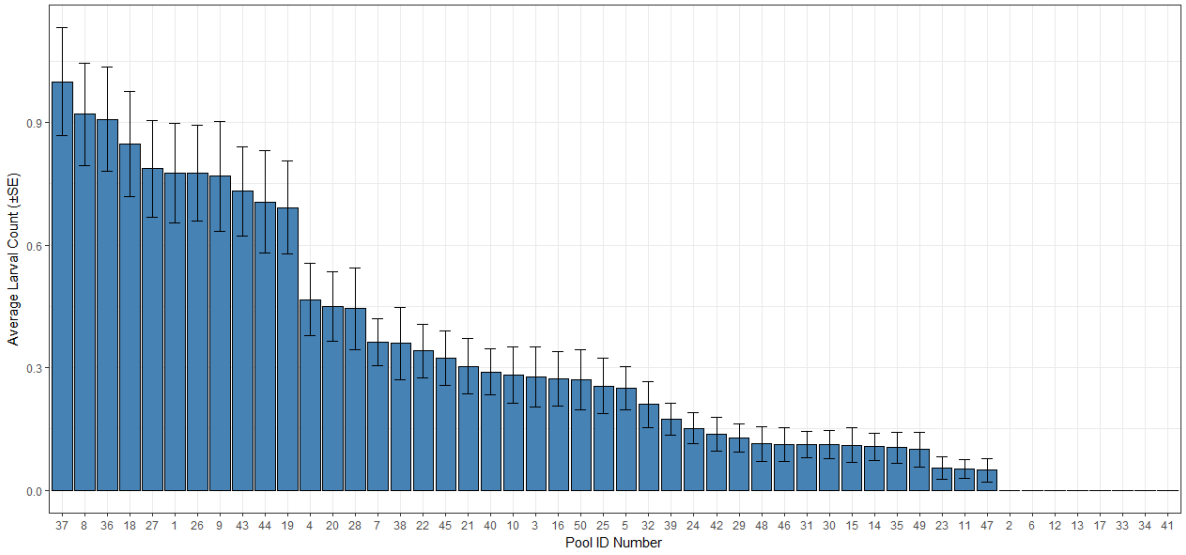

Figure 3.13: Log_{10} of the average total catch (±SE) from each of the fifty pools across the 2018 sampling period.

As observed in both 2016 and 2017, the largest mosquito catches came from the smaller pools sampled (Figure 3.14). Once again, a linear model demonstrated that this relationship was significant $P<0.001$.

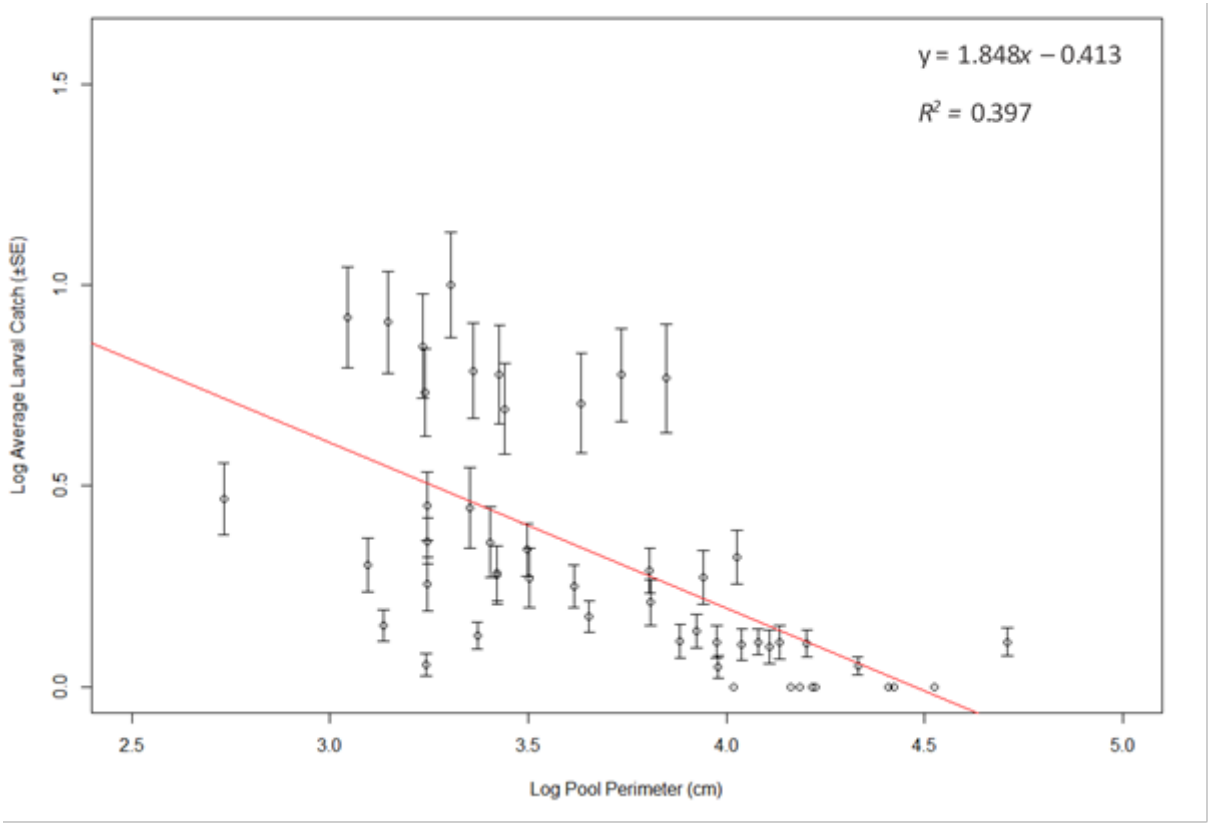

Figure 3.14: Log_{10} of the average larval catch vs. size of the pool (estimated of the perimeter in cm) for the 50 different pools sampled in 2018.

3.5.2 Modelling the Impact of Meteorological Variables and Tidal Flooding on Larval Abundance

The larval catch data across two years of sampling (2017 and 2018) was combined to determine how a range of meteorological variables (temperature, humidity and rainfall) and tide height impacted on the abundance of *Ae. detritus* larvae. Details of the meteorological and tidal variables are discussed in Chapter 2. Each year was first modelled individually before being combined.

A Poisson model was first fitted to the data followed by a negative binomial model. In all instances, the negative binomial model provided the best fit to the data as determined by the AIC values. Both temperature and humidity were found to be significant predictors of mosquito catch (Table 3.1). As temperature increased the number of *Ae. detritus* larvae collected decreased. In contrast to this, increases in humidity resulted in increases in the number of *Ae. detritus* larvae collected. Despite previous studies showing a relationship between tidal flooding and the ecology of *Ae.*

detritus, tide height was not found to be a significant predictor of larval abundance in the present study.

Table 3.1: Regression coefficients with 95% confidence intervals and standard error for the minimal adequate model.

	Estimate (95% CI)	Standard Error
(Intercept)	-2.403 (-4.628; -0.179) *	1.135
Temperature	-0.147 (-0.192; -0.101) ***	0.023
Humidity	0.041 (0.018; 0.064) ***	0.012

***p<0.001, *p<0.05

To further explore the impact of temperature and humidity on mosquito abundance, the average larval catch from both 2017 and 2018 was plotted against averages of temperature and humidity (Figure 3.15). Given that tide has previously been highlighted as a predictor of the abundance of *Ae. detritus* larvae, the average catches were also plotted against tide height (Figure 3.15).

There appears to be a temperature threshold (approximately 16^{o}C) above which the abundance of *Ae. detritus* decreases (Figure 3.15). There is a less evident relationship between larval abundance and humidity (Figure 3.15). However, given that this is a coastal sampling area, humidity remains relatively consistent across the two years of sampling. There is no clear relationship between tide height and larval abundance (Figure 3.15).

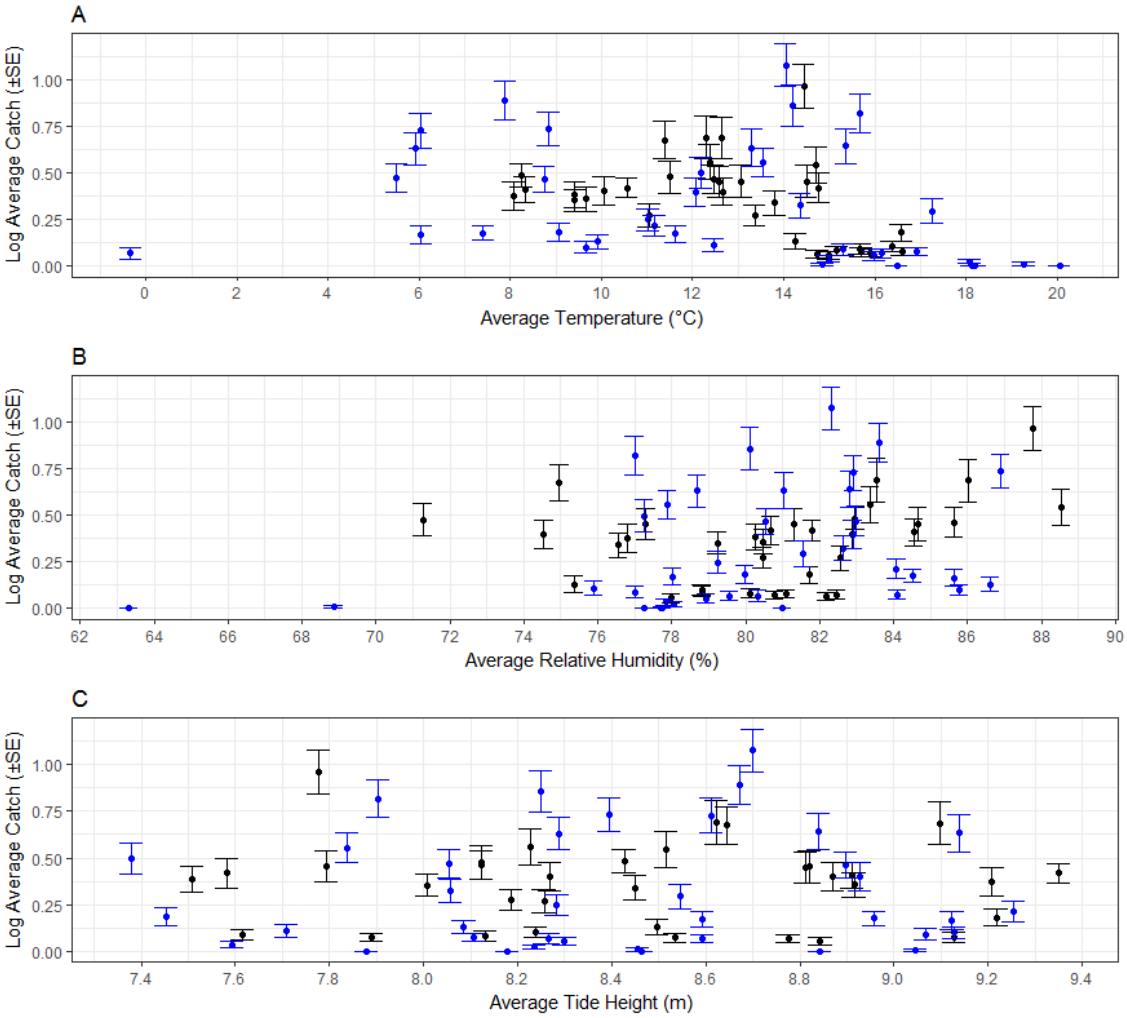

Figure 3.15: Log_{10} of the average weekly larval catches from both 2017 (black) and 2018 (blue) plotted against average weekly climatic variable. A: Temperature, B: Relative Humidity, C: Tide Height.

3.5.3 2018 Drone Surveys

Drone surveys were successful and maps of the mosquito breeding sites on the Dee estuary salt marsh were produced (Figure 3.16).

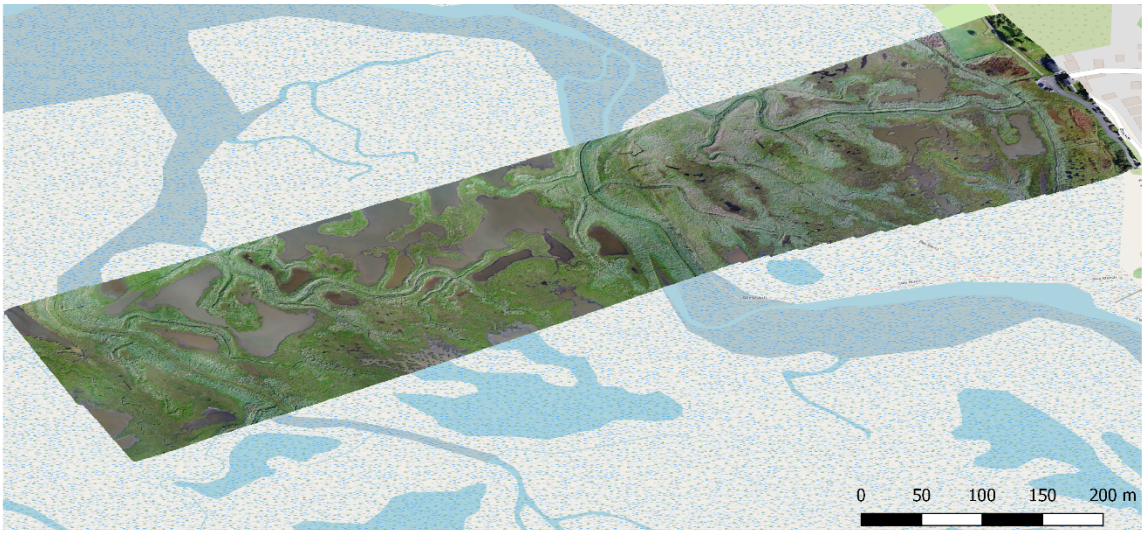

Figure 3.16: Map of the pools on the Dee estuary salt marsh. This map was produced from drone flights in September 2018. Basemap: © OpenStreetMap contributors, CC-BY-SA (https://www.openstreetmap.org/copyright).

Once created, orthomosaic images were classified using QGIS (Figure 3.17). The vegetation on the Dee estuary was classified as 'green' whilst waterbodies were classified as blue. Pools were split into two different categories. The larger shallow pools, were classified light blue. As previously seen, these larger pools contain very few, if any, mosquito larvae. The small deeper pools, which were the most prolific breeding sites, were classified in dark blue. Areas where the marsh has begun to dry out in warmer periods are shown in brown.

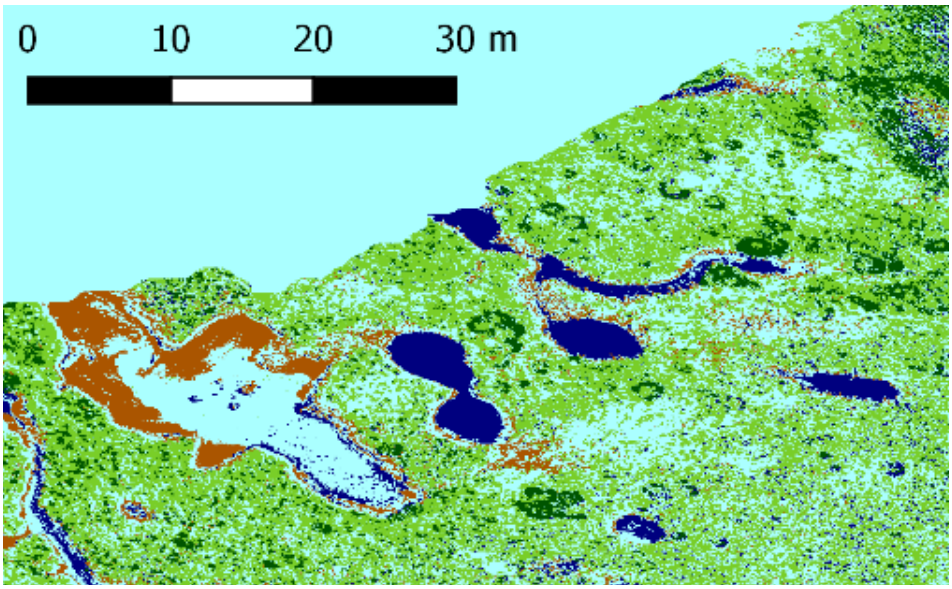

Figure 3.17: Example of the image classification output from QGIS. Water bodies are shown in blue with vegetation in green and dry areas in brown.

Over the course of the year, there are large changes in this salt marsh habitat. In the warmer summer months, the marsh begins to dry out, with several pools disappearing entirely. In contrast to this, in the wetter spring and autumn months the pools on the salt marsh are full, and, in some instances, the entire marsh is covered in a shallow layer of water as a result of flooding from the high tides. Figure 3.18 displays some images of how the marsh changes over time. In the images from July, the marsh had more extensive dry areas around pools beginning to show in the image classification. At times, there were problems with the classification of the drone images. For example, in the October images, the marsh has recently flooded and there is a shallow layer across the majority of the habitat. The image classification tools are therefore struggling to differentiate between the vegetation and waterbodies.

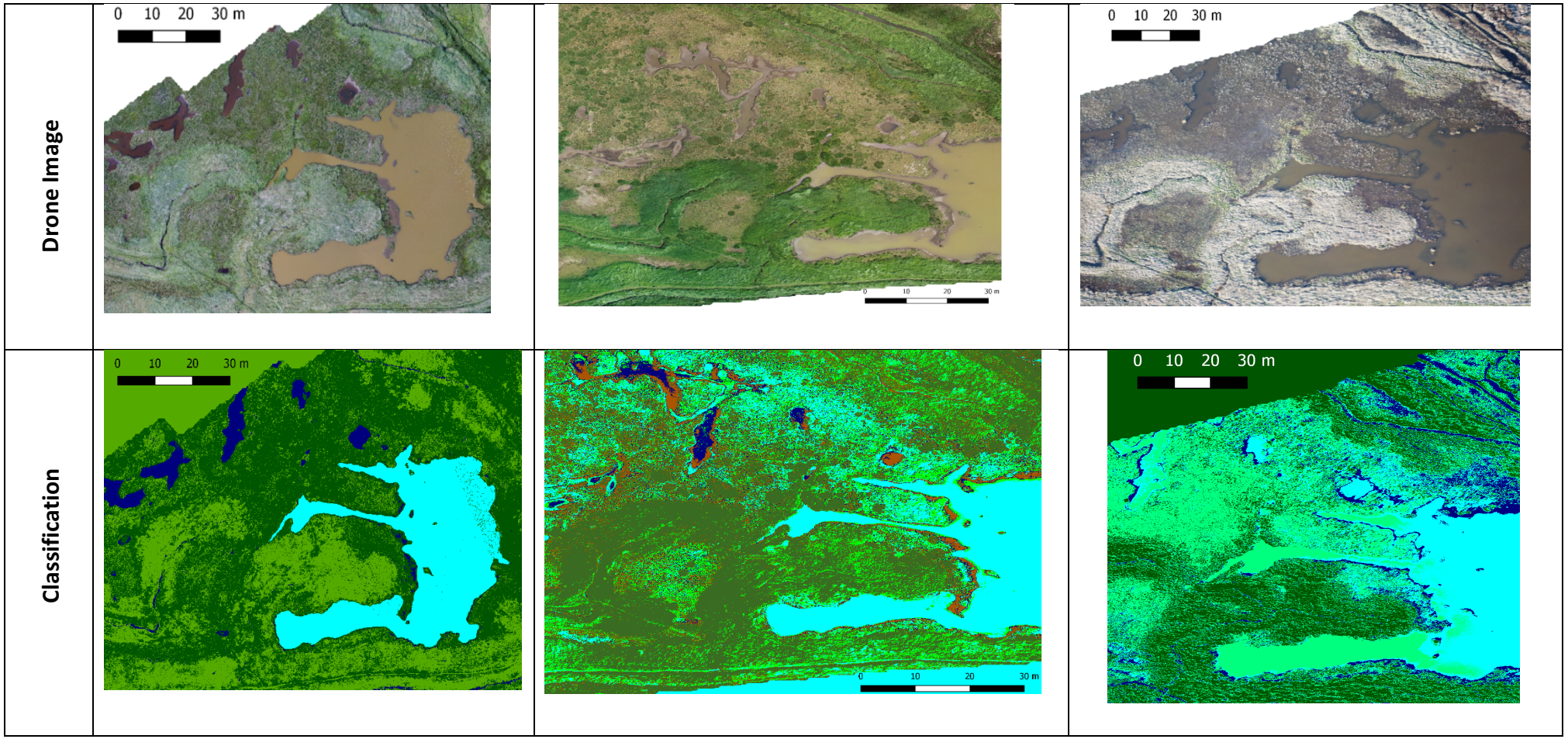

Figure 3.18: Comparison of drone maps and image classification during 2018.

From the image classification it was possible to determine the percentage of each image that was made up of the different classes of land cover (vegetation, large pools, small pools, dry areas). The percentage cover was averaged over the months' image collection to produce a breakdown of how the composition of the marsh changed over the sampling period (Figure 3.19). During the warmer summer months, the marsh begins to dry out. This can be seen by the appearance of 'dry patches' in the image classification output (Figure 3.19). In these areas, the edges of the pools have begun to dry out and, in some instances, entire pools disappear due to desiccation. The overall size of both the large and small pools decreases during this time (Figure 3.19). In July, the total area of pools on the marsh is at its lowest. This corresponds with a reduction in the number of larvae that are collected on the marsh during this time of year. In contrast to this, in September when seasonal high tides have flooded the marsh, the pool size increases corresponding with the increases in the number of *Ae. detritus* larvae present in these pools at this time of the year.

As previously mentioned, the smaller pools are the most prolific *Ae. detritus* breeding sites. In March when *Ae. detritus* is highly abundant, 9.4% of the total area mapped is made up of these small productive breeding sites (Figure 3.19). In contrast to this, in July when there are very few *Ae. detritus* larvae present, only 2.5% of the total area is made up of these smaller pools (Figure 3.19).

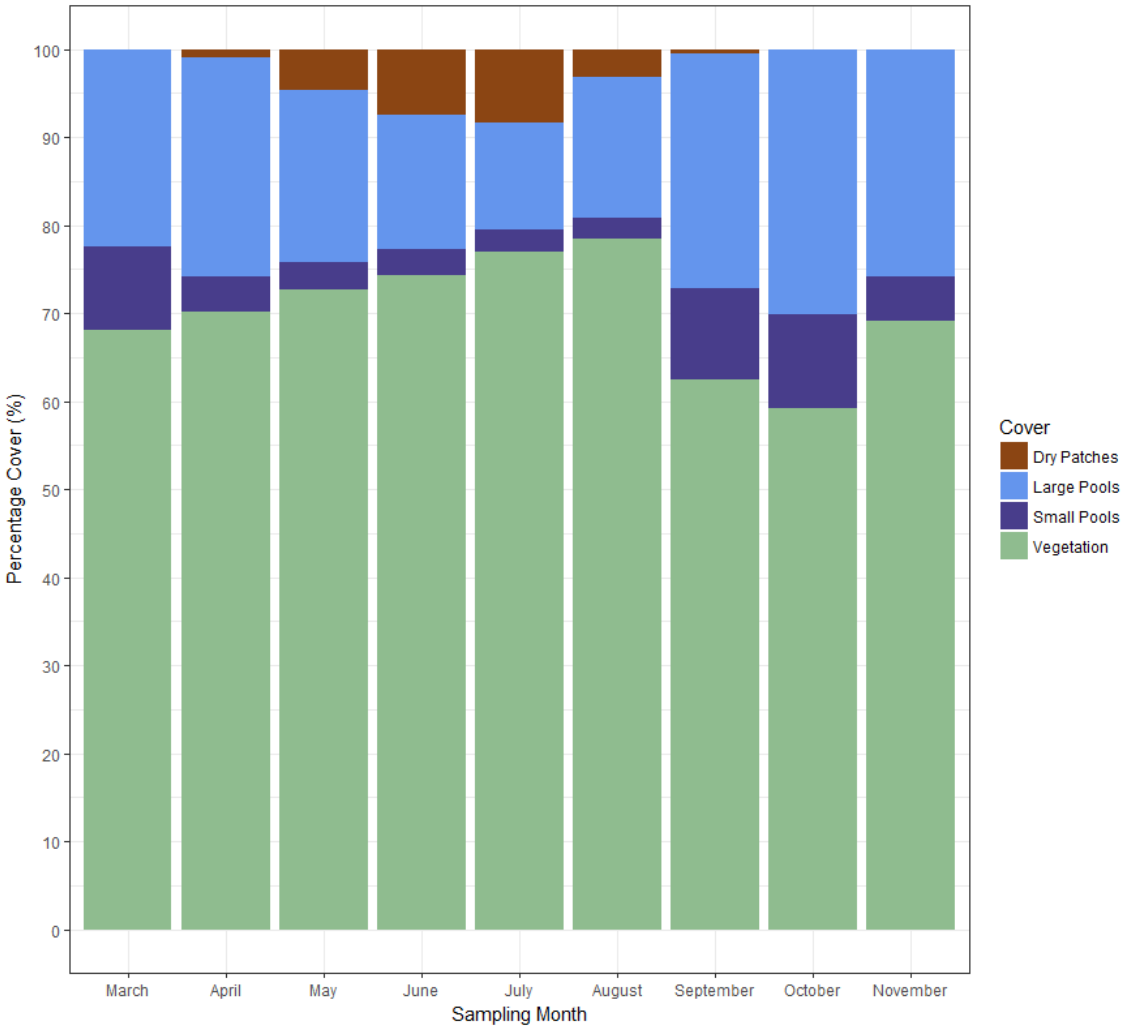

Figure 3.19: Percentage of each of the four landcover classifications over the nine months of drone surveys.

3.6 Discussion

Across all three years of sampling, two clear peaks in the abundance of *Ae. detritus* can be seen in the pools of the Dee estuary salt marsh. The first peak in abundance occurs in early May and the second larger peak occurs in September/October. During the summer months, the number of mosquito larvae drops to almost zero across all pools. There is a negative correlation between pool size and the number of mosquito larvae collected. As pool size increases the number of *Ae. detritus* larvae in pools begins to decrease. When larval surveys were taking place, the presence of predators in pools was noted. In these larger pools, predators appeared to be more abundant. However, further work should be conducted to make a detailed assessment of the presence and abundance of predators in the pools and explore how this impacts larval abundance.

Results from the larval sampling across all three years have demonstrated that as temperature increases, the number of *Ae. detritus* larvae decreases. Plots of abundance vs. temperature have shown that there appears to be a threshold temperature (~16^{o}C) above which larval abundance decreases. This is a result of the breeding sites drying out during these warmer periods. Previous work by Service, has shown that higher temperatures speed up the development of *Ae. detritus* larvae (Service, 1968). However, these period of higher temperatures, were also associated with increased desiccation of the respective breeding sites (Service, 1968).

Ae. detritus is a competent laboratory vector of several different arboviruses (Blagrove *et al.,* 2016, Mackenzie-Impoinvil *et al.,* 2015, Lumley *et al.,* 2018). For these studies, incubation temperatures between 20^{o}C and 28^{o}C have been used (Blagrove *et al.,* 2016, Mackenzie-Impoinvil *et al.,* 2015, Lumley *et al.,* 2018). The result of the larval surveys show that as the average temperature approaches 16^{o}C, the abundance of *Ae. detritus* larvae in the breeding sites declines which, in turn, impacts adult numbers. However, as seen from the data collected in Chapter 2, adult abundance does not decline to zero during these warmer periods. If *Ae. detritus* was to act as an arbovirus vector in the future, the greatest risk of virus transmission

would occur during these warmer periods despite the overall mosquito numbers being lower.

The weekly larval collection data for both 2017 and 2018 was combined with the *Ae. detritus* adult data to determine how the abundance of the two life stages changes over each year of sampling (Figure 3.20 and Figure 3.21).

In 2017, larval abundance first peaks at the end of March with the subsequent peak in adults occurring approximately six weeks later in May. As the number of larvae in the pools drops to almost zero from the end of June, the number of adults collected also begins to decline. The fewest adults are collected at the end of July following a month of very low numbers of larvae in the breeding sites. Larval numbers begin to increase again in August reaching a peak in abundance at the end of September. The subsequent peak in adult abundance occurs just one week later in the first week of October. Larval numbers decrease somewhat after this peak before remaining at a relatively constant level until the end of sampling. *Ae. detritus* overwinters as 4th instar larvae therefore by the end of sampling in November the larvae in the pools are likely to be preparing to overwinter (Snow, 1990).

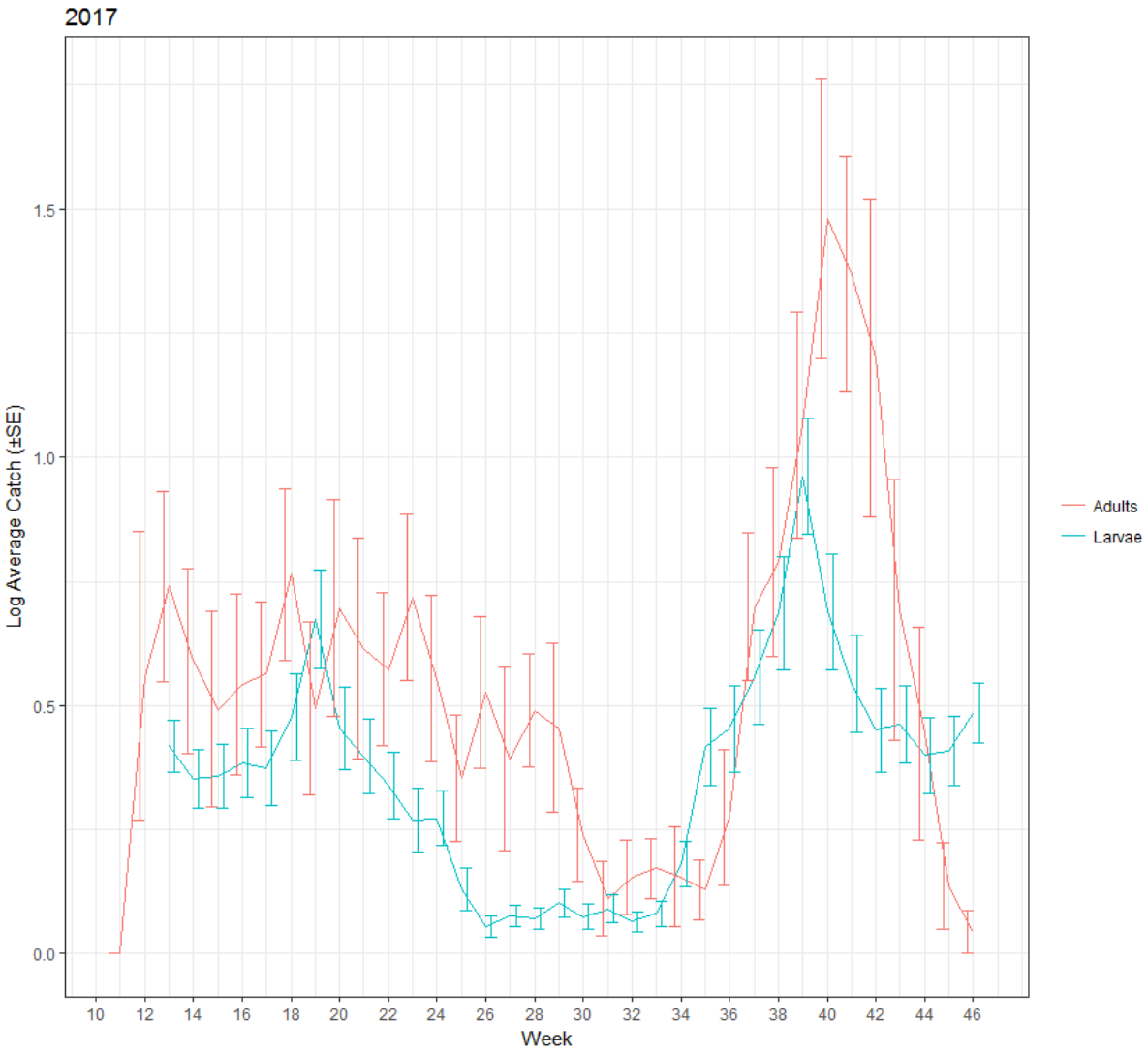

Figure 3.20: Log of the average catch weekly abundance (±SE) of both the larval and adult stages of *Ae. detritus* during 2017.

In 2018, larval abundance first peaks during the second week in April with adult abundance peaking six weeks later in the middle of May. The number of larvae collected in the pools drops from this point, with several weeks of zero catches occurring in July. The number of adults collected also declines during this time with the lowest catches occurring at the end of July. Larval numbers begin to increase again in August reaching a peak abundance in mid-September. The subsequent peak in adult abundance occurs three weeks later at the start of October. Larval numbers decrease from this point onwards before remaining at a relatively constant level until the end of sampling as they begin to overwinter in the breeding sites.

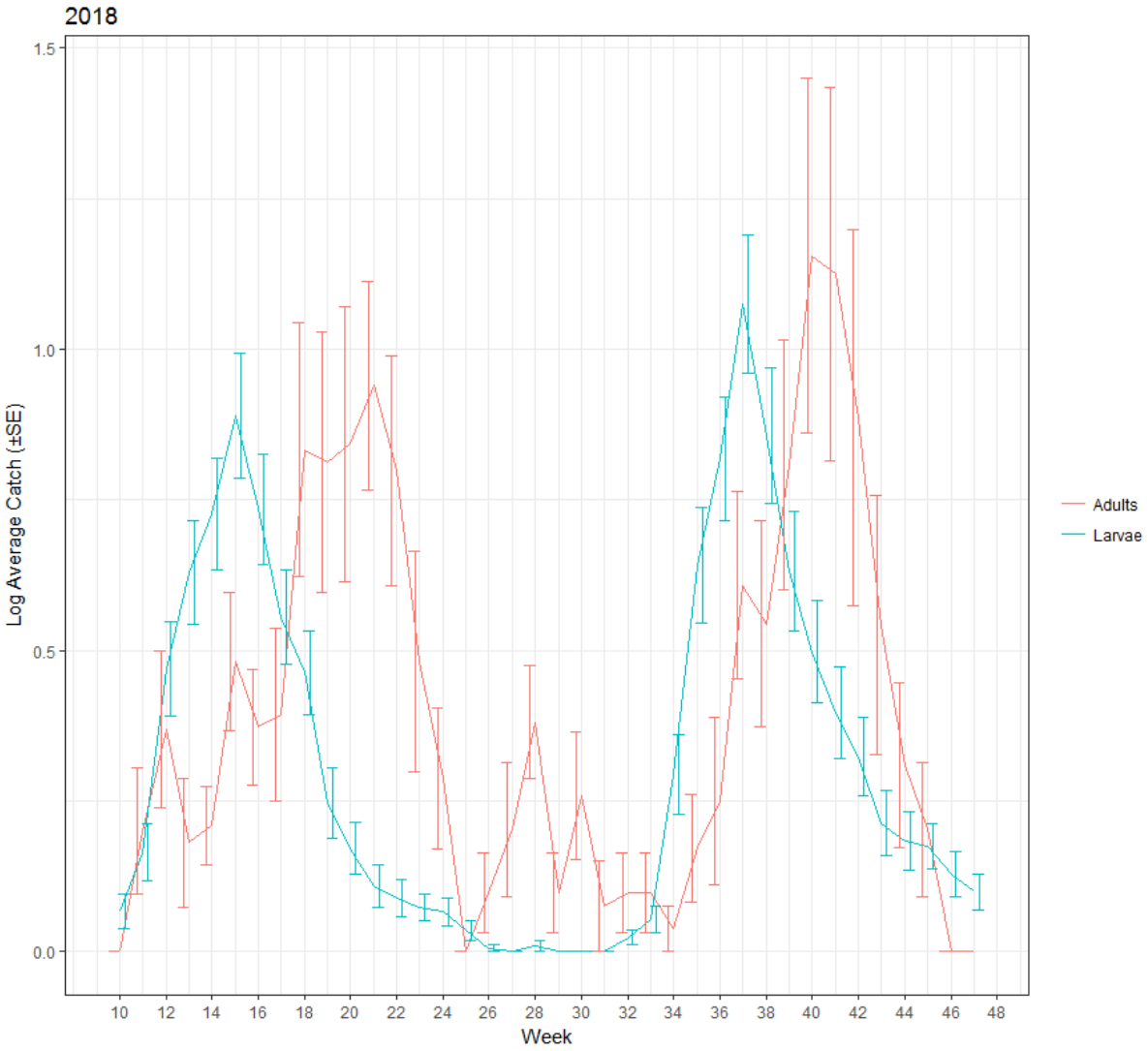

Figure 3.21: Log average catch of the weekly abundance (±SE) of both the larval and adult stages of *Ae. detritus* during 2018.

GLMMs were fitted to the combined 2017 and 2018 larval abundance data. The results of these models indicate that both temperature and humidity were significant predictors of the abundance of *Ae. detritus* larvae in the pools of the Dee estuary salt marsh. As the temperature increases, there is a negative effect on the abundance of *Ae. detritus*. Literature has shown that increases in temperature can speed up the development rate of the immature stages of *Ae. detritus* (Service, 1968). However, from the analysis of the drone images, the desiccation of the marsh, and subsequent breeding sites, during the warmer summer months can be clearly seen. Similar results

to the present study have been reported in Australia for the salt marsh mosquito species *Aedes camptorhynchus (Ae. camptorhynchus)* (Williams *et al.,* 2009). A decline in the population of *Ae. camptorhynchus* occurs during the summer months as temperatures increases and rainfall decreases resulting in the desiccation of the breeding sites of this mosquito species (Williams *et al.,* 2009).

Results of the modelling in the present study suggest that increases in the relative humidity have a positive impact on the abundance of *Ae. detritus*. Despite studies suggesting that tidal flooding plays an important role in the ecology of *Ae. detritus*, no clear evidence for this was seen in the model outputs in the present study (Service, 1968). A lag period of up to two months was applied to the tidal data to explore the impact of historical tidal flooding on the ecology of *Ae. detritus* although no significant effect was ever observed in the models. Further studies could be conducted to explore the impact of tidal flooding on *Ae. detritus* ecology in other parts of the UK.

A drone was successfully used to produce high resolution maps of the breeding sites of *Ae. detritus* on the Dee estuary salt marsh. Waterbodies were successfully classified to provide a detailed picture of how the abundance of suitable breeding sites changes over the *Ae. detritus* breeding season. From the images and subsequent classification, it is possible to observe how this habitat changes over the year. In the warmer months there are more dry areas with a reduction in the number and size of pools on the marsh. This reduction coincides with seasonal reductions in tide height. The ecology of *Ae. detritus* means that eggs that have been laid on the ground are not soaked and therefore do not hatch (Service, 1968). However, once the pools are replenished, eggs are soaked and therefore begin to hatch resulting in the larval, and subsequent adult numbers, increasing again.

Some issues with image classification were encountered when the marsh had been recently flooded. When this was the case, the marsh is almost entirely covered with a shallow layer of tidal flood water. The image classification software therefore found classifying the different macroclasses of land cover difficult. Additionally, some of the most prolific breeding sites were almost entirely covered by vegetation at certain timepoints. Once again, the automatic image classification struggled to identify these

habitats with them often being classified solely as vegetation. In order to overcome this limitation, an additional sensor could be fitted to the drone to provide a more detailed picture of the salt marsh habitat that *Ae. detritus* are breeding in. Carrasco-Escobar et al. (2019) used a drone with a Parrot Sequoia sensor (Parrot, France) to collect Green, Red, Red Edge and Near Infrared multispectral imagery. The addition of this sensor successfully allowed for identification of breeding sites that were covered with vegetation (Carrasco-Escobar *et al.,* 2019).

The area of salt marsh mapped using the drone in the present study was relatively small (approximately 0.411 km^2), it would be informative to use these techniques to map a wider expanse of the Dee estuary to determine how the dynamics of *Ae. detritus* breeding sites changes over this large expanse of habitat. This would be especially important in the areas, such as Parkgate, where *Ae. detritus* poses a significant biting nuisance as the imagery could be used by local authorities to plan vector control programmes (Clarkson and Setzkorn, 2011). The information could also provide warnings to local communities on the predicted level of nuisance; adult abundance peaks approximately six weeks after the peak in larval abundance.

Within Europe, where there is ongoing transmission of pathogenic arboviruses, larvicide treatment of mosquito breeding sites is one of the mainstays of vector control programmes (ECDC, 2017). The detailed maps generated within the present study provide an insight into the habitats that are important breeding site of *Ae. detritus* at different timepoints during the year. These maps could be used to inform vector control programmes by providing a targeted approach to vector control. For example, knowledge of which pools to treat with *Bti* and when this application would be timely, could save local authorities money. Along the Dee estuary, it would be more beneficial to treat breeding sites in March when the pools have high numbers of larvae in rather than in the summer months when the risk from mosquito nuisance is often thought to be at its greatest. Additionally, as demonstrated in the present study, the larger pools within the Dee estuary salt marsh are not prolific *Ae. detritus* breeding sites. Habitat manipulation has previously been used along the Dee estuary to control *Ae. detritus;* the maps generated in this study could be used to inform future initiatives to control mosquitoes in this manner (Clarkson and Setzkorn, 2011,

Medlock *et al.,* 2012a). As mentioned in the introduction to this chapter, OMWM is successfully used in other parts of the world to control mosquito populations (Rey *et al.,* 2012). Drones could be used to provide high resolution, real time imagery to help inform these control initiatives. Should *Ae. detritus* become involved in arbovirus transmission within the UK in the future, it will be important to implement effective control strategies. Real time mapping of breeding sites using drones would be a significant aide to planning control initiatives to ensure that they have the maximum impact possible.

3.7 Conclusions

Clear seasonal trends in the abundance of *Ae. detritus* larvae can be seen on the Dee estuary. As observed with the adult catch data, there are two clear peaks in the abundance of the mosquito larvae. There are large amount of heterogeneity between the breeding sites on the Dee estuary. Larger shallow pools contain very few mosquito larvae, whilst the smaller deeper pools contain high numbers.

For the first time, a drone was successfully used to map the breeding sites of *Ae. detritus* in the UK. Drones offer up the opportunity for flexible data collection which is ideal for observing seasonal changes in a dynamic and ever changing environment. The drone imagery provides a detailed picture of how this habitat changes over the course of the year and, when combined with the on foot larval sampling, this information can be used to determine how changes in this habitat impacts on the abundance and distribution of mosquito larvae. Should *Ae. detritus* become involved in virus transmission, control programmes will be especially important. Mapping of breeding sites using drones could be an extremely useful tool to ensure that larval control is as successful as possible.

Chapter 4 - Quantifying the Biting Rates of *Aedes detritus* and Other Mosquito Species on Humans

4.1 Abstract

As mosquito borne pathogens and invasive mosquito species continue to spread within Europe, it is important that we have a detailed understanding of the tools available for mosquito surveillance and control in the UK. This is especially important given the repeated detection of *Ae. albopictus* in southern England. To this end, field studies were undertaken to compare methods of sampling mosquitoes in the UK with particular reference to *Ae. detritus*.

Using Latin square experimental designs, catches from Mosquito Magnets, HLC, CDC light traps, BG Sentinel traps and the newly developed Host Decoy Trap (HDT) were compared. The CDC light traps, BG Sentinel and HDTs were all baited with dry ice to produce carbon dioxide. To quantify the behavioural effect of thermal cues on mosquito landing responses, the proportion of attracted mosquitoes that landed on a warm (~40^{o}C) or cool (~15^{o}C) HDT were quantified using electric grids (0.5 m x 1.0 m). Finally, to quantify mosquito responses to colour, catches from targets (0.3 m wide x 0.2 m high) coloured black, grey, white, yellow, blue, red and transparent were compared. These targets were also baited with dry ice and covered with clear sticky plastic to collect mosquitoes as they landed.

Several mosquito trapping methods were found to offer alternative approaches to HLC for sampling human biting *Ae. detritus.* No significant difference between the catches from the Mosquito Magnet and BG Sentinel traps, compared to HLC, were detected ($p>0.05$). However, the CDC light trap caught significantly fewer mosquitoes ($p<0.001$). Heat is an important behavioural cue for *Ae. detritus* with significantly more mosquitoes caught by hot HDTs compared to cold traps ($p<0.001$). When CO_2 is removed from the HDT, the mosquito catch drops significantly ($p<0.001$). Black and blue coloured sticky targets caught significantly more *Ae. detritus* compared to other colours, specifically yellow ($p<0.01$). These studies have provided insight into the tools available for mosquito surveillance and have highlighted the importance of olfactory, thermal and visual stimuli in mosquito trap design.

4.2 Introduction

Within the UK, surveillance programmes are currently ongoing to explore the ecology of our native mosquito populations and to detect invasive mosquito species (Vaux and Medlock, 2015, Medlock *et al.,* 2007, Medlock *et al.,* 2018). These programmes are especially important given the increased incidences of mosquito borne diseases in mainland Europe and potential risk of these diseases reaching the UK (Akiner *et al.,* 2016, Tomasello and Schlagenhauf, 2013, Medlock *et al.,* 2018).

4.2.1 Methods of Sampling Mosquito Populations

There are several different methodologies that can be used to sample mosquito populations as both immatures and adults. Sampling of the immature population (both larvae and pupae) requires direct sampling, often using an aquatic dipper (Silver, 2007). When sampling the adult population most methods exploit the host seeking behaviour exhibited by female mosquitoes. Some traps are also designed to be attractive to gravid females offering them an oviposition site (Silver, 2007). Given that male mosquitoes do not exhibit host seeking behaviour it can be difficult to get an accurate representation of the male mosquito population (Silver, 2007).

Human Landing Catches

The current 'gold standard' for collecting anthropophilic mosquito species is a HLC (Briët *et al.,* 2015, Silver, 2007). This method involves volunteers exposing a limb (arm/leg) and collecting mosquitoes that land using an aspirator before they begin to feed (Figure 4.1). This technique provides a quantitative measure of the species and number of mosquitoes attracted to humans. However, HLC exposes the human volunteers to the potential risk of disease and irritation from mosquito bites. It is also subject to observer bias and the method cannot be used to quantify numbers of mosquitoes attracted to non-human hosts (e.g. livestock and wild animals) (Silver, 2007). There are currently no mosquito-borne human diseases in the UK meaning that there are fewer ethical constraints surrounding HLC compared to disease

endemic countries. However, ethical approval is still required as the volunteers are exposed irritation as a result of mosquito bites.

Figure 4.1: Human landing catch volunteer.

Sweep netting

Mosquitoes resting outdoors are often found amongst the vegetation and can be sampled by disturbing this vegetation and capturing mosquitoes using a handheld net (Silver, 2007). Given that this technique targets resting mosquitos, male populations can be sampled using this technique. Sweep netting can also be used to sample swarming mosquitoes (Service, 1994). Dallimore et al. (2017) report the collection of a single male *Ae. aegypti* in northern England whilst sweep netting. However, sweep netting may result in specimen damage making identification more difficult (Silver, 2007). Further to this, sweep netting can be an extremely time-consuming process and may not give an accurate representation of the range of species in a particular habitat.

Given the challenges presented by both HLC and sweep netting, traps can also be used to sample the adult mosquito population. Traps exploit the host seeking behaviour of female mosquitoes, often using a combination of attractants (heat, odours, visual cues). The efficiency of different trapping methods is highly variable and different trapping methods differ in their ability to attract different species of

mosquito (Hesson *et al.,* 2015, Lühken *et al.,* 2014, Huffaker and Back, 1943). Different attractants, climatic conditions and the timing of an experiment can all impact trap efficacy (Hesson *et al.,* 2015, Silver, 2007, Roiz *et al.,* 2012, Baldacchino *et al.,* 2015). These factors need to be considered when planning mosquito surveillance.

Animal Baited Net Traps

Animal baited net traps can be used to sample host seeking mosquitoes with a preference for feeding on animals (Silver, 2007). Mosquitoes are attracted to the animal bait and, after a set period of time, the net is dropped trapping the mosquitoes (Nepomichene *et al.,* 2015, Silver, 2007). Chapman (2017) used horse baited net traps in the UK to evaluate the efficacy of commonly used mosquito repellents. Four different species of mosquito were collected during the study three of which were found to contain a blood meal from horses (Chapman, 2017). It is also possible to use net traps with a human bait to collect anthropophilic mosquito species (Nepomichene *et al.,* 2015). There are however limitations to the use of net traps for sampling mosquito populations. Principally, a proportion of the catch will be missed as there is no holding mechanism for mosquitoes until the net is dropped (Silver, 2007).

Light Traps

A light source, which is produced by traps such as the CDC light trap (John W Hock, Florida, USA) (Figure 4.2), is attractive to insects (Silver, 2007). Once close enough to these traps, a fan, powered by a 6 volt battery, draws insects into a collection net (Silver, 2007). Whilst these traps do collect mosquitoes, they also attracts moths, including rare species, which can limit their use in conservation areas. Additionally, the presence of moths in the collection nets, can result in damage to the mosquitoes rendering them unidentifiable (Li *et al.,* 2015). Light traps can be used in conjunction with CO_2, to increase the mosquito catch (Silver, 2007). However, this means that dry ice or a gas bottles are required in a sampling area which can create logistical problems (Newhouse *et al.,* 1966, Mcphatter and Gerry, 2017). CDC light traps have

been previously used to sample UK mosquito populations where they were particularly effective at collecting *Cx. pipiens* (Hutchinson *et al.,* 2007). Along the Dee estuary, CDC light traps, with no CO_2, bait were used by the local authority in an attempt to reduce nuisance biting in private residences (Clarkson and Setzkorn, 2011). However, these efforts were unsuccessful with no mosquitoes caught using this trapping method (Clarkson and Setzkorn, 2011). CDC light traps have also successfully been used to sample UK mosquito populations in southern England where they collected 12 different mosquito species (Brugman, 2016). CDC light traps are easily transportable, weighting approximately 2 kg, and are relatively inexpensive making them useful research tools (Silver, 2007).

Figure 4.2: A CDC light trap with dry ice to provide CO_2.

Biogents Mosquito Traps

Biogents produce several different mosquito traps with the BG Sentinel and BG Mosquitaire two of the most commonly used designs.

BG Sentinel traps (Figure 4.3) use a counter-flow system, along with an octenol based lure, to attract and collect mosquitoes (Williams *et al.,* 2006). These traps can also be used in conjunction with carbon dioxide to further increase the mosquito catch (ECDC, 2014). The traps are light weight, easily transportable and inexpensive although the battery does require recharging regularly and the mosquitoes are subject to desiccation while in the trap which can make identification more difficult. BG Sentinel traps have previously been used unsuccessfully to collect mosquitoes along the Dee estuary (Clarkson and Setzkorn, 2011). However, PHE have used BG

Sentinel traps to great effect during in the invasive mosquito surveillance programmes within the UK (Vaux and Medlock, 2015, Medlock *et al.,* 2017).

Figure 4.3: The BG Sentinel trap.

The BG Mosquitaire trap (Figure 4.4) is similar in design to that of the BG Sentinel trap but is made of tough plastic and has the option to be powered using mains electricity (Biogents, 2017). This trap can also be used in conjunction with octenol based lures and CO_2 to improve the mosquito catch. BG Mosquitaire traps are also used by PHE in their invasive mosquito surveillance programmes (Medlock *et al.,* 2017). For example, following the detection of *Ae. albopictus* eggs in Kent, BG Mosquitaire traps were used to monitor for adults of the same species (Medlock *et al.,* 2017).

Figure 4.4: BG Mosquitaire trap with a CO_2 canister.

Mosquito Magnets

Although a relatively recent research tool, Mosquito Magnets (Figure 4.5) have been shown to collect large numbers of UK mosquito species (Vaux *et al.,* 2015, Hutchinson *et al.,* 2007). When compared to a CDC light trap, the Mosquito Magnet caught 2.7 times more mosquitoes as well as a wider range of species (Hutchinson *et al.,* 2007). The traps are used with a propane gas bottle which creates CO_2 and heat. An octenol based lure (1-Octen-3-ol (55.15%)) can also be used as an attractant. Mosquitoes attracted to these odours are sucked into the large collection net and are kept in suitable condition for identification (Hutchinson *et al.,* 2007). The drawback of using Mosquito Magnets as a research tool is their cost (approximately £900 per unit). Additionally, Mosquito Magnets are prone to malfunctions requiring repair which can interrupt ongoing studies. Mosquito Magnets have previously been used successfully to sample mosquito populations along the Dee estuary with several different species collected using these traps (Clarkson and Setzkorn, 2011, Chapman, 2017).

There is some suggestion that Mosquito Magnets may be used as an alternative to HLC (Maliti *et al.,* 2015). This is especially important in disease endemic countries where there are ethical implications of performing HLCs. However, further research is required to determine if Mosquito Magnets are an appropriate alternative to HLC.

Figure 4.5: A Mosquito Magnet trap.

Host Decoy Trap

The host decoy trap (HDT) was developed and manufactured by the University of Greenwich and Biogents (Hawkes *et al.,* 2017). This novel trap design exploits a mosquitoes host seeking behaviour in order to collect adult mosquitoes (Hawkes *et al.,* 2017, Abong'o *et al.,* 2018). The trap consists of a black, insulated container (height 36 cm, diameter 38 cm), filled with boiling water which allows the trap to remain hot for several hours (Figure 4.6). The outside of the trap is wrapped in a clear sticky plastic Fics Film (Barrettine Environmental Health, Bristol, UK). Mosquitoes that are attracted to the trap land on this sticky surface and remain stuck. The sticky plastic can then be removed, and the glue dissolved to allow for mosquito identification. The trap can also be odour baited. For example, the HDT has previously been used successfully in the tropics to sample malaria vectors using both human and cattle odours (Hawkes *et al.,* 2017, Abong'o *et al.,* 2018). Host odour is drawn from the source (a human or animal host in a tent), to the HDT allowing the odour to be present without visual cues (Hawkes *et al.,* 2017, Abong'o *et al.,* 2018). The ability of the HDT to collect mosquitoes in the UK is yet to be determined.

Figure 4.6: The Host Decoy Trap (HDT).

Resting Boxes

Typically, mosquito traps collect host seeking mosquitoes meaning that very few blood fed mosquitoes are caught. Blood fed mosquitoes are of interest as subsequent blood meal analysis can be performed giving an indication of host preference (Takken and Verhulst, 2013). Resting boxes mimic the natural resting places of mosquitoes which are normally dark, moist and cool environments (Panella *et al.,* 2011). Resting boxes have been shown to increase the numbers of blood fed mosquitoes caught in the wild compared to other trapping methods (Pombi *et al.,* 2014). However, the resting boxes need to 'compete' with the high number of natural resting sites that are available for mosquitoes in the wild meaning that the number of blood fed mosquitoes can still be relatively low (Burkot *et al.,* 2013). It is often necessary to optimise the design of the resting boxes to ensure that high numbers of blood fed mosquitoes are caught in the various habitats. Chapman et al. (2016) was unsuccessful in using resting boxes to collect blood fed mosquitoes in the UK. However, in southern England, resting boxes were successfully used to collect several species of blood fed mosquito (Brugman *et al.,* 2017a).

Oviposition traps

Oviposition traps can be used to collect eggs to provide an indication of which mosquito species are present in a particular habitat (Silver, 2007). These traps are particularly effective at sampling *Aedes* mosquitoes which lay their eggs inside containers (ECDC, 2014). Oviposition traps are cheap, consisting of a black, water filled, container and an oviposition support (Silver, 2007, ECDC, 2014). However, egg identification can be extremely difficult and in some cases it might be necessary to rear the eggs through to larval or adult stages to confirm identification (ECDC, 2014). In September 2016, 37 eggs of *Ae. albopictus* were collected in a oviposition trap in Kent during routine mosquito surveillance carried out by PHE (Medlock *et al.,* 2017).

4.2.2 Electric Grids as Research Tools

Electric grids (E-Grids) are electrocution devices, similar to the ultra-violet (UV) baited electric grids used in fresh food stores to lure and kill household flies, but, in this case, the UV lure is replaced with a synthetic odour or visual bait, or an actual host. E-grids are a valuable research tool enabling a range of different behaviours to be observed: attraction to baits, alighting, feeding behaviour and interactions with traps (Vale *et al.,* 2015). Although commonly used tools for monitoring tsetse fly behaviour, E-grids have also been used to monitor the behaviour of tropical mosquito species (Vale *et al.,* 2015, Torr *et al.,* 2008, Dugassa *et al.,* 2012, Dugassa *et al.,* 2014, Knols *et al.,* 1998).

A 2008 study in Zimbabwe used E-Grids to study the behaviour of *An. arabiensis* and *An. quadriannulatus* in the field (Torr *et al.,* 2008). The E-Grids used in this study were 1 m high by 0.5 m wide with 0.2 mm copper wires spaced 8 mm apart (Torr *et al.,* 2008, Vale, 1974). E-Grids can produce sparks, especially when touched by an insect and there was evidence to suggest that sparking may have had a detrimental effect on the numbers of mosquitoes caught (Torr *et al.,* 2008). However, the E-Grids successfully caught high numbers of mosquitoes many of which were still suitable for identification (Torr *et al.,* 2008).

E-Grids have also been adapted to produce traps for sampling mosquito populations (Maliti *et al.,* 2015). A recent study in Tanzania developed the Mosquito Electrocuting Trap (MET) (Figure 4.7) designed to sample host seeking malaria vectors as an

alternative to HLC (Maliti *et al.,* 2015). A volunteer is required to place their feet into the MET box and then mosquitoes that are attracted to the host odour are electrocuted and collected (Maliti *et al.,* 2015). The MET was successful at collecting mosquitoes although further optimisation of the trap is needed before the MET can be utilised as an alternative to HLC.

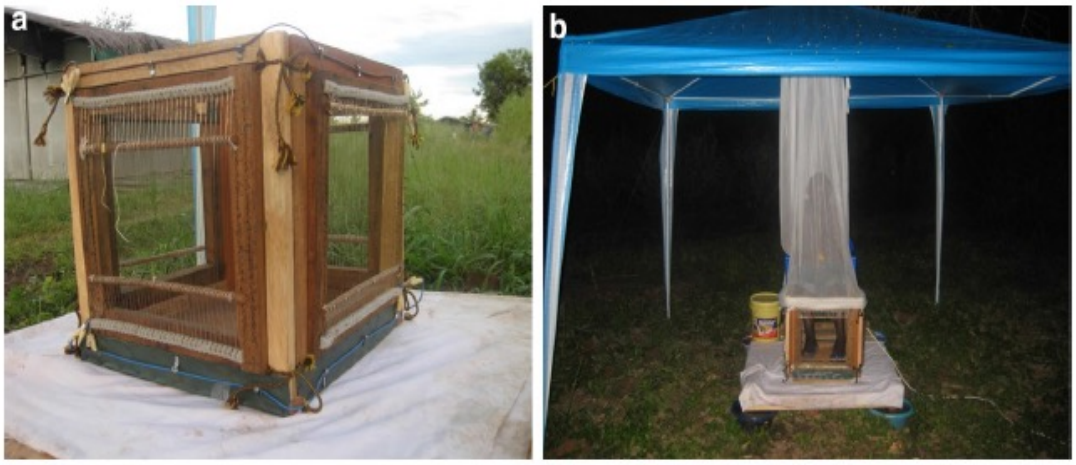

Figure 4.7: a) the design of the MET; b) the MET in operation with a human as an attractant with legs in the trap (Unadapted from (Maliti *et al.,* 2015)).

4.2.3 The Importance of Colour in Trap Design

Vision, along with other stimuli such as odour, is important for allowing blood sucking insects to locate their host (Lehane, 2005). Vision is an important close range stimulus to haematophagous insect species, including mosquitoes. Unlike human vision, insects are sensitive to UV light and insensitive to the parts of the spectrum above 650 nm (e.g. red) (Lehane, 2005).

Colour has previously been shown to be an important consideration in trap and target design for several different species of haematophagous insect. For example, blue is the most effective colour for targets to control tsetse flies (Green and Flint, 1986, Lindh *et al.,* 2012, Green, 1989). For UK midge species, white and clear sticky traps caught significantly more midges compared to yellow and blue traps suggesting that visual cues are important to *C. obsoletus* (Thompson *et al.,* 2014). A 1938 study comparing the attractiveness of different coloured cloth to *Ae. aegypti* found yellow to be a repellent colour while dark colours, specifically black, were found to be

attractive (Brett, 1938). For *Aedes polynesiensis* (*Ae. polynesiensis*), significantly more mosquitoes were found to land on coloured targets compared to white controls with the greatest number of mosquitoes landing on the black and navy blue coloured targets (Chambers *et al.,* 2013). Bright colours, with a low reflection factor, are reported to be less attractive to mosquitoes (Brett, 1938, Hoel *et al.,* 2011). Colour has also been shown to be an important consideration in the design of ovitraps (Hoel *et al.,* 2011). Black and blue ovitraps were found to be more attractive to *Ae. albopictus* compared to orange or white traps (Hoel *et al.,* 2011). There are no published records of the impact of trap colour being evaluated for UK mosquito populations.

4.3 Aim and Objectives

This chapter aims to quantify the relationship between trap catches and the number of mosquitoes biting humans in the UK.

The objectives of this chapter were to:

1. To compare mosquito catches from commonly used mosquito trapping methods to HLC to determine whether traps can be used as a proxy for HLC when sampling the human biting mosquito population in the UK.
2. To assess the importance of host seeking stimuli in the catches of *Aedes detritus.*
3. To determine the impact of trap colour on mosquito collections with particular reference to the host decoy trap.

4.4 Materials and Methods

4.4.1 Sample Sites

All studies were conducted within Ness Gardens with a total of eight locations chosen as suitable locations to place the different mosquito traps under evaluation (Figure 4.8).

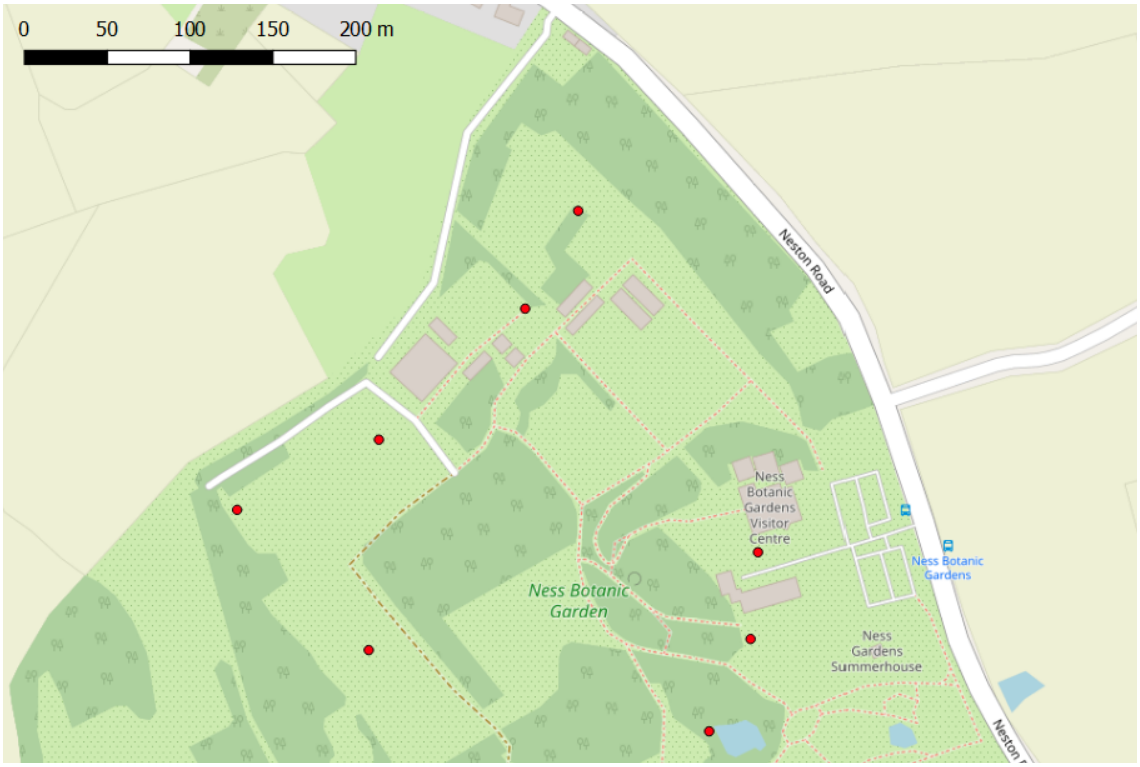

Figure 4.8: Map of Ness Gardens showing the eight different locations within the gardens were the mosquito traps were placed. Basemap: © OpenStreetMap contributors, CC-BY-SA (https://www.openstreetmap.org/copyright).

4.4.2 Comparison Studies

Four different comparison studies were conducted over a three year period. Details of each of these four studies are described below.

Study 1: Comparison of the mosquito catch from three mosquito traps to HLC.

A 4x4 Latin square study was conducted in October 2016 to compare the catches from the Mosquito Magnet, BG Sentinel and CDC light traps to HLC. Each rotation of the Latin square was repeated six times, a value obtained from sample size

calculations to ensure that a significant different was detected. All mosquito trapping commenced one hour prior to sunset and lasted for a total of two hours.

Study 2: Comparison of the mosquito catch from the HDT and the Mosquito Magnet.
Two 4x4 Latin square studies were conducted. The first in October 2017 and the second in June 2018. The following trap combinations were evaluated:

i) Hot, CO_2 baited HDT vs. Mosquito Magnet

ii) Cold, CO_2 baited HDT vs. Mosquito Magnet

iii) Hot, non-CO_2 baited HDT vs. Mosquito Magnet

All traps were set up one hour prior to sunset and left running over night.

Study 3: Comparison of the mosquito landing rates on the HDT using E-grids.
In October 2018, a 4x4 Latin square study design was conducted. This experiment, examined the behavioural basis of heat on the catch of the HDT. It was hypothesized that mosquitoes were attracted to the vicinity of the HDT by the odour of CO_2 and that the heat elicited a landing response. To test this hypothesis, we compared the catches of a 'hot' and 'cold' HTD with an E-grid placed adjacent were compared. The proportion of mosquitoes caught by the E-grid and the HDT provided a relative measure of the numbers that (i) circled or (ii) landed on the HDT.

The trap combinations under evaluation were:

i) Mosquito Magnet

ii) Three HDTs:

- Hot, odour baited with E-grid
- Cold, odour baited with E-grid
- Hot, odour baited alone

All experiments started 90 minutes prior to sunset and were left to run for two hours.

Study 4: Comparison of the mosquito catch from different coloured sticky traps.
Two Latin square studies were conducted each with two replicates. The first in October 2017, compared five different colours: black, blue, yellow, white and clear. The second in June 2018, compared eight different coloured traps: black, white, blue, yellow, red, light grey, dark grey and clear.

The experiments were set up each evening one hour prior to sunset and left to run overnight.

4.4.3 Spectral Analysis

The absorbance spectra of each of the coloured traps was measured using an Ocean Optics USB4000 Spectrometer with an Ocean Optics PX-2 light source at the University of Aberystwyth with the assistance of Dr Roger Santer. The following parameters were set for spectral readings: integration of 150 ms, strobe period of 50 ms, boxcar width of five and 25 scans to average. A non-unity correction was also applied.

For each coloured sample, two measurements were taken: the first of the coloured card only and the second of the coloured card covered in the clear sticky plastic. For the clear sticky plastic, the absorbance was measured over a white tile. The absorbance of each sample was measured three times and the average taken.

4.4.4 Trap Running and Operation

Six different mosquito trapping methods were used in the aforementioned studies. Details of each of the trapping methods are summarised below.

CDC Light Trap
A 6 volt battery was used to power a 6.3 volt LED and fan in the CDC light trap (John W Hock, Florida, USA). Traps were placed ~1 m above the ground and were baited with 1 kg of dry ice.

BG Sentinel Trap
The BG Sentinel traps were powered using a 6 volt battery. Traps were placed on the ground and baited with 1 kg of dry ice.

Mosquito Magnet

Mosquito Magnet traps (Independence Model), were powered using a rechargeable battery (changed weekly). Traps were baited with 1-octen-3-ol (0.5 mg/h) dispensed from Mosquito Magnet branded sachets (Midge Free Zone, Argyll, UK). New sachets were used at the start of each study and if required were changed after one month of use.

Host Decoy Trap

The HDT was filled with 15 L of boiling water which allowed the trap to remain above ambient temperature overnight. For experiments where a cold HDT was required, the trap was filled with 15 L of cold water. The outer surface of the trap was wrapped with the sticky plastic Fics Film. The traps were baited with 2 kg of dry ice and a small fan used to disperse this CO_2 odour.

Electric Nets

Electric nets (E-grid) (1 m high x 0.5 m wide) comprised of a parallel grid of fine copper wires (0.2 mm diameter) spaced 8mm as described in Torr et al. (2008) and Vale (1974) (Figure 4.9). Alternate wires were charged from a 12 volt car battery via a transformer (spark box). The transformer was set to pulse at intervals of 15 ms an energy of 175 mJ as per Vale et al. (2015). E-grid were placed above a sheet of sticky plastic to collect mosquitoes that were electrocuted (Figure 4.9).

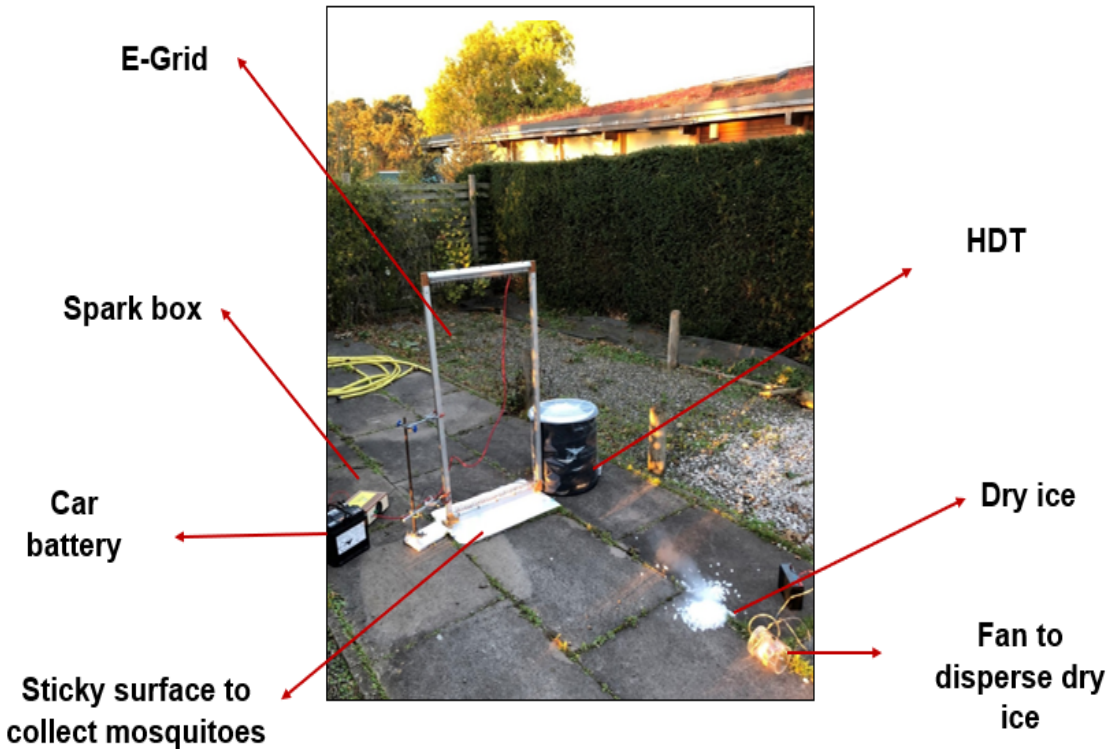

Figure 4.9: Set up of the E-Grids alongside the HDT.

Coloured Sticky Targets

A piece of coloured A4 card was held in place using two wooden supports. Seven different colours were evaluated (Figure 4.10). This card was covered with Fics Film sticky plastic to create a coloured sticky trap. To act as a control, a clear trap was also evaluated. For the clear trap, just the sticky Fics Film was used. The traps were all baited with 1 kg of dry ice.

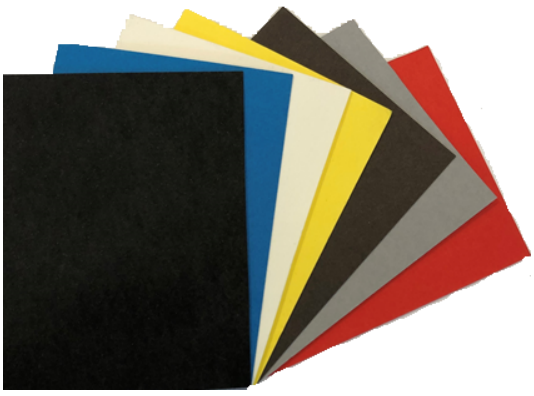

Figure 4.10: Different coloured targets to be evaluated.

4.4.5 Human Landing Catches

A total of six volunteers, including the author, were recruited to participate in the HLCs. Ethical approval was obtained prior to the start of the study from the LSTM research ethics committee, reference number 16-012 (Appendix A). Participants were recruited on a voluntary basis, provided full informed consent and were free to withdraw from the study at any time. Participants had to be 18 years of age, but no other eligibility criteria were defined.

HLCs were conducted in October 2016. The collection period commenced one hour prior to sunset and continued for two hours. Volunteers were asked to roll up one trouser leg and collect mosquitoes attempting to land on the exposed limb. Volunteers were provided with either a mechanical or mouth aspirator to collect the mosquitoes. Mosquitoes were to be placed into pre-prepared paper cups. Collections were split into 30-minute intervals therefore each volunteer was provided with four collection cups.

4.4.6 Mosquito Identification

Mosquitoes collected by a trap utilising a collection net (Mosquito Magnet, CDC light trap, BG Sentinel) were knocked down by placing these nets into a freezer. Mosquitoes captured by a sticky trap (e.g. HDT and E-Grid) were first removed from the sticky surface using Romax glue solvent (Barrettine Environmental Health, Bristol, UK) and left to dry for 24 hours before being identified. All mosquitoes were identified using morphological keys (Cranston *et al.,* 1987, Snow, 1990).

4.4.7 Data Analysis

For each of the studies, GLMMs were fitted to the data sets using R, version 3.3.1., in the 'glmmADMB' package (Appendix B). Trap location and day of sampling were included in the model as random effects and trap type was a fixed effect. Mosquito catch was the response variable. AIC values were used to assess the goodness-of-fit of each model. Final models were generated following a series of stepwise deletions of non-significant factors dependent on the change in AIC.

4.5 Results

4.5.1 Study 1: Comparison of the Mosquito Catch from Three Mosquito Traps to HLC

A total of 336 mosquitoes were collected during the trap comparison study. Two different species of mosquito were collected: *Ae. detritus* which constituted 99% of the total catch and *Cs. annulata.* Only the catch data for *Ae. detritus* was used in the subsequent analysis (Figure 4.11).

The CDC light trap caught significantly fewer mosquitoes compared to the other trapping methods ($p<0.001$) cathcing an average of less than one mosquito per evening (Figure 4.11) (Table 4.1). Although, on average, the BG sentinel trap caught more mosquitoes than the HLC and Mosquito Magnet these differences were not significant.

Table 4.1: Regression coefficients with 95% confidence intervals and standard error for the minimal adequate model.

	Estimate (95% CI)	Standard Error
MM-HLC	-0.444 (-1.117; 0.229)	0.268
CDC-HLC	-2.197 (-3.171; -1.224)***	0.388
BG-HLC	0.116 (-0.556; 0.788)	0.268
CDC-MM	-1.754 (-3.084; -0.423)**	0.530
BG-MM	0.560 (-0.397; 1.516)	0.381
BG-CDC	2.313 (1.136; 3.491)***	0.469

***p<0.001, **p<0.01

MM – Mosquito Magnet, HLC – Human Landing Catch, CDC – CDC light trap, BG – BG Sentinel,

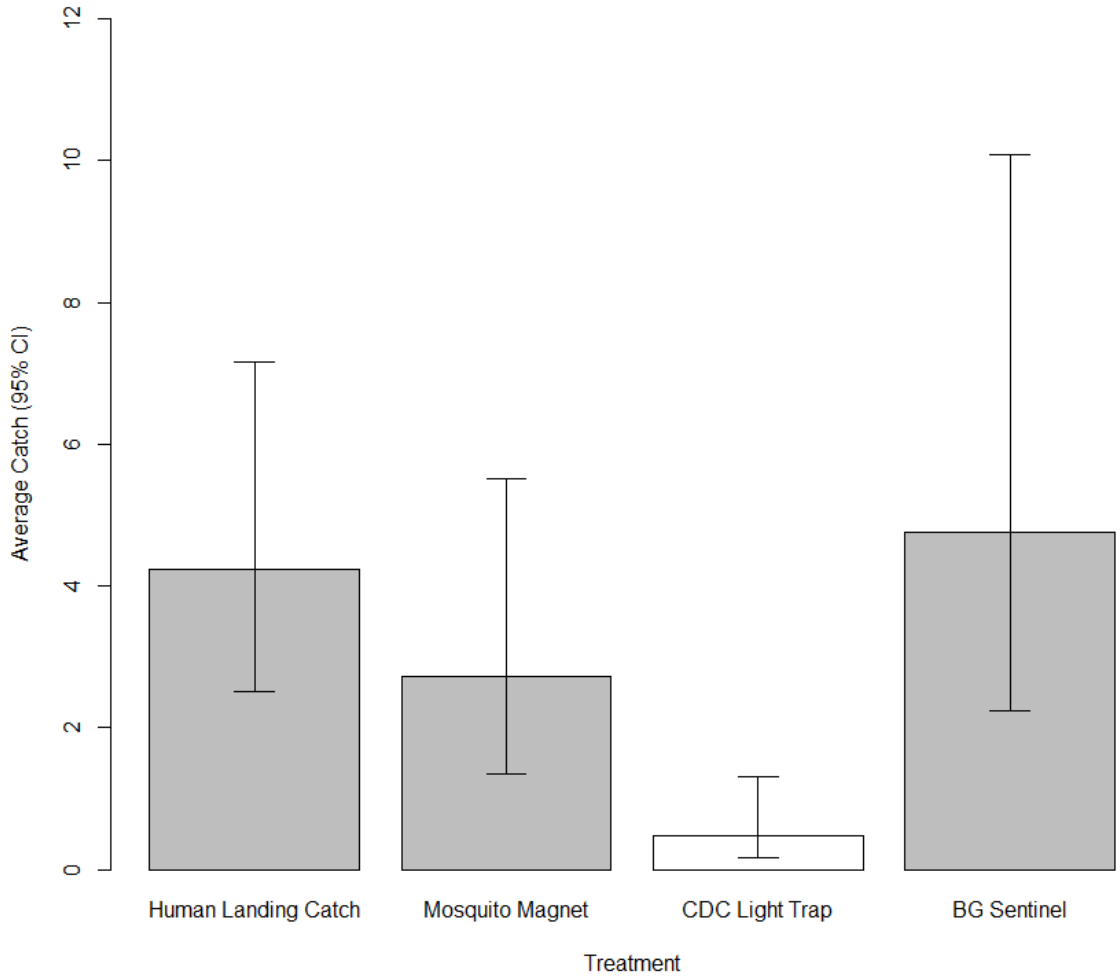

Figure 4.11: Average catch (± 95% CI) from the four different trapping methods. Bars of the same colour indicate no signficant difference between the trappign methods.

Given that there was no significant difference between the catches from the Mosquito Magnet and HLC it was concluded that the Mosquito Magnet can be used as a proxy for HLC in future studies.

Biting Rates Relative to Sunset

The catches from the HLC were split into four different sampling periods to determine the biting rates relative to sunset (Figure 4.12). Significantly fewer mosquitoes were caught one hour post sunset compared to both the catch periods prior to sunset ($p<0.05$). The peak of mosquito activity occurred 30 minutes prior to sunset.

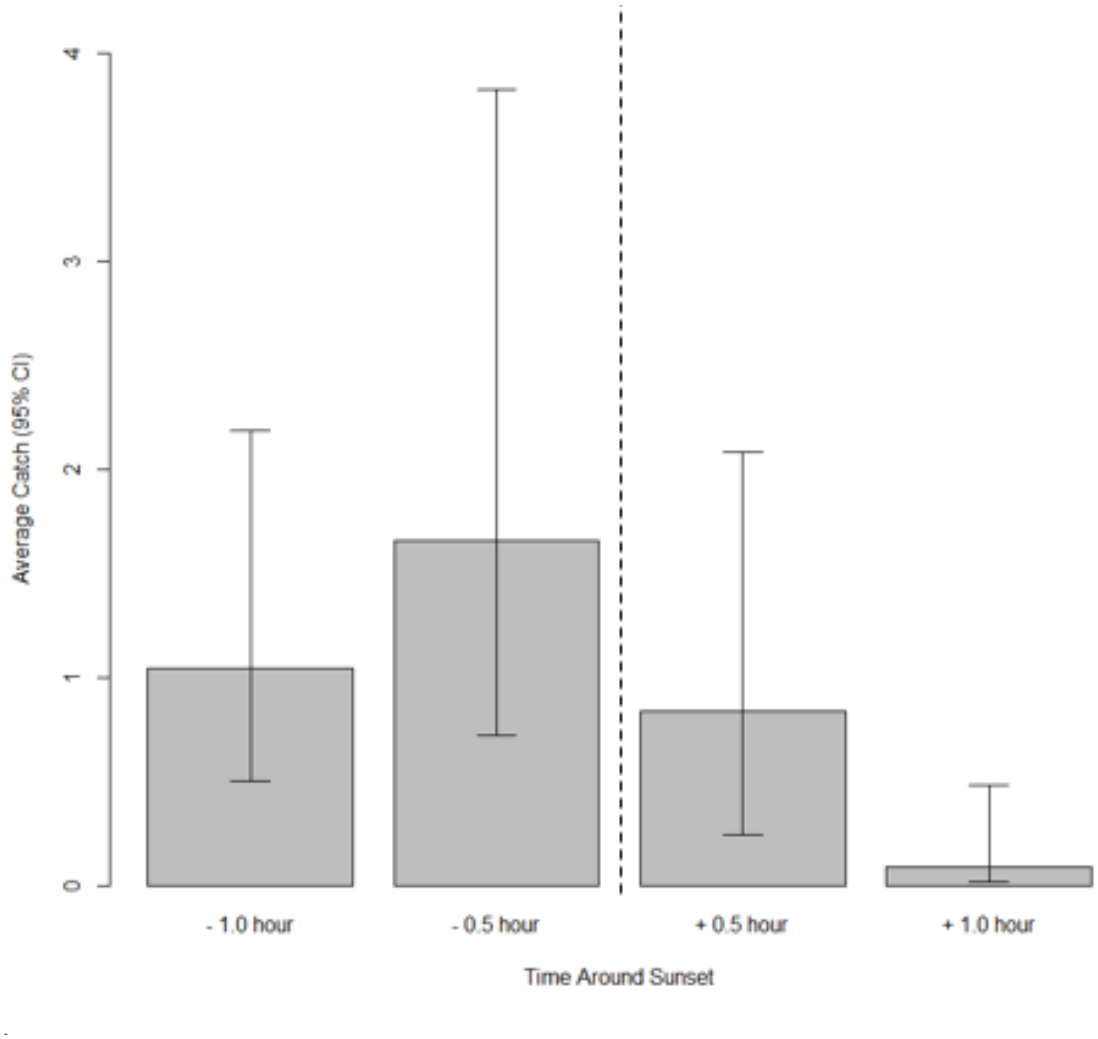

Figure 4.12: Average catch (± 95% CI) from each of the four 30 minute sampling periods. The time of sunset is indicated by the black dashed line.

4.5.2: Study 2: Comparison of the Mosquito Catch from the HDT and the Mosquito Magnet

2017

A total of 3,099 mosquitoes were collected during the 2017 comparison study (Table 4.2). All mosquitoes collected during this experiment were identified as *Ae. detritus.*

Table 4.2: Total number of mosquitoes collected from the HDT and Mosquito Magnet across the different experiments.

Treatment	HDT catch	Mosquito Magnet Catch
Hot, CO_2 baited	1,425	965
Cold, CO_2 baited	53	580
Hot, non-CO_2 baited	0	76

The hot, CO_2 baited HDT caught more mosquitoes (1,425) compared to the Mosquito Magnet (965). However, this difference was not significant $P>0.05$ (Figure 4.13).

The Mosquito Magnet caught approximately ten times more mosquitoes (580) than the cold CO_2 baited HDT (53). There was a significant difference ($p < 0.001$) between the catches of the cold CO_2 HDT and the Mosquito Magnet (Figure 4.13).

The hot, non CO_2 baited HDT caugh no mosquitoes compared to the Mosquito Magnet which caught 76. There was a significant difference between the catches of the non CO_2 baited HDT and the Mosquito Magnet $p < 0.001$ (Figure 4.13).

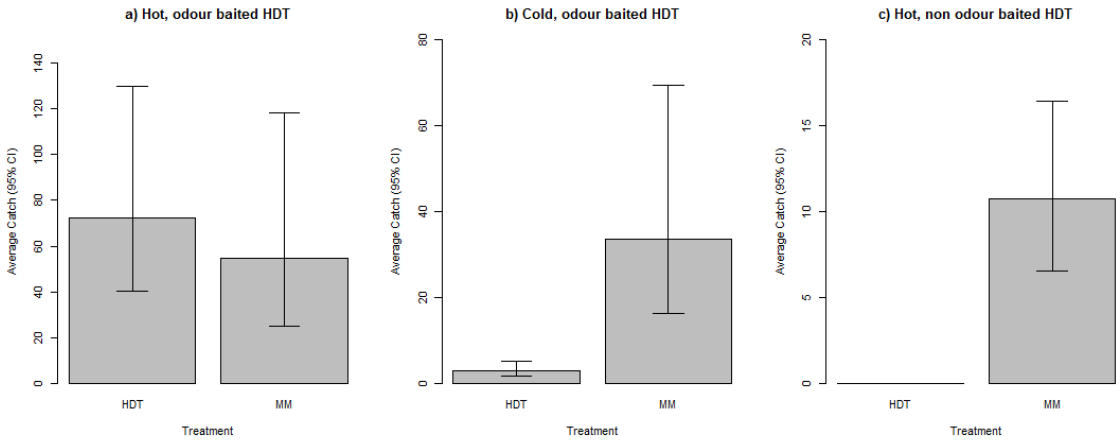

Figure 4.13: Average nightly catch (± 95% CI) from each of the three Latin Sqaure studies. HDT – Host Decoy Trap, MM – Mosquito Magnet.

2018

A total of 831 mosquitoes, from four different species, were collected (Table 4.3). *Ae. detritus* was the most abundant species collected consisting of 67.3% of the total catch. *An. claviger* was the next most abundant species (23.6%) followed by *Ae. rusticus* and *Cs. Annulata* (5.2% and 3.6% respectively).

Table 4.3: Total numbers of mosquitoes collected from the four different trapping methods.

Species	Trap			
	Hot, CO_2 baited HDT	Cold, CO_2 baited HDT	Hot, non CO_2 baited HDT	Mosquito Magnet
Ae. detritus	284	44	6	225
An. claviger	87	19	4	86
Ae. rusticus	15	3	0	25
Cs. annulata	12	4	0	14
Unidentified	2	1	0	0
Total	400	71	10	350

The combined results for all species were analysed as well as the catch data for just *Ae. detritus* (Figure 4.14). For all species, there was no significant difference between the hot, odour baited HDT and Mosquito Magnet catch ($P>0.05$) (Figure 4.14). When CO_2 was removed but the HDT remained hot, the mosquito catch decreased

significantly (p<0.001) (Figure 4.14). The same trends were also observed when just the *Ae. detritus* catch data was analysed (Figure 4.14).

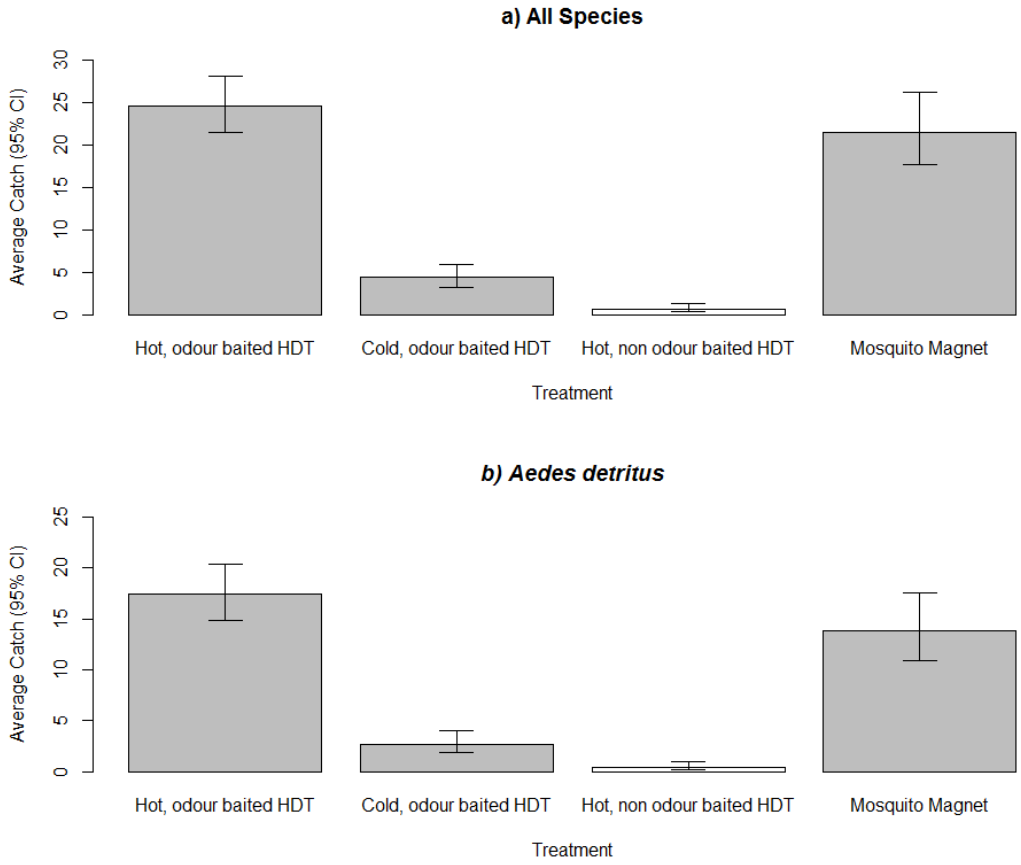

Figure 4.14: Average nightly catch (± 95% CI) from each of the different trapping methods for all species (a) and *Ae. detritus* (b). Bars of the same colour indicate no signficant difference between the trappign methods.

Overall, a combination of both heat and a CO_2 significantly increases the mosquito catch compared to removing either one of these components from the HDT (Figure 4.13 and Figure 4.14). A combination of both heat and CO_2 were required to achieve catch rates where there was no significant difference between the HDT and Mosquito Magnet catches. CO_2 is especially important with a significant reduction in the number of mosquitoes caught by the HDT when this is removed (p<0.001). During the 2017 experiment, when CO_2 was removed, the mosquito catch dropped to zero (Figure 4.13).

4.5.3: Study 3: Comparison of the Mosquito Landing Rates on the HDT using E-grids

A total of 3,589 mosquitoes were collected during this study 73% of which were *Ae. detritus* (Figure 4.15). *An. claviger* and *Cs. annulata* were also caught making up 21% and 6% of the remaining catch respectively. Only the catch data for *Ae. detritus* is used in the subsequent analysis. The combined catch of the hot, CO_2 baited, HDT paired with the E-Grid caught significantly more mosquitoes compare to the other three trapping methods ($p<0.001$) (Figure 4.15). When heat was removed from the HDT, but CO_2 remains, there was a significant difference between the E-Grid and HDT catch $p<0.001$ (Figure 4.15). The Mosquito Magnet caught significantly fever mosquitoes that both the hot, CO_2 baited HDT with E-Grid and hot, CO_2 baited HDT alone $p<0.01$ (Figure 4.15).

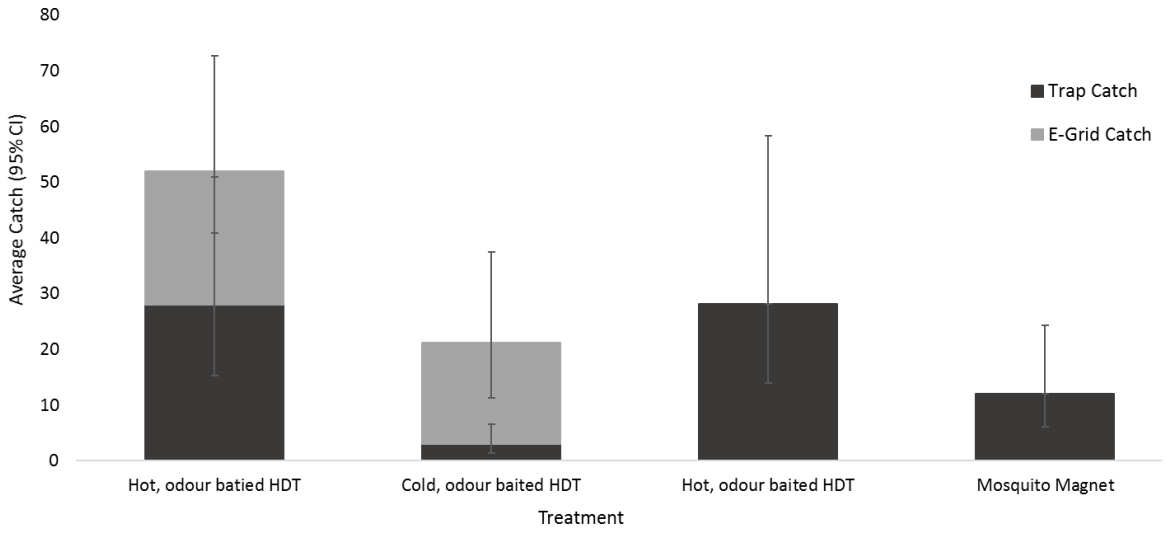

Figure 4.15: Average nightly catch (± 95% CI) from each of the trapping methods. Dark grey bars show the catch from the different trap types with light grey bars showing the mosquito catch from the E-grids.

Heat is clearly important to elicit a landing response from *Ae. detritus* with significantly fewer mosquitoes caught by the cold HDT. When heat is removed from the HDT, significantly more mosquitoes are caught circling the trap than landing on it ($p<0.001$).

4.5.4 Study 4: Comparison of the Mosquito Catch from Different Coloured Sticky Targets

2017

A total of 248 mosquitoes were collected using the coloured sticky targets (Figure 4.16). All mosquitoes were identified as *Ae. detritus*. The black targets caught significantly more mosquitoes than the white, yellow and clear targets ($p<0.001$). The blue target caught significantly more mosquitoes than the yellow target ($p<0.001$). No other significant differences were detected.

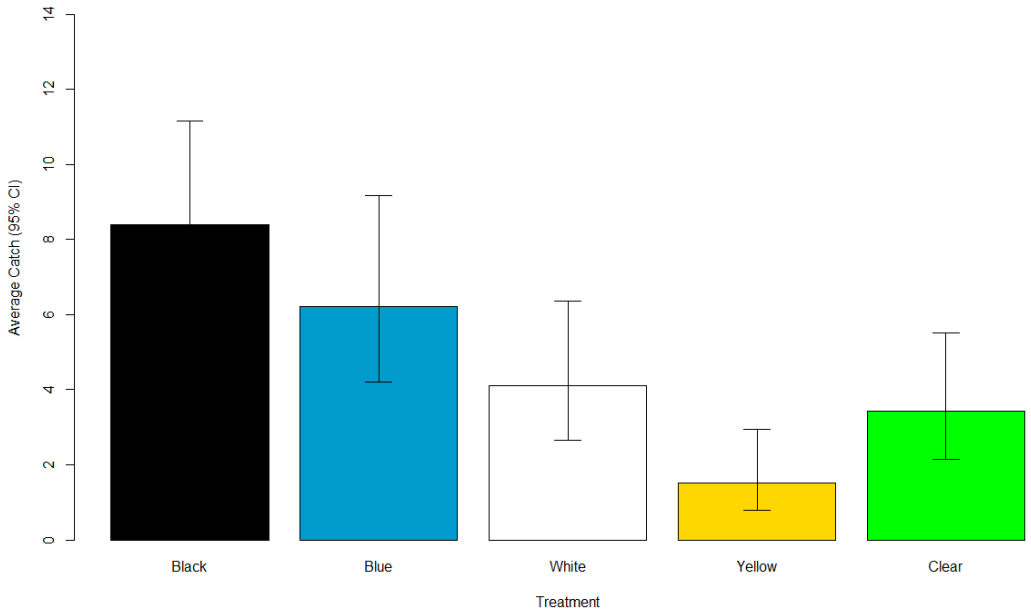

Figure 4.16: Average nightly catch (±95% CI) for each of the five different colour treatments.

2018

A total of 393 mosquitoes from two different species were collected using the coloured sticky targets (Table 4.4). *Ae. detritus* made up 83.2% of the total catch and for the purpose of this study, only catch data for this species is used in the subsequent analyses.

Table 4.4: Total number of mosquitoes collected from the eight different coloured targets from the 2018 study.

Colour	Species	
	Aedes detritus	*Anopheles claviger*
Black	88	14
Blue	74	20
White	33	7
Yellow	13	3
Clear	31	8
Dark Grey	45	8
Light Grey	24	3
Red	19	3
Total	327	66

Both the black and blue sticky targets caught significantly more mosquitoes compared to the other coloured traps ($p<0.01$), with the exception of the dark grey (Figure 4.17). The dark grey sticky targets also caught significantly more mosquitoes than the yellow target ($p<0.01$) (Figure 4.17). No other significant differences between the different colour targets were observed.

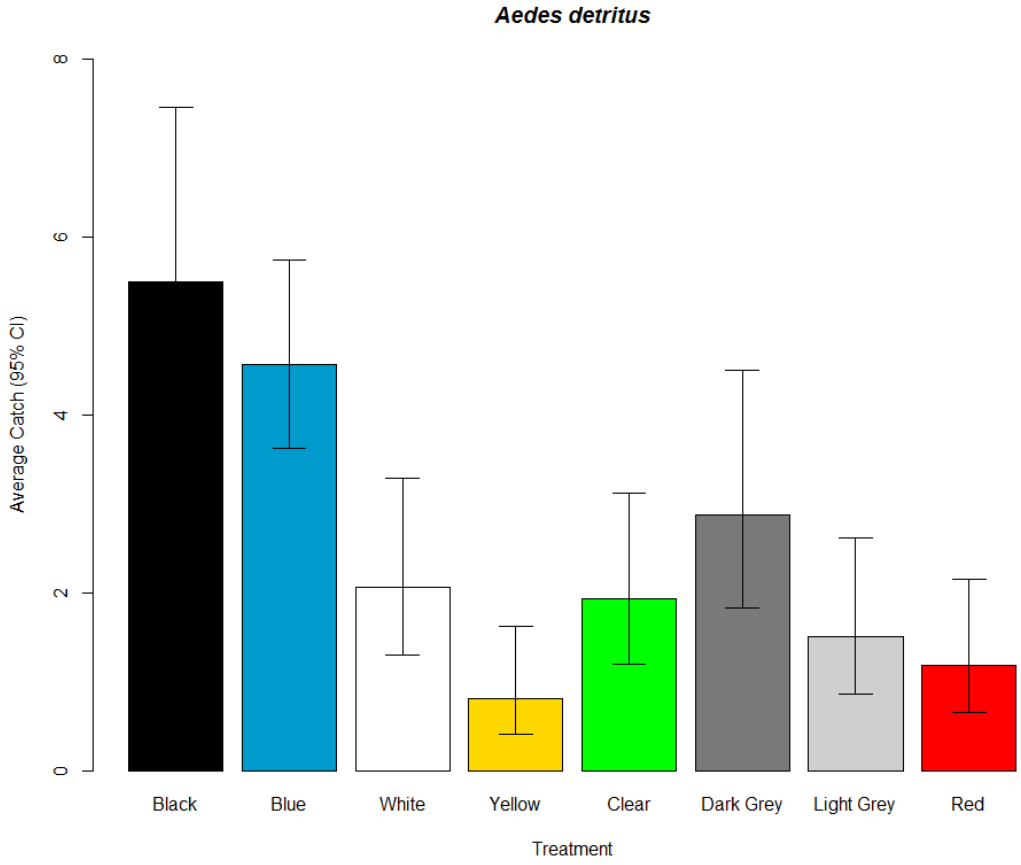

Figure 4.17: Average nightly catch for *Ae. detritus* with 95% CI for each of the different colour treatments.

The results of the colour studies demonstrate a true colour response in *Ae. detritus* with black and blue targets collecting significantly more mosquitoes than the yellow target ($p<0.01$) (Figure 4.16 and Figure 4.17).

Spectral analysis

The reflectance of each of the coloured card samples, and the coloured card wrapped in Fics Film, was measured (Figure 4.18). These two measurements were taken as the sticky Fics Film is known to absorb light at shorter wavelengths. It is clear that there were differences in the reflectance readings across each of the samples. There is very low reflectance values recorded for the black coloured targets (Figure 4.18). For the blue coloured targets, there is a clear peak in reflectance at 450 nm (Figure 4.18).

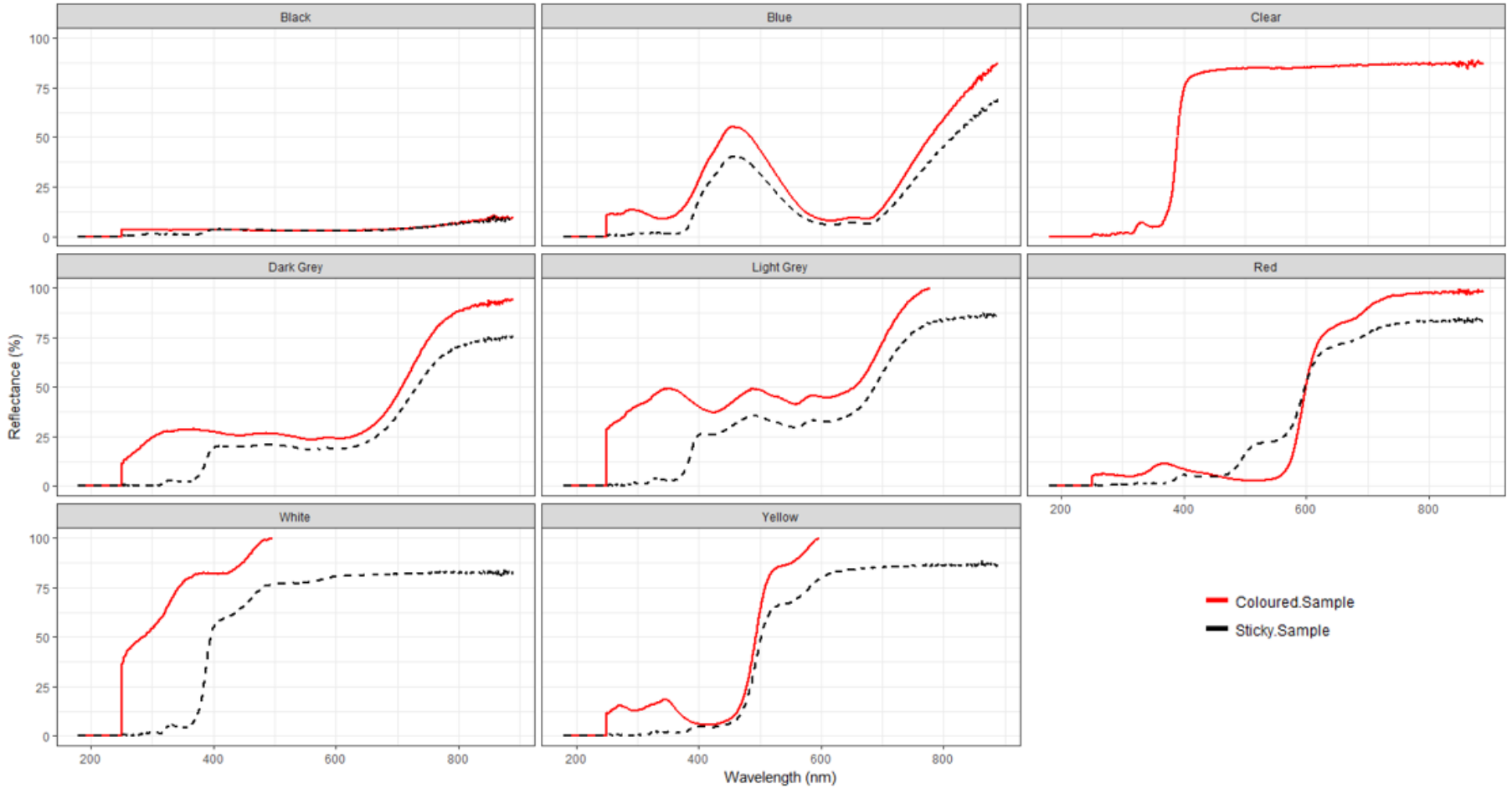

Figure 4.18: Reflectance values for each of the eight samples measured. Red lines show the values for each of the coloured samples with the black dashed lines showing the values for the sticky coated coloured samples.

4.6 Discussion

Trap comparison studies have previously been conducted to determine the efficacy of different methods to sample mosquito populations. Several studies have been conducted in America and mainland Europe (Lühken *et al.,* 2014, Dennett *et al.,* 2004, Irish *et al.,* 2008, Farajollahi *et al.,* 2009, Hoel *et al.,* 2009). However, limited research into the efficacy of different trapping methods in the UK has been conducted. A 2007 study conducted by Hutchinson *et al.,* compared the ability of CO_2 baited CDC light traps and a Mosquito Magnet for mosquito surveillance in the UK (Hutchinson *et al.,* 2007). The study found that the Mosquito Magnet caught significantly more mosquitoes compared to the CDC light trap (Hutchinson *et al.,* 2007). The Mosquito Magnets were particularly efficient when mosquito densities were low (Hutchinson *et al.,* 2007). Several other studies also report that the Mosquito Magnet performs better than other trapping methods when compared in Latin square studies (Lühken *et al.,* 2014, Chaves *et al.,* 2014, Hoel *et al.,* 2009).

The trap comparison experiments conducted in the present study, were the first to evaluate the ability of different mosquito trapping methods to collect *Ae. detritus* mosquitoes in the UK. *Ae. detritus* was the species of interest for these trap comparison studies due to the fact that it is found in extremely high numbers in the chosen sample site. Additionally, it has previously been shown to a competent laboratory vector for several arboviruses (Blagrove *et al.,* 2016, Mackenzie-Impoinvil *et al.,* 2015, Lumley *et al.,* 2018, Chapman, 2017).

The majority of these experiments took place during a seasonal peak in *Ae. detritus* abundance as identified by the longitudinal study described in Chapter 2. The experiments were conducted at this time to ensure that a true difference in the mosquito catches was detected by the Latin square design. However, during the peak in *Ae. detritus* abundance, few other species are active. In order to overcome this limitation, these studies should be repeated at additional timepoints during the year to ensure that other mosquito species of interest are active.

4.6.1 Alternative Methods for Sampling Anthropophilic Mosquitoes in the UK

The results of the experiments to compare mosquito trap catches to HLC have demonstrated that traps can offer up alternative methods to sampling the human biting *Ae. detritus* mosquito population. HLCs are the current gold standard when looking to sample the anthropophilic mosquito population (Silver, 2007). Given that there was no significant difference between the catches from the HLC and Mosquito Magnet or BG Sentinel trap, the results of this experiment demonstrate that these traps can be used as a proxy for HLC obviating the requirement for future HLC experiments to sample *Ae. detritus*. Ethical approval is required before human volunteers can be used in HLC studies. This approval can be difficult to obtain in areas with ongoing disease transmission. Given the potential for the introduction of a mosquito borne pathogen, such as WNV, into the UK, it is important that an understanding of how the anthropophilic mosquito population can be sampled without the use of human volunteers is obtained (Medlock *et al.,* 2018).

The UK is currently at risk from invasive *Aedes* species and PHE have a range of surveillance programmes in place to ensure that these species are detected should they reach the UK (Medlock *et al.,* 2018). A range of trapping methods are currently used in these surveys including Mosquito Magnets and BG Mosquitaire traps (Vaux *et al.,* 2015, Medlock *et al.,* 2017). The results of the present study suggest that both these trapping methods are suitable for collecting human biting *Aedes* species within the UK. Over the last three years, the eggs of *Ae. albopictus* have been collected using ovitraps in Kent (Medlock *et al.,* 2018, Medlock *et al.,* 2017). In response to these incursions, increased numbers of adult traps have been placed within the proximity of the eggs in an attempt to also collect adult *Ae. albopictus* (Medlock *et al.,* 2017). So far, no adults have been collected (Medlock *et al.,* 2017). However, continued surveillance using the best trapping methods available is especially important to ensure that *Ae. albopictus* does not spread within the UK.

The CDC light trap caught significantly fewer mosquitoes compared to the other trapping methods evaluated. Guidelines published by the European Centre for Disease Prevention and Control (ECDC) state that the CDC light trap can perform poorly when sampling *Aedes* mosquitoes in Europe, including *Ae. detritus* (ECDC,

2014). Additionally, previous studies have demonstrated that the CDC light trap can underestimate the number mosquitoes in a population (Giberson *et al.,* 2007, Hesson *et al.,* 2015). However, Brugman (2016) reports good numbers of mosquitoes collected, including *Ae. detritus*, using CDC light traps in the UK. It is possible that the locations in which the CDC light traps were placed limited their suitability for sampling mosquitoes in Ness Gardens. For example, when CDC light traps are hung in trees, this can increase the catch of *Cx. pipiens* (Hutchinson *et al.,* 2007).

This trap comparison study compared the catches of three commonly used mosquito traps to HLC. However, there are several other mosquito trapping methods, such as the BG Mosquitaire, that could be evaluated. Future studies could be conducted to compare the mosquito catches from HLC and the BG Mosquitaire.

4.6.2 Exploring the Impact of Olfactory, Thermal and Visual Cues on the Host Seeking Behaviour of *Ae. detritus*

HDT

Experiments using the HDT and E-Grids were conducted to explore the impact of olfactory and thermal cues on the host seeking behaviour of *Ae. detritus*. The results of these experiments demonstrate the importance of both thermal and odour stimuli when sampling *Ae. detritus*. When the HDT was baited with both CO_2 and heat there was no significant difference between the Mosquito Magnet and HDT catches. However, if either stimulus was removed, then the Mosquito Magnet caught significantly more mosquitoes compared to the HDT. Previous studies have demonstrated that both heat and odour are important host seeking cues for haematophagous insects (Lehane, 2005). In the present study these two factors are working synergistically to increase the catch of *Ae. detritus*. Carbon dioxide is an extremely important odour that activates the host seeking behaviour in mosquitoes (Gillies, 1980, Gibson and Torr, 1999). CO_2 is a universal attractant to mosquitoes (as well as many other biting diptera) and has been shown to increase the number of mosquito caught by a trap on several occasions; including the present study (Gibson and Torr, 1999, Takken and Kline, 1989, Takken and Knols, 2010). When CO_2 is

removed from the HDT the mosquito catch drops to almost zero. Further work could be conducted to evaluate the impact of different odours, such as 1-octen-3-ol, on the ability of the HDT to catch mosquitoes in the UK.

Heat is an important close range a cue for host seeking mosquitoes and clearly elicits a landing response in the present study (Takken and Knols, 2010). This is evident from the results of the HDT and E-grid studies. When the HDT is heated, high numbers of mosquitoes are caught on the surface of the HDT with equally high numbers circulating (as indicated by the E-grid catch). However, when heat is removed, the number of mosquitoes caught on the surface of the HDT drops significantly whilst high numbers continue to circle the trap.

As previously discussed, the Mosquito Magnet can be used as a proxy for HLC when sampling *Ae. detritus.* Given that there was no significant difference between the catches from the hot, CO_2 baited HDT and Mosquito Magnet, this study suggests that the HDT also offers an alternative trapping method for sampling anthropophilic mosquitoes. One of the limitations of the Mosquito Magnet as a research tool is cost (~£900 per unit). In contrast to this, the simple design of the HDT, is much cheaper. However, the HDT requires frequent revisits (at least once per day) to top up the hot water levels unlike the Mosquito Magnet which can be left for up to a month. This limits the ability of the HDT to be used in long term surveillance programmes. Once again, these experiments focused on one mosquito species, *Ae. detritus.* Further work should be conducted to explore the ability of the HDT to sample other mosquito populations. Additionally, the ability of the HDTs to collect mosquitoes from different locations within the UK should be determined.

In the present study, the HDT was baited with CO_2 in the form of dry ice. True host odour comprises several other olfactory stimuli such as lactic acid, ammonia and acetone (Lehane, 2005). The HDT has proven hugely successful at collecting malaria vectors in Africa using both human and cattle odour (Abong'o *et al.,* 2018, Hawkes *et al.,* 2017). Further studies could be conducted within the UK to explore the impact of host odours on the mosquito catches from the HDT. For example, the WNV vector *Cx. modestus,* which is now established within the UK readily feeds on humans, horses and birds (Golding *et al.,* 2012). It would be interesting to used the HDT to

determine how these different host odours impact on the catches of the *Cx. modestus*. This information could provide important insight into odour combinations that could be used to bait traps to control this mosquito species should it become involved with future disease transmission within the UK.

Coloured Targets

Several different coloured targets were used to determine the impact of colour on the landing behaviour of *Ae. detritus*. Each of the coloured targets was baited with dry ice to produce CO_2, which acts as an orientation stimulus to haematophagous insects (Lehane, 2005). The CO_2 provided with each of the coloured target will have acted as a long range stimulus to these mosquitoes, with the colour of the target becoming important at a much shorter range.

The results of the colour comparison studies provide evidence for a true colour response in *Ae. detritus*. Significantly more mosquitoes were collected by the black and blue traps compared to the yellow traps. This is a similar result to that seen for other day biting haematophagous insects such as tsetse flies (Green and Cosens, 1983, Lindh *et al.,* 2012, Torr, 1989). As discussed in the introduction to this Chapter, bright colours, including yellow, have previously been reported to be less attractive to mosquitoes (Brett, 1938, Hoel *et al.,* 2011).

During the setup of the HDT, the trap is wrapped in black cotton thus creating a black trap. However, it would be possible to use different coloured material during this step to produce different coloured HDTs. Hawkes et al. (2017) compared the mosquito catches between a black, high contrast HDT, and an almost clear (Fics Film wrapped around a wire frame), low contrast HDT, in Burkina Faso. The results demonstrated that significantly more mosquitoes were caught by the black high contrast trap (Hawkes *et al.,* 2017). Further experiments could be conducted to explore the impact different coloured HDTs on mosquito catches in the UK.

4.7 Conclusions

The present study has demonstrated that several mosquito trapping methods, which are routinely used in surveillance programmes, provide reliable estimates of the human biting *Ae. detritus* mosquito population along the Dee estuary. This is especially important given the potential for future transmission of mosquito borne viruses, such as WNV and USUV, in the UK. The impact of olfactory, thermal and visual stimuli on mosquito catches were assessed using the HDT and coloured targets. The results demonstrate that a combination of both olfactory and thermal stimuli are important for increasing the catch rates of *Ae. detritus*. Additionally, colour was found to be an important factor that influences *Ae. detritus* landing rates.

Chapter 5 - Vector Competence of *Aedes detritus* to Japanese Encephalitis Virus

5.1 Abstract

Vector competence is a measure of a vector's ability to become infected with a pathogen and to subsequently become infective. There are several factors that can influence this process, including the extrinsic incubation period (EIP) when the pathogen develops within the vector. The EIP is temperature dependent and there is a threshold temperature below which the pathogen does not develop.

Previous studies have assessed the vector competence of UK mosquito species, including *Ae. detritus* for a variety of pathogenic viruses. However, all these studies have used constant incubation temperatures. In the field, mosquitoes are exposed to daily temperature cycles and there is evidence suggesting that these temperature cycles can impact on a mosquito's vector competence. This study aimed to quantify the impact of daily temperature fluctuations on the vector competence of *Ae. detritus* for JEV.

The laboratory studies carried out in this chapter confirmed the vector competence of *Ae. detritus* for JEV under constant and cycling temperature conditions. *Ae. detritus* mosquitoes were maintained at a constant temperature of 19^{o}C. Under the cycling regime, the average temperature was also 19^{o}C, but the temperature fluctuated between 15-24^{o}C to mimic daily temperature cycling. *Culex quinquefasciatus* controls were also shown to be competent vectors under both constant and cycling temperature regimes. However, there was no significant effect of incubation temperature conditions for either *Ae. detritus* or *Cx. quinquefasciatus*.

5.2 Introduction

Vectorial capacity is a measure of a vector's ability to transmit a pathogen. Vectorial capacity is described by an equation where: *m* is the ratio of mosquitoes to hosts, *a* is the host biting rate, *p* is the likelihood that a mosquito survives one day and *n* is the extrinsic incubation period (EIP) (Brady *et al.,* 2016). All components of the vectorial capacity equation are somewhat driven by temperature (Vogels *et al.,* 2017b, Danforth *et al.,* 2016).

$$Vectorial\ Cacity = \frac{m\ a^2\ p^n}{(-\ log_e\ p)}$$

The EIP is the time period between ingestion of a pathogen during feeding and the ability for further transmission of said pathogen (Higgs and Beaty, 2005). The EIP can provide important information on the vector competence of a species: the vector in question must survive the EIP of the pathogen in order to be a competent vector (Samuel *et al.,* 2016, Kramer and Ebel, 2003, Xiao *et al.,* 2014). The EIP is intrinsically linked to temperature with numerous studies exploring the relationship between the two. Temperature has been shown to both increase and decrease the EIP of several mosquito species to a range of arboviruses (Hardy *et al.,* 1983, Chamberlain and Sudia, 1961, Kramer *et al.,* 1983). For example, a 1983 study investigating the vector competence of *Culex tarsalis* for western equine encephalitis virus found that increasing temperature increased the length of the EIP in this mosquito species of the virus (Kramer *et al.,* 1983). In contrast to this, a 2008 study of *Cx. pipiens* and WNV found that increasing temperatures reduced the length of the EIP (Kilpatrick *et al.,* 2008). The 2008 study also demonstrated that temperature can differentially impact the EIP of different strains of the same virus (Kilpatrick *et al.,* 2008). The difference in EIP between strains of WNV is thought to be one of the reasons behind the rapid spread of WNV in the United States (Moudy *et al.,* 2007, Ebel *et al.,* 2004). This newly introduced WNV strain (WN02) had an EIP four days shorter than the EIP of the previous WNV strain (NY99) (Moudy *et al.,* 2007).

The majority of studies exploring the links between temperature, EIP and vector competence use only constant temperatures during their incubation period. However, in the field, mosquitoes are exposed to daily temperature fluctuations. Environmental temperature is known to affect arboviral infections in mosquitoes though both the EIP (Kilpatrick *et al.,* 2008, Dohm *et al.,* 2002) and through the proportion of mosquitoes able to transmit said virus (Zouache *et al.,* 2014, Richards *et al.,* 2012, Dohm *et al.,* 2002, Kilpatrick *et al.,* 2008). Studies investigating daily temperature cycling have demonstrated that this does alter the ability of vectors to transmit a range of pathogens (Lambrechts *et al.,* 2011, Carrington *et al.,* 2013b, Carrington *et al.,* 2013a, Paaijmans *et al.,* 2010, Danforth *et al.,* 2016, Alto *et al.,* 2018). One such study, conducted in 2016, compared the impact of daily temperature fluctuations and a constant temperature on the EIP of *Cx. tarsalis* infected with WNV (Danforth *et al.,* 2016). The results of the study did not demonstrate a difference in EIP between the two temperature conditions although mosquitoes were shown to be competent vector under both temperature regimes (Danforth *et al.,* 2016). Further studies have explored the effects of daily temperature cycling around both low and high average temperatures for both DENV and malaria (Lambrechts *et al.,* 2011, Carrington *et al.,* 2013b, Carrington *et al.,* 2013a, Paaijmans *et al.,* 2010). For DENV infections in *Ae. aegypti,* it appears that a large diurnal temperature range (DTR) (±20^{o}C), around a mean temperature of 26^{o}C, reduced the susceptibility of the mosquitoes to DENV as well as reducing mosquito survival (Lambrechts *et al.,* 2011). However, a large DTR cycling around a low average temperature (20^{o}C) was shown to increase the numbers of infected mosquitoes (Carrington *et al.,* 2013a). Under these experimental conditions the EIP of DENV was reduced by over a week compared to the control group at a constant temperature (Carrington *et al.,* 2013a). For CHIKV infection rates in *Ae. aegypti,* no significant effect of temperature regime (constant vs. small and high DTRs) was found (Alto *et al.,* 2018). In contrast to this, in the same experiment results show that both small and high DTRs had a significant negative effect on the rates of CHIKV infection rates in *Ae. albopictus* (Alto *et al.,* 2018). The differing results of the above experiments highlight the need for more studies to further our understanding of the impact daily temperature cycling has on virus transmission (Alto *et al.,* 2018).

5.2.1 Globalisation, climate change and arbovirus transmission in Europe

Over the last 50 years, there has been a huge change in the geographic distribution of mosquito borne arboviruses, notably DENV, CHIKV, WNV and Zika (Wilder-Smith *et al.,* 2017, Chancey *et al.,* 2015). This change in virus demographics has been attributed in part to both climate change and globalisation (Wilder-Smith *et al.,* 2017, Ooi and Gubler, 2009).

WNV outbreaks in southern Europe have been increasing over the last 20 years with the largest outbreak to date occurring in 2018 (Holt, 2018). During the 2018 outbreak, transmission of the virus began several weeks earlier than previous years (Haussig *et al.,* 2018). The climate in 2018 is thought to have been one of the drivers behind the shift in WNV transmission: periods of high temperatures and rainfall were then followed by extended dry spells (Holt, 2018). These climatic conditions were conducive to mosquito breeding leading to high numbers of WNV vectors being present earlier in the year (Holt, 2018). The WNV outbreaks in Europe have, thus far, been limited to central and southern Europe (Vogels *et al.,* 2017b, Barrett, 2018). Vector competence studies with European vectors have demonstrated that increased temperatures increases WNV transmission rates (Vogels *et al.,* 2016, Vogels *et al.,* 2017a). The results of these studies suggest that temperature is one of the factors limiting the spread of WNV further north in Europe (Vogels *et al.,* 2017b, Fros *et al.,* 2015). However, changes in global temperatures are predicted to drive WNV into more northerly latitudes as well as extending the transmission season of this virus (Paz, 2019).

As WNV increases and moves through Europe, questions have been raised about the risk this virus poses to the UK. The WNV vector *Cx. modestus* is established in southern England (Golding *et al.,* 2012, Medlock and Vaux, 2012). Investigations are ongoing to detect WNV in both UK bird and mosquito populations (Phipps *et al.,* 2008, Vaux *et al.,* 2015) with no positive samples detected to date. Surveillance for WNV transmission within the UK is considered a priority and ongoing efforts are in place to ensure that we are prepared should this virus reach the UK (Medlock *et al.,* 2018).

Globalisation and climate change have also been attributed to the global spread of invasive mosquito species (Medlock *et al.,* 2012b, Tatem *et al.,* 2006, Baylis, 2017). *Ae. albopictus* for example has spread globally through the tyre and lucky bamboo trade with more localised spread via the road networks (Medlock *et al.,* 2012b). This mosquito species also has the ability to adapt to a range of climatic conditions allowing it to survive in northern latitudes (Medlock *et al.,* 2012b). *Ae. albopictus* is a known vector for several arboviruses and this species has been linked to locally transmitted cases of DENV and CHIKV in Europe (Tomasello and Schlagenhauf, 2013). Modelling has demonstrated that the climate in the UK is suitable for *Ae. albopictus* to become established (Medlock *et al.,* 2006, Caminade *et al.,* 2012). Whilst the abundance of *Ae. albopictus* is likely to be lower in the UK than in Europe, there is still the potential of localised arbovirus transmission in the event of infected travellers returning to the UK (Caminade *et al.,* 2012, Medlock and Leach, 2015, Baylis, 2017). For the last three years, the eggs of *Ae. albopictus* have been found in southern England (Medlock *et al.,* 2018, Medlock *et al.,* 2017). In response to these incursions, increased surveillance, training and planning has been underway to prepare local authorities for future introductions of this mosquito species into the UK (Medlock *et al.,* 2018).

5.2.2 Assessment of the vector competence of UK mosquito species

Given the risk posed to the UK from climate change and invasive mosquito species it is especially important that a full understanding of the vector competence of UK mosquito species in ascertained. Several studies exploring the vector competence of UK mosquito species to a range of different viruses have previously been conducted (Table 5.1).

Table 5.1: Vector competence of UK mosquito species for a range of arboviruses incubated at different temperatures.

Mosquito Species	Virus	Virus family	Incubation Temperature (^{o}C)	Competent Y/N
Ae. detritus	JEV[1]	Flavi	23, 28	Y
	WNV[2]	Flavi	21	Y
	DENV[2]	Flavi	21	N
	CHIKV[2]	Alpha	21	N
	RVF[3]	Bunya	20, 25	Y
	VEEV[4]	Alpha	18, 21, 24	N
	RRV[4]	Alpha	21, 24	Y
	ZIKV[5]	Flavi	19, 21, 24, 27, 31	Y
Cx. pipiens s.l.	RVF[3]	Bunya	25	Y
	USUV[6]	Flavi	25	Y
	JEV[4]	Flavi	18	Y
	ZIKV5	Flavi	19, 21, 24, 27, 31	N
Ae. caspius	RVF[3]	Bunya	20, 25	Y
Ae. rusticus	RVF[3]	Bunya	20, 25	N
Ae. cantans/annulipes	RVF[3]	Bunya	20, 25	N
Cs. annulata	VEEV [4]	Alpha	24	N
	JEV[4]	Flavi	21, 24	Y
	ZIKV[5]	Flavi	19, 21, 24, 27, 31	N

	WNV[5]	Flavi	19, 21, 24, 27, 31	Y
Ae. punctor	JEV[4]	Flavi	21	Y

Japanese encephalitis virus (JEV), Rift Valley fever (RVF), Venezuelan equine encephalitis virus (VEEV), Ross River Virus (RRV), Usutu virus (USUV), Zika virus (ZIKV).[1](Mackenzie-Impoinvil *et al.,* 2015), [2](Blagrove *et al.,* 2016), [3](Lumley *et al.,* 2018), [4](Chapman, 2017), [5](Blagrove, Personal Communication, 2019), [6](Hernández-Triana *et al.,* 2018).

The majority of vector competence studies with UK mosquito species have been conducted using *Ae. detritus*. This species has previously been shown to be a competent laboratory vector for JEV (Mackenzie-Impoinvil *et al.,* 2015). During this experiment, two different incubation temperatures were compared: 23°C and 28°C. A higher percentage mortality (65%) was observed at the higher temperature although the greatest transmission rate (25%) was also recorded at this higher temperature (Mackenzie-Impoinvil *et al.,* 2015). In the UK, it is unlikely that *Ae. detritus* would encounter sustained periods of time (up to 21 days) with an average temperature of either 23°C or 28°C. Infection of *Ae. detritus* with DENV appears to have a significant impact on the survival of this mosquito species and further studies into the impact of this virus on *Ae. detritus* need to be conducted (Blagrove *et al.,* 2016). *Ae. detritus* was not found to be a competent vector, under laboratory conditions, for the alphaviruses CHIKV or VEEV (Blagrove *et al.,* 2016, Chapman, 2017). However, it was found to be a competent vector of RRV, also an alphavirus (Chapman, 2017). A single study exploring the vector competence of *Ae. detritus* from mainland Europe has been conducted. A French population of *Ae. detritus* were shown to be competent vectors of RVF under laboratory conditions (Moutailler *et al.,* 2008).

Given that climate has been shown to influence the distribution and seasonality of arbovirus outbreaks (Alto *et al.,* 2018), it is especially important that the links between temperature and vector competence of UK mosquito species is further explored. However, in all of the above experiments, mosquitoes were incubated at constant temperatures (Mackenzie-Impoinvil *et al.,* 2015, Blagrove *et al.,* 2016, Chapman, 2017, Lumley *et al.,* 2018, Hernández-Triana *et al.,* 2018). As previously

discussed, daily temperature fluctuations have been shown to have an impact on vector competence in other laboratory studies. The impact of daily temperature fluctuations has not been determined for any UK mosquito species.

5.3 Aim and Objectives

This chapter describes a series of laboratory experiments with the aim of determining the impact of daily temperature fluctuations on the vector competence of *Ae. detritus* to Japanese encephalitis virus.

The objectives of this chapter were to:

1. Infect a wild population of *Ae. detritus* with JEV incubated under two different temperature conditions:
    a. Constant temperature,
    b. Daily temperature cycling.
2. Compare the infection rates in *Ae. detritus* to a control mosquito species, *Cx. quinquefasciatus*.

5.4 Materials and Methods

5.4.1 Mosquito Collections and Rearing

Aedes detritus

Ae. detritus larvae were collected from pools on the salt marsh at Little Neston (53°16'34.44"N; 3° 4'1.07"W). Larvae were then returned to the insectaries at Leahurst, University of Liverpool. Larvae were reared under ambient conditions in trays along with water collected from the breeding site. No additional food source was provided. Pupae were removed from the trays and placed into pots inside 30 x 30 x 30 cm BugDorm cages (BugDorm, Taichung, Taiwan). Adults were maintained at ambient conditions and were provided with 10% sugar solution on cotton wool. Adults were sugar starved for 24 hours prior to being used for infection studies.

Culex quinquefasciatus

Culex quinquefasciatus (*Cx. quinquefasciatus*) (MUHEZA strain) were supplied from the Liverpool Insect Testing Establishment (LITE). They were reared following the LITE SOPs in an insectary maintained at 28^{o}C with at 12:12 light:dark photoperiod at a humidity of 70%. Adults were sugar starved for 24 hours prior to use in infection studies.

5.4.2 Japanese Encephalitis Virus

JEV was supplied from the culture stocks at the Institute of Infection and Global Health at the University of Liverpool (strain CNS138-11). The stock titre of the virus was 1x10^{6} pfu/ml. However, once the virus was thawed and aliquoted it was not possible to determine final virus titre as the equipment required is currently not in place within the CL3 laboratory in LSTM.

5.4.3 Temperature and Humidity Conditions

Two different temperature conditions were applied to the incubators that the mosquitoes were kept in. Field collected temperature data (see Chapter 2) from the same location that the *Ae. detritus* larvae were collected from was used to define the

incubator temperatures. First, a constant temperature of 19°C (mean July temperature). Second, a daily temperature cycle of the average hourly temperatures throughout July was used to define the temperatures of a cycling incubator (Figure 5.1).

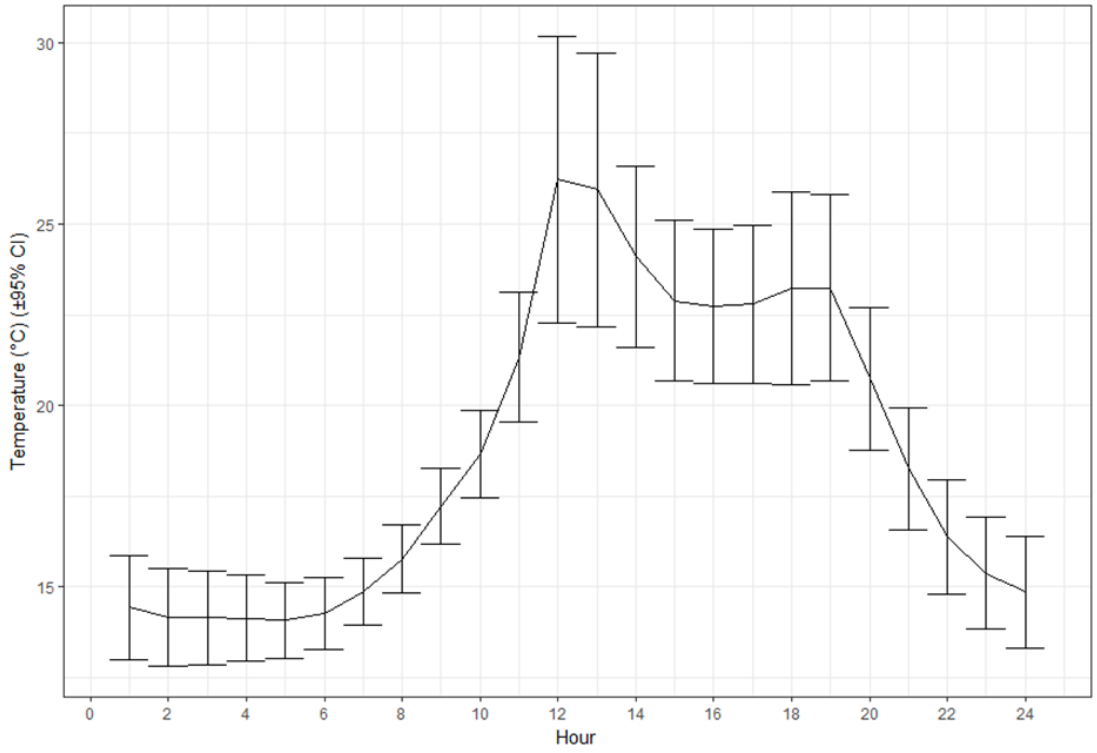

Figure 5.1: Average hourly temperature (±95% CI) throughout July. Data was gathered from a weather station located within Ness Gardens.

The model of the incubator available (Sanyo MIR 153) does not allow a continuous cycle of temperatures over a 24 hour period to be programmed and so a three-step temperature profile which resembled the cycle shown in figure 5.1 was programmed (Figure 5.2).

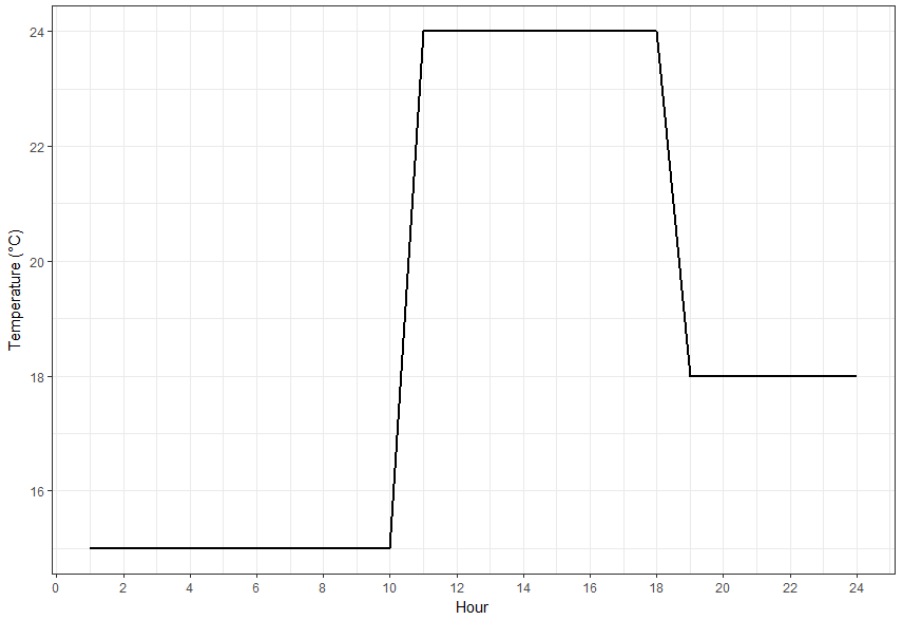

Figure 5.2: Temperature regiment to be followed over 24 hours by the cycling incubator for *Ae. detritus.*

For the control mosquitoes (*Cx. quinquefasciatus*) a constant temperature of 28°C as used in previous infection studies (Mackenzie-Impoinvil *et al.,* 2015) was set. This is also the temperature of the insectary that these mosquitoes are reared in. The temperatures of the cycling incubator are shown in Figure 5.3 and averaged 27.4°C.

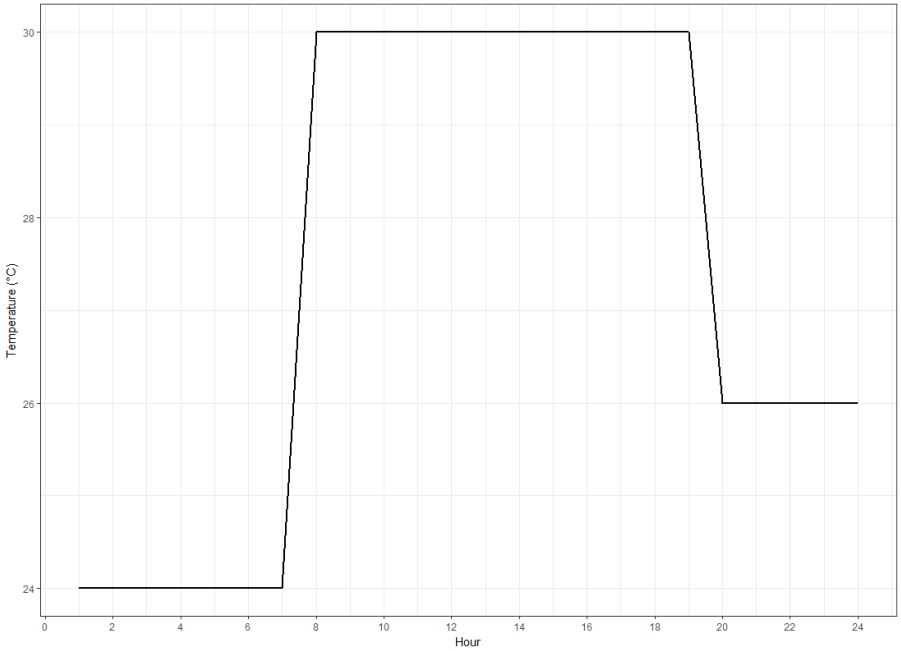

Figure 5.3: Temperature regiment to be followed over 24 hours by the cycling incubator for *Cx. quinquefasciatus.*

In order to maintain a constant relative humidity within the incubator, trays of water were kept at the bottom of each incubator. These were replenished as required to prevent them drying out.

TinyTag data loggers were placed within the incubators to record temperature and humidity data through the duration of the experiments. These data loggers are able to accurately measure temperature to ±0.4^{o}C and relative humidity to ±3.0%

5.4.4 Virus Infections

Mosquitoes for the infection studies were transferred into 1 L Dispo-safe containers (The Microbiological Supply Company, Luton, UK) with a mesh covering (maximum of 30 per container). Infectious blood meals (heparinised human blood, NHS transfusion service, Speke) were provided to the mosquitoes inside a glove box using a Hemotek membrane feeding system (Hemotek Ltd, Blackburn, UK). The mosquitoes were left to feed under low light conditions for two hours. To encourage feeding low levels of CO_2 were provided at the beginning of the feed. Post feeding, fed and unfed mosquitoes were separated. Fed mosquitoes were placed into incubators at either the constant or cycling temperature regime. Mosquitoes were kept with 10% sugar solution which was changed daily. Mosquito survival was recorded daily.

5.4.5 Saliva Extractions

Mosquitoes were immobilised with triethylamine (FlyNap, Carolina Biological Supply Company, Burlington, North Carolina, USA). The saliva was extracted by inserting the proboscis of the mosquito into a capillary tube containing mineral oil for one hour. The saliva and the mosquito body were then stored in separate 1.5 ml tubes containing 200 µl TRIzol Reagent (Thermo Fisher Scientific).

5.4.6 RNA extraction

RNA was extracted using TRIzol Reagent. Firstly, samples were homogenised in 200 µL of TRIzol using metal beads and a homogeniser for one minute. A plate spinner was used to spin the sample at 1000 rpm for five seconds after which the samples

were left to incubate at room temperature for five minutes. 40 µl of chloroform was added and the samples inverted for 15 seconds to mix. Following a two-minute room temperature incubation, samples were centrifuged for 15 minutes at 4°C. The aqueous layer was removed to a new 1.5 ml Eppendorf tube and 100 µl of isopropanol added. The tube was inverted ten times to mix and the samples left to incubate at room temperature for ten minutes. Samples were then centrifuged at 16400 rcf for five minutes at 4°C. The supernatant was removed leaving a gel-like pellet of RNA at the bottom of the tube. 1 ml 75% of ethanol was added, the sample vortexed briefly then centrifuged at 16400 rcf for five minutes at 4°C. Again, 1 ml of ethanol was added and the sample centrifuged at 16400 rcf for five minutes at 4°C. Samples were then centrifuged briefly, and the remaining ethanol removed using a 20 µl pipette. Pellets were left to air dry for 5-10 minutes before RNase-free water was added 20 µl for mosquito bodies and 10 µl for saliva samples. The RNA was dissolved by pipetting the sample up and down. Finally, the total volume was split into two new Eppendorf tubes before being stored at -80°C.

After extraction, the concentration of RNA in the sample was determined using the Thermoscientific NanoDrop and 1 µl of sample. RNase free water was used to blank the machine before the RNA concentration in ng/µl, the 260/280 ratio and the 260/230 ratio were recorded. All extracted RNA was stored at -80°C until use.

5.4.7 Primers and Probe

The primers and probe used for the qPCR reaction are shown in Table 5.2.

Table 5.2: Primers and probe used for the qPCR.

Virus	Sense Primer	Probe	Antisense Primer	Reference
JEV	5' ATCTGGTGYG GYAGTCTCA3'	5'Fam-CGGAACGCGA WCCAGGGCAA-Tamara3'	5'CGCGTAGATG TTCTCAGCCC3'	(Lindahl *et al.,* 2013, Pyke *et al.,* 2004)

5.4.8 qRT-PCR

The QuantiFast one-step RT-PCR kit (Qiagen) was used to detect the presence of viral RNA. Each reaction was made up to a total volume of 25 µl and contained: QuantiFast Probe RT-PCR Master Mix, probe (150 nM), primers (400 nM), Quantifast RT mix, 50x ROX Dye Solution, 2 µl of template RNA and RNase-free water.

The thermocycler conditions were: ten minutes at 50^{o}C, five minutes at 95^{o}C followed by 40 cycles of 95^{o}C for ten seconds and 70^{o}C for 30 seconds. The Agilent Mx3005P real time cycler was used for all reactions (Agilent Technologies, Santa Clara, California).

Both a positive control (RNA from neat virus) and negative control (nuclease-free water) were run alongside each qPCR.

5.4.9 Data Analysis

A sample was considered to be positive for JEV if it had a CT value of ≤40. If a mosquito body tested positive for viral RNA this was considered an infected individual. Only saliva samples positive for viral RNA were considered to be mosquitoes with a transmissible JEV infection. Generalised linear models (GLM) with a binomial error distribution were used to determine whether day post infection and/or temperature condition had statistically significant effects on infection and transmission rates. All analyses were performed in R, version 3.3.1 (R Core Team 2016).

5.5 Results

A total of 1,740 mosquitoes were brought into the laboratory and offered a virus infected blood meal over four repeat experiments. After feeding, the fed and unfed mosquitoes were separated with the total number of fed mosquitoes split between the two temperature conditions (Table 5.3). A total of 840 *Ae. detritus* were offered an infectious blood meal of which 429 (51.1%) fed successfully. The 429 *Ae. detritus* were split between the two temperature conditions with 214 incubated at a constant temperature of 19^{o}C and 215 incubated at the cycling temperatures. A total of 900 *Cx. quinquefasciatus* were offered an infectious blood meal of which 583 (64.8%) fed successfully. The blood fed *Cx. quinquefasciatus* were split between the two temperature conditions with a total of 283 incubated at 28^{o}C and 300 under cycling temperature conditions. A chi-squared test was performed to determine whether there was a difference in feeding rate between the two species (Table 5.3). The results of this test ($\chi^2 = 33.5$, $p<0.001$) indicate that there was a significant difference in the feed rates of the two species.

Table 5.3: Rates of blood feeding from both *Ae. detritus* and *Cx. quinquefasciatus.*

Mosquito species	Temperature conditions	Total, *n*	Blood fed mosquitoes, *n* (%)
Ae. detritus	Constant (19^{o}C)	420	214 (51.0)
	Cycling	420	215 (51.2)
Cx. quinquefasciatus	Constant (28^{o}C)	450	283 (62.9)
	Cycling	450	300 (66.7)

NB these are pooled totals across four repeat experiments

In total, 627 mosquitoes from both species survived their respective incubation periods (Table 5.4). A total of 225 *Ae. detritus* survived: 121 at the constant temperature and 104 at the cycling temperatures. A total of 367 *Cx. quinquefasciatus*

survived: 186 at the constant temperature and 181 at the cycling temperatures. A chi-squared test was performed to determine whether there was a difference in the survival rates between *Ae. detritus* and *Cx. quinquefasciatus* (Table 5.4). The results of this test ($\chi^2 = 87.4$, $p<0.001$) indicate that there was a significant difference in the survival rates between the two species.

Table 5.4: Survival rate of both *Ae. detritus* and *Cx. quinquefasciatus.*

Mosquito species	Temperature conditions	Initial Total, *n*	Surviving incubation period, *n* (%)
Ae. detritus	Constant (19°C)	214	121 (56.5)
	Cycling	215	104 (48.4)
Cx. quinquefasciatus	Constant (28°C)	283	186 (65.7)
	Cycling	300	181 (60.3)

NB these are pooled totals across several repeat experiments

5.5.1 Vector Competence of *Ae. detritus* to JEV

Constant temperature

A total of 20 *Ae. detritus* were collected seven days post infection, 35 on days 14 and 21 and 31 on the final timepoint of 28 days (Table 5.5).

Table 5.5: Summary of the infection and transmission rate of *Ae. detritus* to JEV at 19°C.

Days post infection	Total, *n*	Body positive	Saliva positive	% infected	% transmitting	Transmission rate (%)
s7	20	14	0	70.0	0	0
14	35	12	4	34.3	11.4	33.3
21	35	18	8	51.4	22.9	44.4
28	31	11	2	35.5	6.5	18.2

Daily Temperature Cycling

A total of 20 *Ae. detritus* were collected seven days post infection, 30 on days 14 and 21 and 24 on the final timepoint of 28 days (Table 5.6).

Table 5.6: Summary of the infection and transmission rate of *Ae. detritus* to JEV at cycling temperatures.

Days post infection	Total, *n*	Body positive	Saliva positive	% infected	% transmitting	Transmission rate (%)
7	20	11	1	55.0	5.0	9.1
14	30	14	3	46.0	10.0	21.4
21	30	20	10	66.7	33.3	50.0
28	24	8	2	33.3	8.3	25.0

For the *Ae. detritus* that were incubated at the constant temperature, the greatest proportion of mosquitoes infected with JEV was on day seven post infection (70%) (Figure 5.4). There is a drop in the proportion infected in this group after this time point. In contrast to this, the greatest proportion of *Ae. detritus* infected under the cycling temperature conditions is seen on day 21 post infection (66.7%) (Figure 5.4). Across both temperature groups, the proportion of *Ae. detritus* with transmittable JEV infections reaches its peak at day 21 post infection (Figure 5.4). Peaks of 44.4% and 50% are reached in the constant and cycling temperature groups respectively. There is a drop in the transmission rate for both groups of *Ae. detritus* at 28 days post infection.

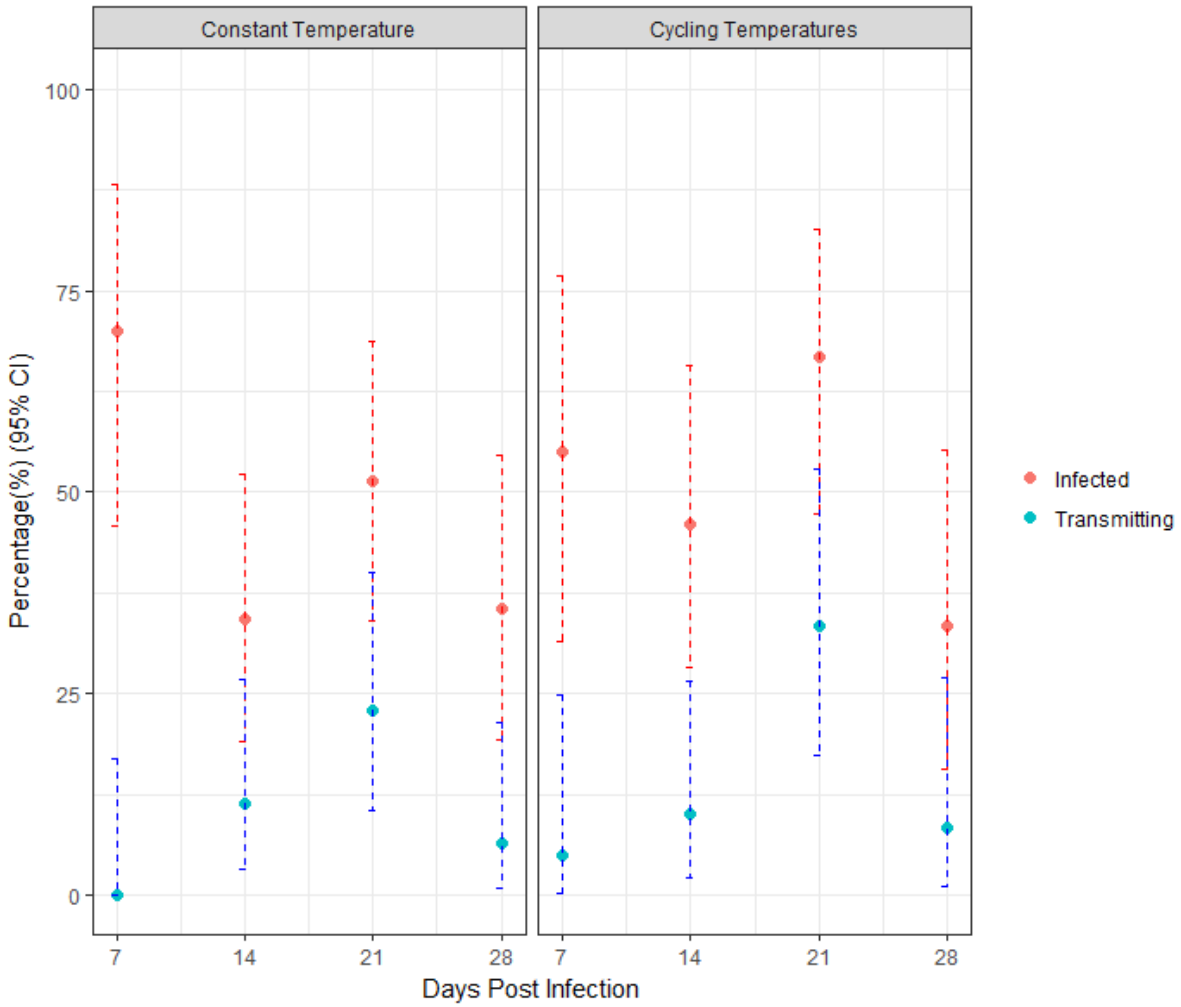

Figure 5.4: Percentage (±95% CI) of *Ae. detritus* infected with and transmitting JEV over the four different time points under the two different temperature conditions.

Fitting GLMs to the results showed that day post infection had a significant impact on the number of body positive mosquitoes. Significantly fewer mosquito bodies were infected on days 14 and 28 compared to day seven ($P<0.05$). There was no significant difference between the number of body positive mosquitoes on day 21. Additionally, there was no significant difference between the two temperature conditions on the number of body positive mosquitoes ($P=0.463$). The GLM also indicated that day post infection had a significant effect on the number of saliva positive mosquitoes. Significantly more mosquitoes were saliva positive on day 21 compared to day seven ($P<0.01$). Once again, there was no significant effect of temperature conditions on the number of saliva positive mosquitoes ($P=0.365$).

5.5.2 *Ae. detritus* Temperature and Humidity Recording

A TinyTag was used to measure the temperature and humidity in both the constant and cycling incubators that *Ae. detritus* were kept in (Figure 5.5, Figure 5.6 and Figure 5.7).

Constant

The incubator set to run at a constant temperature of 19°C failed to maintain this temperature throughout the duration of the experiment (Figure 5.5). The temperature recorded by the TinyTag was consistently lower than the pre-programmed temperature. The average temperature of this incubator was 18.8°C.

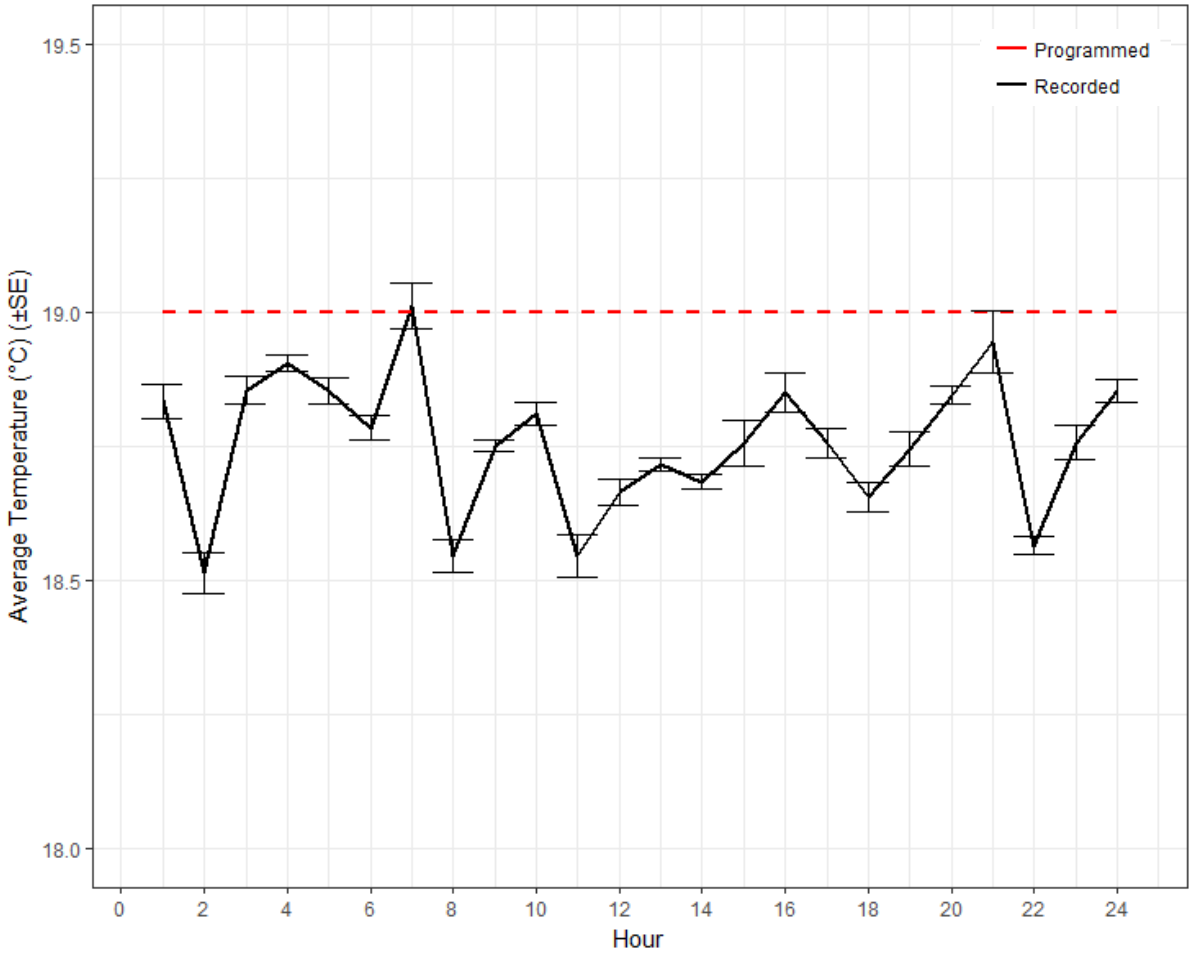

Figure 5.5: The average temperature ±SE recorded in the constant temperature incubator is shown in black with the pre-programmed temperature shown in red.

The humidity within the incubator kept at 19°C remained relatively constant at an average of 76.3% throughout the duration of the experiment.

Cycling

It is clear that the incubator failed to follow the pre-programmed temperature regime under cycling temperature conditions (Figure 5.6). At times, there was a discrepancy of up to 4.5°C between the programmed and recorded temperatures (Figure 5.6). At steps one (15°C) and three (18°C), temperature was consistently recorded higher than expected. At step two (24°C), temperature was consistently lower than expected. The average temperature from the pre-programmed conditions was 19°C. The average temperature from the actual temperatures recorded was 20.1°C.

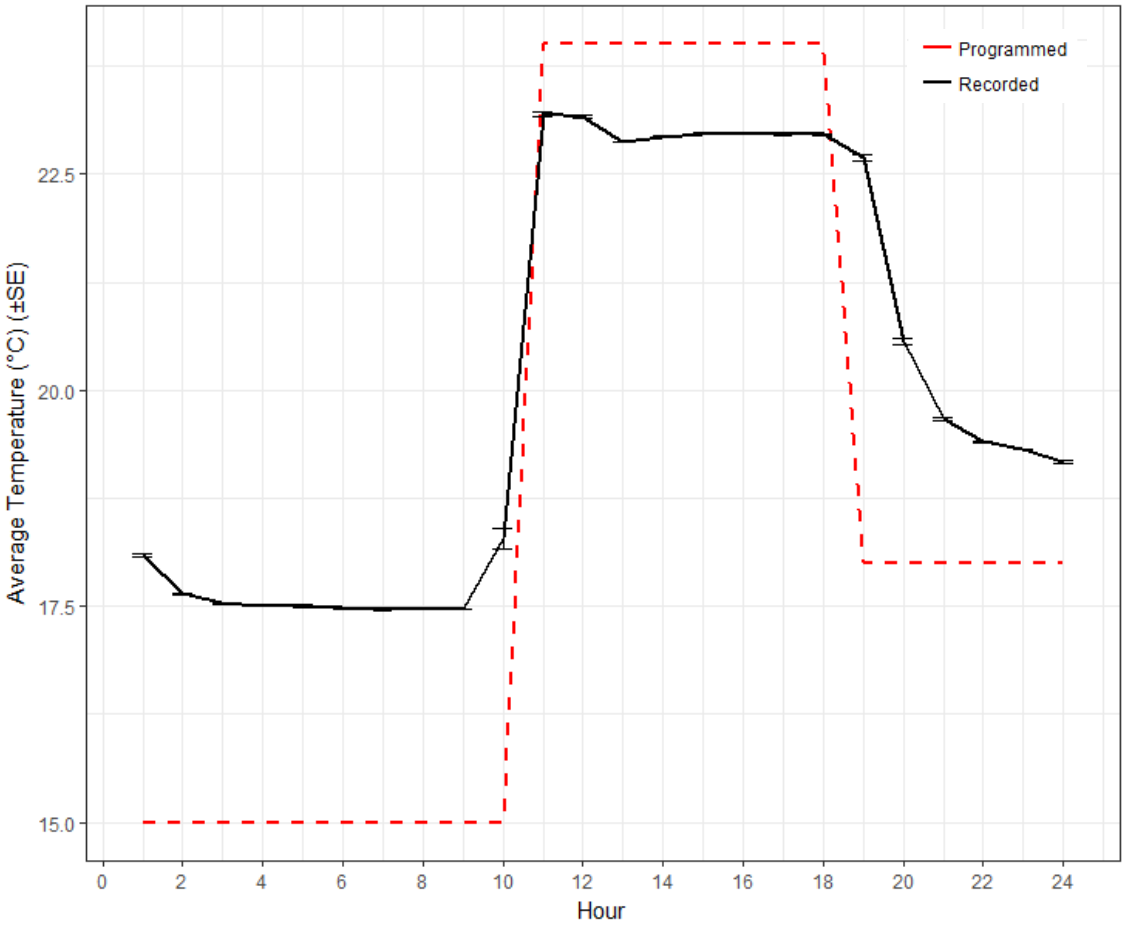

Figure 5.6: The average temperature ±SE recorded in the cycling incubator is shown in black with the pre-programmed temperature profile show in red. Step 1: 15°C, Step 2: 24°C, Step 3: 18°C.

The incubator programmed to cycle the temperature also failed to maintain a constant humidity reading over a 24 hour period (Figure 5.7). At the higher temperatures, the humidity is successfully maintained at >70% however, as the incubator changes temperature and at the lower temperatures the humidity drops to approximately 35%.

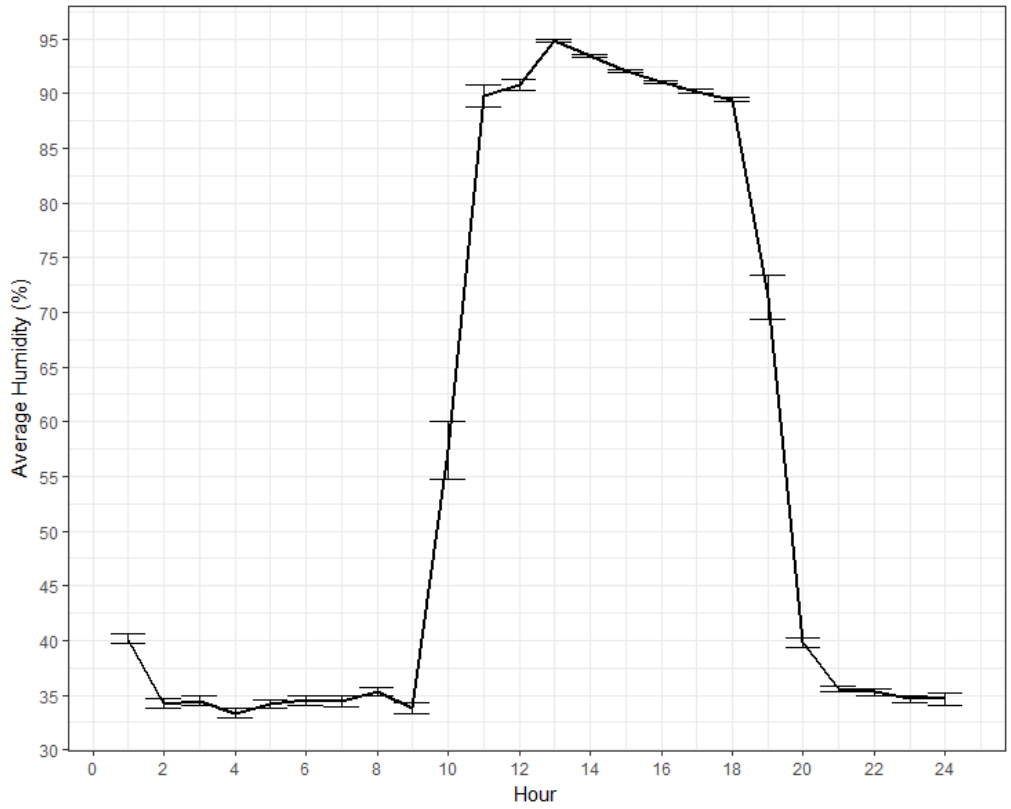

Figure 5.7: The average humidity ±SE recorded in the cycling incubator.

5.5.3 Vector Competence of *Cx. quinquefasciatus* for JEV

Constant temperature

A total of 45 *Cx. quinquefasciatus* were collected from the seven day timepoint, 50 on day 14, 48 on day 21 and 43 on the final timepoint of 28 days (Table 5.7).

Table 5.7: Summary of the infection and transmission rate of *Cx. quinquefasciatus* to JEV at 28°C.

Days post infection	Total, *n*	Body positive	Saliva positive	% infected	% transmitting	Transmission rate (%)
7	45	11	0	24.0	0	0
14	50	31	9	62.0	18.0	29.0
21	48	27	13	56.3	27.1	48.1
28	43	16	8	37.2	18.6	50

Cycling temperature

A total of 45 *Cx. quinquefasciatus* were collected from the seven day timepoint, 55 on day 14, 47 on day 21 and 34 on the final timepoint of 28 days (Table 5.8).

Table 5.8: Summary of the infection and transmission rate of *Cx. quinquefasciatus* to JEV at cycling temperatures.

Days post infection	Total, *n*	Body positive	Saliva positive	% infected	% transmitting	Transmission rate (%)
7	45	18	0	40.0	0	0
14	55	21	6	38.0	10.9	28.6
21	47	28	15	59.6	31.9	53.6
28	34	14	9	41.2	26.5	64.3

For the *Cx. quinquefasciatus* that were incubated at the constant temperature, the greatest proportion of mosquitoes infected with JEV is seen 14 days post infection (62%) (Figure 5.8). After this time point, the proportion of *Cx. quinquefasciatus* infected with JEV declined. In contrast to this, the greatest proportion of *Cx. quinquefasciatus* infected under the cycling temperature conditions was seen on day 21 post infection (59.6%) (Figure 5.8). Across both temperature groups, the proportion of *Cx. quinquefasciatus* with transmissible JEV infections increases to reach a peak at day 21 post infection (Figure 5.8). Peaks of 27.1% and 31.9% are reached in the constant and cycling temperature groups respectively. There is a drop in the transmission rate for both groups of *Cx. quinquefasciatus* at 28 days post infection.

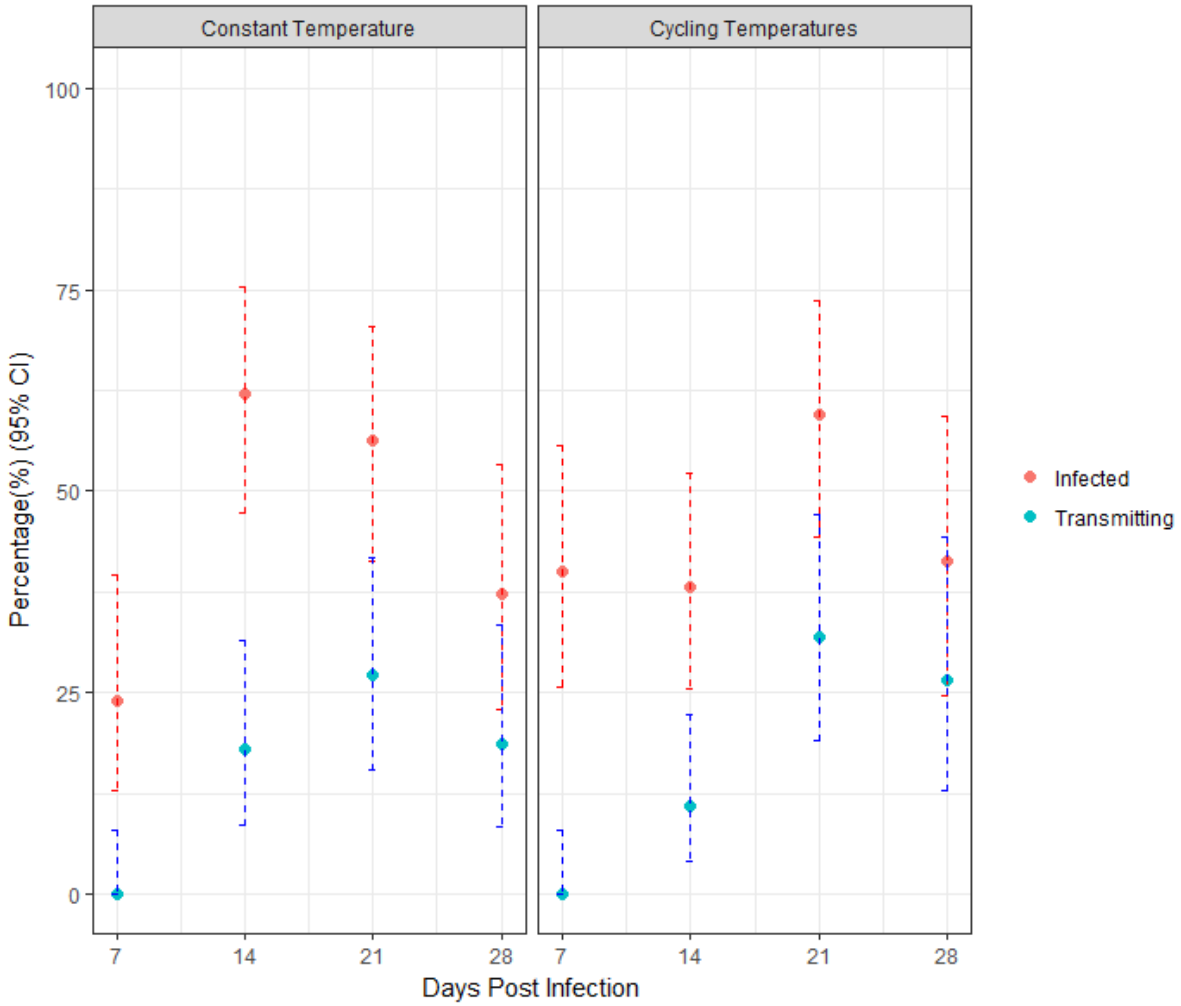

Figure 5.8: Percentage (±95% CI) of *Cx. quinquefasciatus* infected with and transmitting JEV over the four different time points under the two different temperature conditions.

The result of the GLM indicate that day post infection had a significant impact on the number of body positive mosquitoes. Significantly more mosquito bodies were infected on days 14 and 21 compared to day seven ($P<0.05$). There was no significant difference between the number of body positive mosquitoes on day 28. Additionally, there was no significant difference between the two temperature conditions on the overall number of body positive mosquitoes ($P=0.798$). Days post infection did not impact the number of saliva positive mosquitoes. Once again, there was no significant effect of temperature conditions on the number of saliva positive mosquitoes ($P=0.817$).

5.5.4 *Cx. quinquefasciatus* Temperature and Humidity Recording

A TinyTag was used to measure the temperature and humidity in both the constant and cycling incubators (Figure 5.9, Figure 5.10 and Figure 5.11).

Constant

The temperature data collected from the TinyTag shows that despite being programmed to run at 28^{o}C the incubator was consistently running below this temperature at an average of 27.8^{o}C (Figure 5.9).

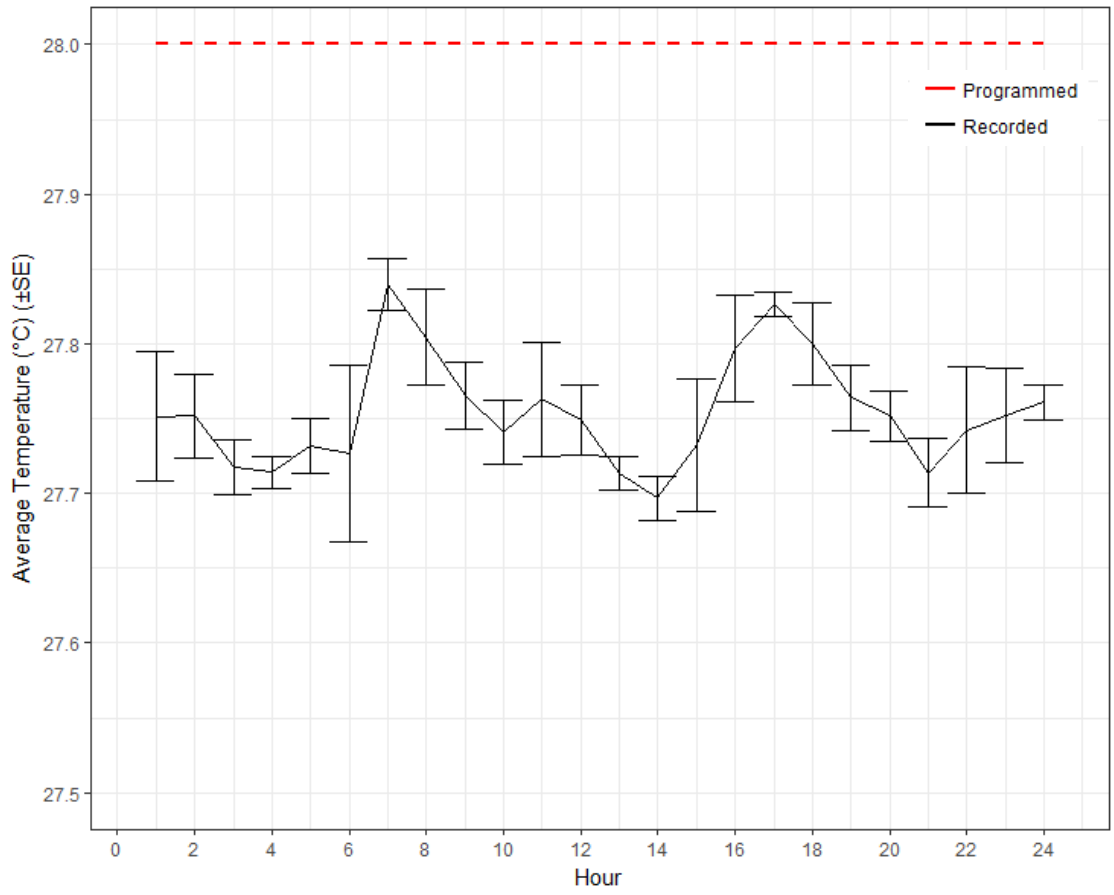

Figure 5.9: The average temperature ±SE recorded in the constant temperature incubator is shown in black with the pre-programmed temperature shown in red.

The humidity within the incubator at a constant temperature remained relatively constant at an average of 84.4% throughout the duration of the experiment.

Cycling

The incubator set to the cycling temperature regime failed to follow the pre-programmed temperature conditions (Figure 5.10). Temperature data generated from the TinyTag shows that the incubator was consistently running at a lower temperature over the first two programmed temperature steps (24°C and 30°C). However, the maximum discrepancy between the programmed and recorded temperatures was only 1.1°C.

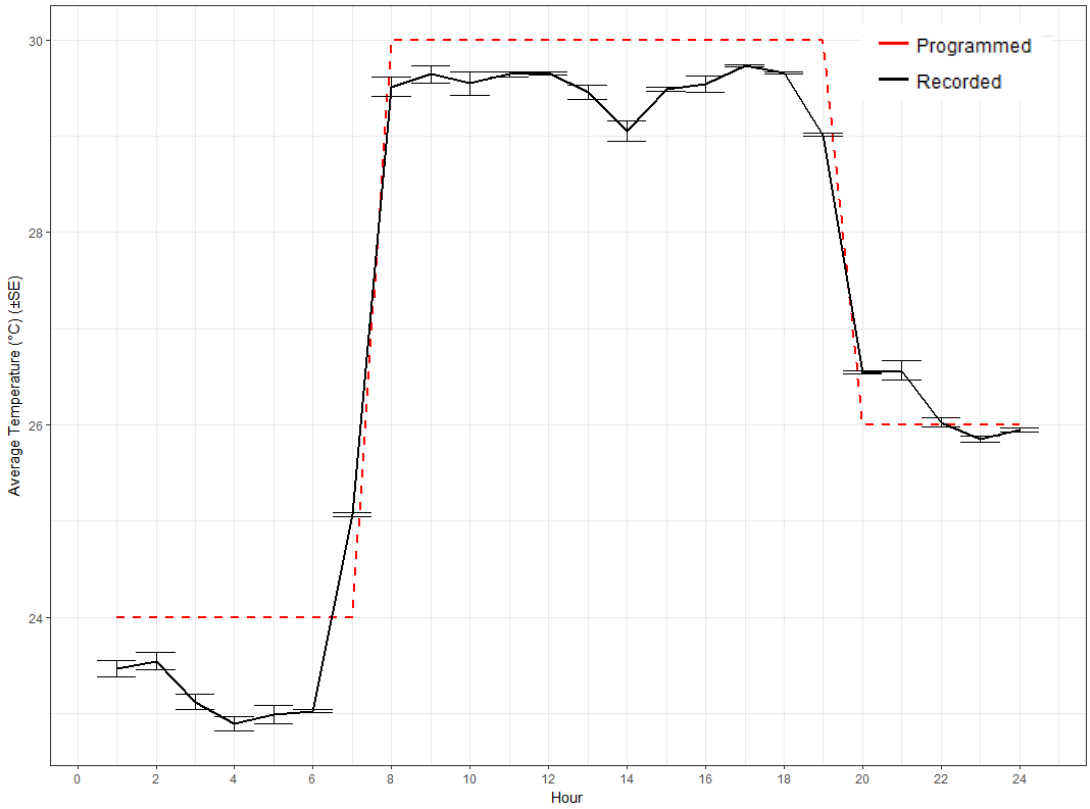

Figure 5.10: The average temperature ±SE recorded in the cycling incubator is shown in black with the pre-programmed temperature profile show in red. Step 1: 24°C, Step 2: 30°C, Step 3: 26°C.

Once again, the incubators failed to maintain a constant humidity under the cycling temperature regime (Figure 5.11). At the highest temperature setting of 28°C, the humidity remains constant at approximately 90%. However, when the temperature changes, the humidity drops and remains at a constant level (approximately 45%).

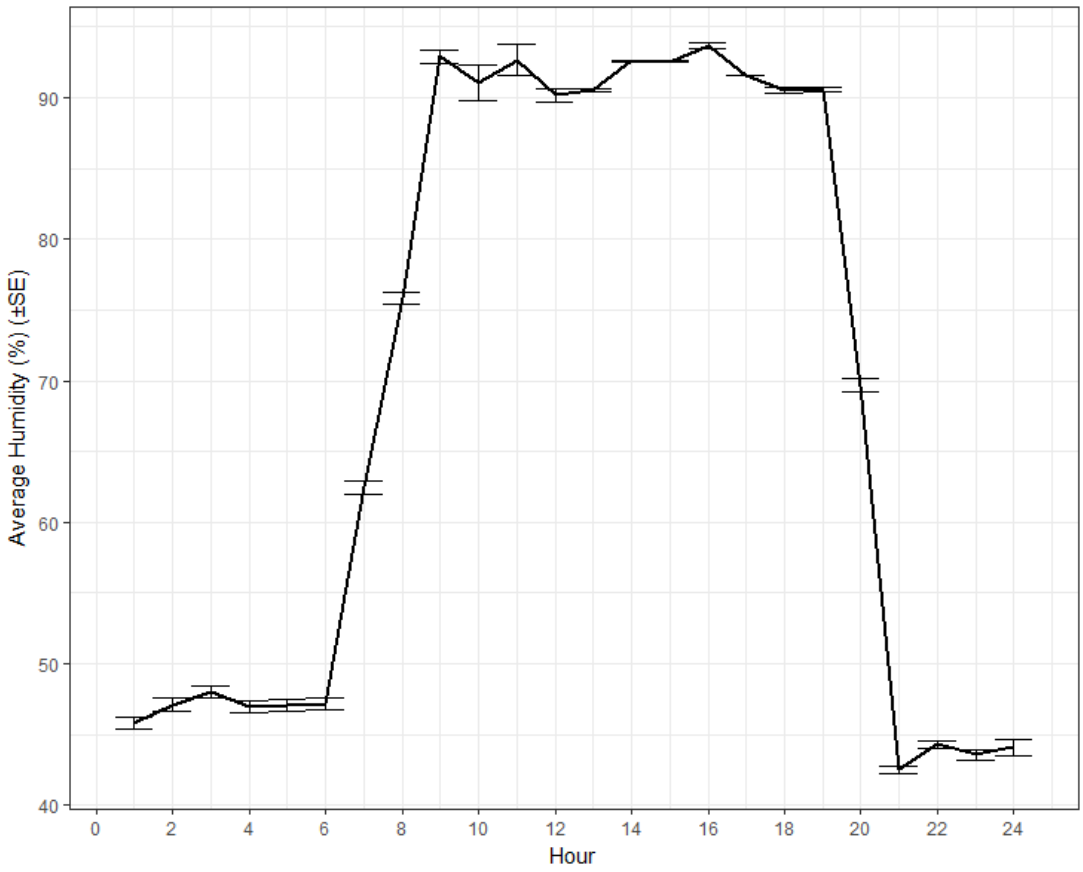

Figure 5.11: The average humidity ±SE recorded in the cycling incubator.

5.6 Discussion

The present study was the first to explore the impact of daily temperature fluctuations on the vector competence of UK mosquito species. *Ae. detritus* has previously been shown to be a competent vector of JEV (Mackenzie-Impoinvil *et al.,* 2015). However, a lower average temperature, 19^{o}C, was used in the present study. This temperature is based on real time field data from the same location the *Ae. detritus* larvae were collected. As previously shown in Chapter 2 of this thesis, *Ae. detritus* has a long period of activity along the Dee estuary and while mosquito numbers decline during the summer months, *Ae. detritus* is still active. The temperature in July was selected as this is the warmest time of the year. Given that the EIP of a virus is temperature dependent, it was necessary to select a temperature during which the virus would successfully replicate within the mosquito. Whilst the abundance of *Ae. detritus* is lower during the summer months; it is during these warmer periods when this mosquito species would most likely be a vector of an arbovirus should they arrive in the UK.

In the present study, a qPCR was used to determine whether or not a mosquito sample was positive for JEV (CT value of ≤40). Ideally, a plaque assay using Vero cells would have been used to determine whether or not mosquito and saliva samples were virus positive (detailed method described in Mackenzie-Impoinvil *et al.,* 2015). Within the scope of the present study, this methodology was not available. However, additional work could have been conducted to further inform the findings of this study. A standard curve for the qPCR could have been produced using a 10-fold serial dilution of the JEV virus stocks to provide a semi-quantitative estimate of virus concentration.

In this laboratory-based study, *Ae. detritus* was found to be a competent vector of JEV. Saliva positive mosquitoes were obtained from both *Ae. detritus* incubated at a constant temperature and cycling temperatures. GLMs demonstrated that the number of days post infection was significant for both the number of mosquitoes infected with and able to transmit the virus. As the number of days post infection increased the number of mosquitoes infected with JEV decreased. In contrast to this, significantly more mosquitoes had a JEV RNA positive saliva three weeks post

infection compared to one week. Comparison between the number of *Ae. detritus* with transmittable JEV infections across temperature regimes showed no significant difference between the two groups. A previous study reports JEV transmission rates in *Ae. detritus* of 67% at 23^{o}C and 33% at 28^{o}C at 21 days post infection (Mackenzie-Impoinvil *et al.,* 2015). In the present study transmission rates of 44% at the constant temperature and 50% at the cycling temperature 21 days post infection were found.

Despite no significant different between the two temperature regimes being detected, the results from the *Ae. detritus* incubated under cycling temperature warrant further deliberation. Although not significant, the transmission rate was 6% higher in the *Ae. detritus* incubated under daily temperature cycles compared to the constant temperature group. The temperature was programmed to cycle around an average of 19^{o}C with a range of temperatures between 15-24^{o}C representing a typical day in July in northwest England. During this temperature cycle, nine hours per day were spent at 24^{o}C. A transmission rate of 50% was detected at 21 days post infection under these conditions. Mackenzie-Impoinvil et al. (2015) report a transmission rate of 67% in *Ae. detritus* incubated at a constant temperature of 23^{o}C 21 days post infection. Further work should explore whether there is a number of hours above a threshold temperature that can increase vector competence of a given mosquito/virus pairing. Within a UK setting, where the average daily temperature may not be sufficient for high infection rates, this is especially important. During the warmer summer months, high daytime temperatures might mean that sufficient time is spent above a threshold temperature to have an impact on virus EIP and therefore vector competence.

The control mosquito group, *Cx. quinquefasciatus,* were also found to be competent laboratory vectors of JEV with transmission rates of 50% at the constant temperature and 64.3% at the cycling temperatures 28 days post infection. However, there was no significant effect of temperature condition found for this mosquito group. In a previous study, a transmission rate of 70% 21 days post infection was found for *Cx. quinquefasciatus* 21 days post infection incubated at a constant temperature of 28^{o}C (Mackenzie-Impoinvil *et al.,* 2015).

JEV is not currently transmitted within Europe, although RNA of the virus has been detected in *Cx. pipiens* mosquitoes collected in Italy (Ravanini *et al.,* 2012). Additionally, JEV RNA has been reported in wild bird populations in Italy (Platonov *et al.,* 2012). If JEV were to be introduced into the UK, it is likely to arrive via: the importation of infected mosquitoes, animal trading or through infected migratory birds (Mackenzie-Impoinvil *et al.,* 2015). There are no studies reporting JEV positive bird populations within the UK. Host preference studies report *Ae. detritus* feeding on humans, other mammals and birds indicating that this species may act as a bridge vector for several arboviruses (Service, 1969, Service, 1971a). The ecology of *Ae. detritus,* along with the results of the present, and other vector competence studies, demonstrate the need to further studies to explore the potential of this species to act as a vector of JEV and other arbovirsues should they arrive in the UK.

Low rates of *Ae. detritus* taking a blood meal were encountered in the present study (51.1%), a rate which is similar to other vector competence studies using this species (Mackenzie-Impoinvil *et al.,* 2015). Given that the adults of *Ae. detritus* were reared from wild-collected larvae, these mosquitoes were not adapted to taking artificial blood meals unlike colony controls, *Cx. quinquefasciatus* (64.8% feeding rate). Further to this, low *Ae. detritus* survival rates were also encountered: 26.8% survived their respective incubation period. Previous studies report mortality rates of *Ae. detritus* between 12.4% and 65% (Mackenzie-Impoinvil *et al.,* 2015, Blagrove *et al.,* 2016). Given the low feeding and survival rates, several repeats (under the same conditions) were required in order to obtain sufficient sample sizes across the four timepoints for each temperature condition. Future work could further explore *Ae. detritus* feeding and survival rates within the CL3 environment to improve study design and aid reproducibility.

The incubators available within the CL3 laboratory limited the design of the present study. It was only possible to programme a three step temperature regime within a 24 hour period. Additionally, the incubators did not maintain the cycling profiles they were programmed to follow. This meant that the cycling temperature regime that *Ae. detritus* were incubated did not match precisely the field collected temperature data. Nonetheless, the average temperature only differed by +1.1^{o}C and the

experiment did produce data that allows comparison of constant vs. variable temperatures. Previous studies have demonstrated that small changes in temperature can impact on the transmission and infection rates of mosquitoes (Kilpatrick *et al.,* 2008). Whilst the true effect of the +1.1^{o}C temperature change is not fully understood, it is feasible that the rates of virus infection and transmission in this group of mosquitoes is higher than it might have been if the correct temperature profile had been followed.

The present study was designed to explore the impact of daily temperature cycling on the vector competence of UK mosquito species. Temperature is known to be an important driver of JEV transmission under field conditions (Impoinvil *et al.,* 2011, Lin *et al.,* 2017). JEV was selected as the virus of choice for the present study due to the fact that it has previously been demonstrated to be transmitted in the laboratory by *Ae. detritus* and that it is possible to get good rates of virus infection in this species (Mackenzie-Impoinvil *et al.,* 2015). A common limitation with studies of the vector competence of wild-collected mosquitoes is sample size. Despite collecting 840 mosquitoes, this study was also unable to achieve sample sizes that would provide a statistically robust study of vector competence in *Ae. detritus*. Going forward, it would be prudent to explore the impact of temperature cycling on other arboviruses which pose a risk to the UK, such as WNV or USUV.

5.7 Conclusions

Field collected *Ae. detritus* have been shown to be competent laboratory vectors of JEV when incubated at 19^{o}C. Further to this, these mosquitoes were also competent vectors under daily temperature fluctuations (range 17.5-23^{o}C). Although there was no significant difference in the infection rates between the mosquitoes exposed to these two different temperature regimes, further studies are warranted to ascertain the full extent of daily temperature cycles on the vector competence of UK mosquito species.

Chapter 6 - General Discussion

This thesis aimed to explore entomological aspects of the risk posed to the United Kingdom by mosquito-borne pathogens. First, the seasonality and distribution of mosquitoes along an 18 km sampling area was determined during a three year longitudinal study. Two, highly predictable, peaks in mosquito abundance were recorded annually. These peaks in abundance appear to be unaffected by seasonal changes in meteorological variables. The larval ecology of one of the most abundant mosquito species, *Ae. detritus,* was further explored over the three years with a drone used to produce maps of the unique habitat in which this species breeds. Two peaks in the larval abundance of this species were observed with temperature and humidity found to be significant drivers of abundance. Maps to observe how the dynamics of the breeding sites of *Ae. detritus* change over the course of the year were successfully produced. Considering the potential need to future mosquito control programmes within the UK, an assessment of the suitability of a range of mosquito traps for sampling the human biting mosquito population was also made. The Mosquito Magnet and HDT were found to be suitable traps for sampling the anthropophilic mosquito population. Finally, the impact of daily temperature fluctuations on the vector competence of *Ae. detritus* was assessed. Whilst no significant effect of temperature was observed during the studies, *Ae. detritus* was found to be a competent laboratory vector for JEV at a constant temperature of 19^{o}C and a cycling temperature with an average of 19^{o}C.

In a recent publication by PHE (Medlock *et al.,* 2018), the following areas were highlighted as the main risks posed to the UK from mosquito borne diseases:

i) The spread of invasive *Aedes* species in Europe;

ii) Detection and spread of West Nile virus and its *Culex* vectors;

iii) Other areas of concern: malaria and other arboviruses.

The findings presented in this thesis are discussed in the context of these three risk factors.

6.1 The Spread of Invasive *Aedes* Species in Europe

As previously discussed in the opening chapter of this thesis, there are ever growing concerns about the spread of invasive *Aedes* mosquitoes across Europe. This risk includes the UK, and the eggs of the invasive species *Ae. albopictus* have already been detected in southern England on several occasions (Medlock *et al.,* 2017, Medlock *et al.,* 2018). Although these currently appear to be isolated incidences, there are concerns that this species may become established within the UK in the future. *Ae. albopictus* is a prolific nuisance biter as well as a competent vector of several viruses (Baldacchino *et al.,* 2015). Climate models have been produced suggesting that the UK climate is sufficient for several months of *Ae. albopictus* activity especially as the UK climate continues to change (Caminade *et al.,* 2012, Metelmann *et al.,* 2019). Other species, such as *Ae. aegypti* and *Ae. japonicus* are also of concern. *Ae. japonicus* is now reported as established in northeast Italy and has the potential to continue spreading in Europe (Montarsi *et al.,* 2019). *Ae. aegypti* is less likely to become established within the UK due to climate restrictions, with predictions that an annual average temperature of 15^{o}C needs to maintained for this species to survive (Medlock *et al.,* 2018).

Given the current risk to the UK from invasive species, it is especially important that we have as full and detailed a knowledge of local mosquito population as possible. Chapter 2 of this thesis describes a three year longitudinal study of the abundance of mosquitoes along the Dee estuary. No invasive mosquito species were detected during the three year sampling period. Two clear peaks in mosquito abundance were observed annually. The first peak in abundance occurs in May/June with large numbers of *An. claviger* being collected at this time. During the summer months, mosquito numbers declined but there was a greater diversity of mosquito species caught during this time. The second peak in abundance occurs in September/October and consisted predominantly of *Ae. detritus.* Despite modelling meteorological variables, it appears that mosquito abundance is highly correlated with the week of the year. This is evident over the three-year study period where peaks in mosquito abundance occur within a few weeks of each other annually. Going forward, the results of the three-year longitudinal study can be used to provide reliable annual

estimates of mosquito abundance along the Dee estuary. These estimates could prove valuable should mosquito-borne pathogens, particularly arboviruses, reach the UK. National bodies, such as PHE, and local authorities will be able to predict mosquito abundance and therefore plan targeted vector control strategies and health warnings to local communities as necessary. It is important that future studies should continue to monitor the abundance of mosquito species within the UK to ensure that the seasonal dynamics of local mosquito populations are fully understood. Further to this, we will be able to detect invasive mosquito species should they arrive in the UK.

The use of drones to successfully map mosquito breeding sites within the UK was demonstrated in Chapter 3 of this thesis. Drones are becoming increasingly popular within ecological research and offer unique data collection opportunities (Carrasco-Escobar *et al.,* 2019). One of the most significant advantages of drone use is the ability to collect and process imagery in real time and overcome the problems of cloud cover that affect remotely sensed imagery produced by satellites (Carrasco-Escobar *et al.,* 2019). Following the detection of *Ae. albopictus* in southern England over the past three years, a huge amount of time, effort and resources have been put into place to monitor for the spread of this mosquito species around the initial detection site (Medlock *et al.,* 2017, Medlock *et al.,* 2018). Given that this is a container breeding mosquito species, successful identification of breeding sites can be extremely difficult (Baldacchino *et al.,* 2015). It is possible that drones could be used to assist with planning of control initiatives. For example, drones could be used to identify high risk breeding sites. In Switzerland, investigations following a series of nuisance biting complaints, found a large population of *Ae. japonicus* breeding in flower vases in cemeteries (Schaffner *et al.,* 2009). If such a scenario occurred within the UK, drones could be used to produce maps to predict how a mosquito population might spread and to identify which breeding sites would require surveillance and or treatment.

Within mainland Europe, traps are commonly used in surveillance programmes to monitor for invasive mosquito species and to determine how these species are spreading (ECDC, 2017, Suter *et al.,* 2016, Canali *et al.,* 2017). For *Ae. albopictus*

surveillance in Europe, traps either target host-seeking or gravid females (Baldacchino *et al.,* 2015). The incursions of *Ae. albopictus* in the UK have been detected through the use of oviposition traps (Medlock *et al.,* 2017). It is important that we are aware of the different methods of sampling mosquito populations and understand which traps are best suited to sampling the population of interest. Chapter 4 reviews some of the most commonly used mosquito traps and determines the ability of these traps to sample the human biting mosquito population along the Dee estuary. No significant difference between the catches from HLC and BG sentinel or Mosquito Magnet traps were identified. To this end, it was concluded that the Mosquito Magnet offers an alternative to HLC for sampling human biting mosquitoes. Since this study was conducted, new mosquito traps have become available such as the BG Mosquitaire which has been specifically designed to sample *Aedes* populations. Further work should be conducted to assess the suitability of these new designs to sample UK mosquito populations. No significant difference between the catches of the Mosquito Magnet and a novel trap design, the HDT, were found. Mosquito Magnets can be expensive to purchase whilst the HDT is a simple and cheap trap design (Hawkes *et al.,* 2017). Within the tropics, the HDT was found to collect significantly more mosquitoes when compared to HLC (Hawkes *et al.,* 2017, Abong'o *et al.,* 2018). In the present study this trap design also collected high numbers of *Aedes* mosquitoes. Experiments should be conducted to assess the ability of the HDT to sample *Ae. albopictus* mosquitoes to determine whether or not this trap could be further developed for use as a future control or surveillance method.

Within Europe, *Ae. albopictus* has been responsible for outbreaks of CHIKV and DENV (Calba *et al.,* 2017, Venturi *et al.,* 2017, Akiner *et al.,* 2016). There is the potential that *Ae. albopictus* mosquitoes infected with an arbovirus could reach the UK. Whilst the temperature within the UK is likely to be a limiting factor for ongoing arbovirus transmission, it is important that we have a detailed understanding of the ability of our native mosquito species to transmit these arboviruses (Medlock *et al.,* 2006, Medlock *et al.,* 2018). To this end, serval studies have been conducted to assess the vector competence status of British mosquitoes to a range of different arboviruses including both CHIKV and DENV. The vector competence of *Ae. detritus* has

previously been assessed for both CHIKV and DENV (Blagrove *et al.,* 2016). The results of these experiments concluded that this mosquito species was not a competent laboratory vector for either virus (Blagrove *et al.,* 2016). Further vector competence studies should be conducted to determine if there are other species of mosquito present in the UK that may have the potential to act as a disease vectors in the future. Given the models predictions that there could be several months of *Ae. albopictus* activity sustained within the UK, it is especially important that future work evaluates the ability of viruses, such as CHIKV and DENV to be transmitted during this period of activity (Medlock *et al.,* 2018).

6.2 Detection and Spread of West Nile Virus and its *Culex* Vectors

There was a large increase in the number of WNV cases reported in Europe in 2018 with WNV spreading rapidly during the transmission season. In 2018, the first human WNV cases were notified at the end of June (ECDC, 2018e). At the time of writing, the latest 2019 reports (4th July 2019) show zero cases of WNV in Europe (Figure 6.1) (ECDC, 2019).

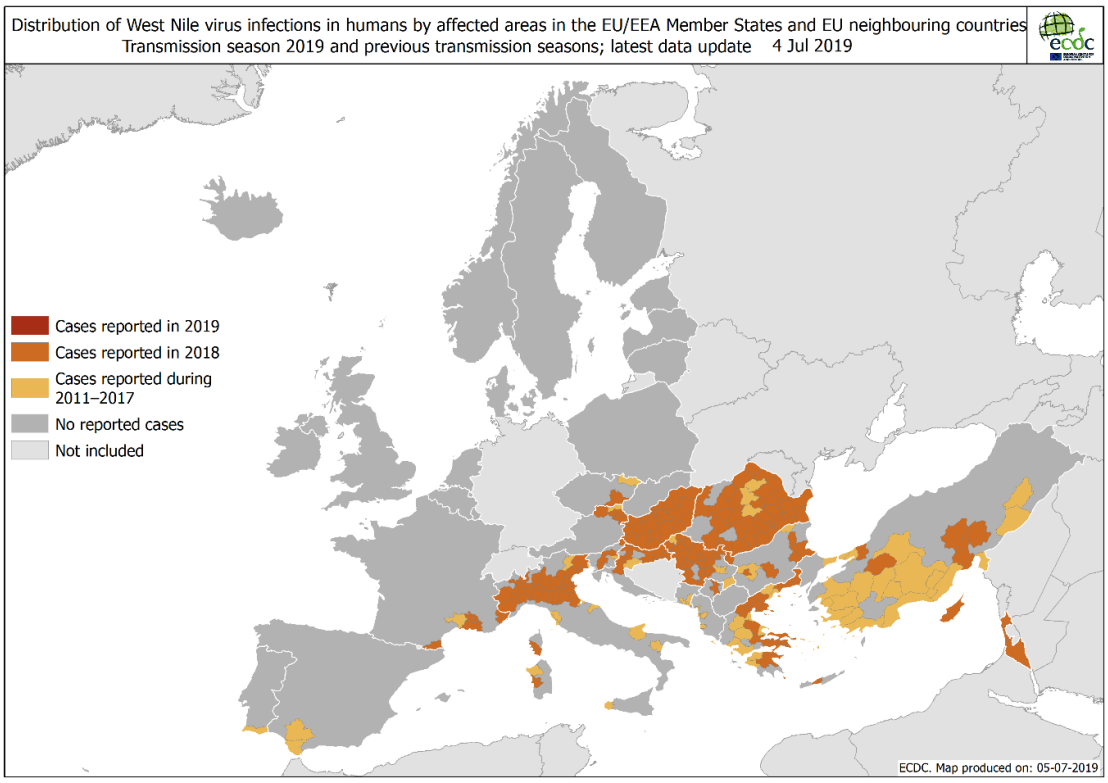

Figure 6.1: Distribution of WNV in Europe as of 4th July 2019 (ECDC, 2019).

Given the spread of WNV in Europe, there are concerns that this virus has the potential to reach the UK (Medlock *et al.,* 2018). There have previously been reports of WNV antibodies circulating in migratory bird populations within the UK (Buckley *et al.,* 2003). However, no further evidence for this has been found (Phipps *et al.,* 2008).

During the three year longitudinal study described in Chapter 2, low numbers of *Culex* mosquitoes were collected. This may have been a result of the trapping methods used (Mosquito Magnet) rather than 'true' low numbers. For example, previous studies in this same sampling area have collected large numbers of *Cx. pipiens* when using CDC light and BG Sentinel traps (Clarkson and Setzkorn, 2011).

The WNV vector *Cx. modestus* is now established in southern England with no further accounts of this species being found further north (Golding *et al.,* 2012). There was no evidence of *Cx. modestus* circulating along the Dee estuary. However, this mosquito species is spreading; potentially facilitated by bird populations (Medlock *et al.,* 2018) (Figure 6.2). Ongoing efforts to monitor the spread of this mosquito

species in the UK are important to ensure that the extent of its distribution is fully understood.

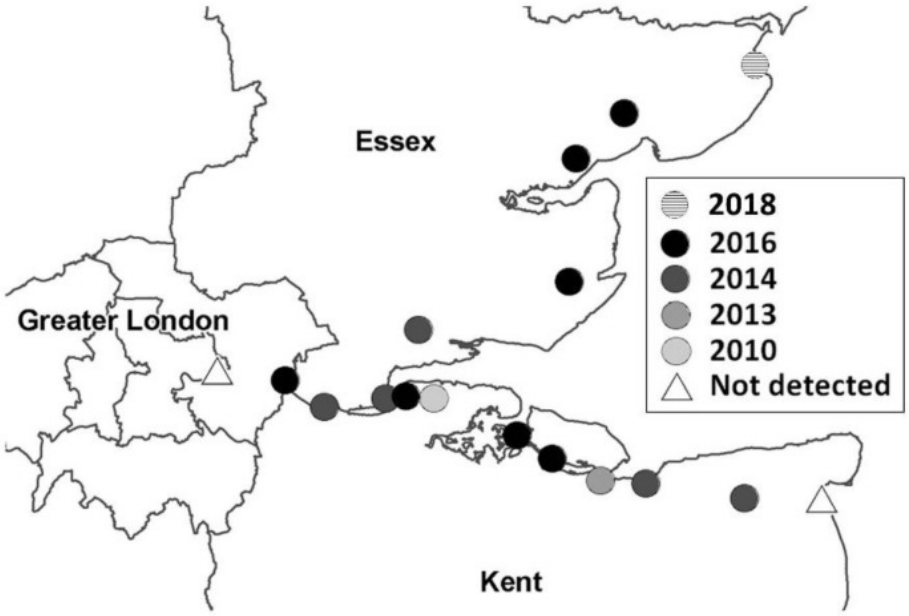

Figure 6.2: The distribution of *Cx. modestus* in southern England (unadapted from (Medlock *et al.,* 2018)).

Given that *Cx. modestus* is a vector of WNV in other parts of Europe, there are concerns that this species could act as a WNV vector should this virus reach the United Kingdom (Golding *et al.,* 2012). A review by (Medlock *et al.,* 2005) discusses the mosquito species that may have a role to play in future WNV transmission in the UK. Of the species discussed in the review, three were found in high numbers during the longitudinal study described in Chapter 2: *Cs. annulata, Ae. cantans/annulipes* and *Ae. detritus. Ae. cantans* is a known vector of WNV in Europe and this species has been highlighted as a potential bridge vector should WNV establish in the UK (Labuda *et al.,* 1974, Medlock *et al.,* 2005). Both *Cs. annulata* and *Ae. detritus* feed on humans and birds and are therefore also implicated as potential bridge vectors of WNV (Medlock *et al.,* 2005). It is important that the ecology of mosquito species that may be involved in future WNV transmission is understood. The seasonality of *Cs. annulata, Ae. cantans/annulipes* and *Ae. detritus* along the Dee estuary is described in Chapter 2. *Cs. annulata* has the longest period of activity with specimens being

caught throughout the three year sampling period. A single peak in the abundance of *Ae. cantans/annulipes* is recorded each year in June. *Ae. detritus* was the most abundant species collected during the longitudinal study with two clear peaks in the abundance of this species occurring annually. Chapter 3, further explores the ecology of the immature stages of *Ae. detritus* species. Two clear seasonal peaks in the abundance of the immature stages of this species were also identified. The peaks in larval abundance occur approximately six weeks prior to the peaks in adult density with temperature appearing to be an important driver in the abundance of both the immature and adult stages of *Ae. detritus*. Although caught in low numbers during the longitudinal study, *Cx. pipiens s.l.* is also circulating along the Dee estuary. This species is an important WNV in mainland Europe (Medlock *et al.,* 2005).

As discussed in Chapter 5, it is important that we have a detailed understanding of the ability of UK mosquito species to transmit arboviruses such as WNV. This is especially important for mosquito species such as *Ae. detritus* and *Cs. annulata* which have been identified as potential WNV bridge vectors. One such study, reports that *Ae. detritus* is a competent laboratory vector of WNV at 21^{o}C (Blagrove *et al.,* 2016). Additionally, *Cs. annulata* has been shown to be a competent laboratory vector of WNV (Blagrove, Personal Communication, 2019). Temperature plays an important role in facilitating a mosquitoes ability to transmit viruses. Studies to explore impact of daily temperature fluctuations on the vector competence of UK species to WNV should be conducted.

The tools developed in Chapter 3 to map the breeding sites of *Ae. detritus* could be used to map the breeding sites of several other mosquito species, including *Cx. modestus*. The north Kent marshes, where *Cx. modestus* is established, would be a suitable habitat to trial the use of drones to map breeding sites of this species (Golding *et al.,* 2012). Should WNV enter the UK, and *Cx. modestus* become implicated in virus transmission, drones could be used to produce high quality, real time habitat maps to help inform vector control programmes or, to produce maps of environmental factors associated with disease risk.

Chapter 4 reviewed several of the methods available to sample mosquito populations, as well as assessing the suitability of a novel mosquito design for

sampling human biting mosquitoes in the UK. Should WNV reach the UK, an understanding of the tools available for surveillance and control of mosquito populations will be especially important. The catches from commonly used mosquito traps were compared to HLC to determine whether traps could be used as an alternative method for sampling the human biting mosquito population. Ethical constraints of performing such experiments in areas with localised WNV transmission are likely to impede research. Therefore, conducting these experiments prior to WNV establishment in the UK is important. Results have demonstrated that there are a range of tools available to researchers to collect human biting mosquitoes in the UK. However, it should be noted that low numbers of *Culex* mosquitoes were caught in the present study, despite knowledge that these mosquitoes are present in the sampling area (Clarkson and Setzkorn, 2011). Other methods may be better suited to sampling the *Culex* populations. For example, bird-baited traps have successfully been used to collect *Culex* mosquitoes in Southern England (Brugman *et al.,* 2018b). There is also evidence that the height that a trap is placed off the ground can increase the catch of *Culex* mosquitoes given that these mosquitoes feed on birds in the tree canopy (Marshall, 1938). Hutchinson *et al.* (2007) reports increased catches of UK populations of *Culex pipiens s.l.* in CDC light traps that are located 5m off the ground compared to 1m and 2.5m. Further work should be carried out to determine the best methods of trapping *Culex* mosquitoes within the UK. However, Golding et al. (2012) did collect high numbers of *Cx. modestus* using Mosquito Magnets traps in Kent. Although low numbers of *Culex* mosquitoes were collected in the present study, high numbers of *Ae. detritus* were collected. As mentioned, *Ae. detritus* has been listed as a potential WNV bridge vector (Medlock *et al.,* 2005). Should WNV arrive in the UK, it is important that methods of controlling *Ae. detritus* are understood in the event that this species becomes involved with virus transmission.

6.3 Other Areas of Concern: Malaria and Other Arboviruses

6.3.1 Malaria

Despite historical transmission of malaria, there is currently no transmission of human malaria in the UK. However, since 1987 there have been almost 40,000 cases

reported in the UK from travellers returning from disease endemic areas (Smith *et al.,* 2008, Behrens *et al.,* 2008, Moyo *et al.,* 2018). There are currently six UK mosquito species that have the potential to transmit malaria parasites: *An. atroparvus, An. algeriensis, An. claviger, An. daciae, An. messeae* and *An. plumbeus* (Lindsay *et al.,* 2010a). Of these six species, two were collected during the three year longitudinal study (*An. claviger* and *An. plumbeus*). *An. claviger* was the second most abundant species over the three years with over 4,000 adults collected. *An. plumbeus* was caught in much lower numbers (*n* = 94). Since 1990, there have been several instances of locally transmitted malaria in European countries including Germany, the Netherlands and Greece (Piperaki and Daikos, 2016, Hertig, 2019). These cases have arisen due to local mosquito populations acquiring malaria infections from infected travellers (Piperaki and Daikos, 2016). Models have predicted that both *Plasmodium falciparum* (*P. falciparum*) and *Plasmodium vivax* (*P. vivax*) could be transmitted in the UK in the future under different climate change scenarios (Lindsay *et al.,* 2010a, Caminade *et al.,* 2014, Kuhn *et al.,* 2003). The risk of *P. falciparum* transmission is considered extremely low with one model predicting transmission in the UK by 2080 (Caminade *et al.,* 2014). It is therefore important that the UK remains alert to the potential threat of malaria transmission.

As discussed within Chapter 4, there are several different method of sampling mosquito populations. The Mosquito Magnet collects high numbers of *An. claviger* and this trap would therefore be a suitable option for monitoring populations of this mosquito species. Unlike several of the mosquito-borne arboviruses, there is an effective treatment for malaria. It is therefore possible that localised control of a malaria outbreak in the UK could focus on treatment of infected individuals rather than on vector control alone.

Larval source management (LSM) is one of the mainstays of malaria vector control in disease endemic countries (Tusting *et al.,* 2013). The use of drones to identify water bodies for LSM has previously been demonstrated by Hardy et al. (2017) and was also demonstrated in Chapter 3 of this thesis. Whilst identification of mosquito breeding sites using drones might be possible for some UK anopheline species, this would not be an option for other species such as *An. plumbeus* which breeds in treeholes.

6.3.2 Other Arboviruses

There is the potential for the transmission of other arboviruses within the UK. For example, USUV, SINV and TAHV are all transmitted within northern Europe (Rijks *et al.,* 2016, Hubálek, 2008, Jöst *et al.,* 2011). The mosquito vectors for these arboviruses are also present in the UK (Medlock *et al.,* 2007).

Several species of mosquito collected during the three year longitudinal study described in Chapter 2 have the potential to be involved with the transmission of the aforementioned arboviruses within the UK. A detailed picture of the seasonal activities of mosquitoes with the potential to act as vectors of arboviruses is important. This will allow us to fully comprehend the risk posed by certain mosquito species. For example, *Ae. cantans,* which acts as a bridge vector of SINV in mainland Europe, has a single peak in abundance lasting approximately one month between May and June (Medlock *et al.,* 2005). In contrast to this, *Cs. annulata*, which is involved with TAHV transmission, is active for several months with no clear peaks in abundance (Lundström, 1999). Control efforts against *Ae. cantans* are therefore likely to last up to a month whilst control would need to continue all year round for *Cs. annulata. Cx. pipiens,* which acts a vector of several arboviruses within mainland Europe, was also collected during the longitudinal study although further work is required to get a detailed picture of the seasonality of this species along the Dee estuary.

As reviewed in Chapter 5, the vector competence status of several UK mosquitoes species to a range of different arboviruses has been determined. However, only three studies have focused on the arboviruses that pose the greatest risk to the UK. Two of these studies assessed WNV and have previously been discussed. The third study explored the vector competence status of *Cx. pipiens* to USUV virus (Hernández-Triana *et al.,* 2018). Only one mosquito was found to be positive for the virus and further studies, with a larger sample size, are required to fully understand the potential for UK *Cx. pipiens* to act as vectors for USUV (Hernández-Triana *et al.,* 2018). There is a lack of published research exploring the vector competence status of British mosquitoes to both SINV and TAHV. Future vector competence studies should target these viruses to ensure that we understand the risk they pose to the UK.

6.4 Conclusions

Increased incidences of disease transmission, coupled with the spread of invasive mosquito species in Europe, means that improving our knowledge of the risk posed to the United Kingdom from these factors is especially important. This thesis aimed to provide insight into these risks through the collection of data relating to the mosquito populations in the North West of England. Ongoing research into the ecology of native mosquitoes is especially important. This research allows the potential role these mosquitoes could play in future disease transmission to be assessed. Additionally, surveillance for invasive mosquito species and research into the methods of controlling these species needs to continue.

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Appendix A
Ethical Approval

LSTM
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Aislinn Currie-Jordan
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Thursday, 24 March 2016

Dear Miss Currie-Jordan,

Re. Research Protocol (16-012) 'Quantitative analysis of human attraction and feeding behaviour of UK mosquito species'

Thank you for your correspondence of the 21 March 2016 responding to the action points raised by the committee. The protocol now has formal ethical approval from the Chair of LSTM Research Ethics Committee.

The approval is for a fixed period of three years and will therefore expire on 23 March 2019. The committee may suspend or withdraw ethical approval at any time if appropriate.

Approval is conditional upon:

- Notification of all amendments to the protocol for approval before implementation.
- Notification of when the project actually starts.
- Provision of an annual update to the Committee. Failure to do so could result in suspension of the study without further notice.
- Reporting of all severe unexpected Adverse Events to the Committee
- Reporting of new information relevant to patient safety to the Committee
- Provision of Data Monitoring Committee reports (if applicable) to the Committee

Failure to comply with these requirements will result in withdrawal of approval and may result in disciplinary action. The Committee would also like to receive copies of the final report once the study is completed.

Yours sincerely,

Dr Angela Obasi
Chair
Research Ethics Committee

Appendix B

Template R Script for Running a GLMM

```
#load required packages

require(bbmle)

require(coefplot)

require(glmmADMB)

require(coda)

require(lme4)

require(reshape)

require(coefplot2)

require(vcd)


# load data

data<-read.csv("data.csv")


#set year and location as factors

yearf<-factor(year)

locf<-factor(location)


# model1: Poisson distribution #

model1<-glmmadmb(catch~(1|yearf)+(1|locf)+temp+rain+tide+humid,data=data,
zeroInflation=FALSE, family="poisson")

summary(model1)


#model 2: negative binomial
```

```
model2<-glmmadmb(catch~(1|yearf)+(1|locf)+temp+rain+humid+tide,data=data,
zeroInflation=FALSE, family="nbinom")

summary(model2)


#compare the AIC values

AIC(model1,model2)

#proceed with the model with the better AIC value


#deletion testing using the drop1 function to identify non-significant predictors of
catch#

model2.2<-drop1(model2,test="Chisq")

modelmm2.2


#update the model to remove non-significant predictors of catch. E.g. rain

model3<-update(model2.2,.~.-rain)

modelmm3


#Continue to run the drop1 and update functions until all the non-significant
predictors have been removed


#Run the final minimally adequate model#

modelf<-glmmadmb(catch~(1|yearf)+(1|locf)+temp+humid,data=data,
zeroInflation=FALSE, family="nbinom")

summary(modelf)
```

Appendix C

How to Build an Orthomosaic using Agisoft Photoscan

1. Open Agisoft and select the *Workflow* menu.
2. Import the images from your drone flight of interest.
3. From the *Workflow* menu select *Align Photos*.
4. Set the parameters to the following:
    a. Accuracy: *High,*
    b. Pair -preselection: *Reference,*
    c. Constrain features by mask: *Disabled,*
    d. Key point limit: *40,000,*
    e. Tie point limit: *10,000.*
5. Select OK to run.
6. Save the newly aligned set of images.
7. From the *Workflow* menu select *Build DEM* to build your Digital Elevation Model.
8. Use the default parameters set within the programme.
9. Select OK to run.
10. From the *Workflow* menu select *Build Orthomosaic.*
11. Make sure the *Surface* is set as *DEM*
12. Select OK to run.
13. From the *File* menu, select the *Export Orthomosaic* option and export your orthomosaic as a *TIFF File.*

Full detailed instruction are available from (Agisoft, 2017).

Appendix D

Image Classification in QGIS

1. Open QGIS and rasta file (orthomosaic) of interest.
2. If not already installed install the two required plugins:
    a. *SEMI AUTOMATIC CLASSIFICATION*
    b. *Value tool*
3. Select the *Preprocessing* tab from the *SEMI AUTOMATIC CLASS* plugin. Select split rasta bands. Save and run.
4. Three new images will be loaded into Layers. You can deselect band four as this does not contain anything.
5. From the *Preprocessing* tab select *Band Set*. Add the three rasta files from Step 4 to *Band set definition*. Save and run.
6. Your new rasta layer *band_set* will have appeared in the *Layers* panel.
7. Right click on the new *bant_set* rasta and select *Properties.* Switch to the *Style* tab and change the *Band Rendering* of the *Red, Green* and *Blue* bands to be in the correct order. Click *Apply*.
8. Select the *SCI input* tab from the *SCP Dock* panel
9. Under the *Training input* tab select *Create a new training input* and save this file.
10. From the *SEMI AUTOMATIC CLASSIFICATION* plugin select the *Create and ROI polygon* function to enable you to draw polygons around different areas of land cover (right click to close the polygon).
    a. Set different *macroclasses* for the different types of landcover. E.g. water, or vegetation.
11. After each new polygon, select the *Save temporary ROI to training output button* to save the polygon. If this polygon represents a new *Macroclass* define it as such at this stage.
12. Continue to add in the various polygons until you have defined the obviously different regions on the rasta file.
13. Use the *Preview* function in the tool bar to assess how well the image classification is going.

14. Once happy with the previews, select the *Classification algorithm* tab. Set the *Algorithm to Maximum Likelihood.*
15. Select the *Classification output* tab and run select run.
16. To access the *Classification report,* select the *Postprocessing* tab from the *SEMI AUTOMATIC CLASS* plugin. Select *Classification report* and run.