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RESEARCH ARTICLE

# An update on the ecology, seasonality and distribution of *Culex modestus* in England

*A.G.C. Vaux*[1,2,*], *A.J. Abbott*[1,3], *C.J. Johnston*[1], *F.M. Hawkes*[3], *R.J. Hopkins*[3], *B. Cull*[1], *G. Gibson*[3], *R.A. Cheke*[3], *A. Callaghan*[2] *and J.M. Medlock*[1,4]

[1]Medical Entomology & Zoonoses Ecology (MEZE), Centre for Climate and Health Security, UK Health Security Agency, Porton Down, Salisbury SP4 0JG, United Kingdom; [2]School of Biological Sciences, University of Reading, Whiteknights, Reading, RG6 6EX, United Kingdom; [3]Natural Resources Institute, University of Greenwich at Medway, Central Avenue, Chatham Maritime, Kent ME4 4TB, United Kingdom; [4]Health Protection Research Unit in Environmental Change and Health, UKHSA, Porton Down, United Kingdom; *alexander.vaux@ukhsa.gov.uk

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## Abstract

*Culex* (*Barraudius*) *modestus* is an important vector of West Nile virus (WNV) in Europe and it has the potential to play a bridge vector role in future WNV transmission in the UK. Here we provide an update on the known distribution of the species in England based on adult and larval data, characterise the preferred *Cx. modestus* larval habitats, and present adult and larval data from sites where the species is known to occur. *Culex modestus* is primarily found in the south-east of England, particularly in North Kent, the Thames Estuary, and along the Essex coast, and as far east as Orford Ness, Suffolk, and in Rainham Marshes, Essex, in the West. Adult numbers peak in mid-late July, with larval numbers highest in late August / early September. Preferred habitats in North Kent are warm, shallow, narrow ditches, with an abundance of marginal, submerged, and floating vegetation. Such environmental data on the distribution, seasonality and habitat preference of *Cx. modestus* are critical for informing WNV surveillance programmes, identifying at risk areas (associated with this species) and providing information for a targeted control strategy in the event of WNV transmission.

## Keywords

mosquito larvae – West Nile virus – seasonality – vector – United Kingdom

## 1 Introduction

Vector-borne disease is an ever-changing field of public health, driven primarily by changes in the distribution of key vectors, and consequently the circulation of pathogens. In Europe, the principal vectors of public health concern are ticks and mosquitoes, both resident and non-native species. In the wider European region, the last three decades have seen many changes in the distribution and emergence of mosquito-borne diseases in Europe. The non-native mosquito species *Aedes* (*Stegomyia*) *albopictus* (Skuse, 1895) established in Italy in the early 1990s (Romi *et al.*, 2008; Sabatini *et al.*, 1990) and has now expanded its distribution to approximately thirty European countries (Medlock *et al.*, 2012; Osório *et al.*, 2018; Schaffner *et al.*, 2013)

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resulting in the subsequent autochthonous transmission of dengue virus (DENV) and chikungunya virus (CHIKV) (Angelini *et al.*, 2007; ECDC, 2017, 2018, 2019; Schaffner *et al.*, 2013), and also Zika virus (ZIKV) (Giron *et al.*, 2019). Circulation of mosquito-borne viruses by resident European mosquito species, particularly West Nile virus (WNV) and Usutu virus (USUV) are now more frequent, and other viruses (Batai virus [BATV], Inkoo virus [INKV], Lednice virus [LEDV], Sindbis virus [SINV], Tahyna virus [TAHV]) have been detected in recent years in Europe (Camp and Nowotny, 2020; Hubálek, 2008; Medlock *et al.*, 2007; Napp *et al.*, 2018; Zeller and Schuffenecker, 2004).

Since WNV was first detected in Europe in France in 1968 (Panthier *et al.*, 1968), it has circulated between birds and ornithophagic mosquitoes in southern and central Europe including the Czech Republic, France, Germany, Greece, Italy, the Netherlands, Romania, Russia, and Spain, and there have been outbreaks of WNV in north Africa (Algeria, Egypt and Morocco), and Israel (Chancey *et al.*, 2015; Murgue *et al.*, 2001; Nikolay, 2015). *Culex pipiens* L., 1758, and *Cx.* (*Barraudius*) *modestus* Ficalbi, 1890 are considered to be the predominant vectors (Hannoun *et al.*, 1964; Mouchet *et al.*, 1970), with other species potentially also having a role in transmission: *Anopheles* (*Anopheles*) *maculipennis sensu lato* (*s.l.*) Meigen, 1818, (Filipe, 1972), *Ae.* (*Ochlerotatus*) *caspius* (Pallas, 1771) (Labuda *et al.*, 1974), *Ae.* (*Aedimorphus*) vexans (Meigen, 1830) (Chaskopoulou *et al.*, 2016), *Cs.* (*Culiseta*) *annulata* (Schrank, 1776) (Petrić *et al.*, 2017), and *Cx.* (*Culex*) *perexiguus* Theobald, 1903, (Ferraguti *et al.*, 2021). There appears to be a high degree of variability between populations of mosquitoes across Europe in their role in WNV transmission, with *Cx. pipiens* from Switzerland reported as showing infection but not competence for WNV lineage 1, whilst those from France and the Netherlands have been shown to be competent (Leggewie *et al.*, 2016; Martinet *et al.*, 2019).

Prior to 2010, it was considered that without the presence of abundant bridge vectors together with migratory birds and particularly an absence of *Cx. modestus* there was a low risk of WNV in the UK as the incidence of human biting by *Culex* mosquitoes was limited and only occurred in foci where *Cx. pipiens* biotype *molestus* (Forskal, 1775) were present (Medlock *et al.*, 2005; Medlock *et al.*, 2007). Detection of a population of *Cx. modestus* in wetlands of the Thames estuary in 2010 meant that populations of potentially suitable bridge vectors for WNV, with the ability to bite humans, were now present. Furthermore, their foci in wetland habitats coincided with areas of wetlands where potentially infected migratory birds spend the summer months and hence these areas were the most likely for future circulation of WNV to humans following importation of the virus by migratory birds (Golding *et al.*, 2012; Medlock and Vaux, 2012). To date *Cx. modestus* is established at wetlands in North Kent and coastal Essex, and there have been isolated reports in the Cambridgeshire Fens and Poole Harbour (Cull *et al.*, 2016; Marshall, 1945; Medlock and Vaux, 2012; Medlock *et al.*, 2014; Vaux *et al.*, 2015). In North Kent, targeted virus screening of *Cx. modestus* in 2013 found no evidence of WNV, with subsequent analysis of samples from 2016–2023 finding no confirmed evidence of WNV circulation (J. Medlock and A. Vaux, unpublished data). There have been no reported human cases of locally acquired mosquito-borne disease in Britain in the last decades (Johnston *et al.*, 2023; Medlock *et al.*, 2018; Vaux *et al.*, 2015).

In the UK, the aquatic habitats of *Cx. modestus* are known to occur in ditches of coastal grazing marsh habitat, characterised as periodically inundated pasture or meadow, usually bounded by permanent ditches used to manage water levels within the pasture (BRIG, 2011; Medlock and Vaux, 2012; Vaux *et al.*, 2015). These aquatic habitats also often support breeding waders including migratory wildfowl, and are found extensively in the Thames Estuary, as well as other low-lying coastal areas (BRIG, 2011; England, 2018). Some evidence of the presence of *Cx. modestus* larvae in aquatic habitats have also been recorded in fen habitat at Wicken Fen (Medlock and Vaux, 2012), and in a permanent ditch adjacent to fen habitat in Ely (Welch, 2022). In Europe, typical reported aquatic habitats include swamps with dense vegetation, rice fields and reedbeds (Becker *et al.*, 2010; De Wolf *et al.*, 2021; Rudolf *et al.*, 2020b; Schaffner *et al.*, 2001; Votýpka *et al.*, 2008), which suggests a broader range of habitats than currently reported in the UK.

This paper provides an update on the distribution of *Cx. modestus* in the UK, reporting on extensive surveys conducted in England between 2016 and 2022, including specific targeted surveillance for *Cx. modestus.* For completeness this paper also brings together previously published records on the species' distribution. It also presents extensive new adult and larval data from sites where it is known to occur, and using larvae abundance data, aims to characterise the preferred aquatic habitat of *Cx. modestus* in England.

## 2 Materials and methods

Field data from different sources were assembled, from surveillance and research projects. Adult surveillance data presented include results from nationwide surveys in England as well as targeted surveillance in parts of the country where *Cx. modestus* has been detected. Larval surveillance data presented here include data generated during field studies with the aim of understanding the distribution of the species and developing knowledge on the aquatic niche of the species in wetlands in the North Kent Marshes.

***Nationwide adult mosquito survey***

The Medical Entomology Group (MEZE) at UK Health Security Agency (previously part of Public Health England and the Health Protection Agency) has run a programme of mosquito surveillance (the Nationwide Mosquito Survey) at sites across England, with data from 2010 to 2021. The survey deployed adult mosquito traps (Mosquito Magnet® Independence and Executive models (Woodstream Corporation, St. Joseph, MO, USA) baited with octenol lures) to record diversity, distribution, abundance and seasonality of native mosquitoes (Vaux and Medlock, 2015). Data relating to the presence/absence of *Cx. modestus* in the adult trap at these nationwide sites were assembled and are presented here. These data incorporate additional data collected at some of the sites surveyed as part of the WetlandLIFE project under the Natural Environment Research Council (NERC) Valuing Nature Programme by entomologists from MEZE and/or University of Greenwich. These sites were at Alkborough Flats, Lincolnshire (2018), Arne, Dorset (2017), Priory Country Park and Fenlake Meadows Nature Reserve in Bedford, Bedfordshire (2017), Chippenham Fen, Cambridgeshire (2017), Otter Estuary, Devon (2017–2018), Hurcott Pool and Woods, Worcestershire (2018), Northward Hill, Kent (2017), Shapwick Heath, Somerset (2017–2018), Steart Marshes, Somerset (2018), Radipole Lake, Dorset (2018), and Greywell Moors, Hampshire (2018) (Hawkes *et al.*, 2020; Medlock *et al.*, in press). Sites were chosen to reflect a broad range of aquatic habitats that may be suitable for mosquitoes, and included managed wetlands, brackish and freshwater areas, wetlands in urban and rural environments, and recently created or restored wetland habitats.

At all sites, traps were operated by volunteers on behalf of MEZE, drawing upon the local capacity provided by the network of nature reserve wardens and environmental health officers. The traps were deployed on alternate weeks for 4 trap nights each week from calendar week 14/15 (early April) to week 42/43 (mid-October). At the end of each survey week, Mosquito Magnet® catch bags were posted to the MEZE laboratory for identification using morphological keys (Becker *et al.*, 2010; Cranston *et al.*, 1987; Snow, 1990). The total number of trap nights over the year varied due to trap malfunction, gas delivery issues, or project volunteer availability. Adult density is reported as the mean number of adult females captured per trap night.

***Targeted adult surveillance***

In 2019, additional surveillance targeting *Cx. modestus* was conducted at three sites in North Kent: Cliffe Mead Wall, Northward Hill, and Chetney Marshes. The trap site at Cliffe Mead Wall is approximately two kilometres north-east of the Nationwide trap site at Cliffe Fort. The sites were chosen based on previous published work showing established populations to be present (Vaux *et al.*, 2015), and the aim was to understand the seasonality and abundance of *Cx. modestus* at those locations. Owing to occasional operational restraints, traps were run for a varying number of trap nights, and therefore are reported separately from the Nationwide project adult data.

*Targeted immature surveillance*

In some of the known endemic areas as directed by both adult mosquito surveillance and larval distribution surveys, namely Cliffe Marshes, Northward Hill, and Chetney Marshes, larval surveys were conducted in 2019 (July to October) with the aim of defining the seasonality and abundance of larvae during the active season. Surveys were conducted using 3×200 ml dips (using a standard 200 ml dipper; John W. Hock Company, Gainesville, FL, USA), every five metres along the edge of ditches and pools. Mosquito larvae were collected and identified in the laboratory to species and instar using morphological keys (Becker *et al.*, 2010; Cranston *et al.*, 1987; Snow, 1990). Larval density is reported as number of larvae per litre.

***Adult and larval seasonality***

Larval count per litre data (North Kent 2015–2016) and adult trap night data (Nationwide mosquito survey 2016–2021) from sites where *Cx. modestus* were present were analysed using the rGAI package (Dennis, 2021), developed for the analysis of seasonal count data (Dennis *et al.*, 2013, 2016). A range of models were fitted based on number of larval generations (1–3; referred to as multiple broods in the package), distribution (Poisson, negative binomial, and zero-inflated Poisson), and seasonal flight pattern, and the model with the lowest AIC was chosen.

### *Distribution of* Culex modestus

In order to better understand the extent of the distribution of *Cx. modestus* within its known range, as informed by the adult mosquito surveillance, immature mosquito sampling was conducted to identify additional wetlands where *Cx. modestus* occurs. In July 2018, September 2020, and September 2023 immature mosquito surveys were conducted to determine the northernmost limit of *Cx. modestus* distribution. Sites were selected based on habitat suitability (presence of ditches in coastal marshes), starting from the most northerly coastal known site where *Cx. modestus* had previously been reported in Essex and heading northwards through Essex, Suffolk and Norfolk. The timings of these surveys were based on data from previous surveys in which larval *Cx. modestus* were found to be abundant in July, and remain in good numbers into September (Golding *et al.*, 2012; Vaux *et al.*, 2015). Extensive surveys for immature stages were conducted by three entomologists, each using a 200 ml dipper multiple times at ditches, ponds, and flooded habitat. All mosquito larvae and pupae were collected and identified in the laboratory to species and instar using morphological keys (Becker *et al.*, 2010; Cranston *et al.*, 1987; Snow, 1990).

A distribution map of *Cx. modestus* was produced using data from these immature surveys and from the Nationwide Mosquito Survey sites (2010–2022) (Vaux *et al.*, unpublished data), Wetland Life sites (2017–2018) (Hawkes *et al.*, 2020; Medlock *et al.*, in press) and all published records of *Cx. modestus* (Cull *et al.*, 2016; Golding *et al.*, 2012; Medlock and Vaux, 2012, 2013, 2015; Medlock *et al.*, 2014; Vaux *et al.*, 2011, 2015; Welch, 2022).

### *Defining* Culex modestus *aquatic habitat*

Immature surveys were conducted (July-November 2015, July-October 2016) on the Hoo peninsula, Isle of Sheppey and neighbouring marshes in order to define habitats occupied by *Cx. modestus* in North Kent. Survey locations (2015 and 2016: Cliffe Pools, Cliffe Village, Rye Street Farm and Swigshole; 2016: Chetney marshes and Isle of Sheppey) were chosen given their proximity to known *Cx. modestus* populations. Sites were randomly chosen within each location (2015: 4 locations each with 20 sites; 2016: 6 locations each with 10 sites), and visited once per fortnight from 22 June 2015 (wk 26) until 31 November 2015 (wk 53), and from 27 June 2016 (wk 26) until 3 November (wk 44). During each visit, larval sampling (5×200 ml dips using a standard dipper) was conducted and additional variables recorded (Supplementary Table S1). Mosquito larvae were identified in the laboratory to species and instar using morphological keys (Cranston *et al.*, 1987; Snow, 1990).

Data were 'top and tailed' to remove data collected before the first appearance of *Cx. modestus* and after the last occurrence of *Cx. modestus* in both 2015 and 2016. The rationale for this was that it was not possible to tell whether a site was negative because it was unsuitable, or because *Cx. modestus* was not present in the environment at this time. Data collected when the ditches had dried out were also removed, as no *Cx. modestus* larvae could be present at this time. DAFOR values were summed across ecologically similar variables, using the sum of the midpoints of DAFOR divisions (values rounded up: 88%, 63%, 38%, 19%, 6% and 0%), to create three new variables: (Abundance of reeds and reedmaces = reed (ditch), reed (bank), reedmace (ditch), reedmace (bank); Abundance of sedge = sedge (bank), sedge (ditch); Abundance of vegetation in the water = free-floating, submerged vegetation, algae).

To test the relationship between *Cx. modestus* larval abundance against the recorded variables, generalised linear mixed regression models (GLMMs) were fitted and analysed in R (R Core Team, 2021). Variance inflation factors were calculated using the *car* package (Fox and Weisberg, 2018) to assess explanatory variables for any collinearity, and variables with a VIF score above 4 (Reed [bank], height vegetation far side) were discarded from the models. Model selection were performed using the *dredge* function from the MuMIN package (Bartoń, 2022), and the DHARMa package (Hartig, 2019) used to check for violation of model assumptions (overdispersion, zero inflation). Models were evaluated using Akaike Information Criterion (AIC) and test for differences in abundance trends using analysis of variance tests (ANOVA). Negative binomial GLMMs was used to investigate *Cx. modestus* larval abundance in relation to the ecological variables collected at each sample point, as detailed in Supplementary Table S1. Sample points and the fortnight of collection were included in the model as random effects in order to account for any autocorrelation. A dendrogram using the Bray-Curtis method was used to examine mosquito species co-occurrence.

## 3 Results

***Nationwide adult mosquito survey***

*Culex modestus* was recorded at six out of a total of 70 nationwide sites: Cliffe Fort, Mucking, Northward Hill (all in Kent), Rainham Marshes, Wallasea Island and Wat Tyler (all in Essex) during the years 2016–2021 (Supplementary Figure S1; Table 1). Across all years, the highest total number of female mosquitoes trapped was recorded at Rainham Marshes (n = 3,278 [2021], trap nights = 40), and the lowest reported was at Cliffe Fort (n = 50 [2017], trap nights = 24). Total numbers of *Cx. modestus* females recorded over a year varied from one (Mucking [2017]) to 1,041 (Wat Tyler [2018]).

Numbers of adult female *Cx. modestus* varied at Cliffe Fort (Supplementary Figure S1) across the years (2017–2019), with highest densities recorded in 2018. However, abundances of *Cx. modestus* were low and represented less than 10% of total adult specimens in each year (Table 1). At Mucking over the same years (Supplementary Figure S1) *Cx. modestus* numbers were higher than at Cliffe Fort, particularly in 2018 (n=336), representing 29% of the total catch that year, with the highest number per trap night during week 30. At Northward Hill the trap operated only in 2017 as part of the Nationwide mosquito project, with 6 *Cx. modestus* out of a total of 1,311 adult mosquitoes recorded over the season (53 trap nights). *Culex modestus* was trapped at the highest densities at Rainham Marshes in 2021, at 71.75 females per trap night, recorded in week 27 (5–9 July). This was the highest density recorded at the site for the whole period (2016–2021) and 2021 was also the year

TABLE 1 Adult trapping (Nationwide sites), by site and by year, showing number of trap nights over the season, total number of all species trapped, mean number of mosquitoes per trap night (TN), total number of *Culex modestus* adult females, number of mosquitoes of other species, *Cx. modestus* as proportion of total catch, and mean number of *Cx. modestus* per trap night.

| Site | Year | No. TN | Total no. all spp. | Mean/TN all spp. | No. *Cx. modestus* | No. other spp. | *Cx. modestus* as proportion of total | Mean/TN *Cx. modestus* |
|---|---|---|---|---|---|---|---|---|
| Cliffe Fort | | | | | | | | |
| | 2017 | 24 | 50 | 2.08 | 5 | 45 | 0.10 | 0.21 |
| | 2018 | 59 | 175 | 2.97 | 11 | 164 | 0.06 | 0.19 |
| | 2019 | 54 | 117 | 2.17 | 2 | 115 | 0.02 | 0.04 |
| Mucking | | | | | | | | |
| | 2017 | 24 | 270 | 11.25 | 1 | 269 | 0.00 | 0.04 |
| | 2018 | 51 | 1,149 | 22.53 | 336 | 813 | 0.29 | 6.59 |
| | 2019 | 50 | 799 | 15.98 | 37 | 762 | 0.05 | 0.74 |
| Northward Hill | | | | | | | | |
| | 2017 | 53 | 1,317 | 24.85 | 6 | 1,311 | 0.00 | 0.11 |
| Rainham Marshes | | | | | | | | |
| | 2016 | 32 | 581 | 18.16 | 3 | 578 | 0.01 | 0.09 |
| | 2017 | 54 | 100 | 1.85 | 6 | 94 | 0.06 | 0.11 |
| | 2018 | 54 | 424 | 7.85 | 140 | 284 | 0.33 | 2.59 |
| | 2019 | 55 | 272 | 4.95 | 35 | 237 | 0.13 | 0.64 |
| | 2020 | 28 | 260 | 9.29 | 129 | 131 | 0.50 | 4.61 |
| | 2021 | 40 | 3,278 | 81.95 | 352 | 2,926 | 0.11 | 8.80 |
| Wallasea | | | | | | | | |
| | 2016 | 48 | 1,067 | 22.23 | 60 | 1,007 | 0.06 | 1.25 |
| | 2017 | 66 | 795 | 12.05 | 730 | 65 | 0.92 | 11.06 |
| | 2018 | 52 | 1,400 | 26.92 | 515 | 885 | 0.37 | 9.90 |
| | 2019 | 36 | 76 | 2.11 | 5 | 71 | 0.07 | 0.14 |
| Wat Tyler | | | | | | | | |
| | 2018 | 42 | 1,521 | 36.21 | 1,041 | 480 | 0.68 | 24.79 |
| | 2019 | 50 | 1,194 | 23.88 | 260 | 934 | 0.22 | 5.20 |

with the highest number of mosquitoes recorded over the season (n=3,278). The year with the second highest abundance of all mosquito species (n=581) at Rainham Marshes was 2016, with only 3 *Cx. modestus* recorded. Higher proportions of *Cx. modestus* were recorded in subsequent years, particularly in 2020 (50% *Cx. modestus*). At Wallasea, *Cx. modestus* was trapped at the highest densities in 2017 (137.5 females / TN) in week 28 (10–14 July), representing 92% of the total mosquitoes across the season. The following year (2018), the species was also recorded in comparatively large numbers, with highest densities reported in week 30 (58 females / TN; 23–27 July). It was also recorded in 2016 and 2019, but in much lower numbers (total 2016 = 60; total 2019 = 5). *Culex modestus* was recorded at Wat Tyler in 2018–2019, with highest densities in week 30 (23–27 July), 2018 (167 females / TN). Due to COVID-19 restrictions in the UK it was not always possible to run traps in the years 2020 or 2021.

***Targeted adult surveillance***

During 2019, adult traps were run over five trapping periods from calendar week 31 (31/7/2019) to week 43 (25/10/2019). Targeted surveys at three sites (Chetney, Cliffe Mead Wall and Northward Hill) in 2019 found highest densities of *Cx. modestus* adult females at Cliffe Mead Wall, peaking in calendar week 31–33 (31/7–15/8/2019) with 111.4 females / TN (Figure 1). Densities were lower in subsequent trap weeks: week 34–36 (15/8–5/9/19 = 42.19 females / TN; week 39 (23/9–1/10/19 = 9.86 females / TN. The trap at Chetney recorded a peak of 27 females / TN (week 34–36), whilst peak densities at Northward Hill were recorded during the same trap week (11.1 females / TN). *Culex modestus* represented over 88% of all specimens caught, with similar rates at all three sites (Table 2). Operational constraints impacted the number of times the traps could be visited resulting in a varying number of trap nights per catch (Figure 1). Despite efforts to site traps in sheltered positions where possible, a lack

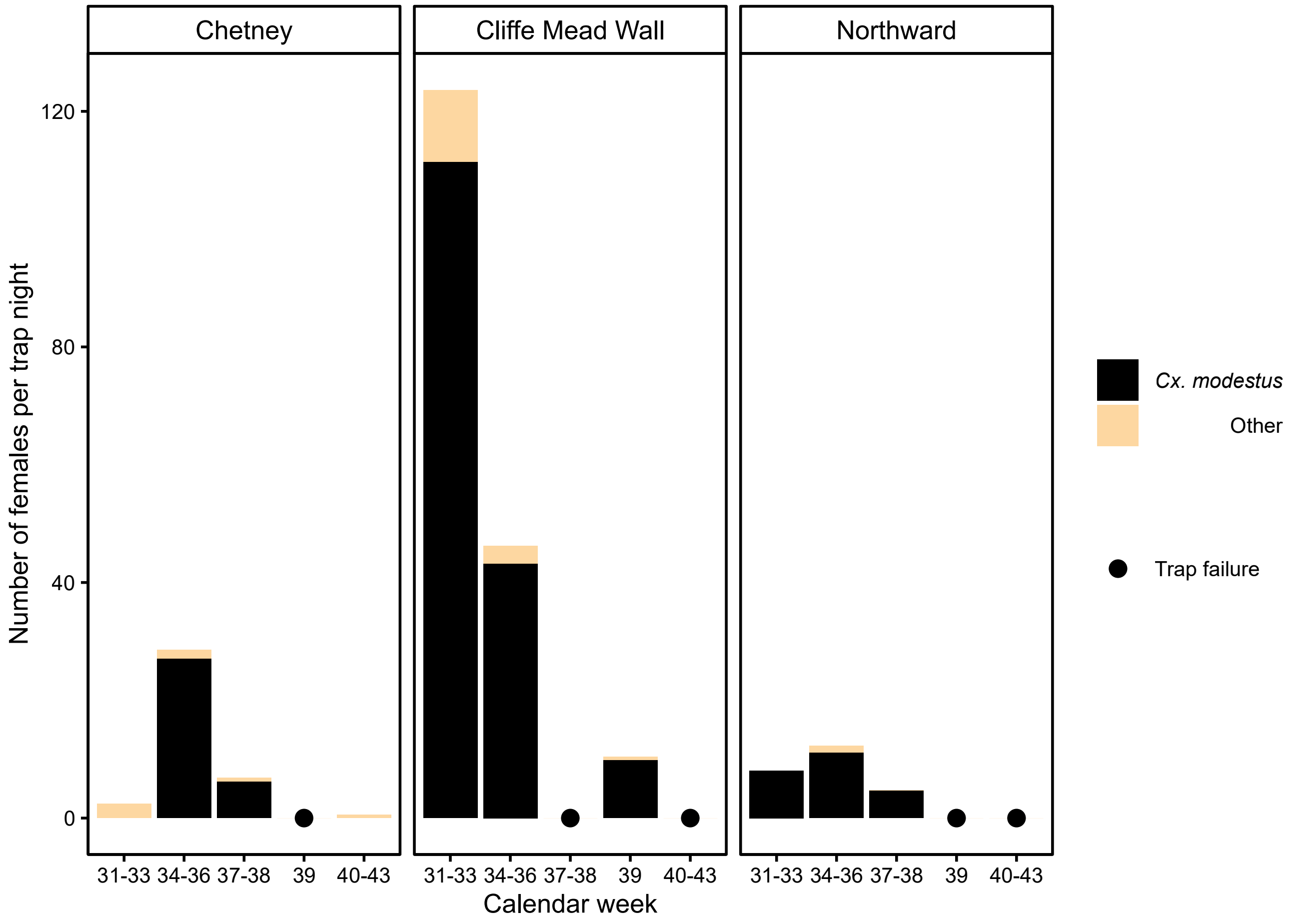


FIGURE 1 Number of females per trap night (*Culex modestus*; all other species grouped as 'Other') for Chetney, Cliffe Mead Wall, and Northward Hill in 2019 shown by calendar week. Symbol indicates trap failure. Number of trap nights (TN) varied: calendar week 31–33 = 15 TN; 34–36 = 21 TN; 37–38 = 15 TN; 39 = 7 TN; 40–43 = 24 TN.

TABLE 2 Targeted surveillance adult trapping at three sites (Chetney, Cliffe Mead Wall, Northward Hill), showing number of trap nights over the season, total number of mosquitoes of all species trapped, mean number of mosquitoes per trap night, total number of *Culex modestus* adult females, number of other species, *Cx. modestus* as proportion of the total number of adult female mosquitoes, and mean number of *Cx. modestus* per trap night.

| Site | No. TN | Total no. all spp. | Mean/TN all spp. | No. *Cx. modestus* | No. other spp. | *Cx. modestus* as proportion of total | Mean/TN *Cx. modestus* |
|---|---|---|---|---|---|---|---|
| Chetney | 75 | 752 | 10.03 | 659 | 93 | 0.88 | 8.79 |
| Cliffe Mead Wall | 43 | 2,899 | 67.42 | 2,647 | 252 | 0.91 | 61.56 |
| Northward Hill | 51 | 451 | 8.84 | 423 | 28 | 0.94 | 8.29 |

of vegetation at the trap sites resulted in trap failure at each of the sites on one or more occasion.

***Targeted immature surveillance***

Immature sampling was conducted on 3/7/2019 (week 27; Cliffe Mead Wall & Northward Hill), 4/7/2019 (week 27; Chetney), and at all three sites on 31/7/2019 (week 31), 15/8/2019 (week 33), 5/9/2019 (week 36), 17/9/2019 (week 38), and 2/10/2019 (week 40). A similar number of litres of water were sampled at Chetney (49.8L) and Northward Hill (56.4L), with a larger volume sampled at Cliffe Mead Wall (162L) owing to the greater length of ditches present and therefore more opportunity for sampling (Table 3). The mean number of larvae per litre of all species ranged from 2.75 to 3.31, and *Cx. modestus* larvae were at a similar density across all three sites (0.95–1.08 larvae per litre). *Culex modestus* accounted for 31–39% of the total mosquito larvae fauna across the sites. The highest densities of *Cx. modestus* larvae were recorded at Cliffe Mead Wall and Northward Hill in week 31, and the species was recorded from weeks 27–38 (Figure 2). At Chetney, *Cx. modestus* was first recorded in week 31, with highest density in week 33, and was last recorded in week 40. No first instar or pupae were recorded, and instar stage II–III were more abundant earlier in the season (Figure 3).

***Adult and larval seasonality***

The earliest *Cx. modestus* larvae were recorded in early July (wk 27), and the latest record was in late October (wk 44) with II and III instar larvae present from weeks 27 to 38. Larval abundance peaked in mid-September (wk 34–36). The parameters chosen in the best-fit model were a mixture model, multiple broods (i.e. multiple generations), and a negative binomial distribution. The predicted mean count per week is shown in Figure 4.

The earliest record of an adult female *Cx. modestus* was from Wat Tyler Country Park, Essex (2019) in week 14 (1–5 April). High numbers of adult females were found in weeks 30–32 (late July to early August) at most sites, with the highest abundance reported at Wat Tyler Country Park in week 30 (23–27 July 2018). The latest record was from Mucking, Essex, in week 42 (15–19 October 2019). The parameters chosen in the best-fit model were a mixture model, multiple broods (i.e. multiple generations), and a negative binomial distribution. The predicted mean count per week is shown in Figure 5.

***Distribution of* Culex modestus**

Enhanced larval surveys in July 2018 began at Fingringhoe Wick (Essex), where *Cx. modestus* was known to be present in 2015 (Cull *et al.*, 2016) and was again confirmed to be present during these surveys in 2018. Surveys then proceeded northward and were conducted nearby at Mersea Island and Wivenhoe and despite reports of nuisance biting at Wivenhoe submitted with an adult *Cx. modestus* to the MEZE's Mosquito Recording Scheme in June 2018 (Johnston *et al.*, 2023), no larvae were found at either location. The species was recorded in ditches at Horsey Island, but not at ditches at nearby Walton Hall Marshes. *Culex modestus* was not found at any other site surveyed (from south to north) at Wivenhoe Ferry Marsh, Boyton Marshes, Aldeburgh Marshes and Sizewell Belts.

In September 2020, during repeat and extended visits, *Cx. modestus* larvae were again confirmed as present at Fingringhoe Wick but were not found during larval surveys conducted at marshes in (from south to north) Wrabness, Cattawade, Ramsholt, Hollesley, Snape, Dingle, Smear, Benacre and Carlton. Surveys in September 2023 confirmed *Cx. modestus* (larvae and

TABLE 3 Targeted surveillance larvae sampling at three sites (Chetney, Cliffe Mead Wall, Northward Hill), showing total number of larvae collected, total number of litres sampled, number of *Culex modestus* larvae, number of *Cx. modestus* larvae per litre, *Cx. modestus* larvae as proportion of total larvae, number of larvae of all other species, number of larvae of all other species per litre, number of larvae of all other species as proportion of total larvae.

| Site | Total no. of larvae | Total litres sampled | Larvae per litre | *Cx. modestus* larvae | *Cx. modestus* per litre | *Cx. modestus* as proportion of total | No. larvae of all other species | No. larvae of all other species per litre | No. larvae of all other species as proportion of total |
|---|---|---|---|---|---|---|---|---|---|
| Chetney | 165 | 49.8 | 3.31 | 54 | 1.08 | 0.33 | 111 | 2.23 | 0.67 |
| Cliffe Mead Wall | 501 | 162.0 | 3.09 | 154 | 0.95 | 0.31 | 347 | 2.14 | 0.69 |
| Northward | 155 | 56.4 | 2.75 | 61 | 1.08 | 0.39 | 94 | 1.67 | 0.61 |

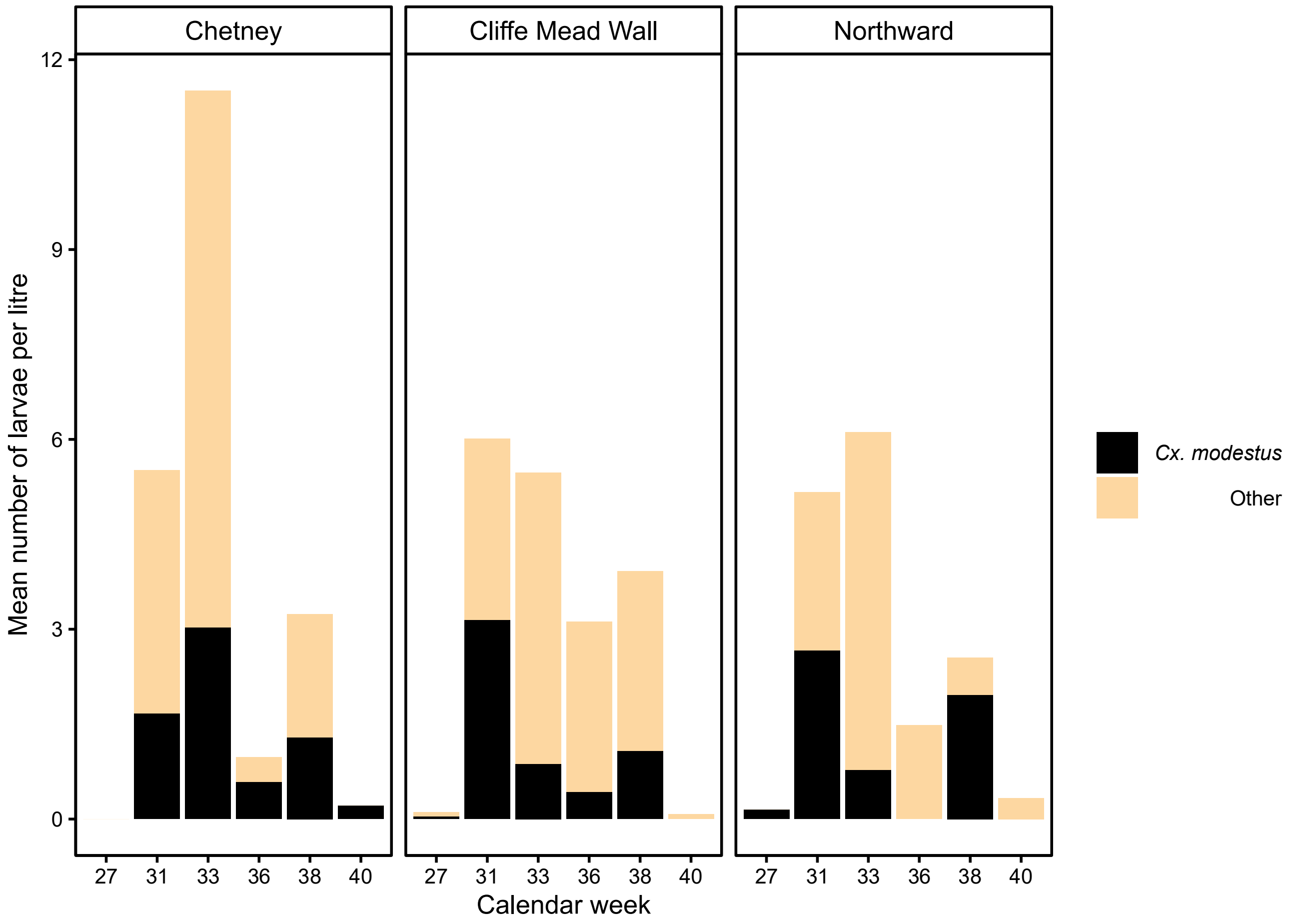


FIGURE 2 Mean number of larvae per litre (*Culex modestus*; all other species grouped as 'Other') for Chetney, Cliffe Mead Wall, and Northward Hill in 2019 shown by calendar week.

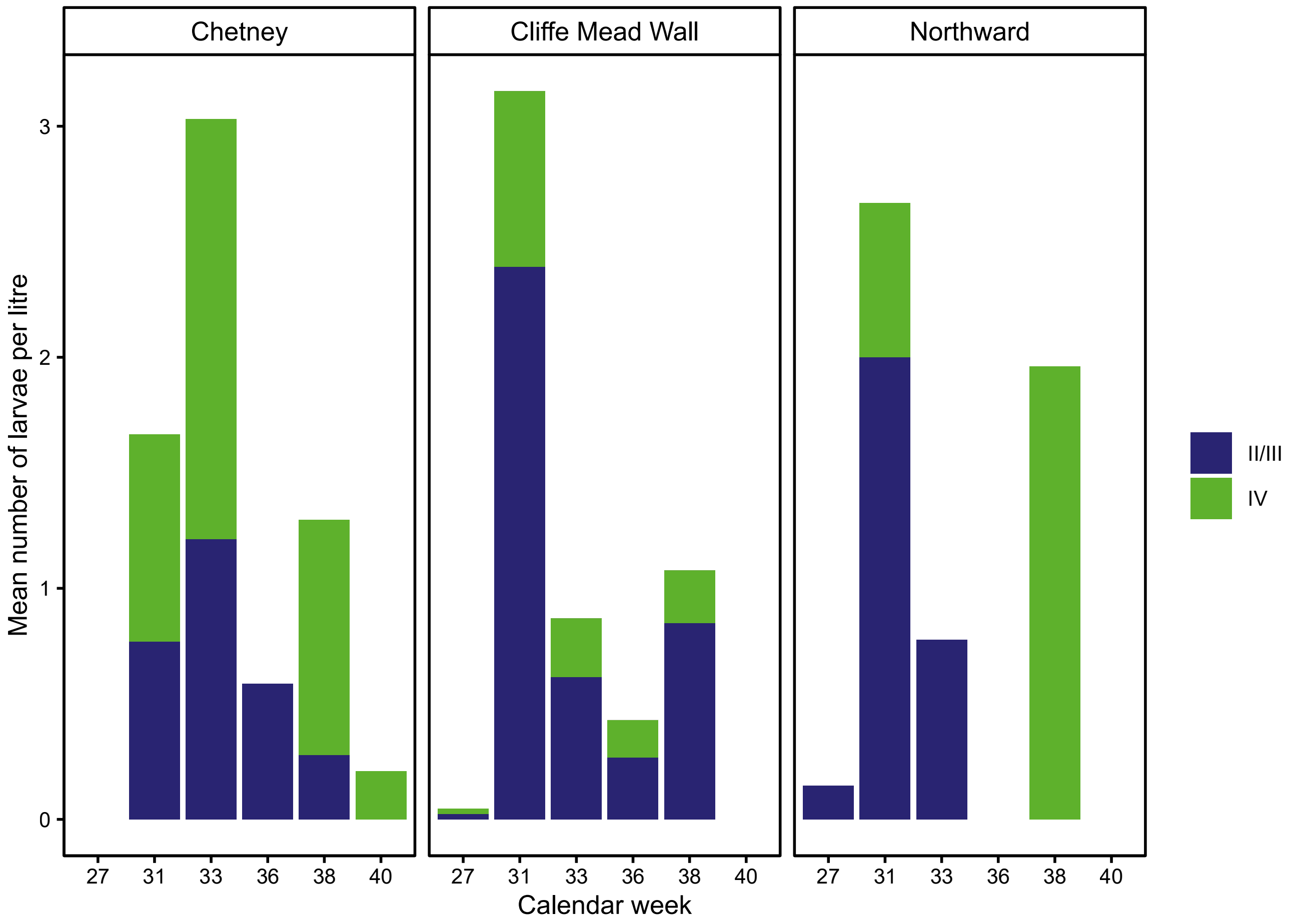


FIGURE 3 Mean number of *Culex modestus* larvae per litre by instar (II/III instar and IV instar) for Chetney, Cliffe Mead Wall, and Northward Hill in 2019 shown by calendar week.

adults) to be present at Orford Ness, but were not found at Cattawade, Ramsholt or Boyton Marshes.

Data were compiled from a range of sources (Supplementary Table S2) and mapped (Figure 6). *Culex modestus* was found for the first time at Sandwich Haven during adult trapping in 2022, and in the following year, *Cx. modestus* larvae were found at Orford Ness, making this the most easterly British record.

### *Defining* Culex modestus *aquatic habitat*

The top five models with the lowest AIC scores (Supplementary Table S3) were compared using pairwise comparisons (ANOVA). The p-values for these comparisons were greater than 0.05, providing no evidence of a significant difference between the models.

The GLMM with the lowest AIC score explaining *Cx. modestus* larval abundance contained the random effects of fortnight and site, and fixed effects of depth of the ditch, width of the ditch, water temperature, visibility through the water, slope angle of the ditch, abundance of sedge, abundance of vegetation in the water (Supplementary Table S3, Table 4, Figures 7–8). There were significant positive correlations between *Cx. modestus* abundance and water temperature (estimate = 0.6364, CI = 0.3973, 0.8755, $P$ = 0.001); abundance of vegetation in the water (estimate = 0.2478, CI = 0.0835, 0.412, $P$ = 0.003) and visibility through the water (estimate = 0.2971, CI = 0.0114, 0.5829, $P$ = 0.042). Depth of the ditch (estimate = -0.612, Cl= -1.027, -0.1968, $P$ = 0.004) and the angle of the slope in the water (estimate = -0.29045, CI = -0.497, -0.0839, $P$ = 0.006) were significantly negatively correlated.

No significant correlation was found between either the abundance of sedge (estimate = 0.2039, CI = -0.0188, 0.4265, $P$ = 0.073), or the width of the ditch (estimate = -0.2263, CI = -0.5050, 0.0524, $P$ = 0.112); however these were retained in the best fit model.

Bray-Curtis analysis showed clustering between the presence of immature stages of *Cs. annulata, Cx. modestus*, *An.* (*Ano.*) *claviger* (Meigen, 1884) and *An. maculipennis s.l.*, with *Cx. modestus* falling between *Cs. annulata*, and the two *Anopheles* species (Figure S2). A separate

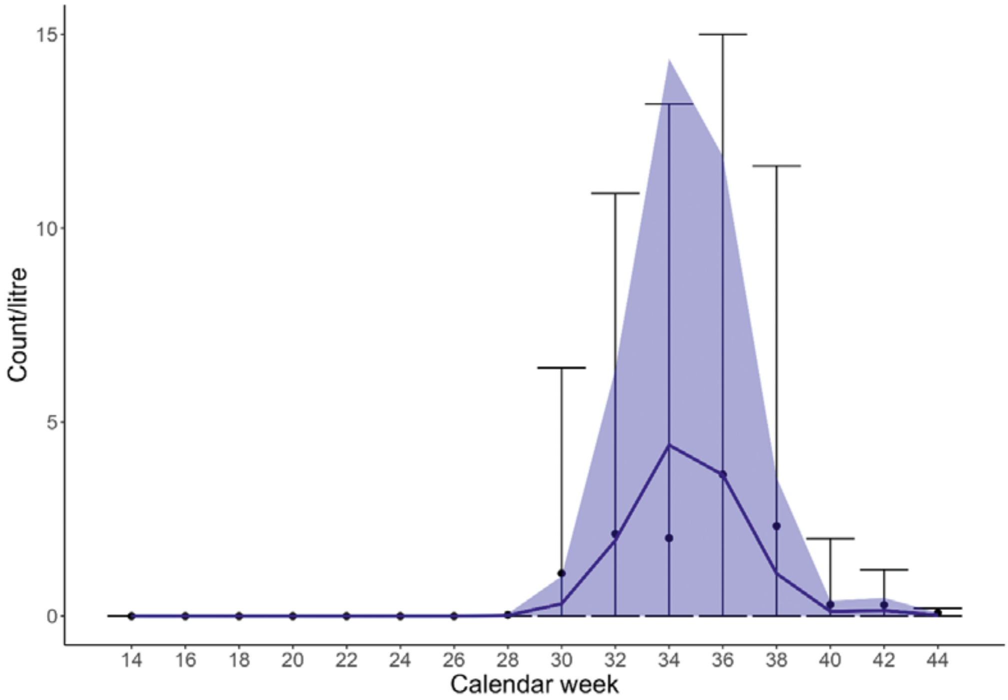


FIGURE 4 Observed mean larval count per litre (black circles) with 5% and 95% quantiles of all observed counts shown as error bars for *Culex modestus* (North Kent, 2015–2016). Predicted mean count per week is shown as a blue line, with shaded blue showing predicted quantiles. April (week 14) to October (week 44).

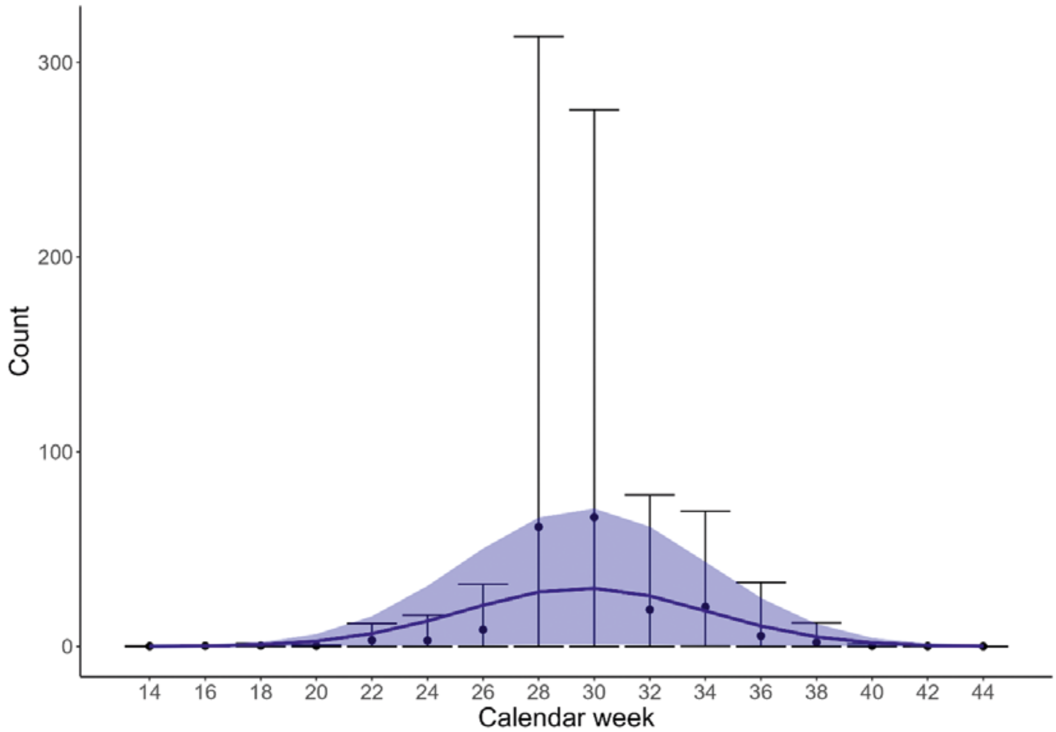


FIGURE 5 Observed adult mean count per trap week (4 trap nights) (black circles) with 5% and 95% quantiles of all observed counts shown as error bars for *Culex modestus* adult females (Nationwide mosquito project, 2016–2022). Predicted mean count per week is shown as a blue line, with shaded blue showing predicted quantiles. April (week 14) to October (week 44).

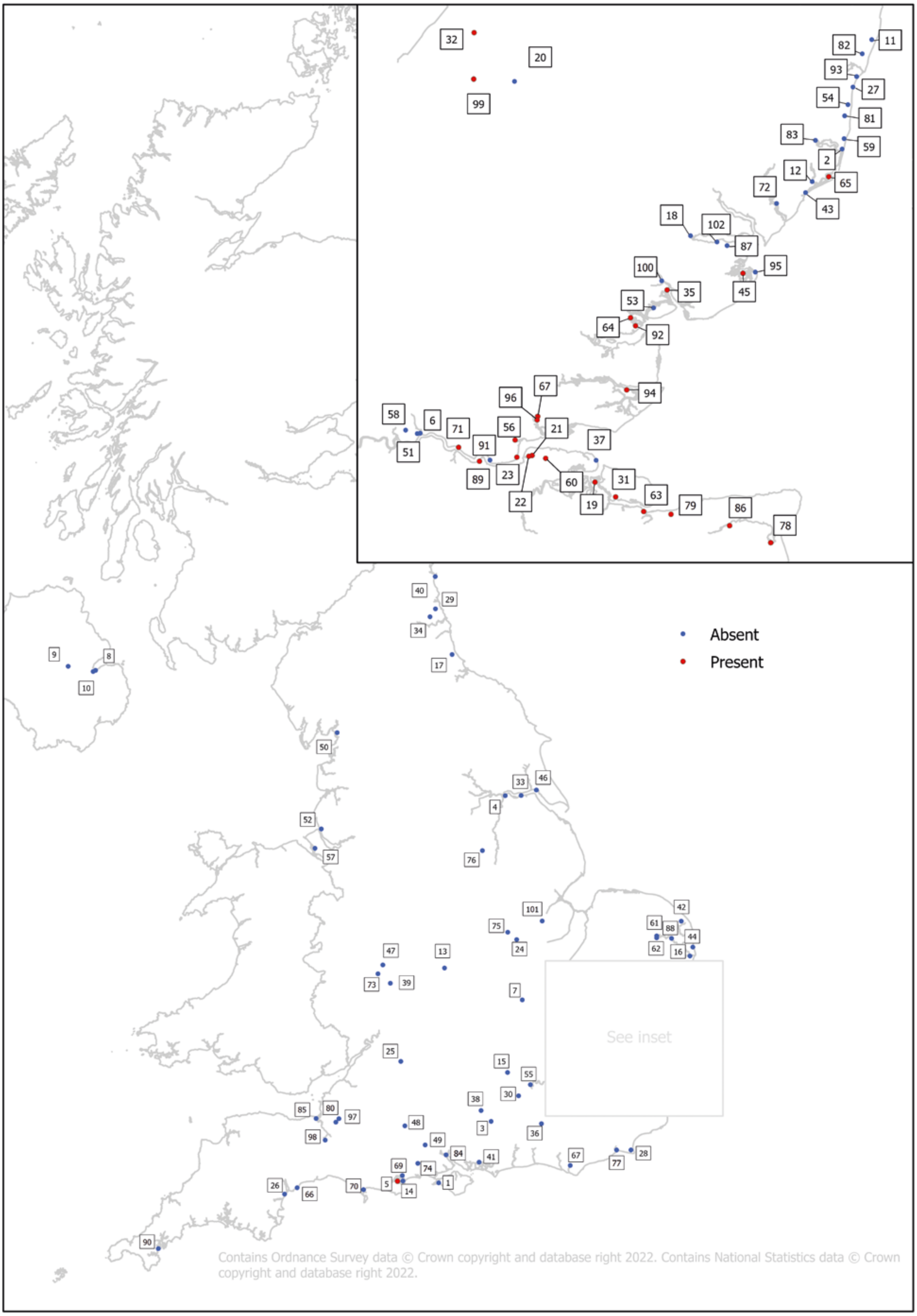


FIGURE 6 Locations of studies referenced in Supplementary Table S2 recording presence / absence of *Culex modestus*. Numbers refer to the ID number (Supplementary Table S2). Red symbols indicate recorded presence, blue symbols indicate absence.

TABLE 4 Model-averaged coefficients from all models explaining the fixed effects on *Culex modestus* larval abundance.[1]

| | Estimate (log) | SE | z-value | *P*-value |
|---|---|---|---|---|
| Intercept | –1.291 | 0.344 | 3.746 | <0.001*** |
| Depth | –0.657 | 0.221 | 2.969 | 0.003** |
| Abundance of sedge | 0.153 | 0.140 | 1.084 | 0.279 |
| Slope angle of the ditch | –0.284 | 0.110 | 2.582 | 0.009** |
| Temperature | 0.627 | 0.126 | 4.968 | <0.001*** |
| Visibility | 0.286 | 0.155 | 1.844 | 0.065 |
| Abundance of vegetation in the water | 0.258 | 0.086 | 2.983 | 0.002** |
| Width | –0.152 | 0.157 | 0.965 | 0.335 |
| Salinity | –0.075 | 0.124 | 0.601 | 0.547 |
| Height on near bank | –0.080 | 0.140 | 0.514 | 0.588 |
| pH | 0.014 | 0.050 | 0.283 | 0.777 |
| Reed and reedmace abundance | –0.041 | 0.115 | 0.355 | 0.723 |
| Water colour | 0.238 | 0.070 | 0.340 | 0.734 |
| Slope angle of the bank | –0.014 | 0.055 | 0.250 | 0.803 |
| Total dissolved solids (TDS) | 0.005 | 0.038 | 0.121 | 0.904 |
| Ditch bearing | 0.015 | 0.097 | 0.162 | 0.871 |
| Shade over the water | –0.001 | 0.018 | 0.076 | 0.940 |

[1]Random effects were 'site' and 'fortnight'. Significance values given: *= *P* <0.05, ** = *P* <0.01, *** = *P* <0.001.

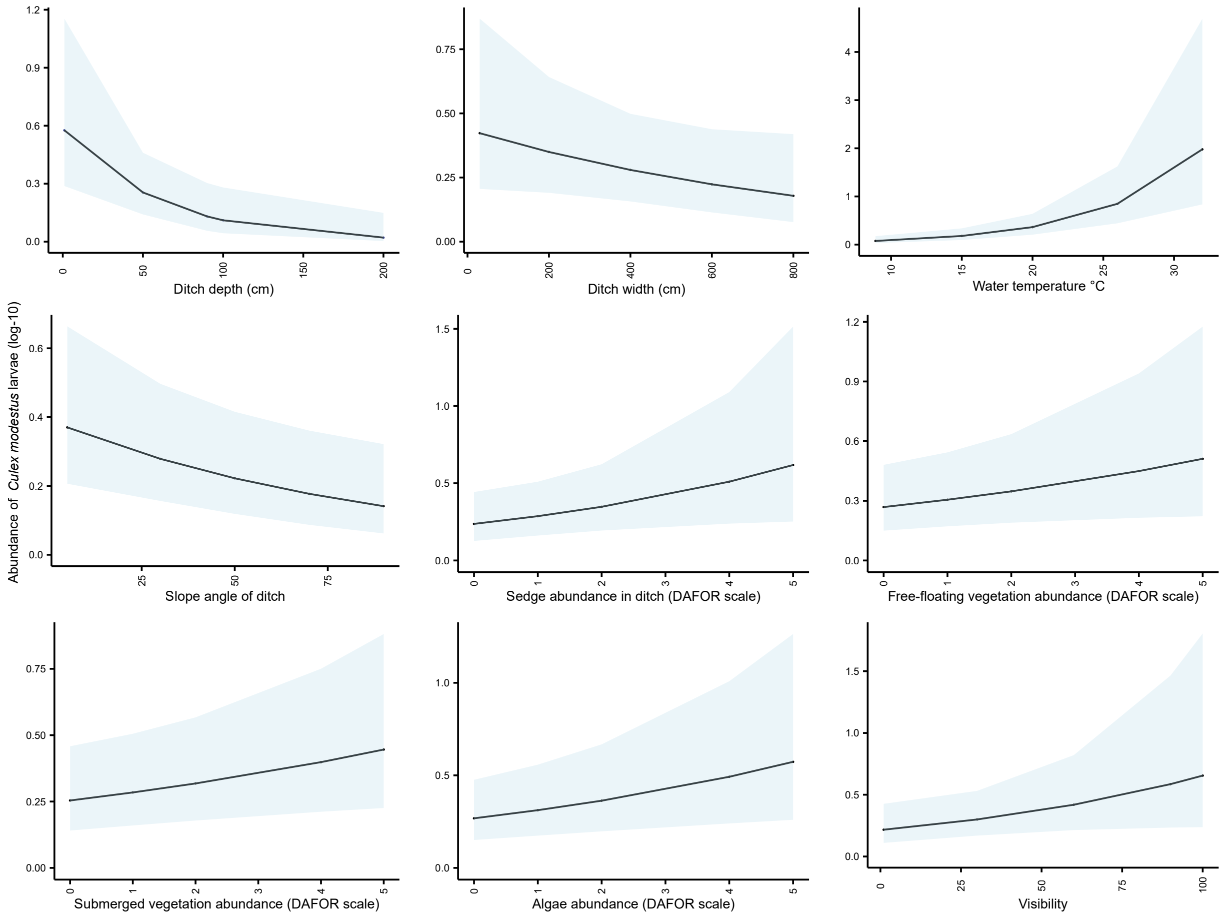


FIGURE 7 Abundance (log) of *Culex modestus* against variables included in the model with the lowest AIC score. Shaded area represents 95% confidence area.

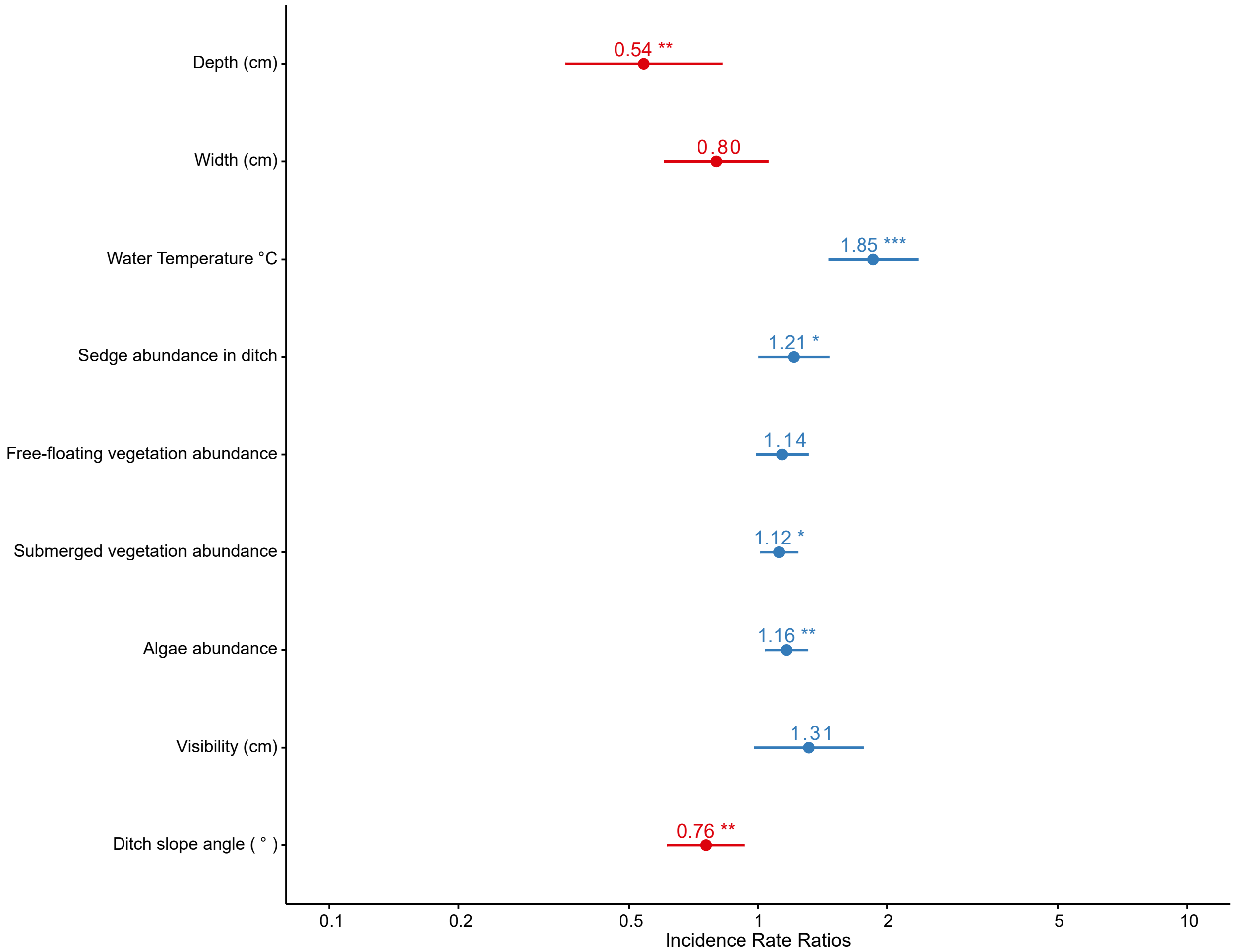


FIGURE 8 Plot of incident rate ratios from the GLMM with the lowest AIC, for the effect of ecological variables on *Culex modestus* larvae abundance. Red variables demonstrate an inverse relationship, blue indicate a positive relationship. Significance: * = $P<0.05$, ** = $P<0.01$, *** = $P<0.001$.

cluster contained *Cx. pipiens*, and *Cx.* (*Neoculex*) *territans* Walker, 1856, however few *Cx. territans* (Walker 1856) were collected.

## 4 Discussion

This paper presents the latest data on the seasonality, distribution and habitat preferences of *Cx. modestus,* an important putative vector of WNV in England. Since its detection in 2010, the species continues to be present in large numbers at permanent ditches in grazing marsh along the North Kent coast. At coastal sites in the south-east of England, the species has been found as far east as Sandwich in Kent, to Rainham Marshes, Essex, in the west, and as far north as Orford Ness, Suffolk. Whilst *Cx. modestus* has been found on multiple occasions in larval searches at Fingringhoe Wick, it has not been detected in similar wetland habitat at nearby sites at Wivenhoe Ferry marsh, likewise whilst present at Horsey Island, it has not been detected at the nearby Walton Hall Marshes. This may indicate that it has been recently introduced to the area and has not yet become established. It may also indicate that *Cx. modestus* has a limited flight range and requires movement via other means for population dispersal, such as at the egg stage on the feet of birds. It is also possible that the surrounding areas in these locations are simply not suitable for *Cx. modestus*. The recent finding at Sandwich in 2022 is the first record of the species in adult mosquito trapping during continuous trapping at the site between 2010–2022. Recent records from further afield from the Kent/Essex foci are few, consisting of the record on the south coast in Arne and from fens at Wicken and Ely (Medlock and Vaux, 2012; Welch, 2022), with larvae found in Cambridgeshire and only a single adult female in the Dorset site despite targeted larval searches (J. Medlock and A. Vaux, unpublished data). This suggests the species may not be established in Poole harbour, as there have been no further records at Arne during subsequent years of larval and adult sampling at this site (Medlock *et al.*, in press).

The activity of adult and immature stages reported here is similar to previous published records (Golding *et al.*, 2012; Vaux *et al.*, 2015) suggesting that there has been little additional expansion over recent years although the broader range of data presented here allows for a fuller appraisal of the seasonality of larval and adult activity, which is important when considering disease risk and mitigation. A previous study at Cliffe Mead Wall reported 97% (2,509/2,550) of all female mosquitoes caught at a Mosquito Magnet trap in August and September were *Cx modestus* (Vaux *et al.,* 2015), and in this study proportions of *Cx. modestus* were similarly high (91%; 2,647/2,899), showing little change in the assemblage of adult mosquitoes at this site. Whilst activity and peak abundances showed some variability across sites and years, sites in North Kent in 2019 showed consistently highest larval abundances in week 31 (late July – early August), although high larval densities were also recorded in late September on one occasion in Essex. Across all years, immatures were recorded from the second week of July, with the latest record at the end of October. The earliest adult record was from the first week of April, and the latest from mid-October. Predicted count data, modelled using larval data from 2015–2016 and Nationwide mosquito project data (2016–2022) showed highest adult counts in late July, and highest larval counts in early September. The data presented here support the literature on the life-history of the species, given its overwintering strategy of diapausing as inseminated adult females (Rudolf *et al.*, 2020a). It is likely that the April adult records are those of overwintered females, recently emerged from their hibernacula and in search of a bloodmeal, before oviposition and subsequent larval development. Larval development appears to be continuous until the end of the season, suggesting oviposition through the season by recently emerged females. Few adult females are recorded at traps later in the season post larval peak, suggesting that those recently emerged adults are not host-seeking, but instead likely to be mating, followed by females seeking hibernacula for the winter. This raises the possibility of a temperature or daylight hour threshold at which emerged females cease host-seeking and oviposition. The data presented here shows that the species maybe univoltine in the UK.

The analysis of ecological characteristics at sites where *Cx. modestus* occurs in North Kent indicate five variables that showed a significant correlation with larval abundance, and two further variables were included in the analysis to improve model fit to the data. In the predicted data, *Cx. modestus* showed a preference for shallow, narrow ditches, with a gentle slope angle, an abundance of vegetation and warmer water temperatures. *Culex modestus* larvae were only found in ditches less than a metre in depth, the majority were found in ditches less than 60 cm deep, and in ditches of between 1–4 metres in width. Temperature was a significant factor at all sample dates and narrow, shallow ditches, with gentle slope angles may heat up faster than wider, deeper ditches, and the results suggest that in North Kent, the species has a preference for warmer aquatic habitats. Gentle slope angles also provide a range of depths for emergent vegetation to establish, and this may also be an important factor for *Cx. modestus* larvae, perhaps by providing shelter from larval predation, or perhaps provision of larval food sources. A non-significant inverse relationship could also be found between *Cx. modestus* and the amount of shade over the ditch. Water temperature has been previously reported to be significantly associated with the presence of some Anopheline species (Fillinger *et al.*, 2009; Shililu *et al.*, 2003) and given its high abundances in Mediterranean countries, it is not unexpected that *Cx. modestus* would be more likely to be found in warmer waters.

Previous studies have reported *Cx. modestus* to be associated with reedbeds and rice fields, which contrast with this study where no significant relationship was shown with the presence of reeds. The positive association between *Cx. modestus* abundance and the abundance of vegetation in the water (free-floating, submerged and/or algae vegetation) is consistent with previous work (Golding *et al.*, 2015). It maybe that water vegetation allows the sun to heat up a layer of the water on the surface, providing an additional thermal input favouring *Cx. modestus* larvae. The UK does not have rice fields, however the main sedge species found in the study area was *Bolboschoenus maritimus*, which has been reported as a weed species in rice fields in India (Cook, 1996) which would indicate these sites share some characteristics with rice fields. *Culex modestus* may be being influenced to some degree by the structure of the vegetation. *Bolboschoenus maritimus* and rice both grow in clumps, forming densely vegetated patches with large gaps between these clumps; the clumped, dense vegetation may provide protection from predators, as is provided by other abundant free-floating, submerged and/or algae vegetation in *Anopheles* (*Ano.*) *pseudopunctipennis* Theobald, 1901 larvae (Bond *et al.*, 2005). This contrasts with reeds which are single stemmed, so only provide the same level of protection from predators when densely packed. Whilst sedge was not found to be significantly associated with *Cx. modestus*, this could have been because abundances were combined between the bank and the ditch, whilst in reality only sedge vegetation in the ditch would have any direct impact on larvae.

It is interesting to note that pH did not appear to have a significant effect on the abundance of *Cx. modestus*. The pH of the water varied across the sites, with *Cx. modestus* being found in waters with a pH of 7.2 (neutral) – 10.2 (alkaline). Since few sample points were visited with a pH outside of this range, it is possible that *Cx. modestus* could also be found in sites with a higher or lower pH. No significant correlation was found between *Cx. modestus* and salinity, with larvae found in waters ranging from 0.2 ppt – 7.2 ppt (though only two sites were above 4.2 ppt). The findings suggest that *Cx. modestus* is tolerant of both freshwater and brackish waters, as previously reported (Becker *et al.*, 2010). Further work would be required to determine whether C*x. modestus* is tolerant of higher salinities.

The data presented here can used to make recommendations for entomologists targeting *Cx. modestus* larvae. Water temperature had the strongest relationship with larval abundance, followed by depth, slope angle, and water vegetation. Entomologists targeting *Cx. modestus* in the UK should therefore focus on warm water habitats (+25 °C), shallow slope angle (<10°), shallow depth (<50 cm), and an abundance of vegetation on the water (>75% coverage).

The grouping of *Cx. modestus* close to *An. maculipennis s.l.* fits with the functional groups concept of both species found in permanent ditch habitat and supports previously published species associations (Golding *et al.*, 2015). Whilst often co-located with *Cx. pipiens* in ditches, larvae of *Cx. modestus* are rarely found in transient temporary waterbodies utilised by *Cx. pipiens*, although *Cx. modestus* have been reported from wet grassland adjacent to flooded ditches in Wicken Fen (Medlock and Vaux, 2012).

This paper includes new data from adult traps at sites across England, including those targeting *Cx. modestus* specifically, larval abundance data at sites where the species is known to be present, and characterises the preferred aquatic habitat. In North Kent, *Cx. modestus* shows a preference for warm, permanent, shallow, well-vegetated ditches, and this can be used to predict suitable habitat in other regions of England. However, it is notable that the species was not recorded at 60 out of 74 sites where a Mosquito Magnet trap was run, and whilst some of those sites had little in the way of suitable habitat, many of them did, supporting the map presented here as likely to represent the actual distribution of the species.

The location of significant populations of *Cx. modestus* is an important factor in assessing the human risk of WNV in the UK, given the likely role the species would play as a bridge vector. Provision of up-to-date maps allow clinicians in areas where the species is present to be mindful of increased risk of WNV transmission. Current distribution maps also provide data to local authorities who in the event of a WNV outbreak may need to enact mosquito control measures targeting key vectors such as *Cx. modestus*. The success or failure of mosquito control interventions is contingent on knowledge of the preferred aquatic habitats of the species, so that operators can focus application of larvicidal control in specific locations. In this case, focusing application of larvicide in narrow, shallow, vegetated permanent ditches would, in North Kent, enable the majority of the population to be controlled. Key to this would be timing of the intervention, and the data presented here show that early application of larvicide in late-April – early June, would enable the targeting of early season larvae, thus preventing the population developing further through the summer, and minimising the amount of time spent and product used. Furthermore, activity by larval instars showed that stages II–III were active from week 27–38, meaning that focussing treatment using *Bacillus thuringiensis israelensis* in those weeks would be most effective at controlling the species, given that stage IV larvae are less severely impacted due to lower feeding rates. The potential for WNV transmission is enhanced by the alignment of the peak of *Cx. modestus* activity and warmest summer temperatures in July and August, and and maybe further enhanced by the increasing frequency of heatwaves and warmer summers.

## Supplementary material

Supplementary material can be found online at https://doi.org/10.6084/m9.figshare.25398670.

**Table S1.** List of variables recorded at each site (2015/2016), shown with measurement units, and instruments used.

**Table S2.** Locations of recorded presence (shown in bold) / absence of *Culex modestus* at sites shown in Figure 7, where adult sampling (Mosquito Magnet, MM), incidental landing catch (LC) or larval sampling (L) was conducted.

**Table S3.** The five models with the highest AICc score.

**Figure S1.** Mean number of females per trap night (*Culex modestus*; all other species grouped as 'Other') for Cliffe Fort, Mucking, Northward Hill, Rainham Marshes, Wallasea, and Wat Tyler, shown by calendar week for years 2016–2021.

**Figure S2.** Dendrogram showing clustering of mosquito species.

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The authors would like to thank landowners, environmental health officers, wetland and nature reserve managers and volunteers who participated in arranging access or running mosquito traps as part of this study; in particular those from Natural England, the Royal Society for the Protection of Birds, and the Wildlife Trusts. AV was part-funded by a Wilke Calvert PhD Studentship through the University of Reading.

#### Conflict of interest

Jolyon Medlock is associate editor of the Journal of the European Mosquito Control Association; he had no influence in the review process and decision making on this manuscript. The other co-authors declare no conflict of interest.

#### Data availability

The detailed data that supports the findings of this study is available upon contacting the authors.

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