The factors which influence the breeding and number of *Aedes detritus* in the Neston area of Cheshire, UK, the production of a local mosquito forecast and public bite reporting.

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Abstract: *Aedes detritus* is an important cause of biting nuisance in the Neston area of Cheshire, UK and the Dee estuary is the site of the breeding mosquitoes. We report on a study of the ecology of *Aedes detritus* on the Dee estuary for the 9 years from 2011 to 2019. We describe the annual, seasonal and geographical distribution of the adult and immature mosquitoes, with peaks in late Spring and early Autumn, and how these are explained by the characteristics of the small breeding pools on the upper levels of the marshes, and their filling by high spring tides. We demonstrate that pool excavation to enlarge and deepen pools greatly reduces breeding. From these studies, we developed an online weekly forecast of the local biting nuisance, and report on the temporal and geographical distribution of the bite reports produced by the public in response. ***Journal of the European Mosquito Control Association*** **38: 17–32, 2020**

Keywords: *Aedes detritus,* seasonal influences, pool excavation, biting nuisance and forecast

Introduction

Neston is a civil parish in the county of Cheshire, UK, and consists of Neston, Little Neston, Parkgate and Ness (population circa 15,000). The tidal estuary of the River Dee forms the western boundary (Figure 1a) and is a United Kingdom Site of Special Scientific Interest and a European Union Special Area of Conservation, largely due to its flora and fauna and especially the enormous populations of birds, many migratory. Most of the marsh area is owned or managed by the Royal Society for the Protection of Birds as part of their Dee Estuary Nature Reserve. (Figure 1).

The nuisance caused by biting mosquitoes has been recognised in the Neston area since 1983, described by Davies (1995) who stated that the species responsible was *Aedes (Ochlerotatus) detritus*. He also described the measures taken to attempt to control the problem by spraying extensive areas of the Dee estuary, which had become increasingly silted up, giving rise to a large number of pools which were ideal for the mosquitoes to breed. Clarkson and Setzkorn (2011) described the results of surveys over the 4 years from 2007 to 2010 of the adult mosquitoes of this area using different types of traps and from specimens caught by residents. This showed that *Ae. detritus* was the most common species of mosquito associated with gardens, homes and schools and was virtually the only mosquito responsible for biting nuisance. These workers analysed the complaints received by the Local Authorities responsible for Neston and showed that these numbers varied from year to year and seasonally throughout the year. In general, the majority of complaints were received in September and October, though in one year, 2005, a significant number were received in May.

Since it is known that the only breeding sites in the Neston area for these mosquitoes, which require salt water pools, is on the Dee estuary, a logical step forward in the study was to examine the immature stages in these pools throughout the year. This study reports on the numbers and species of larvae and pupae of mosquitoes, predominantly *Ae. detritus*, in identified sites over the 9 years of 2011 to 2019, and more broadly in other areas of the marsh, and examines the influence of weather (temperature and rainfall), tides and marsh pool topography on these numbers. Trapping of adult mosquitoes was done in different sites in the Neston area and the results from these traps are also presented, and preliminary work on assessment of the biting nuisance to the local human population.

Materials & Methods

The topography of the marshes and their tidal channels of varying sizes and filling/drainage patterns are such that there is a wide variation in the size, depth and cross-sectional shape of the pools at any one time, let alone the variation which comes with time and in particular following recent very high tides and any persistent heavy rain. Though the marshland looks quite flat to the naked eye, variations in the height of the ground (and so also the bottoms, "lips" and filling channels of the pools) are readily apparent from observations of the filling that comes with tides of different heights.

In summary, there are three main categories of pools (Figure 2):

A. Well-delineated pools which have vertical edges, which tend to be deep (up to about 40cm) and usually large (e.g. at least 10 x 30m) (Figure 2a). When the content of these pools was increased by the tide or rainfall, their area did not increase significantly but the depth of the pools increased. These often do not dry out completely in the summer. only when hot dry weather persists for some time (Figure 2b).
B. Pools were not so clearly delineated, so that they increased in area when they were filled by tide or rainfall as well as increasing in depth. They have

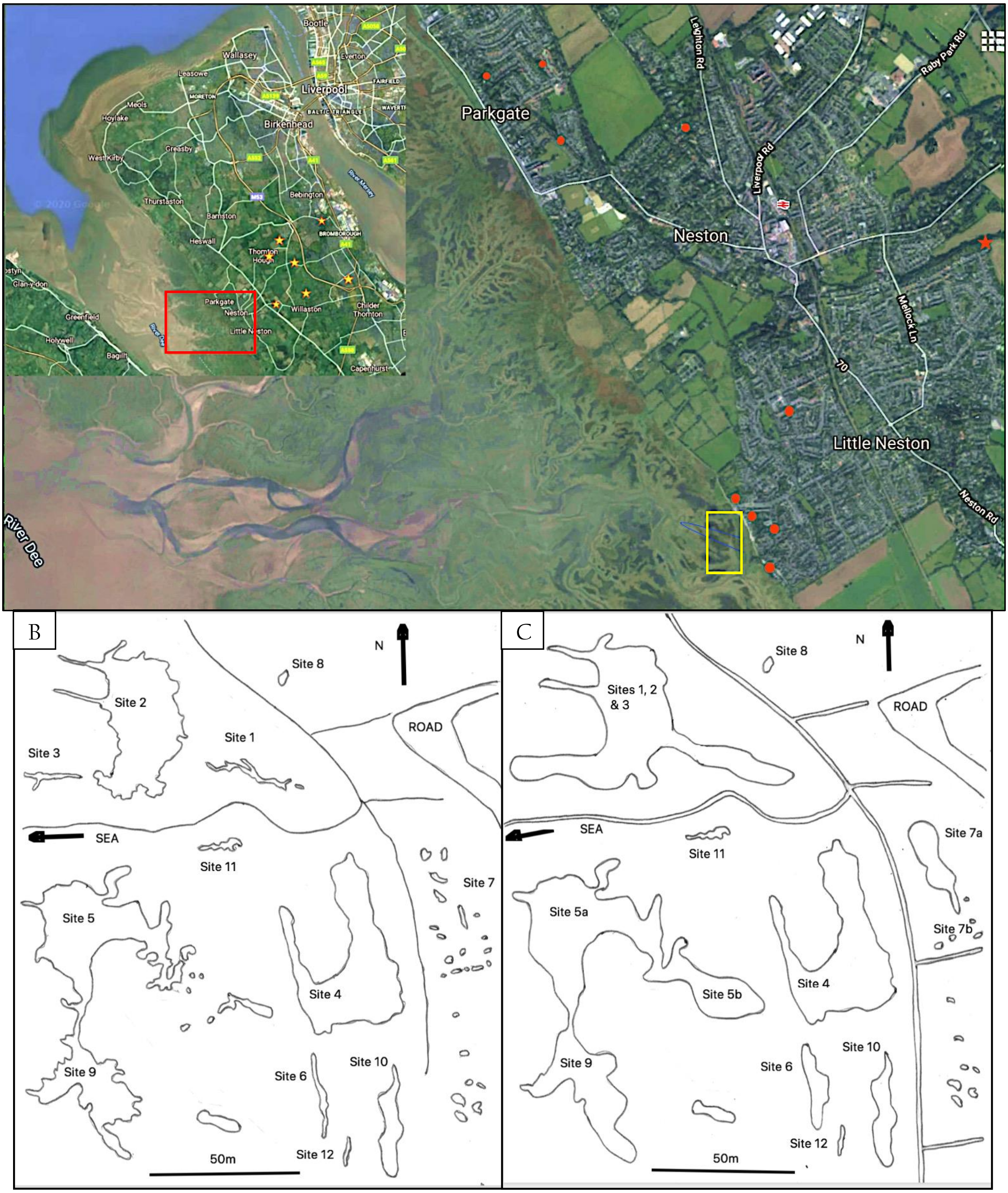

Figure 1: (A) Map showing Dee estuary at Parkgate, Neston and Little Neston, with location of Study Pools indicated by yellow rectangle, and adult trap sites by red circles, and constant site by red star. Inset map shows smaller scale map of Wirral peninsula between River Dee and River Mersey, with Parkgate, Neston and Little Neston area of larger scale map shown in red rectangle. The yellow stars show more remote sites of traps in 2014. Line drawings of Study Pools before (B) and after (C) excavation work.

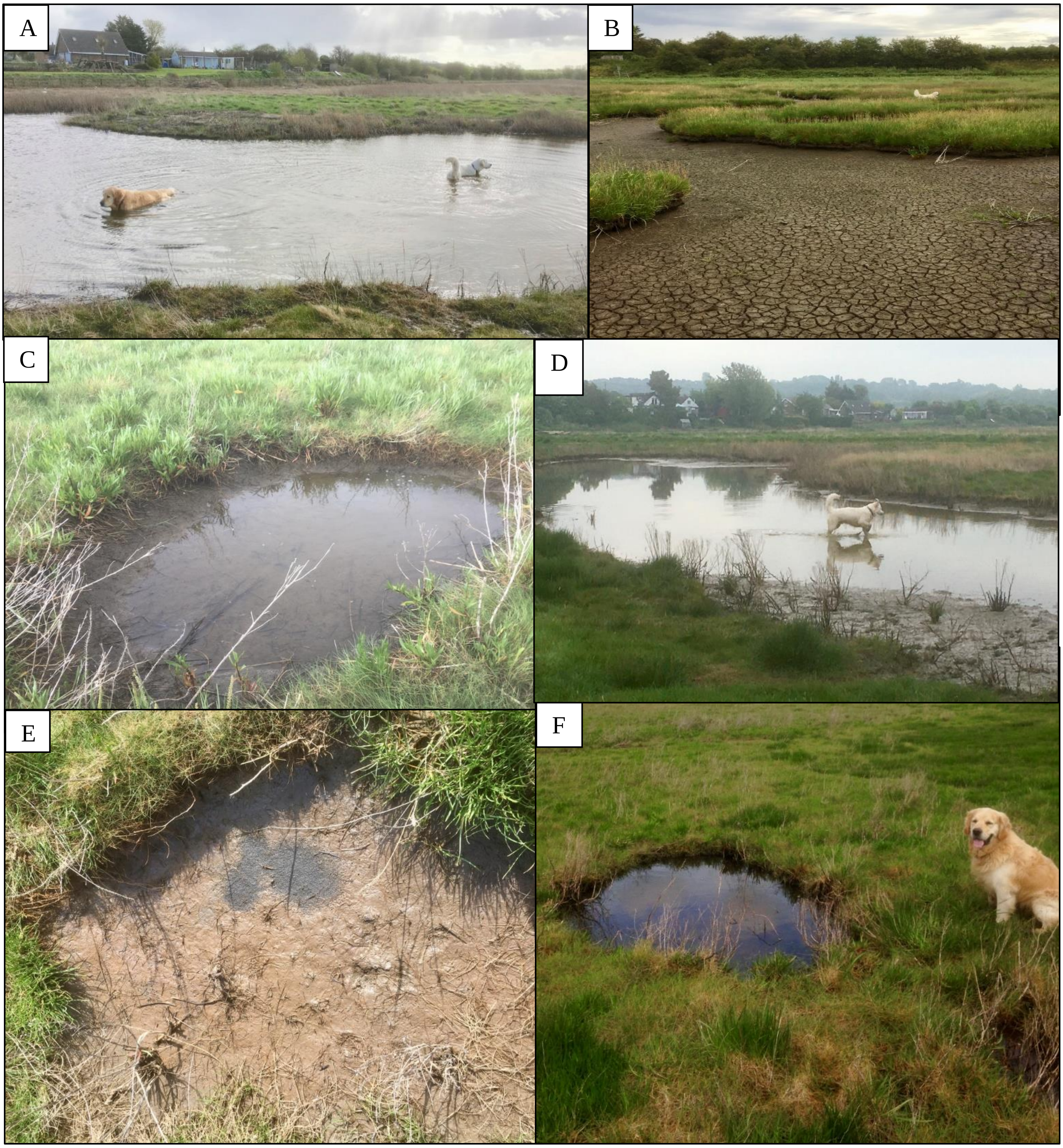

Figure 2: A: category A pool, deep and large. B: category A pool, dried out after prolonged summer drought. Note flat bottom and steep sides. C: same category B pool as in f, beginning to dry out. Note shallow and sloping sides exposing mud as it dries. D: category C pool, shallow sheet of water, easily drying out even in autumn. E: category B pool which has dried out too soon for larvae to mature. Black area is the lowest point of the pool where the larvae concentrated before desiccation. F: same category B pool as in C, full after a 10m high tide.

sloping edges and bottoms, and tend to be small (e.g. 1 x 2-3 m) (Figure 2c & 2f). If situated in the higher marsh areas, these tend to fill only with the very high tides of Spring and Autumn (Figure 2f), or after very heavy rain. They may dry out between these tides (Figure 2e), exposing mud (upon which eggs may be deposited) and then be re-filled by the next spring tides of Spring or Autumn or heavy rain, up to a depth of about 25cm.

C. Much bigger expanses (e.g. 30 x 20 m) of shallow (e.g.10-20cm) water which arise only after very high tides or heavy rainfall (Figure 2d). These invariably dry out quickly (days to 2 - 3 weeks) unless sustained by rain. Some may have Category B pools within them.

The sites, designated 'Study Pools', were selected to represent different types of pools in order to identify the sites which were most suitable for the development of the immature stages of *Ae. detritus*. Although the initial intention was to use the same sites throughout the study, this proved to be impossible as alterations were carried out by the Local Authority in attempts to reduce the suitability of the area for breeding mosquitoes. However, similar sites were added each year whenever significant changes were made to the ecology of the pools, and there were some that could be studied for all nine years. All sites throughout the study were in the same area of the marsh at its extreme east end adjoining a road and houses (Grid 53.278031, -3.068987). The total area was approximately 200 x 175 metres. The situation and sizes of all the sites are indicated on Figure 1 before (Figure 1b) and after (Figure 1c) the main local authority excavations of 2012, and summarised in Table 1. Other sites on the marsh which were not dipped so regularly or systematically were designated 'Other Pools': they included many Category A and B pools but also large shallow expanses of water shortly after very high tides which usually rapidly evaporated before immature forms could pupate (Category C pools).

In April 2011, six sites were selected, numbered 1 to 6 on the map. Sites 1 and 3 were of the Category B and connected with site 2 which was of Category A. However, the channels joining sites 1 and 3 to site 2 were shallow and frequently dried out, when they became separate sites. Site 4 was Category A. Site 5 had an irregular outline with a main part similar to sites 2 and 4 as Category A and other pools radiating from it which became separate pools when they commenced to dry and were then Category B pools. Site 6 was Category B. Site 7 was added in August 2011 and consisted of 12 separate small pools, parts of which were well-delineated but all had significant parts which had shallow margins so that they increased in size and also in depth if they were filled so were considered to be Category B pools. The pools in all sites varied in depth depending on tide and rainfall and had variable depth across their contours so that they dried out in an irregular fashion but generally around the edges and progressively towards the centre. The depths were a maximum of 40 cm to a minimum of 10 cm when they were full of water.

Table 1: Summary of "Study Pool" Sites 2011-2019. 'A' indicates category A pool. 'B' indicates category B pool. Shaded areas indicate pool dipped that year. Black vertical lines indicate excavation conducted.

Pool Site no.	2011	2012	2013	2014	2015	2016	2017	2018	2019
1	B joined by shallow channel	B joined by shallow channel	A	A	A	A	A	A	A
2	A joined by shallow channel	A joined by shallow channel	A	A	A	A	A	A	A
3	B	B	A	A	A	A	A	A	A
4	A	A	A	A	A	A	A	A	A
5	A + irregular side B pools	A + irregular side B pools	A + irregular side B pools	A joined to Pool Site 9 and others to form 5a (Cat. A) & 5b (Cat. B)	A joined to Pool Site 9 and others to form 5a (Cat. A) & 5b (Cat. B)	A joined to Pool Site 9 and others to form 5a (Cat. A) & 5b (Cat. B)	A joined to Pool Site 9 and others to form 5a (Cat. A) & 5b (Cat. B)	A joined to Pool Site 9 and others to form 5a (Cat. A) & 5b (Cat. B)	A joined to Pool Site 9 and others to form 5a (Cat. A) & 5b (Cat. B)
6	B	B	B	A	A	A	A	A	A
7	12 small B pools	12 small B pools	12 small B pools	One large A pool = Pool 7a	One large A pool = Pool 7a	One large A pool = Pool 7a	One large A pool = Pool 7a	One large A pool = Pool 7a	One large A pool = Pool 7a
				6 remaining small B pools = Pool 7b	6 remaining small B pools = Pool 7b	6 remaining small B pools = Pool 7b	6 remaining small B pools = Pool 7b	6 remaining small B pools = Pool 7b	6 remaining small B pools = Pool 7b
8		B	B	B	B	B	B	B	B
9			A	A joined to Pool Site 5	A joined to Pool Site 5	A joined to Pool Site 5	A joined to Pool Site 5	A joined to Pool Site 5	A joined to Pool Site 5
10			B	B	B	B	B	B	B
11			B	B	B	B	B	B	B
12									B

In 2012, all 7 sites were examined regularly and site 8, a Category B pool, was added in September, 2012. In June 2012, over 1000m of the main tidal channel and side channels and gutters were dug out to a width of 2m and a depth of 1m to cause the pools to be flushed out more easily by the tides. These excavations made it difficult and sometimes dangerous to reach sites 1 to 3 and observations on them were discontinued in October 2012. In March 2013, site 9, a pool like site 2, site 10, a pool like site 3, and in September 2013, site 11, a pool like site 3, were added to the sites examined. In November 2013, the Local Authority carried out more extensive excavations which resulted in larger deeper pools in several areas and necessitated amendments to the sites examined in 2014 and 2015 (see Figures 1b and 1c). Sites 1 and 3 were excavated so that they formed part of site 2. Site 4 was unchanged but site 9 was combined with site 5 and excavated into a deep pool, designated site 5a and several branch pools, designated site 5b. When these pools were filled 5a and 5b were contiguous but as the water level fell, they became separated and formed distinct pools. Site 6 was excavated so it became a deeper pool with vertical sides. Six of the small pools which were part of site 7 were combined and excavated with vertical sides, designated site 7a, while the remaining pools were unchanged and designated 7b. Sites 8, 10 and 11 were unchanged.

The Study Pools were examined weekly during April to October inclusive and occasionally during the rest of the year. In addition, the pools were often visited at the times that high tides were forecast to see how far the sea reached into each pool.

The water in each pool was examined for larvae and pupae by sampling with a 500 ml dip in five different parts of each pool. The numbers of larvae and pupae were counted separately and the results from the five samples pooled and allocated into four categories i.e. 0 (zero), where no immature stages were seen; 1, with one to nine stages; 2, with 10 to 100; and 3 when more than 100 immature stages were counted. These semi-quantitative methods allowed a rapid estimate of the numbers of larvae and pupae to be made for each site. Comments were included in the recording sheets concerning the quantity of water in the pool and the appearance of any muddy areas at each site. From 2014, an estimate of the larval instars was made, based on the size of the larvae. Virtually all assessments were performed by one examiner throughout (MC). For analysis and weighting to produce a "score" for graphical purposes summing across sites, 1 was scored as 5, 2 as 50 and 3 as 100.

Assessment of other areas of the marsh

Other areas of the marshes up to 3km around were studied less systematically by regularly walking at all times of the year to study other pools and in particular their degree and method of filling by the tide at different times, building up an extensive knowledge of the marsh. Depth of water could be assessed by wading depth (or sometimes swimming) of two large Golden Retriever dogs. At least 70 pools were studied in this way. Examination for larvae and pupae was usually undertaken, and samples for breeding to adult stages for identification and other purposes were taken from 28 different pools in addition to the Study Pools.

Sampling of larvae & pupae

In 2019, sampling included collection of larvae and pupae which were bred to adults for identification purposes. They were kept in their original water, sometimes topped up with rainwater, and fed on yeast, in an unheated garage with a north-facing window. Emerged adults were caught in netting and placed in a domestic deep freeze prior to identification.

Trapping of adult mosquitoes

Adult mosquitoes were caught throughout the study period in different places in the Neston area (Figure 1a) using Mosquito Magnet traps with octenol attractant tablets. One trap location was constant throughout (marked by red star in Figure 1, latitude 52.2894, longitude -3.0480) about 2km from the marsh. Others were placed at different locations in different years, but with always one within 200m of the edge of the marsh (except 2016). In 2018 and 2019, two additional traps were added in Parkgate, one 50m and another 1.2km from the marsh.

Mosquito identification

Adult mosquito species were identified with the keys in Cranston et al (1987) and Snow (1990). For *Culiseta litorea* and *Cs. morsitans* species, the distinction between them was in some cases supplemented by identification of males by their genital morphology. No attempt was made to separate *Culex pipiens* from *Cx. torrentium* in the trapped specimens and are referred to as *Cx. pipiens* s.l. However, most but not all adults identified as *Cx, pipiens* which were bred from larvae were subjected to conventional PCR and an enzyme digestion protocol following the protocol proposed by Hesson et al (2010), with some modifications, to distinguish *Cx. pipiens pipiens* and *Cx. torrentium* (data to be presented later).

Sources of other data

Tidal data were obtained for confirmed tide heights at Liverpool Gladstone Dock from databases of The British Oceanographic Data Centre. Weather data were obtained from the database of National Centre for Atmospheric Science at Ness Botanic Gardens, (Elev: 43 meters, Lat: 53-16.20 N Long.: 003-03.00 W), about 1.5km from the marsh sampling site.

Table 2: Numbers (and percentages) of trapped mosquitoes each year. Others include *Ae. rusticus, An. maculipennis* s.l, *Ae. geniculatus, Ae. cantans*

Year	*Ae. detritus*	*Ae. caspius*	*Culex pipiens* s.l.	*Culiseta annulata*	*An. plumbeus*	*An. claviger*	Others	Total
2012	3013 (82)	90 (2)	21 (1)	320 (9)	12 (0)	194 (5)	39 (1)	3689
2013	999 (94)	13 (1)	18 (2)	11 (1)	10 (1)	17 (2)	12 (1)	1068
2014	639 (94)	17 (3)	2 (0)	2 (0)	17 (3)	0 (0)		677
2015	569 (86)	77 (12)	0 0)	2 (0)	12 (2)	2 (0)	2 (0)	662
2016	248 (89)	4 (1)	4 (1)	14 (5)	5 (2)	3 (1)		278
2017	175 (69)	47 (19)	3 (1)	21 (8)	4 (2)	2 (1)	1 (0)	252
2018	855 (88)	14 (1)	4 (0)	41 (4)	44 (5)	9 (1)	5 (1)	967
2019	947 (82)	10 (1)	67 (6)	23 (2)	45 (4)	58 (5)		1150
TOTALS	7445 (85)	272 (3)	119 (1)	434 (5)	162 (2)	272 (3)	39 (1)	8743

Results

Adult mosquitoes

Number and species of trapped mosquitoes

In total, 8743 mosquitoes were trapped (Table 2). The vast majority (85%) were female *Ae. detritus*. There were some *Ae. caspius, Cx. pipiens* s.l., *Cs. annulata, An. plumbeus and An. claviger*, and a few *Ae. rusticus, Ae. geniculatus, Ae. cantan*s and *An. maculipennis* group. No *Cs. litorea* or *Cs. morsitans* were caught.

Annual variation in trapped mosquitoes

The number and location of trap sites, and the duration and annual timing of trapping sessions were too varied to express meaningful year-to-year comparisons in total numbers. However, one site (marked by red star in Figure 1, latitude 52.2894, longitude -3.0480) was constant throughout the years 2013 to 2019: Figure 3a shows that although there is an autumn peak every year, the time it appears varies each year from week 33 (mid-August) to week 43 (mid-October). Similar year-to-year variation was seen at other individual trap sites used for fewer years.

In all years, *Ae. detritus* was the greatly predominant species at every site.

Seasonal variation in trapped mosquitoes

Each year, there were marked month-to-month variations in the numbers of mosquitoes trapped. Less numerous species are considered later, but the numbers of *Ae. detritus* usually showed few mosquitoes in summer months but consistent surges in Spring and late Summer/early Autumn, both in individual (Figure 3) and in aggregated trap results (Figure 4a). As will be demonstrated later, this distribution was also evident in the pool dipping results for larvae and pupae, both for individual pools and aggregated results (Figures 7 & 8). Superimposed upon these Spring and early Autumn peaks, in particularly wet summers, there were significant numbers trapped between these peaks (Figure 4b).

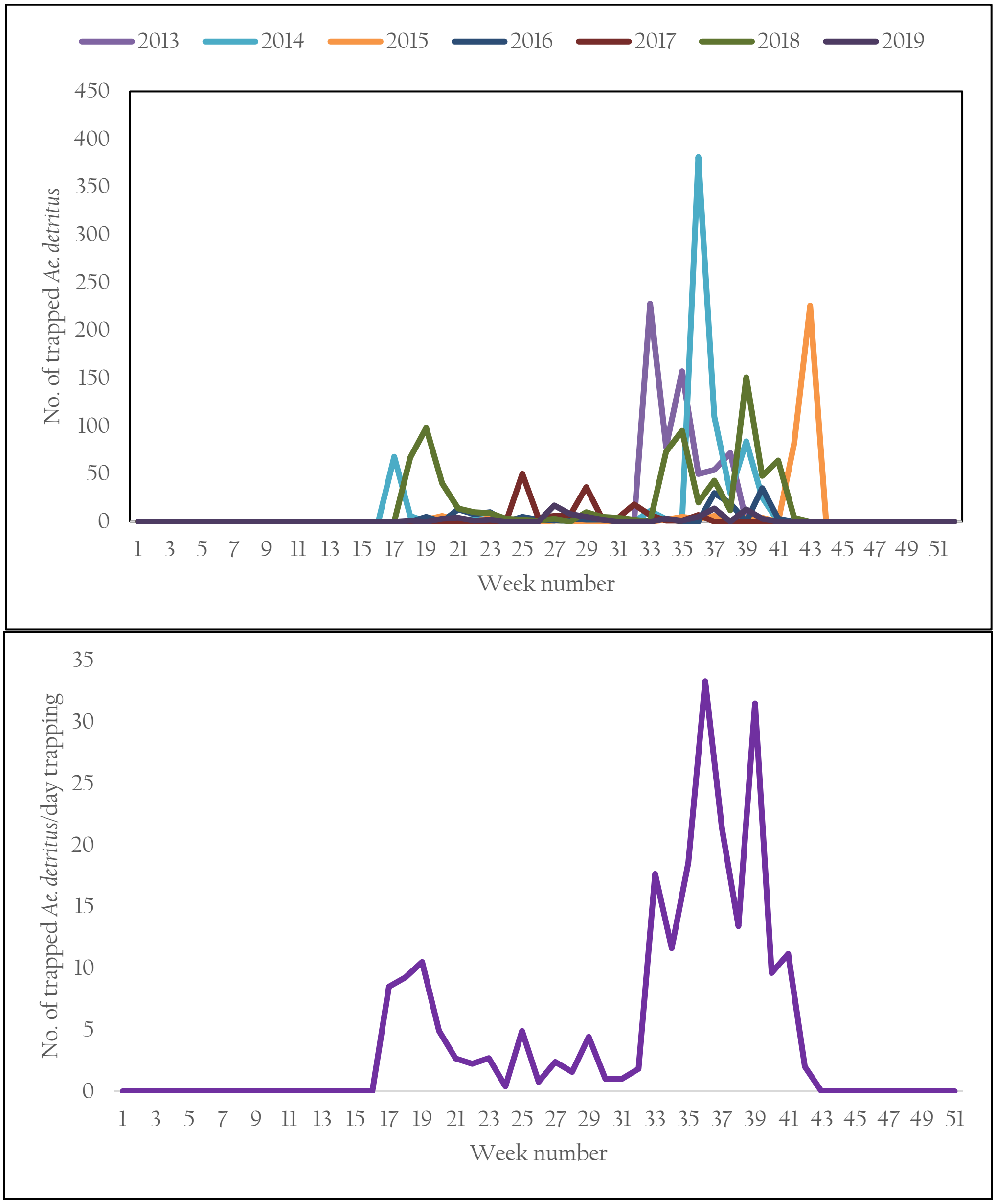

Figure 3: Number of *Aedes detritus* trapped in one trap (Trap 2) in years 2013-2019, showed by year (above), and from all data from 2013-2019.

Number of mosquitoes trapped at different sites

Traps located 1-2 kilometre away from the marshes collected more mosquitoes than those within 30-200m of the edge of the marsh. Over the period between 2013 and 2019, the number of *Ae. detritus* trapped in sites near the marshes was 1096 compared to 3870 trapped over the same days in sites 1-2km from the edge of the marsh. This was evident in every year except 2016, when there was no trap run near the marsh. Comparing numbers in individual weeks between the sites, the number of *Ae. detritus* in sites near the marsh exceeded those distant from the marsh in 40 weeks, whereas the number in the distant trapping sites exceeded those near the marsh in 77 weeks (Figure 5). This may vary at different times of the year but will require further study to confirm and clarify.

Distance travelled by mosquitoes

Over two weeks in September 2014, traps were run on consecutive days at different sites for 24 hours each at increasing distances from the marsh in a north-easterly direction (yellow stars, Figure 1a). *Ae. detritus* were caught in every trap to Bebington, 8 km north-east of Neston. As far as we are aware, there are no *Ae. detritus* breeding sites north of Parkgate on the Dee estuary, and none on the Wirral or Liverpool shores of the Mersey estuary where there are twice daily tides and no static pools. It is therefore concluded that *Ae. detritus* can travel at least 8 kilometres (presumably on the prevailing south-westerly wind).

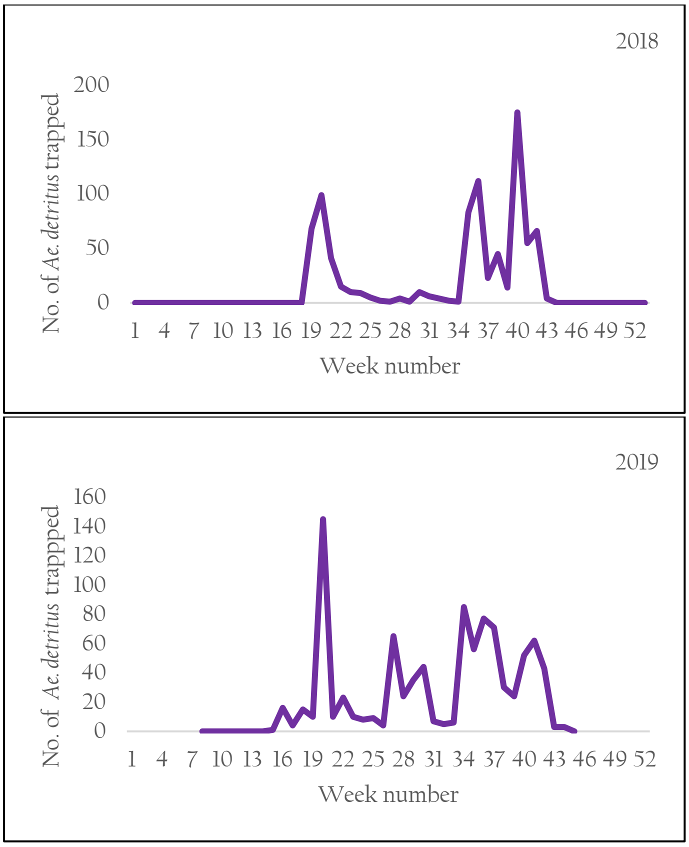

Figure 4: Distribution of trapped *Aedes detritus* each week in all traps 2018 (dry summer; above), and 2019 (wet summer; below).

Immature stages of mosquitoes

Number and species of larvae and pupae

The scoring system used for counting larvae and pupae was semi-quantitative and allowed comparisons of numbers with time rather than representing absolute numbers of larvae in the Study Pools (often in the hundreds or even thousands in each pool), let alone the many hundreds of marsh pools where mosquitoes may breed.

The pool dipping and larval breeding work in 2019 established the species of mosquitoes breeding on the marshes in general and in the Study Pools in particular and an estimate of their percentages (Table 3). These percentages vary slightly with time of year and whether pools on the very edge of the marshes (which may have freshwater streams nearby) were included. However, in all estimates, *Ae. detritus* is overwhelmingly predominant: for all the pools dipped across the marshes ('the other pools') in 2019, 85% were *Ae. detritus* whilst in the Study Pools, *Ae. detritus* were 92% of all adults emerging from larvae samples. In a few pools some *Ae. caspius* were also found in summer months, always mixed with *Ae. detritus*. Other species occurred at certain times of the year in a few pools at the very edge of the marsh where freshwater streams off the land may have contributed and presumably reduced salinity: in winter, *Cs. annulata*, *Cs. litorea*, and occasional *Cs. morsitans* and *An. claviger*; in late summer, *Cx. pipiens pipiens* and a few *Cx. torrentium* (distinguished by PCR by Dr. Hernandez Colina, personal communication). No *Ae. detritus* or *Ae. caspius* were ever found in samples from 26 freshwater pools away from the marshes (up to 8km away).

Table 3: Numbers (and percentages) of trapped and bred mosquitoes 2019.

Year	*Ac. detritus*	*Ae. caspius*	*Culex pipiens* s.l.	*Cs. annulata*	*An. plumbeus*	*An. claviger*	Others	Total
Trapped	947 (82)	10 (1)	67 (6)	23 (2)	45 (4)	58 (5)		1150
Bred: all sampled marsh pools	2331 (85)	116 (4)	259 (9)	30 (1)	0	9 (0.3)	13 (0.5)	2752
Bred: study pools only	1631 (92)	79 (4)	64 (4)	0	0	0	0	1779

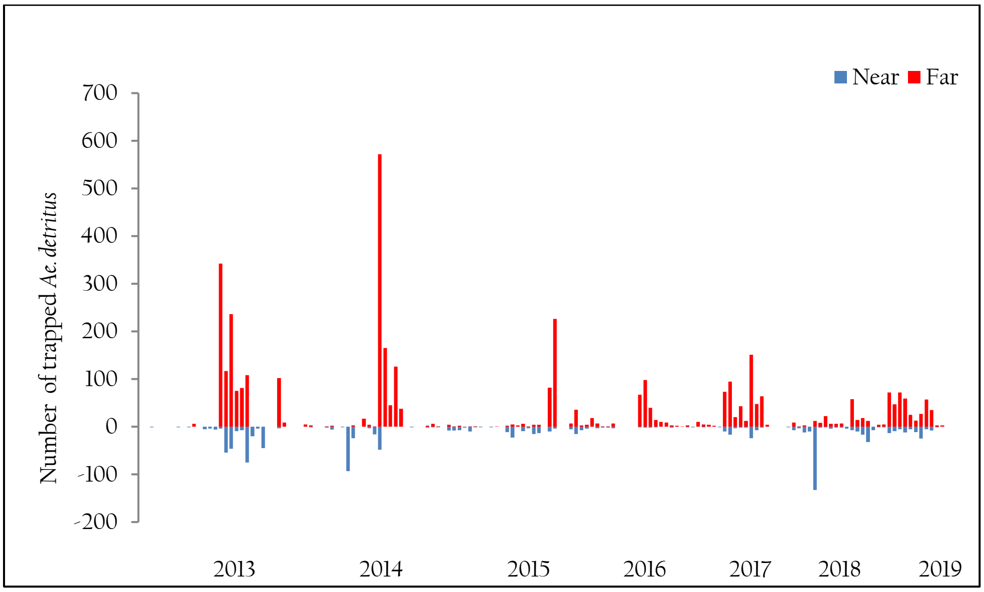

Figure 5: Number of *Aedes detritus* trapped at sites near to (<200 m) and far from (1-2 km) the marsh across different years (no near trap in 2016).

The percentages of the species bred from larvae are similar to the percentages of marsh-derived mosquitoes trapped as adults (Table 2), providing validation of the relevance of the larval and pupal data to study of the adults caught in the traps.

Annual variation in larvae and pupae

As outlined in Methods, the number and location of pools sampled had to change with time due to access problems secondary to ditch excavation by the local authority, and was also influenced by some of the pool excavations (see below). However, some individual pools were sampled in all years, and demonstrate year-to-year variation (Figure 6), as was also observed for the aggregate of pools.

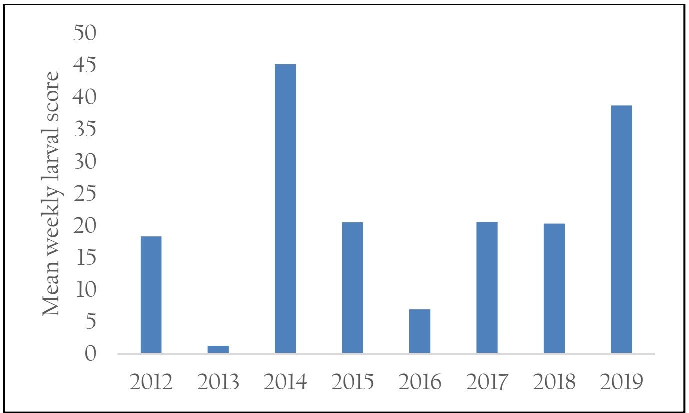

Figure 6: Variation in larval numbers in one pool (Site 8) across the years 2013-2019.

Seasonal variation in larvae and pupae

Both for individual pools and the group of the Study Pools, there was a prominent seasonal variation, with peaks in Spring and late Summer-early Autumn (Figure 7). In the months between these peaks, numbers would depend on whether there was heavy rainfall to sustain the pools (Figure 8a&b).

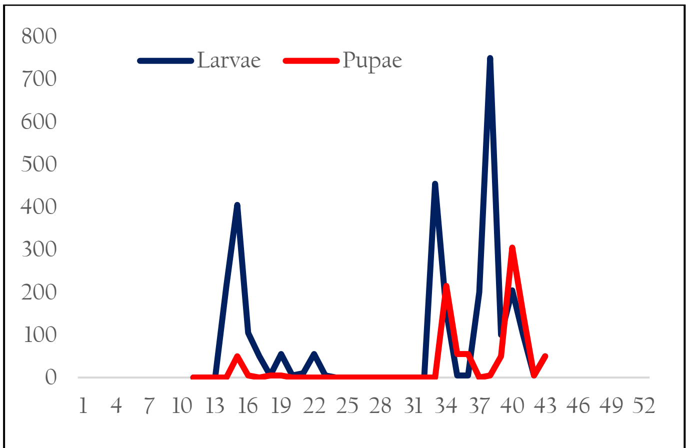

Figure 7: Distribution of larvae & pupae in Study Pools in 2015.

Breeding pools: their location, morphology and filling under the seasonal influences of tides and weather

Observations of the Study Pools and pools elsewhere on the marshes revealed a consistent but complex interplay between various factors:

1. Breeding pools were located in the upper levels of the marsh, near the landward edge, above the usual tidal range and so isolated from routine tidal flushing. Observations on the Study Pools over many years at the time of high tides showed that an actual tidal height of 9.75m as recorded at Gladstone Dock, Liverpool was needed to fill these pools. This height was routinely exceeded in the spring tides of January to April, but rarely during the months of May to July. Depending on the year, it was always exceeded in August or September and occasionally in July. Other pools nearby required a 9.9m tide to reach the threshold for tidal filling.
2. The most productive pools were category B. It was consistently observed that large deep pools (category A) contained few, if any, larvae even on those infrequent occasions when they had been partly or completely dried out in summer followed by refilling by autumnal spring tides. Category C pools sometimes contained early stages after filling by heavy summer rains, but rarely existed long enough in summer for later larval stages or pupae to be found, unless there was a category B pool within them.
3. The most productive pools were those with intermittent filling and drying, a pattern produced at certain times of the year by a combination of very high tide (spring tides) and/or weather (temperature, sunshine, wind and rainfall). There were clearly many possible permutations of these throughout the year, but the annual pattern described below could be distinguished.

Over the winter, all pools remained full after the autumnal spring tides, sustained by rainfall and low evaporation. Category B pools (but not A or C) were observed to contain larvae throughout the winter. First instar larvae could be found as late as November. Pupae were never observed in December or January, and very rarely in February. Larvae appeared to remain without developing to more advanced stages (diapause) between about November to March. Larvae collected in December and kept in an unheated garage would pupate and emerge in February or March. Larvae could obviously survive in pools in which the entire surface was iced over and could be found after breaking the pool's surface ice.

In Spring, the number of larvae in category B pools increased. Some were developing overwintering larvae, but first instars began to appear in March usually, though mild or cold weather could advance or delay this. However, inundation by a spring tide also appeared to trigger a mass synchronised hatching, even in previously filled pools. Larvae were very largely restricted to category B pools where the high tide exceeded the threshold required (see above), as would routinely occur with the spring tides of February to April. This tide height was rarely exceeded during the months of May to July and so pools would start to dry out at a rate depending on the weather and the competing influences of rainfall on the one hand and wind, sunshine and temperature on the other. Category C pools (shallow and extensive) would tend to dry out completely and quickly; category B pools completely over a few weeks; and category A pools far more slowly and incompletely. Larvae could only develop to adults if the pools remained wet long enough, and often were lost to desiccation.

Pools were next routinely filled by the spring tides of late August or early September, and were repeatedly inundated by spring tides in September and October. These tides led to mass hatching of eggs in the category B pools with the production of large numbers of first instar larvae. These larvae developed synchronously and usually led to the production of large numbers of adults some 3 weeks later. It was consistently observed that immature stages were rarely if ever seen in category A pools, even if they had been partly or completely

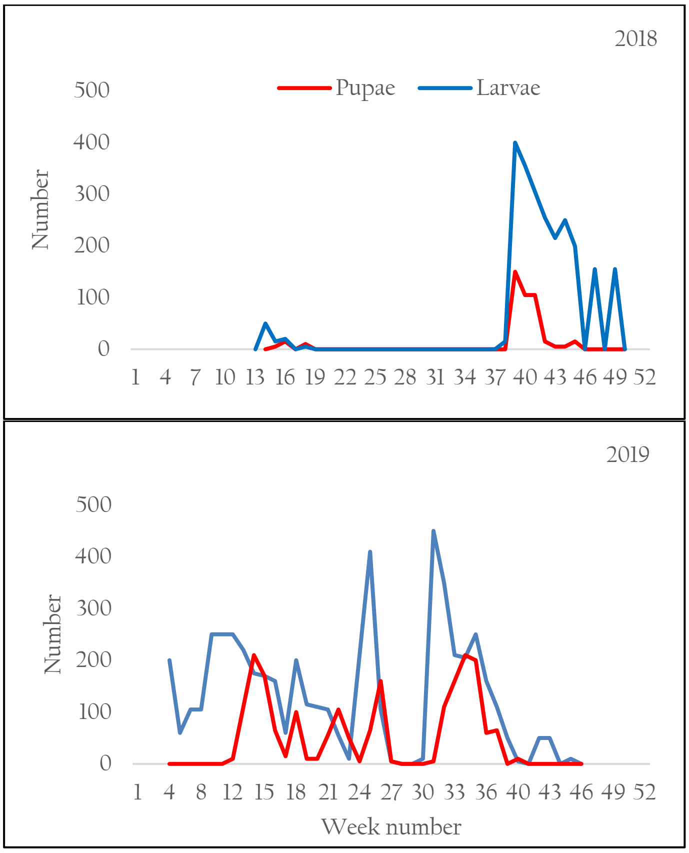

Figure 8: Distribution of larvae and pupae scores across the year in 2018 (typical dry summer; top) and in 2019 (very wet summer; bottom).

dried out (thus revealing their muddy bottoms). The timing of these high tides led to the timing of the peak of immature stages in the autumn and varied each year. During the nine years of the study, four (2011, 2013, 2015 and 2018) showed this pattern where all the category B pools dried out for many weeks during the summer and gave rise to the synchronous development of immature stages after their inundation by the autumn high tides.

In some years, exceptionally high rainfall in the summer months could fill dry pools and cause a similar mass hatching of eggs. Weather permitting, these pools may remain wet for sufficient time for adults to emerge. There were two years (2012 and 2019) when rain fell sufficiently regularly and frequently during the summer for this to occur on more than one occasion. When further rain did not fall, the pools could dry up again before adults were produced and so the lifecycle would be broken. This was occasionally seen in some of the Study Pools in 2014, 2016 and 2017 and was the usual fate of immature stages in the category C pools.

This combination of high tides, rainfall and drying out of pools makes for a complex interplay of factors and makes quantitative description and assessment extremely difficult. Figures 9a & 9b illustrates the relationships between tides, rainfall, and immature stages of *Ae. detritus* in the dry summer of 2018 and the wet summer of 2019.

A synchronised hatching of eggs in the mud, triggered by filling of a dry category B pool by a high tide or persistent heavy rain, could produce thousands of first instar larvae. Their number decreased with time and development, reflecting a natural loss. The time to emergence of adults varied according to the weather: in warm summer weather, the time to pupation could be as little as 17 days. If there was a mixed population of *Ae. detritus* and *Ae. caspius*, obviously triggered by the same filling of a previously dry pool, the emergence of the *Ae. detritus* lagged behind the *Ae. caspius* by about 2 days.

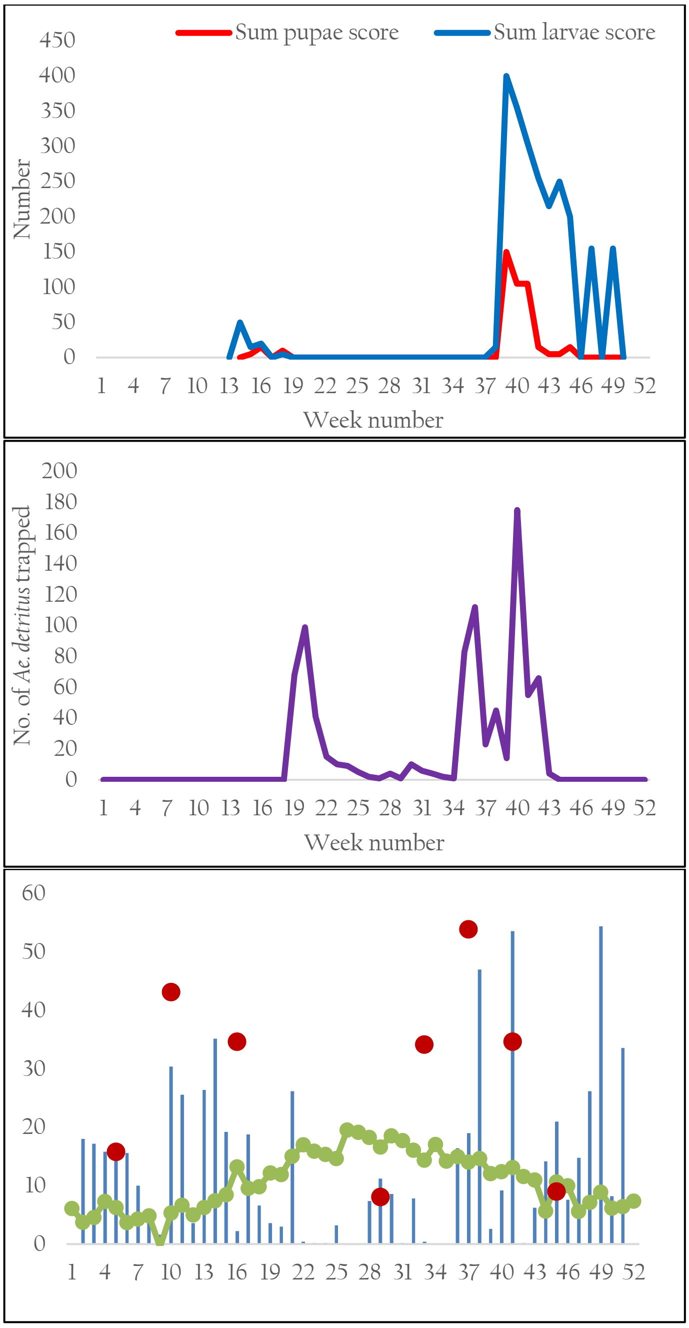

Figure 9a: The influence of very high tides (>9.75m) and weather on numbers of larvae, pupae and trapped *Ae. detritus* throughout 2018. Top = Larvae and pupae 2018. Middle = Trapped *Aedes detritus* each week 2018. Bottom = Rain, temperature, and tide heights >9.75m 2018. (Blue = weekly rain [mm]; Green = mean weekly temp (°C); Red = tide height >9.75 m)

Occasionally, a combination of spring tides (in Spring or Autumn) and strong westerly winds would produce storm surges of exceptionally high tide which would devastate the larval population, washing them out of the pools, presumably dispersing them and leaving them stranded when the tidal waters receded. Remarkably some larvae did survive inundation by even half a metre or more of tidal water on such occasions, especially in those pools with overhanging long grass at the edges.

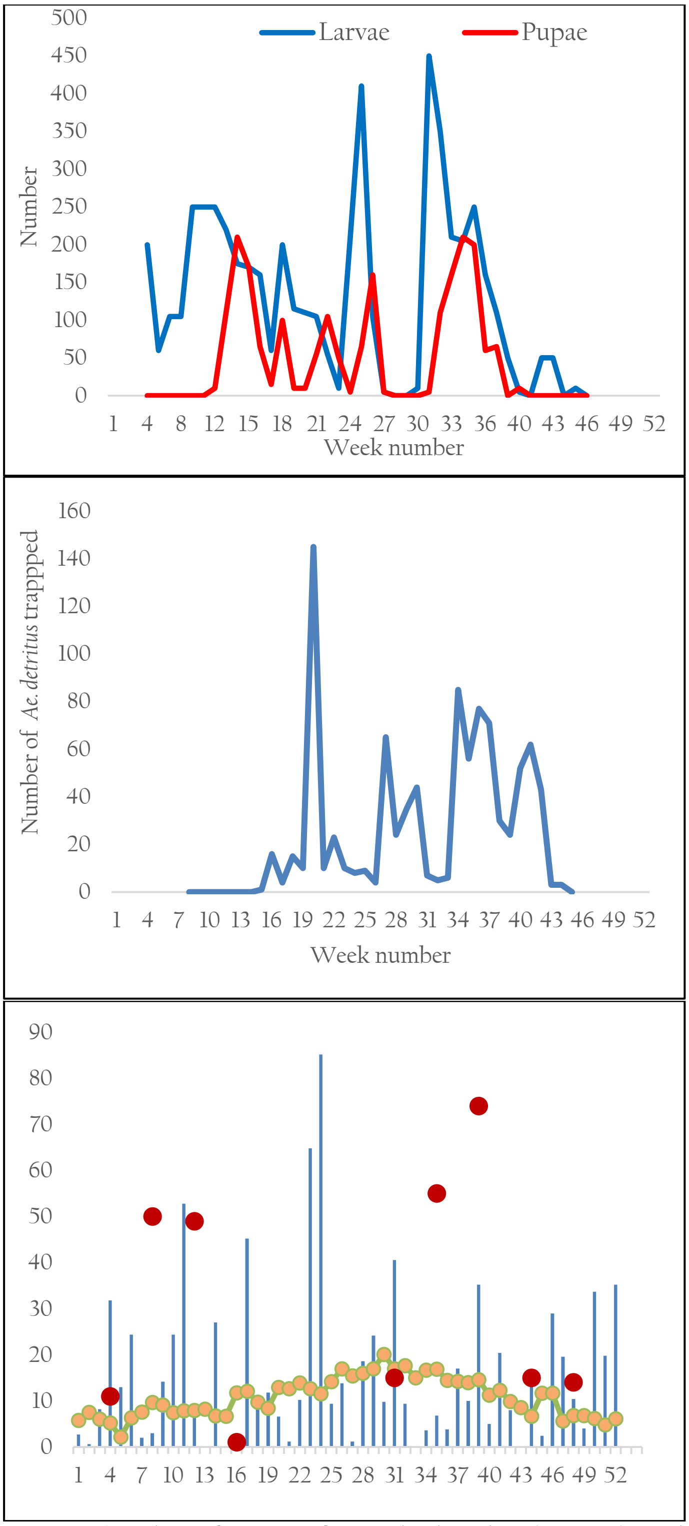

Figure 9b: The influence of very high tides (>9.75m) and weather on numbers of larvae, pupae and trapped *Ae. detritus* throughout 2019. Top = Trapped *Aedes detritus* each week 2019. Middle = larvae and pupae 2019. Bottom = Rain, temperature, and tide heights >9.75m 2019. (Blue = weekly rain [mm]; Green = mean weekly temp (°C); Red = tide height >9.75 m)

The relationship between larvae and pupae results and trap result

The peaks in larvae, pupae and trapped adults generally followed the expected temporal sequence (Figures 7 & 9b), though many factors can prevent a larval peak transforming to a later pupal peak (e.g. drying out of pool or its inundation by a storm surge) and others may influence the pupae-adult relationship (e.g. wind speed and direction, temperature).

Effects of pool excavation

Observations that large, deep and steep-sided pools rarely contained larvae resulted in the local authority changing its annual excavations strategy. Small shallow pools with sloping sides (category B pools) were dug out and amalgamated to create much larger, deeper pools with vertical sides (category A pools). Pools 5 and 9 were amalgamated, and likewise pools 1, 2 and 3 (which also became inaccessible because a major ditch was enlarged). However, two excavations enabled comparisons of larvae and pupae numbers before and after. Pool 6 was dug out as above and the effect on numbers of larvae and pupae found thereafter is shown in Figure 10a. Pools 7 consisted of a series of small category B pools over a 50 x 22m area. About half (those in the north western area) were dug out and amalgamated, and became Pool 7a, measuring 33 x 9m (a category A pool). However, the other half in the south-eastern area were left untouched and were renamed Pools 7b (still category B pools). Figure 10b shows the larvae and pupae counts that followed. Clearly, excavation of small shallow pools with sloping sides into deeper, larger steep-sided pools greatly reduced or eliminated larval and pupal numbers over the following years.

Mosquito forecast & biting reports

There is an understandable local public interest in the biting nuisance, which facilitated engagement with the community and local authority (to whom we also gave advice about marsh excavation). A web-based weekly forecast is produced. Starting in 2016, this is now published weekly from April to November using a traffic light (red, amber, green) indication of the biting nuisance predicted over the coming week based upon a combination of the trap counts, pool dipping (especially pupal assessments) and the weather forecast. This is available, with other mosquito information, on both the Neston Town Council website (2423 page views in 2019, 1148 in 2018) (https://www.neston.org.uk/information/mosquito-monitoring/latest-mosquito-update/) and also through a free "Neston Life" mobile phone app run by "About My Area" (https://www.aboutmyarea.co.uk/Cheshire/Neston/CH64) (6931 page views in 2019). Both are interactive and users are asked to report mosquito bites which can then be mapped by location, date and time of day (https://www.neston.org.uk/information/mosquito-monitoring/report-a-mosquito-bite/). Figure 11 summarises the forecast, mosquito numbers and bite reports (209 were received in 2019). Studies continue but various points regarding the biting nuisance locally have emerged:

1. There is no obvious correlation between the number of bite reports and distance from the marshes (within 3km).
2. There is a positive correlation between the numbers of bites reported and the numbers of mosquitoes caught in the traps in 2019 (Figure 12a); this is modest but significant with a Pearson coefficient of 0.40 ($p=0.01$). Figure 12b shows that if one exceptional week is excluded (the spike in bite reports in week 35 during the Bank Holiday weekend as discussed below) the correlation coefficient rises to 0.66 ($p<0.001$). Furthermore, if the unprecedented, never repeated and unexplained spike in mosquito numbers trapped at one site 20m from the edge of the marsh in week 20 is also excluded (Figure 12c), the correlation coefficient rises further to 0.79 ($p<0.001$).

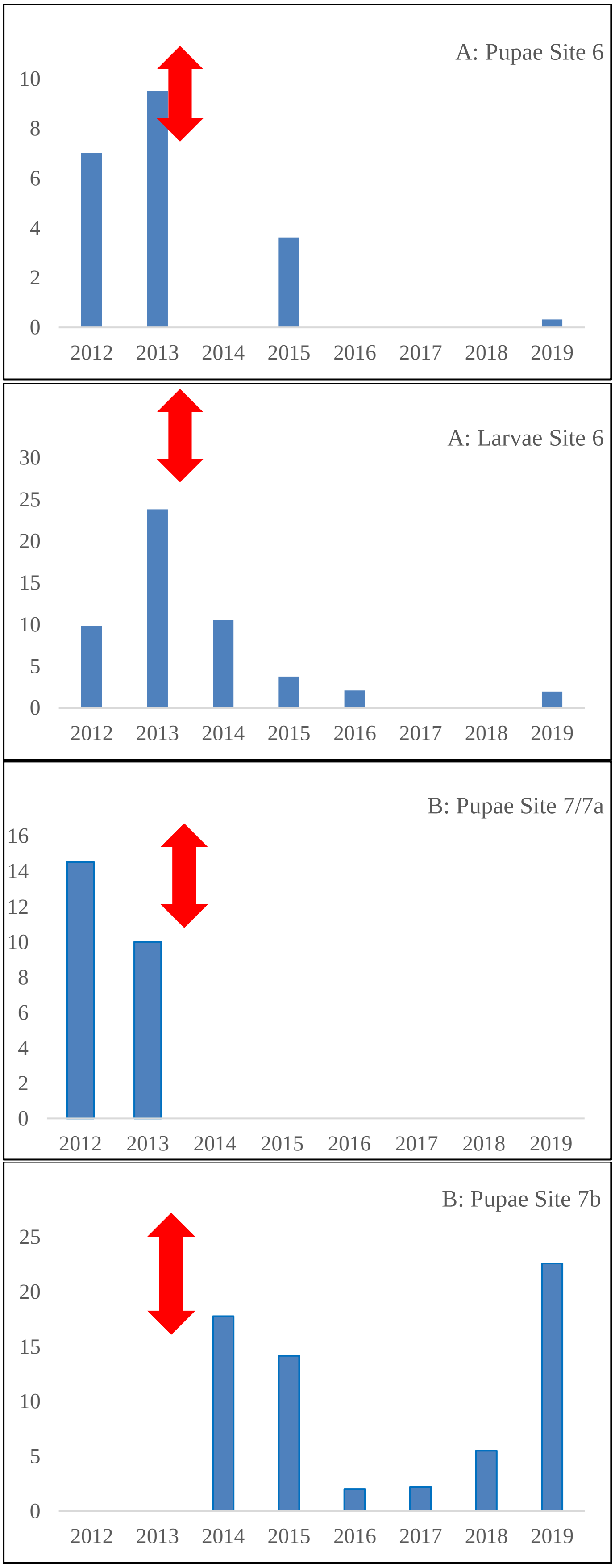

Figure 10: Effect of pool excavation (red arrow) in November 2013 on numbers of (A) larvae & pupae at Site 6; (B) on pupae numbers at Site 7. In 2012 and 2013, a collection of small pools was collectively called Pool 7. In November 2013, about half of these were excavated and joined together into one large pool, subsequently called Site 7a. The other small pools were not excavated and remained as Site 7b.

3. There was a huge spike in bite reports on the August Bank Holiday weekend 2019, though the number of mosquitoes (as assessed by numbers caught in traps) was just as high if not higher in the week before and after (Figure 11). There were exceptional human factors to help explain this surge in bite reports (see Discussion).

Discussion

We have extended the previous study (Clarkson and Setzkorn, 2011) of the temporal pattern of adult mosquitoes in the area of Neston (Neston, Parkgate and Little Neston) for a further 9 years. We have also broadened it to survey the larval and pupal development in the Study Pools and then the wider marshes, and to understand the factors influencing mosquito breeding across the Dee estuary marsh. Furthermore, we have developed the work to include a forecast of the biting nuisance and its measurement, so covering the whole life cycle and breadth of the mosquito problem in the area.

The population of mosquitoes under study

The array of species trapped were as expected from previous trapping studies (Clarkson & Setzkorn, 2011). The percentages of species caught in the traps in the area (Table 2) were similar to the percentages of the various species of adults bred from larvae taken from the Study Pools and also to the percentages bred from the other, more widespread marsh pools sampled (Table 3). The larvae in the Study Pools would therefore seem representative of the marsh in general and of the mosquito population caught in our traps, and so also of the local biting nuisance. The small exception is that no *Cs. litorea* or *Cs. morsitans* were ever caught in the traps but these were bred from larvae taken from pools at the edge of the marsh in winter months. Service (1994) pointed out that these *Culiseta* are not attracted to carbon dioxide traps, as used in our work.

Furthermore, the temporal distribution of counts throughout the year of larvae, pupae and adults were similar, and demonstrated the expected temporal progression of larvae to pupae to adults.

Overall, it seems clear that the different branches of this work are studying the same mosquito population, overwhelmingly *Ae. detritus*. Clarkson & Setzkorn (2011) showed that this species is responsible for the vast majority of the biting nuisance in the area and this is borne out here by study of the temporal pattern of the biting reports to the local authority (Figures 11 and 12).

Larval & pupal studies

The scoring system for numbers of larvae and pupae found in the pool dipping studies was not an accurate count but was designed to be quick and reproducible (all were done consistently by one investigator over the years), and to give a semi-quantitative measure for comparison week-to-week and year-to-year. It fulfilled its purpose adequately and the temporal correlation between the pool results using these methods and the trap results and biting reports supports this (Figures 9 and 12).

Mosquito breeding pools

Our work studied the same set of pools (the Study Pools) over many years, and was latterly extended to a more widespread examination of the type and location of pools in which mosquitoes breed in other areas of the marsh, and to observe their filling patterns at different times of the year. These pools would be considered to be in the "high marsh", above the

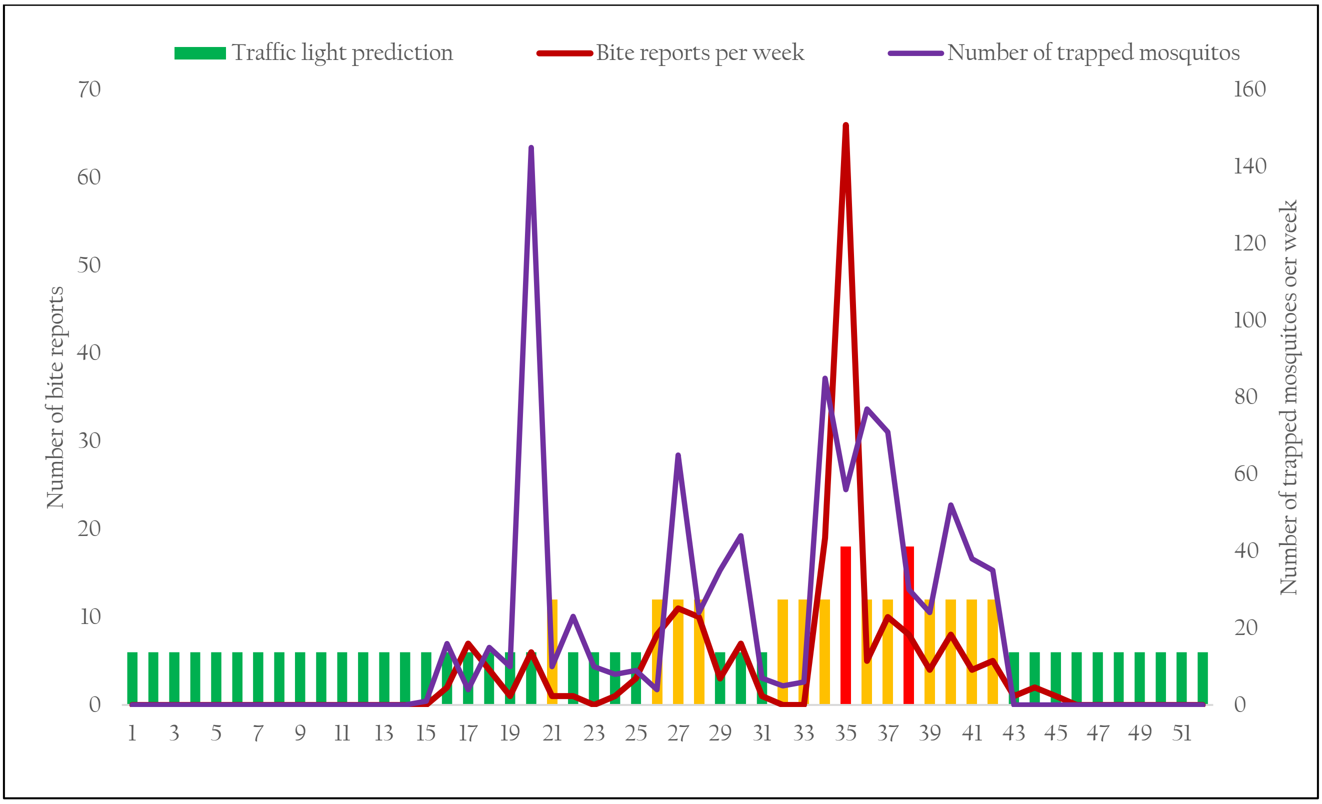

Figure 11: Weekly "traffic light" forecasts of biting nuisance, numbers of trapped mosquitoes and the number of biting reports in 2019.

usual intertidal zone, and only tended to fill with unusually high tides through overflow over the banks of main ditches or by flow through shallow, long serpiginous channels with mouths near the top of the banks of main tidal channels. Importantly, such pools were filled only after the tide reached a threshold by over-flowing into certain channels and so did not empty or even decrease in depth when the tide receded. We have observed that this threshold tide height was 9.7-9.8m (as measured at Liverpool Gladstone Dock) to fill the Study Pools, whereas other pools a few hundred metres away required a 9.9m tide. In important contradistinction to this mode of tidal filling, and as found by others (Service, 1968; Medlock & Vaux, 2013), the few pools (in this area of the marsh) which flushed with lower high tides were not found to contain larvae. We have not surveyed far out on the marshes where it is possible that pools also exist which are isolated from tidal channels by silting and so which may only fill intermittently.

It was clear from observation and from our results that the numbers of larvae hatching and developing is largely dependent upon the cycle of pool filling, then drying out and then refilling. Each stage depends greatly on the height of the tides, usually requiring spring tides in Spring and early Autumn, to trigger egg hatching by filling pools; this has been recognised previously in other locations (Service, 1968; Medlock & Vaux, 2013). The other less obvious factor which was observed sometimes to influence larval numbers greatly was when a very high tide (particularly storm surges) inundated a pool already full of larvae, which were washed out and presumably left stranded as the tide receded. The numbers observable in the pool for future development were greatly diminished immediately afterwards. Superimposed upon these influences, it was repeatedly noticed that the number of larvae surviving from the early to later stages fell spontaneously, presumably due to predation or mortality from chemical, temperature or microbiological factors: this spontaneous depletion of the early stage larvae was noted and discussed in the seminal work by Service (1968).

The height of each pool and its mode of filling varies from one place on the marsh to another. The pools at the highest point of the marsh were the ones particularly likely to have the cycle of intermittent filling, drying out and refilling which is recognised to be essential for breeding (Medlock & Vaux, 2013). However even in these areas, it was notable that different types of pools had very different larval numbers. The size and cross-sectional shape of the pool were two of the obvious factors in determining this, though it is not clear why. This relationship between mosquito breeding and small pool size has been recognised previously (e.g. Medlock & Vaux's results on the salt marsh habitat at Seymer's Marsh, on Brownsea Island). It is likely from our observations that the extent to which the pools dry out and its timing may be important factors, exposing mud at a time when females need to oviposit whilst sometimes leaving a residual central area of water in which larvae could still survive. Preferential oviposition in mud or between tufts of grass at the edge of a pool, which expanded as it filled, could contribute to explaining why the small pools contained much greater concentrations of larvae, since smaller pools would have greater edge-length to volume ratios (in the case of a hypothetical circular pool, the circumference to volume ratio would be inversely proportional to the radius). Service's (1968) egg studies would tend to support this preferential distribution of *Ae. detritus* eggs, with the vast majority of the hatched eggs occurring in the bare mud near the edge of the pools (44.7%) or the mud from within the Spartina/Junctus just at the edge (48.4%). His study clearly describes a pool with gradually sloping sides, so this Spartina /Junctus would be immersed as the pool filled, and our observations about productive pools having sloping sides and expanding in size as they filled would be consistent with this. Service explains how this distribution may be optimal: eggs more centrally posited may hatch with smaller volumes of water entering the pool, and so the resulting

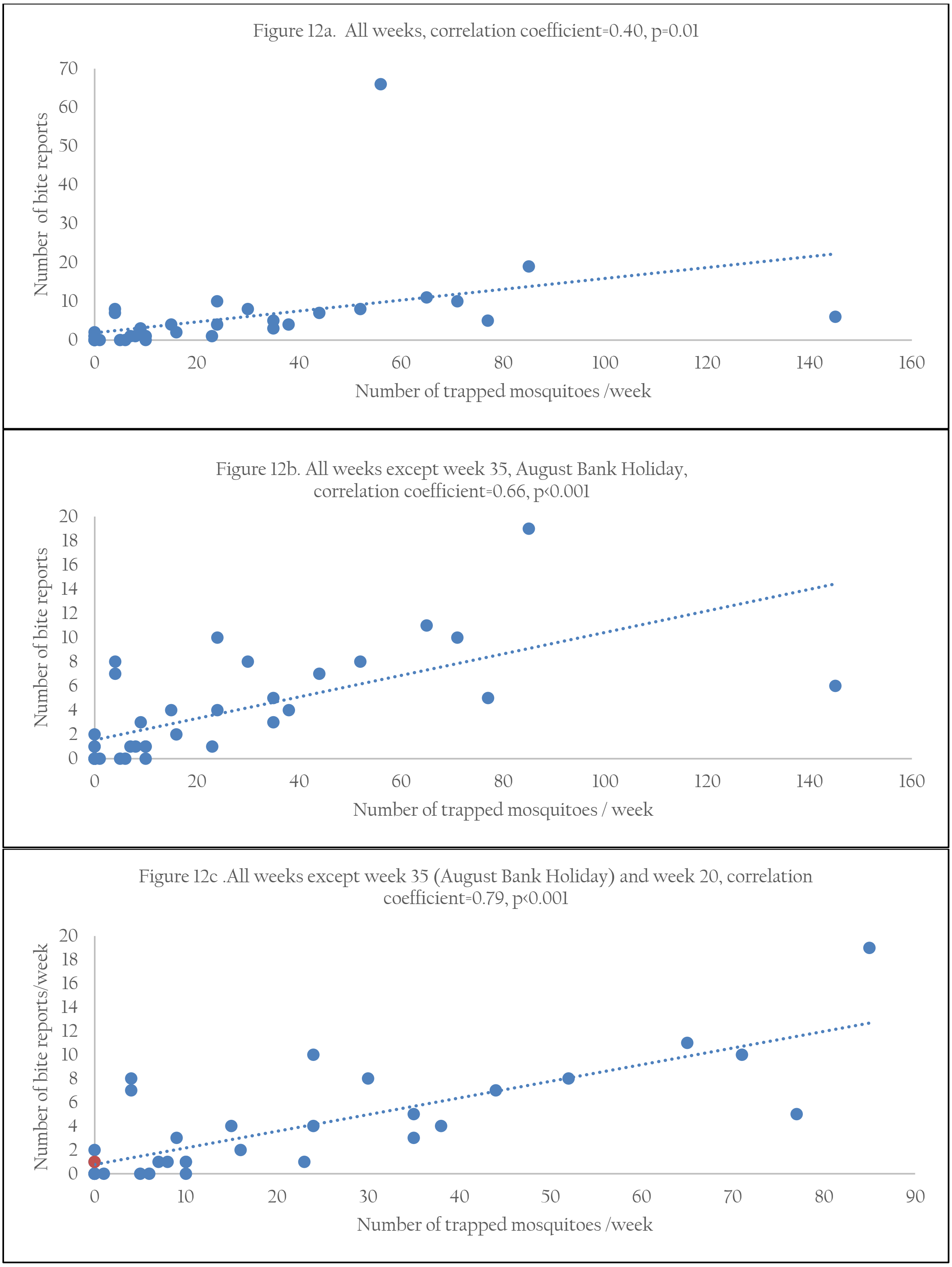

Figure 12: Scatter plots of number of weekly bite reports and number of trapped mosquitoes.

larvae be more susceptible to desiccation, whilst those peripherally posited would only be covered by water, and so hatch, with larger volumes of water which would be sufficient to prevent desiccation before completion of the development to adult emergence. Another consideration may be the timing of pools drying out: the larger and deeper pools were observed to dry out only after long periods of dry, hot, sunny weather; even when they did then re-fill with water, larvae were seldom found in them, perhaps because there would have been very few adult females around after the prolonged dry, hot, sunny weather to oviposit in the mud exposed in those pools only at these times. In contrast, the mud at the edges of category B pools often became available for oviposition within a week or two of emergence of adults. Other possibilities include a difference in the temperatures between the small and large pools, or in their biomes. The persistence of water in large deep pools may also allow the persistence and population increase of larval predators (Medlock & Snow, 2008), and/or development of significant amount of larvicidal bacteria and/or other micro-organisms (Service, 1968). As a likely reflection of a different biome between the various categories of pool, it was a common observation to find large wading birds such as herons and egrets feeding in these larger pools (categories A and C) but not the small pools. We cannot comment upon the type of vegetation since all our Study Pools were surrounded by a variety of grasses, and only one had significant reed growth within it. Elsewhere on the marsh, some of the most prolific breeding pools indeed were reed-filled, but these were in the minority of pools in this area of the marsh.

Annual distribution of mosquitoes

The annual distribution of mosquitoes, with a peak in April and May, and a much larger peak in late August, September and October, is largely dictated by the high spring tides occurring at these times filling breeding pools and triggering egg hatching. Components of the weather, namely rainfall, temperature and wind, can modify whether the pools are wet for long enough for mosquito development to occur. Marshall (1938) recognised that egg hatching and larval development may occur under a wide range of salinity and during wet summers, when persistent heavy rainfall may fill previously dry pools, mosquitoes would develop in the intervening months (for example in 2019).

Geographical distribution of trapped mosquitoes

Traps located a kilometre or two from the edge of the marshes caught more mosquitoes than those situated within 100m (Figure 5). Potential reasons for this could be the prevailing westerly winds, which tend to be more brisk on the shoreline, or that there is a delay in female mosquito development to the stage where blood-seeking behaviour and biting is initiated (time presumably spent feeding and mating), thus requiring time from emergence before being attracted to carbon dioxide and octenol released by the traps. This may also explain the poor correlation between the biting nuisance and distance from the marsh, at least within about 3km. It is recognised that *Ae. detritus* can travel much further from its origin than other British mosquitoes (Marshall, 1938) and indeed we report it being trapped as far as 8 km from the nearest known breeding site.

Practical implications of pool characteristics for mosquito control

Our observations about the type of pool used for mosquito reproduction allowed the local council's annual excavations to be targeted in such a way as to enlarge pools or amalgamate small shallow pools, and deepen them, producing steep sides and flat-bottomed large pools. Our quantitative results demonstrated the effectiveness of this.

Open marsh water management has been shown to be an effective way of controlling mosquito breeding populations (Wolfe, 1996; Meredith and Lesser, 2007; James-Pirri et al, 2009; Russell, 2010; Lesser, 2011). It may involve three components, tidal ditches, pond radials and ponds, and the most appropriate application of these depends on whether the area is in the low marsh/intertidal zone or high marsh, above the ordinary intertidal zone. Particularly relevant for the high marsh are the creation of permanent large ponds, to which smaller pools (or "depressions") may be linked by radials if needed. Often ponds are created by amalgamating multiple small pools, and this is effectively what occurred in several of our Study Pools (e.g. site 7, Figure 10), whilst in other cases pools were linked by broad channels (sites 5 and 1/2/3) or pools were simply deepened and enlarged (e.g. site 6). The results from our dipping work shows how effective this is in controlling mosquito breeding. Our work guiding the excavations of the local council may be viewed as a much smaller scale (and cheaper) version of open marsh water management as practised elsewhere, and can be continued annually (so far none of the pool excavations have been reversed by natural re-silting, unlike ditch digging). There may be restrictions on the size of pools created by the high conservation status of the Dee estuary marsh by both UK and EU regulations.

There is a long history of the local authority conducting mosquito control measures on the Dee estuary (Davies, 1995; Clarkson and Setzkorn, 2011; Medlock et al, 2012). In past decades, this was by means of spraying, which was quite labour-intensive and at times dangerous for council workers (Davies, 1995). It continued intermittently until mid-2012. As well as the practical difficulties encountered in the past (Davies, 1995), the use of larvicidal preparations such as Bti (*Bacillus thuringiensis subspecies israelensis*) has major biological limitations. It is only effective for a few days and so has to be applied repeatedly (even every few weeks) at certain times of the year. It is only effective against larval stages since it has no effect on pupae or on eggs: in view of the survival of dormant eggs for at least a year (Marshall, 1938; Service, 1968), its use would need to be continued for some years. Our work demonstrates its use in large deeper pools would be an expensive waste of resources whilst its application to small pools would be compromised by their number and dispersion. The gradual year-by-year creation of more ponds which had been made deeper and larger by aggregation or linking of small shallow breeding pools would seem a more attractive proposition in controlling the mosquito biting nuisance and also encouraging more birdlife (which is already a tourist attraction for the area), and so important for the local authority and the RSPB, which is the manager of the marsh.

The biting nuisance, its geographical and temporal distribution, and its weekly forecast

With the exception of the Bank Holiday weekend in late August (Figure 11), the biting nuisance (as judged by the number of bite reports) largely varied in 2019 according to the number of adult mosquitoes, as judged by the number caught in the traps. Other factors must also influence this but we await the results of ongoing studies to comment further. Nevertheless, there was a significant correlation between the number of bite reports to the council (using the online form) and the number of mosquitoes caught in our four traps around the area in 2019. Indeed, this correlation is remarkably strong if one excludes one or even both obvious outliers in the data (Figure 12).

We are unaware of any weekly forecast of the likely mosquito biting nuisance elsewhere. The forecast is issued using the weekly data from the trapped adults, the pool dipping (and in particular the number of pupae found) and the weather forecast for the forthcoming week. The availability of the forecast on a mobile phone app has increased its viewing within the local population, but further study of this and the bite reporting continues with development of the app and data collection.

Public reporting of bites is an example of passive mosquito surveillance involving "citizen science" (see Kampen et al, 2015 for review), utilising the intense local interest. It has been encouraged by deliberately keeping the process and the online form simple. Of course, there is no confirmation that biting has been due to a mosquito (as opposed to other biting insects), though the biting reports do generally follow the annual temporal distribution (with the Bank Holiday exception). The biting reports data have illustrated two important points. Firstly, that the biting nuisance does not appear to be correlated with proximity to the marshes, at least within 3 km, which may be consistent with the findings on trapped mosquito numbers at different distances from the marsh. Secondly, although at most times of the year the number of bites reported reflected the number of adult mosquitoes (as judged by the number caught in the traps), it was clear from the report around the August Bank

Holiday 2019, that human behaviour influences the extent of the biting nuisance hugely, and possibly more than the number of mosquitoes in the neighbourhood. There was a huge spike in reports on that August Bank Holiday weekend when there was hot, humid, still weather and numerous mosquitoes. These circumstances occurred also in the week before and after but with only modest bite numbers. However, over the Bank Holiday weekend there was a huge spike in bite reports which was observed to coincide with greatly increased human presence outdoors, with prolonged and extensive skin exposure and often at family barbeques, involving active, sweaty children and hot adults, quenching their thirst in groups long into the evening.

Culex species breeding on the marshes

Though the major source of *Cx. torrentium* found locally was in a tree hole, some were found in pools at the edge of the marsh, along with more numerous *Cx. pipiens pipiens*. The salinity of the water in those pools is unknown, but each is inundated by the tide several times each year, and so, when dry, the mud must have a significant salt content which will then be dissolved by summer rainwater filling the pool later in the year. It is known that *Cx. pipiens pipiens* may breed in brackish water, and our results confirm that *Cx. torrentium* can also do so, as suggested in an early report by Lever (1954). Overall, of *Cx pipiens* s.l. bred from larvae collected from different freshwater and marsh sites in the Neston area, about 25% were *Cx. torrentium* (Enevoldson & Hernandez Colina, unpublished). Both typically bite birds rather than humans or mammals.

Importance of local mosquitoes as potential arbovirus vectors

There are no human mosquito-transmitted diseases in the UK, and presently the importance of local mosquitoes is limited to their biting nuisance. Both *Culex* species above are known to be potential vectors for the transmission of arboviruses (Medlock et al, 2005; Medlock et al, 2007; Mackenzie-Impoinvil et al, 2015; Brugman et al, 2017; Jansen et al, 2019), and the close proximity of both migratory and non-migratory birds on the Dee estuary adds extra significance to their presence. Other bridge vectors could then be of relevance, and this includes *Ae. detritus* which has been recognised as a potential vector for a range of arboviruses (Mackenzie-Impoinvil et al, 2015; Blagrove et al, 2016; Lumley et al, 2018). Its local prevalence, its flight range and its propensity to bite humans, other mammals and birds (Marshall, 1938; Medlock et al, 2005; Medlock et al 2007), suggest that studies of the local mosquito population may be of even greater importance in the future.

Acknowledgements

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