Diversity of Chironomidae (Diptera) breeding in the Great Stour, Kent: baseline results from the Westgate Parks non-biting midge project

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Diversity of Chironomidae (Diptera) breeding in the Great Stour, Kent: baseline results from the Westgate Parks Non-biting Midge Project

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Abstract

Chalk rivers and streams are of conservation importance due their ecological diversity, historical relevance and economic value. With more than 200 chalk watercourses, England is considered unusual in having the most chalk rivers in the world. However, due to increasing anthropogenic activities, many English chalk rivers and streams are becoming badly degraded. The non-biting midges or chironomids (Diptera, Chironomidae) are considered key-stone taxa in aquatic food webs, and have been used as ecological indicators of freshwater quality and environmental stress. Here we determined the generic richness, diversity, and community structure of Chironomidae across six sites in the mid-section of the Great Stour in Kent, a chalk river for which concern has been expressed regarding both water and habitat quality. Based on the morphological identification of 1336 insect larvae from the six sites (four in Westgate Parks, Canterbury, and two at nearby locations upstream and downstream from Canterbury City), a total of 20 genera of Chironomidae were identified, including some taxa indicative of freshwater habitats with low levels of organic pollution. There were different levels of generic richness and diversity among sites, and while there was little variation in the community composition among the sites within Westgate Parks, there were noticeable generic differences among Westgate Parks sites compared with those upstream and downstream, showing the highest complementarity and Beta diversity values. Overall, the results were comparable with other studies on chironomids in chalk rivers and other river systems. Although spatially limited to a small stretch of river, this represents the first study on chironomids in the Great Stour and provides baseline information on the diversity and structure of this important insect group with aquatic larvae, useful for the objective interpretation of any future biological assessments and monitoring programmes on the Kentish Stour, and also for comparisons with other chalk rivers.

Keywords

Kentish Stour, community structure, diversity, aquatic larvae, insects

Introduction

The aquatic larvae of non-biting midges or chironomids (Diptera, Chironomidae) occupy a wide range of habitats, including inland and coastal areas, and different levels of salinity, temperature, pH, oxygen concentration and water flow regimes (Armitage et al. 1995, Frouz et al. 2003). This is possibly due to remarkable physiological and behavioural adaptations, and dispersion and colonisation capacities of this biologically diverse insect group (Frouz et al. 2003). Considered as key-stone taxa in aquatic food webs, chironomids belong to different trophic guilds, functioning as commensals, predators, grazers/scrapers, filter-feeders and detritivores, and are important prey for many invertebrate and vertebrate carnivores (Armitage et al. 1995). Chironomids are well known as excellent indicators of environmental conditions, including water quality and chemical change, in a wide range of freshwater systems, including ponds, lakes, streams and rivers (Pinder 1977, 1989, Porinchu & MacDonald 2003, Wilson & Ruse 2005, Nicacio & Juen 2015). They have also been widely used as palaeo-climatological indicators over periods up to two hundred thousand years in this context (Brooks 2006, Axford et al. 2009).

The utility of chironomids as indicators of freshwater quality lies in their near ubiquity in aquatic systems, high abundance and/or high diversity, complementary relationships with other indicators, short generation times (several generations in one year in some species), relative ease of sampling and taxonomic identification by experts, at least some species have narrow ecological optima (stenotopic), while the adults are able to disperse widely and rapidly as a response to environmental change (Brooks et al. 2007).

Arguably, because of the reasons cited above and because there are few freshwater niches not occupied by at least one species of chironomid, a survey of chironomids can provide as much information about a freshwater system as all the other freshwater invertebrates put together (Wilson & Ruse 2005). Furthermore, the

identification of pupal exuviae for determining chironomid distribution, taxa composition and relative abundance in fresh waters can be useful for assessing and monitoring environmental quality and stress. This has been done applying the Chironomid Pupal Exuvial Technique (CPET) by Wilson and Ruse (2005), a four-point scale of tolerances based on organic pollution stress and covering most of the chironomid genera that occur in Britain and Ireland, advantageous as a broad-brush approach for biological assessment and monitoring of lakes, large stretches of river, canals and small streams. However, pupal exuviae may have originated further upstream or upwind from the collection site, making this technique unsuitable for studying chironomid diversity in defined habitats or in a small stretch of river (Wilson & Ruse 2005). Sampling and identifying chironomid larvae, although more time-consuming than CPET, provides fine-scale knowledge of their abundance, diversity, distribution patterns and microhabitat preferences.

Chalk rivers and streams are watercourses dominated by groundwater discharge from underlying chalk geology (Berrie 1992, Mainstone 1999, Smith et al. 2003, Ladle & Westlake 2006), characterised by having clear water, relatively stable flows and abundant wildlife, including many species of conservation importance (Environment Agency and English Nature 2004, O'Neill & Hughes 2014). With more than 200 chalk watercourses, England is considered unusual in having the majority of chalk rivers and streams in Europe, possibly even the world, warranting attention for their conservation as a key habitat and as Sites of Special Scientific Interest (Mainstone 1999, Environment Agency and English Nature 2004, Ladle & Westlake 2006, O'Neill & Hughes 2014, Pearce 2014, Visser et al. 2019). However, due to increasing water extraction for human use and other anthropogenic activities, many English chalk rivers and streams are becoming badly degraded (Wright & Berrie 1987, Sanders et al. 2007, O'Neill & Hughes 2014, Westwood et al. 2017, Sampson et al. 2019, Visser et al. 2019).

The Kentish Stour, together with some of its tributaries, is considered to be the major chalk river system in the county of Kent (Fig. 1, Supplementary information), and like other chalk streams and rivers it has been important in the regional economy as a site for corn mills, paper making, electricity generation, communication, fishing, and leisure activities (Berrie 1992 Environment Agency 2004, O'Neill & Hughes 2014).

The section of the Great Stour from Ashford to Canterbury, designated by the Kent Wildlife Trust as a (non-statutory) county 'Wildlife Site' (Kent Biodiversity Action Plan 1997, Biodiversity Action Reporting System 2011), is the stretch primarily responsible for the chalk stream classification of the river. The water in this section is significantly augmented by up-welling from aquifers in the underlying Seaford Chalk Formation, by which route most of the drainage from the surrounding North Downs eventually enters the river (rather than directly as side-streams). Between its headwaters and Canterbury City, the Great Stour receives road and farmland run-off, and at Lenham, Ashford (Bybrook), Chartham and elsewhere, treated wastewater discharge – which add pollutants of various sorts, including phosphates, considered especially inimical to a chalk stream ecosystem.

Concern has been expressed by the Kent County Council (KCC) regarding both water and habitat quality of the Great Stour (KCC 2005). Water flow has also been low at times in the recent past, notably spring 2012 and again for periods in early 2018 and May 2019, causing other problems for wildlife as well as more temporary declines in water quality. In line with the National Rivers Authority's (NRA) Kentish Stour Catchment Management Plan Consultation Report (NRA 1994) and the more recent South East river basin district River basin management plan (Environment Agency 2016), local councils including Canterbury City Council (CCC) and KCC would wish to see, in terms of the most recent Water Framework Directives (WFD-UK-TAG 2012; Department for Environment, Food & Rural Affairs – DEFRA 2014),

not only water quality improvement (KCC 2005) but also enhanced habitat connectivity (CCC 2003). This is seen to be desirable for biodiversity as well as amenity values, including angling – a significant leisure pursuit on the Great Stour (KCC 2005). Thus, CCC's Local Plan states: "The Council will seek to ensure that every opportunity is taken to enhance existing aquatic environments and ecosystems. This will include the restoration of natural river features (including riverbanks) and removal of barriers to fish passage when appropriate opportunities arise" (policy CC12: CCC 2017).

The aims of the study were to determine the chironomid larvae generic richness and diversity as well as the community structure across selected sites in the Great Stour which could be used as a baseline for future ecological or environmental projects. Baseline information on chironomids is valuable for the early detection of perturbations to ecosystems and to derive objective interpretation from monitoring programmes (Pinder & Morley 1995, Wood & Petts 1999).

We hypothesised that there would be differences in the generic richness, diversity and composition of chironomid larvae across the sites in the Great Stour, as expected from this diverse group of insects with larvae that can occupy a wide range of aquatic habitats but with stenotopic species. The non-biting midge project always had the primary objective of discovering and then monitoring chironomids present in the riverbed at fixed sampling stations, with a view to recording and assessing changes in water quality over the long term. This includes an intention to move increasingly to DNA-based techniques both to improve resolution over purely morphological evaluation of only semi-quantitative sampling of larvae, and facilitate rapid and accurate, species-level identification. There is also an intention to expand the work in the future, if feasible, to establish sampling stations along the entire length of the Kentish Stour system, to get an understanding of chironomid diversity of this locally important waterway from source to sea, and assess how this relates to or

reflects water quality at different stretches of the river. The present work is a first step toward both the current and longer-term goals.

Materials and Methods

Sample collection

A total of six sites along the Great Stour were selected for sampling chironomid larvae (Fig. 1). This included four sites previously chosen during a restoration project in Westgate Parks by the UK Environment Agency (EA), namely: Rheims Way EA1 (Site 1), Westgate Gardens EA3 (Site 2), Westgate Towers EA4 (Site 3) and Bingley Island EAB (Site 4; a side-stream of the Great Stour), and two other sites, namely: Horton EAH (Site 5; a site 3 km upstream from Westgate Parks and a long-term monitoring site for water quality) and Kingsmead Field K020 (Site 6; a site 1 km downstream from Westgate Parks). Site 1, Site 2 and Site 3 were sampled during spring (May) 2011 and 2012, and in 2013, samples were also taken during spring and autumn (September); Site 4 was sampled during spring (May) 2012, and in 2013, samples were also taken during spring and autumns (October); Site 5 was sampled during spring (April) 2012, and in 2013, samples were also taken during spring and autumn (October); and Site 6 was only sampled in summer (August) 2015. Substrate composition data was available for sites 1-5 showing similar composition with various ranges (65-80% pebbles and cobbles, 20-34% gravel and sand, and 2-4% silt) but data was not available for Site 6; therefore, substrate composition was not considered in the analysis.

Three samples per site across the width of the river were obtained by kick-sampling for 3 min using a pond net and following guidelines for sampling river macroinvertebrates, e.g. Stark et al. (2001) for river sampling protocols. To dislodge the macroinvertebrates, the substratum was disturbed by kicking directly upstream of the net which was held firmly on the substrate and facing upstream, and samples were collected in the net. The samples were first inspected on site to remove and

return any vertebrates or unwanted invertebrates back into the river, and then taken to the laboratory for further inspection. Chironomids were recognised by eye using a magnifying lens and were separated and placed in individually labelled tubes containing 70% IMS until analysis.

Sample preparation

Using dissection needles and forceps under a binocular microscope, the head capsules of preserved chironomid larvae were dissected from the body. The head capsule and body for each specimen were mounted onto microscope slides following a slide preparation technique (Smith 1989) using Hydromount Histology Mounting Media (National Diagnostics) (for details see Supplementary information). In total, 1336 specimens were mounted onto microscope slides. The specimens were then identified to genus or to species morphotype level (whenever possible) by SJB using the key works for larval identification by Brooks et al. (2007), Cranston (1982) and Wiederholm (1983), and a dataset of chironomid larvae for the Great Stour was generated. The slides are currently stored at Canterbury Christ Church University (CCCU) for reference and are potentially available upon request.

Generic richness and diversity

The sampling data were considered as semi-quantitative (an index of relative abundance) because the sampling time was standardised, and although the sampling area was not standardised, the river and substrate conditions across all sites were very similar. All the ecological diversity analyses were performed at genus level using Primer 6 version 6.1.16 (Clarke & Gorley 2006) unless stated.

The total number of genera per site and the abundance per genus per site, simply obtained by counting the number of occurrences, was obtained by pooling the data per year (Table 1). Based on this genus-level dataset, the richness (total number of genera G and Margalef's d), equitability (Pielou's evenness; a measure of equitability

indicating how evenly the individuals are distributed among the different genera) and diversity indexes (Shannon's H and Simpson's indices) per site were calculated.

A genus accumulation curve was used to plot the cumulative genus count against sample number, where sample order was permuted (999 maximum permutations) to obtain the mean observed genus counts, G (observations), per sample. The Michaelis-Menten asymptotic curve was fitted to the observed genus curve and it was used to estimate the total number of genera as Gmax = G(n) + BG(n)/n , where G(n) is the expected number of genera on the last sample (i.e. there was a total of six samples in this study) based on the fitted asymptote curve, B is the sampling effort needed to detect 50% of those genera, and n is the number of samples (Colwell et al. 2004, Williams et al. 2007). Also, the Chao1 and Jackknife1 estimators were used to calculate the genus accumulation curve and genus richness, because they have been shown to be appropriate for abundance data and performed better than asymptotic functions (Williams et al. 2007).

A dominance analysis was done to rank the genera in order of importance and to estimate their percentage contribution to the total dominance per site. The cumulative dominance per genus (as a percentage) was then plotted against the genus rank to visualise their contributions to total dominance per site.

Community structure

To evaluate similarities among sites in the Great Stour, the Bray-Curtis dissimilarity matrix among sites was calculated using the relative (percentage) abundance data. The matrix was then used to perform a hierarchical agglomerative cluster analysis using the unweighted pair group method with arithmetic mean (UPGMA), and the results were plotted as a dendrogram.

The 2D non-metric Multi-Dimensional Scaling (NMDS) analysis, an ordination technique, was performed using in this case the Bray-Curtis dissimilarity matrix to visualise the level of dissimilarity among the sites. Twenty-five restarts and a

minimum Kruskal's stress value = 0.01 were selected as parameters to generate the final 2D configurations. Principal Components Analysis (PCA) with relative abundance data, carried out in PAST version 4.02 (Hammer et al. 2001), was used to find hypothetical variables (principal components) that account for as much as possible of the variance in the multivariate data, and to find the eigenvalues and eigenvectors of the variance-covariance matrix. Two-way indicator species analysis (TWINSPAN) was used to construct a classification of the sites and order the genera according to their site of preference, and to find indicator genera. This was done in WinTWINS version 2.3 (Hill & Šmilauer 2005).

Beta diversity (β) measures the difference in the composition at a certain taxonomic level (i.e. the spatial turnover) between two or more local assemblages, or between local and regional assemblages, and is useful for understanding the spatial aspects of biodiversity (Koleff et al. 2003). Using a presence-absence matrix for the genera found in the Great Stour, the global and pairwise Whittaker's β_W (representing a broad-sense measurement of taxon turnover), and Harrison's β_{-2} and Williams' β_{-3} (representing two narrow-sense measurements of taxon turnover) were calculated representing taxa turnover across sites and compositional change.

We also assessed complementarity (Vane-Wright et al. 1991; Justus & Sarkar 2002) across the six sampling sites. Complementarity is a diversity measurement based on features x areas matrices in which the identities of the features are not 'lost' by reduction to dimensionless numbers, but are manipulated by addition and subtraction of the identified features present in each area. Summation of the features (e.g. species, higher taxa, vegetation types) over all areas under consideration (which can be equal or unequal in extent but must be discrete and non-overlapping) determines the overall or collective set. In a complementarity analysis each component area is compared with every other area, or with area combinations, in terms of complements – those features (or elements), if any, that the area in question has that are not present in each other area or combination of areas (including, at the

limit, the overall set). The main application of complementarity has been in conservation area network selection. In practice, although this metric has proved very significant in this regard, such analyses typically involve numerous other criteria (e.g. Pressey et al. 1993; Margules & Sarkar 2007; Leménager et al. 2014).

Complementarity can also be applied, however, to the comparison of areas simply in terms of their taxonomic or labelled feature diversity (typically presence/absence data only – but more sophisticated manipulations are possible). Each pair of sites was compared in terms of proportional overlap of shared complements. Thus, for example, if two sites have at total of 10 genera and all are represented at both, their shared complement would be 100%; if none of the genera at the two sites (say 7 at one and 3 at the other) was the same, then their shared complement would be zero. Partial overlaps result in intermediate scores. Thus, in such a simple pairwise comparison, high values indicate sites that are very similar, and low values sites that are very dissimilar. The proportion of the total complement represented by individual sites or any combination of sites can also be calculated and compared – here we have explored values for the six individual sites, and all 15 pairings of sites.

Results

Generic richness and diversity

A total of 1336 chironomid larvae were collected, and 20 genera of Chironomidae were identified morphologically across the six sites (Table 1) belonging to the subfamilies Chironominae, Orthocladiinae, Prodiamesinae, and Tanypodinae (Table S1). For some of these subfamilies, a total of 28 species morphotypes were identified (Table S1). In terms of generic richness (Table 2, Fig. 2a), Site 5 (15 genera – 75% of total set) was the richest, while Site 4 was the least rich (6 genera – 30%); however, in terms of generic diversity, Site 6 was the most diverse and with highest evenness value followed closely by Site 5 for diversity, while Site 4 was the least diverse and with lowest evenness value. The number of genera identified increased

with increasing sample size and only the Chao1 curve reached an asymptote (Fig. S1). With the Michaelis-Menten model, 20 genera in the 6th sample were estimated, resulting in a Gmax = 25.31 genera (B = 1.71), while all other models estimated the presence of more than 20 genera in the 6th sample (Fig. S1).

There were different dominance plots for all sites (Fig. 2b), with only five genera reaching ≥20% abundance at any site. Site 4 and Site 2 were clearly dominated by one genus (*Cricotopus*) [authors and dates for all genera recorded here are given in Table 1 and under *Diversity and Ecology* (Supplementary Information)], which represented more than 60% of the total number of larvae sampled at these two sites. At Site 3, more than 50% of the total number of specimens belonged to *Eukiefferiella*. For Site 1, the most abundant genera were *Rheotanytarsus* (39%) and *Eukiefferiella* (35%). At Site 5 the most dominant genus was *Tvetenia* (37%) followed by *Cricotopus* (21%) and *Eukiefferiella* (19%). At Site 6 the dominance was less evident, no genus surpassing 30% abundance. Six genera were only found at one site and with <5% abundance, from which four (*Epoicocladius*, *Rheocricotopus*, *Synorthocladius* and *Thienemanniella*) were only found at Site 5, one (*Orthocladius*) at Site 1, and one (*Macropelopia*) at Site 6.

Community structure

The average Bray-Curtis dissimilarity index among sites was 58% (Table S2). The dendrogram (Fig. 3a) showed Site 6 to be the most different, followed by Site 5, while Sites 2 and 4 and Sites 1 and 3 in Westgate Park clustered together.

The 2D NMDS plot (Fig. S2, Table S3) showed a similar pattern to that obtained with the dendrogram, where Site 6 and Site 5 were the two most dissimilar sites under the first and second dimensions, respectively. The minimum stress = 0.02 indicated that the final configuration was close to the actual dissimilarities among sites. Site 6 versus all other sites showed the greatest dissimilarity along the first dimension, Site 5 versus all other sites showed the greatest dissimilarity along the

second dimension. In the PCA, the first three PCs explained 93.3% of the variance, and the score plot based on PC1 and PC2 showed Sites 5 and 6, Sites 2 and 4, and Sites 1 and 3 in separate clusters and in different directions (Fig. 3b); the loading plot showed *Eukiefferiella*, *Rheotanytarsus* and *Micropsectra* with positive PC scores associated with Sites 1 and 3, *Cricotopus* with negative PC1 and positive PC2 scores associated with Sites 2 and 4, and *Prodiamesa*, *Paratendipes*, *Polypedulum*, *Tanytarsus* and *Tvetenia* with positive PC1 and negative PC2 scores associated with Sites 5 and 6, while all other genera having minor influence on the first two PCs. TWINSPAN classification showed two main groupings: one including all Westgate Park sites and another one including Site 5 and Site 6, which was probably due to the distribution and abundance of some chironomid genera, many of which appeared only in Site 5 and in Site 6 and were absent for Westgate Parks sites (Table S4).

There was low global β diversity in the total sample for all estimates ($\beta_W = 1.11$). Site 6 and Site 5 had the highest pairwise β_W value (Table 3).

For the six sites in the Great Stour, Table 4 shows the pairwise complementarity values for the midge genera, scaled from 0 to 1, as well as the raw data. Under this analysis, Site 2 + Site 4 were the two most similar sites (score of 0.67), closely followed by Site 1 + Site 2 (score of 0.64), Site 3 + Site 4 (score of 0.63), and Site 2 + Site 3 (score of 0.60) – these all being pairings among the four Westgate Parks sites. The most dissimilar pairings were Site 1 + Site 6 and Site 2 + Site 6 (with scores of 0.25), followed by Site 3 + Site 5 (score of 0.29) and Site 4 + Site 5 (score of 0.31) – these being parings of Westgate Parks sites with either Kingsmead or Horton. The pairwise comparison of Site 5 and Site 6, the two most distant sites geographically, gave an intermediate value (score of 0.44). Listing each site in sequence with its most similar/most dissimilar site(s) we obtained the following: 1) Site 1 (Site 2/Site 6), 2) Site 2 (Site 4/Site 6), 3) Site 3 (Site 4/Site 5), 4) Site 4 (Site 2/Site 5), 5) Site 5 (Site 1 = Site 2/Site 4), and 6) Site 6 (Site 5/Site 1 = Site 2).

Table 5 presents the proportion of the total complement of 20 genera represented by the six sites, and all 15 pairings of sites. For the pairings, unsurprisingly the highest representation (18 genera – 90%) was given by Site 5 + Site 6. Based on the available data, there was only one single additional site, Site 1, where both of the 'missing' genera (*Orthocladius* and *Phaenopsectra*) were found. The pair of sites that gave the lowest representation was Site 4 + Site 3, which between them have only 40% of the total complement.

Discussion

Due to increasing water extraction for human use, and pesticide and fertilizer runoff, many English chalk rivers and streams are becoming badly degraded, endangering this unique aquatic ecosystem and the ecological and economic services provided (Wright & Berrie 1987, Environment Agency and English Nature 2004, O'Neill & Hughes 2014, Westwood et al. 2017). Ecological studies are needed to determine a baseline or reference point to which future ecological or habitat management activities could be measured and compared. Here, we presented the first account of the generic richness, diversity and community structure of chironomids in the Great Stour to be used as a baseline study toward the biological monitoring, environmental assessment and preservation of this type of freshwater habitat.

Chironomid larvae were readily sampled in all sites, as expected from this highly diverse and abundant group in freshwaters (Pinder 1986) and specifically in chalk streams (Wright & Symes 1999). The 20 genera identified here represents about 14.1% of the total number of genera in Britain and Ireland (142 full genera are listed by Chandler 2020), a substantial fraction considering the focal sampling in a small stretch in the Great Stour. The genus accumulation curves and Gmax (expected number of genera) suggest that there could be at least five more chironomid genera in this stretch of river, which if found would represent almost 18% of all non-biting midge genera found in Britain and Ireland.

There are few studies of chironomid diversity in chalk streams, but our results are comparable to those, for example, obtained at Tadnoll Brook. In a study of this chalk stream in South Dorset, UK, Pinder (1977) reported 19 genera from which 12 were also found in our study – a similar generic richness but very different composition than the one found here (based on the available raw data, the pairwise complementarity value for Tadnoll Brook/Great Stour is 7/32 = 0.22 – lower than any pairwise comparison presented in Table 4). In the River Kennet, a chalk stream in southern England, six species belonging to five genera of chironomids (Orthocladiinae) were found in association with bulrush (Drake 1983); and in Örvényesi Creek in Hungary, a semi-natural calcareous stream with highly heterogeneous aquatic habitats, 31 genera were found across seven sites (Móra & Szivák 2012). Studies in other river systems have also showed similar results; for example, Sealock and Ferrington (2008) reported 30 genera of chironomid pupal exuviae from eight sites along >10 km stretch in Hardwood Creek, Minnesota, where 10 genera were found using a dipnet method and 20 using pan-and-sieve method; Syrovátka and Brabec (2006) reported a total of 15 chironomid genera in pool and riffle mesohabitats along Svratka River, Czech-Moravian Highlands; and Prat et al. (2016) reported 21 chironomid genera from three sites in River Ter (Girona) and 13 genera from three sites in River Llobregat (Barcelona) in Catalonia, northeast Spain.

In the Great Stour, there were several chironomid genera with low CPET tolerance ratings (A) found across all sites, along with genera with other tolerance ratings (B, C and D), indicating that this stretch of river has low levels of organic pollutants (Environment Agency and English Nature 2004, Wilson & Ruse 2005). Somewhat surprising was the absence of the genus *Chironomus* (bloodworms) in the community composition, one of the most species-rich groups of chironomids in Britain and Ireland (over 30 species listed in Chandler 2020), and with high tolerance for organic pollution (Pinder 1986); however, this genus was identified based on DNA sequence similarity to GenBank (National Center for Biotechnology Information) data in an

exploratory DNA barcoding study of chironomids in Westgate Parks using partial sequences of the mitochondrial gene cytochrome oxidase subunit I (McConkey 2017).

Although the observed differences in generic richness and diversity among sites and different tolerance ratings for morphotypes could reflect unexplored microhabitat conditions in the Great Stour, our taxonomic resolution and unbalanced sampling across different years and in different seasons could account for these differences. However, if so, the sampling effort would reflect the number of genera found per site and this does not seem to be the case; for example, Site 6 (Kingsmead Field) was sampled only once in 2015, had the lowest sample size, but showed the highest generic diversity and the second highest generic richness among all sites, while Site 4 (Bingley Island) had the largest sample size but lowest richness and diversity. Our findings thus suggest that there could be different microhabitat suitability in the Great Stour affecting the chironomid community structure, even at a fine-scale and short stretch of the river. Thus, for example, although not measured, Bingley was the most shallow of the sites, and almost certainly had the lowest and slowest water flow.

If slightly different environmental conditions are present in the Great Stour, this could explain the different percent genus dominance per site even among nearby locations along the river (Puntí et al. 2007, Rae 2013). The dominance plots for the four main (and closest) sites in Westgate Parks (Site 1, Site 2, Site 3 and Site 4) showed that one or two genera accounted for >60% cumulative dominance, involving either *Cricotopus*, *Eukiefferiella* or *Rheotanytarsus*, while the other genera always showed <20% dominance. The site further upstream (Site 5) was dominated by *Tvetenia*, *Cricotopus* and *Eukiefferiella* (78% cumulative dominance), and the site further downstream (Site 6) was dominated by a different set of genera including *Polypedilum*, *Rheotanytarsus*, *Paratendipes* and *Micropsectra* (69% cumulative dominance). Furthermore, the four main sites in Westgate Parks showed greatest generic similarity as evidenced by the Bray-Curtis dissimilarity matrix and

dendrogram, and TWINSPAN two-way classification, whereas the PCA and 2D-NMDS plots showed sites 1 and 3 and sites 2 and 4 from Westgate Parks to have great similarity and distant from sites 5 and 6. The distant position of Site 5 and Site 6 in the PCA and 2D-NMDS plots shows that these sites are both unique and different from each other in terms of generic composition. These results were echoed by the Beta diversity and complementarity analyses, with sites in Westgate Parks showing low Beta diversity (i.e. low genus exchange) and low complementarity (i.e. high generic overlap) among sites, most clearly in pairwise comparisons with Site 4, while pairwise comparisons with Site 5 and Site 6 showing high Beta diversity and high complementarity. Based on these results, in terms of conservation of chironomid biodiversity in the Great Stour, efforts should focus on maintaining stable environmental conditions along the length of the river instead of focusing on just one site; for example in this study, selecting Site 5, Site 6 and any site in the main river stretch in Westgate Parks (Site 1, Site 2 and/or Site 3) would warrant the highest generic richness, diversity, exchange and complementarity.

Environmental variables affecting the distribution of chironomid larvae and other aquatic macroinvertebrates in chalk rivers requires further study to understand the importance of factors structuring their communities, particularly in relation to temporal flow changes and anthropogenic activities. Based on other studies (e.g. Puntí et al. 2007, Casas & Langton 2008, Rae 2013, Syrovátka et al. 2009, Móra & Szivák 2012), it would be expected to differentiate groups of chironomids in relation to the influence of environmental variables in headwater sites with lower temperature and higher water quality, middle sites with permanent water regime and marked seasonal variation, and lower sites with higher water temperature and/or tidal influence. Here, four of the genera detected (20%; *Paratendipes*, *Phaenopsectra*, *Polypedilum* and *Prodiamesa*) have been shown to be exclusively indicative of river sites with minor current and high amount of particulate organic matter, and four (20%; *Eukiefferiella*, *Orthocladius*, *Rheotanytarsus* and *Tvetenia*) were typical of sites with runs and riffles

with mineral substrate with aquatic vegetation (Syrovátka et al. 2009); furthermore, the mixed nature of chironomid tribes found in the Great Stour (Othocladiinae, Tanytarsini and Chironomini) is similarly characteristic of a middle section of Örvényesi Creek, Hungary showing longitudinal zonation and habitat heterogeneity (Móra & Szivák 2012).

Chalk streams and rivers are a valuable and rare habitat, economically important for water extraction, for trout and salmon fisheries, and for leisure and other industrial activities (Berrie 1992, Mainstone 1999), but they are also ecologically important (Berrie 1992, Mainstone 1999, O'Neill & Hughes 2014), having many invertebrates including rare species and streamside vegetation significant for terrestrial adults (Wood & Petts 1999, Harrison & Harris 2002), as well as historically relevant (Berrie 1992, Mainstone 1999, O'Neill & Hughes 2014). Therefore, the early detection of perturbations and biodiversity information in this freshwater ecosystem is warranted (Pinder 1989, Mainstone 1999, Wood & Petts 1999, O'Neill & Hughes 2014). Although identifying chironomids requires expert knowledge of taxonomy and classification, other cost-effective techniques like DNA barcoding (Ekrem et al. 2007) or environmental DNA (eDNA) metabarcoding (Czechowski et al. 2020) could be employed. However, for the molecular characterisation of chironomids or other freshwater invertebrates in the Great Stour, taxonomic identification and generation of voucher specimens is needed to generate an adequate DNA library. In this respect, this study provided information on the chironomid diversity in the Great Stour useful for any future biological assessments and monitoring programmes including those using molecular tools. Future needs include the sampling and biological characterisation of chironomid diversity and community structure along other parts of the Kentish Stour, from source to sea, as well as consistent, scientifically driven biological monitoring for the early detection of ecological perturbations.

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Declaration of interest

The authors have no declaration of interest

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Figure captions

Figure 1. Location of the Great Stour in Kent, UK, and six sampling sites of Chironomidae genera in Westgate Parks and nearby areas. (a) Map of the UK with Kent county highlighted. (b) Kent county showing the Great Stour divided into the Upper Great Stour, the East Stour and the main Great Stour with the sampling locations in the city of Canterbury. (c) Sampling locations, including Rheims Way (Site 1), Westgate Gardens (Site 2), Westgate Towers (Site 3), Bingley Island (Site 4; a side stream of the Great Stour), Horton (Site 5; a site 3 km upstream from Westgate Parks) and Kingsmead Field (Site 6 a site 1 km downstream from Westgate Parks).

Figure 2. (a) Genus percent abundance per site, and (b) genus dominance plot per site in the Great Stour in Kent, UK.

Figure 3. (a) Dendrogram showing the Bray-Curtis dissimilarities among sites, and (b) Principal Components Analysis (PCA) and biplot of Chironomidae genera for sites in the Great Stour in Kent, UK.

1 **Tables**

Table 1. Abundance data of Chironomidae genera per site in the Great Stour in Kent, UK (percent abundance per site between parentheses). See Fig. 1 for site locations.

	Site					
Genus	Rheims Way EA1 (Site 1)	Westgate Gardens EA3 (Site 2)	Westgate Towers EA4 (Site 3)	Bingley Island EAB (Site 4)	Horton EAH (Site 5)	Kingsmead K020 (Site 6)
Brillia	-	-	-	-	1 (0.4)	1 (1.9)
Conchapelopia	1 (0.6)	3 (2.0)	-	1 (0.2)	3 (1.)	2 (3.9)
Cricotopus	27 (17.2)	93 (62.4)	6 (9.7)	387 (60.7)	59 (21.2)	-
Epoicocladius	-	-	-	-	1 (0.4)	-
Eukiefferiella	55 (35.0)	27 (18.1)	33 (53.2)	38 (6.0)	53 (19.1)	-
Macropelopia	-	-	-	-	-	1 (1.9)
Micropsectra	8 (5.1)	6 (4.0)	7 (11.3)	13 (2.0)	3 (1.1)	5 (9.6)
Microtendipes	-	-	-	-	1 (0.4)	1 (1.9)
Orthocladius	1 (0.6)	-	-	-	-	-
Paratanytarsus	1 (0.6)	1 (0.7)	-	-	1 (0.4)	-
Paratendipes	-	3 (2.0)	3 (4.8)	-	-	8 (15.4)
Phaenopsectra	1 (0.6)	1 (0.7)	-	-	-	-
Polypedilum	-	2 (1.3)	1 (1.6)	1 (0.2)	6 (2.2)	12 (23.)
Prodiamesa	-	-	1 (1.6)	-	-	3 (5.8)
Rheocricotopus	-	-	-	-	13 (4.7)	-
Rheotanytarsus	62 (39.5)	13 (8.7)	11 (17.7)	198 (31.0)	25 (9.0)	11 (21.2)
Synorthocladius	-	-	-	-	2 (0.7)	-
Tanytarsus	1 (0.6)	-	-	-	2 (0.7)	4 (7.7)
Thienemanniella	-	-	-	-	3 (1.1)	-
Tvetenia	-	-	-	-	105 (37.8)	4 (7.7)

Total per site	157	149	62	638	278	52
The sites names EA1, EA3, EA4, EAB, EAH and K020 were the original notation used on the slide preparations stored at CCCU, and the correspondence to sampling sites is indicated in Fig. 1 and in Supplementary Information.						

1

1

Table 2. Diversity indices (based on Chironomidae genus data) from the Great Stour in Kent, UK.

Site	G	N	d	H(Ln)	J'	1- λ
Site 1	9	157	1.582	1.350	0.614	0.689
Site 2	9	149	1.599	1.228	0.559	0.567
Site 3	7	62	1.454	1.394	0.717	0.660
Site 4	6	638	0.774	0.934	0.521	0.532
Site 5	15	278	2.488	1.754	0.648	0.765
Site 6	11	52	2.531	2.093	0.873	0.851

G = the number of genera in each sample, N = the number of individuals in each sample, d = Margalef's d = (G-1)/Log(N), H(Ln) = Shannon's index = $- \Sigma(p_i(Ln(p_i)))$ where p_i is the proportion of individuals of each genera, J' = Pielou's evenness = H'/Ln(G), 1- λ = Simpson's index of diversity = $1 - \Sigma(p_i^2)$ where p_i is the proportion of individuals of each genera.

2

3

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Table 3. Pairwise β diversity values (Broad-sense measurements; Whittaker β_W) of Chironomidae genera among sites in the Great Stour in Kent, UK (low β indicates similarity, high β indicates dissimilarity).

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Site 1						
Site 2	0.2222					
Site 3	0.5000	0.2500				
Site 4	0.3333	0.2000	0.2308			
Site 5	0.4167	0.4167	0.5455	0.4286		
Site 6	0.6000	0.5000	0.4444	0.5294	0.3846	

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Table 4. Pairwise complementarity values of Chironomidae genera among six sites in the Great Stour in Kent, UK (raw values above the diagonal; similarities below the diagonal, scaled 0 to 1). For the fractions the numerator equals number of genera shared by both sites; the denominator indicates the total number of genera found at the two sites combined.

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Site 1		7/11	4/12	5/10	7/17	4/16
Site 2	0.64		6/10	6/9	7/17	5/15
Site 3	0.33	0.60		5/8	5/17	5/13
Site 4	0.50	0.67	0.63		6/15	4/13
Site 5	0.41	0.41	0.29	0.40		8/18
Site 6	0.25	0.25	0.38	0.31	0.44	

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Table 5. Representation of the total complement as percentages for the genera of Chironomidae at six sites in the Great Stour in Kent, UK (in bold, diagonal), and by all 15 pairings of the six sites (below the diagonal). Raw values for the pairings are given above the diagonal. The denominator in all cases is the total complement of genera (20) for all six sites combined (Table 1). The minimum and maximum values for pairings are indicated by an asterisk.

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Site 1	**45**	11/20	12/20	10/20	17/20	16/20
Site 2	55	**45**	10/20	9/20	17/20	15/20
Site 3	60	50	**35**	8/20	17/20	13/20
Site 4	50	45	40*	**30**	15/20	13/20
Site 5	85	85	85	75	**75**	18/20
Site 6	80	75	65	65	90*	**55**

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