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

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Supplementary information

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Notes on the Great Stour

The source of the Kentish Stour lies under the southern escarpment of the North Downs, between the village of Lenham and the hamlet of East Lenham. At Ashford, about 18 km to the south-east, these headwaters known as the Great Stour (or Upper or West Great Stour), are joined by several other brooks, including the East Stour, Ruckinge Dyke, Brook Stream and Whitewater Dyke, to form the main section of the river. Up to this point the waterflows of the Great Stour are somewhat erratic, and flash floods can occur. The Great Stour then continues mainly north-eastwards through Wye, Canterbury and Stodmarsh. At Plucks Gutter, *ca* 16 km downstream from Canterbury, the Great Stour joins the Little Stour, and below this confluence the river is termed simply The Stour. The river discharges into the English Channel at Pegwell Bay, approximately 50 km east of its source at Lenham.

Amongst the waterways exclusive to Kent, the Kentish Stour, with a length of some 85 km from Lenham to the sea (ignoring the shortening effected by the Stonar Cut, Richborough), is considered to have a catchment area second only to that of the River Medway. The geology of the river system, with special reference to the Upper Cretaceous chalk which underlays the whole area, has been reviewed by Aldiss et al. (2004). This aquifer water has a very low phosphorous content.

Notes on the Westgate Parks project

In 2013, the UK Heritage Lottery Fund (HLF) made a substantial award for a five-year ‘Parks for People’ project. Although most of the funds were earmarked for infrastructure improvement of the parks, a community-based activity programme, including volunteering, was an essential element of the programme. At that time, the UK Environment Agency (EA), with responsibility for water quality of the river and a desire to see improvements to the ecology of the river as it passed through Canterbury, were active stakeholders in the Friends of Westgate Parks group.

This study originated from the Westgate Parks Project developed in 2011 by the community group *Friends of Westgate Parks* supported by CCC and the UK Heritage Lottery Fund (HLF) (see Greenslade et al. 2013). The funds were sought to improve four contiguous public open spaces in Canterbury City, now known as Westgate Parks, which run upstream from the medieval city centre Westgate Towers for just under 1 km. Upstream, the Great Stour divides into two branches at the western end of Bingley Island (one of the four spaces). Downstream from this point the branches are separated by up to 300 m. Bingley Island and Tannery Field lie between the two branches, Westgate Gardens is divided by the larger of the two streams, while Toddler's Cove adventure playground is on the north side of this main stream.

It was decided that volunteers should be engaged to separate chironomid midge larvae from the regular kick-samples EA were making at the time (together with recent samples that the EA had retained, including some from the 3 km upstream site at Horton), with the prospect of later, in-depth research with respect to water quality as revealed by non-biting midge diversity. In the event, most of the volunteers were undergraduates at Canterbury Christ Church University (CCCU, see acknowledgments). Due to financial cuts, the EA sampling programme was subsequently reduced, and then ended altogether. Volunteers connected with CCCU continued the sampling for a period, including two additional sites (the side stream at Bingley Island, and 1 km downstream at Kingsmead Field). All the samples reported on here were taken within the period 2011–2015.

Chironomid larvae mounting technique

Chironomid larvae were collected and stored in 70% IMS. To start the mounting process, the larvae were first passed to 20% IMS in a cavity block and then sorted under a low-power binocular microscope. Up to four larvae (from the same site and sample) were placed in a tube containing 10% KOH and incubated at 70°C using a dry block heater for 25 min. The larvae were then removed using stainless-steel

forceps and placed in distilled water for 5 min. In a fume cupboard, the KOH was neutralised with glacial acetic acid for a minimum of 5 min. Up to four chironomids per microscope slide were mounted on the microscope slides (one at a time) by placing a small drop of Hydromount Histology Mounting Media (National Diagnostics) for each specimen. Under the low-power microscope, the head was separated from the rest of the body using mounted needles (or cataract scissors if available). The head of the larva was oriented in the right position, with larval pelt on side, then, using clean forceps, a circular cover slip (10 or 13 mm) was put in place and pressed down carefully with blunt instrument to correctly spread the mandibles. This process was repeated until all positions on the slide were used, leaving standard space for labelling. Slides were stored in suitable slide trays. For a detailed protocol for the preparation of insect larvae, see Smith (1989).

Original site notation recorded on slide labels

In this paper the notation Sites 1–6 for the six river bed locations investigated is used throughout. During the study, however, a more complex notation based on an original Environment Agency (EA) numbering scheme was employed – and it is this original numbering scheme that appears on the printed slide labels, as given in the following list:

Site 1 (Rheims Way) = EA1

Site 2 (Westgate Gardens) = EA3

Site 3 (Westgate Towers) = EA4

Site 4 (Bingley Island) = EAB

Site 5 (Horton) = EAH

Site 6 (Kingsmead Field) = KO20

Taxonomic notes on the 20 genera of chironomids from the Great Stour

The 20 genera of Chironomidae found at the Great Stour sampling sites are listed in Table 1 (see also Supplementary information Table S1). All eight subfamilies represented in the Britain and Ireland non-biting midge fauna are shown – but only four are represented in the data set. The numbers in parentheses after subfamilies indicates the number of genera in the British and Irish fauna, and after genera, the number of species (based on Chandler 1998 and updates). The Chironominae are divided into three tribes (two represented) and the Tanypodinae into seven (two represented).

Diversity and ecology of the 20 chironomid genera from the Great Stour

These brief accounts of chironomid genera from the Great Stour are presented alphabetically:

1) *Brillia* Kieffer, 1913. Two species in Britain and Ireland. Wing length *ca* 3–4.5 mm (Coe et al. 1950). According to Cranston (1982), in Britain the larvae of both species breed in flowing water – those of *B. longifurca* Kieffer, 1921 [= *flavifrons* auctt. nec. Johannsen, 1905 – see Chandler (2020)] often being found grazing on the surface of submerged wood. Murray et al. (2018) also list various lentic habitats, such as pools, ponds and the edges of lakes.
2) *Conchapelopia* Fittkau, 1987. Six British and Irish species (Chandler 2020). Wing-length *ca* 3–5 mm. Found in a variety of habitats including running water (Rufer & Ferrington 2007). *C. melanops* Meigen, 1818 is widespread in Britain (Langton 1984).
3) *Cricotopus* van der Wulp, 1874. Following Chandler (2020) the British and Irish species are divided into four subgenera – *Cricotopus* s.s. (22 spp.), *Isocladius* Kieffer, 1909 (12 spp.), *Nostococladius* Ashe & Murray, 1980 (1 sp.), and *Paratrichocladius* Santos Abreu, 1918 (3 spp.). Wing-length *ca* 1.5–4 mm. Found in still and flowing waters, many species are epiphytic grazers on submerged macrophytes, grazing on diatoms and other algae (Cranston 1982).

4) *Epoicocladius* Sule & Zavřel, 1924. A single species, *E. ephemerae* Kieffer, 1924 with wing-length of about 2 mm. Apparently always associated with mayfly larvae, notably *Ephemera danica* Müller, 1764, grazing on the cuticle (Cranston 1982).
5) *Eukiefferiella* Thienemann, 1926. Chandler (2020) lists 14 species for Britain and Ireland. Wing-length *ca* 1.2–2.5 mm (Coe et al. 1950). Mostly associated with running water, but occasionally in springs or slow-flowing streams (Cranston 1982).
6) *Macropelopia* Thienemann, 1916. Four species for Britain and Ireland are listed by Chandler (2020), one of them not formally named; Murray et al. (2018) list five species-level taxa. Wing-length 4–6 mm (Coe et al. 1950). Their predaceous larvae occur in streams (Hildrew et al. 1985). According to Murray et al. (2018) the larvae of *Macropelopia* are usually encountered in fine sediments of springs, streams, bog pools, drains and lake margins. The larvae of *M. nebulosa* Meigen, 1804 occur in muddy rivers and lakes (Wilson & Ruse 2005) and are widespread in Britain (Langton 1984).
7) *Micropsectra* Kieffer, 1909. Some 17 species in the Britain and Ireland fauna (Chandler 2020). Wing length about 2–3.5 mm. The larvae occur in springs, rivers, lakes and ponds in soft sediments (Wilson & Ruse 2005). Several species in the genus are widespread in Britain although some are associated with cool northern regions (Langton 1984). The larvae are found in lakes or running water (Säwedal 1982).
8) *Microtendipes* Kieffer, 1915. Chandler (2020) lists nine species for Britain and Ireland. The adults are relatively large (wingspan 3.5–5 mm: Coe et al. 1950). The genus is widespread and common in Britain (Langton 1984). The larvae occur in rivers, ponds and lakes often amongst moss (Wilson & Ruse 2005; Murray et al. 2018). According to Rufer & Ferrington (2007), writing about Minnesota, they breed in “littoral to sublittoral sediments of large lentic water bodies” and “in submerged mosses in running water.”

9) *Orthocladius* van der Wulp, 1874. Six subgenera are recognised among the British and Irish species: *Eudactylocladius* Thienemann, 1935 (3 spp.), *Euorthocladius* Thienemann, 1935 (5 spp.), *Orthocladius* s.s. (9 spp.), *Pogonocladius* Brundin, 1956 (1 sp.), *Mesorthocladius* Sæther, 2005 (1 sp.), and *Symposiocladius* Cranston, 1982 (3 spp.). Wing-length about 2–4 mm. Associated in most cases with running water (Cranston 1982), but *Orthocladius* larvae can also occur in lakes, ponds, marshes, on wet rocks and even in soils (Murray et al. 2018).

10) *Paratanytarsus* Thienemann & Bause, 1913. The Britain and Ireland list includes 14 species. The adults have wing-length of *ca* 2–3 mm. According to Säwedal (1982) the larvae occur in shallow standing waters – but Rufer & Ferrington (2007) indicate flowing waters as well as lakes. Wilson & Ruse (2005) state the genus can be found in most aquatic habitats. Murray et al. (2018) indicate a variety of lotic and lentic habitats, including bogs, marshes and brackish ponds. The genus is widely distributed in Britain (Langton 1984).

11) *Paratendipes* Kieffer, 1911. Three species in Britain and Ireland. Adult wing-length less than 2 mm up to 3.5 mm. The genus is widespread in Britain (Langton 1984). The larvae occur in rivers, lakes, streams, and ponds where they are associated with sandy and silty substrates (Wilson & Ruse 2005, Murray et al. 2018). Rufer & Ferrington (2007) state that they are found in both “standing and flowing waters in soft sediments and sandy bottoms.”

12) *Phaenopsectra* Kieffer, 1921. Two species on the British and Irish list, with adult wing length 3–4.5 mm (Coe et al. 1950). The genus is widespread in Britain (Langton 1984). The larvae occur in stream and ponds bottoms with sandy silt (Wilson & Ruse 2005, Rufer & Ferrington 2007).

13) *Polypedilum* Kieffer, 1912. The British and Irish fauna is divided into four subgenera: *Pentapedilum* Kieffer, 1913 (3 spp.), *Polypedilum* s.s. (6 spp.), *Tripodura* Townes, 1945 (7 spp.), and *Uresipedilum* Oyewo & Sæther, 1998 (2

spp.). Wing length about 1.75–4 mm. The genus is widespread in Britain (Langton 1984). Larvae occur in almost all types of water bodies, including ephemeral habitats (Wilson & Ruse 2005, Rufer & Ferrington 2007, Murray et al. 2018).

14) *Prodiamesa* Kieffer, 1906. Two species on the British and Irish list. Wing-length *ca* 4–5.5 mm. Known to occur in running waters. The larvae are eurytopic occurring in eutrophic springs, ponds, rivers and the littoral of lakes (Wilson & Ruse 2005), and are said to be “moderately tolerant of pollution” (Murray et al. 2018). *P. olivacea* Meigen, 1818 is widespread in Britain (Langton 1984).

15) *Rheocricotopus* Brundin, 1956. Two subgenera recognised for the British and Irish species: *Psilocricotopus* Saether, 1985 (4 spp.) and *Rheocricotopus* s.s. (2 spp.). Wing-length about 2.5 mm (Coe et al. 1950). The larvae mainly occur in streams and rivers (Cranston 1982), living on aquatic vegetation; less frequently in lake margins (Murray et al. 2018).

16) *Rheotanytarsus* Thienemann & Bause, 1913. Ten species are listed for Britain and Ireland (Chandler 2020). Adult wing-length *ca* 2–3 mm. The larvae occur in springs and streams (Säwedal 1982), rivers (Wilson & Ruse 2005), including the lower reaches, and the margins of lakes where there are currents (Murray et al. 2018). Species such as *R. photophilus* Goetghebuer, 1921 are widespread in Britain but others are more restricted (Langton 1984).

17) *Synorthocladius* Thienemann, 1935. One species of the British and Irish list, with a wing-length of 2.2–2.5 mm (Coe et al. 1950). Occurring widely in streams and rivers, including the River Thames, the larvae can be found on the surface of submerged stones (Cranston 1982).

18) *Tanytarsus* van der Wulp, 1874. A current total of 42 species on the British and Irish list. Wing-length *ca* 1.75–3 mm. The larvae of the many species occur in a wide variety of aquatic habitats, including brackish waters (Wilson & Ruse 2005).

19) *Thienemanniella* Kieffer, 1911. Seven British and Irish species. Small midges, 1–1.8 mm wing-length (Coe et al. 1950). The larvae occur in running waters (Cranston 1982), from fast rivers to slow streams and even ditches (Murray et al. 2018).

20) *Tvetenia* Kieffer, 1922. Four species on the British and Irish list. Wing-length about 1.8–2.4 mm. In Britain the larvae occur in flowing waters (Cranston 1982).

References

Aldiss DT, Bloomfield JR, Buckley DK, Doran SK, Evans DJ, Hopson PM, Royse KR, Woods MA. 2004. A geological model of the chalk of East Kent, Volume 1 of 2: Report. Keyworth: British Geological Survey.

Chandler PJ. 1998. Checklist of insects of the British Isles (New Series). Part 1: Diptera. Handbooks for the Identification of British Insects. 12:1-234.

Chandler PJ. 2020. An update of the 1998 Checklist of Diptera of the British Isles. [updated 15 January 2020]. Available at https://www.dipterists.org.uk/sites/default/files/pdf/BRITISH%20ISLES%20CHECKLIST%202020_01.pdf

Coe RL, Freeman P, Mattingly PF. 1950. Diptera 2. Nematocera: families Tipulidae to Chironomidae. Handbooks for the identification of British Insects. 9:121–206.

Cranston PS. 1982. A key to the larvae of the British Orthocladiinae (Chironomidae). Ambleside: Freshwater Biological Association Scientific Publication (45):1–152.

Greenslade AFC, Barclay MVL, Hammond PM, Booth RG, Bantock TM, Percy D, Osborne D, Ware C, Shelton J, Weekes J, Ponsonby D, Cameron-Fleming I, Vane-Wright RI. 2013. The beetles (Coleoptera) of Bingley Island: a preliminary checklist, with additional notes on history, ecology, bugs (Hemiptera) and flowering plants. Entomologist's Gazette. 64:146–165.

Hildrew AG, Townsend CR, Hasham A. 1985. The predatory Chironomidae of an iron-rich stream: feeding ecology and food web structure. Ecological Entomology. 10:403–413.

Langton PH. 1984. A key to pupal exuviae of British Chironomidae. Published privately by PH Langton.

Murray DA, O'Connor JP, Ashe PJ. 2018. Chironomidae (Diptera) of Ireland – a review, checklist and their distribution. Occ Publ Irish Biogeog Soc. (12): x–404.

Rufer MM, Ferrington LC. 2007. Key to the Chironomidae pupal exuviae in the twin cities metro area lentic waters. Unpublished Master's thesis, University of Minnesota, St. Paul, USA. https://www.rmbel.info/wp-content/uploads/2013/07/Rufer_pupalkey.pdf [accessed 19/08/2020]

Säwedal L. 1982. Taxonomy, morphology, phylogenetic relationships and distribution of *Micropsectra* Kieffer, 1909 (Diptera: Chironomidae). Insect Systematics and Evolution. 13:371–400.

Smith KGV. 1989. An introduction to the immature stages of British flies; Diptera larvae, with notes on eggs, puparia and pupae. In: Dolling WR, Askew RR, editors. Handbooks for the identification of British insects, Vol. 10, Part 4. London: Royal Entomological Society of London.

Wilson RS, Ruse LP. 2005. A guide to the identification of genera of chironomid pupal exuviae occurring in Britain and Ireland (including common genera from northern Europe) and their use in monitoring lotic and lentic fresh waters. Ambleside: Freshwater Biological Association.

Table S1. The 20 genera and species morphotypes of Chironomidae found at one or more of the six sampling sites in the Great Stour in Kent, UK, listed by subfamily and tribe (where applicable), and arranged alphabetically within these groups. Values in parentheses indicate total number of genera within subfamilies, and species within genera, in Britain and Ireland. Tolerance ratings and trophic guild from Wilson and Ruse (2005): A = intolerant of organic stress, B/C = intermediate, D = tolerant, Comm = commensal, Detr = detritivore, Filt = filter-feeder, Graz = grazer, Pred = predator. For original authors and dates for morphotype names see Chandler (1998, 2020).

Subfamily	Tribe	Genera in study	Morphotype	Tolerance rating and trophic guild
Buchonomyiinae (1)	[No tribal division]	None		
Chironominae (47)	Chironomini	*Microtendipes* (9)	*pedellus*	A Detr
			rydalensis	A Detr
		Paratendipes (3)	*albimanus*	A Detr
		Phaenopsectra (2)	*flavipes*	A Detr
		Polypedilum (18)	*nubeculosum*	A Detr
	Tanytarsini	*Micropsectra* (17)	Undetermined	B/D Detr
			contracta	B Detr
			pallidula	B Detr
		Paratanytarsus (14)	*austriacus*	A Graz
			penicillatus	A Graz
		Rheotanytarsus (10)	Undetermined	B Filt
		Tanytarsus (42)	*chinyensis*	A Detr
			mendax	A Detr
Diamesinae (7)		None		
Orthocladiinae (53)	[No tribal division]	*Brillia* (2)	*longifurca*	D Graz
			bifida	C Graz
		Cricotopus (38)	Undetermined	C/D Graz

			sg. *Isocladius*	C/D Graz
			bicinctus	D Graz
			fuscus	C Graz
			pulchripes	C Graz
			tremulus	C Graz
			triannulatus	C Graz
			trifascia	C Graz
		Epoicocladius (1)	*ephemerae*	A Comm
		Eukiefferiella (14)	Undetermined	A/C Graz
			claripennis	C Graz
			devonica	A Graz
			ilklyensis	A Graz
		Orthocladius (22)	Undetermined	A/B Graz
		Rheocricotopus (6)	*chalybeatus*	B Graz
			fuscipes	B Graz
		Synorthocladius (1)	*semivirens*	C Graz
		Thienemanniella (7)	*sp. B*	C Graz
		Tvetenia (4)	*calvescens*	B Graz
Podonominae (3)	[No tribal division]	None		
Prodiamesinae (3)	[No tribal division]	*Prodiamesa* (2)	Undetermined	D Detr
Tanypodinae (26)	Macropelopiini	*Macropelopia* (5)	Undetermined	A/D Pred
	Pentaneurini	*Conchapelopia* (6)	Undetermined	C/D Pred
Telmatogetoninae (2)	[No tribal division]	None		

1

2

1

Table S2. Pairwise Bray-Curtis dissimilarity matrix among sites in the Great Stour in Kent, UK based on (relative) abundance data of Chironomidae genera.

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Site 1						
Site 2	0.5002					
Site 3	0.3245	0.5609				
Site 4	0.4362	0.2231	0.6443			
Site 5	0.5203	0.4807	0.5957	0.6244		
Site 6	0.7248	0.8188	0.6458	0.7650	0.7756	

Site 1 = Rheims Way, Site 2 = Westgate Gardens, Site 3 = Westgate Towers, Site 4 = Bingley Island (a side stream of the Great Stour), Site 5 = Horton (a site 3 km upstream from Westgate Parks), Site 6 = Kingsmead Field (a site 1 km downstream from Westgate Parks).

2

3

1

Table S3. Absolute pairwise distances in the 1st Dimension (below diagonal) and in the 2nd Dimension (above diagonal) among sites in the Great Stour in Kent, UK

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Site 1		0.12	0.3	0.52	0.99	0.06
Site 2	0.53		0.18	0.4	1.11	0.18
Site 3	1.09	0.56		0.22	1.29	0.36
Site 4	0.07	0.46	1.02		1.51	0.58
Site 5	0.36	0.17	0.73	0.29		0.93
Site 6	2.57	2.04	1.48	2.5	0.93	

Site 1 = Rheims Way, Site 2 = Westgate Gardens, Site 3 = Westgate Towers, Site 4 = Bingley Island (a side stream of the Great Stour), Site 5 = Horton (a site 3 km upstream from Westgate Parks), Site 6 = Kingsmead Field (a site 1 km downstream from Westgate Parks).

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Table S4. Two-way indicator species analysis (TWINSPAN) used to construct a classification of the sites and order the chironomid genera according to their site of preference. Two site divisions (0/1) and four genera divisions were found for Chironomidae genera in the Great Stour in Kent, UK.

	Site						
Genus	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Genera divisions
Orthocladius	1	-	-	-	-	-	000
Phaenopsectra	1	1	-	-	-	-	000
Cricotopus	2	3	1	5	3	-	001
Eukiefferiella	3	2	2	2	3	-	001
Rheotanytarsus	3	1	1	4	2	1	001
Micropsectra	1	1	1	1	1	1	010
Paratanytarsus	1	1	-	-	1	-	010
Paratendipes	-	1	1	-	-	1	010
Conchapelopia	1	1	-	1	1	1	011
Polypedilum	-	1	1	1	1	1	011
Prodiamesa	-	-	1	-	-	1	10
Tanytarsus	1	-	-	-	1	1	110
Brillia	-	-	-	-	1	1	111
Epoicocladius	-	-	-	-	1	-	111
Microtendipes	-	-	-	-	1	1	111
Rheocricotopus	-	-	-	-	1	-	111
Synorthocladius	-	-	-	-	1	-	111
Thienemanniella	-	-	-	-	1	-	111
Tvetenia	-	-	-	-	4	1	111
Macropelopia	-	-	-	-	-	1	111
Site divisions	0	0	0	0	1	1	

Site 1 = Rheims Way, Site 2 = Westgate Gardens, Site 3 = Westgate Towers, Site 4 = Bingley Island (a side stream of the Great Stour), Site 5 = Horton (a site 3 km upstream from Westgate Parks), Site 6 = Kingsmead Field (a site 1 km downstream from Westgate Parks).

2

3

Supplementary figures

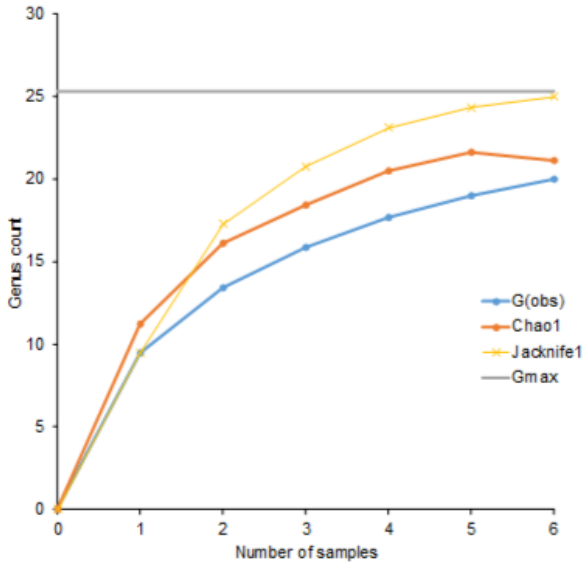

Figure S1. Genus accumulation curve of chironomid larvae found in six sites in the Great Stour in Kent, UK, showing the cumulative genus count against sample number, where sample order was permuted (999 maximum permutations) to obtain the mean observed genus counts, G(observations), per sample. The Chao1 and Jackknife1 estimators were used to calculate the genus accumulation curve and genus richness. The Michaelis-Menten asymptotic curve was fitted to the observed genus curve and it was used to estimate the total number of genera (Gmax).

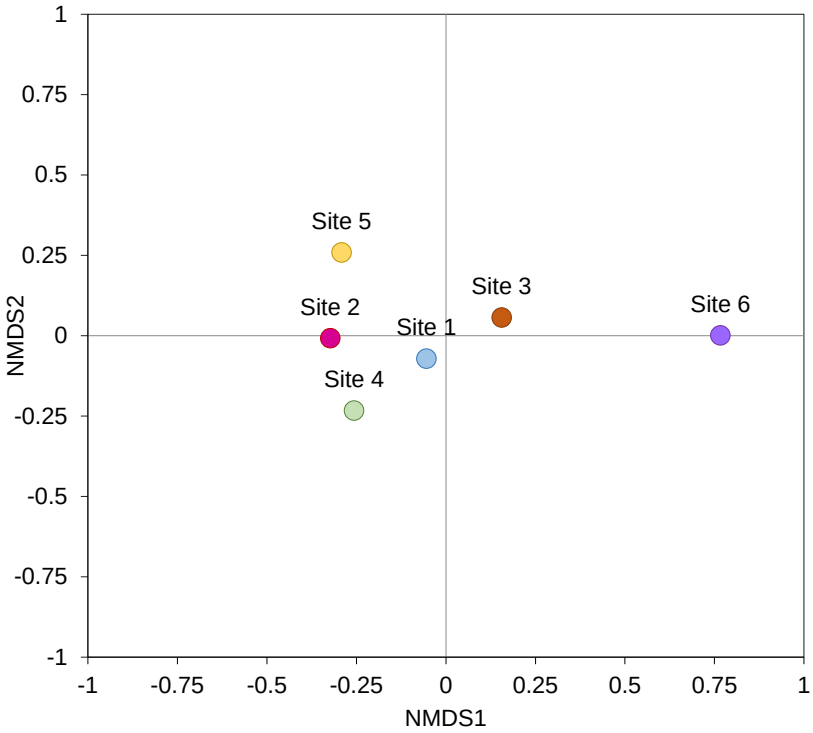

Figure S2. 2D non-metric Multidimensional Scaling (NMDS) plot of Chironomidae genera for sites in the Great Stour in Kent, UK.