Ecological characteristics of pre-imaginal stages of blackflies (Diptera: Simuliidae) in southern England

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Abstract

After adult blackflies were found in traps deployed in mosquito surveys, rivers and streams close to the traps' locations were examined for the presence of pre-imaginal stages. The data obtained were supplemented by additional surveys and analysed in relation to environmental factors. Fourteen taxa from 29 locations were recorded. Of these, seven are of medical and/or veterinary importance: *S. equinum*, *S. erythrocephalum*, *S. intermedium sensu lato* (*s.l.*, a complex of species), *S. lineatum*, *S. morsitans*, *S. noelleri* and *S. ornatum sensu lato* (a complex of species). Analyses of a variety of environmental factors recorded at the insects' breeding sites showed that, by considering larvae and pupae together, the differences in the assemblages of blackfly species were explained by four variables: river depth, temperature, conductivity and elevation

Keywords: Simuliidae; Environmental variables; Multivariate analysis; Medical and veterinary importance; England.

Introduction

In 2017, entomologists working as part of the WetlandLIFE project investigated the biodiversity of mosquito species in England by conducting larval and pupal surveys and trapping adults (Hawkes et al. 2020). During this research, the traps deployed to attract and capture adult specimens of mosquitoes also captured adult blackflies (Diptera: Simuliidae) that were attracted by the carbon dioxide released by the combustion of butane gas and heat produced by the traps (Cheke et al. 2018). The following haematophagic and anthropophilic species were caught (*Simulium* (*Eusimulium*) *aureum* Fries, 1824, *Simulium* (*Wilhelmia*) *equinum* (Linnaeus, 1758), *Simulium* (*Wilhelmia*) *lineatum* (Meigen, 1804), *Simulium* (*Simulium*) *noelleri* Friederichs, 1920 and *Simulium* (*Simulium*) *ornatum s.l.* (Meigen, 1818) (López-Peña et al. 2021). *Simulium lineatum* and members of the *S. ornatum* complex are also implicated as vectors of the pathogenic agents responsible for bovine onchocerciasis and *S. equinum* is known as a cause of "sweet-itch" in livestock, so new ecological information on Simuliidae species in general and on haematophagic species in particular will contribute to a better understanding of their biology and possible use for control purposes. Previous research on the distribution of the larvae and pupae of Simuliidae highlighted the role of abiotic factors in structuring species assemblages. Among these factors, current speed (Morin and Peters 1988; McCreadie and Colbo 1993; Figueiró et al. 2008; Cheke et al. 2017), temperature (McCreadie et al. 2005; Cheke et al. 2017), and elevation (Ya'cob et al. 2016; Cheke et al. 2017) are the most important. Nevertheless, the ecological characteristics of the breeding sites of many species are not yet known, although it has long been recognized that closely related species – *i.e.* belonging to the same subgenus - can exhibit marked differences in their ecological associations (Day et al. 2008), as well as varying in their medical or veterinary significance.

Sampling took place in summer and early autumn when simuliid larvae and pupae are typically observed in high numbers (Maitland and Penney 1967). Abundance data on blackfly assemblages and associated environmental variables were gathered from each of the sampled points in order to investigate environmental factors associated with distributions of blackfly assemblages in southern England.

2. Methods

Study area and sampling design

The study was restricted to England, with samples collected in the Counties of Bedfordshire, Dorset, Hampshire and Worcestershire (Table 1). Twenty-nine samplings were carried out in the study area (Figure 1) at 18 locations in 11 watercourses in the River Avon, River Great Ouse, River Wey, River Whitewater, River Loddon (and two of its tributaries: the River Lyde and the River Row), Bickerley Millstream, Bow Brook and Wannerton Brook (a tributary of the River Stour). In addition, an artificial pond, permanently oxygenated by an electric pump continuously forcing air into the water through plastic tubes and “air stones” was included amongst the samples since pre-imaginal stages of simuliids were found attached to the tubes. Restrictions due to the coronavirus pandemic limited the extent of the study area.

Most of the samples were collected between 22 July and 19 October 2020, dates constrained by the mobility restrictions in the UK due to the coronavirus pandemic. In addition, data from two samples from one of the locations that had been sampled in 2013 were included in the study. Sampling sites ranged widely in latitude, from the Wannerton Brook in the north to the River Wey in the south, but with little variation in elevation (1-80 m above sea level; masl). At each sampling point, several environmental variables were measured *in situ* including elevation (m) and geographical coordinates (degrees °, minutes ', seconds ") with a Geographical Positioning System (GPS; Garmin Montana 610) and relative humidity (%) based on dry and wet bulb ambient temperature (ºC) readings using a compact whirling hygrometer (Casella, London). Physico-chemical water variables were also recorded and included pH (HANNA CLASSIC pHep; Hanna Instruments (Mauritius) Ltd.), total dissolved solids (TDS; ppm) and water temperature (ºC), both with a TDS meter (HM Digital TDS-4TM; www.hmdigital.com), conductivity (μs cm^{-1}) with a conductivity meter (HANNA

DiST HI98303; www.hannainst.com), and the river width and depth (m) with a 30m tape measure (Tradeline TP986689). In addition, the surface velocity ($m.sec^{-1}$) was measured by timing how long it took a one-metre length of nylon string to unravel. The result was converted by multiplying by a velocity correction factor of 0.8 to estimate the overall velocity of the water for later discharge estimates (Murdoch et al. 2001). Water samples were collected from which concentrations of dissolved oxygen [O_2] ($mg\ l^{-1}$) were estimated with a kit (Tetra Test O_2; www.tetra.net) *ex situ*, once in the laboratory, and, following Murdoch et al. (2001), later converted to percentage (%) using a DO % calculator (https://floridadep.gov/sites/default/files/DO%20Saturation%20Calculator7.xlsm). The concentrations of ammonia [NH_3] (ppm), nitrite [NO_2^-] (ppm) and nitrate [NO_3^-] (ppm) were also estimated from the water samples with a kit (API Freshwater master test kit). Other local environmental variables such as the condition of the riparian vegetation, the riverbed substrate type (sandy, stony, rocky, muddy), weather conditions (sunny, cloudy, windy, rainy), amount of sunshine (none, partial, sunny), gradient (flat, moderate, steep), and habitat anthropic level (urban, suburban, rural, mixed), were registered since these factors have been useful in predicting aquatic insect distributions in streams (Vinson and Hawkins 1998). All of these data were used to compile the environmental variables dataset, to be used in the analyses.

Biological sampling and species identification

To collect the samples, established protocols (McCreadie and Colbo, 1991) were used. These involved walking from one bank of the river to the other, whilst checking for pre-imaginal blackflies attached to helophyte plants, riverside trees with trailing vegetation, submerged macrophytes, boulders, cobbles and pebbles. The time invested at each sampling point consisted of 15 minutes (5 minutes on the right bank, 5 minutes in the centre of the water body and 5 minutes more on the left bank). Substrates with attached pre-imaginal stages were recorded (vegetation, stones, wood, metal, plastic, other) and kept individually in hermetically sealed plastic bags to transport the specimens within a cool box. Once in the laboratory, larvae and pupae were detached from their substrates (usually leaves, branches or stems), then fixed in 80% ethanol before the specimens were identified, counted and stored. Identifications were based on morphological characteristics (González 1990; Bass 1998 for mature larvae and pupae; Davies 1966 for adults that emerged from pupae) using a stereomicroscope (Wild Heerbrugg M5A) with cool light from a lamp (KL 1500-T SCHOTT).

Data analyses

Correlations between the environmental variables were checked and only those in pairs for which Pearson's *r* was lower than 0.8 were retained for Principal Components Analysis (PCA), which was used to explore the differences between sampling sites on standardized values of the environmental variables measured.

We performed a canonical ordination analysis (Borcard et al. 2011) using abundance data of mature larvae (immature larvae could not be identified morphologically with sufficient taxonomic resolution) and pupae. As a preliminary step, we used detrended canonical correspondence analysis (DCCA) to determine species gradient lengths with respect to the environmental variables in PCA, and therefore to assess whether unimodal (for further Canonical Correspondence Analysis, CCA) or linear-based (for further Redundancy Analysis, RDA) models underlay the responses of pre-imaginal stages of blackfly species to environmental variables (Birks, 1995). DCCA was performed on the log-transformed data of the abundance of mature larvae and pupae per site and revealed a dominance of unimodal gradients in all cases (maximal length of the first two axes of each DCCA ordination > 3 SD; Leps and Smilauer 2003). Therefore, further analyses were based on CCA, for which we followed a forward selection procedure to identify the main environmental variables that best explained the variability in assemblages of blackfly pre-imaginal stages. This step-by-step approach allows the examination of relationships between abundance data with only the most relevant environmental variables. The significance of the environmental variables introduced at each step was inferred from Monte Carlo permutation tests (999 permutations, p-value < 0.05). Model performances were assessed by adjusted-R^2 values.

Finally, we used Weighted Average (WA) regression (Birks et al. 1990) to estimate the distribution of the pre-imaginal stages of the blackfly species identified in the study along each of the environmental gradients of the variables selected in the previous sequential CCA analyses. WA regression estimates the value with the highest probability of occurrence with respect to a quantitative environmental variable for a given species based on the weighted average of the values of the variable of interest in those sites where the species is present, using the species' relative abundances as weights.

A permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) was performed to assess for significant differences in the blackfly pre-imaginal assemblages among the different substrates sampled (emergent macrophytes, submerged macrophytes, terrestrial plants, wood, rocks). The Bray-Curtis distance among samples was used for this purpose. Finally, Indicator Value (IndVal) analysis (Dufrêne and Legendre 1997) was used to determine the most representative blackfly species among the different substrate categories sampled. The IndVal would take its maximum value (probability equals 1) when all larvae and pupae of a species are found in just one type of substrate (*i.e.*, maximal specificity) and when the species is present in all samples of that type (*i.e.*, maximal fidelity). Here, we followed the criteria of Dufrêne and Legendre (1997) by considering a threshold level of 0.25 for the index to be accepted as relevant, which means that pre-imaginal stages of a given simuliid species are present in more than 50% of the samples of a substrate and with a relative abundance in this substrate type of more than 50%. For the assessment of the significance of a given simuliid species being characteristic of a particular substrate, IndVal was tested by randomization (999 permutations of samples among sample groups).

All statistical analyses were carried out using the free software R version 3.3.3 from The R Foundation for Statistical Computing (R Development Core Team 2017; https://cran.r-project.org). PCA was performed using the *prcomp* function from package “stats”. DCCA and CCA analyses were performed using the *decorana*, *cca* and *ordistep* functions from the “vegan” package (Oksanen et al. 2019). The functions *optima*, *tolerance* and *caterpillarplot* within the “analogue” package (https://cran.r-project.org/web/packages/analogue/index.html) were used in WA regression to depict the values with highest probability of occurrence and ecological amplitude for environmental variables of the pupae of the identified blackfly species. PERMANOVA analysis was performed by means of the function *adonis*, also from the “vegan” package. Finally, IndVal of each species and the respective significance levels per substrate were obtained using the *indval* function from the “labdsv” package (Roberts 2015).

Results

Geographical distribution and occurrence of pre-imaginal stages of blackfly species

Simuliids were present in 26 of the 29 samples. Numerous breeding sites of blackflies were found in our survey, from which many pre-imaginal specimens were obtained (a total of 5604 immature and mature larvae, and 1437 pupae). From them, 811 mature larvae and 1432 pupae were identified, belonging to ~~14~~ 12 species and 2 species complexes included in five subgenera (*Boophthora, Eusimulium, Nevermannia, Simulium* and *Wilhelmia*) of the genus *Simulium*. The species were as follows: *Simulium* (*Eusimulium*) *aureum* Fries, 1824; *Simulium* (*E.*) *angustipes* Edwards, 1915; *Simulium* (*Nevermannia*) *angustitarse* (Lundström, 1911); *Simulium* (*Wilhelmia*) *equinum* (Linnaeus, 1758); *Simulium* (*Boophthora*) *erythrocephalum* (De Geer, 1776); *Simulium* (*Simulium*) *intermedium s.l.* Roubaud, 1906; *Simulium* (*W.*) *lineatum* (Meigen, 1804); *Simulium* (*N.*) *lundstromi* (Enderlein, 1921); *Simulium* (*S.*) *morsitans* Edwards, 1915; *Simulium* (*S.*) *noelleri* Friederichs, 1920; *Simulium* (*S.*) *ornatum s.l.* Meigen, 1818; *Simulium* (*S.*) *petricolum* (Rivosecchi, 1963); *Simulium* (*S.*) *trifasciatum* Curtis, 1839 and *Simulium* (*E.*) *rubzovianum* (Sherban, 1961) (misidentified as *Simulium* (*E.*) *velutinum* (Santos Abreu, 1922), in some previous publications on UK simuliids, Adler (2020)). Table 1 shows information regarding the date, coordinates and elevation of every sample and Table 2 shows the occurrences of pre-imaginal stages (larvae and pupae, separately) of blackfly species in each river studied. The River Whitewater harboured the highest number of taxa (13), while the rivers Row and Avon had the fewest, with just three taxa.

The by-species analysis revealed that *S. aureum* was the least prevalent species, as its pre-imaginal stages were only found in two of the ten rivers studied. On the other hand, larvae or pupae of *S. intermedium s.l.* and *S. morsitans* were found in eight of the ten rivers. *Simulium intermedium s.l.* occurred with the highest abundance of larvae (465, > 65% specimens in the pupal stage), followed by *S. lineatum* (395), being particularly concentrated at sampling point 15 in the River Avon (with 214 pupae and 55 mature larvae; Table 2).

Simulium ornatum s.l. was detected in 13 of 18 sampling points (72.2%), followed by *S. intermedium s.l.* and *S. morsitans* (11 points, 61.1%). With regard to species occurrence per sampling site and visit, 13.8% of the samples did not retrieve any blackfly species and no singleton was ever found. Co-occurrence in samples averaged 4.8 ± 2.9 species (average ± standard deviation), but up to ten species were found in point 4(A). *Simulium intermedium s.l.* was the species complex which co-occurred with other blackfly species with the greatest frequency, at sixteen sampling points (88.9%).

Ecological distinctiveness of larval and pupal habitats

Data on the physico-chemical measurements taken are provided in the Supporting Information (Table SI1). Not all of the samples could be used for the multivariate analyses owing to some data being missing. The first two axes of the PCA on environmental variables explained 25.9 and 20.6% of the total variance, respectively, among the study sites. As the contribution of the subsequent axes sharply decreased, only the first two axes of the PCA were kept for visual inspection (Figure 2). The first component explained was positively related to sandy substrates (loading = 0.493) and negatively to muddy ones (loading = -0.404), and elevation (loading = -0.333). The second component was positively related to depth (loading = 0.555), temperature (loading = 0.387) and nitrite concentration (loading = 0.331), and negatively to stony substrates (loading = -0.415).

The CCA explained a considerable percentage of total inertia (53.7%) in the variability in blackfly assemblages (F = 3.071; *df* = 12, 9; *p*-value = 0.001). Further, a small subset of environmental variables was enough to retain most of the explained variability after applying forward selection. Hence, blackfly pre-imaginal assemblages were mainly explained by a first CCA axis positively related to elevation and negatively to depth, and a second CCA axis positively related to conductivity and negatively to temperature (Figure 3). Figures 3 taxon scores are placed at the centroids (weighted average) of the sampling points where each taxon occurs. *Simulium lineatum, S. erythrocephalum* and *S. equinum* occurred typically at sites with water depths that were higher than the average. These three species were also typically found at low elevations. On the other hand, *S. lundstromi* occurred most frequently at higher elevation and was found in shallower waters. The other ten blackfly taxa recorded in our study had close to average values of depth and elevation, with no significant deviations.

The specific values for the four environmental variables previously identified in the CCA (elevation, depth, temperature and conductivity) at which taxa occurred with highest probability and their ecological amplitude provided information regarding niche specificity of the pre-imaginal stages (Figure 4). Regarding elevation (Figure 4a), *S. aureum* occurred with highest probability at ~75 masl with narrow deviations, indicating that the pupae of these species were restricted to upper reaches in England. On the other hand, *S. lineatum* was restricted to lower elevations (~15 masl). Regarding ecological amplitude, *S. erythrocephalum*, *S. intermedium s.l.*, *S. angustitarse* and *S. rubzovianum* displayed wider elevation ranges than the rest of the taxa. Depth was the main environmental gradient in our study (Figure 4b), with *S. lineatum* occurring with highest probability at the deeper sites (> 0.5 m). The depth distribution of this species clearly differed from those of the other species, whose depth ranges overlapped to a greater extent. As shown in Figure 4d, there was considerable overlap regarding temperature ranges of the larvae and pupae. Overall, we observed wide ranges for temperature in which pre-imaginal stages were found, with *S. aureum* standing out from the rest (range: 8.8-16.9º C). This species also exhibited the widest range regarding conductivity values (278.6-835.8 μS cm^{-1}). The PERMANOVA analysis revealed no significant differences in the simuliid pupal assemblages between substrates ($F_{4,33} = 0.856$, $p = 0.62$). Indeed, we did not find any significant association between particular species and substrates ($p > 0.05$ in all cases). *Simulium aureum* is the species that exhibits the widest range of electrical conductivity, but also has a narrow tolerance for elevation, being restricted to upper reaches in England. *Simulium lineatum* is restricted to areas of low elevation where it exhibits its maximum occurrence. In addition, its river depth distribution also clearly differs from the rest of the species, being found in rivers with greater depths. *Simulium erythrocephalum*, *S. intermedium s.l.*, *S. angustitarse* and *S. rubzovianum* show wider elevation ranges than the rest of the taxa.

4. Discussion

The geographical distributions of larvae and pupae of a variety of simuliid species in southern England were investigated in this study. Previous studies in the region have reported similar simuliid species to those reported here (Bass 1990; Crosskey 1985), but this is the first to investigate their associations with environmental factors using multivariate methods. It is important to understand the factors associated with blackfly species' breeding habitats, above all of those with biomedical and veterinary significance, since, from an applied point of view, knowing the ecology and ethology of these species can be necessary when designing monitoring, prevention and control programmes (Machtinger et al. 2015).

The results of this study support a conclusion that the distribution and species assemblages of pre-imaginal stages of southern English blackflies are significantly associated with a suite of environmental variables including water depth, conductivity, temperature and elevation. Regarding elevation, previous studies have also shown this to be one of the most important abiotic conditions associated with blackflies' distribution patterns (Martínez and Portillo 1999; Crosskey and Crosskey 2000). Moreover, other studies have also highlighted the role of climatic factors such as temperature and topographic variables such as elevation, in shaping assemblages of simuliids' pre-imaginal stages (McCreadie et al. 2005; Ya'cob et al. 2016; Cheke et al. 2017; López-Peña et al. 2020).

Figure 5 shows the values for water velocity (cm/s), TDS (ppm) and oxygen availability (%) at which species occurred with highest probability and their ecological amplitude. These variables were excluded from the ordination analyses because they were highly correlated with other variables found to be more informative or because they were not always measured. However, these variables have been found to be useful for discriminating between the pre-imaginal niches of blackfly taxa in other regions such as Eastern Spain (López-Peña et al. 2020). We can compare our results on water velocity conditions (Figure

5a) in which the larvae and pupae of the identified blackly taxa were found with the results of previous studies in other regions. Thus, *S. lineatum* is shown to be one of the most rheophile species in this and other studies (Bernotienë 2006; López-Peña et al. 2020). Our results also illustrate the broad tolerance to water current velocity conditions of taxa such as *S. rubzovianum* and *S. intermedium s.l.* (Gallardo-Mayenco and Toja 2002; López-Peña et al. 2020) and provide evidence that *S. angustitarse* and *S. trifasciatum* are found across the widest ranges of current velocities. For those taxa, common in this study and that of López-Peña et al. (2020) in the Iberian Peninsula, for which more ecological information is available, the rank distribution regarding water velocity was quite similar (Spearman's r = 0.636). *Simulium lineatum* was found in higher occurrence at lower values of TDS (Figure 5b), while in López-Peña et al. (2020) this was found to be the case for *S. carthusiense* (unrecorded in the UK), while *S. equinum* showed the widest TDS range but in López-Peña et al.'s study (2020) this was true (for turbidity) of *S. bezzi* (unrecorded in the UK). Finally, regarding oxygen, *S. petricolum* and *S. rubzovianum* were found in the widest ranges, but in López-Peña et al.'s study (2020) *S. equinum* had the widest range. In contrast, *S. lineatum* was found only at well oxygenated sites (Figure 5c).

Our study revealed a suite of variables significantly associated with simuliid larvae and pupae assemblage composition. These variables provide comprehensive information regarding niche specificity of the pre-imaginal stages of simuliid species.

Our study was not comprehensive as some taxa would have been missed, given that some occur as larvae and pupae only in certain periods of the season (e.g., univoltine species that pupate during late spring or summer) when we were not able to sample. Besides, regarding the environmental variables, some authors have demonstrated that simuliid species composition varies throughout rivers, streams and rivulets according to elevation (McCreadie et al. 2005; Mantilla et al. 2018; Jitklang et al. 2020). Moreover, the water temperature is a very well-known environmental variable associated with Simuliidae species distribution (McCreadie et al. 2005; Bernotienë 2006, Nascimento et al. 2007; Jitklang et al. 2020).

Most of the species that we found were not unexpected, but the widespread distribution of *S. petricolum*, which we found at six locations, is of interest since this species has only recently been reported from the UK. Day et al. (2010) considered *S. petricolum* to be rare in the UK and reported it from only two sites in Sussex and one in Buckinghamshire.

This study provided information about the distribution patterns and abundance of pre-imaginal stages (regarding mature larvae and pupae) in a selection of English watercourses and showed that the diversity of blackfly species and their assemblages mainly change according to variations in elevation, river depth, water temperature and conductivity. We suggest future studies should take a closer look at whole year distributions and a wider geographical range to obtain enough data to confirm or refute our conclusions.

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Author Contributions

All authors of the present paper declare that have made substantial contributions to the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the manuscript, revising it critically for important intellectual content, and final approval of the version to be submitted.

Data Availability Statement

Most of the data that support the findings of this study are given in the Tables and Supporting Information but other details are available from the authors, upon reasonable request.

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Table 1. Code numbers, names, dates, geographical coordinates and elevations of the watercourses sampled.

Sampling point numerical name	**Water's body name**	**Locality and County**	**Date**	**Latitude**	**Longitude**	**Elevation (m)**
1 (A)	Artificial pond	Bramley (Hampshire)	22/7/20	51° 19' 28.7" N	01° 04' 35.4" W	67
1 (B)	Artificial pond	Bramley (Hampshire)	25/7/20	51° 19' 28.7" N	01° 04' 35.4" W	67
1 (C)	Artificial pond	Bramley (Hampshire)	21/8/20	51° 19' 28.7" N	01° 04' 35.4" W	67
1 (D)	Artificial pond	Bramley (Hampshire)	10/11/13	51° 19' 28.7" N	01° 04' 35.4" W	67
1 (E)	Artificial pond	Bramley (Hampshire)	16/8/13	51° 19' 28.7" N	01° 04' 35.4" W	67
2 (A)	Bow Brook	Bramley (Hampshire)	26/7/20	51° 19' 16.7" N	01° 04' 37.1" W	57
2 (B)	Bow Brook	Bramley (Hampshire)	22/8/20	51° 19' 16.7" N	01° 04' 37.1" W	57
3 (A)	Bow Brook	Bramley (Hampshire)	28/7/20	51° 19' 11.3" N	01° 04' 19.1" W	58
3 (B)	Bow Brook	Bramley (Hampshire)	22/8/20	51° 19' 11.3" N	01° 04' 19.1" W	58
4 (A)	River Whitewater	Greywell (Hampshire)	29/7/20	51° 15' 22.4" N	00° 58' 03.1" W	46
4 (B)	River Whitewater	Greywell (Hampshire)	9/9/20	51° 15' 22.4" N	00° 58' 03.1" W	46
5 (A)	River Whitewater	North Wapnborough (Hampshire)	29/7/20	51° 15' 53" N	00° 57' 00" W	73
5 (B)	River Whitewater	North Wapnborough (Hampshire)	9/9/20	51° 15' 53" N	00° 57' 00" W	73
6 (A)	River Loddon	Sherfield on Loddon (Hampshire)	4/8/20	51° 19' 12.5" N	01° 01' 15.1" W	47
6 (B)	River	Sherfield on	6/9/20	51° 19' 12.5" N	01° 01' 15.1" W	47

	Loddon	Loddon (Hampshire)				
7 (A)	River Lyde	Sherfield on Loddon (Hampshire)	4/8/20	51º 19' 23.8" N	01º 01' 18.7" W	44
7 (B)	River Lyde	Sherfield on Loddon (Hampshire)	6/9/20	51º 19' 23.8" N	01º 01' 18.7" W	44
8 (A)	River Row	Old Basing (Hampshire)	15/8/20	51º 16' 55.1" N	01º 02' 13.5" W	79
8 (B)	River Row	Old Basing (Hampshire)	6/9/20	51º 16' 55.1" N	01º 02' 13.5" W	79
9	River Wey	Weymouth, Radipole (Dorset)	16/9/20	50º 37' 11.0" N	02º 27' 47.0" W	1
10	River Wey	Weymouth, Radipole Lake (Dorset)	16/9/20	50º 37' 10.5" N	02º 27' 57.1" W	1
11	Wannerton Brook	Blakedown, Kidderminster, Hurcott Pool and Woods Nature Reserve (Worcestershire)	20/9/20	52º 23' 56.0" N	02º 12' 34.3" W	50
12	Wannerton Brook	Blakedown, Kidderminster, Hurcott Pool and Woods Nature Reserve (Worcestershire)	20/9/20	52º 24' 00.7" N	02º 12' 00.8" W	50
13	River Great Ouse	Bedford, Fenlake Meadows and Priory Country Park (Bedfordshire)	20/9/20	52º 07' 45.7" N	00º 26' 26.9" W	21
14	Bickerley Millstream	Ringwood (Hampshire)	30/9/20	50°49' 58"N	01°47' 49"W	5
15	River Avon	Ringwood (Hampshire)	30/9/20	50º 50' 08.5" N	01º 47' 26.6" W	9
16	River Wey	Radipole (Dorset)	19/10/20	50º 37'50.0" N	02º 28' 31.5" W	1
17	River Wey	Radipole (Dorset)	19/10/20	50º 38' 15.8" N	02º 28' 41.9" W	5
18	River Wey	Radipole (Dorset)	19/10/20	50º 37' 55.0" N	02º 28' 46.4" W	1

Table 2. Blackfly species found as larvae (L) and pupae (P) according to watercourses. Numbers in parentheses are sample sizes (total n= 29).

Species	**Artificial pond (5)**	**Bow Brook (4)**	**Whitewater (4)**	**Loddon (2)**	**Lyde (2)**	**Row (2)**	**Wey (5)**	**Wannerton Brook (2)**	**Bickerley Millstream (1)**	**Avon (1)**
S. aureum	9P		4P							
S. angustipes	41L, 38P	58L, 20P	20L, 3P		1L, 2P			2L, 48P	1L, 1P	
S. angustitarse		1P	2L, 1P		1L		1L			
S. equinum			5L	72L, 65P	24P	1P	1P		6L, 5P	2P
S. erythrocephalum		1L	3P	1L, 5P					16P	6P
S. intermedium		10P	40L, 75P	4L, 1P	10L, 84P	7L	69L, 82P	25L, 49P	4L, 4P	
S. lineatum			2L	1L, 1P	22P				8L, 88P	55L, 214P
S. lundstromi		2L	1P		5P			1L, 8P		
S. morsitans	2P	1P	36P	5P	57P		1L, 34P	92P	2P	
S. noelleri	31L, 32P	83L, 2P		1P	7P		16P	2L, 26P		
S. ornatum		1L	62L, 31P	14L	35L, 9P	10L	16L	34L, 4P		
S. petricolum	11L, 19P	7L, 26P	2P		3L, 3P		6L, 3P		5L, 3P	
S. trifasciatum		2L, 4P	17L, 15P	4P	20P		6L, 3P	14L, 8P		
S. rubzovianum	1L, 33P	6L, 6P	2L, 22P				2L, 3P			

Figure captions

Figure 1. Map of the study area, showing the locations of the sampling points. The insets show enlargements of the areas sampled together with code numbers for the sites as listed in Table 1.

Figure 2. Principal components analysis (PCA) biplot showing the sampling points with presence of simuliids pre-imaginal stages in relation to environmental variables.

Figure 3. Canonical correspondence analysis (CCA) ordination biplot of simuliid pre-imaginal assemblages. Sampling sites are represented by grey circles, while scores for simuliid taxa are represented by black crosses. Environmental variables were selected using forward selection and Monte Carlo permutation tests (see ‘‘Methods’’).

Figure 4. Values of highest occurrence (modes) and ranges of distribution (error bars) for the four most important environmental variables a) elevation, b) river depth, c) temperature, and d) conductivity for the pre-imaginal stages of the 14 simuliid taxa ~~species~~ found in this study. Species are arranged on the y-axis by increasing optima for each variable.

Figure 5. Values of highest occurrence (modes) and ranges of distribution (error bars) for the environmental factors a) velocity, b) TDS and c) oxygen for the pre-imaginal stages of the 14 simuliid taxa found in this study.

Fig. 1.

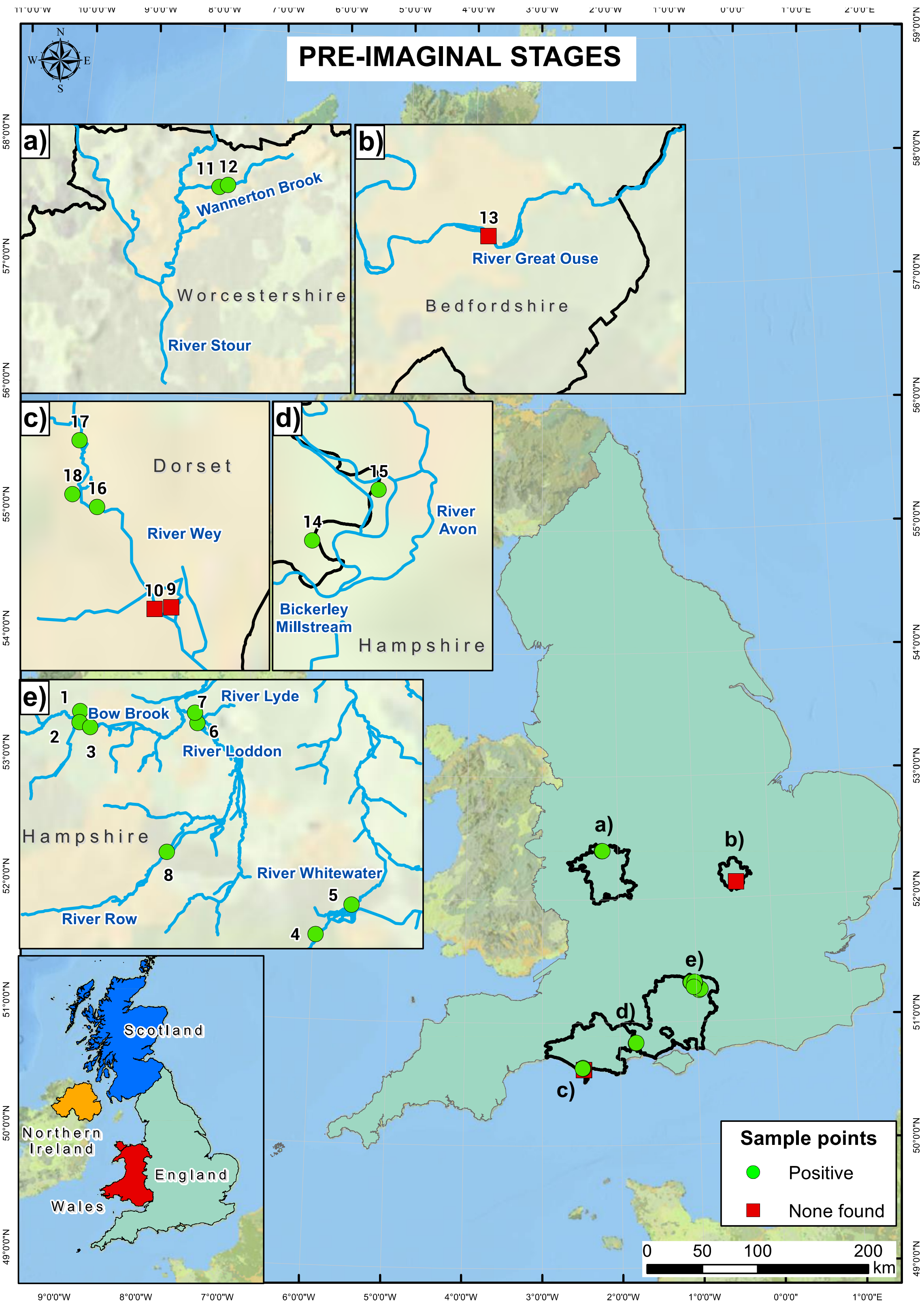
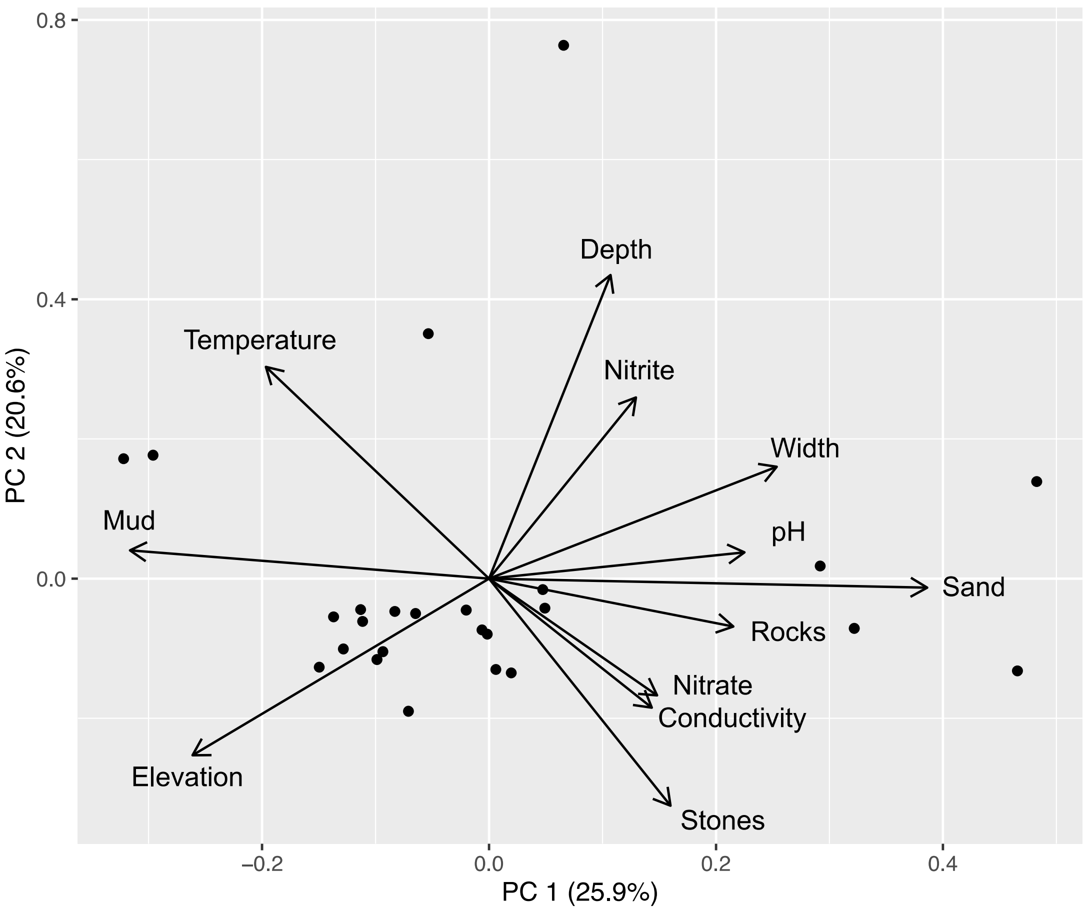

PRE-IMAGINAL STAGES
a)
11 12
Wannerton Brook
Worcestershire
River Stour
b)
13
River Great Ouse
Bedfordshire
c)
17
18 16
Dorset
River Wey
10 9
d)
15
14
River Avon
Bickerley Millstream
Hampshire
e)
1
Bow Brook
2
3
7
River Lyde
6
River Loddon
Hampshire
8
River Whitewater
5
River Row
4
Scotland
Northern Ireland
England
Wales
Sample points
Positive
None found
0 50 100 200 km

Fig. 2.



0.8
0.4
0.0
−0.2
0.0
0.2
0.4
PC 2 (20.6%)
PC 1 (25.9%)
Depth
Temperature
Nitrite
Width
Mud
pH
Sand
Rocks
Nitrate
Conductivity
Elevation
Stones

Fig. 3.

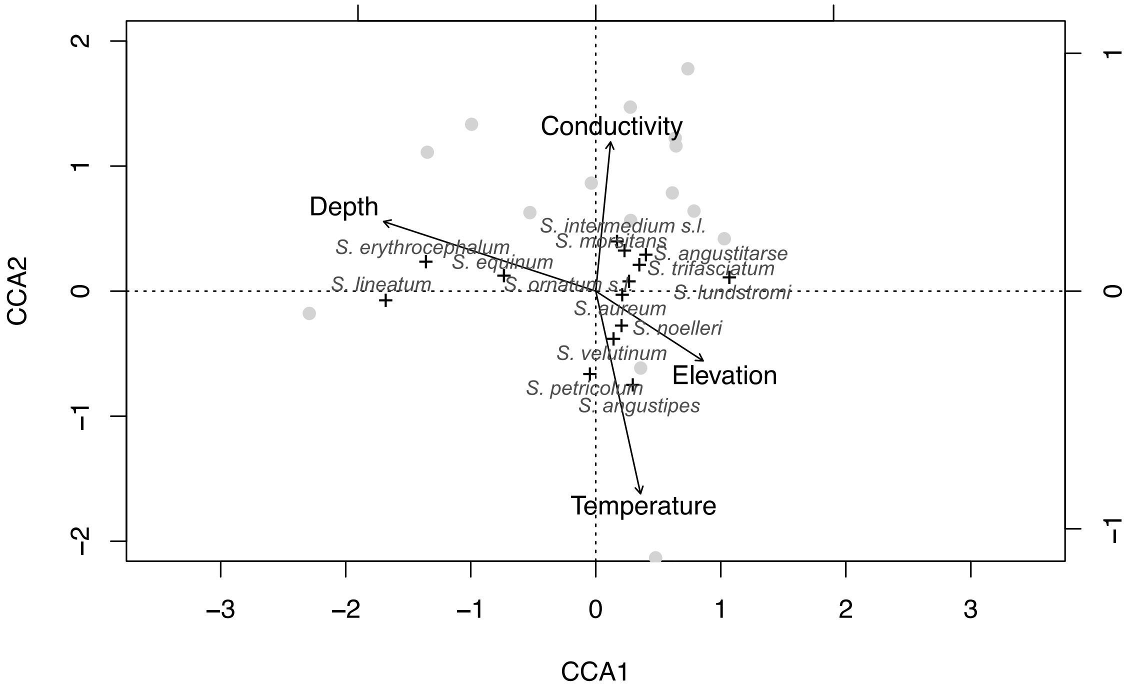

Conductivity
Depth
S. intermedium s.l.
S. monticola
S. erythrocephalum
S. equinum
S. angustitarse
S. trifasciatum
S. lineatum
S. ornatum s.l.
S. lundstromi
S. aureum
S. noelleri
S. velutinum
Elevation
S. petricolum
S. angustipes
Temperature
CCA1
CCA2

Fig. 4.

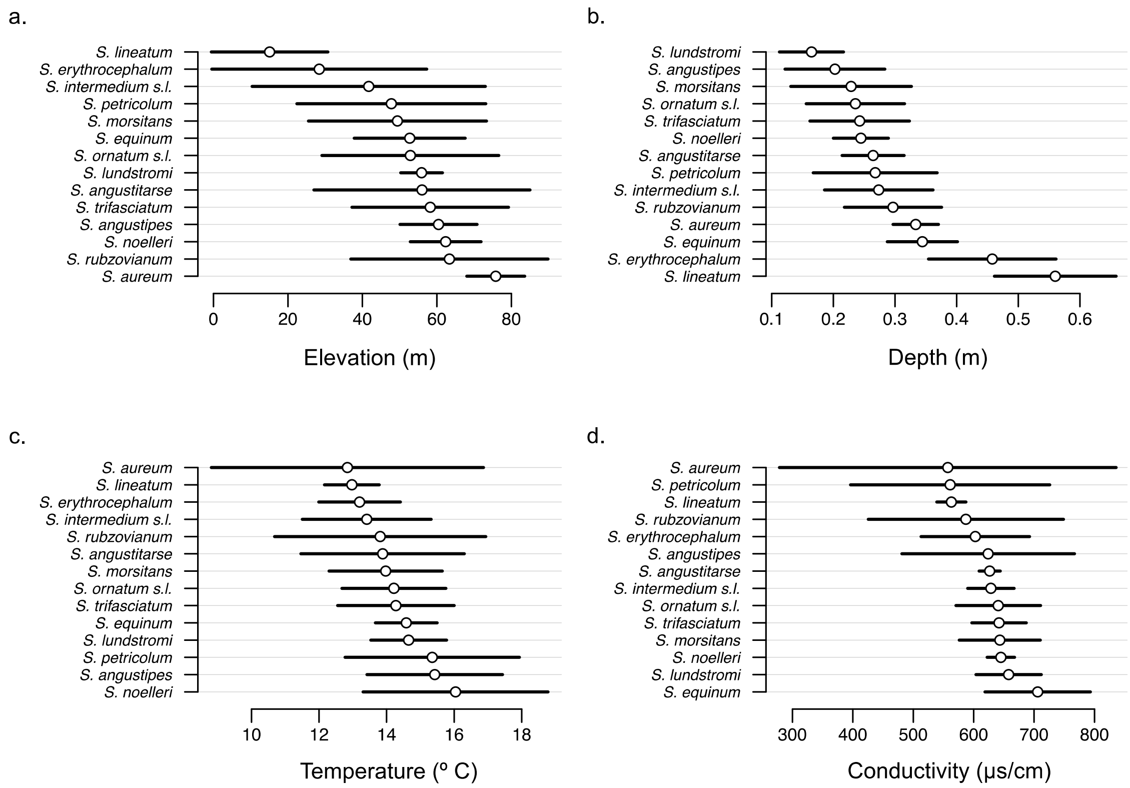
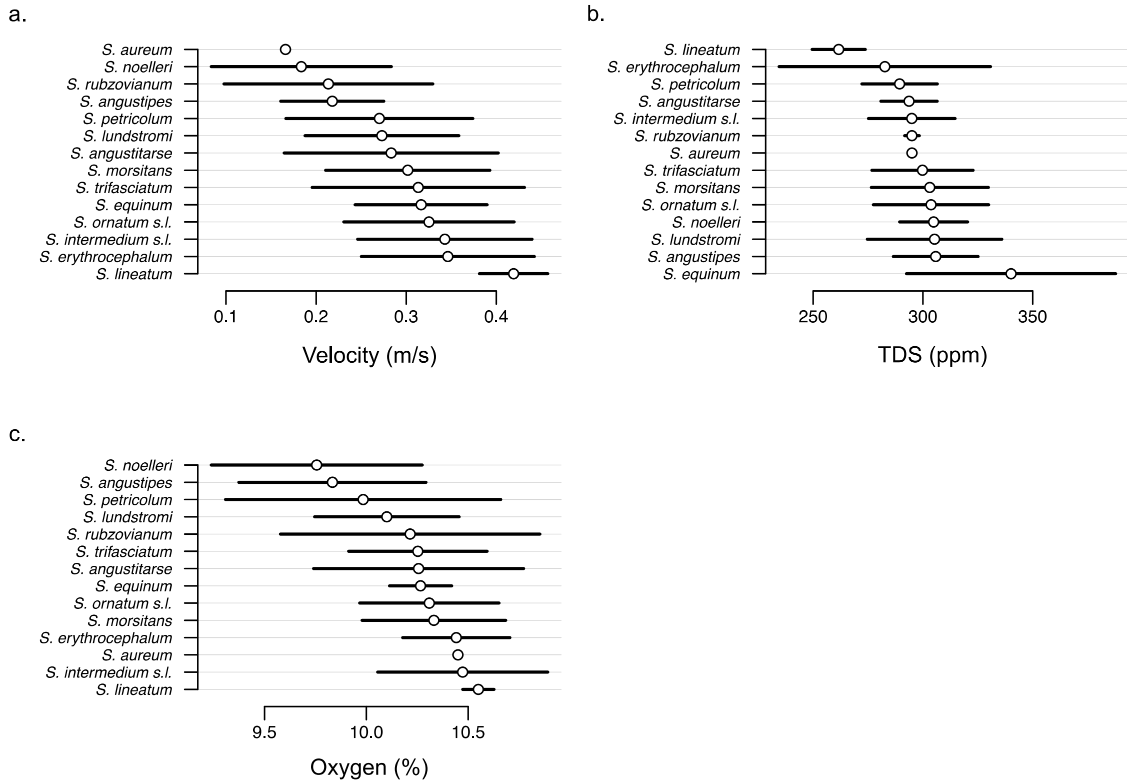

a.
S. lineatum
S. erythrocephalum
S. intermedium s.l.
S. petricolum
S. morsitans
S. equinum
S. ornatum s.l.
S. lundstromi
S. angustitarse
S. trifasciatum
S. angustipes
S. noelleri
S. rubzovianum
S. aureum
0 20 40 60 80
Elevation (m)
b.
S. lundstromi
S. angustipes
S. morsitans
S. ornatum s.l.
S. trifasciatum
S. noelleri
S. angustitarse
S. petricolum
S. intermedium s.l.
S. rubzovianum
S. aureum
S. equinum
S. erythrocephalum
S. lineatum
0.1 0.2 0.3 0.4 0.5 0.6
Depth (m)
c.
S. aureum
S. lineatum
S. erythrocephalum
S. intermedium s.l.
S. rubzovianum
S. angustitarse
S. morsitans
S. ornatum s.l.
S. trifasciatum
S. equinum
S. lundstromi
S. petricolum
S. angustipes
S. noelleri
10 12 14 16 18
Temperature (º C)
d.
S. aureum
S. petricolum
S. lineatum
S. rubzovianum
S. erythrocephalum
S. angustipes
S. angustitarse
S. intermedium s.l.
S. ornatum s.l.
S. trifasciatum
S. morsitans
S. noelleri
S. lundstromi
S. equinum
300 400 500 600 700 800
Conductivity (µs/cm)

Fig. 5.


a.
S. aureum
S. noelleri
S. rubzovianum
S. angustipes
S. petricolum
S. lundstromi
S. angustitarse
S. morsitans
S. trifasciatum
S. equinum
S. ornatum s.l.
S. intermedium s.l.
S. erythrocephalum
S. lineatum
0.1
0.2
0.3
0.4
Velocity (m/s)
b.
S. lineatum
S. erythrocephalum
S. petricolum
S. angustitarse
S. intermedium s.l.
S. rubzovianum
S. aureum
S. trifasciatum
S. morsitans
S. ornatum s.l.
S. noelleri
S. lundstromi
S. angustipes
S. equinum
250
300
350
TDS (ppm)
c.
S. noelleri
S. angustipes
S. petricolum
S. lundstromi
S. rubzovianum
S. trifasciatum
S. angustitarse
S. equinum
S. ornatum s.l.
S. morsitans
S. erythrocephalum
S. aureum
S. intermedium s.l.
S. lineatum
9.5
10.0
10.5
Oxygen (%)